NORTH-WESTERN JOURNAL OF ZOOLOGY 17 (1): 29-33
Article No.: e201507

http://biozoojournals.ro/nwjz/index.html

Scinax fuscovarius (Anura, Hylidae) diet assessment at Serra da Bodoquena, Mato Grosso do Sul, Brazil

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Received: 22. August 2019 / Accepted: 08. July 2020 / Available online: 15. August 2020 / Printed: June 2021

Abstract. Amphibians control the population size of various invertebrate species and are food sources for other predators. Their diets can be influenced by characteristics that facilitate prey discovery according to foraging strategies that vary between anuran species. The diets of seventy-five *Scinax fuscovarius* specimens collected through active nocturnal searches at the Estância Mimosa farm, in the municipality of Bonito, Mato Grosso do Sul, Brazil were assessed. Thirty-nine of the stomachs (52%) contained food items, comprising 94 items and 28 prey categories, divided into three groups (insects, arachnids and crustaceans). Orthoptera was the most frequent prey item, while Araneae was the most abundant prey and Hemiptera Cicadellidae was the most voluminous prey category. The results indicate that *S. fuscovarius* is a generalist and opportunistic predator, using a "sit and wait" foraging strategy. Prey volume was not associated with individual size, but the number of preys per individuals was related to frog size. Variation in diet composition throughout the different life stages along with reproductive activity may explain the low similarity found in the diets of male, female and juvenile individuals.

Key words: amphibians, food items, predator, prey.

Introduction

Amphibians occupy important trophic positions in terrestrial and aquatic ecosystems as they control the population size of several invertebrate species and are food sources for other predators, such as reptiles, birds and mammals (Toledo et al. 2007, Wells 2007). Amphibian diets represent the importance of this group in trophic networks, since this group helps control the population size of the invertebrates that are abundant in certain habitats and during certain periods (Attademo et al. 2005, Duré et al. 2009).

Many factors are determinant of amphibian diets, which are generally influenced by morphological, physiological and behavioral characteristics that facilitate prey discovery, identification and ingestion (Bellocq et al. 2000, Rodrigues et al. 2004). Foraging strategies vary among anuran species, permitting guild classification, and may also directly influence the types of prey ingested (Strüssmann et al. 1984, Van Sluys & Rocha 1998). Most anurans are generalists and opportunistic consumers, ingesting a wide variety of food items according to environmental prey availability (Toft 1981). Dietary variations also reflect the composition of food items present in a particular area (Rodrigues et al. 2004). Differences in the dietary composition among individuals of a certain population associated with prey selection behaviors can reduce intraspecific competition by sharing available resources (Van Sluys & Rocha 1998, Bonansea & Vaira 2007, López et al. 2007).

Although the trophic ecology of several anuran species has been studied (e.g., Moser et al. 2017), the food items comprising the diets of many species, while abundant and widely distributed, have not been assessed. *Scinax fuscovarius* (Lutz, 1925) occurs in the Midwest, and Southeast regions of Brazil, as well as in northern Argentina, Paraguay and Bolivia (Uetanabaro et al. 2007, Haddad et al. 2008, Frost 2019). This species is arboreal and found in open Cerrado areas and close to dams during its reproductive period (Rossa-Feres & Jim 2001, Melo et al. 2007). Dietary information on this species is limited to feeding reports about its tadpole stage, with one study carried out in Argentina and one in São Paulo State, Brazil (Rossa-Feres et al. 2004, Echeverría et al. 2007). However, as adults, *Scinax* species are considered generalist feeders, displaying mainly arthropod-based diets comprised of arachnids, blattarids, beetles and hemipterans (Astwood-Romero et al. 2016, Solé et al. 2005).

Given the scarcity of information about the trophic ecology of *S. fuscovarius*, the diet composition of this species in a population from the Serra da Bodoquena region in Mato Grosso do Sul, Brazil, is described herein. The specific aims of this study were to (i) identify the most important prey items present in *S. fuscovarius* diet; (ii) verify if predator size is related to prey volume or abundance and (iii) evaluate if similarities in prey composition between groups (males, females and juveniles) are correlated to individual sizes.

Material and Methods

A total of 75 *S. fuscovarius* specimens (38 males, 31 females and 6 juvenile specimens) were collected in October and November of 2015 and April and May of 2016, corresponding to this species' reproductive season in the region (Rodrigues et al. 2005). The study area is located in the Serra da Bodoquena region, at the Private Natural Heritage Reserve Estância Mimosa Farm, Bonito municipality, in the south-central region of Mato Grosso do Sul State (20°58'49"S, 56°30'32"W; *datum*: WGS84). The region is characterized by vegetation that varies according to relief and soil type, which presents several forest phytophysiognomies, and is represented by its altitude and the division between the Paraguay River basin and the Apa and Miranda sub-basins (Boggiani 1999).

The specimens were collected through active nocturnal searches (Heyer et al. 2014). They were then sacrificed with Xylocaine® 5% ointment, rubbed over the abdomen and absorbed into the skin, according to the collection license provided by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio: 50586-1). The stomach flushing technique was not used because the examined anurans were also used for another study, which required euthanasia to obtain samples.

Snout-vent length (SVL) were measured for each specimen with a digital caliper (0.1 mm precision), and sex was defined at necropsy of the specimen, by visualizing its gonads. The stomachs of each specimen were removed through a small abdominal incision and fixed in 70% ethyl alcohol for immediate digestion termination. The anurans were fixed in 4% formaldehyde, preserved in 70% alcohol, and deposited into the Mato Grosso do Sul Federal University Zoological Reference Collection (ZUFMS-AMP 04110–04184).

All stomach contents were processed for dietary composition assessments using a stereomicroscope. Each food item was measured (length and width) with a digital caliper (0.1 mm precision), quantified and identified to the lowest taxonomic level (Maddison & Schulz 2007, Rafael et al. 2012), however, different prey classified into the same order were divided into morphospecies. We considered the following as differentiation items: structural characteristics such as wings, mouth parts, size, body shape and color patterns. The partially digested food items were compared to intact individuals from the same taxonomic level, and highly digested prey were not included in the analyses.

The volume (mm^3) of each prey was estimated through the ellipsoid formula: $V = 4/3\pi \times (length/2) \times (width/2)^2$ (Dunham 1983). The frequency of occurrence (*FO%*), number frequency (*FN%*) and volumetric frequency (*FV%*) of each prey were calculated. The frequency of occurrence is given by the number of stomachs that contained prey divided by the total number of analyzed stomachs multiplied by 100. The number frequency is calculated by the number of prey of a certain morphospecies in all stomachs, divided by the total number of prey in all stomachs multiplied by 100, while the volumetric frequency is given as the total value of prey volume divided by the volume of all prey in all stomachs, multiplied by 100. These frequency values were used to calculate the Index of Relative Importance (*IRI*), which verifies the participation of each prey in a certain diet. The IRI is calculated by adding up the number and volumetric frequency percentages multiplied by the frequency of occurrence percentage (Pinkas et al. 1971).

A simple linear regression was applied using the volume of the largest prey and the total number of prey per stomach related to predator SVL, to verify if larger anurans feed on larger and/or more prey. To assess size differences in males, females and juveniles, the Kruskal-Wallis (*H*) non-parametric test was applied followed by the Dunn post-test (Kruskal & Wallis 1952). This test was also used to verify if the number of preys were different between males, females and juvenile individuals. The PERMANOVA test was used to assess possible differences in the prey composition between these groups. In order to analyze the variation in the prey composition consumed by males, females and juvenile individuals, a non-metric multidimensional scaling (*nMDS*) was applied, with an association matrix obtained through Euclidean distance measurement (Clarke & Warwick 1994). The level of significance for all statistical tests was set at $p < 0.05$, and the analyzes were performed in R software version 3.1.1 (R Core Team 2014), using the package 'vegan' (Oksanen et al. 2013).

Results

Of the 75 analyzed individuals, 36 (48%) had empty stomachs (21 males, 14 females and 1 juvenile specimen), while 39 (52%) contained stomach contents (17 males, 17 females and 5 juvenile specimens). Ninety-four food items represented by twenty-eight types of prey belonging to three categories were found: twenty-one insects, six arachnids, and one crustacean. Hemiptera Cicadellidae presented the highest percentage of FV, Orthoptera the highest FO, and Araneae III the highest FN. The latter was also the food item that presented the highest Index of Relative Importance (IRI = 130.58, FO = 5.33, FN = 24.46, FV= 0.04), followed by Orthoptera (IRI = 90.82, FO = 10.66, FN = 8.5, FV= 0.02) (Table 1).

The SVL of *S. fuscovarius* was not related to respective maximum volume of the prey item consumed ($r2 = 0.0008$, $F_{1,\ 26} = 0.8617$, $p = 0.8675$), however, frog size was significantly related to the total number of prey per stomach ($r2 = 0.1008$, $F_{1,\ 73} = 0.0462$, $p = 0.0488$). In addition, PERMANOVA test was not significantly similar in the diet compositions of male, female and juvenile individuals ($F_{2,\ 18} = 1444$, $p = 0.1116$), and despite the absence of similarity in the PERMANOVA analysis, there was great overlap among part of the individuals according to NMDS (Figure 1; Stress = 0.09). Males were larger than juvenile individuals ($H = 13.14$, d.f. = 1, $p = 0.0003$), however, we did not find significant differences between the SVL of males (39.89 ± 6.02 mm; minimum = 31.06; maximum = 46.93) and females (36.44 ± 6.01 mm; minimum = 19.19; maximum = 47.33) and there were also no significant differences between females and juveniles (30.17 ± 5.14 mm; minimum = 28.57; maximum = 35.2). Moreover, there were no differences in the number consumed prey items between the three groups ($H = 2.44$, d.f. = 2, $p = 0.25$).

Discussion

Scinax fuscovarius fed on a wide variety of invertebrates, including arthropods and crustaceans, but its diet consisted predominantly of insects. Orthoptera was the most frequent item in the diet and Araneae III presented the highest relative importance value. The size of the predator determined the abundance of captured prey, but the largest predators did not consume larger preys. These findings indicate that *S. fuscovarius* is a generalist and opportunistic predator, due to its varied diet composed of several different food items.

About half of the analyzed *S. fuscovarius* individuals consumed different prey categories, while several of them (mostly males) had empty stomachs. A large proportion of empty stomachs has also been reported in other studies with different anuran species (Menin et al. 2005, Solé & Pelz 2007, Solé et al. 2009). The absence of stomach contents in many male individuals may be related to factors such as alternating calling nights with feeding nights (Menin et al. 2005). Some species feed before their reproductive period, thus storing energy reserves to endure periods without feeding (Anderson et al. 1999), so they can focus their time on vocalizing and territory defense in search of females (Solé & Pelz 2007, Oliveira et al. 2017).

Insects were predominant in the diet composition of *S. fuscovarius* as observed for many anuran species in different regions (Hirai & Matsui 1999, Rodrigues et al. 2004, Hirschfeld & Rödel 2011, Kittel & Solé 2015). Regarding foraging strategy, it is suggested that *S. fuscovarius* displays a

Table 1. Prey categories consumed by *Scinax fuscovarius* at the Serra da Bodoquena region, Mato Grosso do Sul, Brazil. *N* = number of individuals; *FN* = number frequency; *O* = occurrence; *FO* = frequency of occurrence; *V* = total volume occupied by prey (in mm³); *FV* = volumetric frequency (*FV*%); *IRI* = index of relative importance.

Prey categories	N	FN (%)	O	FO (%)	V	FV (%)	IRI
Arachnida							
Araneae I	2	2.1	2	2.66	53.87	1.31	9.07
Araneae II	2	2.1	2	2.66	16.94	0.41	6.67
Araneae III	23	24.46	4	5.33	1.72	0.04	130.58
Araneae IV	4	4.2	4	5.33	225.50	5.50	51.70
Araneae V	2	2.1	2	2.66	141.14	3.44	14.73
Scorpiones	1	1.06	1	1.33	50.17	1.22	3.03
Sum	34	–	15	–	489.34	–	–
Crustacea							
Isopoda	1	1.06	1	1.33	18.25	0.44	1.99
Insecta							
Blattaria I	5	5.3	4	5.33	222.54	5.43	57.19
Blattaria II	2	2.1	2	2.66	756.7	18.48	54.74
Collembola	2	2.1	2	2.66	13.65	0.33	6.46
Coleoptera I	1	1.06	1	1.33	4.30	0.10	1.54
Coleoptera II	2	2.1	2	2.66	30.50	0.74	7.55
Coleoptera III	2	2.1	2	2.66	14.74	0.36	6.54
Diptera Brachycera Muscidae I	2	2.1	2	2.66	25.45	0.62	7.23
Diptera Brachycera Muscidae II	1	1.06	1	1.33	0.16	<0.03	1.41
Hemiptera I	1	1.06	1	1.33	10.46	0.25	1.74
Hemiptera II	1	1.06	1	1.33	6.21	0.15	1.60
Hemiptera Cicadellidae	5	5.3	2	2.66	873.4	21.3	35.37
Hymenoptera I	4	4.2	3	4	118.8	2.90	28.4
Hymenoptera II	4	4.2	3	4	113.13	2.76	27.84
Hymenoptera III	1	1.06	1	1.33	5.60	0.13	1.58
Hymenoptera Formicidae	5	5.3	4	5.33	97.31	2.37	40.88
Isoptera	1	1.06	1	1.33	1.67	0.04	1.46
Lepidoptera (adult)	8	8.5	3	4	1.54	0.03	34.12
Lepidoptera Pyralidae (larvae)	2	2.1	2	2.66	105.53	2.57	12.42
Mantodea	1	1.06	1	1.33	26.73	0.65	2.27
Megaloptera	1	1.06	1	1.33	39.91	0.97	2.69
Orthoptera	8	8.5	8	10.66	1.118	0.02	90.82
Sum	59	–	47	–	2469.448	–	–
Total	94	–	–	–	4093.92	–	–

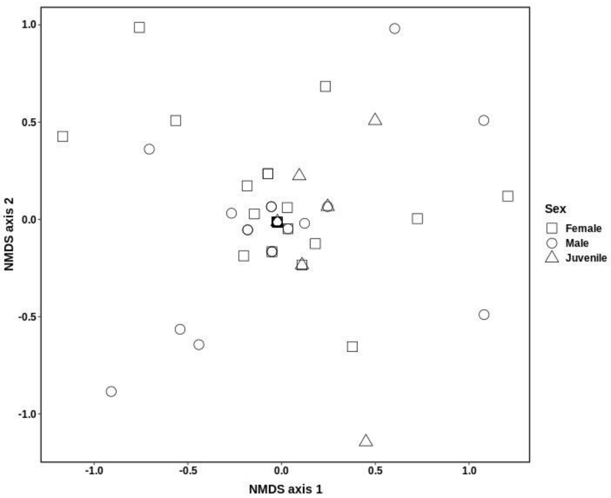

Figure 1. Non-metric multidimensional scaling (*nMDS*) based on the diet composition of male, female and juveniles of *Scinax fuscovarius* from Serra da Bodoquena, Mato Grosso do Sul, Brazil

"sit and wait" type of foraging behavior, with fast moving prey present in most stomachs, such as Araneae, Blattaria, Coleoptera, Hymenoptera, Lepidoptera and Orthoptera arthropods. These prey categories are typically consumed by anuran species that display this type of foraging strategy (Solé et al. 2002, Solé et al. 2009), strongly associated with the arboreal habit of this predator, which can perch and wait for prey on branches and trunks for long periods of time (Melo et al. 2007). However, sedentary preys, such as Diptera and larvae of Pyralidae (Lepidoptera), were also observed and could be associated with active opportunistic foraging (Leivas et al. 2018). Thus, *S. fuscovarius* may explore two foraging strategies based on environmental prey availability (López et al. 2007, Sabagh et al. 2010).

Orthoptera was detected with the highest frequency of occurrence, and this item is commonly found as a primary item in the diet of other *Scinax* species, such as *S. garbei* and *S. ictericus*; while *S. pedromedinae* and *S. ruber* have diets that consist mostly of Araneae and Coleoptera (Duellman 2005).

The category Araneae III showed the highest index of relative importance and the highest numerical frequency, being the most numerous preys in the diet of *S. fuscovarius*. These results should be attributed to prey availability, one of the main determining factors for amphibian diets, associated with the opportunistic behavior of most species, which directly reflects the available resources in a predators' environment (Kittel & Solé 2015, Moser et al. 2019).

The size of the predator is known to be an influential factor for anuran diets, as larger anurans consume a greater number of and larger prey (Sugai et al. 2012, Teles et al. 2018). Nevertheless, predator size is also a factor that selects and limits the prey that the predator can ingest, which may result in diets specialized in certain resources (Toft 1980, Forti et al. 2011, Silva et al. 2019). According to Rodrigues et al. (2005), *S. fuscovarius* males are significantly smaller than females, however, no differences in relation to sex were found herein, but between males and juveniles there was a difference. Larger individuals of *S. fuscovarius* did not prey on the bulkiest prey; however, they consumed many preys. These results have also been confirmed in other studies, supporting that larger frogs generally have larger jaws which facilitates the capture of numerous prey types, thus, they are able to ingest on a larger number of prey (Biavati et al. 2004, López et al. 2007, Quiroga et al. 2009, Pacheco et al. 2017).

Ontogenetic changes in anuran diets are associated with the size of frogs, since they can feed on larger and more numerous preys as they grow, which results in a positive energy balance in adulthood (Martins et al. 2010). This ontogeny in relation to the size and number of preys consumed is associated with changes resulting from alterations in microhabitat, foraging strategies and prey capture efficiency during different life stages (Lima & Magnusson 2000, Hirai 2002). These reasons may explain the low similarity in the composition of the stomach content between male, female and juvenile individuals; this slight overlap of food items in the diet may indicate a reduction in intraspecific competition for these specimens (Anderson et al. 1999). Several prey categories were unique and occurred only in one individual, which is probably due to the generalist-opportunistic feeding behavior of *S. fuscovarius* and the availability of these items in the study environment, since species can feed according to local prey availability (López et al. 2009). Therefore, the findings reported herein indicate that *S. fuscovarius* dietary habits reflect food generalism and opportunism. The ontogenetic variation in the diet composition in association with the reproductive activity of males may explain the low similarity in the prey composition in male, female and juvenile individuals.

Acknowledgement. The authors thank the entire Estância Mimosa Farm staff for allowing anuran sampling. We thank Eduardo Oliveira Pacheco, Leandro Alves da Silva, and Priscila Santos Carvalho who assisted and hugely collaborated in the animal sampling. This work was supported by the Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul under Grant number 59/300.328/2015 and Conselho Nacional de Desenvolvimento Científico e Tecnológico under Grant number 311492/2017-7. We thank two anonymous reviewers for improving the manuscript.

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