Ecological observations on the leaf-litter frog *Adenomera marmorata* in an Atlantic rainforest area of southeastern Brazil

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We analysed the diet, pattern dispersion, calling activity and microhabitat use of the leptodactylid frog *Adenomera marmorata* at an Atlantic rainforest site on Ilha Grande, in southeastern Brazil. *Adenomera marmorata* is endemic to the Brazilian Atlantic rainforest biome, occurring in the leaf litter of forests from Rio de Janeiro state to Santa Catarina state, and this is the first ecological study of the species. It has a clumped pattern of dispersion along the forest floor. Calling activity extended from dusk to dawn, although on rainy days some individuals remained active during the daylight period. The individuals collected for diet analysis were most frequently found on the leaf-litter surface and under the leaf litter of the forest floor. Of the six potential microhabitat categories we recorded, *A. marmorata* used only two (leaf litter and fallen branch). We conclude that in the Atlantic forest of Ilha Grande, *A. marmorata* possesses crepuscular–nocturnal calling activity, and is exclusively associated with the leaf litter of the forest floor where it feeds predominantly on isopods, ants and insect larvae.

Key words: Anura, calling activity, diet, dispersion pattern, Leptodactylidae, microhabitat use

INTRODUCTION

In tropical forests, amphibians and reptiles are important components of the communities of the leaf litter of the forest floor (Fauth et al., 1989; Allmon, 1991; Vitt & Caldwell, 1994, Van Sluys et al., 2001). Several factors influence the composition and abundance of leaf-litter frogs, including the depth of the leaf litter layer, humidity and degree of anthropic disturbance (Scott, 1976; Lieberman, 1986; Heinen, 1992). Amphibians, in general, are carnivorous and feed mainly on arthropods (Toft, 1980a,b; Duellman & Trueb, 1986). They are usually considered as opportunistic predators, for which the prey consumed reflect the availability of food items of appropriate size in the environment (Duellman & Trueb, 1986, Lima & Moreira, 1993). According to Toft (1980a), three main feeding strategies are recognized among anurans: ant-specialists, non-ant specialists and generalists. Leptodactylids, according to Toft (1980a,b, 1985), are assigned to the non-ant specialists guild.

The genus *Adenomera* Steindachner, 1867 (Leptodactylidae) is found only in cisandean South America and currently contains eight nominal species of small terrestrial frogs (Frost, 2004). One of these species, *Adenomera marmorata*, is endemic to the Brazilian Atlantic rainforest biome, occurring in the forests from the states of Rio de Janeiro to Santa Catarina (Izecksohn & Carvalho-e-Silva, 2001; Frost, 2004). These frogs are commonly found in the leaf litter of forested areas, but can also be found in urbanized areas (Heyer et al., 1990; Izecksohn & Carvalho-e-Silva, 2001; pers. obs. by the authors). Despite the relatively wide distribution of the species and its relatively high abundance in the areas of ocurrence (Heyer et al., 1990; Izecksohn & Carvalho-e-Silva, 2001), detailed information about most aspects of the ecology of *A. marmorata* is lacking. Available information is restricted to morphological characteristics and to some annecdotal information regarding calling activity, microhabitat, reproduction and tadpole development (Heyer et al., 1990; Izecksohn & Carvalho-e-Silva, 2001).

In the present study we analysed different aspects of the spatial distribution, calling activity, diet and microhabitat of *A. marmorata* in an Atlantic forest area of Rio de Janeiro state. To our knowledge, this is the first ecological study of this species.

METHODS

Study area

The study was carried out from September 2001 to March 2002 in the Atlantic rainforest of Ilha Grande (23º11'S, 44º12'W), an island approximately 150 km south of the city of Rio de Janeiro in Rio de Janeiro state, southeastern Brazil. The forest exhibits different levels of regeneration from disturbances caused by human activities in the last centuries, which ceased with the transformation of the area into a state park (Araújo & Oliveira, 1988). Some remnants of primary forest can still be found in the most inaccessible areas of the island. Annual rainfall is about 2200 mm and mean annual temperature is about 22.5 °C (NUCLEN, 1996). The study area, located between Vila Dois Rios and the Parnaioca beach, on the oceanic side of the island, is characterized by a 15-year-old patch of regenerating forest.

Collecting methods and analysis

To evaluate the pattern of dispersion of *A. marmorata* in the area we established one grid with ten parallel lines 20 m apart. Along each line we established sampling points every 20 m (*n*=10), totalling 100 points for the grid. Each

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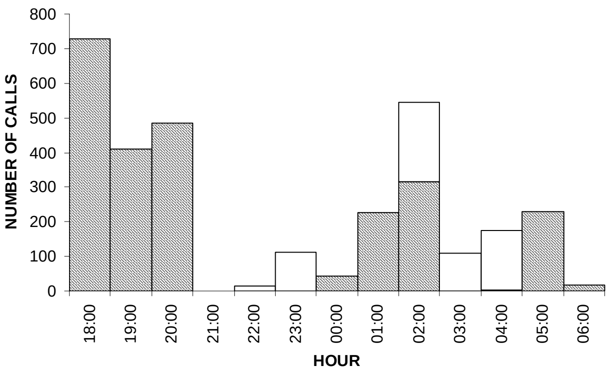

Fig. 1. Total number of calls (over three months) emitted by males of *Adenomera marmorata* in the Atlantic rainforest of Ilha Grande, Brazil at hourly intervals. Hatched bars: calls emitted during hourly intervals without rainfall; open bars: calls emitted during hourly intervals with rainfall.

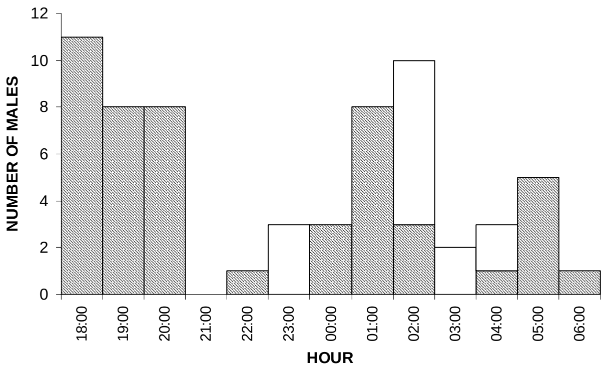

Fig. 2. Total number of males (over three months) of *Adenomera marmorata* engaged in calling activity in the Atlantic rainforest of Ilha Grande, Brazil. Hatched bars: males calling during hourly intervals without rainfall; open bars: males calling during hourly intervals with rainfall.

month (one day per month), between December 2001 and March 2002, all grid points were sampled by the observers, from dusk onward. Each point was sampled only once per month, to ensure no calling individuals were counted more than once. At each grid point the number of active *A. marmorata* (i.e. number of males vocalizing) was recorded for a period of 5 mins. For an estimate of the dispersion pattern, we used the Morisita index of dispersion: Id = $n\Sigma X^2$-N/N(N-1), where n is the number of points sampled, N the total number of individuals counted in all points, and X the number of individuals counted at each point (Brower & Zar, 1984). If the dispersion is random, $0<Id\leq 1.0$; if uniform, Id=0; and if aggregated, Id >1. Additionally, from September to November of 2001 we recorded male calling activity in the area through a continuous 24 h period on one day per month. For this, at three fixed points in the forest, for periods of 5 mins at hourly intervals, the number of individuals calling and the number of emitted calls were recorded. During observation days, we also recorded if it rained.

We analysed diet composition in terms of the number, volume (in mm^3) and frequency of occurrence of each prey type. The collection of specimens of *A. marmorata* for stomach content analysis was carried out in areas of the forest different from those where we recorded calling activity and dispersion pattern. All individuals collected (*n*=21) were humanely killed and dissected, and their stomach contents were analysed under a stereomicroscope. The snout–vent length (SVL) and mouth width of the frogs and the length and width of each prey item were measured (to the nearest mm) with callipers. To estimate the volume of each individual prey item, we used the formula for an ellipsoid: $V=4/3\pi(L/2)(W/2)^2$, where L is the length and W the width of each prey item (Dunham, 1983). For each prey category in the diet of *A. marmorata*, we calculated the index of importance I_x=(%N+%V+%F)/3, where N = number, V = volume and F = frequency of occurrence of each prey type in the diet (Powell et al., 1990). To evaluate if the width of the frogs' mouths affected the size or volume of prey that a frog could consume (average length of the three longest prey items in each stomach and average volume of the three largest prey items in each stomach, respectively) we used simple regression analysis (Zar, 1999). For the regression analysis we only considered individuals that had three or more prey items in their stomachs. We also used simple regression analysis to evaluate if the body size of *A. marmorata* individuals affected the number of prey items consumed. Before we proceeded with the statistical analysis, we tested the normality of the distribution of the data and, when the data were not normally distributed, we used a non-parametric test.

Also, we estimated the frequencies of usage of each microhabitat type by *A. marmorata*. For each individual found, we recorded the substrate type it was using at the moment of its first sighting. To estimate if the species uses the different microhabitats according to their frequencies in the environment, we sampled the distribution of abundances of the available microhabitats in the area in order to compare with the frequencies of those used by the frogs. In the present study, we used a combination of a transect sampling method and the random point technique proposed by Marcum & Loftsgaarden (1980) for estimating the availabilities of potential microhabitats in the study area. However, estimates by this technique could contain a sampling error component, as the number of sampling points is a crucial factor in estimating the resource availability (Thomas & Taylor, 1990). Along ten transects approximately 50 m long in the same area where we recorded the microhabitats of the individuals sampled, we recorded the microhabitats available at each point on the ground every 5 m along the transect. The frequencies of microhabitats for the 78 points sampled provided us with an estimate of the frequencies of available potential microhabitats for the frogs. The differences between the distribution of frequencies of microhabitats used and available were tested with the Kolmogorov-Smirnov test for two independent groups (Zar, 1999).

Table 1. Number (N), volume (V; mm³) and frequency of occurrence (F; %) of prey consumed by *Adenomera marmorata* (n=21) in the Atlantic rainforest of Ilha Grande, Brazil. I_x = Index of importance. *Includes larvae of the orders Coleoptera, Diptera and Lepidoptera.

Prey category	N (%)	V (%)	F	I_x
INSECTA				
Hymenoptera (ants)	30 (33.3)	6.8 (2.7)	33.3	0.231
Insect larvae*	17 (18.8)	46.9 (18.7)	42.8	0.268
Collembola	2 (2.2)	0.2 (0.1)	9.5	0.039
Coleoptera	1 (1.1)	12.7 (5.1)	4.7	0.036
Orthoptera	1 (1.1)	5.6 (2.2)	4.7	0.026
Diptera	1 (1.1)	0.9 (0.4)	4.7	0.020
Isoptera	1 (1.1)	0.2 (0.1)	4.7	0.019
ARACHNIDA				
Araneae	2 (2.2)	1.51 (0.6)	9.5	0.041
Acari	2 (2.2)	1.3 (0.05)	9.5	0.039
CRUSTACEA				
Isopoda	30 (33.3)	125.6 (50.1)	66.7	0.500
MOLLUSCA				
Gastropoda	2 (2.2)	3.5 (1.4)	9.5	0.043
CHILOPODA				
Chilopoda	1 (1.1)	0.6 (0.3)	4.7	0.020
Unidentified arthropod remains		57.8 (23.1)		
TOTAL	90	250.3		

RESULTS

The value of the dispersion index (Id) of *Adenomera marmorata* was 2.49 in December 2001 (n=28 calling individuals) and 2.60 in January 2002 (n=54). In February and March 2002 it was not possible to estimate the dispersion index because the number of individuals found (n=4 in February and n=3 in March) was reduced. The calling activity of males extended from dusk to early morning (Figs 1 and 2) with maximum activity usually occurring from 1800 to 2000. When it rained, there was some intensification of calling activity by *A. marmorata* (Figs 1 and 2). The number of calling individuals was significantly related to the number of calls emitted by these males (r^2=0.887; $F_{1,10}$=86.539; P<0.001).

Adenomera marmorata consumed 12 different groups of prey, mostly arthropods (Table 1). In terms of number of prey, Hymenoptera (Formicidae) (33.3%) and Isopoda (33.3%) were the predominant prey types. In terms of volume, Isopoda prevailed (50.1%), followed by insect larvae (18.7%). The most frequent items in the diet were Isopoda and larvae, which were present in 67% and 43% of the stomachs, respectively. The most important items of the diet according to the index of importance were Isopoda (I_x=0.500), larvae (I_x=0.268) and Hymenoptera (ants) (I_x=0.231). As the values of the mean volumes of the three largest prey items ingested by each individual did not follow a normal distribution (Kolmogorov-Smirnov: D_{max}=0.271, P=0.015), the data were log-transformed before we carried out the regression analysis. The width of the frogs' mouths did not influence the mean prey size (r^2=0.002; $F_{1,10}$=0.022; P=0.884) nor the mean prey volume (r^2=0.069; $F_{1,10}$=0.740; P=0.410). Similarly, the SVL of *A. marmorata* did not influence the number of prey consumed (r^2=0.013; $F_{1,19}$=0.246; P=0.626).

All individuals of *A. marmorata* were found on the ground and associated with the leaf litter of the forest floor. Most observed frogs were on (n=13; 62%) or under (n=7; 33.3%) leaves of the leaf litter (Fig. 3). The distribution of frequencies of microhabitats used by the frogs differed significantly from those available in the habitat (KS: D_{max} 0.833; P=0.009) (Fig. 3).

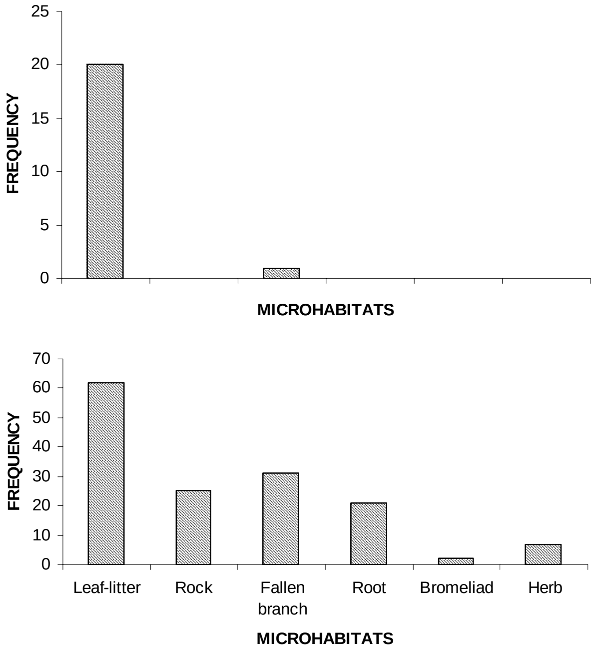

Fig. 3. Distribution of frequencies of microhabitat categories: above, those used by *A. marmorata* (n=21); below, those potentially available in the habitat in the Atlantic rainforest of Ilha Grande, Brazil (n=78 points).

DISCUSSION

Our data indicate that in the forest of Ilha Grande, *Adenomera marmorata* has a clumped dispersion pattern. In tropical forests the leaf-litter layer of the forest floor is not homogeneous, but very variable, both in quantitative (e.g. litter depth) and in qualitative terms (e.g. types of structural elements associated with the litter layer, such as leaves, branches, stones, etc.). As *A. marmorata* is an anuran that, in natural habitats, lives associated with the leaf-litter layer (Heyer et al., 1990; Izecksohn & Carvalho-e-Silva, 2001; present study), the dispersion pattern found in our study probably reflects the variation in small-scale characteristics of the leaf litter layer in the study area.

Heyer et al. (1990) reported *A. marmorata* as having a diurnal activity pattern in the Atlantic forest of Boracéia, in São Paulo state, with males calling in the late afternoon, usually in association with showers. However, our data indicated that at Ilha Grande the calling activity of *A.*

marmorata males is crepuscular and nocturnal, occurring from dusk till dawn. However, during rainy days, *A. marmorata* males may start their calling activity earlier, during the daylight period. On some occasions, we recorded that outside the observation period, when it started to rain, some individuals started calling before the crepuscular period. Also, during its usual calling period (i.e. dusk and night), this frog species shows an intensification of calling activity when it rains. These data are consistent with the idea that rainfall should be an important parameter affecting calling activity of *A. marmorata* and concurs with the observation of Heyer et al. (1990). It is possible that the diurnal activity of *A. marmorata* reported by Heyer et al. (1990) in Boracéia could result from rainy conditions during their observations.

At Ilha Grande, *A. marmorata* has a diet composed of small arthropods, mainly isopods, insect larvae and ants. As a leptodactylid, it would have been expected to fit the non-ant specialist guild (*sensu* Toft, 1980a). However, our results suggest that this generalization does not apply to this species, since ants form a substantial part of its diet. Its diet is similar to those of other sympatric litter-dwelling anurans such as *Zachaenus parvulus* (Van Sluys et al., 2001) and *Eleutherodactylus parvus* (Marra et al., 2004). Although it eats several types of arthropods, *A. marmorata* can be considered an important predator of isopods, an unusual trend among leaf-litter frogs (Duellman, 1978; Toft, 1980a; Vitt & Caldwell, 1994; Caldwell, 1996). A similarly large consumption of isopods was found in the diet of the sympatric *Zachaenus parvulus* (Van Sluys et al., 2001). However, at this time it is not clear if this was a result of the relative availability of this prey in the area or of prey selection.

In anurans, in general, there is a positive relationship between prey size and body size and/or mouth width, as they tend to ingest their prey whole (Duellman & Trueb, 1986; Lima & Moreira, 1993; Van Sluys & Rocha, 1998; Van Sluys et al., 2001). However this trend did not appear in the present study. The lack of relationship between *A. marmorata* mouth width and the mean size of prey consumed may be due to the high consumption of small colonial insects such as ants, as has been suggested for the lizard *Cnemidophorus littoralis*, for which the absence of this relationship seems to be due to the large consuption of termites (Teixeira-Filho et al., 2003). As the ants consumed were all of similar sizes, this resulted in a decrease in the variation of the size of prey. Nevertheless, there was also no relationship between frog SVL and the number of prey ingested.

Our data showed that *A. marmorata* does not use the microhabitats potentially available in the forest in the same proportion at which they are available. Of the six microhabitat categories recorded, *A. marmorata* used only two, mostly the leaf litter (95.2%), which suggests that, in the forest of Ilha Grande, this is the microhabitat most used by this frog species. However, as the number of sampling points (*n*=78) used to estimate the microhabitat availability was reduced, it is possible that the result contained a sampling error component (Thomas & Taylor, 1990). The data indicated that *A. marmorata* uses the habitat exclusively in a horizontal way. These data agree with the generic information available on the habitat of this species (Heyer et al. 1990; Izecksohn & Carvalho-e-Silva, 2001).

We conclude that in the Atlantic forest of the Ilha Grande, *A. marmorata* possesses crepuscular-nocturnal activity, and is exclusively associated with the leaf-litter of the forest floor where it feeds predominantly on isopods, ants and insect larvae.

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