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# Diet of *Hypsiboas leptolineatus* (Braun and Braun, 1977) (Amphibia: Anura: Hylidae) during the breeding season

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**Abstract.** We studied the diet of *Hypsiboas leptolineatus* in a protected area in Rio Grande do Sul state, Brazil and compared our results with available data from other species within the genus *Hypsiboas*. Stomachs from 41 specimens were flushed and the contents analysed. An index of relative importance of preys was calculated showing that the most important items for this species are Lepidoptera larvae, Coleoptera and Araneae. Our results bring novelty as we retrieved stomach contents from all studied individuals, whose capture was done while they were vocalizing. This is the first description of *H. leptolineatus* diet, contributing to the knowledge on this species ecology not only because of the trophic resources used, but also because we found evidence that they do feed during the breeding season.

**Keywords.** Araucaria forest, stomach flushing, diet, predation.

## Introduction

Trophic relations in amphibians are often investigated by analysing stomach content (Maneyro et al., 2004; López et al., 2005). Evaluation of stomach content and identification of preys can help comprehending characteristics of species (Pough et al., 2004) and understanding trophic niches. Amphibians can be classified as either active or passive (sit-and-wait) foragers; and specialist, intermediate or generalist predators (Toft, 1980 and 1981; Duellman and Lizana, 1994; López et al., 2003), according to the species and abundance of preys found in their stomachs. The information given by the diet is important to understand natural history and population fluctuations (Anderson et al., 1999).

Amphibians are important components of the food chain because they are a significant part of animal biomass in ecosystems, being a source of food for several animal groups (Toledo et al., 2007; Oliveira et al., 2013*)* and their diet is basically composed by insects and other arthropods, such as spiders and mites (Simon and Toft, 1991; Duellman and Trueb, 1994; Eterovick and Sazima, 2004).

Hylidae is the largest family of anurans. Diet composition among hylids can vary according to morphologic and physiologic aspects of the species (Toft, 1981). *Hypsiboas leptolineatus* is a tree-frog found on the Araucaria plateau of Rio Grande do Sul (Brazil), whose males measure 26 to 34 mm, while females reach 30 to 36 mm. They present longitudinal yellow or brown lines, with small dots along the body, and bigger ones on the dorsal area (Braun and Braun, 1980). This specie is found mainly around lentic environments. This preference may be related to their reproductive habits, where a gelatinous clutch is attached to vegetation in the water and tadpoles scrap among stones (Kwet and Di-Bernardo, 1999; Deiques et al., 2007). *H. leptolineatus* reproduce throughout the year, interrupting calling activity only during very low temperatures (Reinke and Deiques, 2010). Thus, they may feed during breeding, as already shown for *H. pulchellus,* another hylid with a prolonged reproductive activity (Solé and Pelz, 2007). As an alternative strategy, males from several anuran species stop feeding during the breeding season in order to focus their energy on calling activity (Duellman and Trueb, 1994). This study characterised diet and trophic

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niche breadth of males of *H. leptolineatus* during vocalisation, based on stomach content.

## Material and methods

Specimens were captured manually at night (Crump and Scott Jr, 1994), on January 10, 2014 (from 23:30 to 00:30) near a pond inside a reserve (Centro de Pesquisas e Conservação da Natureza – Pró-Mata, (29.1612° and 29.2100°S; 50.0448° and 50.0900°W) in the Brazilian state of Rio Grande do Sul. The pond is surrounded by grasses and shrubs inside an area of regeneration of *Araucaria* forest. Snout-vent length (*SVL*) was measured using a digital calliper. Body mass (*BM*) was measured using a digital balance. Each specimen had its stomach flushed following the methodology proposed by Solé et al. (2005) and specimens were released at the capture site at the same night, about four hours after being captured. Stomach contents were transferred to vials, fixed in 70 % ethanol and later analysed under a stereomicroscope. Prey items were classified morphologically at the order level. The volume ($mm^3$) occupied by each prey was calculated using a gridded paper, in which each item was uniformly spread keeping height in 1 mm (Hellawell and Abel, 1971).

Data on number (N), frequency of occurrence (F) and volumes (V) of the items were recovered. An index of Relative Importance (IRI) was calculated following the formula $IRI = (\%N + \%P)(\%FO)$, where $\%N$ is the relative abundance of each prey in the sample set; $\%P$ is the percentage of weight of each prey in the sample set; $\%FO$ is the relative frequency of occurrence of each prey in the sample set (Pinkas et al., 1971; Krebs, 1989). The higher the IRI, the higher the importance of a given prey category. In order to evaluate the trophic niche breadth, the Levins index (*B*) was used (Krebs, 1989): $B = 1/\Sigma pi^2$, where *p* is the fraction of items in the food category *i*. This index allows the measurement of breadth of the diet, considering, mainly, the quantitative distribution of each prey. The Standardised Levins Index (*Bsta*) range from 0 to 1, according to $Bsta = (B-1) / (n-1)$, where *n* represents prey orders. Values close to 0 indicate narrow trophic niche (Krebs, 1989).

## Results

A total of 41 specimens measuring from 30.11 mm to 37.11 mm *SVL* (mean ± SD 32.89 ± 1.41 mm) and weighing from 1.2 to 2.1 g (1.55 ± 0.18 g) were captured. We observed individuals calling continuously during capture. Eighty-two items were found within 11 prey categories and vegetal material (Table 1). Among the preys, Coleoptera (n = 20) and Araneae (n = 17) were the most numerically abundant, while the most representative groups regarding volume were Lepidoptera (V = 1,677 $mm^3$) and Lepidoptera larvae (V = 854 $mm^3$). Araneae (F = 14) and Coleoptera (F = 13) were the most frequent items. Among 1 % of the total stomach content was vegetal and 6 % were preys that could not be identified since digestion was already

**Table 1.** Prey categories consumed by *Hypsiboas leptolineatus* from an Araucaria plateau in Rio Grande do Sul, Brazil. *N* = number of individuals; *V* = total volume of preys ($mm^3$); *F* = frequency of occurrence of each prey category; (*%*) = percentage related to total; *IRI* = Index of relative importance.

| Preys (order) | N (%) | V (%) | F (%) | IRI |
|---|---|---|---|---|
| Coleoptera | 20 (24.39) | 645 (12.30) | 13 (31.71) | 1163.49 |
| Araneae | 17 (20.73) | 304 (5.80) | 14 (34.15) | 905.94 |
| Lepidoptera Larvae | 12 (14.63) | 854 (16.29) | 11(26.83) | 829.71 |
| Lepidoptera | 6 (7.32) | 1677 (31.99) | 6 (14.63) | 575.25 |
| Diptera | 14 (17.07) | 306 (5.84) | 10 (24.39) | 558.80 |
| Trichoptera | 3 (3.66) | 420 (8.01) | 3 (7.32) | 85.40 |
| Opiliones | 4 (4.88) | 310 (5.91) | 3 (7.32) | 78.96 |
| Blattaria | 3 (3.66) | 306 (5.84) | 3 (7.32) | 69.48 |
| Hymenoptera | 1 (1.22) | 16 (0.31) | 1 (2.44) | 3.72 |
| Collembola | 1 (1.22) | 2 (0.04) | 1 (2.44) | 3.07 |
| Acarina | 1 (1.22) | 1 (0.02) | 1 (2.44) | 3.02 |
| Plant material | -- | 75 (1.43) | 2 (4.88) | -- |
| Other | -- | 326 (6,22) | 17 (41.46) | -- |

advanced. Based on the Index of Relative Importance (IRI) we found that the most important item in the diet was Coleoptera, followed by Araneae and Lepidoptera larvae. Among the least important preys were Acarina, Collembola, and Hymenoptera (Table 1). The Levins Index of standardised trophic niche breadth (*Bsta*) was 0.51.

## Discussion

Diet composition of males of *H. leptolineatus* revealed Coleoptera as the most important prey category, as has already been observed in other studies with different species from the same genus (Araújo et al., 2007; Solé and Pelz, 2007; Sabagh et al., 2010). This order is known to be among the most abundant, mainly during warmer months (Teixeira et al., 2009). However, as we did not survey the available resources in our study, we cannot infer if this is the most abundant preys in the environment. The occurrence of Araneae in *H. leptolineatus* diet can be related to the fact that predator and prey use the same microhabitat suspended on shrubs, which may facilitate encounters, similarly to *H. albopunctatus*, a species with similar habits (Araújo et al., 2007). Vegetal material found in the stomachs can have its origin in accidental ingestion during prey capture (Teixeira et al., 2002; Santos et al., 2004; Mahan and Johnson, 2007). In a study conducted with *Hypsiboas raniceps* in Mato Grosso do Sul, a high trophic niche breadth was found (Bsta = 0.64; Sabagh, et al., 2010). Also, compared to the niche breadth obtained for *H. puchellus* (Bsta = 0.31) in wetlands in southern Brazil (Oliveira, M. unpublished data), *H. leptolineatus* presented a medium to high trophic niche breadth (Bsta = 0.51), which can be explained by the uniform consumption of six prey categories (Coleoptera, Araneae, Lepidoptera Larvae, Lepidoptera, and Diptera), as demonstrated by the Relative Importance Index. This indicates that the species is a generalist and opportunist consumer, possibly feeding on the most available preys in the environment.

Retrieving stomach contents from 100 % of the individuals was an unexpected result, as significant smaller percentages of stomach content were found in a previous study (Solé and Pelz, 2007). This previous study (Solé and Pelz, 2007) was carried out during calling activity in the same site (Pró-Mata, São Francisco de Paula, RS), and the diet of five other species of tree-frogs were analised. Significant stomach content was found only for one species (*Hypsiboas pulchellus*). Moreover, Duellman and Trueb (1986) described that many anuran species interrupt feeding during calling activity. These controversial results may be related to the fact that *H. leptolineatus*, as well as *H. pulchellus*, are active throughout the year (Achaval and Olmos, 2007), also feeding during calling activity.

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