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Diet of *Rhinella arenarum* (Anura, Bufonidae) in a coastal habitat in southern Brazil

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Abstract. Amphibians are good models for the study of trophic ecology because they occupy different trophic levels during their development. In this study, we evaluated the diet of *Rhinella arenarum* (Hensel, 1867) during breeding season in a marine–freshwater transitional habitat in southern Brazil. Based on the analysis of stomach contents, we recorded five groups of invertebrates (Araneae, Coleoptera, Diptera, Hymenoptera and Orthoptera). Despite of their low palatability, Coleoptera was the most representative group by both numeric and volumetric evaluation. The studied population had a smaller number of items in their diet when compared with to previous studies. These results suggest an example of a specialization in the diet during *R. arenarum* at this habitat, being Coleoptera the most common prey.

Keywords: Behaviour, diet composition, sand dunes

Amphibians are good models for the study of trophic ecology because they occupy different trophic levels of food webs (Duré et al., 2009). Despite the prevalence of insects in the diet of frogs, it can also include other invertebrates and vertebrates (Duellman and Trueb, 1994). Some bufonids, as *Melanophryniscus,* have a level of dietary specialization that exhibit preferences for ants or mites (Bortolini et al., 2013). Dietary specialization on predating beetles and ants was observed for some species of *Rhinella* (Lajmanovich, 1995; Peltzer et al., 2010). However, there is uncertainty about this specialization, since beetles and ants are often among the most abundant invertebrates at the terrestrial substrate surface and their consumers include generalist and/or opportunistic predators (Sabagh et al., 2012). Knowledge on the level of dietary specialization is important to detect regional and local differences on feeding habits, as well during different periods of the year (e.g. breeding season), of populations from different geographic regions (Duré et al., 2009). *Rhinella arenarum* (Fig. 1) is a relatively abundant species throughout its distribution area, which extends from the coastal region of southern Brazil to Uruguay, Argentina, and Bolivia (Frost, 2017). There are a few diet studies for the species, and they were performed only in some areas of occurrence known for the species (e.g. Quiroga et al., 2009; Attademo et al., 2005; Isacch and Barg, 2002).

In addition, in the extreme southern region of Brazil, there is a particular "continental land narrowing", generating a transitional habitat formed by a relatively small extension of land (approximately 10-30 km in width), surrounded by freshwater and marine systems

Figure 1. Adult individual of *Rhinella arenarum*. (Photo by Tozetti A. M.).

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(coastal sand dunes; Wollmann and Simioni, 2013). This environmental configuration (transitional habitat) may influence the composition of prey species that are likely to drive the specific dietary patterns of local consumers (Loebmann and Vieira, 2007). Thus, the objective of our study was to evaluate the diet *R. arenarum* in a transitional habitat (freshwater-marine water) in southern Brazil.

Materials and Methods

The diet of *Rhinella arenarum* was studied by analysing the stomach contents of 19 individuals captured in a coastal grassland habitat between November and December 2012. The study area encompasses a mosaic of sand dunes and grass fields associated with freshwater (Mirim Lagoon) and marine systems formed by a sand dune beach (Wollmann, and Simioni, 2013), at the municipality of Rio Grande in the state of Rio Grande do Sul (32.5069°S, 52.5842°W). The individuals were visually detected (see Crump and Scott, 1994) during the day and searched over all of the available microhabitats in the area. The specimens were euthanized using topical anaesthetic (Xylocaine) and then fixed with 10% formaldehyde before stomach content analysis. The captured specimens were collected under the permission of Brazilian wildlife regulatory service (SISBIO#35187-1) and deposited in the herpetological collection of Laboratório de Ecologia de Vertebrados Terrestres, of the Universidade do Vale do Rio dos Sinos (see Appendix I).

For the dietary evaluation, the specimens were taken to the laboratory and dissected to remove the contents from their stomach. We focused on the stomach because it was not possible to identify any solid prey fragments in other parts of the digestive tract. The contents of the stomach were preserved in 70% alcohol and then processed. Food items were identified and then quantified. We standardized the prey identification at order level (e.g., Araneae, Coleoptera, Diptera) because it is usually the lowest taxonomic level possible considering the consuming level of the prey. We also recorded plant material and unidentifiable fragments. We calculated the area (mm²) of each item using a millimetre graph paper. This measurement was obtained by spreading each item on a Petri dish to cover its surface and to maintain a regular height of 1 mm (Hellawell and Abel, 1971). The contents of each specimen's stomach were taken as a single sample. For each item (prey category) we calculated the number, volume, and frequency of occurrence in both absolute and percentage values. The volume (V) of each item was obtained by multiplying the area occupied by the item by the height. For this purpose, each item was crushed and spread evenly on a Petri dish with bottom graph, keeping the height standardized in 1mm (Hellawell and Abel, 1971). No empty space was left between the crushed materials. We then calculated the Index of Relative Importance (IRI), from Pinkas et al. (1971), to determine the relative importance of each prey item in the diet using the following formula: 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 of the entire samples (Krebs, 1999). Higher values of IRI indicate a greater importance of the prey category in the diet. To analyse the degree of feeding specialization the niche breadth size using Levin's Measure of Niche Breadth (B) was calculated (Krebs, 1999). This measure allows calculation of the amplitude of the diet, particularly considering the quantitative distribution of each prey item. To facilitate comparisons with other studies, we calculated Levin's standardized measure of niche breadth (Bsta), which limits the value on a scale from 0 to 1 according to the following equation: Bsta = (B-1) / (n-1), where n represents the number of resources (prey species) registered. Values closer to 0 are attributed to a specialist diet, while values closer to 1 represent a generalist diet (Krebs, 1999). Plant material and unidentifiable fragments categories were not quantified in number of individuals. Therefore, we did not calculate the IRI for them and these categories were not used to calculate the niche breadth.

Results

We recorded five prey categories: Araneae, Coleoptera, Diptera, Hymenoptera, and Orthoptera. According to numerical and volumetric measures, Coleoptera was the most representative group (N = 86.8%, V = 77.6%). In addition, the IRI noted that Coleoptera was the most important prey category (IRI = 16447.3). The remaining prey categories (Tab. 1) were registered with values at least ten times smaller than Coleoptera, regardless of the type of metric observed (number of individuals, volume or frequency of occurrence). Hymenoptera was the second most representative prey category (N = 6.6%, V = 0.3%, IRI = 145.5), followed by Araneae (N = 4.6%, V = 0.7%, IRI = 111.7). Diptera (N = 1.3%, V = 0.02%) and Orthoptera (N = 0.7%, V = 0.5%), which showed the lowest values. All the Hymenoptera found were ants. The niche breadth of the species was 0.08.

Table 1. Prey categories found in the stomachs of *R. arenarum* in a transition freshwater-marine habitat in southern Brazil. N = number of individuals registered; V = volume occupied by prey item in entire sample (in mm³); F = frequency of occurrence of prey category (%); IRI = Index of Relative Importance.

Prey	N (%)	V (%)	F (%)	IRI
Coleoptera	132 (86.8)	31509 (77.6)	19 (100)	16447.3
Hymenoptera	10 (6.6)	135 (0.3)	4 (21.1)	145.5
Araneae	7 (4.6)	285 (0.7)	4 (21.1)	111.7
Diptera	2 (1.3)	10 (0.02)	1 (5.3)	7.1
Orthoptera	1 (0.7)	204 (0.5)	1 (5.3)	6.1
Plant Material	--	994 (0.7)	10 (52.6)	--
Unidentified	--	7451 (18.4)	13 (68.4)	--

We also recorded plant material and unidentifiable fragments as a significant fraction of the stomach content (Tab. 1). The plant material was found in a relatively high frequency of occurrence value (F = 52.6%), as well as the amount of unidentified material, which presented relatively high values for both frequency of occurrence value (F = 68.4%) and volume (V = 18.4%).

Discussion

We registered five prey categories in the diet of *Rhinella arenarum*, which was lower than the number of prey categories (orders) observed for this species in studies conducted in Argentina: 18 (Quiroga et al., 2009); 11 (Isacch and Barg, 2002); and nine (Attademo et al., 2005). An equal number were obtained for *Rhinella icterica* (five orders) in southeastern Brazil (Sabagh et al., 2012). Whereas Sabagh and Carvalho-e-Silva (2008) were able to find a broader set of prey categories for *R. icterica*, reaching up to 21 different orders of prey. A wide variety of food items were also recorded for *Rhinella schneideri* (12 orders; Duré et al., 2009). Regional variations in niche breadth have already been recorded for other *Rhinella* species. In *R. icterica*, for example, Bsta values ranged from 0.03 to 0.20, according to the habitat studied (Sabagh et al., 2012).

The dietary importance of Coleoptera to *R. arenarum* has already been observed in Argentina (Quiroga et al., 2009; Cossovich et al., 2011; Sabagh et al., 2012). However, in this region, Hymenoptera was an important prey category as well, in contrast to our study. To Sabagh and Carvalho-e-Silva (2008), Formicidae (Hymenoptera) was the most relevant category to a population of *R. icterica* (IRI = 97.47) and *R. crucifer* (IRI = 125.86).

Great discrepancies in the proportions with which different prey are consumed are not common in the genus (Lajmanovich, 1995). The predominance of Coleoptera and Hymenoptera (Formicidae) in the diet of arthropods eaters, such as anurans, can be associated with the fact that beetles and ants are often abundant in most habitats (Clarke, 1974; M.O. pers. observ.). The predominant ingestion of the most abundant prey in the environment (opportunistic diet) has been reported for some *Rhinella* species (Evans and Lampo, 1996) and for other bufonids (Bonansea and Vaira, 2007). Another explanation for the differences in the food preference is that the variation in dietary composition between studies might be related to differences in the period of the year when the animals were studied (Sabagh and Carvalho-e-Silva, 2008). However, if the diet was exclusively directed by capturing the most abundant prey, we would expect a greater importance of Hymenoptera in our samples, as ants are one of the most abundant terrestrial invertebrates in environments similar to our study area in southern Brazil (Gantes and D'Incao, 2011).

Despite their high representation in the samples, the plant material may have been unintentionally consumed within the substrate during prey ingestion (Van Sluys et al., 2001). However, some studies suggest that the consumption of plant material may help in eliminating active digestion of prey exoskeletons (Anderson et al., 1999), which should be useful for predators with high levels of Coleoptera ingestion.

The population of *R. arenarum* studied seems to be supported by small prey richness, where only one prey category (order Coleoptera) is significantly more important than all the other categories. It is expected that species have broad trophic niches in certain regions and narrower trophic niches in others. Here, this difference may be related to regional variations in prey availability. Regardless of the reason, our results show a certain trophic plasticity of the species.

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Appendix I.

The *Rhinella arenaum* specimens used in this study are deposited at UNISINOS University (Universidade do Vale do Rio dos Sinos), in the Herpetological Collection of the Terrestrial Vertebrates Ecology Laboratory (CHLEVT). Voucher numbers: CHLEVT 84, 85, 86, 87, 88, 89, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, and 393.

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