Diet and helminths of *Enyalius brasiliensis* (Lacertilia, Iguania, Leiosauridae) in an Atlantic Rainforest remnant in southeastern Brazil

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Abstract

Our study aimed to add information about the diet and endoparasites of *Enyalius brasiliensis* from an Atlantic Rainforest remnant in the state of Rio de Janeiro, southeastern Brazil. Regarding diet, *E. brasiliensis* consumed arthropods, with caterpillars and beetles being the most important preys. Regarding helminth parasites, overall prevalence was low (9.5%), with 238 nematodes of the genus *Physaloptera* found in the stomach of one specimen and one nematode of the genus *Rhabdias* inside the lung of another. Our results corroborate the observations of previous studies that indicate that lizards of the genus *Enyalius* tend to feed mainly on relatively large-bodied arthropods and to harbour depauperate endoparasite fauna.

Keywords: feeding habits, forest habitat, lizard, Nematoda, parasitism

Dieta e helmintos de *Enyalius brasiliensis* (Lacertilia, Iguania, Leiosauridae) em um remanescente de Mata Atlântica do sudeste do Brasil

Resumo

Nosso estudo teve como objetivo adicionar informações sobre a dieta e endoparasitas de *Enyalius brasiliensis* em um remanescente de Mata Atlântica do estado do Rio de Janeiro, sudeste do Brasil. Em relação à dieta, *E. brasiliensis* consumiu artrópodes, sendo que lagartas e besouros foram as presas mais importantes. Em relação ao parasitismo por helmintos, a prevalência foi baixa (9,5%), com 238 nematóides do gênero *Physaloptera* encontrados no estômago de um espécime, e um nematóide do gênero *Rhabdias* infectando o pulmão de outro. Nossos resultados corroboraram as observações de estudos anteriores que indicaram que lagartos do gênero *Enyalius* tendem a se alimentar principalmente de artrópodos de grande porte e abrigar uma fauna depauperada de endoparasitas.

Palavras-chave: hábitos alimentares, habitats florestais, lagarto, Nematoda, parasitismo

1. Introduction

The genus *Enyalius* Wied, 1821 (Iguania, Leiosauridae) currently encompasses nine species of diurnal medium-sized lizards (up to 115 mm snout-vent length) (Rodrigues et al., 2006). These lizards generally exhibit arboreal habits, but may occasionally be encountered on the forest floor leaf litter (Zamprogno et al., 2001, Van Sluys et al., 2004, Sousa and Cruz 2008). Although some species occur frequently or predominantly in open habitats (Jackson 1978, Rodrigues et al., 2006), most of the members of this genus are restricted to forested areas and seem to be sensitive to habitat fragmentation (Teixeira et al.,

2005). Studies regarding ecological aspects of species in this genus demonstrated that these lizards are generalist predators that feed predominantly on arthropods (Vanzolini 1972, Vitt et al., 1996, Zamprogno et al., 2001, Marques and Sazima 2004, Van Sluys et al., 2004, Teixeira et al., 2005, Rautenberg and Laps 2010, Barreto-Lima and Souza 2011), and may present inter-sexual differences in diet composition (Sousa and Cruz 2008, Sturaro and Silva 2010). There is also some information regarding helminths associated with lizards of the genus *Enyalius* (Sousa et al., 2007, Vrcibradic et al., 2007, 2008, Ávila

and Silva 2010, Barreto-Lima et al.,, 2012), but data are still lacking for most species.

Enyalius brasiliensis (Lesson 1828) is a species endemic to the Atlantic Rainforest biome, occurring in the states of Rio de Janeiro and Espírito Santo, in southeast Brazil (Jackson 1978). Lizards of this species are sexually dichromatic, with males predominantly green and females brown with a row of dark rhomboid marks along the middorsum. Although this species may be reasonably abundant in some areas, there is currently scarce information about its ecology (Van Sluys et al., 2004, Teixeira et al., 2005). Herein we analyse the diet and associated helminth fauna of E. brasiliensis from an Atlantic Rainforest remnant in the state of Rio de Janeiro, aiming to increase the available information on this species and on the genus Envalius as a whole.

2. Material and methods

The lizards were collected within two conservation units: the Reserva Ecológica de Guapiaçu (hereafter REGUA; 22°24' S, 42°44' W) and the Parque Estadual dos Três Picos (within a private property, Fazenda Santa Bárbara; 22°25' S, 42°35' W); the two study sites are ca. 16 km apart and located in the municipality of Cachoeiras de Macacu, in the state of Rio de Janeiro, southeastern Brazil. Climate in the region is wet and warm, with annual rainfall varying from 2,000 to 2,500 mm, and mean annual temperature from 18° to 24°C (Rocha *et al*, 2007; Siqueira et al, 2009).

Lizards were collected during herpetological surveys carried out in late October and early November 2006 on the Fazenda Santa Bárbara (at altitudes between 500 and 800 m), and between July 2007 and March 2010 in REGUA (at altitudes between 40 and 500 m). Two sampling methods were employed: visual encounter surveys (hereafter VES; Crump and Scott 1994), and pitfall traps with drift fences (Corn 1994).

The VES method was always performed at night by time-constrained samplings, totalling 1,325 hours of active search (1,250 hours in REGUA and 75 hours in Fazenda Santa Bárbara). In REGUA, each pitfall trap system consisted of eleven 60-litre buckets buried in the ground and set approximately 10 m apart from each other, with soft plastic drift fences about 50 cm high extended between them. A total of 15 pitfall traps systems (165 buckets) were set up (three in the continuous forest of REGUA and 12 in forest fragments in its surroundings) and were surveyed during 30 days totalling 4,950 buckets-days of sampling effort. On the Fazenda Santa Bárbara, three pitfall trap systems were set up, each consisting of ten 30-litre buckets buried in the ground, and were surveyed over 18 days, totalling 540 buckets-days of sampling effort.

In the laboratory, the snout-vent length (SVL) and jaw width (JW, taken at the widest point of the head) of all collected individuals of *E. brasiliensis* were measured with a caliper (precision of 0.1 mm), before they were dissected for analyses of their diet and associated endoparasites. The whole gastrointestinal tract and the lungs of the

lizards were checked carefully for helminths under a stereomicroscope. Stomach contents also were analysed under a stereomicroscope and the preys encountered were identified to Order (to Class for Diplopoda). Each individual prey found in stomachs was measured in it's length (L) and width (W) using a caliper (precision of 0.1 mm), and those measurements were used to estimate prey volume through the formula of the ovoid-spheroid $[V = 4 / 3\pi (L / 4)]$ 2). $(W/2)^2$ (Dunham 1983). The frequency of occurrence of each prey category in the diet was expressed as the proportion of the number of stomachs that contained that category. Additionally, we calculated an Index of Importance Value (I_n) for each prey category, following Howard et al., (1999). To assess whether lizard mouth dimensions affect the size of the prey consumed by the lizards we performed Simple Linear Regression Analysis (Zar 1999) between the mean volume of the biggest prey item ingested per individual and the lizards' JW measurements. The results for the descriptive statistics are presented throughout the text as mean \pm one standard deviation. All data were tested for the normality of distribution and for homocedasticity of variances before performing statistical analyses (Zar 1999). The lizards collected for this study were deposited at the Museu Nacional, Rio de Janeiro (MNRJ 21569-82) and at the Museu de Zoologia, Universidade de São Paulo (MZUSP 98041), and the parasites found inside them were deposited at the Coleção Helmintológica do Instituto de Biociências de Botucatu (Physaloptera sp. - CHIBB 6515; Rhabdias sp. - CHIBB 6696).

3. Results

Seventeen individuals of *E. brasiliensis* were collected using the VES method and four using pitfall traps. Thirteen of the 21 individuals collected were males (= 61.9%), seven were females (= 33.3%), and one (= 4.8%), was a juvenile (SVL = 36.4 mm) whose sex could not be determined. One of the females (SVL = 92.4 mm), collected in REGUA during the wet season (March 2009), had 13 eggs in its oviducts, and two females from Fazenda Santa Bárbara (96.0 mm and 104.4 mm SVL) had vitellogenic follicles (15 and 10, respectively) in their ovaries.

Mean SVL of the 21 *E. brasiliensis* specimens was 80.8 ± 14.4 mm (range: 36.4-104.4 mm) and their JW averaged 14.7 ± 2.2 mm (range: 8.1-17.9 mm). The mean SVL of males was 81.4 ± 3.6 (range: 76.5-89.4 mm; N = 13), and that of females was 86.1 ± 17.3 (range: 58.0-104.4 mm; N = 7).

Three individuals of *E. brasiliensis* (14.3% of the total) had no food in their stomachs. The remaining specimens had consumed predominantly arthropods, and a reduced proportion of plant material (Table 1). In numerical terms, Lepidoptera larvae (27.3% of all items ingested), Hymenoptera (15.9%) and Coleoptera (15.9%) were the most important prey items in the diet (Table 1). Regarding volume, the most important prey categories in the diet of *E. brasiliensis* were Lepidoptera larvae (46.0% of total volume ingested) and Phasmida (13.4%) (Table 1). In terms

of frequency of occurrence Lepidoptera larvae (present in 55.6% of stomachs) and Coleoptera (38.9%) were the most representative prey categories (Table 1). Lepidoptera larvae, Coleoptera and Hymenoptera (mostly wasps) were overall the most important prey categories in the diet, as they had the highest values of Index of Importance (Table 1). The number of identifiable food items per stomach averaged 2.5 ± 1.8 (range: 1-7). Length of ingested prey items averaged 16.2 mm (range: 0.8-64.0 mm), and individual prey volume averaged $1,100.2 \pm 1,804.8 \text{ mm}^3$ (range: 22.5-6,539.7 mm³). There was a positive relationship between the volume of the largest prey ingested per lizard and JW (variables log-transformed; $r^2 = 0.556$, $F_{1.16} = 20.015$, p < 0.001), but no significant relationship between the number of prey ingested per individual and lizard SVL (variables log-transformed; $r^2 = 0.155$, $F_{1,15} = 2.757, p = 0.118$.

Two of the 21 individuals of *E. brasiliensis* analysed were infected by nematodes (overall prevalence = 9.5%). In one of them, a female from REGUA (SVL = 92.4 mm), the stomach contained 238 nematodes of the genus *Physaloptera* Rudolphi, 1819 (mean body length of ten specimens = 8.1 ± 1.2 mm; mean diameter = 0.6 ± 0.7 mm; N = 10). These worms were all immature, and thus could not be identified to species. In the other one, a male from REGUA (SVL = 76.5 mm), we found one adult nematode of the genus *Rhabdias* Stiles & Hassall, 1905 (body length = 3.6 mm; diameter = 0.2 mm) in one of its lungs, but it could not be identified to species either.

4. Discussion

Our data indicate that *E. brasiliensis* is a generalist predator, feeding predominantly on relatively large-bodied arthropods such as caterpillars and beetles. Those prey are

usually among the main components of the diet of species in the genus Enyalius in general, based on data from the literature (see Table 2). Insect larvae (mainly of Lepidoptera) seem to figure prominently in the diets of all species in the genus (except E. bilineatus; Zamprogno et al., 2001, Teixeira et al., 2005) and the available data suggests that those lizards tend to consume relatively large prey items $(\geq 9 \text{ mm of length})$ (see Table 2). Sturaro and Silva (2010) reported mean and maximum prey lengths of only 1.95 mm and 7.3 mm, respectively, for a population of *E. perditus*, but those values apparently represent an error or misprint, as the data on estimated volumes on their Table 2 (e.g. 526 mm³ for one Blattodea) suggest much greater linear dimensions for the larger items. The ingestion of prey that are both large and soft-bodied (such as insect larvae and Orthoptera) is energetically advantageous, considering the low costs of handling and digestion and the amount of energy obtained per item (Pough and Andrews 1985, Grimmond et al., 1994).

Despite the importance of Hymenoptera in the diet of the population of *E. brasiliensis* in our study, we found a low representativeness of social insects (few Formicidae and no Isoptera in stomachs) compared to that of other studied populations of this species (Van Sluys et al., 2004, Teixeira et al., 2005) and of other congeneric species (see Table 2). The diet composition of Atlantic Rainforest lizards can vary as a function of food availability in the environment, as well as some species-specific characteristics such as body size, energy requirements and patterns of habitat use. Studies including samplings of potential prey in the environment have suggested that species of the genus *Enyalius* may present differential electivity for some items and thus have a certain degree of selectivity in their diet, in spite of the wide array of prey types consumed (Sousa and Cruz 2008,

Table 1. Diet composition of *Enyalius brasiliensis* (N = 18) from the Atlantic Rainforest of the Reserva Ecológica de Guapiaçu and its surroundings, in southeastern Brazil. Numerical data (N), volumetric data (V, in mm³), frequency of occurrence (F), and Index of Importance values (Ix) are presented for each prey category. Percentages are shown in parentheses. Categories not considered for the calculation of Ix were represented by (*).

Prey category	N (%)	V (%)	F (%)	Ix
Diplopoda	4 (9.1)	130.7 (0.7)	4 (22.2)	0.064
Arachnida				
Araneae	4 (9.1)	641.2 (3.2)	4 (22.2)	0.072
Hexapoda				
Orthoptera	4 (9.1)	2,413.1 (12.2)	3 (16.7)	0.094
Phasmida	1 (2.3)	2,654.3 (13.4)	1 (5.6)	0.060
Mantodea	1 (2.3)	389.6 (2.0)	1 (5.6)	0.022
Hemiptera	3 (6.8)	1,728.1 (8.7)	2 (11.1)	0.067
Coleoptera	7 (15.9)	1,406.4 (7.1)	7 (38.9)	0.131
Hymenoptera	7 (15.9)	824.4 (4.2)	6 (33.3)	0.113
Lepidoptera (larvae)	12 (27.3)	9,137.1 (46.0)	10 (55.6)	0.322
Diptera (larvae)	1 (2.3)	177.3 (9.0)	1 (5.6)	0.018
Arthropod Remains *		318.6 (1.6)		
Plant Remains *		26.4 (0.1)		
Total	44 (100)	19,847.4 (100)		

Table 2. Dietary data for species of the genus *Enyalius* compiled from the literature, including sample size (N, considering only individuals with food in stomachs), the main prey items on the diet, and mean prey length (PL, with range in parentheses). The absence of data is indicated with (?). The (*) corresponds to the subspecies *Enyalius brasiliensis boulengeri*. The (**) indicate that the values are probably erroneous (see Discussion).

Species	N	Main prey items	PL (mm)	Source
E. bilineatus	45	orthopterans, cockroaches, ants	9.1 (2.1–28.2)	Zamprogno et al., 2001
E. bilineatus	84	ants, orthopterans	13.3 (3.8–35.9)	Teixeira et al., 2005
E. brasiliensis	14	termites, ants, larvae	?	Van Sluys et al., 2004
E. brasiliensis*	25	cockroaches, larvae	15.0 (6.2–32.4)	Teixeira et al., 2005
E. brasiliensis	18	larvae, beetles, wasps + ants	16.2 (0.8-64.0)	this study
E. iheringii	18	beetles, larvae, spiders	31.1 (9.2–57.6)	Rautenberg and Laps 2010
E. leechii	9	termites, larvae	14.9 (11.2–46.9)	Vitt et al., 1996
E. perditus	30	isopods, larvae, ants	? (1.3–32.3)	Sousa and Cruz 2008
E. perditus	28	larvae, spiders, ants	1.9 (0.1–7.3)**	Sturaro and Silva 2010
E. perditus	33	ants, larvae, termites	?	Barreto-Lima and Sousa 2011

Sturaro and Silva 2010, Barreto-Lima and Souza 2011). In the present study, as we did not sample invertebrates in the habitat to estimate the potential availability of prey, we cannot say if *E. brasiliensis* has a negative electivity for colonial insects or if their poor representativeness in stomachs simply reflects their local availability.

Sousa and Cruz (2008) and Sturaro and Silva (2010) suggested that *Enyalius* spp. may forage actively, based on the high representativeness of colonial insects and larvae in their diet. However, Rautenberg and Laps (2010) pointed out that these lizards have the typical characteristics of sit-and-wait foragers and considered them as such. Nevertheless, no studies have so far assessed foraging behaviour of any species of *Enyalius*. Considering this, and the fact that Iguanian lizards, as a group, are typically ambush foragers (Pianka and Vitt 2003), we suggest that *Enyalius* spp. should be considered sit-and-wait foragers until direct evidence of their foraging behaviour is produced.

We found millipedes (Diplopoda) and fragments of dead leaves among the stomach contents of *E. brasiliensis*, which may be indicative that these lizards forage on the forest floor. The proportional volume of plant remains in the stomachs was low (0.2%) and corresponded to dry leaf fragments, suggesting that the occurrence of these items in the diet resulted from accidental ingestion during prey capture on the leaf litter. Although *Enyalius* spp. have been traditionally considered as primarily arboreal lizards (Etheridge 1969), there is evidence that those lizards may also forage on the forest floor (Vanzolini 1972, Sazima and Haddad 1992, Zamprogno et al., 2001, Van Sluys et al., 2004, Sousa and Cruz 2008), and our data for *E. brasiliensis* support this.

We found a positive relationship between the volume of the largest prey ingested per lizard and JW, but no significant relationship between the number of prey ingested per individual and lizard SVL. Probably smaller prey become energetically less advantageous as the lizards increase in body size and the consumption of larger prey

may result in a positive energetic balance. Additionally, a larger body and head size provides stronger bites (Herrel et al., 2001) which could facilitate the intake of larger prey.

Our study recorded the occurrence of nematodes in the stomachs and lungs of E. brasiliensis from REGUA, corresponding to the first records of the genera Physaloptera and Rhabdias in this lizard species. The only species of *Enyalius* previously reported as host for the genus Physaloptera is E. bilineatus, for which two species of that genus (P. retusa Rudolphi 1819 and P. lutzi Cristófaro, Guimarães and Rodrigues 1976) were recorded (Vrcibradic et al., 2007). Besides, nematodes of the genus Rhabdias have been reported infecting the lungs of E. bilineatus (Vrcibradic et al., 2007), E. perditus and E. iheringii (Vrcibradic et al., 2008). We found low prevalence (4.8%) and low infection intensity of Rhabdias sp. (only one nematode) in the lizards analysed. Nematodes of this genus infect the lungs of amphibians and reptiles (Bursey et al., 2003), and have also been previously found occurring with low infection intensities in other species of Enyalius (Vrcibradic et al., 2007, 2008).

Nematodes of the genus *Physaloptera* can use several species of lizards as hosts, typically occupying their stomach and, sometimes, also their intestines (Rocha et al., 2000, Ávila and Silva 2010). Species of *Physaloptera* may occur with high prevalence (over 50%) in some populations of South American lizards of different genera and families (e.g. Vrcibradic et al., 2000, Fontes et al., 2003, Rocha et al., 2003, Bursey and Goldberg 2004). The low prevalence (under 15%) observed in the present study and in that of Vrcibradic et al. (2007) suggest that lizards of the genus *Enyalius* are not the preferred hosts of *Physaloptera* spp. Our data is in agreement with evidences from previous studies (Sousa et al., 2007, Vrcibradic et al., 2007, 2008, Barreto-Lima et al., 2012) which suggest that helminth communities of Enyalius spp. tend to be depauperate in terms of species richness.

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