Diet of bromeliad-frog *Phyllodytes luteolus* (Anura, Hylidae) in Atlantic Forest environments: what have the frogs been eating outside sandy coastal plains?

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**Abstract.** Knowledge about the diet of anurans in different environments is essential to understanding important aspects of their trophic ecology. The bromeliad-frog *Phyllodytes luteolus* inhabits tank bromeliads in sandy coastal plains and lowland forests on the mainland, as well as a continental island in southeastern Brazil. In this work, we describe and analyze the diet of *P. luteolus* in three environments. We obtained the consumed prey items of 92 frogs (32 from sandy coastal plain, 32 from lowland forest and 28 from the island) via a stomach-flushing procedure. We found some variations in consumed prey composition and prey volume across populations, but ants represented the most important consumed prey in all environments. Only ants had a relative importance greater than 50%, which may suggest a specialized diet that transcends the sandy coastal plain environment.

**Key-Words.** Amphibians; Lowland forest; Prey; Restinga; Trophic ecology.

**INTRODUCTION**

Amphibians generally prey on a wide variety of invertebrates (Lima *et al.*, 2010; Cicort-Lucaciu *et al.*, 2011; Solé & Rödder, 2010), vertebrates (Duellman & Lizana, 1994), and, less frequently, fruits (Silva & Britto-Pereira, 2006). They are therefore considered to be generalists in their diet. However, prey consumption by amphibians may vary in composition and size, especially when populations live in different environments (Berazategui *et al.*, 2007; Sabagh *et al.*, 2012; Maia-Carneiro *et al.*, 2013).

The bromeliad frog *Phyllodytes luteolus* (Wied-Newied, 1824) is an endemic species from the Atlantic Forest on the eastern coast of Brazil (Frost, 2016). This frog evolved to use the water stored in tank bromeliads, which are required for reproduction (Peixoto, 1995; Haddad & Prado, 2005). This ecological relationship with bromeliads allows the frog occurrence in environments where fresh water is a limiting resource, namely, in sandy coastal plains (Peixoto, 1995; Ferreira *et al.*, 2012; Mageski *et al.*, 2016; Frost, 2016). However, some populations of *P. luteolus* were recently found in other environments, including lowland forests and a continental island (Peixoto, 1995; Mageski *et al.*, 2014; Mageski *et al.*, 2015).

*Phyllodytes luteolus* was reported to be the most abundant anuran in sandy coastal plains, preying mainly on ants and termites (Eterovick, 1999; Ferreira *et al.*, 2012; Mageski *et al.*, 2015; Motta-Tavares *et al.*, 2016). However, nothing is known about the diet of *P. luteolus* outside the sandy coastal plains. This knowledge is crucial to describing the spectrum of its diet and to understanding the patterns and variations of its prey consumption, positioning in trophic webs and...
foraging strategies (Toft, 1980, 1981; Wells, 2007; Motta-Tavares et al., 2016).

We described and analyzed the diet of three *P. luteolus* populations that inhabit different environments (sandy coastal plains, lowland forest and island) in order to reveal the most important consumed prey item outside sandy coastal plains. Our goal was to assess the variety of consumed prey items (kind, number and volume) at each environment in order to estimate trophic niche overlapping among the populations and to determine the foraging strategy of *P. luteolus*.

### MATERIALS AND METHODS

We sampled three populations of *P. luteolus* in different environments in 2015 (9 and 10 May in the sandy coastal plain, 23 August in the island and 13 and 14 September in the lowland forest), all within Espírito Santo State (Fig. 1). The sandy coastal plain (-20.614780° lat, -40.18049° long, datum WGS84 – Fig. 1), which is greatly influenced by the ocean, is a largely open environment with sandy soil and low vegetation cover (Franco et al., 1984; Mageski et al., 2016). The lowland forest (-19.137466° lat, -40.062733° long, datum WGS84 – Fig. 1) has a mixture of sandy and clay soil, with a high proportion under cover of vegetation (Jacomine, 1996; Paula, 2006). The island (-20.613186° lat, -40.382230° long, datum WGS84 – Fig. 1) is mainly composed of rocky outcrops with ground vegetation and is close to the sandy coastal plain (~3 km).

We searched for frogs randomly in clusters of tank bromeliads during the night (18-23 h). Following capture, we applied a stomach-flushing procedure (Solé et al., 2005) to obtain the stomach contents, which were preserved in 70% ethanol. To prevent recapture, all captured individuals were kept in moist plastic bags until the end of sampling and were then released.

We analyzed the stomach contents under a stereomicroscope. All prey items were identified to Order level, and ants were identified to Family level. The number and frequency of occurrence for each prey item were quantified, and the width and length were measured with digital caliper (0.01 precision). These measurements were required for calculating the ellipsoidal volume *(V = 4/3π * (L/2) * (W/2)^2)*, where L = prey length and W = prey width (Colli & Zamboni, 1999; Biavati et al., 2004), and the index of relative importance *(i = (N% + F% + V%)/3)*, where N% = the number of each prey category relative to the total number of prey ingested, F% = the number of stomachs containing each prey relative to number of stomachs analyzed, and V% = the volume of each prey category relative to the total volume of prey ingested (Biavati et al., 2004). We used the prey volume to calculate Pianka’s similarity index (Pianka, 1973) to test the trophic niche overlap between populations:

\[
O_{jk} = \frac{\sum_{i=1}^{n} p_{ij} p_{ik}}{\sqrt{\sum_{i=1}^{n} p_{ij}^2 \sum_{i=1}^{n} p_{ik}^2}}
\]

where \(n\) is the total number of prey, and \(p_{ij}\) is the proportion of prey \(i\) consumed (number or volume).

Additionally, we performed an analysis of similarity with non-metric multidimensional scaling (NMDS, Motta-Tavares et al., 2016) using Euclidean distance to compare the presence of consumed prey across populations. We used analysis of variance (ANOVA) to test whether the number and volume of ingested prey were different across populations (Motta-Tavares et al., 2016). All statistical analyses were performed in R 3.1.2 (R Development Core Team, 2016), using \(P < 0.05\) as the significance level.

### RESULTS

In all environments, *P. luteolus* were found inhabiting tanks of soil bromeliads in open areas (without vegetation cover). We evaluated the stomach contents of 92 individuals: 32 from sandy coastal plains, 32 from lowland forest and 28 from the island. Three of the individuals collected in the lowland forest did not have any stomach contents. We identified a total of 397 consumed prey items belonging to 12 taxa (Table 1). Ants were the most important prey category in the diet of *P. luteolus* in populations sampled, representing the highest number, frequency and volume relative to the other prey categories (Table 1). However, some consumed prey items were exclusive to a single environment (Table 1 and Fig. 2).

The number of prey items consumed by *P. luteolus* in the lowland forest (mean = 7.65 ± 2.18 SE), sandy coastal plains (mean = 4.69 ± 1.56), and on the island (mean = 2.59 ± 0.57 SE) did not differ (ANOVA, \(F_{2,71} = 2.22, P = 0.11\)), which was confirmed by the high niche overlap between populations (Table 2). However, the volume of consumed prey in the lowland forest (mean = 21.51 ± 5.28 SE) differed from that of the san-

**Figure 1.** Study sites in Espírito Santo State, southeastern Brazil. Dark gray points represent the sampled *Phyllodytes luteolus* populations.
Table 1. Prey ingested by *P. luteolus* in three environments in Espírito Santo State, southeastern Brazil. Number of ingested prey items in all stomachs (N), frequency of prey items in all stomachs (F), volume of prey ingested per category (V) and relative importance index (I).

| Taxon                              | N (%) | F (%) | V (%) | I (%) |
|------------------------------------|-------|-------|-------|-------|
| Sandy Coastal Plains               |       |       |       |       |
| Chilopoda                          | 2 (1.85) | 2 (7.14) | 6.6 (3.86) | 3.5 (3.4) |
| Coleoptera                         | 2 (1.85) | 2 (7.14) | 9.8 (5.67) | 4.6 (4.4) |
| Hymenoptera Formicidae             | 92 (85.18) | 20 (71.43) | 141.8 (82.14) | 84.6 (82.4) |
| Coleoptera                         | 3 (2.78) | 1 (3.57) | 1.9 (1.13) | 1.9 (1.8) |
| Isoptera                           | 7 (6.48) | 1 (3.57) | 7.8 (4.54) | 5.2 (5.3) |
| Larvae                             | 2 (1.85) | 2 (7.14) | 4.5 (2.65) | 2.8 (2.7) |
| Lowland Forest                     |       |       |       |       |
| Araneae                            | 3 (1) | 3 (9) | 17.3 (3) | 7.7 (2.58) |
| Coleoptera                         | 6 (3) | 5 (16) | 25 (4) | 12 (4) |
| Diptera                            | 4 (2) | 2 (6) | 10 (2) | 5.3 (1.7) |
| Hymenoptera Formicidae             | 196 (87) | 27 (84) | 357.5 (58) | 193.5 (64.9) |
| Hymenoptera non-Formicidae         | 11 (5) | 7 (22) | 73.7 (12) | 30.5 (10.2) |
| Hemiptera                          | 4 (2) | 3 (9) | 20.9 (3) | 9.3 (3.1) |
| Odonata                            | 1 (0) | 1 (3) | 117 (19) | 39.6 (13.2) |
| Island                             |       |       |       |       |
| Coleoptera                         | 2 (3.08) | 2 (6.67) | 2.6 (0.95) | 2.2 (1.8) |
| Diptera                            | 4 (6.15) | 4 (13.33) | 8.6 (3.11) | 5.53 (4.5) |
| Homoptera                          | 1 (1.54) | 1 (3.33) | 9.4 (3.39) | 3.8 (3.1) |
| Hymenoptera Formicidae             | 56 (86.15) | 21 (70.00) | 253 (90.49) | 110 (90.5) |

Table 2. Diet overlap index between populations of *P. luteolus* on the island and in mainland ecosystems in Espírito Santo State, southeastern Brazil.

| Environments relations     | Prey number | Prey volume |
|----------------------------|-------------|-------------|
| Sandy Coastal Plains x Island | 0.71         | 0.74         |
| Lowland Forest x Island     | 0.75         | 0.52         |
| Sandy Coastal Plains x Lowland Forest | 0.74         | 0.47         |

dy coastal plains (mean = 7.75 ± 2.91 SE) and the island (mean = 14.08 ± 1.99 SE), presenting smaller niche overlap in relation to those two areas (ANOVA: F2,71 = 3.03, P = 0.05, Tukey HSD: P = 0.04) (Table 2).

**DISCUSSION**

The diet of *P. luteolus* was composed exclusively of arthropods, and ants were the most consumed prey in number, frequency and volume in environments sampled. The high importance of ants in the diet of *P. luteolus* was also observed in other sandy coastal plains in Espírito Santo State (Ferreira et al., 2012; Motta-Tavares et al., 2016) and Bahia (Motta-Tavares et al., 2016; Solé & Loebmann, 2017). Because of the high consumption of ants and termites, *P. luteolus* has been considered to be strongly associated with bromeliads (Lima & Moreira, 1993; Mestre et al., 2001; Juncá & Borges, 2002; Sepka, 2008). These findings corroborate other studies that have suggested that *P. luteolus* rarely leaves the bromeliads to feed (Peixoto, 1995; Ferreira et al., 2012; Motta-Tavares et al., 2016).

Although differences probably reflect the available prey in each environment, all consumed prey items were strongly associated with bromeliads (Lima & Moreira, 1993; Mestre et al., 2001; Juncá & Borges, 2002; Sepka, 2008). These findings corroborate other studies that have suggested that *P. luteolus* rarely leaves the bromeliads to feed (Peixoto, 1995; Ferreira et al., 2012; Motta-Tavares et al., 2016).

We found that in the lowland forest, *P. luteolus* consumed a larger volume of prey and had a lower niche overlap compared to sandy coastal plains and the island. This variability is probably a consequence of the distance between locations and vegetation dissimilarities, differences that are reflected in prey composition (Maia-Carneiro et al., 2013). The sandy coastal plain and lowland forest are very distant to each other (ca. 160 km) and have dissimilar vegetation, whereas the island is nearby the sandy coastal plain (ca. 3 km) and has similar vegetation (Rizzini, 1979; Mageski et al., 2015). Similar patterns were
found in a study comparing *P. luteolus* diet in three sandy coastal plains of southeastern Brazil (Guarapari, Guriri and Prado), which showed that the major differences in consumed prey volume were found between the more distant populations (Motta-Tavares et al., 2016). Another study that compared the diet of *Rhinella ornata* across environments showed no difference in consumed prey volume between similar environments (Maia-Carneiro et al., 2013). Thus, both greater distance and vegetation dissimilarities may influence the variability of prey composition between environments, and that is reflected in the reported differences in consumed prey.

Our work contributes to the understanding of the patterns and variations underlying the diet of *P. luteolus* inhabiting environments outside of sandy coastal plains. We suggest that ants may be the most important component of the diet of *P. luteolus*, due to their high proportion in the frog’s diet, as was observed in the environments studied here. Therefore, it is necessary to perform comparative studies between consumed and available prey items in bromeliads using the electivity index, as proposed by Jacobs (1974), to determine whether the observed dietary preferences of this bromeliad frog truly exist or are merely an artifact of prey availability.

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