Diet composition of two hylid species (*Dendropsophus haraldschultzi* and *D. minutus*) in anthropogenic environments in eastern Amazonia

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ABSTRACT

The diet composition of hylids ranges from generalist to specialist, depending on the relative proportions of different types of prey found in their gastrointestinal tracts and trophic niche. Here, we report the diet composition and compare the niche breadth of two syntopic hylid species in eastern Amazonia, *Dendropsophus haraldschultzi* and *D. minutus*, which use anthropogenic environments during the rainy season. We collected 32 individuals of *D. haraldschultzi* and 30 individuals of *D. minutus*. The most important preys found in the diet of *D. haraldschultzi* were Hemiptera (34.6%), whereas Lepidoptera larvae (63.8%) were predominant in the diet of *D. minutus*. Both *Dendropsophus* species had a similar niche breadth with generalist characteristics. The consumption of mobile and slow-moving prey, and hard-bodied and soft-bodied arthropods, indicated a combined use of both "sit-and-wait" and "active search" foraging strategies.

Key Words: Trophic ecology; generalist; anurans; natural history; urbanization.

Anurans are opportunistic and generalist predators, feeding on any potential prey available in the environment (Ceron *et al.,* 2018; Michelin *et al.,* 2020; Moroti *et al.,* 2021), and their diet composition is closely related to foraging strategies (Toft, 1980; 1981; Huey and Pianka, 1981; Taigen and Pough, 1983). Active foragers are effective in capturing small, slow-moving prey that sting or those generally considered distasteful to predators (e.g., ants and termites) and have specialized feeding habits (Toft, 1981). In contrast, sit-and-wait foragers are effective in capturing actively moving prey (e.g., coleopterans and orthopterans) and have generalized feeding habits (Toft, 1981). However, most species can also adapt their feeding strategy according to food availability (Menin *et al.,* 2005; Petrozzi *et al.,* 2021).

The diet composition of hylids ranges from generalist (Leivas *et al.,* 2018; Moser *et al.,* 2019; Silva *et al.,* 2021) to specialist (Parmelee, 1999; Castro *et al.,* 2016), depending on the relative proportions of different types of prey found in their gastrointestinal tracts and trophic niche (López *et al.,* 2009). Among the Neotropical hylids, *Dendropsophus haraldschultzi* (Bokermann 1962) and *D. minutus* (Peters, 1872) are two small-sized, nocturnal, arboreal species. *Dendropsophus haraldschultzi* can be found perched on leafy branches or aquatic vegetation in floating meadows associated with ponds and large (permanent) rivers that discharge into the Amazon River (Hödl, 1977; Böning *et al.,* 2017; Menin *et al.,* 2020). *Dendropsophus minutus* inhabits a variety of open and forest habitats and can be found in both natural and anthropogenic environments (Abegg *et al.,* 2014; Leivas *et al.,* 2018).

The diet of *D. haraldschultzi* is unknown. The few aspects of its biology and natural history already studied include (i) a brief call description of a population from the Amazon basin (Hödl, 1977), (ii) a possible association with bromeliads and an Amazonian plant species in northern Brazil (Sanches *et al.,* 2019; Figueiredo *et al.,* 2021), and (iii) the tadpole description (Menin *et al.,* 2020). In contrast, previous studies on the diet composition of *D. minutus* were based on populations from northern (Van Sluys and Rocha, 1998), northeastern (Santos *et al.,* 2004), and southern (Leivas *et al.,* 2018) Brazil. Despite this, little is known about the diet of these two species in anthropogenic environments in Amazonia. Here, we report the diet composition and compare the niche breadth of these two hylid species during the rainy season.

We collected the two hylid species during the rainy season, from February to June 2019, using the nocturnal visual search method (Crump and Scott Jr., 1994). Both species were collected in Amapá state, eastern Amazonia, northern Brazil. *Dedropsophus haraldschultzi* was sampled in a seasonally flooded area (regionally referred to as the "ressaca" area) surrounding human settlements in the municipality of Santana (0.0365°S, 51.1626°W). *Dendropsophus minutus* was sampled in an abandoned temporary pool in the urban perimeter of the municipality of Serra do Navio (0.9066°N, 52.0073°W). Specimens were collected under ICMBio/RAN, Institutes of Ministry of Environment, Government of Brazil, permit number 48102-2. This permit was subject to the approval of all procedures for collecting and euthanizing organisms. Hylids were euthanized because this work was part of a research project on anuran-parasite networks, whose methodology requires animal euthanasia. Both species are classified as "Least Concern" according to the International Union for the Conservation of Nature (IUCN, 2022). All individuals are housed at Coleção Herpetológica of the Universidade Federal do Amapá, in Macapá, Brazil with the acronym CECC.

We euthanized the specimens with a topical anesthetic (lidocaine), fixed them in formaldehyde 10%, and preserved them in ethanol 70%. We dissected the preserved specimens to remove the gastrointestinal tract (stomach and intestine). For the diet analysis, we determined the prey items to the taxonomic level or Order by analyzing the material under a stereomicroscope, following the taxonomic key developed by Rafael *et al.* (2012). We measured

the width (w) and length (l) of all prey items to estimate the ellipsoid volume per prey using Griffiths and Mylotte's (1987) formula: $V = (4\pi/3) (l/2) (w/2)^2$. To determine the importance of each prey item, we used the Pinkas *et al.* (1971) importance index through the occurrence percentage (F%), numeric percentage (N%), and volumetric percentage as follows: IRI = F% (N% + V%).

We calculated the amplitude of the trophic niche through the Levins' Trophic Niche Amplitude Index (B) (Krebs, 2004), defined by: $B = 1 / \Sigma pi^2$, in which p is the proportion of individuals of a given resource i (taxon) found in the diet. To facilitate comparisons between the two species, we calculated the standardized Levins' index (Bsta), which limits the index to a scale from 0 to 1 according to the following equation: Bsta = (B-1) / (n-1), where n is the number of resources (prey categories) recorded. Values near zero are assigned to a specialized diet, whereas those closer to 1 are to a generalist diet.

To estimate the richness of prey categories in the diet of each species, we made rarefaction curves based on samples using Estimates 9 (Colwell, 2013), with 1,000 random permutations with no reposition. This analysis treated stomachs as samples and prey types as richness categories (curves were made for each species).

We collected 62 individuals, 32 individuals of *D. haraldschultzi* and 30 of *D. minutus*. Only ten individuals of *D. haraldschultzi* (31.3%) and 14 of *D. minutus* (46.7%) analyzed had gastrointestinal contents. For *D. haraldschultzi*, four prey categories were identified: Coleoptera, Coleoptera larvae, Hemiptera, and Isoptera (Table 1). Hemipterans were the most important prey category (34.6%). *Dendropsophus minutus* exhibited a lower richness of prey categories: Araneae, Diptera, and Lepidoptera larvae (Table 1). Lepidoptera larvae were the most important prey category (63.8%). *Dendropsophus haraldschultzi* (Bsta = 0.82) and *D. minutus* (Bsta = 0.73) displayed a similar niche breadth.

The number of prey categories consumed by *D. haraldschultzi* and *D. minutus* was lower than that reported for congeners (*D. branneri*, Castro *et al.,* 2016; *D. counani*, Sanches *et al.,* 2021; *D. microcephalus*, Fonseca-Pérez *et al.,* 2017; *D. minutus*, Van Sluys and Rocha, 1998; Santos *et al.,* 2004; Leivas *et al.,* 2018; *D. nanus*, and *D. sanborni*, Menin *et al.,* 2005). The lower number of prey categories in the diet of *D. haraldschultzi* and *D. minutus* in anthropogenic areas are consistent with findings of

Table 1. Prey categories found in the gastrointestinal contents of *Dendropsophus haraldschultzi* and *D. minutus* in anthropogenic environments in eastern Amazonia. N = number of individuals, F = frequency of occurrence of prey categories, V = total volume (in mm3) occupied by prey categories, IRI = Index of Relative Importance, (%) = percentage values over the total number of prey items.

Prey categories	*Dendropsophus haraldschultzi* (N = 32)				*Dendropsophus minutus* (N = 30)			
	N (%)	F (%)	V (%)	IRI	N (%)	F (%)	V (%)	IRI
Araneae	-	-	-	-	5 (35.7)	4 (50.0)	23.1 (45.7)	43.8
Coleoptera	4 (18.2)	2 (20.0)	1.9 (13.4)	17.2	-	-	-	-
Coleoptera larvae	3 (13.6)	1 (10.0)	1.5 (10.3)	11.3	-	-	-	-
Diptera	-	-	-	-	2 (14.3)	2 (25.0)	5.2 (10.3)	16.5
Hemiptera	3 (13.6)	3 (30.0)	8.4 (60.0)	34.6	-	-	-	-
Isoptera	10 (45.5)	2 (20.0)	2.3 (16.3)	27.2	-	-	-	-
Lepidoptera larvae	-	-	-	-	7 (50.0)	2 (25.0)	58.8 (116.4)	63.8

previous studies (Menin *et al.,* 2015; Santana *et al.,* 2019; Sanches et al., 2019) and indicate that human alteration of natural areas likely reflects the diet composition of anurans.

Although *D. haraldschultzi* and *D. minutus* exhibited a similar trophic niche breadth with generalist characteristics, the prey rarefaction curve in relation to the number of stomachs analyzed reached the asymptote, indicating that the number of individuals sampled was sufficient to estimate the richness of prey items (Fig. 1).
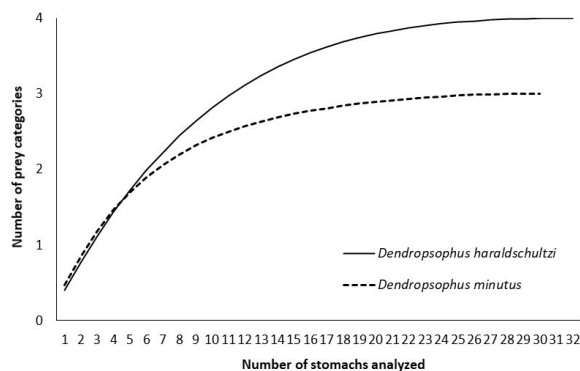

Figure 1. Accumulation curve showing the number of prey categories relative to the number of stomachs containing prey items analyzed in the diet of *Dendropsophus haraldschultzi* and *D. minutus* in anthropogenic environments in eastern Amazonia.

Both *Dendropsophus* species consumed ephemeral resources, such as larvae (coleopterans and lepidopterans) and termites, reinforcing the idea that anurans are opportunistic predators (Toft, 1981). Although the two species were sampled in anthropogenic environments, *D. haraldschultzi* had a higher numerical frequency of prey due to the greater availability of arthropods associated with aquatic macrophytes where this species was sampled (see Menin *et al.,* 2020). On the other hand, the low numerical frequency of prey found in *D. minutus* when compared to natural environments, such as the ombrophilous forest in Amazonia (N = 6 preys; Van Sluys and Rocha, 1998); semi-deciduous rainforest in northeastern Brazil (N = 6 prey; Santos *et al.,* 2004), and ombrophilous mixed forest in southern Brazil (N = 10 prey; Leivas *et al.,* 2018) should be related to the sampled environment, swimming temporary rain pool in an urban area.

The consumption of mobile (e.g., Araneae, Coleoptera, Diptera, and Hemiptera) and small and slow-moving prey (e.g., larvae and termites) and two types of prey in different proportions in their diet: hard-bodied (e.g., Coleoptera and Hemiptera) and soft-bodied arthropods (e.g., Araneae, larvae, Diptera, and Isoptera), indicated a combined use of both "sit-and-wait" and "active search" foraging strategies (Toft, 1980; Huey and Pianka, 1981).

The fact that the two studied hylid species were collected during the rainy season limited the interpretation of our results. Future research focusing on increased sampling during the rainy and dry seasons and assessing prey availability in non-urbanized environments will produce relevant information about the diet composition, foraging strategies, and how species act in trophic webs. Nevertheless, our findings provide a basic description of the diet of *D. haraldschultzi* and *D. minutus* in Amazonia, which can help us understand more aspects of the natural history of these anuran species in urban landscapes.

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