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Feeding pattern and use of reproductive habitat of the Striped toad *Rhinella crucifer* (Anura: Bufonidae) from Southeastern Brazil

Rodrigo B. Ferreira, Rogério L. Teixeira

Museu de Biologia Prof. Mello Leitão, Av. José Ruschi, 4, Centro, 29650-000, Santa Teresa-ES – Brazil. Corresponding author. E-mail: rodrigoecologia@yahoo.com.br

Abstract. Diet composition, foraging mode, and using of reproductive habitat of *Rhinella crucifer* was studied in an artificial pond in Espírito Santo, Brazil. The favored substrate was leaf litter, followed by Cyperaceae/Poaceae. Calling sites, preferred for 23.3 % (n = 7) of the observed toads, were within the water, with only the head not submerged. We analyzed a total of 61 specimens, mainly males (98.5% male and 1.5% female). Seven categories of prey were found in the stomach contents: Coleoptera, Hymenoptera (Formicidae), Isoptera, Lepidoptera, Orthoptera, Gastropoda (Mollusca), Opilionida (Arachnida). Our studies indicate that the diet of *Rhinella crucifer* consists mainly of terrestrial colonial arthropods. Formicidae was the predominant food item in frequency of occurrence, number of prey and weight. Isoptera and Coleoptera were also relevant in terms of weight. Neither large ontogenetic dietary nor seasonal shifts were observed in the population studied. Our results suggest that no intraspecific food resource partitioning occurs in adult or juveniles. *Rhinella crucifer* adults avoid competition inhabiting different home range habitats and seem to be ant-specialist with a wide foraging mode.

Keywords. Amphibian, colonial arthropods, feed, foraging mode, Neotropics.

INTRODUCTION

Rhinella crucifer is found from the northern State of Ceará to the State of Rio de Janeiro, Brazil (Baldissera et al., 2004; Marques et al., 2006), inhabiting both rainforest and disturbed habitats (Aquino, 2004). It is the most common toad in some states of Brazil (Aquino, 2004) whose biology has largely been studied (Haddad et al., 1990; Baldissera et al., 2004; Sabagh and Carvalho-e-Silva, 2008). Many biological aspects, however, remain unstudied. For example, not much is known about the diet, foraging modes and use of reproductive habitat of *R. crucifer*. These studies are particularly useful in providing practical interpretations of species-specific behavioral observations.

Toft (1981) and Strüssmann et al. (1984) found a positive correlation between diet and foraging mode for anurans. Foraging tactics are the central subject of modern behav-

ioral ecology. Predator animals are known to efficiently capture prey, thus maximizing energy under any environmental conditions (e.g. Krebs and Davies, 1997). Bufonidae have been classified as ant-specialists for most of the authors (Toft, 1980; Flowers and Graves, 1995; Isacch and Barg, 2002; Rosa et al., 2002), although other authors have preferred to classify them as generalists (Smith and Bragg, 1949; Evans and Lampo, 1996; Grant, 1996; Sabagh and Carvalho-e-Silva, 2008).

The use of reproductive habitats by Anurans tends to be under specific conditions. Anurans differ in habitat use for breeding, calling site, annual reproductive period, daily period of calling activity, and acoustic features of advertisement call, which are interpreted as important isolating mechanisms (e.g. Wells, 1977; Haddad et al., 1990). Even so, *Rhinella* has been often related to natural interspecific hybridization (Feeder, 1979; Sullivan, 1986; Haddad et al., 1990).

The purpose of this study is to describe the dietary composition, foraging mode and analyze the use of reproductive habitat of the Striped toad, *R. crucifer*.

MATERIAL AND METHODS

Study area

The studied area was Barragem Norte (20°45.580'S, 40°34.250'W, 8 m a.s.l.), situated in the city of Anchieta, State of Espírito Santo, southeastern Brazil. Barragem Norte is an artificial lagoon that is covered by extensive vegetation, both inside the lagoon and along its margins. Vegetation in the area consisted primarily of *Typha* aff. *domingensis* (Thyphaceae), *Eleocharis* sp. (Cyperaceae), *Nymphaea* sp. (Nymphaeaceae), *Lagenocarpus* aff. *rigidus* (Cyperaceae), and species of Poaceae. The selected study area was approximately 1200 m^2.

According to Köppen-Geiger Climate Classification (1936), the Anchieta's climate belongs to *Aw Tropical* type, with high temperatures, rainy summer (December, January, February, and March), and dry winter (June, July, August and September). After 35 years (1971 to 2006) of assessment, the Weather station defined an average rainfall of 1000-1150 mm/year and temperature of 23.5 °C (Incaper, 2006). The region still has a particular dynamic constrained by the air currents *Tropical Atlântica*, which is hot and wet, and the *Polar Atlântica* which is dry and cold, acting mainly in the winter season.

Samples

Striped toad collections were carried out from November 1999 to September 2000 in Barragem Norte, with one field visiting each month. Specimens were collected manually along random transects of about 100 meters, and along the marginal portions of the ponds, by two people spending five hours a day. Toads were sacrificed using ethanol solutions (10%) and transferred to 10% formalin for fixation. To interrupt further digestion of prey items, formalin was also injected intraperitoneally. After a week, the toads were washed and preserved in 70% ethanol.

Analyses

Toad snout vent length (SVL mm) was measured using Vernier calipers (to the nearest 0.01 mm) and weighed using a digital balance (0.1 g precision). Toads were then dissected, sex was

determined and the stomachs were extracted. Stomach contents were spread on Petri dishes and analyzed with a stereo-microscope. Prey items were identified to order level, counted, and measured; maximum length of prey was measured using Vernier calipers (to the nearest 0.01 mm). Frequency of occurrence (F), number of prey (N), and wet weight (W; 0.1 mg) were calculated in order to quantify the importance of each prey type. The frequency of occurrence was defined as the number of individuals that had determined item *i* in the stomachs, divided by the total number of sampled exemplars. Predominant hunting method was estimated by divided all prey orders found into ecological guilds, according to prey taxonomic order. The percentage of individuals found in each guild was used for comparison purposes. The relationship between length and mass of preserved specimens was calculated using type III regression analysis.

Voucher specimens were deposited in the herpetological collection of the Museu de Biologia Prof. Mello Leitão (MBML), Santa Teresa, State of Espírito Santo, Brazil (*R. crucifer*: MBML 4650-4652, 4681, 4682).

RESULTS

Rhinella crucifer was found from January through September with most individuals collected in May. Specimens were frequently collected along the pond margin and occasionally on aquatic vegetation inside the pond, during the months of November, January, March, May, July, and September. Urine release and feigning death were the primary defense mechanisms exhibited. Vocalizations and amplexus were observed only during winter months (June, July, August, and September). Detailed information about calling sites was obtained for 30 individuals. Calling sites ranged from 0 to 500 cm from the pond margin with a mean distance of 21.3 ± 263.0 cm. The calling sites above ground elevation from 0 to 10 cm height with an average of 0.3 ± 1.7 cm. Leaf litter was the favored substrate for reproductive habitats (14 specimens = 46.7%), followed by Cyperaceae/Poaceae (8 specimens = 26.7 %). Seven specimens (23.3%) of the Striped toads chose the water as calling site and one male (3.3 %) was seen calling from wood located in the water.

A total of 68 specimens were collected at Barragem Norte having SVL ranging from 54.0-105.2 mm (mean 70.05 mm ± 7.83 mm). The relationship between SVL and mass was highly significant ($R^2 = 0.92$; $P < 0.01$). The collected specimens were heavily biased toward males. Only one female was collected and it was the largest specimen. Seven categories of prey were identified among the 61 specimens of *R. crucifer* examined. Coleoptera, Hymenoptera (Formicidae), Isoptera, Lepidoptera, Orthoptera, Gastropoda (Mollusca), Opilionida (Arachnida) (Table 1). Seven specimens (4.76%) had empty stomachs. The diet of *R. crucifer* consists mainly of terrestrial, colonial arthropods that usually occur on the ground (Fig. 1). Formicidae was the most predominant items in terms of frequency, number of prey, and weight. Also Isoptera and Coleoptera were relevant in terms of weight. Other food items such as Lepidoptera larvae, Orthoptera, Gastropoda, and Opilionida were found, but not in large amounts. The diet of *R. crucifer* varied little in relation to SVL classes. The three most important food items (Formicidae, Coleoptera, and Isoptera) occurred at high proportions in almost all SVL classes. In the smallest SVL class (50.0-59.9 mm SVL), the proportion between Formicidae and Coleoptera was relatively the same. Formicidae had the highest proportion, except for the group with an SVL > 80.0 mm, in those classes where Isoptera was predominant in relation to weight.

Table 1. Summary of the preys found in the stomachs of *Rhinella crucifer* based on 61 specimens; F= frequency; n = number of prey; W= prey weight in mg. Legend for guilds: THS: terrestrial, hidden, in, on ground; TAC: terrestrial, active, on ground and TC: terrestrial, colonial.

Guilds		F	%F	n	%n	W	%W
Insecta							
Coleoptera	THS	17	27.9	30	6.1	2054.2	18.2
Hymenoptera (Formicidae)	TC	54	88.5	325	66.2	5802.2	51.5
Isoptera	TC	9	14.8	129	26.3	2039.2	18.1
Lepidoptera larvae	THS	1	1.6	1	0.2	484.0	4.3
Orthoptera	TAC	1	1.6	1	0.2	160.7	1.4
Insect remains		3	4.9	-	-	27.5	0.2
Mollusca							
Gastropoda	THS	2	3.3	2	0.4	584.1	5.2
Arachnida							
Opilionida	TAC	2	3.3	3	0.6	107.4	1.0
Total		-	-	491	100.0	11259.3	99.9

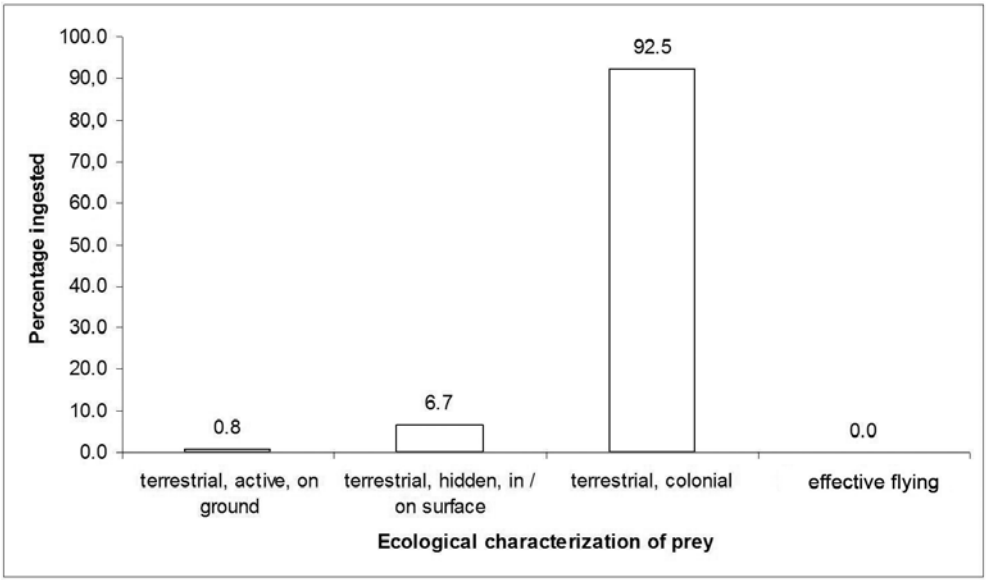

Fig. 1. Percentages of prey items according to ecological prey guilds ingested. Legend: Terrestrial, active, on ground: Orthoptera and Opilionida; Terrestrial, Hidden, in, on surface: Coleoptera, Gastropoda and Lepidoptera; Terrestrial, Colonial: Isoptera and Hymenoptera.

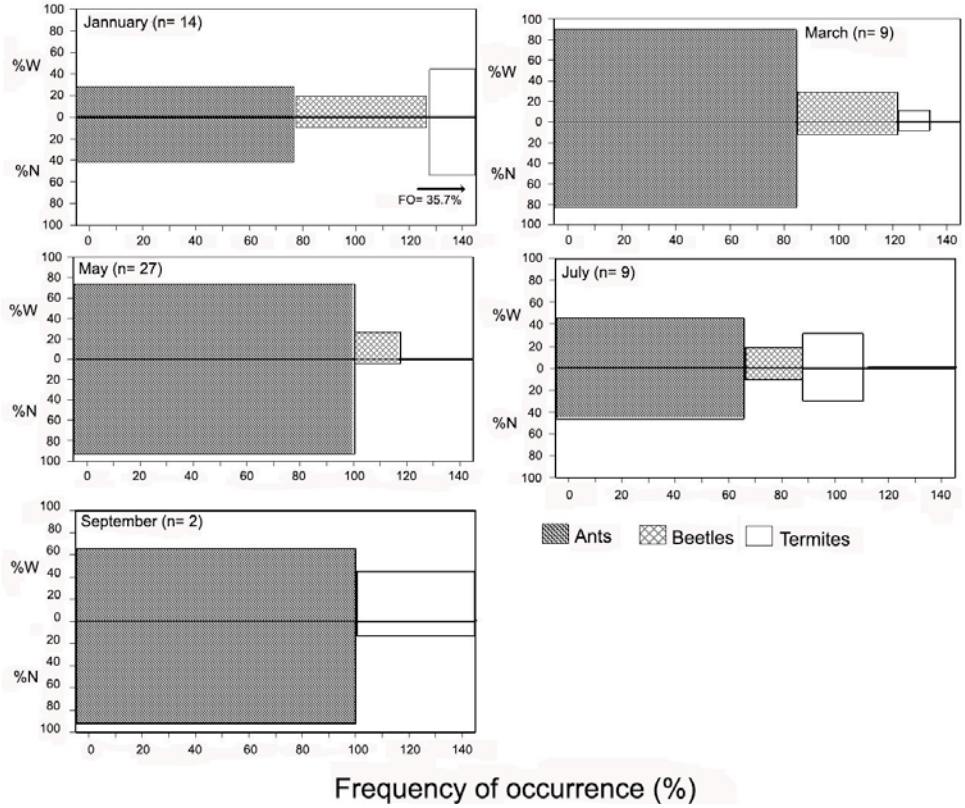

Fig. 2. Main food items found in the stomach contents of *Rhinella crucifer* according to the collected months and based on the prey wet weight (W), number (n), and the frequency. Southeastern Brazil.

Formicidae were the major prey items ingested in all months except for July, when Coleoptera and Isoptera were consumed in relatively equal proportions (Fig. 2).

DISCUSSION

Rhinella crucifer might be classified as a wide forager and an ant-specialist. The classification is justified by having slow-moving locomotion, possessing poisons in the parotid glands, preferencing for small preys, and high frequency and weight of ants founds per stomach. Sabagh and Carvalho-e-Silva (2008) considered this species as generalist since they recorded agile prey, such as cockroaches, crickets, and spiders. Sabagh and Carvalho-e-Silva (2008), however, also found a high proportion of ants within the stomach contents of Striped toad, which resulted in a high Importance relative index (IRI) for this prey.

Inclusion of mobile preys might be due to nutrients balance in toads diet (Clark, 1982), or as response to fluctuations in prey abundance (Donnely, 1991). Moreover, *R.*

crucifer is an active forager. During its locomotion and searching for colonials arthropods, it occasionally encounters and eats other types of prey.

Sabagh and Carvalho-e-Silva (2008) suggested that electivity test is necessary to confirm if *R. crucifer* is an ant-specialist. The positive electivity has been confirmed to Bufonidae by Toft (1980, 1981), Flowers and Graves (1995), and others. Damasceno (2005) found ants' positive electivity studying *Rhinella granulosa* (Spix, 1824) in the Caatinga Biome, Brazil. Isacch and Barg (2002) had with the same results for *Rhinella arenarum* (Hensel, 1867) and *Rhinella dorbignyi* (Duméril and Bibron, 1841) in the Pampas, Argentina. Thus, we consider that electivity test is not essential to state that *R. crucifer* is an ant-specialist. Furthermore, colonial arthropods compose approximately 70% of animal biomass in tropical forest (Hölldobler and Wilson, 1990), being the major food source. Clarke (1974) pointed out that Formicidae and Coleoptera are frequently present in bufonid diet species, the author also stated that could be a consequence of abundance and availability of these arthropods in the soil. Ants and several beetle groups (e.g. carabids and harpalids) are unpalatable to many predators due to formic acids and quinones, respectively (Zug and Zug, 1979). Therefore, specialization on those preys might confer certain advantages. Predators specialized in eating unpalatable preys decrease food competition with others predators. Moreover, thick skin (see Brito Gitirana and Azevedo, 2005) provides more resistance to *R. crucifer* faces ant bites and stings, allowing them to feed on these insects for longer periods (Sabagh and Carvalho-e-Silva 2008). It is possible that those advantages result from ant-specialist feeding selection. Duellman and Trueb (1994) suggested that bufonids, within anurans, would be also morphologically constrained to eat small prey (ants).

Active foraging exposes the frogs to high risks of predation, but most bufonids possesses poisons in parotids glands. Thus, they are avoided by natural predator as snakes (Lulling, 1971), and are considered toxic to birds and mammals (Tokuyama et al., 1969). Toft (1980) stated that foraging behavior and anti-predator tactics are complementary and perhaps coevolved. Ontogenetic dietary shifts are reported for many anuran species (e.g. Woolbright and Stewart, 1987; De Bruyn et al., 1996; Giaretta et al., 1998; Ferreira et al., 2007). These shifts allow for intraspecific resource partitioning which facilitates higher population densities due to less intraspecific competition. If formicids are plentiful in the environment, there is probably limited intraspecific competition. Nevertheless, our data support the absence of ontogenetic dietary shifts in *R. crucifer* as suggested also by Sabagh (2008).

Seasonal differences in diet have been reported for many amphibian species, reflecting availability of prey and seasonal differences in prey selection (Duellman and Trueb, 1994). The large amount of ants found in the diet of *R. crucifer* suggests that this prey may be available throughout the year, favoring ant-specialists habit.

Home range appears to differ among adults of *R. crucifer*, as we never observed two specimens in close proximity to each other, except during amplexus or at calling sites. This behavior may decrease potential interference in the use of the same niche.

Interactions between ecological and evolutionary mechanisms in space may enhance or diminish the potential for local coexistence (Urban and Skelly, 2006). Many species of anurans migrate to temporary ponds for breeding which increases potential for interspecific interaction (Duellman and Trueb, 1994). Seasonal occurrence of *Rhinella* has been shown in several studies. Rossa-Ferres and Jim (1994) found *R. crucifer* and *R. schnei-*

deri at the end of the cold and dry seasons in Botucatu, State of São Paulo. In the same seasons, Pombal (1997) found them in a permanent pond at the Serra de Paranapiacaba, State of São Paulo, and Bernarde and Anjos (1999) in Londrina, State of Paraná. Teixeira et al. (2007) observed both *R. crucifer* and *R. pombali* at three lagoons near Anchieta. In April and May, 2006, both species were observed syntopically calling at ponds located in Vargem Alta and Nova Lombardia, State of Espírito Santo (pers. obs.). These species are phylogenetic close and it is possible that they are able to maintain a natural mating.

The single captured *R. crucifer* female was larger than the males. Differences in sizes between sexes may be the result of sex-specific growth rates and/or sex-specific longevity (Márquez et al., 1997). Several studies have reported larger females than males for amphibians. For example Lee (2001) found adult females of *R. marina* significantly larger than adult male. However, the low number of *R. crucifer* females collected in our study does not allow inferring its analyses.

There are two possible explanations for the abundance of males to females: (i) males arrive at the pond (or the vocalization and feeding site) before the females, suggesting that the latter have a different behavior related to their forage and mating activities. If this is the case, our sampling period did not allow opportunities to encounter females. (ii) The sex-ratio obtained here actually is representative of the population. If this is the case, strong competition would be expected between males for access to females.

Several studies have indicated that bufonids are selective feeders. As previously stated, the more recent studies have reported similar results (e. g. Evans and Lampo, 1996; Moreira and Barreto, 1996; Suazo-Ortuño et al., 2007), with bufonids feeding mainly on ants or beetles. The current study asserts this pattern to *R. crucifer*, which is widely foraging and ant-specialists, feeding almost exclusively on colonial arthropods as Formicidae and Isoptera.

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