

Diet of juveniles of the venomous frog *Aparasphenodon brunoi* (Amphibia: Hylidae) in southeastern Brazil

ROGÉRIO L. TEIXEIRA¹, RICARDO LOURENÇO-DE-MORAES², DÉBORA C. MEDEIROS³, CHARLES DUCA³, ROGÉRIO C. BRITTO⁴, LUIZ C.P. BISSOLI⁵, RODRIGO B. FERREIRA^{3,*}

¹ *In memoriam*

² Laboratório de Herpetologia e Comportamento Animal, Departamento de Ecologia, Universidade Federal de Goiás, Campus Samambaia, 74001-970 Goiânia, GO, Brazil

³ Laboratório de Ecologia de Populações e Conservação, Programa de Pós-graduação em Ecologia de Ecossistemas, Universidade Vila Velha. Rua Comissário José Dantas de Melo, Boa Vista II, 29102-920 Vila Velha, ES, Brazil. *Corresponding author. E-mail: rodrigo-ecologia@yahoo.com.br

⁴ Associação Capixaba em Defesa da Água e da Mata Atlântica (ACADAMA). Rua Lourenço Roldi, 29665-000 São Roque do Canaã, ES, Brazil

⁵ Ello Ambiental. Av. Getúlio Vargas, 29700-010 Colatina, ES, Brazil

Submitted on: 2017, 17th April; revised on: 2017, 29th May; accepted on: 2017, 27st September

Editor: Daniele Pellitteri-Rosa

Abstract. Seventy juvenile individuals of *Aparasphenodon brunoi* were collected on the low parts of tree trunks in an Atlantic Forest remnant. Arthropods were the dominant prey found in their stomachs. Coleoptera (adult and larvae) was the most important prey regarding prey frequency, number, weight, and index of relative importance. Secondary preys included Hymenoptera that was important regarding number of prey and Hemiptera that was important regarding prey weight. Trophic ontogeny was detected. The diversity of prey suggests *A. brunoi* is an opportunistic sit-and-wait predator.

Keywords. Anura, ecology, Hylidae, predation, ontogeny, feeding pattern.

Studies regarding dietary aspects of animals may provide important data for a better understanding of the fundamental niche, position in the food webs, feeding time strategy, and metabolic needs of a species (Christian et al., 2007; Miller et al., 2010). Anurans are usually considered opportunistic predators regarding their feeding habits and most of them are arthropod consumers (Wells, 2007). Generally, the diet of anurans is related to their snout-vent length, ability to detect and capture prey as well as prey availability in the environment (Giaretta et al., 1998; Ferreira et al., 2015).

Aparasphenodon brunoi Miranda-Ribeiro, 1920 is a hylid treefrog that uses bromeliads and hollows of tree trunks as diurnal refuge (i.e., bromelicolous species, *sensu*

Peixoto, 1995) and reproduce in temporary ponds (Wogel et al., 2006). It is endemic to the coastal plain of Brazil (i.e., restinga habitat) from São Paulo to Bahia states (Ruas et al., 2013; Frost, 2016). This species has a highly toxic secretion that may be injected by bony spines on the head (Jared et al., 2015). Furthermore, some studies have shown that the toxicity of a frog is determined by the compounds sequestered from the diet during early development stage (e.g., Daly et al., 1994; Sime et al., 2000).

There is little information on the ecology of juveniles of the venomous treefrog *A. brunoi*, and diet studies on adults have shown that this treefrog is a generalist forager of arthropods (Teixeira et al., 2002; Mesquita et al., 2004). The aim of this study is to describe the diet of juveniles

of *A. brunoi* in an Atlantic Forest remnant from south-eastern Brazil.

The study area is in the coastal plain of Pontal do Ipiranga district, municipality of Linhares, Espírito Santo state, Brazil (19°13'S, 39°43'W). The region is characterized by pastures and remnants of Atlantic Forest. The treefrogs were manually captured at night between September and October 2000. According to Mesquita et al. (2004), individuals of this species smaller than 48 mm are considered juveniles. The individuals were anesthetized using lidocaine cream (5%) and fixed in a solution of formalin 10% for 72 hours and then washed and placed in a solution of alcohol 70%. In laboratory the specimens were measured to the snout-vent length (SVL in mm), weighted (0.01 g precision) and dissected to determine the sex and to analyze the stomach content. The stomach contents were removed, spread in Petri dish and identified to the lowest taxonomic level possible.

Preys were dried on paper towels and the following parameters for each prey category were evaluated: occurrence frequency, number, wet weight (0.0001 g precision), and index of relative importance (IRI). The IRI was calculated according to Pinkas et al. (1971) and the percent IRI (%IRI) according to Cortés (1997). For intraspecific comparison, IRI was calculated for adults of *A. brunoi* using data available on Teixeira et al. (2002). Intact preys were measured to the total length with a caliper (0.1

mm precision). The voucher specimens were deposited at the zoological collection of the Instituto Nacional da Mata Atlântica/Museu de Biologia Professor Mello Leitão (MBML), Espírito Santo State, Brazil.

The data were tested for normality using Kolmogorov-Smirnov test and for homogeneity using Bartlett test. SVL and weight were compared between sexes using the analysis of variance (ANOVA). In this analysis, sex was the independent variable while SVL and weight were the dependent variables (Neter et al., 1990). Cluster analysis based on Euclidean distance was used to evaluate trophic ontogeny based on size classes of *A. brunoi*. Only the weight value of the main prey was used in this analysis and the data were log transformed. The relationship between prey length and anuran SVL was tested by a regression analysis. Mean and standard deviation are provided. The significance level was set to 0.05.

Seventy juvenile individuals of *A. brunoi* were collected on the low parts of tree trunks (< 1m). Adults were on trees canopy and were not collected. Thirty-two individuals were juvenile males and 38 were juvenile females. Juvenile males ranged on SVL from 31.0 to 39.2 mm (mean = 34.3, SD = 2.0 mm) and in weight from 2.0 to 4.4 g (mean = 2.9, SD = 2.0 g). Juvenile females ranged on SVL from 32.1 to 43.6 mm (mean = 36.7, SD = 2.7 mm) and in weight from 2.1 to 6.7 g (mean = 3.6, SD = 2.7 g). The SVL and weight differed significantly between

Table 1. Prey items of juveniles of *Aparasphenodon brunoi* from Espírito Santo state, southeastern Brazil. F = occurrence frequency; N = number of prey; W = weight; IRI = index of relative importance.

Prey	F	%F	N	%N	W	%W	IRI	%IRI
Insecta								
Blattodea	2	3.77	2	3.23	120.1	3.94	27.03	0.77
Coleoptera (Adult)	19	35.85	21	33.87	963.5	31.64	2348.53	67.26
Coleoptera (Larvae)	8	15.09	8	12.90	267.9	8.80	327.45	9.38
Hemiptera	3	5.66	3	4.84	946.5	31.09	203.36	5.82
Homoptera	1	1.89	1	1.61	1.8	0.06	3.16	0.09
Hymenoptera	7	13.21	10	16.13	127.1	4.17	268.16	7.68
Isoptera	1	1.89	1	1.61	19.3	0.63	4.23	0.12
Orthoptera	5	9.43	5	8.06	333.1	10.94	179.17	5.13
Insect remnant	3	5.66	-	-	23.3	0.76		
Arachnida								
Araneae	3	5.66	3	4.84	160.6	5.27	57.22	1.64
Crustacea								
Ostracoda	3	5.66	8	12.90	1.5	0.05	73.30	2.10
Other								
Anuran skin	5	9.43	-	-	80	2.63		
Total	-	-	62	100.00	3044.7	100.00		

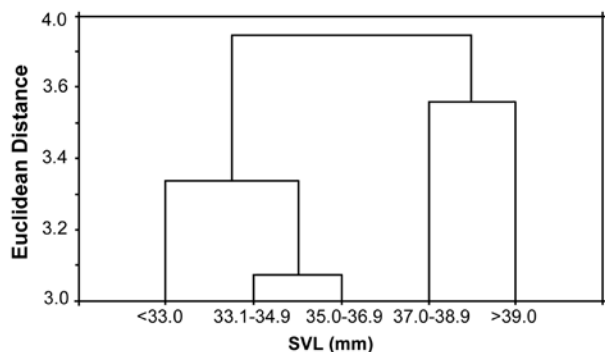


Fig. 1. Cluster analysis based on Euclidian distance shows the trophic ontogeny in juveniles of *Aparasphenodon bruno* from Espírito Santo state, southeastern Brazil.

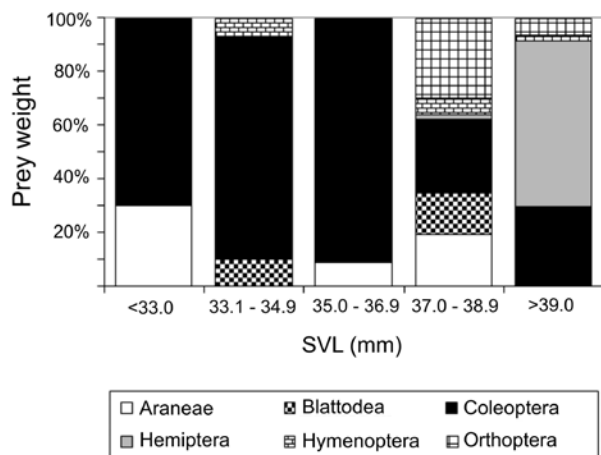


Fig. 2. Prey weight percentage according to snout-vent length (SVL) classes of juveniles of *Aparasphenodon bruno* from Espírito Santo state, southeastern Brazil.

the sexes ($F_{1,68} = 17.3$, $P < 0.01$; $F_{1,68} = 12.6$, $P < 0.01$, respectively). Females were on average larger and heavier than males.

Fifty-three (76%) individuals had food items in their stomach, representing 12 prey categories, mostly arthropods, (Table 1). Coleoptera (adult and larva) was the most important prey regarding prey frequency, number, weight, and IRI. Secondary preys included Hymenoptera that was important regarding number of prey and Hemiptera that was important regarding prey weight. It was detected trophic ontogeny in *A. bruno* (Fig. 1). Specimens less than 36.9 mm had similar diet, possibly because they predated more Coleoptera (Fig. 2). Specimens larger than 36.9 mm had similar diet by feeding mostly upon Hemiptera. The length of intact prey ingested by *A. bruno* varied from 2.8 to 15.9 mm (mean = 8.9, SD 3.7). There was no relation-

ship between total length of prey and anuran SVL ($R^2 = 0.003$, $P > 0.05$).

The variety of prey categories suggests that *A. bruno* is an opportunistic sit-and-wait predator. The diet of *A. bruno* from another two populations had also a variety of prey categories (Teixeira et al., 2002; Mesquita et al., 2004). In fact, the feeding upon small arthropods has been reported in several studies of anurans (Teixeira and Coutinho, 2002; Ferreira and Teixeira, 2009; Ferreira et al., 2012). The presence of ostracods, common on tank bromeliads (Oliveira et al., 1994), indicates bromeliculous habit of *A. bruno* at the studied fragment.

Apparently, juveniles of *A. bruno* feed upon different prey categories compared to adults. In our study, Coleoptera was the most important prey (%IRI = 67.26). In Teixeira et al. (2002), Hymenoptera was the most important prey (%IRI = 20.1) for adults of *A. bruno*. Coleoptera was the least important prey (%IRI = 0.7) for this population of adults. In our study, *A. bruno* feeds on different prey categories according to their SVL. Surprisingly, *A. bruno* SVL was not related to prey size. Changes on diet correlated to anuran SVL have been reported for other species (Giaretta et al., 1998; Teixeira and Vrcibradic, 2003; Ferreira et al., 2007). Trophic ontogeny may be an important mechanism to avoid intraspecific competition and predation. Probably the diet of the adults at tree canopy may strengthen the trophic ontogeny in our studied population. The studied remnant may provide necessary amount of prey for *A. bruno* due to the high percentage of stomachs with prey. We recommend future studies to evaluate the relationship between prey items and *A. bruno* toxicity.

ACKNOWLEDGMENTS

We thank Gladstone I. Almeida, Aílston Anastácio and José A. P. Schneider for fieldwork assistance. PETROBRAS funded this research. IBAMA provided the sampling permit (07/98, 02001.003072/97-21). RLM thanks CNPq for scholarship (process 140710/2013-2; 152303/2016-2). RBF and DCM thank CAPES for scholarships.

REFERENCES

- Christian, K., Webb, J.K., Schultz, T., Green, B. (2007): Effects of seasonal variation in prey abundance on field metabolism, water flux, and activity of a tropical ambush foraging snake. *Physiol. Biochem. Zool.* **80**: 522-533.

- Cortés, E. (1997): A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes. *Can. J. Fish. Aquat. Sci.* **54**: 726-738.
- Daly, J.W., Secunda, S.I., Garrafo, H.M., Spande, T.F., Wisnieski, A., Cover, J.F.J. (1994): An uptake system for dietary alkaloids in poison frogs (Dendrobatidae). *Toxicon* **32**: 657-663.
- Ferreira, R.B., Dantas, R.B., Teixeira, R.L. (2007): Reproduction and ontogenetic diet shifts in *Leptodactylus natalensis* (Anura: Leptodactylidae) from southeastern Brazil. *Bol. Mus. Biol. Prof. Mello Leitão* **22**: 45-55.
- Ferreira, R.B., Teixeira, R.L. (2009): Feeding patterns and use of reproductive habitat of the Striped Toad *Rhinella crucifer* (Anura: Bufonidae) from Southeastern Brazil. *Acta Herpetol.* **4**: 125-134.
- Ferreira, R.B., Schineider, J.A.P., Teixeira, R.L. (2012): Diet, fecundity, and use of bromeliads by *Phyllodytes luteolus* (Anura: Hylidae) in southeastern Brazil. *J. Herpetol.* **46**: 19-24.
- Ferreira, R.B., Beard, K.H., Choi, R.T., Pitt, W.C. (2015): Diet of the nonnative greenhouse frog (*Eleutherodactylus planirostris*) in Maui, Hawaii. *J. Herpetol.* **49**: 586-593.
- Frost, D.R. (2016): Amphibian Species of the World: An Online Reference. Version 6.0, American Museum of Natural History. <http://research.amnh.org/vz/herpetology/amphibia>, accessed at: 2014/12/30. Accessed 30 October 2016.
- Giarretta, A., Araújo, M.S., Medeiros, H.F., Facure, K.G. (1998): Food habits and ontogenetic diet shifts of the litter dwelling frog *Proceratophrys boiei* (Wied). *Rev. Bras. Zool.* **15**: 385-388.
- Jared, C., Mailho-Fontana, P.L., Antoniazzi, M.M., Mendes, V.A., Barbaro, K.C., Rodrigues, M.T., Brodie, E.D. Jr. (2015): Venomous frogs use heads as weapons. *Curr. Biol.* **25**: 2166-2170.
- Mesquita, D.O., Costa, G.C., Zatz, M.G. (2004): Ecological aspect of the casque-headed frog *Aparasphenodon brunoii* (Anura, Hylidae) in a Restinga habitat in southeastern Brazil. *Phyllomedusa* **3**: 51-59.
- Miller, T.W., Brodeur, R.D., Rau, G., Omori, K. (2010): Prey dominance shapes trophic structure of the northern California Current pelagic food web: evidence from stable isotopes and diet analysis. *Mar. Ecol. Prog. Ser.* **420**: 15-26.
- Neter, J., Wasserman, W., Kutner, M.H. (1990): Applied Linear Statistical Models. Regression, Analysis of Variance, and Experimental Designs. Homewood, Irwin.
- Oliveira, M.G.N., Rocha, C.F.D., Bagnall, T. (1994): The animal community associated with the tank bromeliad *Neoregelia cruenta* (R. Graham) L. B. Smith. *Bromélia* **1**: 22-29.
- Peixoto, O.L. (1995): Associação de anuros a bromeliáceas na Mata Atlântica. *Revta. Univ. Rural, Sér. Ciênc. da Vida* **17**: 75-83.
- Pinkas, L., Oliphant, M.S., Iverson, I.L.K. (1971): Food Habits of Albacore, Bluefin Tuna, and Bonito in California Waters. *Calif. Dep. Fish Game, Fish. Bull.* **152**: 1-105.
- Ruas, D.S., Mendes, C.V.M., Del-Grande, M.L., Solé, M. (2013): *Aparasphenodon brunoii* Miranda-Ribeiro, 1920 (Anura: Hylidae): distribution extension and geographic distribution map for Bahia state, Brazil. *Check List* **9**: 858-859.
- Sime, K.R., Feeny, P.P., Haribal, M.M. (2000): Sequestration of aristolochic acids by the pipevine swallowtail, *Battus philenor* (L.): evidence and ecological implications. *Chemoecology* **178**: 169-178.
- Teixeira, R.L., Coutinho, E.S. (2002): Hábito alimentar de *Proceratophrys boiei* (Wied) (Amphibia, Anura, Leptodactylidae) em Santa Teresa, Espírito Santo, Sudeste do Brasil. *Bol. Mus. Biol. Prof. Mello Leitão* **14**: 13-20.
- Teixeira, R.L., Schineider, J.A.P., Almeida, G.I. (2002): The occurrence of amphibians in bromeliads from the southeastern Brazil Restinga habitat, with special reference to *Aparasphenodon brunoii* (Anura, Hylidae). *Rev. Bras. Biol.* **62**: 263-268.
- Teixeira, R.L., Vrcibradic, D. (2003): Diet of *Leptodactylus ocellatus* (Anura; Leptodactylidae) from coastal lagoons of southeastern Brazil. *Cuad. Herpetol.* **17**: 113-120.
- Wells, K.D. (2007): The Ecology and Behavior of Amphibians. The University of Chicago Press, Chicago.
- Wogel, H., Weber, L.N., Abrunhosa, P.A. (2006): The tadpole of the casque-headed frog, *Aparasphenodon brunoii* Miranda-Ribeiro (Anura: Hylidae). *South Am. J. Herpetol.* **1**: 54-60.