Herpetological Conservation and Biology 16(2):251–258.
Submitted: 5 October 2020; Accepted: 3 May 2021; Published 31 August 2021.

Diet of *Melanophryniscus paraguayensis* (Anura: Bufonidae): an Endemic Species to Paraguay

Karina Núñez[1,3], Marta Duré[2], Griselda Zárate[1], Fátima Ortiz[1], and Medes Mendoza[1]

[1]*Universidad Nacional de Asunción, Facultad de Ciencias Exactas y Naturales, Departamento de Biología, Colección Zoológica, San Lorenzo, Paraguay*
[2]*Centro de Ecología Aplicada del Litoral, Consejo Nacional de Investigaciones Científicas y Técnicas (CECOAL, CONICET-UNNE)/ Facultad de Ciencias Exactas y Naturales y Agrimensura (FACENA-UNNE), Corrientes, Argentina*
[3]*Corresponding author, e-mail: ranitapy@gmail.com*

Abstract.—***Melanophryniscus paraguayensis*** **(no common name) is an endemic toad of the central grasslands in the eastern region of Paraguay. Details about its natural history are poorly understood and it is categorized nationally as Vulnerable. This work describes the diet composition of this species and the relationship between toad body size and the number and volume of prey consumed. We analyzed the stomach content of 162 individuals, using the stomach flushing technique, after measuring and weighing them. For each prey category, we calculated the volume, number, and frequency of occurrence, and we estimated the relative importance index (IRI) with these data. We also estimated the standardized Shannon Diversity Index and Levins Niche Breadth Index for prey categories, and we analyzed the correlation between size of the anurans and prey size. Seventy-six individuals had identifiable content, which consisted of 1,357 prey classified into 16 categories, mostly at the order level. Ants and mites were the prey taxa with the greatest contribution in number and frequency and represent the most important prey based on IRI. Volumetrically, ants and beetles predominated. Ticks, spiders, springtails, flies, true bugs, wasps, termites, thrips, larvae and nymphs of insects, centipedes, crabs, and snails were occasional prey. The mean prey volume consumed by toads was positively correlated with toad snout-vent length. The Shannon and Levins indices showed that the composition of the diet was dominated by a few groups of arthropods. This work demonstrates the importance of ants, mites, and beetles as food for *M. paraguayensis*, which is consistent with findings for other species of the genus *Melanophryniscus* and of many species of the Bufonidae family.**

Key Words.—acari; Formicidae; predator-prey; stomach flushing; trophic ecology

Introduction

The regulation of arthropod populations through predation is one of the most recognized services provided by anuran amphibians in ecosystems (Valencia-Aguilar et al. 2013; Hocking and Babbitt 2014; Cortés-Gomez et al. 2015). Trophic composition is essential in the study of the trophic niche and reflects processes related to foraging strategies and prey selection and capture (Wells 2010). Of all the prey available for consumption by a species, those that are actually consumed are conditioned by abiotic and biotic factors and the evolutionary history of both the predator and the prey (Vitt and Caldwell 2014). The importance of studying trophic ecology is to understand the contribution of taxa such as amphibians, arthropods, and other invertebrates in the flow of matter and energy through food chains. This knowledge allows the development of more effective conservation strategies, both for these animals and for the ecosystems of which they are a part.

Melanophryniscus (South American redbelly toads) is a South American genus in the Bufonidae family, currently comprised of 31 species that are distributed in northern Argentina, Paraguay, southeast Bolivia, southeast Brazil, and Uruguay (Frost 2021). Six species occur in Paraguay (Weiler et al. 2013): *M. atroluteus* (Uruguay Redbelly Toad), *M. devincenzii* (Rivera Redbelly Toad), *M. fulvoguttatus* (no common name), *M. klappenbachi* (Klappenbach's Red-bellied Frog), *M. krauczuki* (no common name), and *M. paraguayensis* (no common name). This last species, included in the *M. stelzneri* group, was described by Céspedez and Motte (2007). Individuals of this species measure < 30 mm snout-vent length (SVL), have black coloration with yellow dorsal spots, and ventral red spots (Fig. 1), and are endemic to the grasslands of central Paraguay. The species is categorized as Vulnerable at the national level because its distribution is restricted and its habitat is continually degraded due to urbanization and agricultural and livestock development (Motte et al. 2019).

Although most species of the genus *Melanophryniscus* are categorized under some degree of threat (Motte et al. 2019; International Union for the Conservation of Nature 2020), the trophic ecology of most of them has not yet been well studied and fundamental data on their natural history are unknown. Of the 11 species

Figure 1. Dorsal view of *Melanophryniscus paraguayensis* (no common name) in its habitat in Paraguay (snout-vent length = 21.58 mm). (Photographed by Fátima Ortiz).

in the *M. stelzneri* group, the diets of only four have been described: *M. cupreuscapularis* (no common name; Duré and Kehr 2006; Duré et al. 2009), *M. montevidensis* (Montevideo Redbelly Toad; Mebs et al. 2005), *M. rubriventris* (Yungas Redbelly Toad; Bonansea and Vaira 2007), and *M. stelzneri* (Redbelly Toad; Filipello and Crespo 1994). Of the remaining species of the genus, only the diet of *M. devincenzii* (Bortolini et al. 2013) is known. Recognizing the importance of knowing the aspects of the natural history of the endemic and threatened *M. paraguayensis*, we aimed to provide knowledge on the composition of the diet of this species. We also determined if there was a relationship between the size of these toads and the number and volume of prey consumed, from data recorded in wetland areas in the districts of Asunción, Nueva Italia, and Quiindy in Paraguay.

Materials and Methods

Study sites.—We conducted the study in three sampling sites located in the districts of Asunción, Nueva Italia, and Quiindy chosen for being within the distribution of *M. paraguayensis* (Fig. 2). The first sampling site was an urban park and the last two to the Ypoá Wetland Complex, in the Central and Paraguarí departments. The urban park (25°15'53.3"S, 57°32'42.7"W) is characterized by degraded grassland with abundant exotic grasses, mainly Star Grass (*Cynodon nlemfuensis*). The Nueva Italia sampling site (25º39'24"S, 57º31'00.8"W) was an area impacted by sugarcane crops and livestock. The vegetation included islands of semi-deciduous sub-humid forests, hygrophilous forests, and natural grasslands. In Quiindy, the sampling site (25°56'54.90"S, 57°26'29.17"W) was

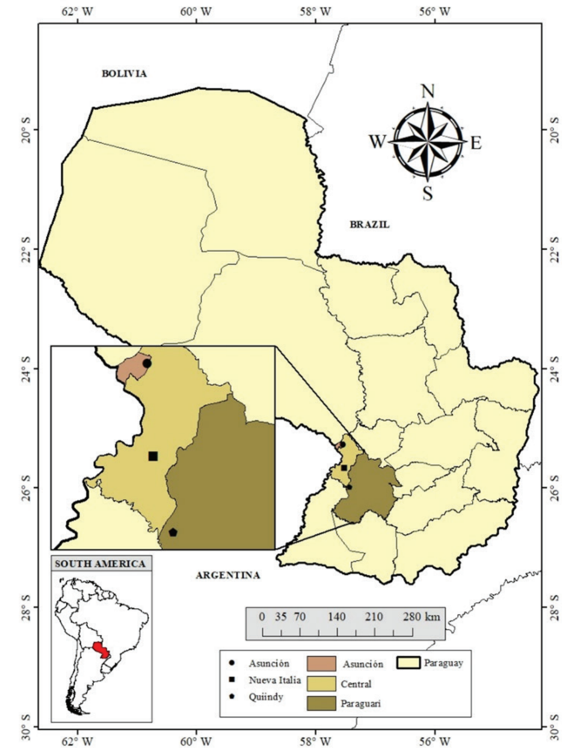


Figure 2. Location of the three sampling sites used in this study in the districts of Asunción, Nueva Italia, and Quiindy in Paraguay. The three darker polygons in the inset map represent departments in Paraguay.

characterized by a mostly rocky soil, with plantings of Bread Grass (*Brachiaria brizantha*) for fattening cattle, sub-humid semi-deciduous and hygrophilous forests, palm savannahs of Caranday Palm (*Copernicia alba*), and wetland savannas with dominance of *Pirizales* (*Cyperus giganteus*) and cattail (*totorales*; *Typha* sp.).

Data collection.—We captured post-metamorphic toads by hand, both during the day and night, between 1000 and 2200, in December of 2017, February, May, August, and September of 2018, and February and May of 2019, using visual encounter surveys (Crump and Scott 1994). We captured toads on days when temperature ranged between 12º C and 32º C. After capture, we took the toads to camp where we measured SVL with a 0.01 mm precision digital caliper, weighed them with a 0.01 g precision digital scale, and determined their sex according to external characteristics such as vocal sac. Afterwards, we applied the stomach flushing technique (Solé et al. 2005), using 20 ml of well water, and we preserved the regurgitated contents in 70% alcohol. We released the individuals at the capture site the same day or the following day. We sacrificed 20 specimens and deposited them in the Zoological Collection of the Facultad de Ciencias Exactas y Naturales – Universidad Nacional de Asunción (specimen numbers: CZCEN 1326, 1336, 1376, 1377, 1381, 1382, 1431, 1432, 1433, 1434, 1442, 1452, 1453, 1454, 1469, 1477, 1494, 1539, 1540, 1583).

We analyzed the stomach contents under a stereomicroscope, and we quantified and identified the food types to the lowest taxonomic level possible, generally Order (Family level in the case of Formicidae), following Hogue (1993), Gullan and Cranston (2010), and Heckman (2011). We classified the Acari subclass into two groups: mites and ticks (Ixodida), and we considered insect larvae as a separate category, due to the difficulty of identification when the diagnostic structures are digested.

We measured the length (L) and width (W) of the prey with stereomicroscope (Carl Zeiss, Oberkochen, Baden-Württemberg, Germany) with built-in camera AmScope MU900 software camera 3.7 (AmScope, Irvine, California, USA), and then we estimated their volume (V; mm^3) using the ellipsoid formula (Dunham 1983):

$$V = \frac{4}{3} \Pi \left(\frac{1}{2} L\right) \left(\frac{1}{2} W\right)^2$$

To avoid volumetric bias, only prey with more than 70% of the body undigested were included in the analysis. In addition, we quantified the frequency of occurrence (F) of each prey in stomachs and the abundance (N) of each prey category. With these variables (N, V, and F), we calculated the Relative Importance Index (IRI; Pinkas et al. 1971):

$$IRI = (N\% + V\%)\ F\%$$

where: N% = numerical percentage of prey, V% = volumetric percentage, and F% = percentage of frequency of occurrence.

To determine the sampling efficiency, we constructed the prey category accumulation curve in relation to the number of stomachs studied with the Estimates 9 software (Colwell, R.K. 2013. EstimateS: statistical estimation of species richness and shared species from samples. Version 9. Available from http://purl.oclc.org/estimates [Accessed 15 May 2020]). We used the Shannon Diversity Index to quantify diet diversity (Shannon and Weaver 1949):

$$H' = -\sum_{i=1}^{s} (p_i \ln p_i)$$

where p_i is the proportion of prey type i in the sample, and s is the total number of prey categories. Additionally, we estimated the Niche Breadth using the index proposed by Levins (1968):

$$Nb = \left(\sum P_{ij}^2\right)^{-1}$$

where P_{ij} represents the probability of finding the item i in the sample j. We standardized both indices (J': evenness measure of the Shannon-Wiener, and B_A: Levins Standardized Niche Breadth) to facilitate comparison between species of the same genus, as described in Krebs (2014):

$$J' = \frac{H'}{\ln n}$$

$$B_A = \frac{Nb - 1}{s - 1}$$

To determine the relationship between the size (SVL) of toads with the abundance of prey and mean prey volume, we used Spearman's non-parametric correlation coefficient (r_s; Zar 2010) with $\alpha = 0.05$ using PAST 3.02 software (Hammer et al. 2001).

Results

We captured 162 post-metamorphic individuals of *M. paraguayensis*, of which 76 had identifiable stomach content (32 females and 44 males), corresponding to 47% of the total captured and analyzed. The mean SVL was 21.67 ± (standard deviation) 2.15 mm (range, 17.00–26.73 mm), and the mean body mass was 0.93 ± 0.29 g (range, 0.33–1.77 g). We identified 16 prey categories in the diet of *M. paraguayensis*, composed of arthropods, including seven taxonomic categories of insects, three of arachnids, and a single taxon of centipedes, crabs, and snails (Table 1). The most numerous prey categories were Hymenoptera - Formicidae (N = 560, N% = 41.27%) and Acari (mites; N = 531, N% = 39.13%). Formicidae was also the taxon with the highest volumetric contribution (V = 113.68 mm^3, V% = 49.20%), followed by Coleoptera (V = 43.28 mm^3, V% = 18.73%). These taxa were also the most frequent: Hymenoptera - Formicidae (F = 69, F% = 90.79%), Acari (mites; F = 42, F% = 55.26%), and Coleoptera (F = 25, F% = 32.89%). Mean richness prey type per stomach was 3.18 (maximum = 10). The number of prey per stomach ranged from 1 to 173 with a mean = 17.86 ± 25.82, and the volume of prey ingested was between 0.06 and 19.96 mm^3 (3.04 ± 3.93 mm^3). We also recorded plant material and sand granules in stomachs.

Based on IRI results, the diet of *M. paraguayensis* was dominated by Hymenoptera - Formicidae (IRI = 82.14), followed by Acari (mites; IRI = 25.9), and Coleoptera (IRI = 7.4). The prey accumulation curve, in relation to the number of stomachs analyzed, showed a tendency to asymptote (Fig. 3). The diversity of the diet of *M. paraguayensis* was $H' = 1.46$ (standardized $J' = 0.53$) and the trophic niche breadth was $Nb = 3.03$ (standardized $B_A = 0.14$). The relationship between the SVL of the toads and the mean prey volume of stomach contents was significant and positive ($rs = 0.30$, $P = 0.009$; Fig. 4). The relationship between SVL and number of prey was not significant ($rs = 0.05$, $P = 0.616$; Fig. 4).

Table 1. Diet composition of *Melanophryniscus paraguayensis* toads (n = 76) from three sites in the Asunción, Nueva Italia, and Quiindy districts of Paraguay. Abbreviations are N = abundance, number of prey; N% = number of prey as percentage of total prey consumed; V = prey volume (mm³); V% = volume of prey as percentage of total prey volume; F = frequency of occurrence of prey category; F% = relative frequency; IRI = relative importance index.

Class	Prey Category	N	N% (%)	V (mm³)	V% (%)	F	F% (%)	IRI
Arachnida	Acari – not Ixodida (mites)	531	39.13	17.88	7.74	42	55.26	25.90
	Acari – Ixodida (ticks)	29	2.14	9.51	4.12	16	21.05	1.32
	Araneae (spiders)	23	1.69	8.57	3.71	19	25.00	1.35
Insecta	Coleoptera (beetles)	51	3.76	43.28	18.73	25	32.89	7.40
	Collembola (springtails)	1	0.07	0.01	0.00	1	1.32	0
	Diptera (flies)	79	5.82	2.49	1.08	14	18.42	1.27
	Hemiptera – Adults (true bugs)	3	0.22	3.08	1.33	3	3.95	0.06
	Hemiptera – Nymph (true bugs)	13	0.96	2.15	0.93	7	9.21	0.17
	Hymenoptera – Non-Formicidae (wasps)	9	0.66	1.00	0.43	8	10.53	0.12
	Hymenoptera – Formicidae (ants)	560	41.27	113.68	49.20	69	90.79	82.14
	Isoptera (termites)	2	0.15	0.91	0.40	1	1.32	0.01
	Thysanoptera (thrips)	18	1.33	4.23	1.83	13	17.11	0.54
	Larvae	30	2.21	14.78	6.40	17	22.37	1.93
Chilopoda	(centipedes)	6	0.44	5.87	2.54	5	6.58	0.20
Malacostraca	Decapoda (crabs)	1	0.07	0.37	0.16	1	1.32	0
Gastropoda	(snails)	1	0.07	3.23	1.40	1	1.32	0.02
	Total	1,357	100	231.05	100			

Discussion

We found 16 categories of prey in the diet of *M. paraguayensis*. Most prey were arthropods (mainly insects and arachnids), although the consumption of gastropod mollusks was also recorded. These results are similar to those obtained for congeneric species, both in the number of prey categories and in the composition of the diet (Filipello and Crespo 1994; Bonansea and Vaira 2007; Duré et al. 2009; Bortolini et al. 2013). *Melanophryniscus paraguayensis* has a diet dominated by ants, mites, and beetles. This is reflected in the IRI results and in the accumulation curve (which asymptotes), so analyzing a greater number of samples would likely yield new categories only for prey consumed occasionally. The evenness measure of diversity index was $J' = 0.53$, lower than values obtained for *M. devincenzii* in Brazil and *M. montevidensis* in Uruguay, and higher than that registered for *M. stelzneri* and *M. cupreuscapularis* in Argentina and *M. admirabilis* in Brazil (Table 2). As in two other species of the genus (Table 2), the trophic niche breadth of *M.*

Table 2. Number of prey categories and indices for trophic diversity and niche breadth in seven species of *Melanophryniscus*. Sample sizes are indicated in parentheses. Prey categories were mostly at the Order level, except as noted. Terms are *H'* = Shannon Diversity Index, *J'* = Evenness measure of the Shannon-Wiener, *Nb* = Levins Niche Breadth, B_A = Levins Standardized Niche Breadth. The asterisk (*) indicates range of values for four populations and the symbol -- = unreported values.

Species	Prey categories	*H'*	*J'*	*Nb*	B_A	Source
Melanophryniscus admirabilis (n = 92)	13	—	0.17	—	—	Thayná Lima, unpubl. data
M. cupreuscapularis (n = 22)	11	0.95	0.39	1.39	0.04	Duré et al. (2009)
M. devincenzii (n = 34)	14	—	0.79	—	—	Bortolini et al. (2013)
M. montevidensis (n = 35)	35 families	—	0.61	—	—	Federico Achaval-Coppes, unpubl. data
M. paraguayensis (n = 76)	16	1.46	0.53	3.03	0.14	This study
M. rubriventris (n = 132)	17	—	—	2.7–4.5*	—	Bonansea and Vaira (2007)
M. stelzneri (n = 37)	15	1.77	0.43	2.34	0.08	Filipello and Crespo (1994)

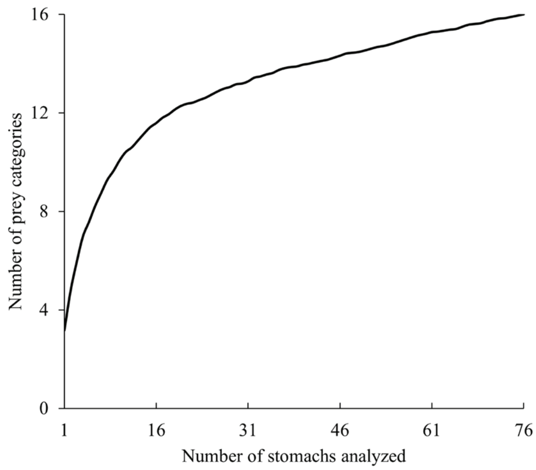


Figure 3. Accumulation curve showing the number of prey categories in relation to the number of stomachs containing prey analyzed in the study of the diet of *Melanophryniscus paraguayensis* (no common name) in Paraguay.

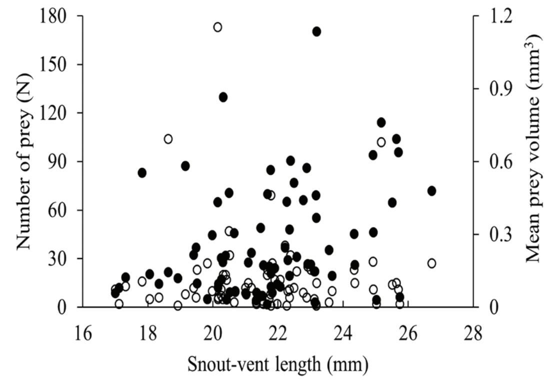


Figure 4. Relationship between snout-vent length (mm) of post-metamorphic *Melanophryniscus paraguayensis* (no common name) in Paraguay in relation to the number (open circles) and mean volume (solid circles) of prey consumed.

paraguayensis was low (B_A close to 0). This would indicate that these species have a diet with a tendency to specialization, which is composed of several prey types but only one (or a few) prey types dominate.

Filipello and Crespo (1994) report the consumption of arthropods and mollusks for *M. stelzneri* from Argentina with a predominance of ants, springtails, mites, beetles, termites, and insect larvae. The consumption of formicids, mites, coleopterans, and collembola was also reported for other species from Argentina such as *M. cupreuscapularis* (Duré and Kehr 2006; Duré et al. 2009) and *M. rubriventris* (Bonasea and Vaira 2007; Daly et al. 2007; Quiroga et al. 2011). These prey categories were also dominant in the diet of *M. devicenzii* from Brazil (Bortolini et al. 2013).

The preferential consumption of coleopterans and ants has been reported for neotropical species of the Bufonidae family (Isacch and Barg 2002; Cossovich et al. 2011; Menin et al. 2015). Some of the species that occur in sympatry with *M. paraguayensis* show this tendency, such as the toad *Rhinella azarai* (no common name), which mainly consumes ants (Ingaramo et al. 2012). The same was reported in another study that analyzed the diet of four *Rhinella* species that live in sympatry with *M. cupreuscapularis* in Argentina (Duré et al. 2009). It was observed that the smallest species (*R. bergi*, *R. major*, and *Rhinella dorbignyi* (=*R. fernandezae*); all no common name) preferentially consumed ants, while the larger *Rhinella diptycha* (=*R. schneideri*; Cururu Toad) adults preferred beetles.

Amphibians produce and accumulate skin secretions that contain a wide variety of defensive chemicals from consumed prey, including peptides, proteins, biogenic amines, bufadienolides, tetrodotoxins, and lipophilic alkaloids (Daly 1998; Daly et al. 2005; Saporito et al. 2012; Hantak et al. 2013; Jeckel et al. 2015). The *Melanophryniscus* diet includes a large number of mites and ants (Filipello and Crespo 1994; Bonansea and Vaira 2007; Quiroga et al. 2011, Garraffo et al. 2012; Bortolini et al. 2013), which constitute the main source of lipophilic alkaloids in the diet of other poisonous frog species such as dendrobatids and mantelids (Clark et al. 2005; Saporito et al. 2009; Hantak et al. 2013). Previous studies report that some *Melanophryniscus* species have sequestered alkaloids (derived from their diet), but they also biosynthesized indolealkylamine bufotenin (Garraffo et al. 1993; Hantak et al. 2013; Jeckel et al. 2015; Mebs et al. 2018). Some arthropods have been indicated as containers for alkaloids, including mites, ants, and some beetles and millipedes (Saporito et al. 2007, 2009). The diet of *M. paraguayensis* has a high proportion of these prey.

Mollusks (gastropods) and myriapods are occasional prey in bufonid species (Duré et al. 2009), as well as the crustacean found in a single individual in the present study. Finally, ingestion of plant material was also reported for *M. devicenzii* (Bortoloni et al. 2013) but was considered as accidental consumption. We agree with this assessment and speculate that the plants we found in *M. paraguayensis* stomachs were ingested together with the arthropods that are found on them.

In addition to the obvious relationship between diet and alkaloids, the selection of a particular type of prey may be linked to other factors. Extrinsic factors that influence predator-prey dynamics include the abundance of each prey type in the environment and competition at the intra or interspecific level; other factors of intrinsic origin are those associated with the physiology of the predator in the consumption and digestion of food during its ontogeny (Duellman and Trueb 1986; Bortolini et al. 2013). Based on this, the strategy used by the predator for detection, capture, ingestion, and digestion of prey involves adjustments in cost-benefit terms. Thus, active foragers, such as the species of the genus *Melanophryniscus*, move about through the environment in search of prey, expending considerable energy in the search phase but little energy in the capture phase because they consume grouped prey (Vitt and Caldwell 2014). *Melanophryniscus* individuals have to capture a high number of prey because, in general, prey are small, of aggregate distribution (anthills and termite mounds), chitinous and, therefore, more expensive to digest (Toft 1981; Dietl et al. 2009).

In relation to ontogenetic changes in diet, Filipello and Crespo (1994) proposed a differential consumption of prey by adults and juveniles of *M. stelzneri*. They argued that there is low overlap in the diet between these stages of development, which may be achieved in part by juveniles using an active foraging strategy in contrast to adults using an intermediate between active foraging and sit-and-wait strategies. Therefore, adults eat mostly Formicidae whereas juveniles prefer Collembola. In our study, post-metamorphic individuals (female and male) were analyzed without incorporating an analysis of the diet by stage of development. The low consumption of Collembola in the diet of the specimens that we analyze may be related to the fact that we only considered post-metamorphic adults specimens, or it could be explained by lower prey availability in the environment, which was not estimated. We found that larger toads of *M. paraguayensis* consumed larger prey, but a relationship between toad size and prey number was not evident. The relationship between size and volume ingested was also found in *Melanophryniscus rubriventris* (Bonansea and Vaira 2007), *M. devincenzii* (Bortolini et al. 2013), but not in *Melanophryniscus cupreuscapularis* (Duré and Kehr 2006).

The batrachofauna of Paraguay consists of 87 species (Motte et al. 2019; Cabral et al. 2020), of which 23 are categorized as threatened (Motte et al. 2019). These authors point out the importance of collecting biological information on the species to develop action plans to mitigate threats. In the case of *M. paraguayensis*, the combination of its restricted distribution with habitats shrinking due to urbanization and agricultural and livestock development could negatively affect its populations, as was shown for the diet of *Elachistocleis bicolor* (Two-colored Oval Frog), which is affected by agricultural land uses (Berazategui et al. 2007). Therefore, it is important to expand knowledge about different ecological and biological aspects of the species. The information provided in this study represents the first contribution to the knowledge of the natural history of this species that is of interest for conservation.

Acknowledgments.—We thank the Consejo Nacional de Ciencia y Tecnología (CONACYT) for financing the field trips with resources from the Fondo para la Excelencia de la Educación y la Investigación and Bolivar Garcete for helping us with the identification of arthropods. Daniela Tabilo kindly revised English writing. This study was carried out under the permit for scientific collection from Ministerio del Ambiente y Desarrollo Sostenible (N° 244/2017).

Literature Cited

Berazategui, M., A. Camargo, and R. Maneyro. 2007. Environmental and seasonal variation in the diet of *Elachistocleis bicolor* (Guérin-Méneville 1838) (Anura: Microhylidae) from northern Uruguay. Zoological Science 24:225–231.

Bonansea, M.I., and M. Vaira. 2007. Geographic variation of the diet of *Melanophryniscus rubriventris* (Anura: Bufonidae) in northwestern Argentina. Journal of Herpetology 41:231–236.

Bortolini, S.V., R. Maneyro, F.C. Achaval, and N. Zanella. 2013. Diet of *Melanophryniscus devincenzii* (Anura: Bufonidae) from Parque Municipal de Sertão, Rio Grande do Sul, Brazil. Herpetological Journal 23:115–119.

Cabral, H., M.D. Casagranda, F. Brusquetti, F. Netto, V. Ferreira, and E. Lavilla. 2020. Multiscale endemism analysis for amphibians of Paraguay. Herpetological Journal 30:35–46.

Céspedez, J.A., and M. Motte. 2007. Una nueva especie de *Melanophryniscus* Gallardo, 1961 de Paraguay (Amphibia: Anura: Bufonidae). Facena 23:31–42.

Clark, V.C., C.J. Raxworthy, V. Rakotomalala, P. Sierwald, and B.L. Fisher. 2005. Convergent evolution of chemical defense in poison frogs and arthropod prey between Madagascar and the neotropics. Proceedings of the National Academy of Sciences of the United States of America 102:11617–11622.

Cortés-Gomez, A.M., C.A. Ruiz-Agudelo, A. Valencia-Aguilar, and R.J. Ladle. 2015. Ecological functions of neotropical amphibians and reptiles: a review. Universitas Scientiarum 20:229–245.

Cossovich, S., L. Aun, and R. Martori. 2011. Análisis trófico de la herpetofauna de la localidad de Alto Alegre (Depto. Unión, Córdoba, Argentina). Cuadernos de Herpetología 25:11–19.

Crump, M.L., and N.J. Scott, Jr. 1994. Visual encounter surveys. Pp. 84–92 *In* Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.C. Hayek, and M.S. Foster (Eds.). Smithsonian Institution, Washington, D.C., USA.

Daly, J.W. 1998. Thirty years of discovering arthropod alkaloids in amphibian skin. Journal of Natural Products 61:162–172.

Daly, J.W., T.F. Spande, and H.M. Garraffo. 2005.

Alkaloids from amphibian skin: a tabulation of over eight-hundred compounds. Journal of Natural Products 68:1556–1575.

Daly, J.W., J.M. Wilham, T.F. Spande, H.M. Garraffo, R.R. Gil, G.L. Silva, and M. Vaira. 2007. Alkaloids in bufonid toads (*Melanophryniscus*): temporal and geographic determinants for two Argentinian species. Journal of Chemical Ecology 33:871–887.

Dietl, J., W. Engels, and M. Sole. 2009. Diet and feeding behaviour of the leaf-litter frog *Ischnocnema henselii* (Anura: Brachycephalidae) in Araucaria rain forests on the Serra Geral of Rio Grande do Sul, Brazil. Journal of Natural History 43:1473–1483.

Duellman, W., and L. Trueb. 1986. Biology of Amphibians. Johns Hopkins University Press, Baltimore, Maryland, USA.

Dunham, A.E. 1983. Realized niche overlap, resource abundance, and intensity of interspecific competition. Pp. 261–280 *In* Lizard Ecology, Studies of a Model Organism. Huey, R., E. Pianka, and T. Schoener (Eds.). Harvard University Press, Cambridge, England.

Duré, M.I., and A.I. Kehr. 2006. *Melanophryniscus cupreuscapularis* diet. Herpetological Review 37:338.

Duré, M.I., A.I. Kehr, and E.F. Schaefer. 2009. Niche overlap and resource partitioning among five sympatric bufonids (Anura, Bufonidae) from northeastern Argentina. Phyllomedusa 8:27–39.

Filipello, A.M., and F.A. Crespo. 1994. Alimentación en *Melanophryniscus stelzneri* (Anura: Bufonidae). Cuadernos de Herpetología 8:18–24.

Frost, D.R. 2021. Amphibian Species of the World: an Online Reference. Version 6.1. American Museum of Natural History. https://amphibiansoftheworld.amnh.org/index.php.

Garraffo, H.M., N.R. Andriamaharavo, M. Vaira, M.F. Quiroga, C. Heit, and T.F. Spande. 2012. Alkaloids from single skins of the Argentinian toad *Melanophryniscus rubriventris* (Anura, Bufonidae): an unexpected variability in alkaloid profiles and a profusion of new structures. SpringerPlus 1:1–51. https://doi.org/10.1186/2193-1801-1-51.

Garraffo, H.M., T.F. Spande, J.W. Daly, A. Baldessari, and E.G. Gros. 1993. Alkaloids from bufonid toads (*Melanophryniscus*): decahydroquinolines, pumiliotoxins and homopumiliotoxins, indolizidines, pyrrolizidines, and quinolizidines. Journal of Natural Products 56:357–373.

Gullan, P.J., and P.S. Cranston. 2010. The Insects: An Outline of Entomology. Wiley, Oxford, England.

Hammer, Ø., D.A. Harper, and P.D. Ryan. 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4:9.

Hantak, M.M., T. Grant, S. Reinsch, D. Mcginnity, M. Loring, N. Toyooka, and R.A. Saporito. 2013. Dietary alkaloid sequestration in a poison frog: an experimental test of alkaloid uptake in *Melanophryniscus stelzneri* (Bufonidae). Journal of Chemical Ecology 39:1400–1406.

Heckman, C.W. 2011. Encyclopedia of South American Aquatic Insects: Hemiptera-Heteroptera: Illustrated Keys to Known Families, Genera, and Species in South America. Springer Science and Business Media, New York, New York, USA.

Hocking, D.J., and K.J. Babbitt. 2014. Amphibian contributions to ecosystem services. Herpetological Conservation and Biology 9:1–17.

Hogue, C.L. 1993. Latin American Insects and Entomology. University of California Press, Berkeley, California, USA.

Ingaramo, M.D.R., J.L. Acosta, V.H. Zaracho, C. Falcione, E.G. Etchepare, R.V. Semhan, and B.B. Álvarez. 2012. Distribución y comentarios sobre la dieta de *Rhinella azarai* (Anura, Bufonidae) en Corrientes, Argentina. Cuadernos de Herpetología 26:55–58.

Isacch, J.P., and M. Barg. 2002. Are bufonid toads specialized ant-feeders? A case test from the Argentinian flooding pampa. Journal of Natural History 36:2005–2012.

International Union for the Conservation of Nature (IUCN). 2020. The IUCN Red List of Threatened Species. Version 2020–3. https://www.iucnredlist.org. melanophryniscus&searchType=species.

Jeckel, A.M., T. Grant, and R.A. Saporito. 2015. Sequestered and synthesized chemical defenses in the poison frog *Melanophryniscus moreirae*. Journal of Chemical Ecology 41:505–512.

Krebs, C.J. 2014. Ecological Methodology. Publishing in Process. University of British Columbia, Vancouver, British Columbia, Canadá.

Levins, R. 1968. Evolution in Changing Environments: Some Theoretical Explorations. 2nd Edition. Princeton University Press, Princeton, New Jersey, USA.

Mebs, D., W. Pogoda, R. Maneyro, and A. Kwet. 2005. Studies on the poisonous skin secretion of individual Red Bellied Toads, *Melanophryniscus montevidensis* (Anura, Bufonidae), from Uruguay. Toxicon 46:641–650.

Mebs, D., W. Pogoda, and S.W. Toennes. 2018. Loss of skin alkaloids in poison toads, *Melanophryniscus klappenbachi* (Anura: Bufonidae) when fed alkaloid-free diet. Toxicon 150:267–269.

Menin, M., R.S. Santos, R.E. Borges, and L. Piatti. 2015. Notes on the diet of seven terrestrial frogs in three agroecosystems and forest remnants in northwestern São Paulo State, Brazil. Herpetology Notes 8:401–405.

Motte, M., V. Zaracho, A. Caballero-Gini, M. Ferreira-Riveros, L.R. Nardelli, D. Coronel-Bejarano, F. Netto, A. Carosini, V. Rojas, D. Bueno, et al. 2019. Estado de conservación y lista roja de los anfibios del Paraguay. Boletín del Museo Nacional de Historia Natural del Paraguay 1:1–62.

Nectoux Culau, C., P. Colombo, L. Azevedo Moura, and R. Ott. 2019. Dieta do sapinho-narigudo-de-barriga-vermelha, *Melanophryniscus macrogranulosus* (Anura, Bufonidae). Anais do IX Congresso Brasileiro de Herpetologia 2:106468.

Pinkas, L., M.S. Oliphant, and I.L.K. Iverson. 1971. Food habits of Albacore, Bluefin Tuna, and Bonito in California waters. Fish Bulletin 152:1–105.

Quiroga, M.F., M. Vaira, and M.I. Bonansea. 2011. Population diet variation and individual specialization in the poison toad, *Melanophryniscus rubriventris* (Vellard, 1947). Amphibia-Reptilia 32:261–265.

Saporito, R.A., M.A. Donnelly, R.A. Norton, H.M. Garraffo, T.F. Spande, and J.W. Daly. 2007. Oribatid mites as a major dietary source for alkaloids in poison frogs. Proceedings of the National Academy of Sciences of the United States of America 104:8885–8890.

Saporito, R.A., M.A. Donnelly, T.F. Spande, and H.M. Garraffo. 2012. A review of chemical ecology in poison frogs. Chemoecology 22:159–168.

Saporito, R.A., T.F. Spande, and H.M. Garraffo. 2009. Arthropod alkaloids in poison frogs: a review of the 'dietary hypothesis'. Heterocycles 79:277–297.

Shannon, C.E., and W. Weaver. 1949. The Mathematical Theory of Communication. University of Illinois Press, Urbana, Illinois, USA.

Solé, M., O. Beckmann, B. Pelz, A. Kwet, and W. Engels. 2005. Stomach-flushing for diet analysis in anurans: an improved protocol evaluated in a case study in Araucaria forests, southern Brazil. Studies on Neotropical Fauna and Environment 40:23–28.

Toft, C.A. 1981. Feeding ecology of Panamanian litter anurans: patterns in diet and foraging mode. Journal of Herpetology 15:139–144.

Valencia-Aguilar, A., A.M. Cortés-Gómez, and C.A. Ruiz-Agudelo. 2013. Ecosystem services provided by amphibians and reptiles in Neotropical ecosystems. International Journal of Biodiversity Science, Ecosystem Services and Management 9:257–272.

Vitt, L.J., and J.P. Caldwell. 2014. Herpetology: An Introductory Biology of Amphibians and Reptiles. 4th Edition. Elsevier, Norman, Oklahoma, USA.

Weiler, A., K. Nuñez, K. Airaldi, E. Lavilla, S. Peris, and D. Baldo. 2013. Anfibios del Paraguay. Universidad Nacional de Asunción, Facultad de Ciencias Exactas y Naturales, San Lorenzo, Central, Paraguay.

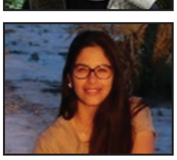
Wells, K.D. 2010. The Ecology and Behavior of Amphibians. University of Chicago Press, Chicago, Illinois, USA.

Zar, J.H. 2010. Biostatistical Analysis. 5th Edition. Pearson Prentice-Hall, Upper Saddle River, New Jersey, USA.

Karina Núñez received a degree in Biological Sciences from the Universidad Nacional de Asunción (UNA), San Lorenzo, Central Department, Paraguay, in 2002 and a Master's degree in Ecology from the Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazônia, Brazil, in 2008. Currently, she is a doctoral student at the Universidad Nacional del Nordeste (FACENA-UNNE), Corrientes, Corrientes, Argentina. Karina is a Professor and Researcher at Facultad de Ciencias Exactas y Naturales at UNA and a categorized Researcher of the Consejo Nacional de Ciencia y Tecnología, Asunción, Capital, Paraguay. Her work focuses on the ecology and conservation of vertebrates, especially anuran amphibians. (Photographed by Karina Núñez).

Marta Duré graduated in Zoology from the Universidad Nacional del Nordeste, Corrientes, Corrientes, Argentina, and she received a Ph.D. in Natural Sciences from the Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina. She is an Adjunct Professor in the Department of Biology at the Universidad Nacional del Nordeste (FACENA-UNNE), Corrientes, Corrientes, Argentina, and Assistant Researcher at the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Corrientes, Corrientes, Argentina. Marta is a specialist in feeding behavior and trophic ecology of anurans, and she has also conducted bioacoustics studies and collaborated in research on parasitism and amphibian conservation. (Photographed by Marta Duré).

Griselda Zárate is a Biologist with a Master's degree in Manejo de Recursos Naturales y Gestión Ambiental del Territorio from the Universidad Nacional de Asunción, San Lorenzo, Central Department, Paraguay. She teaches subjects related to biology and studies vertebrate diversity, ecology, and conservation, mainly of amphibians. Griselda was recently part of the research team of the Ypoá Wetlands Herpetofauna project. (Photographed by Fátima Ortiz).



Fátima Ortiz received a degree in Biology from the Facultad de Ciencias Exactas y Naturales of Universidad Nacional de Asunción (UNA), San Lorenzo, Central Department, Paraguay, in 2015. She is currently studying for a Master's degree in Systematic and Biodiversity (Universidad Nacional de Asunción). Fátima teaches at UNA and works as a researcher in projects related to ecology and zoology of the wetlands of Paraguay, and as an environmental educator. (Photographed by Arnaldo Corvalán).

Medes Mendoza obtained a degree in Biology in 2017 from the Facultad de Ciencias Exactas y Naturales (FACEN) of the Universidad Nacional de Asunción, San Lorenzo, Central Department, Paraguay. He is currently working on a postgraduate degree in Geographic Information Systems and Remote Sensing (Universidad del Pacífico, Asunción, Paraguay). Medes works as an Assistant in the Botanical Laboratory of the FACEN and also in Biodiversity and Conservation projects. (Photographed by Fátima Ortiz).