



Feeding habits of the leaf litter frog *Haddadus binotatus* (Anura, Craugastoridae) from two Atlantic Forest areas in southeastern Brazil

LÍVIA COCO¹, VITOR N.T. BORGES JÚNIOR¹, LUCIANA A. FUSINATTO¹,
MARA C. KIEFER², JANE C.F. OLIVEIRA¹, PABLO G. ARAUJO¹, BIANCA M. COSTA³,
MONIQUE VAN SLUYS¹ and CARLOS F.D. ROCHA¹

¹Instituto de Biologia Roberto Alcântara Gomes, Departamento de Ecologia, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier, 524, 20550-019 Rio de Janeiro, RJ, Brasil

²Instituto de Biologia, Departamento de Biologia Geral, Universidade Federal Fluminense, Caixa Postal 100436, Centro, 24020-971 Niterói, RJ, Brasil

³Associação Educacional de Vitória, Rodovia Serafim Derenzi, 3115, São Pedro, 29030-026. Vitória, ES, Brasil

Manuscript received on September 14, 2012; accepted for publication on April 3, 2013

ABSTRACT

Haddadus binotatus is an endemic anuran of the Brazilian Atlantic Forest and currently, there is no information about the diet of this species. We analyzed the diet of two populations of this anuran in two states in southeast Brazil. Samplings were carried out in 2004 in the state of Rio de Janeiro and in 2009 and 2010 in the state of Espírito Santo. *Haddadus binotatus* presented a rich diet composition, preying 19 prey types. Orthoptera, Coleoptera, and Blattodea were the most important preys in the Rio de Janeiro population, and Orthoptera, Araneae and Hemiptera were the most important in the Espírito Santo population. The diet composition differed numerically between the two localities, but not in terms of volume, which can reflect local differences in the prey availability in the two habitats. The jaw width limited the size of prey, which is expected for predators who swallow the preys without chewing. The proportion of individuals with empty stomachs was higher in the Rio de Janeiro population (39.2%) than in the Espírito Santo population (17.9%), suggesting that the former could be in a lower energy balance. The females of the species were larger than the males, which may result from the production of larger eggs.

Key words: arthropods, diet, generalist feeder, sexual dimorphism.

INTRODUCTION

Leaf litter anurans are widely known to eat mainly arthropods (Toft 1981, Van Sluys et al. 2001, Marra et al. 2004, Dietl et al. 2009, Martins et al. 2010, Almeida-Santos et al. 2011, Sugai et al. 2012). For example, *Zachaenus parvulus* (Cycloramphidae) ate mainly Formicidae and Isopoda (Van Sluys et al. 2001), *Ischnocnema henselii* (Brachycephalidae)

fed mostly on Araneae and Orthoptera (Dietl et al. 2009) whereas *Ischnocnema parva* consumed predominantly ants and isopods (Martins et al. 2010) and *Brachycephalus didactylus* (Brachycephalidae) ate mainly Collembola and Acari (Almeida-Santos et al. 2011). The prey consumption could reflect the local availability of food of appropriate size (Lima and Moreira 1993), resulting in different categories of arthropods as the most important prey for each anuran species.

Correspondence to: Lívia Coco da Silva
E-mail: liviasilva87@yahoo.com.br

For leaf litter frogs, Toft (1980a, 1981) proposed a diet continuum from species specialized in slow moving and hard-bodied arthropods (principally ants or mites) to generalists, for frog species preying on mostly soft-bodied and mobile arthropods (avoiding ants and mites). The feeding behavior of leaf litter frogs can be related to this continuum, with ant/mites specialists being active foragers eating many small preys per unit of time and ant/mites avoiders presenting a sit-and-wait strategy eating few large preys per day (Toft 1980a, 1981). Following this classification, species that formerly composed the genus *Eleutherodactylus* (Leptodactylidae), currently subdivided into 10 genus (Hedges et al. 2008, Padial et al. 2009), including the *Haddadus* (Hedges et al. 2008) (Craugastoridae), have been considered either generalists (e.g. *Ischnocnema henselii* (Brachycephalidae) or ant/mites specialists (e.g. *Ischnocnema parva* (Brachycephalidae) (Dietl et al. 2009, Martins et al. 2010).

Haddadus binotatus (Spix, 1824) (Craugastoridae) is endemic to the Brazilian Atlantic Forest biome, occurring from the states of Bahia to Rio Grande do Sul (Hedges et al. 2008). This species presents direct eggs development, independent of water (Haddad and Sazima 1992, Canedo and Rickli 2006), and is found mainly at dusk and at night (Rocha et al. 2007, 2011). *Haddadus binotatus* is an abundant species in the leaf litter of some areas of Atlantic Rainforest in the state of Rio de Janeiro (e.g. Rocha et al. 2007, Almeida-Gomes et al. 2010), representing an important component of leaf litter communities at these localities. An abundant, and relatively large sized species as *H. binotatus* (Rocha et al. 2007, 2011) may prey on a variety of items of different sizes, representing locally an important source of death to prey, and potentially regulating some prey populations.

Currently, there is no information about feeding habits for any of the two living species in the genus *Haddadus*, including *H. binotatus*. Therefore, we aimed to characterize the diet composition of two

populations of *H. binotatus*, one from an area of Atlantic Rainforest of Rio de Janeiro (hereafter RJP) and another from an area in the state of Espírito Santo (hereafter ESP), in southeastern Brazil. We specifically asked: 1) Which are the food items predominantly consumed by the two populations of *H. binotatus*?; 2) In which extent do *H. binotatus* of distinct populations differ in their diet composition?; 3) Does jaw width of the *H. binotatus* affect the volume of ingested preys?; 4) Does the body size of the *H. binotatus* affect the number of preys per stomach?; and 5) Does *H. binotatus* have sexual dimorphism in body size?

MATERIALS AND METHODS

We carried out the study at three areas of the Atlantic Rainforest in two states of Southeastern Brazil (Rio de Janeiro and Espírito Santo). The two areas, the Estação Ecológica Estadual Paraíso (EEEP) (22°29'S, 42°55'W; elevation 150 – 300 m) and the Reserva Ecológica de Guapiaçu (REGUA) (22°24'S, 42°44'W; elevation 40 – 400 m), were located in the municipality of Guapimirim and Cachoeiras de Macacu respectively, both in the central region of the state of Rio de Janeiro. The climate of EEEP and REGUA is wet and warm, total annual rainfall ranges between 2000 mm and 3000 mm, and daily temperature vary from 14°C to 37°C (Bernardo et al. 2011). The third area was in the region of Serra das Torres (21°00'S, 41°14'W; elevation 1000 m) in the municipality of Atílio Vivacqua, south of the state of Espírito Santo. The climate of the region is wet and warm, with mean annual rainfall of about 1300 mm, and mean annual temperature of about 24.5°C (INCAPER 2010). The two areas from the state of Rio de Janeiro (RJP), EEEP and REGUA, are about nine km apart from each other, whereas the area sampled in Serra das Torres (in the state of Espírito Santo) (ESP) was nearly 230 km away from the localities in Rio de Janeiro.

We did *Haddadus binotatus* samplings during September of 2004 in EEEP and October of 2004

in REGUA, in the state of Rio de Janeiro, and during August, September and December of 2009 and March of 2010 in Serra das Torres, in the state of Espírito Santo. In EEEP and REGUA, we used two sampling methods: large-plot (quadrat) (Jaeger and Inger 1994) and time-constrained visual search (Crump and Scott Jr 1994) for searching the frogs. In Serra das Torres, we only used the large-plot sampling method. For the large-plot method, we established both in REGUA and in EEEP 28 quadrats of 5 x 5 m (25 m²), totaling 56 plots (1400 m² of forest floor sampled), and 180 quadrats of 4 x 4 m (16 m²) in Serra das Torres (2880 m²). We searched each plot for about half an hour and only at night [for a complete description of large-plot method see Rocha et al. (2007)]. For time-constrained visual search, we performed transects of 30 minutes distributed along three periods of the day, of which 79.5 h were of diurnal, 60 h were of crepuscular and 71 h were of nocturnal search, totaling 212.5 hours of sampling effort in each area. During each transect, the observer moved at a slow walking pace, carefully searching the surroundings for the presence of *H. binotatus*. All individuals of *H. binotatus* found during samplings were collected, euthanized with lidocaine ointment (anesthetic) or 10% ethanol, about 3 – 4 hours after the capture, and fixed in 10% formalin. All procedures used to capture and euthanasia followed the guidelines of the Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists (available at: <http://www.asih.org/publications>), aiming to reduce animal suffering, and were authorized by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) and the Instituto Estadual do Ambiente (INEA). We deposited specimens at the herpetological collection of the Museu Nacional, Rio de Janeiro.

We measured specimens in their snout-vent length (SVL) and in their jaw width (JW) with a 100

mm Digital Vernier Calliper (Maplin) (precision of 0.01 mm). We dissected the individuals, removed the stomachs and identified the sex through gonadal analyses. We analyzed their stomach contents qualitatively and quantitatively under a K-700L Stereo Microscope (Motic). We identified all prey items to the taxonomic level of Order and Family, in the case of Formicidae. Arthropod remains that could not be identified were grouped in the category “Arthropod remains”. We analyzed the diet of *H. binotatus* in terms of number, volume (in mm³) and frequency of occurrence of each type of prey. We counted prey items and measured each one in their width (W) and length (L) with a 100 mm Digital Vernier Calliper (Maplin) (precision of 0.01 mm). We estimated the volume (in mm³) for each prey category by using the formula for an ovoid-spheroid: $V = 4/3\pi (L/2)(W/2)^2$, where “L” is the length and “W” the width of a given prey item (Dunham 1983). We obtained the frequency of occurrence of each prey category in the diet dividing the number of stomachs which contained that category by the total number of stomachs analyzed, with the exception of the empty ones. For each item, we also obtained an importance index (I_x) through the mean of number, volume, and frequency of occurrence proportions (Howard et al. 1999).

For data analysis, we considered the samples from the state of Rio de Janeiro (EEEP and REGUA) as one population (RJP), given the proximity of these two localities, which belong to the same mountain range named Serra dos Órgãos. To evaluate the feeding strategy (specialist or generalist) and width niche of *H. binotatus* populations from Espírito Santo and Rio de Janeiro, we did a “specialization tendency diagram” (Amundsen et al. 1996). In the diagram, we considered the proportion of the prey item only for the individuals that consumed it [$P_i = (\sum S_i / \sum S_{ii}) \times 100$] where S_i is the stomach content composed of the prey i , and S_{ii} represents the total of stomach

content in only those predators with the prey i in their stomachs (Y axis) and the frequency of occurrence of the prey items in the diet [$F_i = (N_i/N) \times 100$] where N_i represents the number of predators that had the prey i in their stomach and N represents the total number of predators with stomach contents (X axis). To evaluate if diet composition differed between populations we performed a Kolmogorov-Smirnov test for two independent samples, based on numeric and volumetric proportions of prey items (Zar 1999). We also estimated the trophic similarity between the numerical and volumetric proportions of prey categories of the two populations using the “MacArthur and Levins Similarity Equation” modified by Pianka (1986) [$O_{jk} = \sum p_{ij} p_{ik} / \sqrt{\sum (p_{ij}^2) (p_{ik}^2)}$], where p_{ij} and p_{ik} are the volumetric/numerical proportions of prey item category i present in the diet of populations j and k , respectively. We estimated the correlations between the mean volume of the prey ingested and the JW of the individuals, and between the number of prey per stomach and the SVL of frogs using the Spearman Rank Correlation (Zar 1999). For all diet analyses we excluded plant pieces and arthropod remains. Finally, to test if there was sexual dimorphism in body size (SVL) for each population, we used the one-way analysis of variance (ANOVA) (Zar 1999). We presented the results for the descriptive statistics throughout the text as mean \pm standard deviation. We log-transformed the variable whenever the criteria for normality and homoscedasticity were not achieved (Zar 1999). We performed all statistical analyses using statistic software (SYSTAT 11[®], SYSTAT Software Inc.).

RESULTS

We analyzed 79 individuals of *Haddadus binotatus*, of which 51 were from RJP and 28 were from ESP. Twenty frogs (39.2%) from RJP and five (17.9%) from ESP had empty stomachs. Diets of both populations were composed by arthropods. We identified 19 food items consumed by *H. binotatus* from RJP.

Orthoptera was the most important prey item in terms of number (32.6%), followed by Blattodea (16.3%), Coleoptera and Hymenoptera (Formicidae) (both with 7.0%) (Table I). Volumetrically, the greatest contribution was from Orthoptera (31.2%), Blattodea (17.5%), Plant remains (13.1%) and Coleoptera (9.1%). The most frequent items were Plant Remains (45.0%), Orthoptera (32.3%), Blattodea (16.0%), Coleoptera and Hymenoptera (Formicidae) (both with 10.0%). Orthoptera (32.0%), Blattodea (16.6%) and Coleoptera (8.7%) were the items with the highest values of I_x (Table I). At ESP, we identified 16 food items in the diet of *H. binotatus*. In terms of number, Araneae (15.6%) was the most representative prey item, followed by Orthoptera (12.5%), Acari, Isopoda and Homoptera (all with 9.4%) (Table I). Volumetrically, Orthoptera (18.0%), Chilopoda (15.6%) and Hemiptera (13.4%) dominated. In terms of frequency of occurrence, the most important items were Plant Remains (30.0%), Araneae (22.0%), Orthoptera (17.0%) and Isopoda (13.0%). The items with the highest values of I_x were Araneae (16.0%), Orthoptera (15.8%), Isopoda (10.1%) and Hemiptera (9.6%) (Table I).

The data showed that all categories of prey were consumed by few individuals in both populations. In both graphics, we observed that the two populations of *H. binotatus* had a high between-phenotype contribution to the niche width, both of them with wide trophic niche (Figs. 1, 2). Diet composition differed between populations in terms of numerical proportion ($D_{max} = 0.450$; $P = 0.023$), but not volumetrically ($D_{max} = 0.200$; $P = 0.749$). The trophic similarity between the two populations (O_{jk}) in terms of number was 0.68 and 0.58 in terms of volume.

For the individuals of *H. binotatus* ESP we found a significant positive correlation ($r_s = 0.6025$; $P = 0.0063$, $n = 19$) between the mean volume of ingested preys ($136.3 \pm 181.6 \text{ mm}^3$, range: 3.7 mm^3 - 688.9 mm^3) and JW ($10.6 \pm 4.8 \text{ mm}$, range: 4.0 mm - 19.9 mm) (Fig. 3), but the

TABLE I
Number (N), volume (V, in mm³), frequency of occurrence (F) and Index of Importance (I_x, in %) of each food item in the diet of the anuran *Haddadus binotatus* in Atlantic Rainforest of the states of Rio de Janeiro (RJ) (*n* = 31) and Espírito Santo (ES) (*n* = 23), southeastern Brazil. Percentage values (%) in brackets.

Category of Food Item	RJ				ES			
	N(%)	V(%)	F(%)	I _x	N(%)	V(%)	F(%)	I _x
Arachnida								
Scorpiones	1.0(2.3)	18.3(0.6)	1.0(3.0)	2.0				
Opiliones	1.0(2.3)	2.4(0.1)	1.0(3.0)	1.8	1.0(3.1)	64.7(1.5)	1.0(4.0)	2.9
Araneae	2.0(4.7)	27.7(0.9)	1.0(3.0)	2.9	5.0(15.6)	464.6(10.5)	5.0(22.0)	16.0
Acarineae	1.0(2.3)	151.3(4.8)	1.0(3.0)	3.4	3.0(9.4)	45.8(1.0)	2.0(9.0)	6.5
Pseudoscorpiones					1.0(3.1)	329.6(7.4)	1.0(4.0)	4.8
Malacostraca								
Isopoda	1.0(2.3)	54.3(1.7)	1.0(3.0)	2.3	3.0(9.4)	350.7(7.9)	3.0(13.0)	10.1
Chilopoda	1.0(2.3)	17.9(0.6)	1.0(3.0)	2.0	1.0(3.1)	688.9(15.6)	1.0(4.0)	7.6
Diplopoda	1.0(2.3)	46.8(1.5)	1.0(3.0)	2.3				
Hexapoda								
Orthoptera	14.0(32.6)	978.1(31.2)	10.0(32.3)	32.0	4.0(12.5)	798.4(18.0)	4.0(17.0)	15.8
Dermaptera	1.0(2.3)	23.3(0.7)	1.0(3.0)	2.0				
Mantodea	1.0(2.3)	20.4(0.7)	1.0(3.0)	2.0	1.0(3.1)	20.2(0.5)	1.0(4.0)	2.5
Isoptera	1.0(2.3)	6.0(0.2)	1.0(3.0)	1.8				
Blattodea	7.0(16.3)	548.3(17.5)	5.0(16.0)	16.6	2.0(6.3)	51.4(1.2)	2.0(9.0)	5.5
Hemiptera	2.0(4.7)	97.1(3.1)	2.0(6.0)	4.6	2.0(6.3)	594.8(13.4)	2.0(9.0)	9.6
Homoptera	1.0(2.3)	2.3(0.1)	1.0(3.0)	1.8	3.0(9.4)	49.9(1.1)	2.0(9.0)	6.5
Thysanoptera					1.0(3.1)	< 0.01	1.0(4.0)	2.4
Coleoptera	3.0(7.0)	284.8(9.1)	3.0(10.0)	8.7	2.0(6.3)	119.5(2.7)	2.0(9.0)	6.0
Hymenoptera (Formicidae)	3.0(7.0)	22.8(0.7)	3.0(10.0)	5.9	2.0(6.3)	19.5(0.4)	2.0(9.0)	5.2
Lepidoptera	1.0(2.3)	121.0(3.9)	1.0(3.0)	3.1	1.0(3.1)	33.9(0.8)	1.0(4.0)	2.6
Diptera	1.0(2.3)	151.5(4.8)	1.0(3.0)	3.4				
Plant Remains		409.8(13.1)	14.0(45.0)			571.6(12.9)	7.0(30.0)	
Arthropods Remains		147.5(4.7)	3.0(10.0)			223.1(5.0)	3.0(13.0)	
TOTAL	43(100)	3131.7(100)			32(100)	4426.8(100)		

number of preys per stomach (1.7 ± 0.9 , range: 1.0-4.0) was not significantly correlated ($r_s = 0.1089$; $P = 0.65735$; $n = 19$) with SVL (32.0 ± 12.7 mm, range: 13.0 mm-56.0 mm). For the *H. binotatus* RJP, we did not find significantly correlation ($r_s = 0.1766$; $P = 0.3983$; $n = 25$) between the mean volume of ingested preys ($59.3\text{mm}^3 \pm 67.6$, range: 4.1mm^3 - 273.6mm^3) and the JW of specimens (12.3 ± 3.7 mm, range 7.9 mm-20.3 mm), nor ($r_s = -0.0982$; $P = 0.64041$; $n = 25$) between the number of preys per stomach (1.8 ± 1.2 , range: 1-5) and the SVL of individuals ($32.8\text{mm} \pm 7.9$, range: 21.5 mm-51.1 mm).

The mean number of preys per stomachs for the individuals in *H. binotatus* of ESP (1.7 ± 0.9 , range: 1-4) and RJP (1.8 ± 1.2 , range: 1-5) did not differ significantly (ANOVA: $F_{1,42} = 0.001$, $P = 0.971$). The mean volume of preys per stomachs for the individuals in *H. binotatus* ESP (136.3 ± 181.6 mm, range: 3.8 mm-688.9 mm) and RJP (59.3 ± 67.6 mm, range: 4.1 mm-273.6 mm) did not differ significantly either (ANOVA: $F_{1,42} = 1.395$, $P = 0.244$).

In terms of SVL, males of *H. binotatus* from ESP (29.4 ± 4.7 mm; range: 16.8 mm-37.5 mm; $n = 31$) and RJP (29.5 ± 5.3 mm; 18.6 mm-36.7 mm; $n = 20$) were significantly smaller (ES - ANOVA:

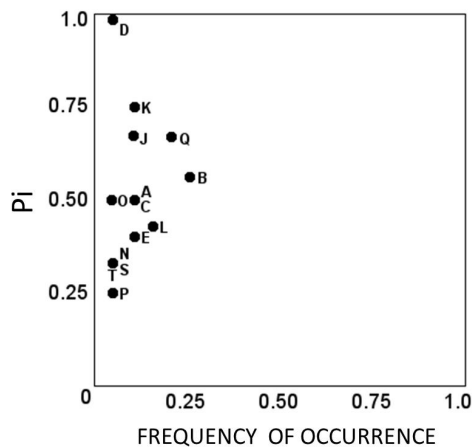


Figure 1 - Specialization diagram of *Haddadus binotatus* population from an Atlantic Rainforest area in the state of Espírito Santo, southeastern Brazil. The Y axis (Pi) shows the proportion of the prey item only for the individuals that consume it. The X axis shows the frequency of occurrence of the prey items in the diet. Each letter inside the diagram represents one prey type (A = Acari; B = Araneae; C = Blattodea; D = Chilopoda; E = Coleoptera; J = Hemiptera; K = Homoptera; L = Isopoda; N = Lepidoptera; O = Mantodea; P = Opiliones; Q = Orthoptera; S = Pseudoscorpiones; T = Thysanoptera).

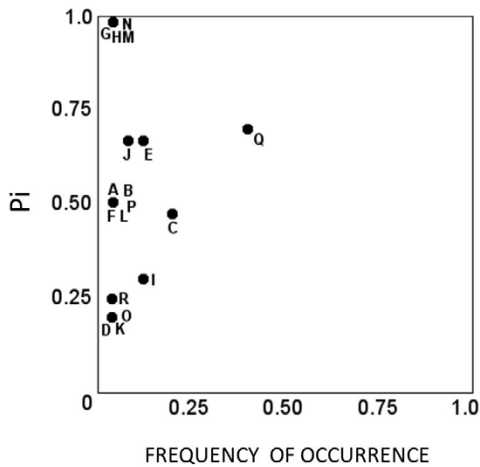


Figure 2 - Specialization diagram of *Haddadus binotatus* population from an Atlantic Rainforest area in the state of Rio de Janeiro, southeastern Brazil. The Y axis (Pi) shows the proportion of the prey item only for the individuals that consume it. The X axis shows the frequency of occurrence of the prey items in the diet. Each letter inside the diagram represents one prey type (A = Acari; B = Araneae; C = Blattodea; D = Chilopoda; E = Coleoptera; F = Dermoptera; G = Diplopoda; H = Diptera; I = Hymenoptera (Formicidae); J = Hemiptera; K = Homoptera; L = Isopoda; M = Isoptera; N = Lepidoptera; O = Mantodea; P = Opiliones; Q = Orthoptera; R = Scorpiones).

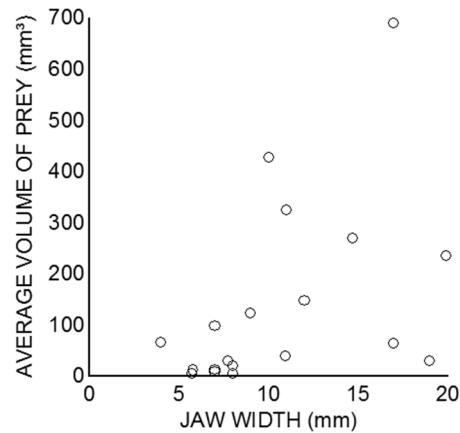


Figure 3 - Relationship between the mean volume of prey (mm^3) and the jaw width (mm) of individuals of *Haddadus binotatus* population from an Atlantic Rainforest area in the state of Espírito Santo, southeastern Brazil ($r_s = 0.6025$; $P = 0.0063$, $n = 19$).

$F_{1,25} = 5.091$; $P = 0.033$; RJ - ANOVA: $F_{1,49} = 4.282$, $P = 0.044$) than females (ES - 38.3 ± 14.3 mm; 8.96 mm–58.82 mm; $n = 12$; RJ - 34.3 ± 9.5 mm; 17.4 mm–59.0 mm; $n = 31$).

DISCUSSION

Haddadus binotatus of RJP and ESP fed on a wide array of arthropods, with 20 prey types identified to the level of Order. Leaf litter frogs of the Atlantic Forest fed upon a variety of prey from different Classes as Arthropoda, Gastropoda, and even vertebrates as anurans (e.g. Van Sluys et al. 2001, Boquimpani-Freitas et al. 2002, Marra et al. 2004, Almeida-Gomes et al. 2007, Dietl et al. 2009, Martins et al. 2010, Almeida-Santos et al. 2011, Klaion et al. 2011, Sugai et al. 2012). The number of prey types registered to the taxonomic level of Order can also differ considerably among leaf litter frog species, varying from six (e.g. Boquimpani-Freitas et al. 2002) to 22 items consumed (e.g. Siqueira et al. 2006, Teixeira et al. 2006). In other biomes, such as the Cerrado and the Amazonia, the number of preys identified to this taxonomic level and consumed by leaf litter frogs, also seemed to be around 22 (e.g. Biavati et al. 2004, Ortega et al. 2005).

The most important preys were Orthoptera, Blattodea and Coleoptera in RJP and Orthoptera and Araneae in ESP. It has been suggested that Blattodea, Coleoptera, and Orthoptera are important prey for large leaf litter frogs, as they present large body sizes and mass, which provide an appropriate amount of food and energy (Boquimpani-Freitas et al. 2002, Klaion et al. 2011). Other arthropods such as Isopoda, Chilopoda and Araneae are also recognized as being an important volumetric contribution to the diet of medium-sized frogs (Van Sluys et al. 2001, Dietl et al. 2009). Although arthropods are the main prey of leaf litter frogs (e.g. Lima and Moreira 1993, Marra et al. 2004, Almeida-Gomes et al. 2007, Almeida-Santos et al. 2011), the diet composition of frog species may be composed by similar prey types but with different relative importance. This fact can be observed in some species that coexist with *H. binotatus* in the forest floor. For example, at Ilha Grande (RJ), Orthoptera was the most important prey of *Proceratophrys tumpinamba* (Cycloramphidae) (Boquimpani-Freitas et al. 2002), whereas Araneae, Isopoda and Formicidae were the main preys of the sympatric and syntopic *Ischnocnema parva* (Brachycephalidae) (Marra et al. 2004). Furthermore, the most important preys of *Stereocyclops incrassatus* (Microhylidae) at a locality in Espírito Santo were Formicidae, Coleoptera, and Isopoda (Teixeira et al. 2006). Similarly, *Rhinella ornata* (Bufonidae) consumed mainly Formicidae, Coleoptera and Orthoptera in EEEP and REGUA (Maia-Carneiro et al. 2013), the same areas of the present study in the state of Rio de Janeiro. However, *Proceratophrys boiei*, a syntopic species with similar size to that of *H. binotatus*, had not only a similar diet, composed mainly by Coleoptera, Blattodea, and Orthoptera, but the prey shared similar importance in their diets (Giaretta et al. 1998, Klaion et al. 2011). The preference for different prey types, in leaf litter frog assemblages, may be evolved as a mechanism to

allow the coexistence of species with similar food and habitat requirements, or simply may reflect the more frequent encountered prey.

In both populations, some individuals of *H. binotatus* had vegetal remains in stomachs but in different proportions. Plant remains were usually represented only by pieces of dead leaves. The occurrence of plant remains has been reported in diet studies of some anuran species (e.g. Marra et al. 2004, Siqueira et al. 2006, Martins et al. 2010, Almeida-Santos et al. 2011), and its ingestion has been usually considered non-intentional and supposed to be ingested accidentally when the anuran swallows the prey among dead leaves on the forest floor (e.g. Van Sluys et al. 2001, Martínez-Coronel and Pérez-Gutiérrez 2011), which seems to be the case of *H. binotatus*. Only for the hyliid *Xenohyla truncata* has the ingestion of plant matter been considered intentional (Silva et al. 1989, Silva and Brito-Pereira 2006). Our results indicated that *H. binotatus* feed mainly on arthropods and has a relatively generalized diet, amongst the richest of the Atlantic Forest in terms of taxonomic level of Order. Furthermore, *Haddadus binotatus* was among the frog species that had the higher density and mass per hectare in some studied sites at the Atlantic rainforest (EEEP) (Rocha et al. 2007, 2011), which suggests that the species can play an important role on its prey populations at least at these sites.

We found a slight difference in number and volume of prey types consumed by the two populations of *H. binotatus*, which can reflect, at least partially, local differences in the prey availability of the two habitats of the Atlantic Rainforest, since invertebrate communities can change among areas (Lacerda et al. 1998, Santos et al. 1998) or as a result of seasonal changes (Toft 1980b, Anderson et al. 1999, Wells 2007) which in the case of our study, can also be a result of the sampling periods.

Mouth size in the *H. binotatus* of ESP explained approximately 36% of the variation in prey volume

consumed, suggesting that prey size can be adjusted and constrained by mouth size of frogs (although this trend did not appear for the RJP). It is expected that animals that cannot handle their prey and need to swallow them whole, tend to concentrate their diet on relatively large prey (Siqueira et al. 2006) as observed for *H. binotatus* in the present study and for other anurans (e.g. Ortega et al. 2005, Martínez-Coronel and Pérez-Gutiérrez 2011), most of them from Atlantic Forest areas (e.g. Boquimpani-Freitas et al. 2002, Dietl et al. 2009, Marra et al. 2004). As a consequence, anurans tend to show an adjustment between prey size and mouth size (Van Sluys et al. 2001, Jordão-Nogueira et al. 2006, Martins et al. 2010, Klaion et al. 2011). In some cases in where this relationship between prey size and mouth size is not clear or does not occur (as may be the case of RJP), usually a high consumption of small prey (low mean volume) or similar-sized prey, as Formicidae (Almeida-Gomes et al. 2007), Collembola, Isopoda and Acari, or the small size of frog (Van Sluys et al. 2006, Almeida-Santos et al. 2011) are involved. In fact a comparatively lower mean volume of prey in frog stomachs was registered for the RJP which could have prevented such relationship to be found. Additionally, we did not find correlation between the number of prey ingested and the frog body size indicating that a large size did not imply in an increase in the number of prey ingested. This result may be expected for frogs that select the prey size, according to their mouth size, as we found at Espírito Santo, and as it is expected for frogs that prefer to ingest larger preys to maximize the cost of capture.

The proportion of individuals having empty stomachs in the population of RJP (39.2%) was more than twice that of the ESP (17.9%) which suggests that the two *H. binotatus* populations could have had differences in their energy balance at the moment of the collection of the individuals. The observed proportion of individuals of a species having empty stomachs has been used as an index of instantaneous energy balance for lizards (Huey et al. 2001) and

fishes (Arrington et al. 2002) in which individuals having empty stomachs are assumed to be in negative energy balance and dependent on reserve stores (Boivin and Power 1990, Huey et al. 2001, Arrington et al. 2002) whereas individuals having food in their stomachs would be gaining energy and thus, would be in positive energy balance (Huey et al. 2001). We still do not know to which extent this energy trends work among frogs but our data is suggestive that, comparatively, the RJP was in lower energy balance when compared with ESP.

Our data showed that there is sexual dimorphism in body size of *H. binotatus*, with females being larger than males. Sexual size dimorphism has been reported to leaf litter anurans (e.g. Woolbright 1989, Boquimpani-Freitas et al. 2002, Lee 2001, Van Sluys et al. 2006), and a positive relationship between the number of eggs and female size (Prado and Haddad 2005, Martins et al. 2010) is usually associated with advantages of females producing more eggs or larger eggs, although anurans with direct development have, in general, smaller clutches with larger eggs (Wake 1978, Zug et al. 2001, Almeida-Santos et al. 2011). As clutch size and female size is not positively related in *H. binotatus* (Canedo and Rickli 2006), the larger sizes attained by females could result from evolutionary forces favoring the production of larger eggs by females.

We conclude that the leaf litter frog *Haddadus binotatus* has a relatively generalized diet (20 prey types) which is composed mainly of arthropods being Orthoptera, Coleoptera, and Blattodea the most important preys among Rio de Janeiro population, and Orthoptera and Araneae the most important among the Espírito Santo population. Populations of this frog may differ somewhat in diet, potentially attributable to differences of the environments they live in. The size of *H. binotatus* mouth affected the size of prey consumed, as it is expected for frogs that swallow preys, according to their mouth size. The sexual dimorphism of

the *H. binotatus* in its size, with females being comparatively larger than the males, could result from advantages of larger females being favored by the production of larger eggs.

ACKNOWLEDGMENTS

We thank A. Pissinati, N. Locke, A. C. Balbino, A. Lacerda and V. Colli for permission to work and logistic help at the Estação Ecológica Estadual Paraíso (EEEP), in Guapimirim municipality, Reserva Ecológica de Guapiaçu (REGUA), in Cachoeiras de Macacu municipality, in the state of Rio de Janeiro, and Serra das Torres, in Atílio Vivacqua municipality, in the state of Espírito Santo. This study was supported by research grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) to C.F.D. Rocha (processes 304791/2010-5 and 472287/2012-5), M.C. Kiefer (process 150353/2003-0), V.N.T. Borges Junior (process 141318/269-0), and L.A. Fusinato (Doctorate fellowship; process 142823/2009-0). C.F.D. Rocha also received a grant from the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) through the “Programa Cientistas do Nosso Estado” (process E-26/102.765/2012). M.C. Kiefer also received research grant from FAPERJ (Process No. E-26/171.168/2006). L. Coco received a fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). L.A. Fusinato also received a sandwich doctorate fellowship from Capes (Process N°. 0378/11-9). This study was partially supported by the Critical Ecosystem Partnership, Conservation Biodiversity Center of Conservation International, and Instituto Biomas. We would also like to thank all the people that helped with the field work and laboratory procedures.

RESUMO

Haddadus binotatus é um anuro endêmico da Mata Atlântica Brasileira e, atualmente, ainda não existem informações sobre a dieta desta espécie. Analisamos a

dieta de duas populações desse anuro em dois estados do sudeste brasileiro. As amostragens foram realizadas em 2004 no estado do Rio de Janeiro e em 2009 e 2010 no estado do Espírito Santo. *Haddadus binotatus* apresentou uma composição de dieta rica, predando 19 tipos de presas. Orthoptera, Coleoptera e Blattodea foram as presas mais importantes na população do Rio de Janeiro, e Orthoptera, Araneae e Hemiptera foram as presas mais importantes na população do Espírito Santo. A composição da dieta diferiu numericamente entre as duas localidades, mas não em termos de volume, o que pode refletir diferenças locais na disponibilidade de presas nos dois habitats. A largura da mandíbula limitou o tamanho da presa, o que é esperado para predadores que engolem as presas sem mastigar. A proporção de indivíduos com estômagos vazios foi maior na população do Rio de Janeiro (39,2%) do que na do Espírito Santo (17,9%), sugerindo que a primeira poderia estar em um menor balanço energético. As fêmeas da espécie foram maiores que os machos, o que pode resultar de uma seleção de tamanhos maiores atingidos pelas fêmeas, favorecendo a produção de ovos maiores.

Palavras-chave: artrópodes, dieta, predador generalista, dimorfismo sexual.

REFERENCES

- ALMEIDA-GOMES M, VAN SLUYS M AND ROCHA CFD. 2007. Ecological observations on the leaf-litter frog *Adenomera marmoratus* in an Atlantic rainforest area of southeastern Brazil. *Herpetol J* 17: 81-85.
- ALMEIDA-GOMES M ET AL. 2010. Anurofauna of an Atlantic Rainforest fragment and its surroundings in northern Rio de Janeiro State, Brazil. *Braz J Biol* 70: 871-877.
- ALMEIDA-SANTOS M, SIQUEIRA CC, VAN SLUYS M AND ROCHA CFD. 2011. Ecology of the Brazilian flea frog *Brachycephalus didactylus* (Terrarana: Brachycaphalidae). *J Herpetol* 45: 251-255.
- AMUNDSEN PA, GABLER HM AND STALDVIK FJ. 1996. A new approach to graphical analysis of feeding strategy from stomach contents data – modification of the Costello (1990) method. *J Fish Biol* 48: 607-614.
- ANDERSON AM, HAUKOS DA AND ANDERSON JT. 1999. Diet composition of three anurans from the playa wetlands of northern Texas. *Copeia* 1999: 515-520.
- ARRINGTON DA, WINEMILLER KO, LOFTUS WF AND AKIN S. 2002. How often do fishes “run on empty”? *Ecology* 83: 2145-2151.

- BERNARDO CSS, LLOYD H, BAYLY N AND GALETI M. 2011. Modelling post-release survival of reintroduced Red-billed Curassows *Crax blumenbachii*. *Ibis* 153: 562-572.
- BIAVATI GM, WIEDERHECKER HC AND COLLI GR. 2004. Diet of *Epipedobates flavopictus* (Anura: Dendrobatidae) in a neotropical savanna. *J Herpetol* 38: 510-518.
- BOIVIN TG AND POWER G. 1990. Winter condition and proximate composition of anadromous arctic charr (*Salvelinus alpinus*) in eastern Ungava Bay, Quebec. *Can J Zool* 68: 2284-2289.
- BOQUIMPANI-FEITAS L, ROCHA CFD AND VAN-SLUYS M. 2002. Ecology of the horned leaf-frog, *Proceratophrys appendiculata* (Leptodactylidae), in an insular Atlantic rainforest area of southeastern Brazil. *J Herpetol* 36: 318-322.
- CANEDO C AND RICKLI E. 2006. Female Reproductive Aspects and Seasonality in the Reproduction of *Eleutherodactylus binotatus* (Spix, 1824) (Amphibia, Leptodactylidae) in an Atlantic Rainforest fragment, Southeastern Brazil. *Herpetol Rev* 37: 149-151.
- CRUMP ML AND SCOTT JR NJ. 1994. Visual encounter surveys. In: HEYER WR ET AL. (Eds), *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*, Washington D.C.: Smithsonian Institution Press, USA, p. 84-92.
- DIETL J, ENGELS W AND SOLÉ M. 2009. Diet and feeding behavior of the litter-frog *Ischnocnema henselii* (Anura: Brachycephalidae) in an Araucaria rain forest on the Serra Geral of Rio Grande do Sul, Brazil. *J Nat Hist* 43: 1473-1483.
- DUNHAM AE. 1983. Realized Niche Overlap, Resource Abundance and Intensity of Interspecific Competition in Lizard Ecology. In: HUEY RD, PIANKA ER and SCHOENER TW (Eds), *Lizards Ecology: Studies of Model Organism*, Cambridge: Harvard University Press, USA, p. 261-280.
- GIARETTA AA, ARAÚJO MS, MEDEIROS HF AND FACURE KG. 1998. Food habits and ontogenetic diet shifts of the litter dwelling frog *Proceratophrys boiei* (wied). *Rev Bras Zool* 15: 385-388.
- HADDAD CFB AND SAZIMA I. 1992. Anfíbios da Serra do Japi. In: MORELATTO LPC (Ed), *História Natural da Serra do Japi: ecologia e preservação de uma área florestal no Sudeste do Brasil*, Campinas: Unicamp e Fapesp, Brasil, p. 188-211.
- HEDGES SB, DUELLMAN WE AND HEINICKE MP. 2008. New World direct-developing frogs (Anura: Terrarana): Molecular phylogeny, classification, biogeography, and conservation. *Zootaxa* 1737: 1-182.
- HOWARD AK, FORESTER JD, RUDER JM, PARMERLEE JR JS AND POWELL R. 1999. Natural history of a terrestrial Hispaniolan anole: *Anolis barbouri*. *J Herpetol* 33: 702-706.
- HUEY RB, PIANKA ER AND VITT LJ. 2001. How often do lizards "run on empty"? *Ecology* 82: 1-7.
- INCAPER - INSTITUTO CAPIXABA DE PESQUISA, ASSISTÊNCIA TÉCNICA E EXTENSÃO RURAL. 2010. Available at: <http://www.incaper.es.gov.br/>. Archived by WebCite at <http://www.webcitation.org/69FzBGrrO> on 18 July 2012.
- JAEGER RG AND INGER RF. 1994. Quadrat sampling. In: HEYER WR et al. (Eds), *Measuring and monitoring biological diversity: standard methods for amphibians*, Washington D.C.: Smithsonian Institution Press, USA, p. 97- 102.
- JORDÃO-NOGUEIRA T, VRCIBRADIC D, PONTES JAL, VAN SLUYS M AND ROCHA CFD. 2006. Natural history traits of *Crossodactylus aeneus* (Anura, Leptodactylidae, Hyloinae) from an Atlantic forest area in Rio de Janeiro State, southeastern Brazil. *S Am J Herpetol* 1: 37-41.
- KLAION T, ALMEIDA-GOMES M, TAVARES LER, ROCHA CFD AND VAN SLUYS M. 2011. Diet and nematode infection in *Proceratophrys boiei* (Anura: Cycloramphidae) from two Atlantic rainforest remnants in Southeastern Brazil. *An Acad Bras Cienc* 83: 1303-1312.
- LACERDA P, SARMENTO A, MARQUES AM AND ROCHA CFD. 1998. Variação sazonal na diversidade, abundância e produtividade de artrópodos em uma área de Mata Atlântica do sudeste do Brasil (Casimiro de Abreu, RJ). *Anais do VIII Seminário Regional de Ecologia* 8: 847-856.
- LEE JC. 2001. Evolution of a Secondary Sexual Dimorphism in the Toad, *Bufo marinus*. *Copeia* 2001: 928-935.
- LIMA AP AND MOREIRA G. 1993. Effects of prey size and foraging mode on the ontogenetic change in feeding niche of *Colostethus stepheni* (Anura: Dendrobatidae). *Oecologia* 95: 93-102.
- MAIA-CARNEIRO T, KIEFER MC, VAN SLUYS M AND ROCHA CFD. 2013. Feeding habits, microhabitat use and daily activity period of *Rhinella ornata* (Anura, Bufonidae) from three Atlantic rainforest remnants in southeastern Brazil. *Northwest J Zool* 9: 157-165.
- MARRA RV, ROCHA CFD AND VAN SLUYS M. 2004. Food habits of *Eleutherodactylus parvus* (Anura: Leptodactylidae) at an Atlantic rainforest area, southeastern Brazil. *Herpetol Rev* 35: 135-137.
- MARTÍNEZ-CORONEL M AND PÉREZ-GUTIÉRREZ M. 2011. Composición de la dieta de *Craugastor lineatus* (Anura: Craugastoridae) de Chiapas, México. *Acta Zool Mex* 27: 215-230.
- MARTINS ACJS, KIEFER MC, SIQUEIRA CC, VAN SLUYS M, MENEZES VA AND ROCHA CFD. 2010. Ecology of *Ischnocnema parva* (Anura: Brachycephalidae) at the Atlantic rainforest of Serra da Concórdia, state of Rio de Janeiro, Brazil. *Zoologia* 27: 201-208.
- ORTEGA JE, SERRANO VH AND PINILLA MPR. 2005. Diet composition and microhabitat of *Eleutherodactylus johnstonei* in an introduced population at Bucaramanga city, Colombia. *Herpetol Rev* 36: 238-241.
- PADIAL JM, CASTROVIEJO-FISHER S AND DE LA RIVA I. 2009. The phylogenetic relationships of *Younganastes* revised (Anura: Terrarana). *Mol Phylogenet Evol* 52: 911-915.
- PIANKA ER. 1986. *Ecology and Natural History of Desert Lizards*. New Jersey: Princeton University Press, 208 p.
- PRADO CPA AND HADDAD CFB. 2005. Size-fecundity relationship and reproductive investment in female frogs in the Pantanal, south-western Brazil. *Herpetol J* 15: 181-189.

- ROCHA CFD ET AL. 2007. A survey of the leaf-litter frog assembly from an Atlantic forest area (Reserva Ecológica de Guapiáçu) in Rio de Janeiro State, Brazil, with an estimate of frog densities. *Trop Zool* 20: 99-108.
- ROCHA CFD ET AL. 2011. Parameters from the community leaf-litter frogs from Estação Ecológica Estadual Paraíso, Guapimirm, Rio de Janeiro State, southeastern Brazil. *An Acad Bras de Cienc* 83: 1259-1267.
- SANTOS HC, ROCHA CFD AND BERGALLO HG. 1998. A produtividade, diversidade e abundância da mesofauna do litter em dois segmentos de Mata Atlântica (Mata de Planície e Mata de Encosta) na Ilha do Cardoso, Cananéia, São Paulo. *Anais do VIII Seminário Regional de Ecologia* 8: 823-836.
- SILVA HR AND BRITO-PEREIRA MC. 2006. How much fruit do fruit-eating frogs eat? An investigation on the diet of *Xenohyla truncata* (Lissamphibia: Anura: Hylidae). *J Zool* 270: 692-698.
- SILVA HR, BRITO-PEREIRA MC AND CARAMASCHI U. 1989. Frugivory and seed dispersal by *Hyla truncata*, a neotropical tree-frog. *Copeia* 1989: 781-783.
- SIQUEIRA CC, ARIANI CV, VAN SLUYS M AND ROCHA CFD. 2006. Feeding ecology of *Thoropa miliaris* (Anura, Cycloramphidae) in four areas of Atlantic rain forest, southeastern Brazil. *J Herpetol* 40: 520-525.
- SUGAI JLMM, TERRA JS AND FERREIRA VL. 2012. Diet of *Leptodactylus fuscus* (Amphibia: Anura: Leptodactylidae) in the Pantanal of Miranda river, Brazil. *Biota Neotrop* 12: 99-104.
- TEIXEIRA RL, VRCIBRADIC D AND GLADSTONE IA. 2006. Food habits of *Stereocyclops incrassatus* (Anura: Microhylidae) from Povoação, Espírito Santo State, southeastern Brazil. *Boletim do Museu Mello Leitão* 19: 53-58.
- TOFT CA. 1980a. Feeding ecology of thirteen syntopic species of Anurans in a seasonal tropical environment. *Oecologia* 45: 131-141.
- TOFT CA. 1980b. Seasonal variation in populations of the Panamanian litter frogs and their prey: a comparison of wetter and drier sites. *Oecologia* 47: 34-38.
- TOFT CA. 1981. Feeding ecology of Panamanian litter anurans: patterns in diet and foraging mode. *J Herpetol* 15: 139-144.
- VAN SLUYS M, ROCHA CFD AND SOUZA MB. 2001. Ecology of the leptodactylid litter frog *Zachaeus parvulus* in Atlantic rainforest of southeastern Brazil. *J Herpetol* 35: 322-325.
- VAN SLUYS M, SCHITTIN GM, MARRA RV, AZEVEDO ARM, VICENTE JJ AND VRCIBRADIC D. 2006. Body size, diet and endoparasites of the microhylid frog *Chiasmocleis capixaba* in an Atlantic forest area of southern Bahia state, Brazil. *Braz J Biol* 66: 167-173.
- WAKE MH. 1978. The Reproductive Biology of *Eleutherodactylus jasperi* (Amphibia, Anura, Leptodactylidae), with Comments on the Evolution of Live-bearing Systems. *J Herpetol* 12: 121-133.
- WELLS KD. 2007. *The Ecology and Behavior of Amphibians*. Chicago: The University Chicago Press, USA, 1400 p.
- WOOLBRIGHT LL. 1989. Sexual Dimorphism in *Eleutherodactylus coqui*: Selection Pressures and Growth Rates. *Herpetologica* 45: 68-74.
- ZAR JH. 1999. *Biostatistical Analysis*, 4th ed., New Jersey: Prentice Hall, USA, 663 p.
- ZUG GR, VITT LJ AND CALDWELL JP. 2001. *Herpetology: An Introductory Biology of Amphibians and Reptiles*, 2nd ed., San Diego: Academic Press, USA, 630 p.