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Diet of five anuran species in a forest remnant in eastern Acre state, Brazilian Amazonia

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Abstract. The diet of anuran species is based on a large variety of prey items, which may be related to the availability of prey in the environment. In Amazonia, studies on the diet of anurans have occurred mainly in primary forests. Because forest fragmentation promotes changes in the environment, it is expected that the diet of anuran species will also be affected. Here we describe the diet composition of anurans captured in a forest remnant at Fazenda Amoty, municipality of Plácido de Castro, state of Acre, Brazil. Stomach contents of 34 specimens of *Allobates femoralis* (Aromobatidae), *Adenomera hylaedactyla*, *Engystomops freibergi* (Leptodactylidae), *Rhinella* sp. (Bufonidae), and *Elachistocleis muiraquitan* (Microhylidae) had 877 prey items belonging to 19 prey categories. The diet of most species were arthropods. Myrmicinae ants (Hymenoptera, Formicidae) was the most abundant and frequent item in *A. hylaedactyla*, *Rhinella* sp. and *A. femoralis* stomachs. Termitidae (Isoptera) was the second most abundant taxon in *Rhinella* sp. and *A. hylaedactyla*, and the only taxon found eaten by *E. freibergi* and *E. muiraquitan*. Most studied species showed generalist diet, which is the habit for most anuran species. Contrarily, *E. freibergi* and *E. muiraquitan* are likely specialized in Isoptera.

Keywords. Diet composition, niche breadth, niche overlap, diet specialization

Introduction

Anurans are carnivorous and feed on a wide variety of arthropods (e.g. Duellman, 1978; Lima and Magnusson, 1998; Sabagh et al., 2012). Formicidae, Isoptera, Coleoptera and Araneae are frequently reported in the diet of anuran species (Batista et al., 2011; Maragno and Souza, 2011; Sabagh et al., 2012), and may be related to the availability of prey in the environment (Menin et al., 2005; Oliveira et al., 2018). Although most species are characterized as generalists, specialization in ants or termites has been reported for species of Bufonidae, Microhylidae, Leptodactylidae and Dendrobatidae (Toft, 1980; Parmelee, 1999; Santana and Juncá, 2007). The relationship between anuran and prey sizes is a factor that can determine morphological restrictions on prey consumption and ontogenetic variation in the diet (Lima, 1998; Lima and Magnusson, 1998).

Syntopic species may overlap in the use of such major resource dimensions of the ecological niche as time, space and food (Pianka, 1994). In this sense, the use of resources may involve competition, but differences in the dimensions of the ecological niche resource base may facilitate species coexistence (Pianka, 1994). This is supported by studies demonstrating that competition for food may be a minor factor in anuran communities, and that syntopic species may have similar niche amplitude, but share prey of different sizes (Menin et al., 2005).

Most studies on the anuran diet in Neotropical region have involved populations found in primary forests (Duellman, 1978; Lima and Magnusson, 1998; Parmelee, 1999), agroecosystems or in artificial water bodies in pasture areas (Attademo et al., 2005; Hoyos-Hoyos et al., 2012; Menin et al., 2005, 2015). However, studies

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of species found in forest remnants are scarce (Silva and Rossa-Feres, 2010; do Couto et al. 2018). The process of forest fragmentation changes the environment, causing variations in some ecological factors and affecting biodiversity (Laurance and Vasconcelos, 2009). Furthermore, forest fragmentation, along with habitat loss, are among the major factors threatening amphibian populations in the world (Cushman, 2006). Moreover, forest fragmentation can also lead to the presence of species that occur in the matrix surrounding the fragment. Anuran species from open areas may use forest fragments as shelter during the day, as well as possibly for foraging (Silva and Rossa-Feres, 2007). Consequently, there may be changes in the composition of the anuran assemblage structure and in species eaten by the anurans, resulting, overall, in changes to the feeding habits of amphibian species. On the other hand, the small set of studies available on anuran species in forest remnants indicates that they have diets similar to those in preserved forests (do Couto et al., 2018), or to that described for the same species in other localities in Brazil, suggesting that the diet may be a conservative characteristic for each species (Silva and Rossa-Feres, 2010).

In the current study we aimed to describe the diet composition of species of anurans in a forest remnant located in a pasture area in the eastern region of the state of Acre, western Amazonia, Brazil. In order to determine the diet composition, we analyzed the stomach content of 49 individuals belonging to four families and five species; we also determined the breadth and overlap of the trophic niche to the most abundant species. The species of terrestrial anurans found in the study area are typical of regional *terra firme* forests (*A. femoralis*, *Rhinella* sp., and *E. freibergi*; Duellman, 1978, 2005; Parmelee, 1999; von May et al., 2009) or open areas (*A. hylaedactyla* and *E. muiraquitan*; Lima et al. 2012; Ramalho et al., 2016), and are commonly found in the leaf litter in such respective habitats. The latter two species could potentially use the forest remnant as diurnal shelter and foraging area, as reported by Silva and Rossa-Feres (2007) for anuran species in southeastern Brazil. This is the first study to evaluate the diet of anurans in a rural forest fragment in Amazonia.

Material and Methods

Study area.— Individuals used in this study were collected in a *terra firme* forest fragment at the Fazenda Amoty (10.338694° S, 67.485056° W; DATUM = WGS84), AC-90 highway, in the municipality of Plácido de Castro, Acre state, Brazil. The region's climate is tropical monsoonal (Am from the Köppen-Geiger system), with precipitation in the driest month ≥ 100 mm (Peel et al., 2007). The studied forest fragment covers eight hectares and has a lotic water body. The surrounding matrix is composed of pasture.

Data collection.—Individuals were captured from August 2014 to August 2015 in pitfall traps placed in two transects. Each transect had 11 traps composed of 500 mL plastic cups. Traps were separated by five meters apart and were situated at least 200 meters inside from the forest border. Transects were 10 meters apart. Specimens were retrieved from traps every 15 days, with a total of 22 samples and 396 days of summed effort. The pitfall traps used in this study were installed to capture edaphic insects, and so are much smaller than those generally used in herpetological field studies. Thus, we captured only juveniles and adults of small-size anurans.

The individuals were fixed with 10% formalin, preserved in 70ºGL alcohol, identified with specialized literature (Duellman, 2005; Funk et al., 2008; Nunes-de-Almeida and Toledo, 2012), and deposited (as accession numbers UFAC 6463 to 6495, 6497 to 6514) in the Herpetological Collection of the Universidade Federal do Acre (UFAC), municipality of Rio Branco, Acre state, Brazil. Species nomenclature follows Frost (2019). For each individual, we measured snout-vent length using digital callipers.

To determine diet composition, 49 specimens were dissected and had their stomach content identified to Order and/or Family level, following the identification keys of Triplehorn and Johnson (2011). Ants were identified to subfamily level following Baccaro et al. (2015). We measured the length and width of each prey item using an ocular micrometer mounted on a Zeiss Stemi SV 11 stereomicroscope.

Data analysis.—We estimated the volume of each item according to spheroid volume formulae: $V = (\pi . length.width^2)/6$ (Colli et al., 1992). Breadth and overlap of the trophic niche for more abundant species was estimated using the Shannon-Wiener diversity index (H', logarithm at the base 10, decits/individual) and the Morisita-Horn index (C_H), respectively (Krebs, 1999). We used a linear regression to test the relation between the snout-vent length of the most abundant anuran species and stomach contents volume (total volume of preys consumed for each individual), prey richness (total number of different prey categories consumed for each individual) and prey abundance (total number of preys in the stomach of each individual). All analyses were made using Systat 12.0 software.

Results

Forty-nine anuran individuals were captured, representing four families (Aromobatidae, Bufonidae, Leptodactylidae and Microhylidae) and five species [*Allobates femoralis* (Boulenger, 1884), *Rhinellla* sp., *Adenomera hylaedactyla* (Cope, 1868), *Engystomops freibergi* (Donoso-Barros, 1969), and *Elachistocleis muiraquitan* Nunes-de-Almeida and Toledo, 2012]. *Adenomera hylaedactyla* was the most abundant species representing 57% (N = 28) of the captured individuals (Table 1).

Thirty-four (69.4%) individuals had stomach content. Two (4.1%) individuals of *Rhinella* sp., eight (16.3%) of *A. hylaedactyla* and five (10.2%) of *E. freibergi* (Table 1) had empty stomachs. In total, we recorded 877 prey items belonging to 19 categories (Table 2). The anuran species preyed mostly on arthropods (Table 2). The number of prey items per stomach varied from one to nine for *A. hylaedactyla*, one to 404 for *E. freibergi*, eight to 55 for *Rhinella* sp., two to five for *A. femoralis* and only one prey item for *E. muiraquitan*.

Myrmicinae (Hymenoptera, Formicidae) was the most abundant and frequent item in the stomachs of *A. hylaedactyla*, *Rhinella* sp. and *A. femoralis* (Table 2). Termitidae (Isoptera) was consumed in high abundance by four studied species, and was the second most abundant prey category in the diet of *Rhinella* sp. and *A. hylaedactyla*, and the only prey item found in the stomachs of *E. freibergi* and *E. muiraquitan* (Table 2).

Termitidae (Isoptera) was the taxon with the highest volume (15.7%) consumed by *A. hylaedactyla*, followed by Coleoptera larvae (9.3%), unidentified insects (8.6%) and Dermaptera (7.7%). Ponerinae (Hymenoptera, Formicidae) (18.7%) was the item with highest volume consumed by *Rhinella* sp., followed by Termitidae (Isoptera) (10.9%). Unidentified immature insects (39.3%) were the most voluminous item in the *A. femoralis* diet.

Niche breadth was highest for *A. hylaedactyla* (H'= 1.03), followed by *Rhinella* sp. (H' = 0.629). Niche overlap was high among these species ($C_H = 0.705$).

There was no relationship between sizes of *A. hylaedactyla* and the prey volume ($F_{1,18} = 0.079$; $p = 0.781$), prey richness ($F_{1,18} = 0.214$; $p = 0.649$) and prey abundance ($F_{1,18} = 0.044$; $p = 0.836$). The size of *Rhinella* sp. was significantly correlated to prey abundance ($F_{1,6} = 6.305$; $p = 0.046$), but not to prey volume ($F_{1,6} = 2.735$; $p = 0.149$) or prey richness ($F_{1,6} = 0.004$; $p = 0.952$).

Discussion

The diet of studied species was composed mostly by arthropods. Myrmicinae ants was the most abundant and frequent item in *A. hylaedactyla*, *Rhinella* sp. and *A. femoralis* stomachs, while Termitidae (Isoptera) was the second most abundant taxon in *Rhinella* sp. and *A. hylaedactyla*, and the only taxon found eaten by *E.*

Table 1. Number of individuals and snout-vent length (SVL; in mm) of five anuran species captured monthly in a forest remnant in Fazenda Amoty, municipality of Plácido de Castro, Acre state, Brazil. Values in brackets are the number of empty stomachs. Values of SVL are means ± standard deviation and range (in parenthesis), except for species with one or two individuals only, for which individual values are given.

Family/Species	Sep/2014	Oct/2014	Nov/2014	Dec/2014	Jan/2015	Feb/2015	Mar/2015	Total N of individuals	SVL (mm)
Aromobatidae									
Allobates femoralis	-	1	-	-	-	1	-	2	20.13–20.69
Bufonidae									
Rhinella sp.	6 [1]	3 [1]	1	-	-	-	-	10	28.94±4.55 (20.47–35.27)
Leptodactylidae									
Adenomera hylaedactyla	4	3 [1]	6 [1]	4 [2]	2	6 [3]	3 [1]	28	22.24±2.22 (17.03–27.02)
Engystomops freibergi	3 [2]	1 [1]	2 [1]	-	-	1	1 [1]	8	30.04±3.87 (25.54–35.45)
Microhylidae									
Elachistocleis muiraquitan	1	-	-	-	-	-	-	1	38.24
TOTAL	14	8	9	4	2	8	4	49	

Table 2. Prey items of five anuran species in a forest remnant at Fazenda Amoty, municipality of Plácido de Castro, Acre state, Brazil. N: number of items; N(%): numerical percentage; F: frequency; F(%): occurrence-frequency percentage; V: volume; V(%): volumetric percentage.

Prey Category/Species	N	N(%)	F	F(%)	V (mm^3)	V(%)
AROMOBATIDAE						
***Allobates femoralis* (N = 2)**						
Diplopoda	1	14.29	1	50.00	32.15	33.66
Diptera	1	14.29	1	50.00	5.07	5.31
Hymenoptera						
Formicidae - Myrmicinae	3	42.86	2	100.00	4.42	4.62
Unidentified insects (fragments)	2	28.57	1	50.00	53.88	56.40
TOTAL	7				95.52	
BUFONIDAE						
***Rhinella* sp. (N = 8)**						
Araneae	4	1.71	3	37.50	371.44	4.46
Coleoptera						
Bostrichidae	1	0.43	1	12.50	6.30	0.08
Ceratocanthidae	1	0.43	1	12.50	13.85	0.17
Lampyridae	1	0.43	1	12.50	352.35	4.23
Unidentified adults	5	2.14	4	50.00	40.73	0.49
Unidentified larvae	3	1.28	3	37.50	346.06	4.15
Diplopoda	1	0.43	1	12.50	24.94	0.30
Diptera	6	2.56	1	12.50	602.61	7.23
Hemiptera	1	0.43	1	12.50	1.10	0.01
Heteroptera	1	0.43	1	12.50	3.11	0.04
Hymenoptera						
Formicidae - Ectatomminae	4	1.71	4	50.00	494.76	5.93
Formicidae - Myrmicinae	124	52.99	6	75.00	1254.59	15.05
Formicidae - Ponerinae	32	13.68	5	62.50	3012.61	36.13
Isoptera						
Termitidae	50	21.37	3	37.50	1812.88	21.74
TOTAL	234				8337.33	
LEPTODACTYLIDAE						
***Adenomera hylaedactyla* (N = 20)**						
Araneae	3	4.84	3	15.00	539.09	7.78
Coleoptera						
Unidentified adults	5	8.06	5	25.00	106.05	1.53
Unidentified larvae	4	6.45	3	15.00	1286.72	18.56
Dermaptera	1	1.61	1	5.00	1059.69	15.29
Diplopoda	5	8.06	2	10.00	73.88	1.07
Diptera						
Unidentified adults	2	3.23	1	5.00	7.13	0.10
Unidentified larvae	3	4.84	1	5.00	193.72	2.79
Hemiptera	1	1.61	1	5.00	1.10	0.02
Hymenoptera						
Formicidae - Ectatomminae	4	6.45	3	15.00	12.76	0.18
Formicidae - Myrmicinae	13	20.97	7	35.00	106.60	1.54
Formicidae - Ponerinae	2	3.23	2	10.00	141.60	2.04
Isoptera						
Termitidae	13	20.97	7	35.00	2176.67	31.40

Table 2. Continued.

Prey Category/Species	N	N(%)	F	F(%)	V (mm^3)	V(%)
Orthoptera	1	1.61	1	5.00	30.87	0.45
Unidentified insects (fragments)	4	6.45	2	10.00	1188.83	17.15
Mollusca						
Gastropoda	1	1.61	1	5.00	6.30	0.09
TOTAL	62				6931.01	
***Engystomops freibergi* (N = 3)**						
Isoptera						
Termitidae	573	100	3	100	12452.73	100
MICROHYLIDAE						
***Elachistocleis muiraquitan* (N = 1)**						
Isoptera						
Termitidae	1	100	1	100	0.66	100

freibergi and *E. muiraquitan*. Diet composition of *A. hylaedactyla*, *Rhinella* sp. and *A. femoralis* indicate generalist and opportunistic feeding strategy. This is reported for most anuran species (Duellman and Trueb, 1994; Parmelee, 1999). *Adenomera hylaedactyla* had the largest niche breadth, probably due to the larger number of specimens analyzed in comparison to the other species studied here. However, we did not find a relationship between *A. hylaedactyla* size and the variables prey volume, abundance and richness. Identity of prey item found in the diet of *A. hylaedactyla*, *Rhinella* sp. and *A. femoralis* were similar to those reported from other regions in Amazonia, and for species of the same genera in other Brazilian biomes. Species of *Adenomera* consume predominantly Formicidae, Isoptera, Araneae, Coleoptera and insect larvae (Duellman, 1978, 2005; Parmelee, 1999; Almeida-Gomes et al., 2007; Astwood-Romero et al., 2016). Studies in Peru (Parmelee, 1999; Duellman, 2005), Ecuador (Duellman, 1978) and Colombia (Astwood-Romero et al., 2016) have reported that, in terms of abundance and volume, Formicidae and Coleoptera were the most important prey items in the diet of *R. margaritifera* species group. This includes the consumption of ants belongs to the subfamilies Ecitoninae and Myrmicinae (Fajardo-Martinez et al., 2013). Prey abundance was proportional to individual size for *Rhinella* sp. in our study: with the largest individuals consuming a higher number of prey, probably due to demands of energy uptake.

We found seven prey items in the stomachs of only two individuals of *A. femoralis*. The diet of *A. femoralis* from Peru (Parmelee, 1999) was more diversified than that found in our study due to the greater number of analyzed specimens; Formicidae and Acari gave the largest number of prey items, while larval insects, Formicidae and Araneae provided the largest volumes (Duellman, 2005). In general, in *Allobates cepedai* (Morales, 2002), *Allobates hodli* Simões, Lima and Farias, 2010, *Allobates juanii* (Morales, 1994), *Allobates sumtuosus* (Morales, 2002), and *Allobates trilineatus* (Boulenger, 1884) studied in different regions of Amazonia, Formicidae, Isoptera and Coleoptera had the highest abundance and volume across preys (Duellman, 1978, 2005; Parmelee, 1999; Juncá and Eterovick, 2007; Simões et al., 2010; Astwood-Romero et al., 2016).

According to Clarke (1974), Coleoptera and Formicidae play an important role in the diet of anurans, being the most frequent prey worldwide. Coleoptera consumption (both larva and adult) is advantageous for anurans because they are generally larger than other arthropods and have a high protein content in all parts, so offering more sustenance to feeding anurans (Anderson and Smith, 1998). Formicidae form potential prey for anurans mostly due their abundance and richness in different sites in arboreal and terrestrial habitats, so that their availability is generally greater than potential prey from other invertebrate groups. Moreover, Myrmicinae is the most diverse Formicidae subfamily, with wide distribution, immense morphological diversity and generally generalist habit, and so easily occupying disturbed areas such as forest fragments (Carvalho and Vasconcelos, 1999; Fernández 2003; Baccaro et al., 2015). Therefore, Myrmicinae represent an important prey base for *A. hylaedactyla*, *Rhinella* sp. and *A. femoralis*. Also, ants from the Ponerinae subfamily are an important prey for *Rhinella* sp. These ants are predators and they are the biggest ants found in forests being a well-known item prey for anurans (Baccaro et al., 2015).

Engystomops freibergi may be Isoptera specialists, but the small sample size prevents further conclusion. In Peru, *E. freibergi* (as *Physalaemus petersi* in Parmelee, 1999) preyed almost exclusively on Isoptera (except by one Coleoptera). Indeed, *E. freibergi*, *Engystomops petersi* Jiménez de la Espada, 1872, *Engystomops pustulosus* (Cope, 1864) and species of the genus *Physalaemus* are considered to be specialists in Termitidae (Isoptera) (Duellman, 1978, 2005; Ryan, 1985; Parmelee, 1999; Giaretta and Menin, 2004; Silva and Rossa-Feres, 2010; Menin et al., 2015). On the other hand, *Engystomops pustulatus* (Shreve, 1941) has a generalist diet, a characteristic that is attributed to the presence of teeth in the species (Narváez and Ron, 2013). Because termites are decomposers in tropical systems, termites are abundant in forests, both in natural landscapes and forest remnants, building their nests mostly in the leaf litter. Termitidae is the commonest family of Isoptera, representing 85% of Brazilian termite fauna living in large colonies with complex nests, which explain their extensive consumption by anuran species (Constantino, 1992, 2012). Only one specimen of *E. muiraquitan* was analyzed and it had consumed a single individual termite. However, information on diet composition of *Elachistocleis* species in other biomes, indicate that Formicidae and Isoptera are the main items in the diet (Solé et al., 2002; Berazategui et al., 2007; López et al., 2007), suggesting phylogenetic relationship may have an influence on the diet of the species in this genus (Marques-Pinto et al., 2018).

The extensive niche overlap observed between the studied species may be the result of species behavioral and morphological characteristics. *Adenomera hylaedactyla* and *Rhinella* sp. are predominantly diurnal leaf litter-living species (Lima et al., 2012) and, in our study, we found captured individuals of these species overlapped in the size (see Table 1). Additionally, the wide overlap in the use of food resources can be related to the high availability in the environment of the exploited prey base types (Menin et al., 2005; Oliveira et al., 2018), or the overall absence of competition because these resources are not in short supply (Pianka, 1994).

In conclusion, most of the species studied had a generalist diet and showed broad overlap in the use of food resources, probably consuming resources according to their availability in the environment. Additionally, we did not detect any differences in diet composition when compared to other areas, including pristine areas, suggesting a conservative diet for each species (Silva and Rossa-Feres, 2010). However, based on our results and information in the literature, we suggest that *E. freibergi* and *E. muiraquitan* are specialized in Isoptera. Specializations in the use of resources can reduce the potential competition in sympatric species, permitting their coexistence (Pianka, 1974), and contributing to species resilience to live in forest fragments.

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