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Original Article

Diet of a bromeligenous species of treefrog in the Brazilian semiarid

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(With 1 figure)

Abstract

Phyllodytes tuberculosus is a bromeligenous anuran species whose geographic distribution is restricted to areas of the Caatinga and to transition zones between Caatinga and Atlantic Forest in the central-south of the state of Bahia. Its existence in close relationship with bromeliads and its occurrence in dry and highly seasonal environments drove us to the supposition that species is opportunistic/generalist regard to diet. We collected the items present in the stomach of 43 *P. tuberculosus* individuals with stomach-flushing method. Of these, 18 stomach contents were obtained during the dry period (April–October), and 25 during the rainy period (November–March). We recorded nine classes of prey, finding the greatest relative importance for the categories Formicidae and Coleoptera in dry season and Blattodea (Termites), Formicidae and Coleoptera in the rainy season. *Phyllodytes tuberculosus* presented a varied, possibly opportunistic diet in semiarid environments. Additionally, we report an apparent temporal differentiation related to the typical Caatinga seasonality. This study adds to the data on the natural history of an anuran species adapted to a semiarid environment with a narrow geographic distribution.

Keywords: caatinga, conservation, lophyohylinae, trophic resources.

Dieta de uma espécie bromelígena de anuro do semiárido brasileiro

Resumo

Phyllodytes tuberculosus é uma espécie de anuro bromelígena cuja distribuição geográfica está restrita a áreas de Caatinga e zonas de transição entre Caatinga e Mata Atlântica no centro-sul do estado da Bahia. Sua relação próxima com bromélias e sua ocorrência em ambientes secos e fortemente sazonais nos levou a supor que a espécie apresenta uma dieta generalista/oportunista. Por meio de lavagem estomacal, coletamos os itens alimentares presentes no estômago de 43 indivíduos de *P. tuberculosus*. Destes, 18 estômagos foram obtidos durante a estação seca (Abril-Outubro) e 25 durante a estação chuvosa (Novembro–Março). Nós registramos nove classes de presas, sendo que as maiores importâncias relativas foram observadas para os itens Formicidae e Coleoptera na estação seca e Blattodea (Termites), Formicidae e Coleoptera no conteúdo recuperado na estação chuvosa. *Phyllodytes tuberculosus* apresentou uma dieta variada, possivelmente oportunista, condizente com a vida em ambientes semiáridos. Ademais, também observamos uma aparente diferenciação temporal relacionada a sazonalidade típica da Caatinga. Este estudo adiciona dados a respeito da história natural de uma espécie de anuro adaptada a ambientes semiáridos e com distribuição geográfica restrita.

Palavras-chave. *caatinga, conservação, lophyohylinae, recurso alimentar.*

1. Introduction

The genus *Phyllodytes* (Wagler, 1830) is composed of 14 species (Frost, 2019). Its representatives present odontoids on the mandible, the principal characteristic that differentiates them from other hylids (Peixoto and Cruz, 1988; Cruz et al., 2006). The species that comprise the genus are small to medium in size, have a greenish-yellow color, with wide variation in their dorsal coloration pattern, and live in close association with bromeliads (e.g. Eterovick, 1999; Peixoto et al., 2003; Caramaschi and Peixoto, 2004); hence their classification as bromeligenous species (*sensu* Peixoto, 1995).

Bromeliads are very particular phytotelmata. In addition to offering shelter and protection to many vertebrate and invertebrate species (Rocha et al., 1997; Richardson, 1999; Juncá and Borges, 2002), they provide ecological and physical-chemical conditions for the development and maintenance of species capable of exploiting this resource (Peixoto, 1995). Another highlight of this phytotelma pertains to ecological parameters—its interior constitutes an entire ecosystem (Picado, 1913; Oliveira et al., 1994; Mestre et al., 2001) in which many associations exist between diverse life forms (Reitz, 1983), a factor that distinguishes the faunistic components within phytotelmas from the ecosystems outside them.

Interactions between bromeliads and the life forms that can be found inside them are particularly interesting in highly seasonal areas. In Caatinga zones, areas characterized by unpredictable rain, with lengthy dry periods in some regions (Krol et al., 2001), this phytotelma can play role of an oasis (Islair et al., 2015), providing water and food to species capable of exploiting this resource, as is the case of *Phyllodytes tuberculosus* Bokermann, 1966, one of the few species of the genus that occurs in Caatinga areas (see Santos & Silva, 2010 *Phyllodytes acuminatus*). The existence of this species in Caatinga, a zone characterized by dry and thorny vegetation and scarce precipitation (Andrade-Lima, 1981; Krol et al., 2001), implies that the species presents ecological, behavioral and physiological adaptations in response to the selective pressures imposed by the prevailing water deficit and high temperatures of this domain (Hillman et al., 2009; Jared et al., 2019). Currently 98 anuran species are known to occur in the Caatinga areas (Garda et al., 2017).

Seasonal environments with limited resources, such as the Caatinga, may favor generalist species with an opportunistic foraging strategy. The cost related to the maintenance of a more specialized diet may be high in such scenario, due to both potential scarcity of food resources and increase physiological stress during the foraging activity (Friedman et al., 2019). Amphibians are considered as generalist predators, with opportunistic foraging behavior, being the availability of prey the determinant feature of their diet (Macnamara, 1977; Pough et al., 1998; Solé and Rödder, 2009). There are few exceptions to this statement; some species may present diet specializations frequently linked to active search foraging strategy (Lieberman, 1986; Simon and Toft, 1991; Lima and Moreira, 1993; Das, 1996), such as the mirmecofagy reported to *P. edelmoi* Peixoto, Caramaschi, and Freire, 2003 by Araujo-Vieira et al. (2018). According to these authors, this specialist feeding behavior could be considered a synapomorphy of the genus.

We believe the use of bromeliads in a strongly seasonal environment would favor generalist species over specialist ones and, in this sense, we predict that *P. tuberculosus* will present a generalist and opportunist diet exploring the most abundant resource available inside the bromeliads. We aimed to contribute to the knowledge about the ecology of *P. tuberculosus* filling a gap that in conjunction with other types of data, could enable an understanding of the ecological demands of the species. Additionally, data about diet may provide interesting insights about taxonomic relationship between closely related species (Sih and Christensen, 2001).

2. Material and Methods

This study was conducted in an area surrounding the Barragem da Pedra dam in the village of Santa Clara (13°55'24"S 40°23'57"W), district of Monte Branco, in the municipality of Jequié, central-southern Bahia. It is a region subject to alterations resulting from human occupation (real estate speculation) and the construction and operation of the west–east railway, the Ferrovia de Integração Oeste–Leste (FIOL). The local vegetation is described as shrub-arboreal Caatinga and contains patches of evenly spaced bromeliads. The climate is classified as semiarid steppe climate (BSh) (Peel et al., 2007) with precipitation below 500 mm (SEI, 2003).

Thirteen field excursions took place between October 2016 and December 2017 (six in the rainy period and seven in the dry period), each one corresponding to one field day (in which various bromeliad patches were visited). We considered the months from November to March as the rainy period (average monthly rainfall of 82.4 mm), and from April to October as the dry period (average monthly rainfall of 41.6 mm) (values obtained from the platform INPE, 2018).

We used visual and acoustic active search methodology at the species' reproductive sites (Heyer et al., 1994) to locate individuals and collect data. Data collection started around 17:30 h and finished between 23:00 h and 00:00 h. During these field excursions, the captured individuals were measured (snout vent length– SVL; mandible length – ML; head width – HW; body weight – BW) using a caliper with 0.1 mm precision, and weighed using a dynamometer with 0.1 g precision.

The stomach contents were collected following the stomach-flushing protocol, proposed by Solé et al. (2005), using a 3 ml syringe, 4 cm urethral probe, sieve, forceps and filtered water. We standardized the procedure for four repetitions of stomach-flushing, and, in cases where no stomach contents were found, null was given for diet. The procedure was performed while in the field, a maximum of one hour after each individual had been captured. The stomach contents were stored in an eppendorf® tube containing 70% alcohol. The stomach contents were then identified, using a stereo microscope, to the lowest taxonomic level possible. Next, the contents were distributed into food categories, which were quantified using a millimeter (0.1 cm) graph board. For this, we pressed the ingested material until it reaches 1 mm of height, to minimize de effects of items with different sizes on the analysis (see Hyslop, 1980). Then, we counted the number of quadrants (= number of points) occupied by each food category, which corresponded to the number of points for that category. Thus, each category's average number of points (P%) represents a relative subjective measure of its quantity in volume (Hyslop, 1980; Zavala-Camin, 1996). We considered the number of stomachs in which each category was present as the frequency of occurence index. To assess the importance of each prey consumed, we calculated the alimentary importance index (IAi) (Eq. 1), as proposed by Kawakami and Vazzoler (1980) (with modifications), for each evaluated category:

$$IAi = \frac{FOi \ x \ Pi}{\sum_{i=1}^{n} (FOi \ x \ Pi)} \quad (1)$$

Where:
IAi = alimentary importance index
i = 1, 2, ... n = determined food category
FO = frequency of occurrence (%) of the food category i
P = average number of points (%) for each food category i

Eleven individuals were collected, euthanized with lidocaine 5%, fixed in formaldehyde 10%, preserved in 70% alcohol (Heyer et al., 1994), and deposited in the Zoology Collection of the Universidade Estadual do Sudoeste da Bahia (MHNJCH 1099, MHNJCH 1103, MHNJCH 1104, MHNJCH 1106, MHNJCH 1107, MHNJCH 1108, MHNJCH 1109, MHNJCH 1110, MHNJCH 1126, MHNJCH 1132, MHNJCH 1133) under collection permit n° 54629-1.

To determine the correlation between body measurements and the relative volume of the food categories, we performed a non-parametric Spearman's rank correlation tests, confronting each measurement (SVL, ML, HW, body mass) of captured individuals with the number of points of the categories observed in their respective stomachs. For this test we only consider the food categories that occurred in more than five stomachs.

The temporal analysis of the diet was performed according to the periodicity established for the dry season (April–October) and rainy season (November–March). To verify if the food categories differed in order of importance between the seasons, we performed a Spearmann's rank correlation test (Fritz, 1974) using the IAi values obtained for each category in each season. To verify difference in diet activity according to the season (wet and dry) we compare the number of points obtained for each stomach throughout Mann-Whitney test.

We considered significant values of $p < 0.05$.

3. Results

Among the 51 individuals analyzed, only 43 presented stomach contents, 25 of which corresponded to the rainy period and 18 to the dry period. We distributed the stomach contents in nine food categories (Table 1). The IAi showed that Formicidae and Coleoptera were the most important categories, followed by Blattoidea (Termitidae) (Table 1). Plant material was not recorded in the composition of the analyzed stomach contents.

The averages of the morphometric measurements of the *Phyllodytes tuberculosus* individuals were as follows: body mass of 0.96 g (SD ± 0.27, range = 0.40–1.50 g, N = 43), SVL of 24.26 mm (SD ± 2.32, range = 14.0–29.1 mm, N = 43), HW of 9.13 mm (SD ± 0.99, range = 6.0–11.1 mm, N = 43) and ML of 6.78 mm (SD ± 1.14, range = 5.0–10.8 mm, N = 43). There was no significant correlation between the morphometric parameters and the size of each food category (Table 2).

In the temporal analysis, we observed no correlation between the IAi values of the food categories (r = 0.611; p = 0.088), indicating distinct diets in rainy and dry seasons. This finding is due mainly to the food category Blattodea (Termites) having a higher importance value in the rainy period (IAi = 58.78) compared to the dry period (IAi = 12.71), during which the categories Coleoptera and Formicidae were consumed with greater frequency (Table 1) (Figure 1). There was no difference in the diet activity of

Table 1. Percentage values of frequency of occurrence (FO), average number of points (P%) and alimentary importance index (IAi) of each category, representing the composition of the diet of *Phyllodytes tuberculosus*, municipality of Jequié, state of Bahia.

Food Category (Number of analysed stomachs = 43)	FO (%)	P (%)	IAi
Hymenoptera (Formicidae)	58.14	23.06	35.40
Coleoptera	60.47	22.04	35.19
Blattodea (Termitidae)	23.26	43.45	26.69
Blattodea (no Termitidae)	13.95	5.35	1.97
Orthoptera	2.33	1.94	0.12
Lepidoptera	9.30	1.47	0.36
Diptera	4.65	1.05	0.13
Mantodea	2.33	0.89	0.05
Aranae	4.65	0.73	0.09

Table 2. Correlation between morphometric measurements (SVL= snout vent length, HL= head length, ML= mandible length, BW= body weight) and the number of points per food category (rs = Sperman coefficient).

Food Category	SVL		HL		ML		BW	
	rs	p	rs	p	rs	p	rs	p
Hymenoptera (Formicidae)	0.004	0.998	-0.174	0.264	0.135	0.389	0.098	0.533
Coleoptera	0.035	0.822	0.063	0.690	-0.030	0.847	0.013	0.933
Blattodea (Termitidae)	-0.061	0.697	0.083	0.599	0.208	0.181	-0.079	0.613
Blattodea (no Termitidae)	0.269	0.081	0.083	0.599	-0.015	0.923	0.121	0.439
Lepidoptera	0.104	0.508	0.224	0.149	0.060	0.700	0.111	0.479

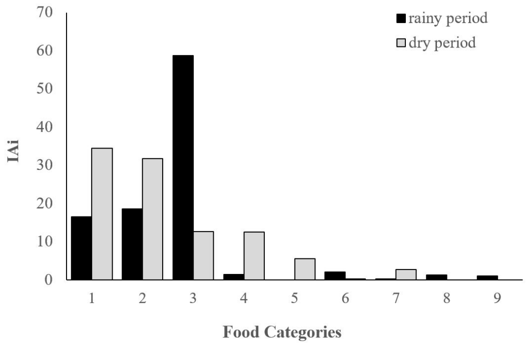

Figure 1. Values of alimentary importance index (IAi) of each food category [1 - Hymenoptera (Formicidae); 2 - Coleoptera; 3 - Blattodea (Termitidae); 4 - Blattodea (no Termitidae); 5 - Orthoptera; 6 - Lepidoptera; 7 - Diptera; 8 - Mantodea; 9 Aranae] for two distinct periods (rainy and dry periods) of Phyllodytes tuberculosus, municipality of Jequié, state of Bahia.

the species according to the season (U= 281,5 P= 0.427). However, we highlight that the number of empty stomachs were three times greater in the dry season (6 stomachs).

All *P. tuberculosus* individuals recorded in this study were observed in bromeliads of the genus *Aechmea*, most of them probably of the same species.

4. Discussion

The association established between fauna and bromeliads is a relationship that benefits both involved parts. The obvious benefits to the fauna cover shelter, foraging area and reproduction (Rocha et al., 1997; Richardson, 1999; Juncá and Borges, 2002). For the bromeliads, the fauna confers nutrients (organic material and the excreta of the individuals that occupy this micro-habitat) (Benzing, 2000) that can be assimilated by the plants (Frank and Lounibos 1983; Kitching 2000, 2001). In a harsh environment such as the Caatinga, this plant-animal relationship is fundamental to the maintenance of different populations even in the scarcity of resources, a natural condition in highly seasonal environments.

Besides the above-mentioned benefits offered by the bromeliads to bromeligenous species, the bromeliad represents for *P. tuberculosus* the additional advantage of being a semi-permanent body of water, used by the species as a site for vocalization, egg deposition and tadpole development (reproductive mode 6, Haddad et al., 2013). It is important to note that bromeliads in Caatinga environments often offer the only water bodies available for many vertebrate and invertebrate species, acting as an oasis, and creating a very particular dynamic of community organization (Islair et al., 2015).

In general, the diet composition of species that live in association with bromeliads presents specific characteristics, such as the presence of fauna that also frequents this plant (Araujo-Vieira et al., 2018). The life restricted to the bromeliad's interior explains the absence of plant material in its diet composition, a common characteristic of the diets of anurans whose lifestyle is associated with the occupation of bromeliads (see Brandão et al., 2003).

We did not observe dipteran larvae in the diet of *P. tuberculosus* adults nor within the bromeliads. Perhaps this result is an artefact stemming from the failure to recover these items. However, the absence of dipteran larvae in the diets of other anuran species has also been observed by other authors (Pinto, 2011; Amado, 2014). Recently, Salinas et al. (2018) confirmed the importance of this food category in the larval diet of another bromeligenous species of *Phyllodytes* (*P. luteolus* (Wied-Neuwied, 1824)), which points to a predator-prey relationship between larvae; this finding represents a promising direction for future ecological studies in the Caatinga.

In general, the species' diet analyzed here is similar to that observed in sand coastal plains by Ferreira et al. (2012) in the states of Bahia and Espírito Santo, and by Motta-Tavares et al. (2016) in the state of Espírito Santo, for *Phyllodytes luteolus*; these studies found a predominance of ants and termites. The huge presence of termites (Termitidae) in the composition of the *P. tuberculosus* diet is due to the location of termite mounds, which are situated above bromeliads (personal observation) and are susceptible to seasonal variation. Termites, as well as other social organisms whose populations undergo seasonal reproductive effects, constitute a food resource that, when available, occurs in abundance (Noirot, 1989). Thus, they must be exploited as a food resource within a short timeframe. These data explain the presence of a large number of termites concentrated in periods favorable to the termite colony (rainy periods).

Pertel et al. (2010) verify that *Ololygon arduous* (Peixoto, 2002), a bromeligenous treefrog species may forage outside the bromeliads. Although we registered some items in the diet that we did not observe inside the bromeliad (i.e. Mantodea), we cannot state that *P. tuberculosus* forage outside the plant, nor we can predicate that the species forage exclusive inside the phytotelma. Likewise we cannot rule out the possibility that items not recorded here may indeed exist within bromeliads but were not found within the plants we analyzed, whether because of the small number of plants analyzed, the period in which the bromeliads were collected or the prey's sporadic use of the plant. However, we must add here that, during this study, we did not observe any *P. tuberculosus* individual outside the phytotelmata.

The alimentary importance index (IAi) allows the most appropriate distinction of relative importance of each food category, even given variations in terms volume and occurrence of categories (Kawakami and Vazzoler, 1980). This analysis dilutes the effects of prey of high volume but low occurrence, and of prey that is frequent but very small in size (see Kawakami and Vazzoler, 1980). In the present study we observed a high proportion of termites in the rainy period but the IAis indicated that the most important food categories in the studied species' diet were coleopterans, followed by ants. Termites were considered only the third most important category (IAi = 0.267), possibly due to the cyclical nature of their occurrence. Leite-Filho et al. (2015) observed the same pattern (higher importance values for coleoptera and ants) for *Leptodactylys caatingae, L. macrosternum, Physalaemus albifrons*, *P. cicada*, *Proceratophrys cristiceps*, *Rhinella granulosa* and *R. jimi* in a Caatinga area in Northeastern Brazil. The same result was also observed for other bromeligenous species (see Pertel et al., 2010). The lack of information about the diet of anurans from Caatinga prevent us to better explore hypotheses about the diet of anurans from this biome or the diet of bromeliads species of Caatinga.

We found no correlation between the morphological parameters of *P. tuberculosus* and the ingested contents, which suggests that the species consumes prey of various sizes. This data, together with the discussed above, suggests that the species diet reflects the composition of resources frequently or occasionally available in the phytotelma, what seems to be a pattern of bromeligenous frogs. *Phyllodytes tuberculosus* enjoys a vast array of food resources which could lead us to question the diet specialization raised by Araujo-Vieira et al. (2018), and in consequence, the use of this source of information as a synapomorphy for the group. These finds corroborate our premise the species would probably exhibit a generalist and opportunistic diet, most advantageous in strong seasonal environments.

The present study offers new data on the diet of *P. tuberculosus*, a species of restricted geographic occurrence, endemic to the Caatinga and transition areas in the interior of Bahia. It is also one of a small group of species with distribution in semiarid areas. The information presented here increases the knowledge about the genus, and it represents the first step to uncover the ecological and historical mechanisms that enable this species to occupy Caatinga environments.

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