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Diet, sexual dimorphism, and fertility aspects of *Melanophryniscus fulvoguttatus* (Mertens, 1937) from Central-East Brazil

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Abstract

Melanophryniscus fulvoguttatus is a small toad that inhabits the Cerrado, Atlantic Forest, and humid Chaco of South America. Due to its aposematic coloration and behavior, it is a species that may present chemical defenses such as alkaloids and a restricted diet similar to other species of the genus. Although it was described in the early 20th century, there is still little information on its natural history and ecology. Here, we described the diet, tested the sexual dimorphism, and calculated the fertility index of *M. fulvoguttatus* based on a sample of eight males and 21 females from a population within the Cerrado. Sexual dimorphism was assessed using 15 morphometric measures; diet was calculated as an index of relative importance by prey item and tested for sex differences; fertility was tested for relations between body size and the number of eggs; and we also estimated the reproductive investment. Our results demonstrate that this population's diet consisted mostly of ant species, with *Pheidole radoszkowskii* being the most important, followed by *Crematogaster* sp. and *Cyphomyrmex transversus*. Regarding sexual dimorphism, we observed that females were significantly larger than males, with a positive relationship between female size and the number of eggs. We did not find significant differences in the diets of males and females. Although the diet and reproductive aspects of *M. fulvoguttatus* are similar to those of its congeneric species, further studies are needed in order to address the relationship between the species diet and its chemical defenses.

Key Words

Bufonidae, food webs, natural history

Introduction

Melanoprsyniscus is a South American genus of small-sized toads whose distribution includes Bolivia, Paraguay, Argentina, Brazil, and Uruguay (Frost 2023). The genus is currently composed of 31 species arranged in three species groups based on warts, snout protuberances, and skin coloration (Cruz and Caramaschi 2003; Frost 2023). Furthermore, *Melanophryniscus* is one of the few genera within Bufonidae with known alkaloids, which is a chemical defense against predators (Wells 2007; Grant et al. 2012). In addition, species of this genus typically display the unken reflex, the defensive behavioral response of arching the back and rising the limbs and head, thus exposing aposematic red fingers and palms, presumably to avoid subjugation against predators (Wells 2007; Toledo et al. 2011; Hantak et al. 2013).

Melanophryniscus fulvoguttatus is a relatively medium-sized toad from the *M. stelzneri* species group typically found in the Cerrado, Atlantic Forest, and humid Chaco from Argentina, Paraguay, and Brazil (Weiler et al. 2003; Frost 2023). *M. fulvoguttatus* is classified as Least

Concern (Baldo and Langone 2004). However, species within this genus are reported to be illegally commercialized; the last conservation assessment took place more than 15 years ago (Baldo and Langone 2004), and it inhabits biomes that face intensive habitat losses due to anthropogenic pressures (Carvalho et al. 2009; Mereles and Rodas 2014; Mohebalian 2022).

Individuals of *M. fulvoguttatus* species present skin spines in their head, dorsum, and flanks, whereas body spines are located in shallow warts (Cruz and Caramaschi 2003). The skin is predominantly dark brown with dorsal yellow spots and ventral yellow and red spots (Weiler et al. 2003). Additionally, its hands and palms are bright red, a coloration pattern that has been associated with the unken reflex (Cruz and Caramaschi 2003; Weiler et al. 2003). Its aposematic coloration and behavior suggest the presence of chemical defenses such as alkaloids, as occurs in several species of Anura, including *Melanoprhyniscus* (Wells 2007; Daly et al. 2008). This pattern is repeated all over the world, including species of Myobatrachidae (Australia; Sague et al. 2023), Mantellidae (Madagascar; Clark 2006), Dendrobatidae, and Bufonidae (Central and South America; Drast et al. 2005; Daly et al. 2007). Additionally, anurans with this kind of defense usually sequester their alkaloids from the diet, making necessary specialist habits and diets that are mostly composed of ants and mites (Darst et al. 2005; Saporito et al. 2007; Saporito et al. 2009a; Walters et al. 2023).

Other variables that affect the quantity of chemical defense acquired by the diet are those related to body size, like snout-vent length and wet skin mass (Jeckle et al. 2015), factors that defer between sexes, such as the fact that usually in anurans females are larger than males (Shine 1975), a common pattern in *Melanophryniscus* (Bidau et al. 2011).

Despite the current panorama of *M. fulvoguttatus* and its potential for the discovery of unknown chemical defenses, multiple natural historical features such as diet, tadpole morphology, advertisement call, reproductive mode, and reproductive strategies remain unknown. Therefore, it is paramount to unveil the multiple aspects of this species natural history in order to assist with future conservation assessments. In order to better understand the natural history aspects of this species, we described the diet, tested the sexual dimorphism, and calculated the fertility index of this conspicuous toad from South America.

Material and methods

A total of 29 individuals (eight males and 21 females) of *Melanophryniscus fulvoguttatus* were collected in a Cerrado area from Bela Vista municipality, Mato Grosso do Sul state, Central-East Brazil (21º59'53.0"S, 56º43'25.0"W; SISBIO license 49080-5). Organisms were euthanized with lidocaine 5%, fixed in formaldehyde 10%, and preserved in alcohol 70%. All samples were housed at the Coleção Zoológica da Universidade Federal de Mato Grosso do Sul (ZUFMS-AMP 15777–15805).

The stomachs of 29 collected specimens were removed by ventral dissection, and they were subsequently preserved in 70% alcohol at ZUFMS-AMP along with the specimens. Samples with empty stomachs (six females and two males) were not considered in the analyses. Diet items were classified by order level, and prey belonging to the Formicidae (Hymenoptera) were identified by genera and species level whenever possible. From there, we calculated an index of relative importance (IRI) per prey category present in the diet following Pinkas et al. (1971): IRI = (N%+V%)*F%, where F% is the relative frequency of an item, N% is the relative number of an item, and V% is the relative volume of an item. To calculate the relative volume, we used a chamber made with microscopy slides nearest to 1 mm³ (Camera et al. 2014; Carrillo et al. 2022). The comparison for possible differences between male and female diets was made through a PERMANOVA analysis using the volume of consumed prey. All statistical analyses were conducted using the vegan package for the R platform (Oksanen et al. 2022; R-Core-Team-D 2022).

All organisms collected were characterized as sexually mature by direct inspection of gonads. We used the 15 morphometric measurements following Heyer et al. (1990) and Cruz and Caramaschi (2003) to assess the sexual dimorphism in size and shape: snout vent length (SVL), head length (HL), head width (HW), inter orbital distance (IOD), inter nostril distance (IND), eye diameter (ED), eye nostril distance (END), nostril snout distance (NSD), upper eyelid width (UEW), thigh length (THL), tibia length (TL), foot length (FL), foot and tarsus length (FTL), hand length (HAL), and forearm length (FAL). Measurements were taken using a caliper with a minimal accuracy of 0.01 mm or under a dissecting microscope when needed. We tested for differences in body size between sexes using a principal component analysis (PCA) and a multivariate analysis of variance (MANOVA) with the two first components of the PCA.

Considering the females collected, we also tested whether larger females are capable of investing more in oocytes. This was done by assessing the relationship between body size and the number of eggs. Eggs were removed using a ventral dissection and preserved in 70% alcohol at ZUFMS-AMP along with the specimens. We compared the female size (SVL) and the number of oocytes using a linear model. The reproductive investment was calculated following Pereira and Maneyro (2018): RI = number of Oocytes/SVL. Oocyte size was considered as the mean of the oocyte diameter per female, using 10 oocytes per female.

Results

The most consumed order was Hymenoptera (Table 1), over Arachnida, Coleoptera, and Diptera. Within Hymenoptera, most prey items belonged to the Formicidae. *Pheidole radoszkowskii* (Formicidae) was the most common species in frequency, volume, and total number, thus being the most important species consumed (IRI = 1088.49), followed by *Crematogaster* sp. (IRI = 233.52) and *Cyphomyrmex transversus* (IRI = 190.11). There were no significant differences in diet composition or in the volume of prey between males and females ($F = 0.60$, $p = 0.55$).

We observed a significant difference in mean body size between males and females of *Melanophryniscus fulvoguttatus* ($F = 3.62$, $p = 0.04$; Fig. 1). Despite overlap in the size ranges of several morphometric measurements (Table 2), on average, females are larger than males (SVL: 22.87 ± 1.31 and 20.91 ± 0.39 mm, respectively).

Females of *Melanophryniscus fulvoguttatus* presented an average of 110.9 ± 79.94 eggs (from 12 to 246 mm). Egg polarization was always well defined. There was a positive relation between the size (SVL) of females and the number of eggs ($F = 14.95$; $p = 0.001$), in which larger females presented more eggs than the small ones (Fig. 2). The mean egg size was 1.10 ± 0.06 mm. The reproductive investment was estimated at 4.89 ± 3.47 (from 0.56 to 10; Fig. 3).

Table 1. Diet of *Melanophryniscus fulvoguttatus* from Bela Vista, Mato Grosso do Sul (Brazil). Number of prey items (N), Frequency (F), Volume in mm³ (V), and index of relative importance (IRI).

	N	N%	F	F%	V	V%	IRI
Arachnida							
Araneae	1	0.24	1	4.76	1	0.24	1.19
Insecta							
Coleoptera	2	0.48	2	9.52	8	1.91	19.1
Diptera	1	0.24	1	4.76	2	0.48	2.39
Hymenoptera							
Formicidae							
Pheidole radoszkowskii	176	42	12	57.14	46	10.98	1088.49
Cyphomyrmex transversus	36	8.59	7	33.33	19	4.53	190.11
Wasmannia auropunctata	6	1.43	1	4.76	1	0.24	1.48
Crematogaster sp.	128	30.55	5	23.81	18	4.3	233.52
Non Formicidae	24	5.73	4	19.05	12	2.86	70.96
Vegetation	2	0.48	2	9.52	5	1.19	11.93
Undetermined	43	10.26	11	52.38	37	8.83	553.18

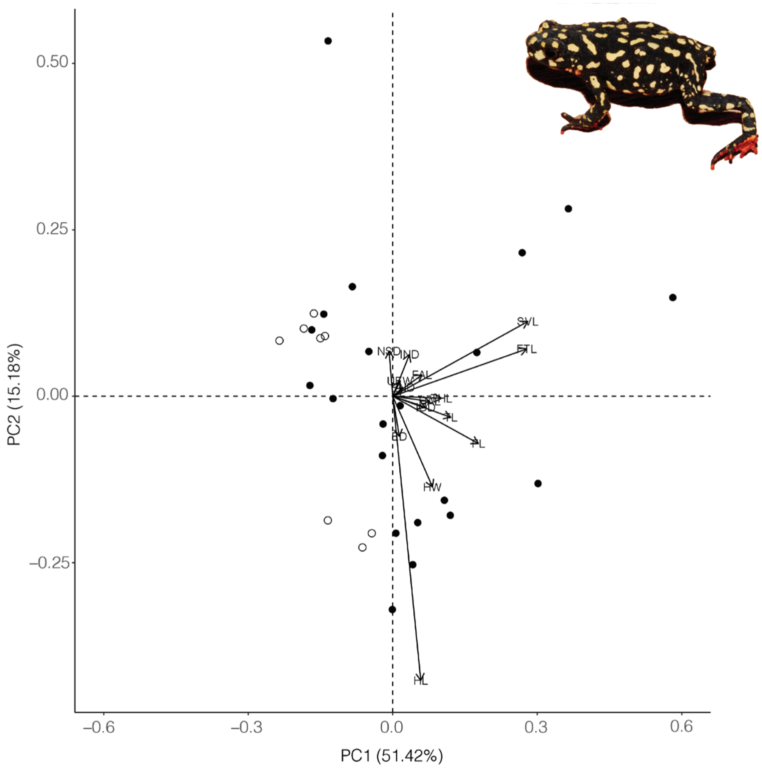

Figure 1. Principal components analysis for male (white) and female (black) body measures. Snout vent length (SVL), head length (HL), head width (HW), inter orbital distance (IOD), inter nostril distance (IND), eye diameter (ED), eye nostril distance (END), nostril snout distance (NSD), upper eyelid width (UEW), thigh length (THL), tibia length (TL), foot length (FL), foot and tarsus length (FTL), hand length (HAL) and forearm length (FAL).

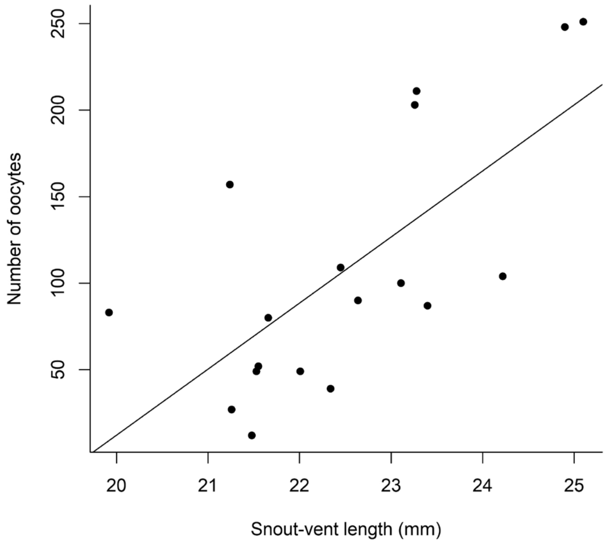

Figure 2. Relationship between female snout vent length and number of eggs of *Melanophryniscus fulvoguttatus*.

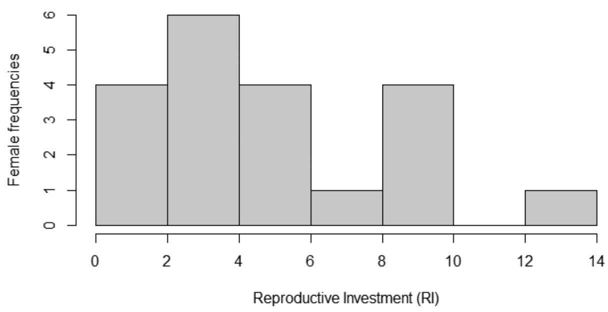

Figure 3. Frequency of the reproductive investment in females of *Melanophryniscus fulvoguttatus*.

Table 2. Mean, standard deviation, and range (mm) of 15 morphometric features of *Melanophryniscus fulvoguttatus* from Bela Vista, Mato Grosso do Sul (Brazil).

	Female (N= 21)	Male (N= 8)
Snout vent length	22.87 ± 1.31 (20.55–25.63)	20.91 ± 0.39 (20.29–21.40)
Head length	5.96 ± 1.15 (3.05–7.90)	5.65 ± 0.92 (4.66–6.95)
Head width	7.07 ± 0.67 (5.52–8.57)	6.67 ± 0.33 (6.14–7.20)
Inter orbital distance	2.70 ± 0.81 (1.56–4.55)	2.99 ± 0.83 (1.67–4.05)
Inter nostril distance	1.68 ± 0.37 (0.90–2.40)	1.45 ± 0.55 (0.66–2.15)
Eye diameter	1.68 ± 0.33 (1.06–2.23)	1.67 ± 0.51 (1.07–2.67)
Eye nostril distance	1.74 ± 0.19 (1.15–2.19)	1.72 ± 0.31 (1.46–2.41)
Nostril Snout distance	1.26 ± 0.33 (0.69–1.80)	1.13 ± 0.18 (0.82–1.33)
Upper eyelid width	2.31 ± 0.46 (0.84–2.87)	2.39 ± 0.34 (1.81–2.92)
Thigh length	7.73 ± 0.69 (6.78–9.44)	6.99 ± 0.55 (6.31–7.65)
Tibia length	7.23 ± 0.53 (6.62–8.40)	6.45 ± 0.41 (5.94–7.17)
Foot length	6.57 ± 1.03 (4.56–9.10)	6.44 ± 0.87 (5.60–8.31)
Foot and tarsus length	10.64 ± 1.42 (8.85–14.67)	9.96 ± 0.81 (8.90–10.94)
Hand Length	5.13 ± 0.41 (4.73–6.25)	4.77 ± 0.31 (4.40–5.40)
Forearm Length	5.49 ± 0.40 (4.98–6.50)	5.24 ± 0.30 (4.86–5.77)

Discussion

All the most important items in the diet of *Melanophryniscus fulvoguttatus* belonged to the Order Hymenoptera (Formicidae), followed by Coleoptera and Diptera. Although species of *Melanophryniscus* typically have a very restricted diet, the most important prey are usually Hymenoptera and Aracnida (Acari), as in *M. devincenzii*, *M. rubriventris*, *M. stelzneri*, *M. klappenbachi* and *M. cupreuscapularis* (Filipello and Crespo 1994; Bustos-Singer and Rodriguez 1997; Bonansea and Vaira 2007; Daly et al. 2008; Quiroga et al. 2011; Bortolini et al. 2013); hence, the results presented herein indicate that *M. fulvoguttatus* may have the most restricted diet within *Melanophryniscus* (Bonansea and Vaira 2007; Bortolini et al. 2013). Although we did not account for prey availability in this study, we highlight that the ant species *Pheidole radoszkowskii*, *Crematogaster* sp., and *Cyphomyrmex transversus* (Formicidae) were the most relevant for the diet of *M. fulvoguttatus*. Similarly to the ants found in *M. klappenbachi*, *M. rubriventris*, and *M. stelzneri*, the ant species reported herein all present chemical defenses and alkaloids (Filipello and Crespo 1994; Bonansea and Vaira 2007; Quiroga et al. 2011; Arias et al. 2021). Additionally, it has been reported that the diet of *M. rubriventris* can change between populations, which may also be the case with *M. fulvoguttatus* (Bonansea and Vaira 2007; Quiroga et al. 2011).

Multiple species of anurans present chemical defense complemented with aposematic colorations, e.g., Bufonidae, Dendrobatidae, and Mantellidae (Saporito et al. 2009a). Some of its congenerics have alkaloids described, i.e., *M. stelzneri*, *M. rubriventris*, and *M. klappenbachi* (Daly et al. 2007, 2008; Mebs et al. 2018; Arias et al. 2021). These species also presented a similar diet to *M. fulvoguttatus*. In fact, diet is a main factor in frogs's chemical defenses; species with alkaloids-rich skins usually consume a very specific diet to sequester those substances from ants and mites; otherwise, toxicity decreases (Mebs et al. 2018). In this context, the diet and coloration of *Melanophryniscus fulvoguttatus* are typical of frog species that use alkaloids as chemical defense, and they need to be confirmed experimentally.

The redescription of *Melanophryniscus fulvoguttatus* (Cruz and Caramaschi 2003) presented a clear difference in size between males and females, with descriptive statistics indicating females are larger than males, which is in agreement with the results presented herein. Moreover, it is worth mentioning that our sample included males and females smaller than the redescription. This may be because we only analyze organisms from one locality, while the redescription included samples from six localities (Cruz and Caramaschi 2003). Nevertheless, sexual dimorphism has been demonstrated in numerous amphibian species (Shine 1975; Wells 2007), including the congeneric *M. montevidensis* (Pereira and Maneyro 2018). Furthermore, these differences may be a pattern that changes between populations or geographically (Yu et al. 2010). Sexual size dimorphism within bufonids is well known and well-established in most of the toads' genera (e.g., Mueses-Cisneros et al. 2012; Peloso et al. 2012; Rojas et al. 2022). Additionally, species from other families such as Ranidae, Hylidae, and Leptodactylidae have shown variation in size and sexual dimorphism due to geographic variation (Yu et al. 2010; Boaratti and Da Silva 2015). Size also may affect the quantity of acquired chemical defenses that can be sex-related, as proven in *Oophaga pumilio* and *Melanophryniscus moreirae* (Saporito et al. 2009b; Jeckel et al. 2015); however, this relation has not been tested in *M. fulvoguttatus*.

Bufonidae species mainly invest in large clutches of small eggs, which, in theory, depend on the availability of environmental energy (Vitt and Caldwell 2014). For instance, *Melanophryniscus krauczuki* has clutches of up to 401 eggs (Baldo and Basso 2004). The females of *Melanophryniscus fulvoguttatus* here analyzed presented similar clutch sizes to other congeneric species of *Melanophryniscus*, such as *M. stelzneri* and *M. montevidensis* (Bustos-Singer and Rodriguez 1997; Cairo et al. 2008; Pereira and Maneyro 2018). Species of *Melanophryniscus* are usually explosive breeders, and reproduction relies on temporary ponds formed after heavy rainfalls (Cairo et al. 2008). Eggs can be laid under water or above the water surface, depending on the species (Bustos-Singer and Rodriguez 1997; Vaira 2005; Goldberg et al. 2006; Cairo et al. 2008). On the other hand, a phytotelmata reproductive mode has also been described for the genus (Langone et al. 2008; Steinbach-Padilha 2008; Bornschein et al. 2015). In this case, female investment is in small clutches like *M. alipioi* with 16 eggs (Bornschein et al. 2015). Now that we know that phytotelmata present smaller clutches, it would be expected to find bigger eggs than those from species from temporary ponds like *M. fulvoguttatus*, but it needs to be tested.

Still, there is no specific information on the reproduction of *M. fulvoguttatus*. Although it belongs to the *M. stelzneri* species group and reproduction may be similar, we do not know if there are variations for *M. fulvoguttatus* in reproductive period, reproductive mode, egg laying, amplexus, advertisement call, or call locations.

Further analyses of the diet should account for prey availability to determine the selectivity of *Melanophryniscus fulvoguttatus* for ant-related diets for chemical defenses such as alkaloid sequestering. It is necessary to describe the skin-acquired chemical defenses to compare with the diet, establish a direct relationship with the chemical defenses presented in the community of arthropods that compose the *M. fulvoguttatus* diet, and study if there are additional factors involved, such as body size or sex. Additionally, the rate of acquisition of chemical defenses is unknown, and the efficiency of chemical defense acquisition may be an interesting ecological feature to explore between the genus.

Reproductive investment from species that reproduce in phytotelmata needs to be described and compared with water egg-laying species. Besides, several reproductive features mentioned above remain unknown, and they need to be described for a better understanding of the ecology and reproduction of the species, thus helping future conservation assessments to enhance species preservation.

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