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## Diet Seasonality and Feeding Preferences of *Brachycephalus pitanga* (Anura: Brachycephalidae)

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**ABSTRACT.**—Miniature toadlet species of the genus *Brachycephalus* are endemic to the Atlantic rain forest of Brazil, and little is known about their biology and ecology. We present data on the diet of *Brachycephalus pitanga* in both rainy and dry seasons and compare the diet to the prey available in leaf litter. We analyzed the stomach contents of 30 specimens: 20 of them collected in the rainy season and 10 in the dry season. Two hundred fifty-nine prey were identified, including spiders, mites, collembolans, ants, hemipterans, dipterans, and coleopterans. In the leaf litter, we identified 1,056 potential prey—676 in the rainy season and 380 in the dry season—belonging to 11 categories, with mites, collembolans, and ants being the most common potential prey. Toadlets consumed a larger number of prey but less volume and less taxonomic diversity in dry season compared to the rainy season. In the rainy season, females ate a larger number of items and a greater diversity than males, but their niche breadths were very similar. The prey items ingested showed greater similarity to the available items in the dry season, with mites and larvae being consumed in greater proportions than expected. Stomach contents collected during the rainy season suggested a preference for collembolans and spiders. Our results represent the first information on the diet of *B. pitanga* and are compared to data available for related species.

**RESUMO.**—As espécies de anuros miniaturizados do gênero *Brachycephalus* são endêmicas da Mata Atlântica do Brasil, e pouco se conhece da sua biologia e ecologia. No presente trabalho nós apresentamos dados sobre a dieta de *Brachycephalus pitanga* nas estações seca e chuvosa e os comparamos à disponibilidade de presas na serapilheira. Analisamos os conteúdos estomacais de 30 espécimes, 20 deles coletados na estação chuvosa e 10 na estação seca. Duzentos e cinquenta e nove itens foram identificados, incluindo aranhas, ácaros, colêmbolos, formigas, hemípteros, dípteros e coleópteros. Na serapilheira nós identificamos 1,056 itens—676 na estação chuvosa e 380 na estação seca—pertencentes a 11 categorias, sendo ácaros, colêmbolos e formigas os itens mais frequentes. Durante a estação seca os indivíduos consumiram um maior número de presas, mas em menor volume e diversidade. Na estação chuvosa, os indivíduos consumiram um menor número de presas, mas em maior volume e diversidade. Na estação chuvosa, as fêmeas consumiram um maior número de itens e em maior diversidade em comparação aos machos, mas a amplitude de nicho dos dois sexos foi similar. A ingestão de presas mostrou uma grande similaridade com os itens disponíveis na estação seca, sendo ácaros e larvas as categorias consumidas em maior proporção que o esperado. Na estação chuvosa, os indivíduos exibiram uma preferência por colêmbolos e aranhas. Nossos resultados são as primeiras informações sobre a dieta de *Brachycephalus pitanga* e foram comparados com os dados disponíveis para outras espécies próximas.

The composition of anuran diet is influenced by the size, mobility, availability, and abundance of prey in the environment, and anuran species can modify their diet according to season, sex, or age (Toft, 1980; Donnelly, 1991; Löw and Török 1998; Dietl et al., 2009). Such differences, both interspecific and intraspecific, play a role in anuran community structure (Toft, 1980). The selectivity in prey consumption and its variations can provide clues about species' foraging behavior. There are two main types of foraging tactics: ambushing, by which predators rely most on their prey's mobility to initiate encounters; and active searching, by which predators continually move to locate prey, with a continuum between the two types (MacArthur and Pianka, 1966; Huey and Pianka, 1981; Greene, 1986). Active foragers feed more on small, slow prey, especially prey that occur in social aggregations, such as ants or termites, whereas ambush foragers typically feed on big and more mobile prey (Dietl et al., 2009).

A relationship between mouth size and prey size was found previously in other anurans and was expected because most anurans eat prey whole (Toft, 1980; Lima, 1998; Martins et al., 2010). Such a relationship can be explained by the tendency to optimize foraging energy (Löw and Török, 1988). From another perspective, Toft (1980) reported that ant specialists feed on smaller prey types than would be expected for their sizes, whereas generalists eat bigger prey.

Although studies of anuran diet are common (e.g., Toft, 1981; Giaretta et al., 1998; Dietl et al., 2009; Martins et al., 2010), there

are few studies of diet for species in the genus *Brachycephalus*. We found published data for *Brachycephalus didactylus* (Almeida-Santos et al., 2011), *Brachycephalus garbeanus* (Dorigo et al., 2012), *Brachycephalus brunneus* (Fontoura et al., 2011), *Brachycephalus ephippium* (Pombal, 1992), and *Brachycephalus pernix* (Wistuba, 1998). Considering the number of prey, the most consumed items for these species were mites, collembolans, and ants. Fontoura et al. (2011), Pombal (1992), and Wistuba (1998) also present data on prey availability, which provide a basis from which to infer whether our target species is a generalist or whether it prefers some sort of prey.

There is a lack of information on the diet of *Brachycephalus pitanga*; therefore, we aimed to answer the following questions: 1) Is prey size correlated with frog jaw width? 2) Does the proportion of consumed prey reflect the availability of prey? 3) Are there seasonal variations in diet and prey preference for type, size, and quantity of ingested items? 4) Are there differences in the diets of males and females?

### MATERIALS AND METHODS

We conducted field studies in an Atlantic forest area at the Núcleo Santa Virgínia (NSV), Parque Estadual da Serra do Mar, São Luiz do Paraitinga, São Paulo State, southeastern Brazil (23°21'09.57"S, 45°07'58.21"W). For the stomach-content analysis, we used 30 frogs: 10 adult specimens collected in the dry season (July 2011) and 20 specimens collected in the rainy season (November 2011). Abundance of toadlets is high at NSV, such that specimens were collected in a 4-m<sup>2</sup> area within each collection period. We collected the toadlets at the same area in

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TABLE 1. Stomach contents of *Brachycephalus pitanga*. *N* = Number, *V* = Volume (mm<sup>3</sup>), *F* = Observed frequency, and *Ix* = Importance index. Percentages are in parentheses. Núcleo Santa Virgínia, São Luiz do Paraitinga, São Paulo, Brazil. \*Remains of the prey could not be measured.

Items	Dry season ( <i>N</i> = 10)				Rainy season ( <i>N</i> = 20)			
	<i>N</i> (%)	<i>V</i> (%)	<i>F</i> (%)	<i>Ix</i>	<i>N</i> (%)	<i>V</i> (%)	<i>F</i> (%)	<i>Ix</i>
Arachnida								
Araneae					2 (1.09)	3.4 (16.3)	2 (10)	0.09
Acari	125 (70.62)	3.1 (58.4)	10 (100)	0.76	141 (77.47)	8.9 (43.2)	17 (85)	0.68
Hexapoda								
Collembola	40 (22.6)	1.4 (26.4)	8 (80)	0.43	23 (12.63)	4.9 (23.7)	8 (40)	0.25
Hymenoptera								
Formicidae	2 (1.13)	0.08 (1.5)	1 (10)	0.04	9 (4.95)	1.2 (6.0)	7 (35)	0.15
Larvae	10 (5.54)	0.7 (13.6)	5 (50)	0.23				
Hemiptera					1 (0.55)	*	1 (5)	
Diptera					3 (1.64)	0.02 (0.09)	3 (15)	0.05
Coleoptera					3 (1.64)	2.2 (10.6)	2 (10)	0.07
Total	177 (100)	5.28 (100)			182 (100)	20.62 (100)		

both seasons. When nonreproductive, gonads of both sexes are difficult to differentiate such that in the dry season, we were unable to identify the toadlets as male or female. Thus, our information for the dry season is not sex specific. However, in the rainy season, it was possible to identify the sexes because eggs were visible in the abdomens of the females. We collected 10 males and 10 females in the rainy season and used a *t*-test to compare the quantity and size of prey items in their diets.

We could not use stomach flushing because of the small size of the toadlets (Solé and Pelz, 2007). Therefore, we anesthetized specimens in 2% xylocain and immediately fixed and kept them in 10% formalin for 24 h, transferring them later to a 70% ethanol solution. Specimens were deposited in Coleção de Anfíbios Célio F. B. Haddad (CFBH), at Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil. While viewing the specimens under a stereomicroscope, we dissected the frogs and removed their stomachs. Males and females were distinguished in wet season, and we measured their snout-vent length (SVL) and jaw width (JW) (measured as the width of the head at the angle of the jaws sensu Toft, 1980) with a Mitutoyo digital caliper.

To assess food availability, we collected the leaf litter of a 50 × 50 cm plot in each season in the place where the toadlets were collected. The samples were screened with a Berlese-Tüllgren funnel (Rodrigues et al., 2008). We considered only items of comparable sizes to those found in the stomach (up to 2.45-mm<sup>3</sup> volume).

Both stomach contents and leaf litter items were counted, photographed, and measured with a Nikon SMZ 1500 stereomicroscope with a coupled Nikon Digital Sight DS-Fi1 camera. We identified the stomach contents to the lowest taxonomic level possible, according to Johnson and Triplehorn (2004). We estimated prey volume using the following formula for ellipsoid bodies (Griffiths and Mylotte, 1987):  $V = 4/3 \times \pi \times (1/2) \times (w/2)^2 \times l$ , where *l* = prey length and *w* = prey width. The relative importance index was calculated with the formula  $I = (F\% + N\% + V\%)/3$ , where *F*% = frequency of occurrence, *N*% = numeric percentage and *V*% = volumetric percentage (Biaavati et al., 2004).

We verified seasonal and sex differences with a student *t*-test or *z*-test. All analyses followed Zar (1984) and were performed with Statistica 7.0, with  $P \leq 0.05$  being considered significant.

The Hulbert niche breadth was calculated according to Krebs (1998) using the following formula:  $B' = 1/\sum(p_j^2/a_j)$ , where, *p<sub>j</sub>* = the proportion of item *j* consumed by the toadlets and *a<sub>j</sub>* = the

proportion of item *j* available in the environment. To calculate the niche breadth overlap between males and females, we used the percentage overlap formula:  $P_{ij} = [\sum(\text{minimum } p_{ij}, p_{jk})]/100$ , where *p<sub>ij</sub>* = the proportion that resource *i* is of the total resources used by males, *p<sub>jk</sub>* = the proportion that resource *j* is of the total resources used by females and *N* = total number of resources states (Krebs, 1989). The proportion of each prey category in the stomach contents was related to the leaf litter items with a forage ratio test using the formula  $FR = r_i/p_i$ , where *r<sub>i</sub>* = the proportion of prey *i* in the stomach contents and *p<sub>i</sub>* = the proportion of prey *i* in the environment and log-transforming the results. The forage ratio values (log *FR*) range from negative infinite to 0 for negative selection and from 0 to positive infinite for positive selection (Jacobs, 1974). The confidence interval was calculated using the formula proposed by Strauss (1979).

## RESULTS

**Stomach Contents.**—Toadlets measured 8.75–13.91 mm in SVL ( $x \pm SD = 11.75 \pm 1.53$  mm), and the jaw width ranged from 2.51–3.77 mm ( $x \pm SD = 3.30 \pm 0.37$ ). These two measurements were correlated positively ( $P < 0.0001$ ;  $r^2 = 0.78$ ; *df* = 28; *N* = 30). We identified 259 different items in the stomach contents. For each toadlet, we found an average of  $12.4 \pm 9.8$  items, with  $18.2 \pm 10.31$  in the dry season and  $9.5 \pm 8.38$  in the rainy season. Food items belonged to eight categories: four of which were found in the dry season (Acari, Collembola, Formicidae, and larvae) and seven in the rainy season (Araneae, Acari, Collembola, Formicidae, Hemiptera, Diptera, and Coleoptera) (Table 1). All larvae were considered together regardless of their taxonomic group. In the dry season, the mean prey size was  $0.58 \pm 1.59$  mm (range 0.2–3.75 mm; *N* = 177), and in the rainy season, it was  $0.86 \pm 1.54$  mm (range 0.4–3.34 mm; *N* = 182). Prey size and prey volume were correlated ( $P < 0.001$ ;  $r^2 = 0.43$ ; *df* = 336). Specimens collected in the rainy season ate larger prey than those collected in the dry season (*t*-test:  $P < 0.001$ ; *df* = 335; *t* = −4.13). Although prey volume did not correlate significantly with jaw width in either season, in the dry season, there was a stronger tendency ( $P = 0.07$ ;  $r^2 = 0.33$ ; *df* = 8; *N* = 10) than in the rainy season ( $P = 0.48$ ;  $r^2 = 0.03$ ; *df* = 16; *N* = 18).

**Sex Differences in Prey Consumption.**—Of the 20 specimens collected in the rainy season, only one male had an empty stomach. We identified 67 items for the males and 115 for females; males consumed fewer prey categories than did females (five and seven, respectively). We found no significant differences

TABLE 2. Small animals obtained using a Berlese-Tüllgren funnel from two litter samples collected where the *Brachycephalus pitanga* toadlets were captured. N = Number, V = Volume (mm<sup>3</sup>), percentages are in parentheses. Núcleo Santa Virgínia, São Luiz do Paraitinga, São Paulo, Brazil.

Item	Dry season		Rainy season	
	N (%)	V (%)	N (%)	V (%)
Arachnida				
Araneae	1 (0.26)	0.01 (0.03)	5 (0.73)	0.1 (0.5)
Acari	195 (51.72)	5.6 (17.7)	518 (76.62)	6.8 (32.7)
Pseudoscorpiones			4 (0.59)	0.1 (0.5)
Hexapoda				
Collembola	120 (31.83)	3.1 (9.7)	71 (10.5)	2.8 (13.4)
Hymenoptera				
Formicidae	44 (11.67)	15.3 (48.2)	58 (8.57)	8.7 (41.8)
Larvae	3 (0.79)	2.3 (7.2)	1 (0.14)	0.4 (1.9)
Hemiptera	1 (0.26)	0.05 (0.1)		0.9 (4.32)
Diptera	2 (0.53)	0.35 (1.1)		
Coleoptera	7 (1.85)	2 (6.3)	12 (1.77)	0.6 (2.9)
Thysanoptera	2 (0.53)	0.1 (0.3)	6 (0.88)	0.1 (0.5)
Orthoptera			1 (0.14)	0.3 (1.4)
Miriapoda				
Chilopoda	2 (0.53)	0.4 (1.2)	0	
Total	377 (100)	31.73 (100)	676 (100)	20.8 (100)

between the volumes for the categories of prey common to males and females ( $P = 0.37$ ;  $df = 182$ ;  $t = 0.88$ ). Additionally, males and females showed a high percentage of niche overlap (94%).

**Leaf-Litter Arthropods.**—We identified 1,056 items in leaf litter: 380 in the dry season and 676 in the rainy season. Regardless of the season, the most common items were mites, collembolans, and ants (Table 2). We found differences in the proportions of items between seasons for mites, collembolans, flies, and chilopods (z-test,  $P < 0.05$ ,  $df = 4$ ); only mites and ants were present in greater proportions in the rainy season. The items' sizes (dry season:  $0.922 \pm 0.943$  mm, 0.14–4.616 mm; wet season:  $0.827 \pm 0.712$  mm, 0.22–3.23 mm), as well as their volumes (dry season:  $0.101 \pm 0.166$  mm<sup>3</sup>, 0.0004–0.832 mm<sup>3</sup>; wet season:  $0.098 \pm 0.146$  mm<sup>3</sup>, 0.001–0.574 mm<sup>3</sup>), differed between seasons (size:  $P < 0.0001$ ;  $df = 1,056$ ;  $t = 9.51$ ; volume:  $P < 0.0001$ ;  $df = 1,056$ ;  $t = 7.50$ ), with the larger items being found in the dry season.

**Selectivity of Prey.**—We calculated the proportion and volume of consumed and available items for both seasons (Fig. 1). The values for niche breadth were higher in the rainy season ( $B' = 0.96$ ) than in the dry season ( $B' = 0.65$ ). The forage ratio test showed differences between dry and rainy seasons (Table 3). Thus, despite the importance of mites in the diet, in the rainy season toadlets ate them in the same proportion in which they were found in the environment. Ants were avoided in both seasons. Collembolans were avoided in the dry season and slightly preferred in the rainy season. We could not reach any conclusions about the consumption of spiders, beetles, or larvae because of the large confidence intervals, probably attributable to the small number of these items in the stomachs.

#### DISCUSSION

Prey observed in the stomachs of *B. pitanga* (mites, collembolans, and ants) were almost the same as reported for other *Brachycephalus* species: *B. brunneus* (Fontoura et al., 2011), *B. didactylus* (Almeida-Santos et al., 2011), *B. ephippium* (Pombal, 1992), and *B. pernix* (Wistuba, 1998). A preference for mites, followed by collembolans, was found for *B. pitanga* and also in *B. brunneus* and *B. pernix*. However, for *B. didactylus* and *B. ephippium*, the preference was collembolans followed by mites. For *B. garbeanus*, mites and ants were the most consumed items (Dorigo et al., 2012).

Mites were the prey with the highest levels of relative importance and were consumed in higher proportion than their occurrence in the environment during the dry season. The importance of mites in the *Brachycephalus* diet seems to be related to the small size of both predator and prey, as observed by Simon and Toft (1991), who found a negative correlation between frog size and the quantity of small prey in their diets. Although we have an indication of the preferred prey of *B. pitanga*, our methods for sampling the leaf litter arthropods do not allow us to rule out the possibility that toadlet behavior or the microhabitats used by some prey influenced our results.

Toft (1981) classified frogs in two major categories according to their diets: "ant specialists" and "non-ant specialists." In the family Brachycephalidae, *Ischnocnema henselii* was classified by Dietl et al. (2009) as a preferential ant eater but not a real specialist. Among the genus *Brachycephalus*, we found different patterns in ant consumption. *Brachycephalus brunneus* ate ants in the same proportion as the ants were found in environment (Fontoura et al., 2011). For *B. garbeanus*, ants were one of the most important items in the diet (Dorigo et al., 2012). However, *B. ephippium* (Pombal, 1992) and *B. pernix* (Wistuba, 1998) avoided ants, similar to what we observed in *B. pitanga*.

The niche breadth values were higher in the rainy season than in the dry season, which indicates that in the rainy season toadlets consume a more generalist diet. Differences in prey consumption between seasons has been reported for anurans and may be related to differences in the seasonal variability in resource abundance (Duellman and Trueb, 1986; Santos et al., 2004; Miranda et al., 2006) or even differences in the behavior. For example, individuals of *B. pitanga* are frequently seen on the leaf litter during the rainy season, but they are rarely seen exposed in the dry season, being observed instead amid the leaf litter where the prey species are different from those on the surface (EGO, pers. obs.). For *B. pernix* (Wistuba, 1998) and *B. garbeanus* (Dorigo et al., 2012), there was no such difference, and the niche breadth values were similar for both seasons. We found fewer stomach items in the rainy season, the opposite of what Dorigo et al. (2012) reported for *B. garbeanus* and Wistuba (1998) reported for *B. pernix*. Our observations suggest that in the rainy season, in addition to foraging, toadlets are engaged in reproduction. We suppose that the time and energy spent

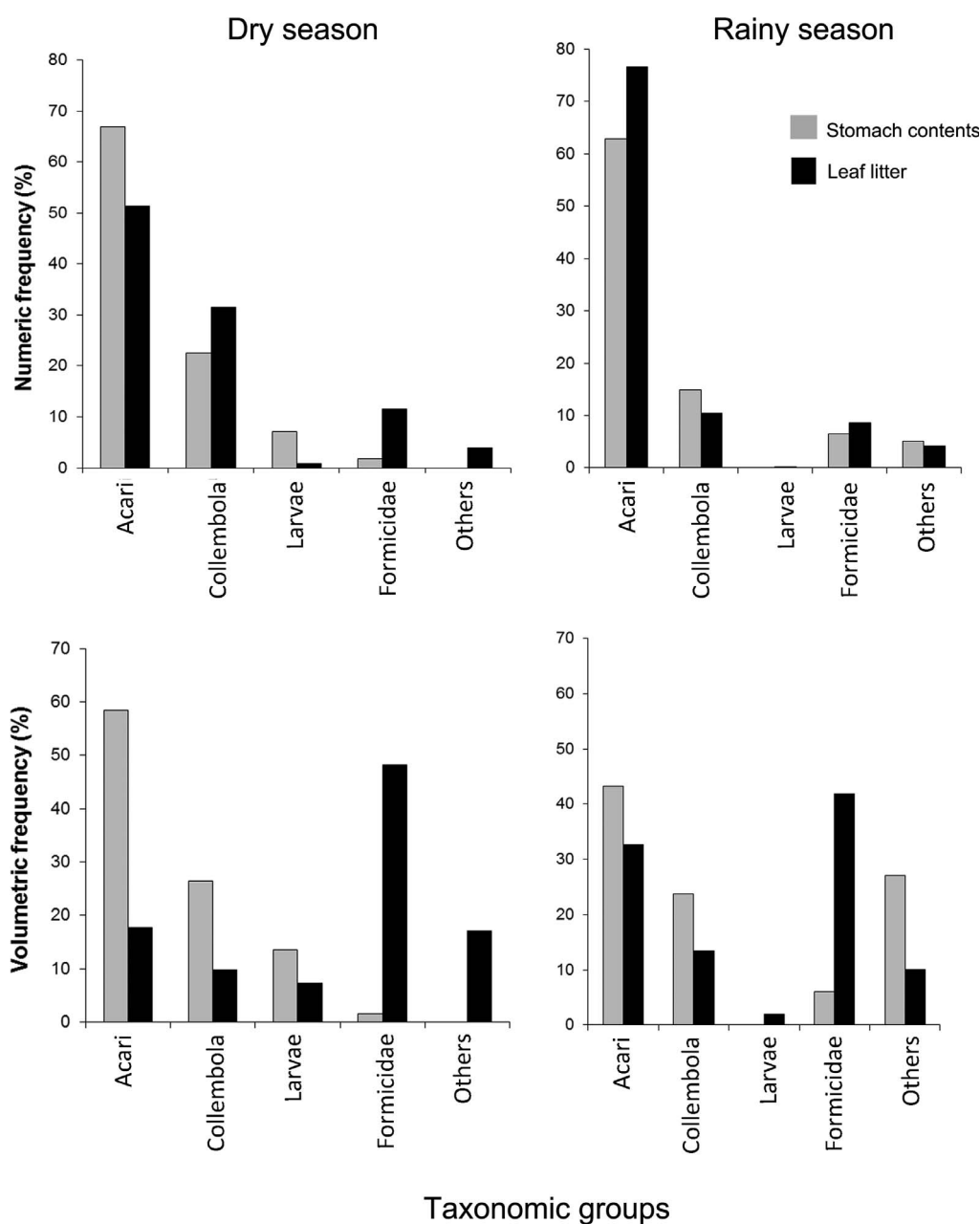


FIG. 1. Proportions for the number and volume of items found in the stomach contents of *Brachycephalus pitanga* (gray bars) and leaf litter (black bars). Núcleo Santa Virgínia, São Luiz do Paraitinga, São Paulo, Brazil. The category "others" refers to all remaining items.

reproducing is compensated for by the ingestion of larger prey in the rainy season, relative to the dry season.

We observed that in the dry season, the jaw width tends to explain the variation in maximum prey size, whereas in the

rainy season, there was no correlation. Such a correlation was observed previously for other species of different genera (e.g., Toft, 1980; Lima, 1998; Löw and Török, 1998; Martins et al., 2010; Forti et al., 2011) and may indicate selection on prey size.

Our data suggest that female *B. pitanga* fed on a higher number and diversity of prey in the rainy season than did males. However, in terms of the proportion of consumed prey, there was no difference between males and females, which was demonstrated by a high niche overlap. Such result was reported previously by Dorigo et al. (2012) for *B. garbeanus*. Differences in the diets of males and females have been observed previously in other species and might be related to dimorphism in size (Camilleri and Shine, 1990) or to the behavioral dimorphism between the sexes (e.g., Donnelly, 1991; Miranda et al., 2006).

Solé and Pelz (2007), studying hylids, observed that the shorter the reproductive period, the less that males will feed

TABLE 3. Forage ratio of the *Brachycephalus pitanga* diet for numeric values. Confidence intervals are in parentheses. Núcleo Santa Virgínia, São Luiz do Paraitinga, São Paulo, Brazil.

	Dry season	Rainy season
Acari	0.13 (−0.05–0.33)	0 (−0.03–0.04)
Collembola	−0.14 (−0.1–0.12)	0.08 (−0.17–0.33)
Larvae	0.85 (−0.38–2.09)	–
Formicidae	−1.01 (−1.46–0.55)	−0.23 (−0.20–0.27)
Araneae	–	0.17 (−0.57–0.92)
Coleoptera	–	−0.03 (−0.47–0.41)

during this period. We observed males of *B. pitanga* feeding and reproducing in the same area and period, but our data on number of ingested prey suggest that they feed less frequently during reproductive activity. Furthermore, at the end of reproductive period, male *B. pitanga* were visibly thinner (EGO, pers. obs.). The loss of mass might also be related to the high energetic cost of reproductive activities in male anurans, such as vocalizations and physical combat (Wells, 2007). Reduced feeding and energetic costs likely explain our observations of thinner toadlets although our data are limited to demonstrate this relationship.

The mobility of the consumed prey and our field observations allow us to infer that *B. pitanga* is an active forager. The higher presence of mites in the diet of *B. pitanga*, which are animals of low mobility, may indicate an active foraging behavior, similar to what was previously observed for *B. pernix* (Wistuba, 1998; Dietl et al., 2009). Our observations also support active foraging (MacArthur and Pianka, 1966) by these toadlets because we observed the toadlets moving through the leaf litter with their heads tipped toward the ground as they searched for prey.

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