

## Diet of *Haddadus binotatus* (Spix, 1824) (Anura: Craugastoridae) in Brazilian Atlantic Rainforest, Bahia state

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**Abstract.** Anurans commonly feed on arthropods, most species being “sit-and-wait” foragers. We studied diet aspects of *Haddadus binotatus*, a craugastorid frog inhabiting Atlantic Forest remnants, in a forest fragment at Michelin Ecological Reserve, southern Bahia, Brazil. Individuals were collected at night from leaf litter, transferred to the nearby laboratory, measured and had their stomach contents retrieved using a flushing procedure. Stomach contents were correlated with morphometric aspects of the frogs. The absence of a significant correlation between these variables indicates that *H. binotatus* does not select prey by its size, even with increasing body size. The most important diet items were Araneae and Orthoptera, unlike described for the genus *Eleutherodactylus*, thus qualifying *H. binotatus* as a “no ant specialist”. The low number of prey items per stomach and an intermediate niche breadth suggest that *H. binotatus* may alternate the foraging strategy or at least show some plasticity. The highly positive electivity index for Orthoptera in relation with the available data for other species of the Craugastoridae family suggests that preference for Orthoptera may be a pattern in this family.

**Key words:** Craugastoridae, leaf litter, stomach-flushing, *Eleutherodactylus*, feeding habits.

### Introduction

Amphibians are described as generalist predators, feeding mainly on arthropods, mollusks, annelids (Solé & Rödder 2010), but exceptions have also been documented, like the consumption of fruits (Silva & Britto-Pereira 2006) and small vertebrates (Duellman & Lizana 1994, Cicort-Lucaciu et al. 2011). Most species are “sit-and-wait” predators, showing no preference for a specific type of prey, while some species are active foragers with a preference for a specific type of prey (e.g. Rödel & Braun 1999, Solé et al. 2002, Berazategui et al. 2007).

The family Craugastoridae includes 115 species in two genera, *Craugastor*, with 113 species, and *Haddadus*, recently relocated from the genus *Eleutherodactylus* (Hedges et al. 2008) with two species, *H. plicifer* and *H. binotatus* (Frost 2011). *H. binotatus* is a direct developing species. Females of this species lay eggs in terrestrial sites, typically on the leaf litter of the forest floor (Canedo et al. 2006). The species is distributed in the Atlantic Rainforest from Rio Grande do Sul to the state of Bahia (Heinicke et al. 2007), although some authors have suggested that *H. binotatus* could represent a species complex formed by at least two different species (Haddad & Sazima 1992, Heyer & Juncá 2003). Despite being a locally common species in the Atlantic Rainforest, little is known

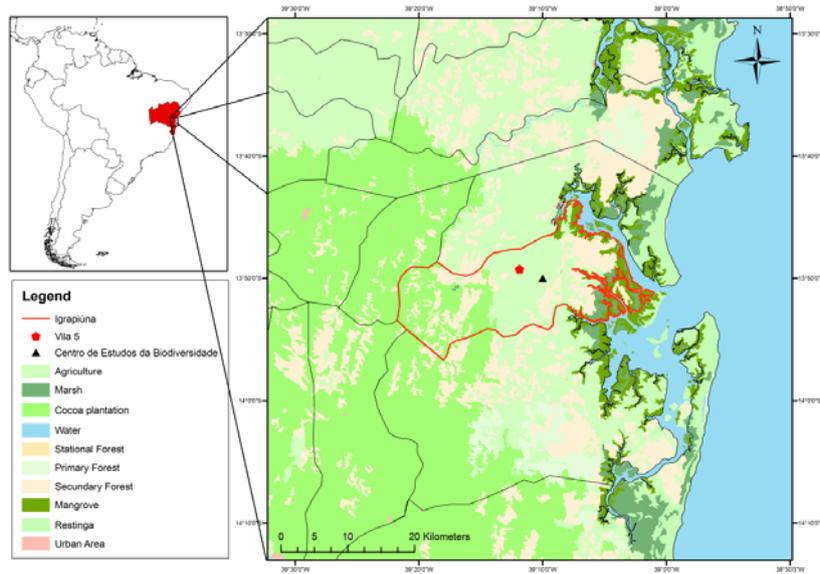
about its natural history and studies published until now have dealt mainly with its advertisement call (Dias et al. 2012, Moura et al. 2012).

Anurans play an important role in ecosystem functionality because they can act as predator and prey (Caldart et al. 2011, Gaiarsa et al. 2012) and change this role according to some life trait aspects (Travis et al. 1985). Due to their biphasic life, they can explore different resources along their life history (Kovács et al. 2007, Ficetola et al. 2011, Brito & Cascon 2012). Moreover, some amphibian diet aspects are directly related to other factors as toxicity and aposematism (Biavati et al. 2004, Valderama-Vernaza et al. 2009, Santos & Grant 2011).

In the present study we aimed to investigate the patterns of trophic resource utilization in *H. binotatus* (Fig. 1) in an Atlantic Rainforest remnant of Bahia State, Brazil.

### Material and methods

The study was conducted at Michelin Ecological Reserve, an area belonging to the Center for Biodiversity Studies, located in the municipality of Igrapiúna, Bahia, with an area of 3,096 ha. The study area was an Atlantic Rainforest fragment, named “Vila 5” (13°49'15.3”S, 39°11'52.1”W), with primary and secondary vegetation (Fig. 2). We searched for individuals of *H. binotatus* and captured them by hand in suitable microhabitats on the forest floor from April 2010 to March 2011 between 21:00 and 01:00, during five days per month.



**Figure 2.** Map showing the Centro de Estudos da Biodiversidade (triangle) and the area where individuals of *Haddadus binotatus* were collected (pentagon).



**Figure 1.** Adult *Haddadus binotatus* from Michelin Ecological Reserve.

At the field station, located approximately 5 km from the studied forest fragment, snout-vent length (SVL) and mouth width (MW) were recorded to the nearest 0.1 mm with a caliper. The mass of the frogs was assessed to the nearest 0.01g with a digital scale. Prey items were retrieved using the stomach-flushing protocol proposed by Solé et al. (2005), and conserved in ethanol 70% immediately after flushing. Individuals were measured and weighted always before flushing; after the procedure were kept in captivity until the end of the field trip and then released at the same site where they had been collected.

Prey items were sorted, counted, and identified to the lowest possible taxonomic category, and had their length and width measured to the nearest 0.1mm with a

caliper under a stereomicroscope. Partially damaged prey items had their approximate length calculated using the regression equations proposed by Hirai and Matsui (2001). The volume of intact prey was estimated using the formula for ovoid-spheroid bodies (Dunham 1983):

$$\text{Prey Volume} = \frac{4\pi}{3} \left( \frac{\text{length}}{2} \right) \left( \frac{\text{width}}{2} \right)^2$$

A Pearson Correlation Test, with a 95% confidence interval was performed in order to verify the correlation between snout-vent length and body mass and ingested volume for each individual, and the correlation between mouth width and the largest prey ingested for each individual.

To assess the importance of each consumed prey category, the index of relative importance (IRI) (Pinkas et al. 1971) was calculated as:

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

Where %O is the occurrence percentage of each prey category, %N is the percentage of the number of items in all stomachs, and %V is the percentage of the volume of items in all stomachs. Niche breadth was calculated using Levin's standardized index (Krebs 1999):

$$B_A = \frac{\left( \frac{1}{\sum p_j^2} \right) - 1}{n - 1}$$

Where  $p_j$  is the proportion in number or volume of each prey category with respect to the total number or volume of prey in each category of *H. binotatus* diet.

A sample of 1 m<sup>2</sup> of leaf litter was collected with the Berleze-Tullgren method (Rodrigues et al. 2008), and selectivity was calculated using an electivity index, as proposed by Jacobs (1974):

$$D = \frac{R_k - P_k}{(R_k + P_k) - (2R_k P_k)}$$

Where  $R_k$  is the proportion of prey category  $k$  in stomach contents, and  $P_k$  is the proportion of prey category  $k$  in the environment. Graphics were obtained and analysis was conducted using the software R 2.13.1 (R Development Core Team 2011).

## Results

We encountered 133 individuals in the field, of which 100 revealed stomach contents after the flushing procedure. Body mass was in mean 2.77 g ( $\pm$  2.71 g, min = 0.18g, max = 11.35g), snout-vent length 29.05 mm ( $\pm$  9.38 mm, min = 13.7 mm, max = 51.4mm) and mouth width 11.72 mm ( $\pm$  4.23 mm, min = 4.9 mm, max = 28.4 mm).

There was no significant correlation between

body mass and ingested volume by each individual ( $t = 0.3908$ ,  $p = 0.6971$ ), such as between snout-vent length and ingested volume ( $t = 0.3524$ ,  $p = 0.7256$ ) (Fig. 3) and between mouth width and the largest prey ingested by each individual ( $t = 0.1961$ ,  $p = 0.8451$ ) (Fig. 4).

The highest IRI was for Araneae (859.33), followed by Orthoptera (682.84) and Isopoda (288.9), and the lowest was for Gastropoda (1.01) and Lepidoptera (1.1) (Table 1).

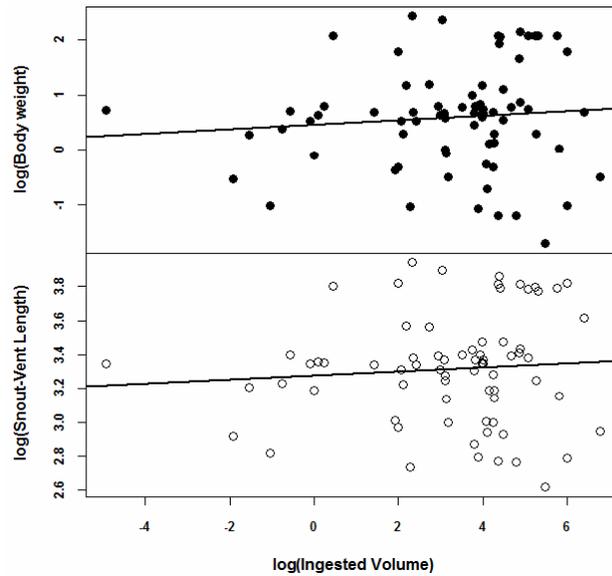
The Levins' Index of Niche Breadth was 0.506, with on average 1.56 items per stomach. In the leaf litter, the most frequent item was Acari (6695 individuals), followed by Collembola (1875 individuals) and Hymenoptera Formicidae (825 individuals). The item with largest relative frequency in stomach contents was Lepidoptera larvae, followed by Araneae and Hymenoptera Formicidae (Fig. 5).

Jacobs' Electivity Index was positive for Orthoptera (0.984), Lepidoptera larvae (0.974) and Odonata (0.915) and negative (-1) for Collembola, Coleoptera larvae, Hemiptera larvae, Diptera,

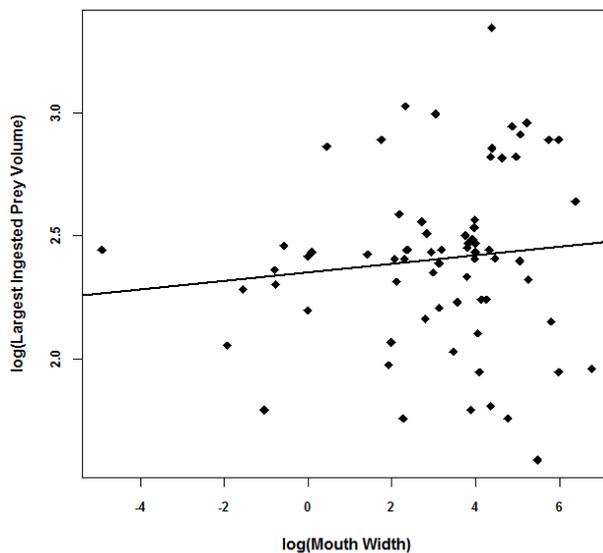
**Table 1.** Stomach contents of analyzed *Haddadus binotatus*.

Prey Category	N	N%	F	F%	V	V%	IRI
Insecta							
Hemiptera (Im)	4	2.99	4	5.41	80.6	1.24	19.84
Hymenoptera Formicidae	19	14.18	10	13.51	269.34	4.14	250.37
Hymenoptera other	3	2.24	2	2.7	596.94	9.19	26.61
Orthoptera	20	14.93	18	24.32	1392.43	21.43	682.84
Coleoptera	4	2.99	4	5.41	506.76	7.8	39.41
Lepidoptera (Im)	1	0.75	1	1.35	8.32	0.13	1.1
Lepidoptera (Lv)	22	16.42	3	4.05	59.96	0.92	81.71
Blattaria	8	5.97	7	9.46	659.04	10.14	117.02
Odonata	1	0.75	1	1.35	20.97	0.32	1.25
Dermaptera	4	2.99	4	5.41	65.81	1.01	19.16
Isoptera	23	17.16	3	4.05	74.85	1.15	89.35
Myriapoda							
Chilopoda	5	3.73	5	6.76	123.76	1.9	32.32
Crustacea							
Isopoda	12	8.96	12	16.22	1042.68	16.04	288.9
Amphipoda	2	1.49	2	2.7	17.95	0.28	4.45
Arachnida							
Araneae	22	16.42	21	28.38	1557.32	23.96	859.33
Acari	5	3.73	2	2.7	22.1	0.34	11.35
Mollusca							
Gastropoda	1	0.75	1	1.35	0.03	0.001	1.01
Total	156				6498.87		

**Legend:**  $N$  is the number of items in all stomachs,  $N\%$  is the relative number for each item in all stomachs,  $F$  is the number of stomachs in which each item was found,  $F\%$  is the relative number of occurrence of each item in all stomachs,  $V$  is the total volume of items in all stomachs,  $V\%$  is the relative volume number per item, and  $IRI$  is the Pinkas's Index of Relative Importance. Im = Imago, Lv = Larvae.



**Figure 3.** Relation between body mass and ingested volume (full circle points) and between snout-vent length and ingested volume (empty circle points) in *H. binotatus*.



**Figure 4.** Relation between volume of largest ingested prey and mouth width in *H. binotatus*.

Scorpiones, Opiliones, Pseudoscorpiones, Diplopoda, Oligochaeta and Nematoda (Fig. 6).

### Discussion

There was no significant correlation between body mass and ingested volume and between snout-vent length and ingested volume, such as between mouth width and volume of the largest prey in-

gested. This can mean that *H. binotatus* does not select prey by size, even with increasing body size. Dietl et al. (2009) verified a significant correlation between mouth width and the largest prey volume for *Ischnocnema henselli*, a species closely related to *H. binotatus* (Frost et al. 2006, Heinicke et al. 2007). These two species do not seem to share the same foraging strategy, consuming larger preys with the increase of body mass, and consequently with increase of mouth width. This was

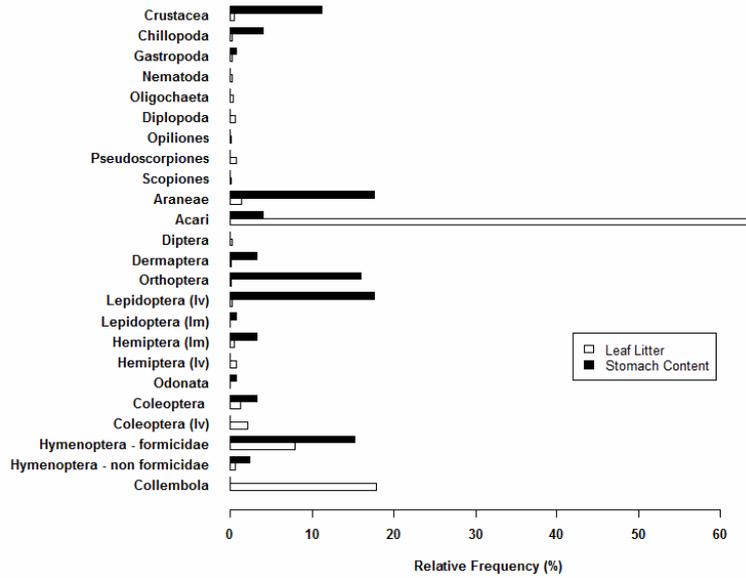


Figure 5. Comparison between Relative Frequency of Leaf Litter Invertebrate Fauna and *H. binotatus* stomach contents.

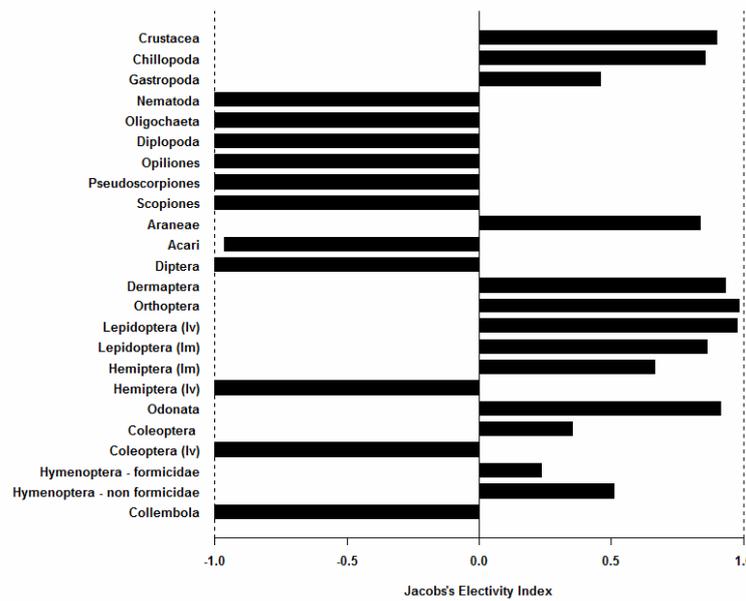


Figure 6. Jacobs' Electivity Index. The index ranges from -1 (negative electivity) to 1 (positive electivity)

also demonstrated by Martins et al. (2010) for *I. parva*, counteracting the idea that larger individuals change their prey selection (Duellman & Trueb 1994). Alternatively, the lack of significance in relationships between prey size and predator size

could also be dependent on the size range of available prey. Lack of this relationship has already been reported for some Hyloinae (Caldart et al. 2012).

According to Toft (1985), two different diet

patterns can be identified for anurans: “ant specialists” and “no ant specialists”. Duellman (1978) exposed that species of the genus *Eleutherodactylus* and related species feed mainly on Hymenoptera Formicidae, and because of this may be considered “ant specialists”. The diet of *H. binotatus* revealed Orthoptera and Araneae as the most important items, which does not qualify them as “ant specialist” despite the close relation with the species of the genus *Eleutherodactylus* (Frost et al. 2006, Heinicke et al. 2007). Our data is in agreement with Lima and Magnusson (1998), who suggest that general classifications on family level is not always appropriate to predict foraging activity. Diel et al. (2009) also reported a large consumption of Araneae for *I. henselli*, but this species also consumes a large number of Hymenoptera Formicidae, as well as *Eleutherodactylus johnstonei* from Barbados (Ovaska 1991). *Adenomera marmorata*, also a leaf litter frog, has Isopoda and Hymenoptera Formicidae as main consumed items (Almeida-Gomes et al. 2007), as well as *Zachaeus parvulus*, another common leaf litter frog from the Atlantic Rain Forest (Sluys et al. 2001), which demonstrates a variation in diet for species that feed in similar environments.

A low number of prey items per stomach together with a large niche breadth is characteristic for species that demonstrate a “sit-and-wait” foraging strategy (Perry & Pianka 1997). *H. binotatus* showed a low quantity of items per stomach, but an intermediate niche breadth index, that can indicate that it may alternate between active foraging and a “sit-and-wait” foraging mode (Huey & Pianka 1981) or at least show some plasticity regarding foraging strategies. Juncá and Eterovick (2007) found similar results for *Allobates marcehianus* and *Anomaloglossus stephensi*, demonstrating that these species alternate the foraging strategy.

Jacobs’ electivity index is larger for Orthoptera and Lepidoptera larvae. The high ingestion of Lepidoptera larvae could indicate an active foraging behavior, and the ingestion of a large number of Orthoptera could evidence a “sit-and-wait” behavior, corroborating the use of these two foraging strategies. The electivity index and relative importance index suggest that feeding on Orthoptera could be a pattern for *H. binotatus* and other species of the family Craugastoridae, although Araneae also showed high electivity and importance values. Martínez-Coronel and Pérez-Gutiérrez (2011) also observed a greater selection for Or-

thoptera by *Craugastor lineatus*. This pattern has not been reported for other related species, as *Eleutherodactylus bransfordi* and *E. vocator*, with Jacobs’ electivity index of -1 and -0.82 for this item, respectively, but is similar for those found for *E. altae* and *E. golmeri* (0.8 and 1, respectively) (Toft 1981).

Our work demonstrated that *H. binotatus* has a generalist feeding strategy referring to prey size, preferring mainly Araneae and Orthoptera as diet. We also suggest that this species can alternate between different foraging strategies or at least may show some plasticity, and should be included in the “no ant specialist” guild because other categories were more important and more selected than Hymenoptera Formicidae (Toft 1981).

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