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## Distribution of frogs in riparian areas of an urban forest fragment in Central Amazonia

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**Abstract:** This study aimed to evaluate how environmental variables (width of valley, stream size, forest edge distance, and leaf-litter depth) affect the abundance and richness of species of frog species in riparian areas of an urban forest fragment in Central Amazonia. The study was conducted at the campus of the Universidade Federal do Amazonas, Manaus, Brazil, between May 2008 and April 2009, when three nocturnal and diurnal samples were taken from ten plots. A total of 17 species belonging to seven families were registered. The number of species recorded per plot varied from seven to 14. We detected a relationship between the edge distance and richness: the plots with lower richness were located near the fragment border. The variables had no significant effect on abundance of each species.

**Keywords:** abundance, Anura, edge effects, diversity, environmental variables.

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**Resumo:** O objetivo do presente estudo foi avaliar os efeitos de variáveis ambientais (largura do baixo, tamanho do corpo d'água, profundidade da liteira e distância da borda) sobre a abundância e riqueza de espécies de anuros em áreas ripárias de um fragmento florestal urbano na Amazônia Central. O estudo foi realizado no campus da Universidade Federal do Amazonas, Manaus, Brasil, entre maio de 2008 e abril de 2009, onde três amostragens diurnas e três noturnas foram realizadas em dez parcelas permanentes. Foram encontradas 17 espécies pertencentes a sete famílias. O número de espécies registrado por parcela variou de sete a 14. Foi detectado um efeito significativo da distância da borda sobre a riqueza de espécies, onde um menor número de espécies foi registrado em parcelas localizadas próximas às bordas do fragmento. As variáveis analisadas não apresentaram efeitos sobre a abundância de cada espécie.

**Palavras-chave:** abundância, Anura, efeito de borda, riqueza, variáveis ambientais.

## Introduction

Riparian areas are environments that can provide shelter for many species from different taxonomic groups (Sabo et al. 2005). For most amphibians, riparian areas are important due to the presence of breeding sites – streams and temporary ponds located near streams –, and the availability of these breeding sites can be the main factor related to species distribution (Zimmerman & Bierregaard 1986). Studies with anurans in riparian areas in Australia, Borneo, and Brazil indicate that the size of the water body and the understory vegetation can be related to the composition of species (Parris & McCarthy 1999, Eterovick 2003, Keller et al. 2009). For other studies made in Central Amazonia, the soil pH, slope and clay proportion were related to the abundance of species that have aquatic reproduction; this species are distributed near water bodies (Menin 2005, Rojas-Ahumada & Menin 2010). However, species with terrestrial reproduction, which have broad distribution in the environmental gradient, showed a relationship with edaphic variables (Menin et al. 2007).

The formation of small forest fragments in urban landscapes affects the ecology and conservation of many species, including amphibians (Cushman 2006, Hamer & McDonnell 2008). The IUCN Red List of Threatened Species (2010) shows that 32% of amphibian species are known to be threatened or extinct, while the percentage of bird and mammal species is 12 and 20%, respectively (Baillie et al. 2004). The most worrisome fact is that the value given for amphibians could be underestimated due to lack of data, representing 25% for this biological group (Vié et al. 2008), and the Amazon rainforest is one of the environments where little is known about this group. Amphibians generally have strong endemism and are therefore sensitive to environmental changes. Due to this reason, habitat loss by anthropogenic activities results in the local extinction of species associated to primary forest (Tocher 1998).

Anthropogenic activities that cause fragmentation processes change the environment on the edges of the forest fragment, and can also cause alterations of some ecological factors, consequently affecting the biodiversity (Laurance & Vasconcelos 2009). Relatively few studies have examined edge effects in amphibian assemblages, despite the expectation that these organisms may be more vulnerable to desiccation in drier environments near edges (Lehtinen et al. 2003). According to Gardner et al. (2007), there is no strong support for the importance of edge effects for amphibians, with a number of studies finding different responses. Studies concerning edge effects on anuran assemblages in different tropical forests indicate reduction in abundance of some species or absence of effects (Gascon 1993, Pearman 1997, Marsh & Pearman 1997, Schlaepfer & Gavin 2001, Toral et al. 2002, Dixo & Martins 2008). Many ecological processes, for example, colonization rate, dispersion and breeding success, depend on the environment around the fragment (Gascon et al. 1999, Laurance & Vasconcelos 2009). Moreover, the fragmentation process reduces genetic diversity even to species with relatively high dispersal abilities, leading to local extinctions (Dixo et al. 2009).

In order to evaluate the distribution and abundance of anurans in riparian areas at an urban fragment we determined: 1) the composition of diurnal and nocturnal anuran species; and 2) the effects of abiotic and biotic variables on the richness and abundance of each species.

## Material and Methods

### 1. Study area

The study was conducted at the campus of the Universidade Federal do Amazonas – campus UFAM (03° 04' 34" S and 59° 57' 30" W), municipality of Manaus, Amazonas, Brazil (Figure 1), and it is part of the Biodiversity Research Programme (Programa

de Pesquisa em Biodiversidade – PPBio: <http://ppbio.inpa.gov.br>). The forest fragment of the campus has an area of about 600 ha of terra-firme forest (a not seasonally flooded forest), secondary forest, campinarana sites (a low, relatively light forest with thin-stemmed trees 10-20 m height) and deforested areas (Borges & Guilherme 2000, Nery et al. 2004). The area has ten first order streams and two second order streams – following Strahler's modification of Horton's scale (Petts 1994). The rainy season extends from November to May, with a dry season between June and October (Marques Filho et al. 1981). The fragment isolation process began in 1971 when immigrants started a massive occupation of the area due to the attractive employment perspective of the emerging Manaus free-zone industrial district. The total isolation of the fragment occurred about 20 years ago.

### 2. Data collection

Three diurnal and nocturnal sampling events were carried out throughout one year (May-July of 2008, November-December of 2008 and March-April of 2009). Data were collected at ten plots located near streams of terra-firme forest (Figure 1; more information is available from <http://ppbio.inpa.gov.br>).

Diurnal surveys lasted about 2 hours per plot and were conducted between 8:00 AM and 4:00 PM by two people walking along a 250 × 1 m (0.025 ha) plot. Observers visually scanned and carefully turned over the leaf litter, detecting individuals by visual encounter (Menin et al. 2007, 2008), and only the individuals located within the plot were recorded. If some calling male was visualized inside the plots, it was counted.

Nocturnal surveys were performed using standardized sampling methods: visual sampling (visual encounter surveys, with the use of a headlight) and auditory survey, simultaneously (Crump & Scott 1994, Zimmerman 1994, Menin et al. 2007, 2008). Each plot was covered by two people walking side by side for about an hour. The samplings were conducted between 6:30 PM and 10:00 PM. After every 5 m, the observers stopped and registered the number of individuals of each species in vocalization activity. At the same time, they searched visually in the leaf litter and vegetation to a height of about 2 m. All frog sightings and/or all calls heard at a distance of approximately 20 m on either side of the 250 m long centreline were counted, totalling about 1 ha plot. All sampling was carried out by the same persons (senior author and field assistant).

The stream size, the depth of the leaf litter layer (recorded in each sampling period), and the width of the valley were recorded by measurements taken every 50 m along the plot trail. For valley width we considered the lowland areas around streams subject to flooding. The size of the stream was determined by measuring the width of the stream, depth of the stream (obtained with measuring tape) and the length of the plot (250 m), which were multiplied. The depth of the leaf litter layer was recorded with a graduated stick (cm) and the mean litter depth was used for each plot in analyses. The distance of the plot to the nearest fragment edge was measured on a map of the campus.

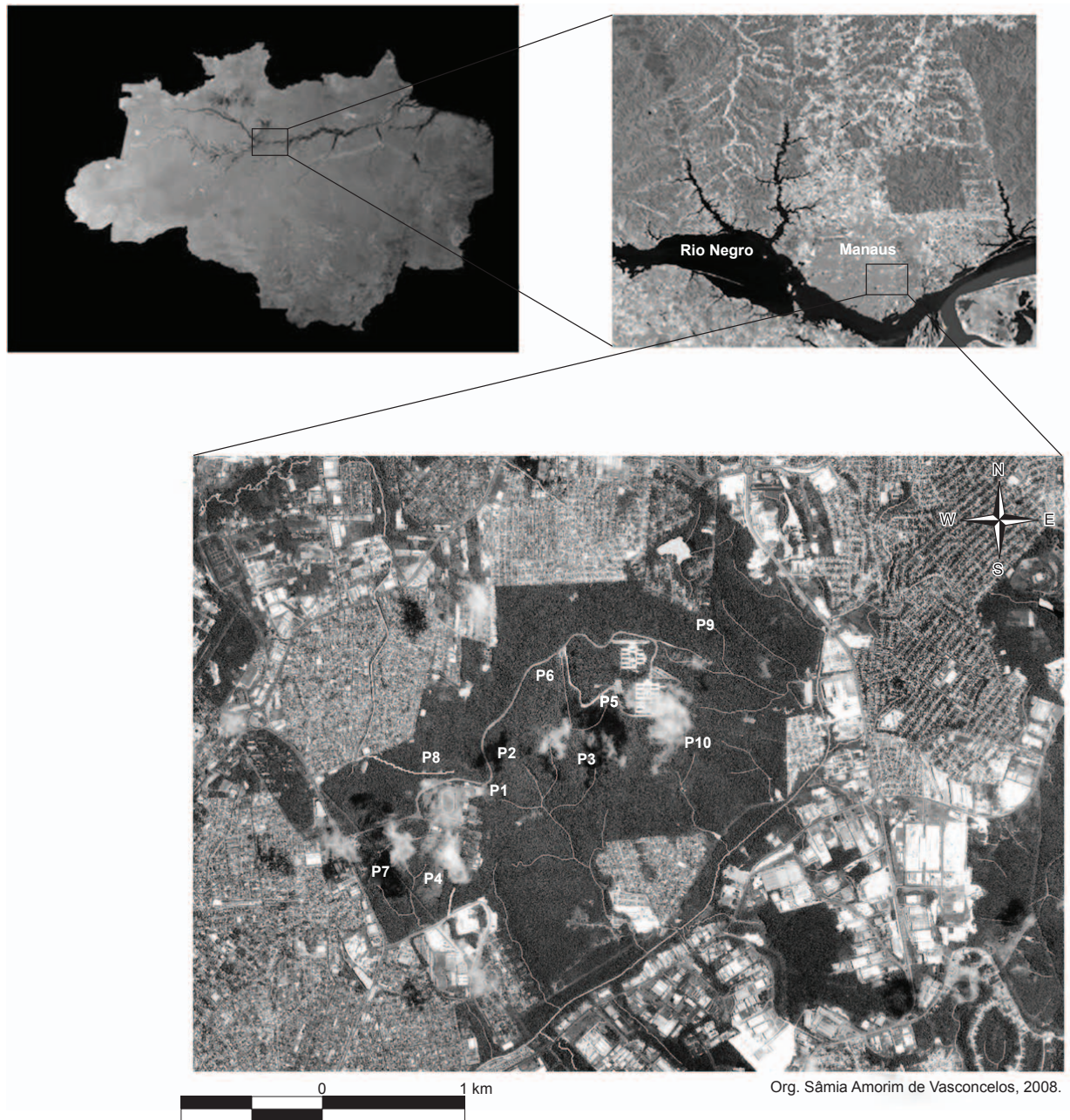
The stream width varied from 1.22 to 6.62 m. The stream depth varied from 0.04 to 0.32 m. The valley width varied from 9.90 to 36.67 m. The leaf litter depth varied from 2.83 to 6.33 cm. The forest edge distance varied from 187 to 972 m (Table 1).

### 3. Data analyses

*Leptodactylus* aff. *andreae*, a crepuscular species, *Dendrophryniscus minutus*, a diurnal species and *Pristimantis fenestratus*, species with diurnal (juvenile) and nocturnal (adults) habits were registered both during the diurnal and the nocturnal sampling. The abundance of the first two species was higher in the diurnal sampling and only the diurnal sampling was therefore



## Frogs in an urban fragment



**Figure 1.** Geographical location of the study area, campus of the Universidade Federal do Amazonas, Manaus, Amazonas State, Brazil. Irregular lines represent streams inside the fragment. Abbreviations (P1 to P10) represent the identification number of each plot. (modified at <http://earth.google.com>).

considered in the analyses. For *P. fenestratus* we considered only the nocturnal sampling.

We tested for spatial auto-correlation of independent variables with Mantel tests, using the R environment for statistical computing (R Development Core Team 2009). We calculated association matrices of independent variables as the difference in the value of the variable between plots. We used the Euclidean Distance coefficient to calculate the geographic distance matrix.

The effects of the independent variables (stream size, depth of leaf litter layer, forest edge distance) on the abundance of each species (data on abundance of each species is available from <http://ppbio.inpa.gov.br/Eng/inventarios/campus/anuros>) and on the number of species in each plot were determined using linear multiple regression (regression model: Species Abundance or Number of Species =  $a +$  Stream size + Leaf Litter Depth + Edge Distance). For the regressions, only species found in at least in 80% of the plots were considered. The colinearity between environmental variables used in the regression model was assessed by the Pearson's correlation matrix, which demonstrate that valley width was collinear to forest edge distance (Pearson correlation  $r = 0.50$ ), and then removed from the analysis. For species from the family Hylidae, we did not use the variable leaf litter depth in the regression model. The value of abundance

considered in the analyses. For *P. fenestratus* we considered only the nocturnal sampling.

considered in this analyses was the higher abundance recorded for each species in three samples in each plot, with values standardized in Log (x+1) before analyses to reduce the effect of extreme values. The stream size measures were not normally distributed and log transformed prior to analysis. The number of species was determined considering the three samples. All analyses and tests were performed with the software MYSTAT 12.0.

## Results

### 1. Species composition

In the ten plots, a total of 17 species of anurans were recorded belonging to seven families (Tables 2 and 3), using all sampling methods and periods. Fifteen species belonging to six families were recorded in nocturnal samples (Table 2). The species *D. minutus*,

**Table 1.** Values of environmental variables to each plot, recorded by measurements taken every 50 m along the plot trail (stream width and depth, valley width, leaf litter depth) or on a map (forest edge distance), campus UFAM, Manaus, Amazonas, Brazil.

Plot	Stream width (m)	Stream depth (m)	Valley width (m)	Leaf litter depth (cm)	Forest edge distance (m)
1	2.38	0.23	11.92	5.33	594
2	3.05	0.19	13.97	5.67	471
3	6.15	0.19	10.21	4.33	353
4	1.22	0.16	11.82	6.33	300
5	1.41	0.15	13.00	3.87	972
6	2.23	0.08	18.33	3.45	664
7	3.40	0.08	36.67	3.38	187
8	2.45	0.09	10.33	3.83	250
9	6.60	0.04	10.43	4.00	663
10	6.62	0.32	9.90	2.83	537

**Table 2.** Number of plots and number of individuals per species of anurans found by two sampling methods conducted in nocturnal periods, campus UFAM, Manaus, Amazonas, Brazil.

Family/species	Number of plots	May–Jul 2008		Nov–Dec 2008		Mar–Apr 2009	
		visual	auditory	visual	auditory	visual	auditory
Bufonidae							
<i>Dendrophryniscus minutus</i> (Melin, 1941)	1	0	1	0	0	0	0
Centronelidae							
<i>Vitreorana oyampiensis</i> (Lescure, 1975)	6	0	1	0	14	0	2
Hylidae							
<i>Hypsiboas cineracens</i> (Spix, 1824)	10	10	180	3	194	2	198
<i>Hypsiboas lanciformis</i> Cope, 1871	9	10	13	2	13	3	25
<i>Osteocephalus oophagus</i> Jungfer and Schiesari, 1995	10	14	42	10	77	1	54
<i>Osteocephalus taurinus</i> Steindachner, 1862	7	4	0	3	1	1	3
<i>Phyllomedusa bicolor</i> (Boddaert, 1772)	2	2	0	0	4	0	1
<i>Scinax boesemani</i> (Goin, 1966)	1	0	1	0	0	0	2
<i>Scinax garbei</i> (Miranda-Ribeiro, 1926)	1	1	0	0	0	0	0
Leptodactylidae							
<i>Leptodactylus</i> aff. <i>andreae</i> Müller, 1923	9	0	0	2	43	0	0
<i>Leptodactylus pentadactylus</i> (Laurenti, 1768)	10	8	0	10	0	15	0
<i>Leptodactylus petersii</i> (Steindachner, 1864)	7	4	12	5	1	0	10
Microhylidae							
<i>Synapturanus mirandaribeiroi</i> (Nelson & Lescure, 1975)	4	0	1	0	35	0	0
Strabomantidae							
<i>Pristimantis fenestratus</i> (Steindachner, 1864)	10	1	41	0	60	2	23
<i>Pristimantis zimmermanae</i> (Heyer & Hardy, 1991)	10	1	27	0	50	0	13
Number of species	-	10	9	7	11	6	10
Total number of species	-	14		13		11	
Number of individuals	-	55	319	35	492	24	331
Total number of individuals	-	374		527		355	

**Table 3.** Number of plots where each species of anuran was recorded and number of individuals in each diurnal sample, campus UFAM, Manaus, Amazonas, Brazil.

Family/species	Number of plots	May–Jul 2008	Nov–Dec 2008	Mar–Apr 2009
Aromobatidae				
<i>Allobates</i> sp.	5	13	0	5
<i>Anomaloglossus stepheni</i> (Martins, 1989)	5	7	6	9
Bufonidae				
<i>Dendrophryniscus minutus</i>	10	102	39	49
Leptodactylidae				
<i>Leptodactylus</i> aff. <i>andreae</i>	10	219	136	166
Strabomantidae				
<i>Pristimantis fenestratus</i>	8	3	13	5
Number of species	-	5	4	5
Number of individuals	-	344	194	234

*Vitreorana oyampiensis*, *Scinax boesemani*, and *Synapturanus mirandaribeiroi* were recorded only in auditory samples, while the species *Scinax garbei* and *Leptodactylus pentadactylus* were recorded only in visual samples (Table 2). *Hypsiboas cineracens* was the most abundant species, representing 46.7% of all the recorded individuals, followed by *Osteocephalus oophagus* (15.7%) and *Pristimantis fenestratus* (10.0%). The higher number of species was recorded in the first sampling period and the higher number of individuals was detected in the second sampling period.

Five species belonging to four families were recorded during the diurnal sampling (Table 3). The most abundant species was *Leptodactylus* aff. *andreae*, representing 67.5% of all recorded individuals, followed by *D. minutus* (24.6%). A higher number of individuals and species were recorded in the first and third sampling periods (May–July 2008 and March–April 2009).

## 2. Spatial auto-correlation

There was no correlation between the geographical distances among plots and the variables stream size, valley width or forest edge distance (Mantel tests:  $P = 0.10$ ,  $P = 0.76$  and  $P = 0.99$ , respectively). There was a significant correlation between the geographical distances between plots and leaf litter depth ( $r^2 = 0.42$ ,  $P = 0.02$ ).

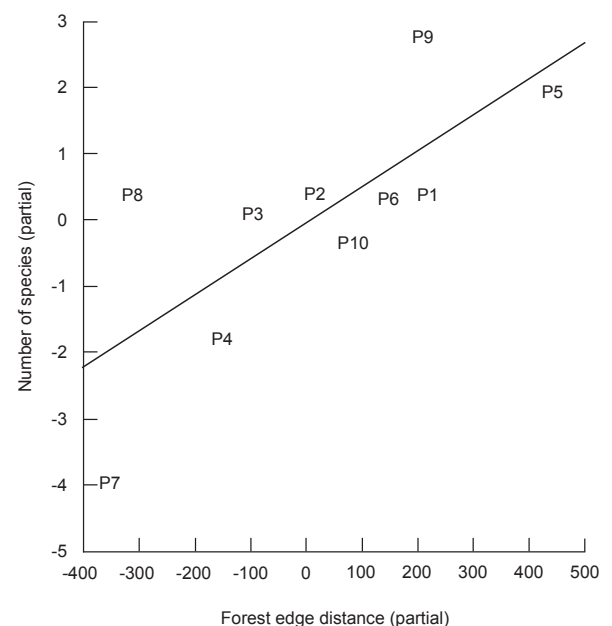
## 3. Effects of environmental variables on richness and abundance

The number of species recorded per plot ranged from 7 to 14. The plots with lower richness were located near the fragment border. The model explained about 53% of the variation in the species richness (Species richness =  $5.643 + 0.572$  stream size +  $0.006$  forest edge distance +  $0.054$  leaf litter depth;  $R^2 = 0.528$ ;  $F_{(3,6)} = 2.233$ ;  $P = 0.185$ ). The richness was related positively to the forest edge distance ( $t = 2.486$ ;  $P = 0.047$ ), which the streams nearest to border of the fragment has a lower number of species (Figure 2).

The abundance of each species was not related to any of the variables (Table 4).

## Discussion

In the present study, the distance to forest edge was related to species richness, but not to the abundance of each species. The relationship found in this study suggests that edge effects are acting on species richness. This response of anurans was expected since these organisms are considered sensitive to environmental changes due to a complex of morphological and physiological features

**Figure 2.** Partial regression for statistically significant effect of predictor variable on number of species of frogs at the campus of the Universidade Federal do Amazonas, Manaus, Amazonas State, Brazil.

(Lehtinen et al. 2003). Edge effects determine biotic and abiotic changes, such as fluctuations in temperature, increase of wind and solar radiation, and decrease of humidity, which could in turn affect the organism optimum (Camargo & Kapos 1995). In spite of the anurans are considered sensitive to environmental changes (Cushman 2006), the studies concerning edge effects on anuran assemblages showed contradictory results (Gardner et al. 2007) and variation in space and time (Lehtinen et al. 2003). For species of *Pristimantis* (Strabomantidae), the studies indicate reduction in abundance of some species on the border of a fragment or absence of effects (Pearman 1997, Schlaepfer & Gavin 2001). For leaf litter frogs in forest fragments of Brazilian Atlantic forest, no effects were detected (Dixo & Martins 2008). Similarly, absence of responses was detected to terrestrial frogs in a cloud forest and in an Andean montane forest, both in Ecuador (Marsh & Pearman 1997, Toral et al. 2002). However,



**Table 4.** Results of multiple regressions between the abundance of seven anuran species and the environmental variables, campus UFAM, Manaus, Amazonas, Brazil.

Species	Constant	Stream size	Forest edge distance	Leaf litter depth	R <sup>2</sup>	F	P
<i>Dendrophryniscus minutus</i>	0.176	0.515	-0.001	0.032	0.340	1.029	0.444
<i>Hypsiboas cineracens</i>	2.849	0.123	0.0001	-	0.338	1.785	0.236
<i>Osteocephalus ooophagus</i>	1.911	0.001	0.001	-	0.196	0.852	0.466
<i>Leptodactylus</i> aff. <i>andreae</i>	1.432	0.381	0.002	-0.264	0.674	4.132	0.066
<i>Leptodactylus pentadactylus</i>	1.818	-0.208	0.0001	-0.003	0.313	0.909	0.490
<i>Pristimantis fenestratus</i>	3.727	-0.201	-0.001	-0.106	0.184	0.452	0.725
<i>Pristimantis zimmermanae</i>	-1.959	0.681	0.001	-0.045	0.406	1.369	0.339

in a study conducted at Madagascar, all amphibian species were edge-avoiders in dry season but different patterns were observed in wet season: one species remained edge-avoider and all other species showed no significant response to edge proximity (Lehtinen et al. 2003). According to Schlaepfer & Gavin (2001), diurnal amphibians may be particularly vulnerable to edge effects because the abiotic conditions (sunny, dry) during the day are unfavourable to the physiological needs of amphibians. These results also suggest that frogs do not respond uniformly to edges because there are many effects involved and each species will interact with these factors in a different manner (Gascon 1993, Schlaepfer & Gavin 2001). In contrast to observed by Gascon (1993), Schlaepfer & Gavin (2001), and Lehtinen et al. (2003), which found that responses to edges are less obvious during wet periods, our study is the first to indicate an edge effect during wet season.

No significant relationships were found between abundance of the most common species and the environmental variables measured. The abundance of terrestrial anuran species was not influenced by depth of the leaf litter layer in several studies carried out in Central Amazonia (Allmon 1991, Menin 2005, Menin et al. 2007). On the other hand, relationships were found between the depth and/or the volume of litter in many studies with communities or species of anurans in Costa Rica forests – Central America (Lieberman 1986), Brazil – Central Amazonia (Tocher et al. 1997), Uganda – Africa (Vonesh 2001) and Brazil – Southeast region (Van Sluys et al. 2007). According to Menin et al. (2007), the contradictory relationships of anuran communities and the leaf litter layer can be related to different methods quantifying litter characteristics (volume, depth, or drymass) in these studies. In addition, the litter quality may vary with topography and/or seasonally, because the litter decomposes faster in the rainy season, often resulting in a shallower litter layer (Luizão & Schubart 1986). In Australia and Borneo, studies showed a relationship between stream size and number or composition of species (Parris & McCarthy 1999, Keller et al. 2009), suggesting that the water duration and water body size may have an influence on the richness of frog species. However, this variable was not found to influence communities in Brazil (Eterovick 2003). The absence of relationships in our study can be explained to small variation among the size of studied water bodies.

Currently, 30 species of anurans have been found in the campus UFAM (Cordeiro & Sanaïotti 2003), of which 21 were found preferentially inside the forest. Seventeen of these species were found in this study. *Hypsiboas lanciformis*, a species common to open areas and forest borders (Lima et al. 2006, Menin et al. 2008), was found in nine of the 10 plots. In the nocturnal sampling, the highest abundance was recorded in the second sampling period, and the lowest number of individuals was recorded in the third sampling period, corresponding

to the beginning and middle of the rainy season, agreeing with data obtained by Menin et al. (2008) to a primary forest near to Manaus, Brazil. The pattern found for diurnal samplings is also similar to that found by Menin et al. (2008), for which the number of species was approximately the same throughout the rainy season.

Some common species found in other areas near to Manaus (e.g. *Rhinella proboscidea* (Spix, 1824) – Bufonidae, *Synapturanus* cf. *salseri* Pyburn, 1975 – Microhylidae; Tocher 1998, Menin et al. 2007, 2008, Rojas-Ahumada & Menin 2010) were not found in our study. In addition, the treefrogs *Phyllomedusa tarsius* (Cope, 1868), *P. tomopterna* (Cope, 1868), *P. vaillantii* Boulenger, 1882 which reproduces in isolated forest ponds (Rodrigues et al. 2010) and *Trachycephalus resinifictrix* which reproduces in small water bodies in tree holes (Schiesari et al. 2003) also were not reported. Despite the absence of sampling design that supports comparisons among forest fragments, some speculative hypotheses are suggested as a stimulus for future studies: 1) the lower number of species was occasioned by the size of the fragment – species-area relationships (MacArthur & Wilson 1967); 2) some species must be sensitive to environmental changes (Neckel-Oliveira & Gascon 2006, Bickford et al. 2010) and so, did not occur at the campus due to alterations caused in the region; 3) the absence of connection with continuous forest, preventing recolonization by species that were locally extinct (Cushman 2006) affecting the interspecific interactions and the coexistence of species (Leibold et al. 2004); or 4) the synergism between these hypotheses. In addition, other factors such as human activities on the borders may be generating deeper effects at the campus UFAM, because on these sites, there has been an accumulation of waste left by people living around the fragment and the streams near the border are now being used for recreation.

In summary, this study showed that the edge effect is acting on richness of anurans species at the studied urban fragment.

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