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Diet of *Odontophrynus americanus* (Duméril and Bibron, 1841) in southern Atlantic Forest of Brazil

William Boavista Machado[1,*], Fernanda Rodrigues de Avila[2], Mateus de Oliveira[1], Patrícia Witt[2], and Alexandro Marques Tozetti[1]

The description of the diet and feeding behaviour is one of the most significant steps to understand the relationship among species, and, in a certain manner, essential to design a species' ecological niche (Sih and Christensen, 2001; Da Rosa et al., 2002). Amphibians, in specially anurans, play a fundamental role in trophic webs since they act as predators of a group of organisms (mostly arthropods) that compose a huge biomass in many ecosystems (Wells, 2007). At the same time, anurans are consumed by many predators (vertebrates and invertebrates as well). Therefore, anurans are potential elements acting as links among trophic chains in many different ecosystems (Duellman and Trueb, 1994; Araújo et al., 2007). In the last decades, there has been an unquestionable improvement in the number of studies about diet or feeding ecology of anurans in the Neotropics. However, even for already evaluated species, more detailed studies are welcome since they favor the evaluation of regional variation in feeding behaviour. Considering this, species with a wide distribution, such as *Odontophrynus americanus* (Duméril and Bibron, 1841), fit as good models for massive and detailed studies of diet. This species occurs in an area extending from central Brazil to Paraguay, Uruguay, and Argentina (Maneyro and Carreira, 2016; Aquino et al., 2010). In addition, available information on the diet of *O. americanus* is limited to studies developed in grassland and agricultural habitats in Argentina (Isacch et al., 2002; Peltzer et al., 2010; Cossovich et al., 2011). The aim of this study was therefore to evaluate the diet of *O. americanus* based on gut content analysis of individuals from Brazilian natural habitats.

We examined animals collected in November 2014 and January 2016 in the southernmost portion of the Brazilian Atlantic Forest. The climate in the study region is subtropical humid with an annual precipitation of 1.300 mm and an average air temperature of 19.4°C (Maluf, 2000). Sampling was performed in the municipalities of Capela de Santana (29°40'S and 51°20'W) and Porto Alegre (30º14'S and 51º05'W), southern Brazil. We used two techniques to capture individuals: active search (Crump and Scott Jr, 1994) and accidental captures. The active search was conducted only in the locality of Capela de Santana. Immediately after the capture, individuals were transferred to a cooler box and kept under a temperature of 15º C to reduce the process of digestion of their gut contents (Oliveira, 2014). No more than four hours after capture, individuals were euthanized by application of a lethal amount of benzocaine on their skin. Specimens from Porto Alegre were collected in a natural reserve called Reserva Biológica do Lami José Lutzenberger (RBLJL). The RBLJL is located in a well-preserved forest formation (Witt, 2017) and, together, both sampled localities represent natural habitats of *Odontophrynus americanus*. We must highlight that the captures of individuals in RBLJL were the result of a by-catch in pitfall traps filled with alcohol solution (Campbell and Christman, 1982), which were installed during a entomological field work. The alcohol guarantees the automatic process of euthanasia and ends the process of digestion, favouring diet evaluation. Those traps were installed in order to evaluate the entomofauna of the RBLJL, and the capture of anurans was not intentional.

Due to the differences in sampling size, as well the similarities in the composition of the diet among individuals of both localities, we pooled all samples

[1] Universidade do Vale do Rio dos Sinos – UNISINOS, Laboratório de Ecologia de Vertebrados Terrestres, São Leopoldo, Rio Grande do Sul, Brazil.
[2] Universidade do Vale do Rio dos Sinos – UNISINOS, Laboratório de Genética e Biologia Molecular, São Leopoldo, Rio Grande do Sul, Brazil.
[*] Corresponding author. E-mail: william.boavista@hotmail.com

as a single sample. All individuals were dissected and gut content was removed and preserved in alcohol solution (70%). We identified prey species using a stereomicroscope and comparing with a reference guide. Preys were identified at the level of order and their contribution to the diet was estimated by the Index of Relative Importance (IRI), expressed in percentage, following Pinkas et al. (1971). Higher values of IRI imply greater relevance of a specific prey in the diet. We also estimated niche breadth through the Levin's Standardized Trophic Niche Breadth Index (Bsta) (Krebs, 1999).

We examined 38 individuals, from which four presented empty stomachs. We identified 13 categories of consumed prey (Table 1). The most important prey items, based on IRI values, were Lepidoptera larvae (IRI = 726.9), Coleoptera (IRI = 612.6) and Isopoda (IRI = 552.8). The same set of prey items revealed greater values based on the number of individuals (Isopoda: %N = 22.8; Coleoptera: %N = 19; Lepidoptera larvae: %N = 16.5). Based on a volumetric measurement, besides Lepidoptera larvae (%V = 24.7), two new prey items exhibited higher values: Orthoptera (%V = 11.6) and Araneae (%V = 9.1). Four prey items revealed higher values in terms of frequency of occurrence: Coleoptera (%FO = 23.5), Isopoda (%FO = 20.6), Lepidoptera larvae (%FO = 17.6) and Gastropoda (%FO = 17.6). About one third of the total volume of prey items in the digestive tract presented a high level of fragmentation, which caused limitations for taxonomic identification. This material was described as "unidentified" and made up 27.3% of the total volume of gut content. Levin's Standardized Trophic Niche Breadth Index (Bsta) was 0.5, suggesting a non-specialized diet.

Our results show that *Odontophrynus americanus* consumed mostly Isopoda, Coleoptera and Lepidoptera larvae as reported in populations from Argentina (Isacch et al., 2002; Peltzer et al., 2010; Cossovich et al., 2011). These prey items represent some of the most often recorded invertebrates in the ground litter of many Brazilian forests (Couri et al., 2009). This suggests that their dominance in the diet would be a result of their high availability in the habitat. In addition, Isopoda has affinities with moist microhabitats, having been observed under logs, rocks and in holes in the ground (Dobson and Postema, 2014), characteristics that are shared with habitats where *O. americanus* is most frequently observed (pers. obs.). Although the dominance of Isopoda in the diet was observed in other populations of *O. americanus* (Isacch et al., 2002;

Table 1. Categories of consumed prey based on gut contents of *Odontophrynus americanus* in southern Brazil. N= number of individuals; V= volume of each prey item (mm³); F= frequency of occurrence (%); IRI= Index of Relative Importance.

	Gut content						
	N	**%N**	**V**	**%V**	**FO**	**%FO**	**IRI**
Annelida	8	10.1	468.0	3.0	2.0	5.9	77.2
Araneae	4	5.1	1421.0	9.1	4.0	11.8	166.8
Blattodea	1	1.3	378.0	2.4	1.0	2.9	10.9
Chilopoda	2	2.5	36.0	0.2	1.0	2.9	8.1
Coleoptera	15	19.0	1099.0	7.1	8.0	23.5	612.6
Diplopoda	2	2.5	464.0	3.0	2.0	5.9	32.4
Gastropoda	8	10.1	783.0	5.0	6.0	17.7	267.3
Hymenoptera	3	3.8	167.0	1.1	3.0	8.8	43.0
Isopoda	18	22.8	634.0	4.1	7.0	20.6	552.8
Lepidoptera (larvae)	13	16.5	3857.0	24.7	6.0	17.7	726.9
Lepidoptera	2	2.5	60.0	0.4	2.0	5.9	17.2
Neuroptera	1	1.3	100.0	0.6	1.0	2.9	5.6
Orthoptera	2	2.5	1808.0	11.6	2.0	5.9	83.1
Unidentified			4264.0	27.4			

Peltzer et al., 2010; Maneyro and Carreira, 2016), this prey item was not consumed in some other localities (Isacch et al., 2002). It is important to highlight that prey mobility would affect their probability of being detected by anurans. Prey types with greater potential for mobility, such as Coleoptera, (Crowson, 1981), are more prone to be found and preyed than less mobile ones. On the other hand, Lepidoptera larvae, supposedly a group with low mobility, were dominant in the diet of the examined anurans. We argue that the gregarious habits of these larvae (Monteiro et al., 2007; Dobson and Postema, 2014) favoured the consumption of a large number of individuals when at least one of them was detected. Although somewhat speculative, we argue that *Odontophrynus americanus* exhibit an opportunistic feeding behaviour. Its non-specialized diet, reinforced by our estimation of niche breadth (0.5), suggests a different pattern from what was observed by Cossovich et al. (2011). The low consumption of Araneae and Gastropoda in our samples diverges from that of other populations (see Peltzer et al., 2010). Considering this, we point out that *O. americanus* might be able to adjust its diet according to the availability of local prey, which also suggests a certain level of opportunism in their feeding behaviour.

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