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Diet and first report of batracophagy in *Leptodactylus podicipinus* (Anura: Leptodactylidae)

Karoline Ceron[a,b], Matheus de Toledo Moroti[a,c], Ronildo Alves Benício[d], Zulia Porcel Balboa[b], Yasmine Marçola[b], Luiza Becker Pereira[e] and Diego José Santana[a,b,c]

[a]Mapinguari – Laboratório de Sistemática e Biogeografia de Anfíbios e Répteis, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Cidade Universitária, Campo Grande, Brazil; [b]Programa de Pós-Graduação em Ecologia e Conservação, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Cidade Universitária, Campo Grande, Brazil; [c]Programa de Pós-Graduação em Biologia Animal, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Cidade Universitária, Campo Grande, Brazil; [d]Programa de Pós-Graduação em Ecologia e Recursos Naturais, Universidade Federal de São Carlos, São Carlos, Brazil; [e]Programa de Pós-Graduação em Ecologia, Universidade Federal de Santa Catarina, Florianópolis, Brazil

ABSTRACT

In this study, we describe the diet of *Leptodactylus podicipinus* in South Pantanal. We analysed the stomach content of 30 individuals collected in September 2017 in the Miranda sub-region, Mato Grosso do Sul. We identified 19 prey categories to Order level, out of which Coleoptera was the most representative group and the most important prey category, followed by Orthoptera and Hemiptera. We recorded a post-metamorphic individual of *Rhinella schneideri* in the diet of *L. podicipinus*, the first record of batracophagy for this species. Our results provide evidence for the opportunistic and generalist feeding behaviour of *L. podicipinus*.

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Knowledge on species feeding habits is crucial for the studies of a community's natural history, interactions and energy flux through the ecosystems [1]. Amphibians play important roles in the food webs of ecosystems because they represent a link between terrestrial and aquatic environments [2,3]. The diet of anurans is generally based on arthropods [4] and is influenced by factors such as prey availability [5,6], habitat changes [7], body size [8–10], seasonality [11,12], hunting strategy [13–16] and evolutionary factors [17]. Although anurans' diet consists basically of insects [1], it can also include vertebrates, as bats [18,19], fishes [20], snakes [21] and other anurans [batracophagy: 14,22–28].

Leptodactylus podicipinus (Cope, 1862) is a widely distributed leptodactylid frog, occupying open formations of Paraguay, Argentina, Bolivia, northwestern Uruguay, and central Brazil [29]. This species is considered small (females 30–54 mm SVL, males 24–43 mm SVL) and is characterized by its light coloured belly with dark spots and a stripe extending from either under midportion of the eye or posterior corner of the eye [30].

The Pantanal is one of the largest wetlands in the world and has a marked seasonal flood. It is subdivided into different sub-regions, according to the magnitude and frequency of floods, the soil and the vegetation, among others [31]. These floods pulses can alter the dynamic of resources and the structure of communities [32], modifying ecological process, as well as the diet composition of anurans. Although the Pantanal is an environment with predominance of flooded areas, which is an important environmental feature for amphibians, it is one of the least studied Brazilian regions [33]. Thus, the objective of our study was to evaluate the diet of *L. podicipinus* in south Pantanal, Midwest Brazil.

The diet of *Leptodactylus podicipinus* was studied by analysing the stomach contents of 30 individuals ($N = 15$ males and 15 females) captured in Base de Estudos do Pantanal (BEP, 19°34′37″S and 57°00′42″W) of the Universidade Federal de Mato Grosso do Sul (UFMS), in the Miranda sub-region [sensu 34], Corumbá, state of Mato Grosso do Sul, Brazil, in September 2017. We collected individuals by visual encounter surveys [35] along swamps available in the area.

The specimens were euthanized using topical anaesthetic (xylocaine 5%) and then fixed with 10% formaldehyde before analysing the stomach content. We removed stomachs through a small abdominal incision and stored the contents in separate vials. We preserved the frog individuals in 70% alcohol. The captured specimens were collected under the permission of Brazilian wildlife regulatory service (SISBIO#56729-1) and housed at Coleção Zoológica de Referência da Universidade Federal de Mato Grosso do Sul (ZUFMS AMP 07800–07829).

CONTACT Karoline Ceron adenomera@gmail.com

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We analysed the stomach content under a stereomicroscope, and identified each item to order level. We standardized prey identification to order level (e.g. Araneae, Coleoptera, Diptera), because it was the lowest taxonomic level possible considering the prey fragmentation level. After measuring the length and width of each prey, we estimated their volumes using the formula of the ellipsoid:

$$V = 4/3\pi * 2(W/2)2 * L/2$$

where, V = volume, W = width and L = length [36]. For each item (prey category) we calculated the number, volume, and frequency of occurrence in both absolute and percentage values. We then calculated the Index of Relative Importance (IRI) to determine the relative importance of each prey item in the diet using the following formula, according to Pinkas et al. [37]:

$$\text{IRI} = (\%N + \%P)\%FO$$

where % N is equal to the relative number of each prey item per sample set, % P is equal to the mass percentage of each prey item in the sample set and % FO represents the relative frequency of occurrence on the entire samples [38]. Higher IRI values indicate a greater importance of the prey category in the diet.

We also analysed the niche breadth using Levin's Measure of Niche Breadth (B) [38]. This measure allows calculation of the amplitude of the diet, particularly considering the quantitative distribution of each prey item. In order to allow comparisons with other studies, we calculated Levin's standardized measure of niche breadth (BA) after Hurlbert [39], which limits the value on a scale from 0 to 1, according to the following equation:

$$BA = \frac{B-1}{(n-1)}$$

where n represents the number of resources (prey species) registered. Values closer to 0 are attributed to specialist diets, while values closer to 1 represent generalist diets [38]. We performed all analyses using the software R, version 3.2 [40].

We found 19 preys categories, out of which Coleoptera was the most representative group (N = 37.01%, V = 26.64%) and the most important prey category (IRI = 1009.6). Based on the IRI and frequency values, Orthoptera were the second most representative prey category, followed by and Hemiptera [Table 1]. Anura, Coleoptera larvae, Collembola, Isoptera, Lepidoptera larvae, Mantodea, Odonata and Thysanura were the least frequent items, with a single occurrence. All stomachs evaluated had contents. The Levin's niche breadth of the species was 0.1.

We also recorded the first case of batracophagy for this species. The stomach content of an adult *L. podicipinus* male (CRC = 32.25 mm, mass = 3.4 g, ZUFMS AMP 07820) presented a post-metamorphic *Rhinella schneideri* (Werner, 1894) (CRC = 11.52 mm, mass = 0.2 g, ZUFMS AMP 07830), shown in Figure 1.

Table 1. Prey categories found in the stomachs of *Leptodactylus podicipinus* in Pantanal, Midwest Brazil. N = Percentage of the number individuals registered; V = Percentage of volume occupied by prey item in entire sample (in mm^3); F = Percentage of frequency of occurrence of prey category; IRI = Index of Relative Importance.

Prey category	*N* (%)	*V* (%)	*F* (%)	IRI
Annelidae	3.1	1.7	3.4	8.7
Anura	0.8	5.4	1.7	5.9
Acari	1.6	0.0	3.4	3.4
Araneae	3.1	2.9	5.1	14.2
Blattaria	2.4	5.6	3.4	16.7
Coleoptera	37.0	26.6	23.7	1009.7
Coleoptera larvae	1.6	2.7	1.7	5.9
Collembola	1.6	0.0	1.7	1.7
Diptera	6.3	0.6	6.8	10.3
Hemiptera	14.2	10.9	11.9	157.14
Hymenoptera	9.4	1.5	10.2	24.6
Isoptera	0.8	0.2	1.7	1.9
Lepidoptera larvae	0.8	2.5	1.7	3.7
Mantodea	0.8	0.4	1.7	2.0
NI	4.7	1.6	8.5	16.2
Odonata	0.8	4.3	1.7	5.1
Odonata larvae	3.9	7.6	6.8	36.8
Orthoptera	6.3	24.7	3.4	158.9
Thysanura	0.8	0.7	1.7	2.3

The number of prey categories that we registered in the diet of *L. podicipinus* (N = 19) was lower than the number of prey categories observed for this species in a study conducted in Pantanal of Abobral (N = 24) [11], and higher than another study conducted in rice fields in Pantanal of Miranda (N = 18) [7]. However, the higher number of prey orders registered by Rodrigues et al. [11] was influenced by the numbers of habitats sampled, which increases the diversity of available preys. In our study, we sampled only one habitat.

Species of the *Leptodactylus* genus are considered sit-and-wait generalist predators, consuming few food items that are large and mobile [41], as shown in the present study by the great abundance of coleopterans in the diet. Coleoptera is the largest order of insects in the world and Brazil [42]. The richness and abundance of this order is influenced by vegetation formations and soil moisture [42], which may explain the high IRI of this order in the diet composition of *L. podicipinus*. Likewise, this pattern can be attributed to differences in prey availability, since diet composition is determined by the arthropods availability in the environment occupied by the population [13,43,44]. This was also observed by Rodrigues et al. [11] and Piatti and Souza [7] for Pantanal, where Coleoptera was the most important order in the diet of *L. podicipinus* during both the wet and the dry season.

The presence of vertebrates in the diet of anurans is mainly restricted to large species, as previously reported for several *Ceratophrys*, *Leptodactylus*, *Lithobates* and *Rhinella* species [22,45–47]. However, this is the first report of batracophagy in the small-sized species *Leptodactylus podicipinus*. Although it is rare, small anurans can also prey other anurans [e.g. 48]. During the

Figure 1. The post-metamorphic *Rhinella schneideri* (left – ZUFMS-AMP07830) preyed by an adult male of *Leptodactylus podicipinus* (right – ZUFMS-AMP07820) in Pantanal, Midwest Brazil.
Note: Scale bar = 1 cm.

night of the fieldwork, we observed a high density of post-metamorphic *R. schneideri* active in the area sampled. The feeding strategy of sit-and-wait may offer possible advantages in situations of high population density or scarce food resources, such as obtaining energy while decreasing intraspecific competition [45,49]. Although the bufotoxin secreted by *R. schneideri* during all life stages causes nausea, vomiting, and even paralysis and death in potential predators [50], these aspects do not seem to have prevented the predation by *L. podicipinus*. Rodrigues et al. [11], suggesting that *Leptodactylus podicipinus* is an opportunistic predator. The post-metamorphic of *R. schneideri* in the stomach of *L. podicipinus* detected in our study may provide further evidence of this type of feeding behaviour.

In this sense, reports on the natural history and diet of anurans emphasize the importance of conducting studies in different habitats to understand the feeding patterns of widely distributed species [6], such as *L. podicipinus*. In the Pantanal of Miranda, the diet of *L. podicipinus* essentially consisted of invertebrates, particularly of Coleoptera. However, studies of food availability are imperative to verify if this result is influenced by prey availability or if it is species-specific preference. To the best of our knowledge, this is the first report of batracophagy in *L. podicipinus*.

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ORCID

Karoline Ceron http://orcid.org/0000-0003-2354-3756
Matheus de Toledo Moroti http://orcid.org/0000-0002-2645-9130
Ronildo Alves Benício http://orcid.org/0000-0002-7928-2172
Luiza Becker Pereira http://orcid.org/0000-0002-1649-811X
Diego José Santana http://orcid.org/0000-0002-8789-3061

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