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A INFLUÊNCIA DOS FRAGMENTOS FLORESTAIS NA DINÂMICA DE POPULAÇÕES DE ANUROS NO NOROESTE DO ESTADO DE SÃO PAULO

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NOROESTE DO ESTADO DE SÃO PAULO**

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*Aos meus pais, Wasington e Maria
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incentivo e amizade,*

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RESUMO GERAL

A configuração da paisagem tem um papel fundamental na estrutura e organização de comunidades. A vegetação da região noroeste do Estado de São Paulo, caracterizada como Floresta Estacional Semidecidual e Savana, se restringe hoje a 4% de sua área original, tendo sido substituída por pastagens, culturas diversas ou áreas urbanas. Contudo, pouco se conhece sobre o efeito desses remanescentes florestais para a diversidade de anuros caracterizados como habitat-generalistas e dependentes de corpos d'água para reprodução. Desta maneira, nosso objetivo neste estudo foi verificar a importância da configuração da paisagem na diversidade de anuros de área aberta na região noroeste do Estado de São Paulo. Para isso, foram realizados dois estudos: i) um estudo experimental, com poças artificiais instaladas a diferentes distâncias dos fragmentos florestais e, ii) inventários em corpos d'água temporários para avaliar a importância de variáveis ambientais do habitat e da paisagem na distribuição da riqueza de espécies e da abundância populacional de anuros associados a paisagem aberta. No primeiro estudo, nós hipotetizamos que poças de reprodução próximas a fragmentos florestais teriam maior riqueza de espécies e maior abundância populacional de anuros que poças distantes dos fragmentos florestais, porque a proximidade diminuiria os riscos associados com desidratação e predação durante deslocamentos no período reprodutivo. No segundo estudo, nós hipotetizamos que corpos d'água temporários com longo hidroperíodo e alta porcentagem de vegetação localizados próximos a fragmentos florestais iriam abrigar uma maior riqueza de espécies e abundância de anuros porque proporcionariam maior número de microhabitats para sítios de vocalização e refúgio, e menor risco à predação para girinos e imagos recém-metamorfoseados. Nossos resultados mostram que a configuração da paisagem têm influência importante sobre a distribuição e abundância de anuros em paisagens agrícolas e sugere diretrizes para a manutenção de configurações favoráveis dos habitats aquáticos e

terrestres para a conservação dessas espécies. Corpos d'água temporários com hidroperíodo longo e alta porcentagem de vegetação localizadas próximas a fragmentos florestais e distantes das rodovias irão abrigar maior número e abundância de espécies de anuros e, portanto, são alvos úteis para a conservação e gestão das comunidades de anfíbios de áreas abertas.

ABSTRACT

Landscape arrangement plays a key role in community structure and composition. The northwestern São Paulo State's vegetation, characterized as Semideciduous Seasonal Forest and Savanna, is restricted to 4% of its original area, having been replaced by grazing, plantations or urban areas. Little is known about the importance of these forest remnants for the diversity of anurans found in this region where most anuran species are characterized as habitat-generalists and dependent on water bodies for reproducing. Thus, the present study's aim was to verify the importance of landscape configuration on the diversity of open-area anurans in northwestern São Paulo State. For this, two studies were performed: i) an experimental study, with artificial pools located at different distances from the forest fragments and, ii) surveys in temporary water bodies to assess the importance of environmental variables of habitat and landscape in the distribution of species richness and abundance of anuran associated with the open-areas. In the first study, we hypothesized that breeding areas near forest fragments would have greater species richness and greater abundance than breeding areas farther from forest fragments because proximity decreases risks associated with dehydration and predation during breeding migrations. While in the second, we hypothesise that temporary pools with long hydroperiod and high percentage of vegetation located near forest fragments will harbor a greater number of species and abundance of anurans because these habitats would have higher number of microhabitats for shelter and calling sites and would provide lower risk of predation for tadpoles and newly metamorphosed. Our results show that landscape configurations has an important influence on frog and toad distribution and abundance in tropical agro-agricultural landscapes and suggest guidelines for maintaining favorable configurations of aquatic and terrestrial habitats for conserving this species rich and imperiled species suite. Temporary pools with long

hydroperiod and high percentage of vegetation located near forest fragments and distant from roads will harbor a greater number of species and abundance of anuran and hence are useful targets for conservation and management of these amphibian communities.

INTRODUÇÃO GERAL

Muitas espécies não são confinadas a um único hábitat, mas movem-se entre habitats ou vivem na fronteira onde dois habitats se encontram. Para essas espécies, os tipos de hábitat que existem em uma escala regional são de importância crucial (Primack & Rodrigues 2001). Os anfíbios anuros são particularmente sensíveis às alterações do hábitat pois, além da baixa mobilidade que limita a dispersão dos indivíduos para áreas favoráveis (Bowne & Bowers 1994), a maioria das espécies apresenta um ciclo de vida complexo envolvendo uma larva aquática e um adulto terrestre (Wilbur 1980), o que os torna dependentes da qualidade destes dois ambientes (Duellman & Trueb 1986).

Recentemente, houve um aumento no número de estudos que avaliam a influência de aspectos da paisagem (tipo de matriz, número, distribuição e conectividade entre as poças, porcentagem de cobertura vegetal nativa, densidade de estradas) sobre a abundância e padrões de distribuição de populações de anfíbios (Gascon *et al.* 1999, Guerry & Hunter 2002, Weyrauch & Grubb 2004). Alguns estudos têm detectado uma relação positiva entre a abundância e riqueza de espécies de anfíbios com a proximidade dos corpos d'água até os fragmentos florestais (Laan & Verboom 1990, Findlay & Houlihan 1997) e com a área de cobertura florestal nativa (Hecnar & M'closkey 1997, Vallan 2000, Guerry & Hunter 2002). Além disso, os fragmentos florestais são de extrema importância para a dispersão dos juvenis de anuros, que evitam as áreas abertas devido ao alto risco de dessecação (Rothermel & Semlitsch 2002, Rothermel 2004) e podem servir de refúgios para os adultos de algumas espécies (Weyrauch & Grubb 2004).

A perda e a fragmentação de hábitat estão entre as maiores ameaças para populações de anfíbios (Cushman 2006). Segundo Knutson *et al.* (1999) e Hazell *et al.* (2001) fragmentos florestais providenciam refúgios necessários para muitas espécies que passam

parte ou toda a época em que não estão em atividade reprodutiva em árvores, arbustos ou na serapilheira. Além disso, apresentam habitats relativamente menos perturbados em comparação com áreas agrícolas e urbanas, proporcionando corredores para migração de anuros entre sítios de reprodução e áreas onde realizam outras atividades como alimentação e estivação (Laan & Verboom 1990, Knutson *et al.* 1999), uma vez que áreas fortemente antropizadas provavelmente constituem barreiras para seu deslocamento (Gibbs 1998, Becker *et al.* 2007). Consequentemente, a descontinuidade entre habitats aquáticos e terrestres força muitas espécies com larvas aquáticas a realizarem migrações reprodutivas de alto risco (Becker *et al.* 2007, Becker *et al.* 2010). Portanto, a configuração espacial dos habitats aquáticos e terrestres é uma influência importante sobre a diversidade e distribuição de anfíbios, influenciando a viabilidade das populações (Marsh *et al.* 2000, Marsh & Trenham 2001, Laan & Verboom 1990, Gibbons 2003).

Na região tropical a fragmentação de habitats florestais é um processo marcante e crescente. Um exemplo perturbador refere-se à vegetação da região noroeste do estado de São Paulo. Caracterizada como Floresta Estacional Semidecidual e Savana, ela se restringe hoje a 4% de sua área original, tendo sido substituída por pastagens, culturas diversas ou áreas urbanas (SMA/IF 2005). Tal impacto coloca a região como a mais desmatada e fragmentada do estado e com a menor concentração de unidades de conservação (Kronka *et al.* 1993). Contudo, pouco se conhece sobre a importância desses remanescentes florestais para a diversidade de anuros dessa região ao quais, na sua maioria são classificados como habitat-generalistas e dependentes de corpos d'água para reprodução.

Durante as amostragens deste trabalho nós registramos duas novas espécies para região: *Chiasmocleis albopunctata* (ANEXO I) e *Dendropsophus melanargyreus* (ANEXO II), sendo esse último, novo registro para o estado de São Paulo (Silva *et al.* 2009, Silva *et al.*

2010). Com esses registros, são quatro novos registros de espécies de anuros para a região nos últimos dois anos (Prado 2009, Silva *et al.* 2009, Silva *et al.* 2010), contribuindo para o aumento da lista de espécies de 33 para 37 espécies. O fato desses registros terem sido efetuados nos últimos dois anos, corrobora a indicação constante nas “Diretrizes para conservação e restauração da Biodiversidade no Estado de São Paulo” (Rodrigues *et al.* 2008) que a região noroeste do Estado de São Paulo é uma área prioritária para inventários biológicos.

Desta maneira, o objetivo geral do presente estudo foi verificar a importância da configuração da paisagem na diversidade e distribuição de anuros de área aberta na região noroeste do Estado de São Paulo.

O presente estudo está estruturado em dois capítulos:

- no capítulo 1, instalamos 120 poças artificiais à diferentes distâncias dos fragmentos florestais para testar a hipótese que poças de reprodução próximas a fragmentos florestais teriam maior riqueza de espécies e maior abundância populacional de anuros do que poças localizadas distantes e no interior dos fragmentos florestais. Este capítulo está submetido para publicação no periódico *Landscape Ecology*;
- no capítulo 2, amostramos 18 corpos d’água para avaliar a importância de variáveis em escala local (habitat de reprodução) e em escala regional (paisagem) na determinação dos padrões de distribuição da riqueza de espécies e da abundância populacional de anuros associados a áreas abertas. A hipótese testada é que corpos d’água temporários com longo hidroperíodo e alta porcentagem de vegetação localizados próximos a fragmentos florestais abrigam maior riqueza de espécies e abundância de anuros que poças com hidroperíodo

curto, localizadas distante de fragmentos florestais. Este capítulo está aceito para publicação após “*major revision*” no periódico Wetlands.

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Capítulo 1

An experimental assessment of landscape configuration effects on frog and toad abundance and diversity in tropical agricultural landscapes of southeastern Brazil

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ABSTRACT

Amphibians are an imperiled group of vertebrate animals that typically have biphasic life histories involving a shift from aquatic larval habitats to terrestrial adult habitats. Habitat loss is the greatest threat to amphibians and the importance of the spatial configuration of terrestrial and breeding habitats upon the landscape in determining amphibian persistence is poorly known. The information gap is particularly acute in tropical landscapes which simultaneously host the greatest and most imperiled amphibian fauna on Earth. We installed 125 artificial ponds at different distances from forest fragments embedded in an agricultural matrix in southeastern Brazil. Constructed ponds attracted 13 anuran species; ponds at the forest fragment-matrix transition hosted a greater abundance and higher species richness of frogs and toads than those installed either far from or well within forest fragments. Forest fragments larger than 70 ha in agricultural areas harbored more individuals than smaller fragments. Our results indicate that landscape configuration has an important influence on frog and toad distribution and abundance in tropical agricultural landscapes and suggest guidelines for maintaining favorable configurations of aquatic and terrestrial habitats for conserving this species rich and imperiled species suite.

Keywords: Brazil, frogs, generalized linear mixed models, isolation, landscape, semi-deciduous Atlantic Forest, terrestrial habitats

1. INTRODUCTION

Globally there are approximately 6,000 known amphibian species (AmphibiaWeb 2010), with the Neotropics sheltering the highest number of frog and toad species (Duellman 1999, Young et al 2004). Over recent decades, widespread species declines and extirpations of populations have been reported (e.g. Alford and Richards 1999; Houlahan et al 2000). Neotropical amphibians are the most threatened (Stuart et al 2004), including Brazilian species of frogs and toads (Silvano and Segalla 2005) that together represent the greatest component of frog and toad diversity in the world (AmphibiaWeb 2010).

Habitat loss and fragmentation are considered the greatest threats to amphibian populations, affecting 89% of all threatened species (Young et al 2004; Cushman 2006). A primary reason is that most amphibians have biphasic life histories that involve migrating to aquatic breeding areas and returning to terrestrial habitats (Duellman and Trueb 1986, Sinsch 1990; Blaustein et al 1994), which provide places of foraging, aestivation, migration and dispersal (Stebbins and Cohen 1995; Marsh and Trenham 2001). Forest fragments have been identified as important habitats that enhance landscape connectivity (Laan and Verboom 1990), and reduction of connectivity through habitat loss may reduce anuran diversity (Lehtinen et al 1999; Becker et al 2010). Forest fragments provide refuge for many species that spend part or all of the time in trees, bushes or in leaf litter when not breeding (Knutson et al 1999; Hazell et al 2001). Because discontinuity between suitable aquatic and terrestrial habitats forces many species with aquatic larvae to perform risky breeding migrations through disturbed environments (Becker et al 2007; Becker et al 2010), the spatial configuration of aquatic and terrestrial habitats could be an important influence on amphibian diversity and distribution by influencing population viability (Marsh et al 2000; Marsh and Trenham 2001; Laan and Verboom 1990; Gibbons 2003).

The complex patterns of habitat use by amphibians pose challenges for conserving them in part because we know relatively little about the importance of habitat connectivity. For example, high temperatures and low humidity in open areas can be limiting for amphibians, which have high risk of desiccation (Rothermel and Semlitsch 2002). Although commonly assumed to be extremely limited, the dispersal ability of amphibians is difficult to study and usually underestimated by conventional, mark-recapture studies (Porter and Dooley 1993); indeed amphibians migrating to distances of 2-15 km have been reported (Alford and Richards 1999; Marsh and Trenham 2001; Funk et al 2005). Moreover, little is known about the factors that influence the dispersal ability of amphibians in different types of landscapes (Rothermel 2004).

Few studies have provided clear insights into relationship between the configuration of aquatic and terrestrial habitats and population persistence in any tropical amphibian and thus it is difficult to design habitat plans to conserve this highly diverse and simultaneously threatened group of vertebrates. For this reason, we installed artificial ponds at different distances from forest fragments to evaluate the interaction between forest fragment size and distance between breeding and terrestrial habitats. We hypothesized that breeding areas near forest fragments would have greater species richness and greater abundance than breeding areas farther from forest fragments because proximity decreases risks associated with dehydration and predation during breeding migrations. Furthermore, because most species recorded in this region breed in ponds located in open areas (“pasture matrix”) but rely on forest fragments for shelter and foraging during the extended non-reproductive period in the dry season (Silva and Rossa-Feres 2007), we expected to find more use of artificial ponds in larger forest fragments.

2. MATERIAL AND METHODS

2.1. Study area

Experimental ponds were constructed in watersheds of Turvo-Grande, São José dos Dourados, Baixo Tietê and Tietê-Batalha in the State of São Paulo, Brazil (20° 33' S/50° 13' W to 21° 35' S, 48° 29' W). The region is one of most altered watersheds in South America (Castro et al 2005) given that the original vegetation of the region — mesophytic semideciduous forest and patches of cerrado (Ab'Sáber 2003) —has been devastated by agricultural activities such that the remaining forest is distributed among a few, small and highly scattered fragments (SMA / IF 2005). The region's hot and humid climate is characterized by two well-defined climatic seasons (IBGE 2009): a rainy season between October and March and a pronounced dry season between April and September during which only 15% of the total annual rainfall of 1100-1250 mm falls.

2.2. Experimental design

We conducted experiments with artificial ponds during two rainy seasons (between 2007 and 2009) when most anuran species in the region breed (e.g. Rossa-Feres and Jim 2001; Vasconcelos and Rossa-Feres 2005; Santos et al. 2007). Natural ponds differ greatly in structure and heterogeneity (Metzger 2003; Cushman 2006) so we used artificial ponds which can permit better controlled experiments on the drivers of amphibian distribution (e.g., Marsh et al 1999). Pools were constructed in association with particular forest fragments that: (1) had pasture area along at least one edge and (2) were separated from other forest fragments by a distance at least twice that to artificial ponds installed away from the focal forest fragment's edge.

2.2.1. Experiment I: Forest fragment proximity effects

We selected six forest fragments well separated from each other (by 40 - 215 km) and in two size categories (Fig. 1): (1) three “medium” forest fragments (67, 95 and 108 hectares) and (2) three “large” forest fragments (1360, 1656 and 2189 hectares). Associated with each forest fragment we installed a set of 20 artificial ponds, totaling 120 ponds. Ponds were created along transects 50 m apart and 200 m in length extending perpendicular to a particular fragment’s edge from the fragment’s interior into the surrounding matrix of pasturelands (Fig. 1). Each transect consisted of five treatments: ponds installed at the forest edge, in the matrix 50 - 100 m from the forest edge, and in the forest fragment’s interior 50 - 100 m from the fragment’s edge (Fig. 1). The ponds were hand dug, 1.5 m long, 1.0 m wide and 0.3 m deep. Each pond’s bottom was covered with plastic sheeting to provide an impervious substrate on which was placed a small soil layer and leaf litter and then allowed to fill with rainfall (Fig. 2). The frog and toad community was characterized in terms of the number of individuals counted within 2 m of each pond’s border and by the presence of tadpoles sampled with hand net (3 mm² mesh). Pool communities were sampled three times for five consecutive days each (December 2007 to February 2008), totaling 15 days of sampling in each forest remnant.

2.2.2. Experiment II: Fragment size effects

To better evaluate the influence of forest tract size within the range of fragment sizes typical of the region (90% of forest fragments in São Paulo State have areas smaller than 40 hectares, Nalon et al. 2008) we performed further sampling in five forest fragments (5, 11, 17.5, 67 and 108 hectares). In each forest remnant we installed a set of three artificial ponds, totaling 15 ponds, of dimensions identical to those in Experiment I (Fig. 3). The ponds were

installed at a given forest's edge and at 100 m and 200 m away within the agricultural matrix (Fig. 1). Each group of ponds was sampled five times for five consecutive days each, during December 2008 to March 2009, totaling 25 days of sampling in each forest remnant, using the methods described in Experiment I except that to verify if frogs and toads were in resident ponds throughout the reproductive season each individual was marked with a cotton string (which would eventually rot off) tied in the inguinal region; capture histories were recorded by individual variation in the number of knots tied in the string.

2.3. Data analyses

To investigate which variables explain the distribution of abundance (number of individuals) and species richness of frogs and toads, we fitted generalized linear mixed models (GLMMs) to the data using the lmer function implemented in the lme4 package (Bates and Maechler 2009) in R 2.10.1 (R Development Core Team 2010); forest fragments (blocks) were considered as random effects and treatment and size of fragments (categorical variables) as fixed effects. Because the response variables (abundance and species richness) were count data, we used a quasi-Poisson error distribution and log link function (Crawley 2007). Akaike's information criterion, corrected for small sample sizes (AIC_c , Burnham and Anderson 1998), was used as the model selection criterion and Akaike weights ($wAIC_c$) were used to evaluate model-selection uncertainty, which express the weight of evidence favoring that model as the best of all those in the model set (Burnham and Anderson 1998). For each model considered, we calculated the percentage deviance explained (%DE) as a measure of goodness-of-fit.

3. RESULTS

3.1. Experiment I

We recorded 52 individuals of 11 frog and toad species using the artificial ponds (Table 1). *Chiasmocleis albopunctata* was the only species recorded in the larval stage, with tadpoles found in an artificial pond installed 100 m within one of the forest fragments. Two individuals of *Pseudopaludicola* aff. *falcipes* and one of *Leptodactylus labyrinthicus* were recaptured in the same artificial pond installed 50 m far from the edge toward the matrix. Species richness (likelihood ratio test, $\chi^2 = 13.25$, $df = 4$, $p = 0.01$) and abundance ($\chi^2 = 34.69$, $df = 4$, $p < 0.000$) differed among distance treatments, but not in relation to fragment size (species richness, $\chi^2 = 1.26$, $df = 1$, $p > 0.26$ and abundance, $\chi^2 = 2.45$, $df = 1$, $p > 0.11$). Higher species richness was estimated in ponds installed at (1) the forest edge and 50 m into the pasture than those (2) 50 m and 100 m within forest fragments and at 100 m into pasture (Fig. 4). The model explaining the number of species that included only the distance treatment was best supported (Table 2, Fig. 4). For abundance the model containing distance only as well as the model including both distance and size of fragments were considered equally well supported (Table 2).

3.2. Experiment II

We recorded 35 individuals of eight anuran species using the artificial ponds (Table 2). Only *Leptodactylus* gr. *latrans* and *Leptodactylus fuscus* were not recorded in artificial ponds located at the edge of the forest fragments, whereas at ponds installed at 100 m and 200 m of the forest fragments we recorded three and two species, respectively (Table 1). The model explaining frog and toad abundance that included both distance treatment and size of

forest fragments was best supported (Table 2, Fig. 5), with both distance treatments ($\chi^2 = 36.51$, $df = 2$, $p < 0.000$) and size of forest fragments ($\chi^2 = 8.01$, $df = 1$, $p < 0.004$)

contributing. In contrast, a model containing only the distance treatment was the best supported in explaining species richness (Table 2, Fig. 6), with distance treatments ($\chi^2 = 8.55$, $df = 2$, $p = 0.01$) showing difference among treatments, but not the size of forest fragments ($\chi^2 = 2.62$, $df = 1$, $p = 0.1$). Ponds installed at the forest fragment edge hosted a higher species richness and abundance of frogs and toads than those located 100 m and 200 m into pasture (Figs. 5 and 6).

4. DISCUSSION

Our results show that breeding areas located both at the edge of and near to (50 m from) forest fragments supported greater abundance and species richness of frogs and toads than breeding areas located 100 and 200 m far from forest fragments in our southeastern Brazil study area. Similarly, many temperate-zone focused studies have reported an increase in abundance and species richness of amphibians in breeding areas near forest fragments (e.g. Loman 1988; Laan and Verboom 1990; Findlay and Houlihan 1997; Herrmann et al 2005). Among the only tropical-based studies ever conducted on this topic, Gascon et al (1999) recorded that species richness of frogs and toads in central Amazonia increased after forest fragmentation with some frogs mainly associated with matrix habitats occasionally invaded fragments, which contributed to the general increase in species richness. Similarly, Dixon and Martins (2008) in a study in Brazilian Atlantic Forest recorded two generalist and non-forest species, *Physalaemus cuvieri* and *Leptodactylus latrans* (also recorded in the present study) at forest edges.

Most frog and toad species recorded in the artificial ponds are generalist species associated with more than one biome in Brazil (IUCN et al 2006). According to Santos et al (2009), the climate of the region, which has a pronounced dry season, favors species with

flexible reproductive modes adapted to dessication resistance (Santos et al 2009). Despite our focal species suite being generalists, our results nevertheless showed that greater abundance and species richness of frogs and toads at the forest edge is not a simple consequence of augmenting species associated with forest with those from the forest interior, as in most studies. Rather forest edge-associated species represent a distinct suite of the region-wide species pool. These results stress that forest fragments despite their greatly reduced occurrence in tropical landscapes heavily transformed by agriculture nevertheless play an important role in sustaining frogs and toads in southeast Brazil.

Becker et al (2010) demonstrated that even species that usually avoid open habitats are forced to cross inhospitable matrix habitats when breeding sites are not present within forest fragments. Accordingly “habitat split” is a widespread phenomenon in fragmented landscapes increasing the chance of extinction for species with aquatic larvae. However, in contrast to the frog and toad species recorded in Brazilian Atlantic Forest by Becker et al. (2010), those recorded in this study do not avoid matrix habitats. The generalist species in this study used both pasture and the forest habitat and are obligated to cross open habitats because pasture despite its inherent risks when traversing it provide high quality habitat for anuran reproduction in the form of artificial water bodies created by agriculturalists for a variety of reasons including as a water source for cattle (Urbina-Cordona et al 2006). Thus, proximity to forest fragments that can provide refuges from heat and water stress likely is likely the main driver influencing the ability of frogs and toads to establish and sustain breeding populations in open land ponds.

Forest size was an important factor in this study affecting frog and toad abundance in some species but not overall species richness. It is widely recognized that large fragments are essential to some anurans dependent on certain microclimate conditions to their reproduction

or physiology (Pearman 1997; Vallan 2000; Haddad and Prado 2005, Cushman 2006). So-called “edge effects” in small fragments are more severe for air temperature, air moisture, soil moisture and light intensity showing higher variation when compared with large forest fragments (Murcia 1995; Laurence 1991). Therefore, because frog and toad species associated with open lands likely use the forest fragments as shelter and foraging (Silva and Rossa-Feres 2007) we emphasize the importance of conservation of forest fragments of adequate size in agricultural areas. Notably, in fragments of Brazilian Atlantic Rainforest, Dixo et al (2009) reported that genetic diversity in *Rhinella ornata* populations, a species with relatively high dispersal capacities, was lowest in the smallest fragments (1-5.5 ha), likely due to decreases in population sizes. These studies together suggest that preservation of forest fragments larger than 70 ha in agricultural areas will harbor a higher number of individuals than smaller ones and consequently contribute to maintenance of more viable frog and toad populations.

5. CONCLUSION

Our results emphasize the value of conserving forest fragments to protect amphibian diversity and abundance in tropical agro-savannah landscapes, even for species typically identified primarily as breeders in ponds of open environments. More specifically, our results identify the importance of proximity between aquatic and terrestrial habitats in determining local patterns of abundance and species richness. Preservation of forest fragments larger than 70 ha in agricultural areas will also generally enhance local population sizes and thereby overall population viability. Furthermore, artificial ponds can be a useful, experimental approach for determining how spatial factors affect the distribution pattern of frogs and toads.

6. ACKNOWLEDGEMENTS

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7. TABLES

Table 1 – Number of species and abundance of frogs and toads recorded in artificial ponds installed at different distances from forest fragments in the northwestern São Paulo State. In Experiment I artificial ponds were placed at the forest edge, -50 and -100 m from the edge toward the interior of the forest fragments and + 50 e + 100 m from the edge toward the matrix. In Experiment II artificial ponds were installed at the edge, 100 and 200 m into the matrix.

	Experiment I					Experiment II		
	- 50	- 100	Edge	+ 50	+ 100	Edge	100	200
<i>Chiasmocleis albopunctata</i>		1 ^a	1 ^b			5 ^b		
<i>Dendropsophus nanus</i>				1 ^b				
<i>Dendropsophus minutus</i>						1 ^b		
<i>Eupemphix nattereri</i>			3			3	1	1
<i>Leptodactylus gr. latrans</i>							1	
<i>Leptodactylus fuscus</i>			8	10	5		1	2
<i>Leptodactylus mystacinus</i>	1		1 ^b					
<i>Leptodactylus mystaceus</i>	1		1					
<i>Leptodactylus labyrinthicus</i>				2				
<i>Pseudopaludicola aff. falcipes</i>				4 ^{b,A}	1			
<i>Physalaemus cuvieri</i>	1	1	1	1	2	6		
<i>Scinax fuscovarius</i>			1	1		4 ^{b,c}		
<i>Rhinella schneideri</i>			3		1	10		
Number of species	3	1	8	6	4	6	3	2
Total abundance	3	2	19	19	9	29	3	3

^a Recorded based on vocalization

^b Recorded in the larval stage

^c Pair recorded mating in the artificial pond

Table 2 – The *a priori* model set used to examine the relationship within tropical agro-savannah landscapes in southeastern Brazil between abundance and species richness of frogs and toads (response variables) in relation to distance treatment and forest remnant size (explanatory variables) based on generalized linear mixed-effects in two experiment with artificial ponds. k = number of parameters, ΔAIC_c = difference in Akaike's Information Criterion for each model from the most parsimonious model, wAICc = AICc weight, LL = negative log-likelihood, and %DE = percent deviance explained in the response variable by the model under consideration.

EXPERIMENT I	k	ΔAIC_c	wAICc	LL	Deviance	%DE
response variable: Abundance						
abundance ~ distance	6	0	0.611	-27.16	54.33	38.97
abundance ~ distance + size	7	1	0.372	-25.94	51.88	41.72
abundance ~ distance + size + distance * size	11	7.2	0.016	-20.27	40.53	54.46
abundance ~ size	3	23.5	<0.001	-43.29	86.57	2.75
response variable: Species richness						
richness ~ distance	6	0	0.649	-18.27	36.54	26.61
richness ~ distance + size	7	2.2	0.218	-17.64	35.28	29.15
richness ~ size	3	3.3	0.126	-24.27	48.54	2.53
richness ~ distance + size + distance * size	11	9.8	0.004	-12.66	25.33	49.13
EXPERIMENT II						
response variable: Abundance						
abundance ~ distance + size	5	0	0.841	-4.58	9.168	82.92
abundance ~ distance	4	3.4	0.157	-8.59	17.19	67.99
abundance ~ distance + size + distance * size	7	13.1	0.001	-4.48	8.973	83.29
abundance ~ size	3	28	<0.001	-22.84	45.68	14.93
response variable: Species richness						
richness ~ distance	4	0	0.585	-4.39	8.79	49.3
richness ~ distance + size	5	2.0	0.211	-3.08	6.16	64.45
richness ~ size	3	2.1	0.204	-7.35	14.71	15.15
richness ~ distance + size + distance * size	7	15.3	<0.001	-3.04	6.08	64.89

8. FIGURES

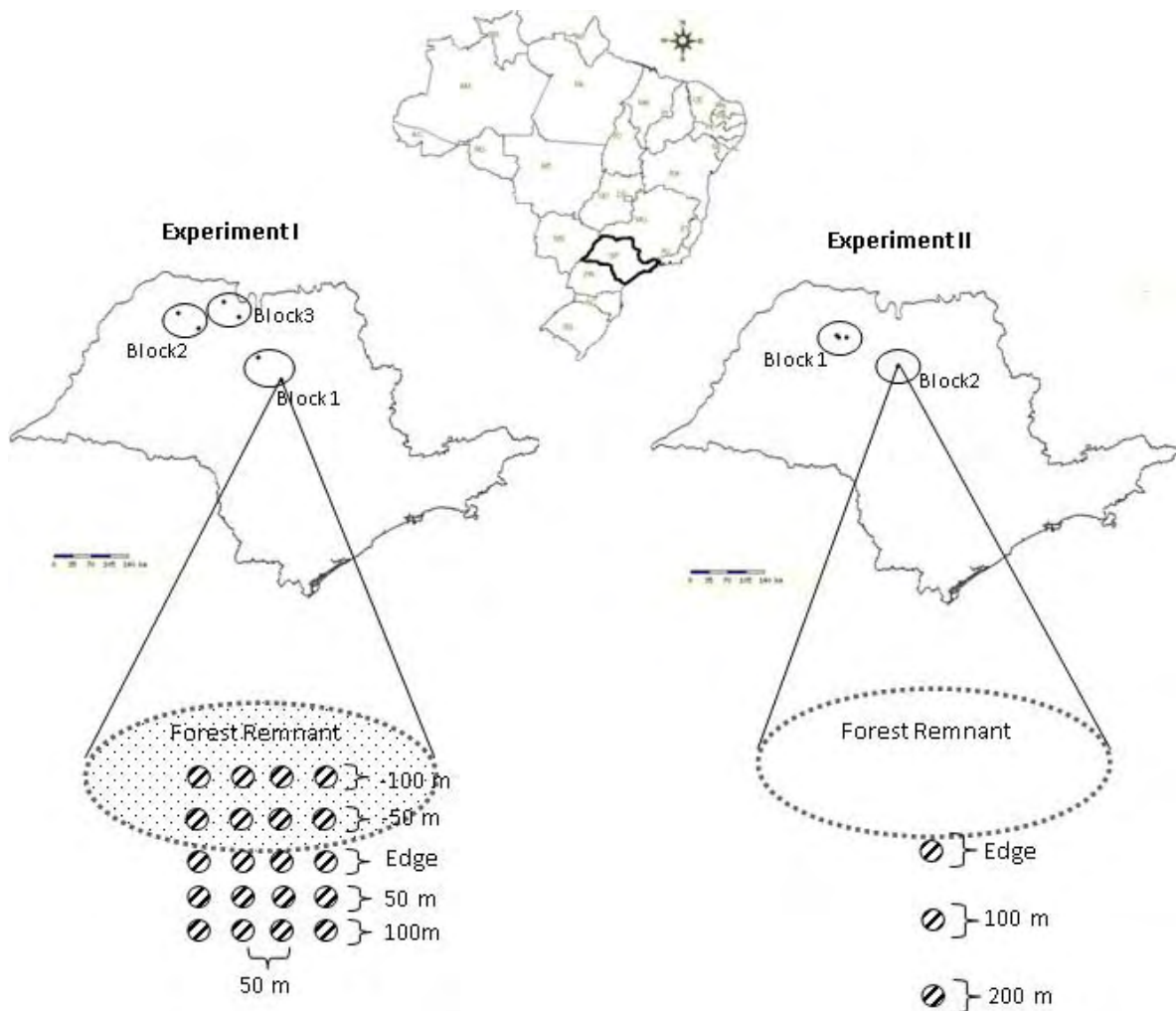


Figure 1 –Location of study area in Brazil. São Paulo State is highlighted showing the locations of the forest fragments (blue circle) grouped according to blocks sampled. Offset: Experimental design (without scale) involving artificial ponds. Experiment I: artificial ponds installed in the edge, 50 and 100 m from the edge toward the matrix and -50 and -100 m from the edge toward the interior of the forest fragments in the rainy season of 2007/2008. Experiment II: artificial ponds installed at the edge, 100 and 200 m of forest fragments in the rainy season 2008/2009.

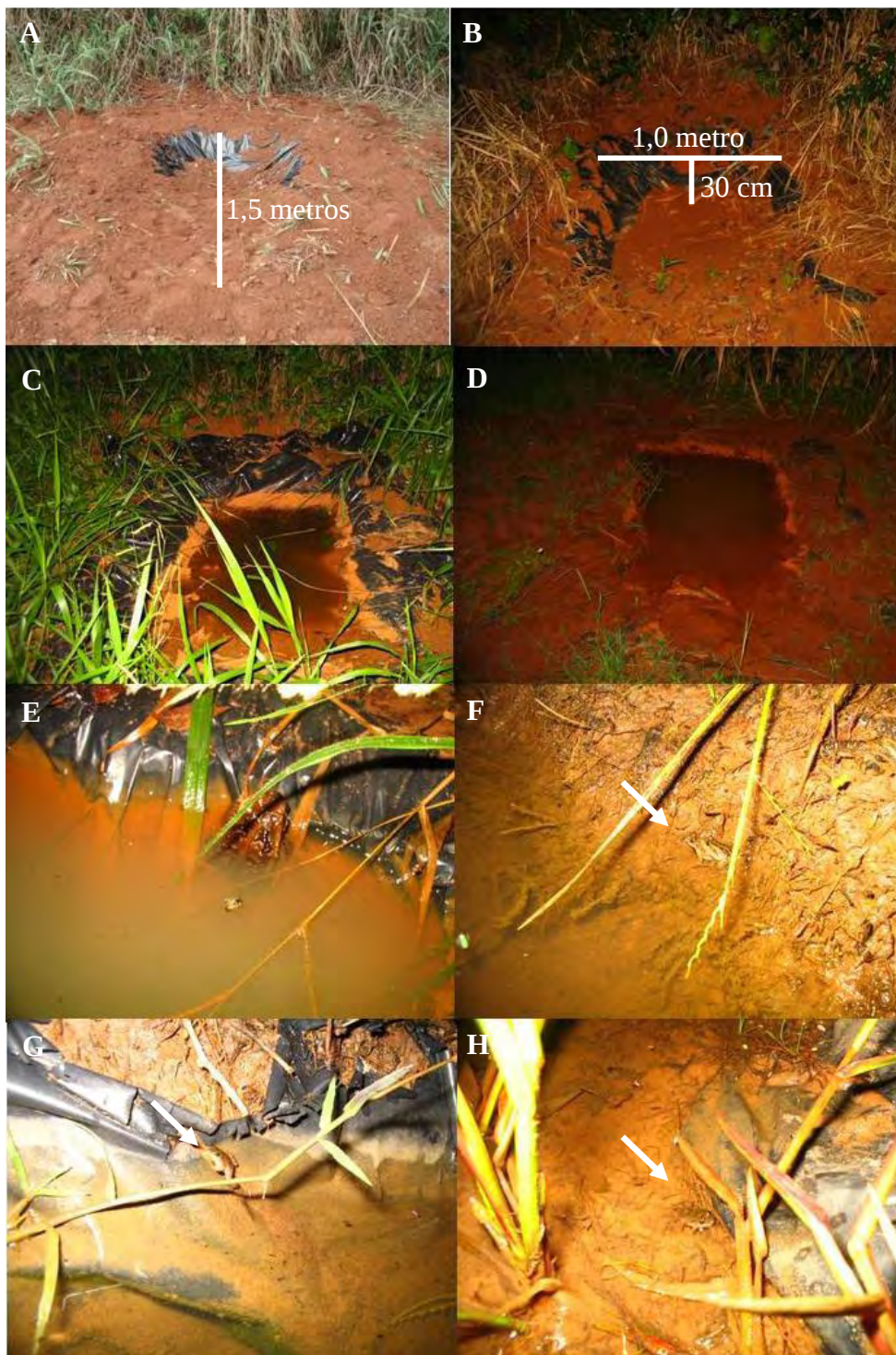


Figure 2 – Artificial ponds in rainy season 2007/2008 (Experiment I): (A) after installation; (B) dried in the end of sampling; (C e D) filled with rainwater; (E) *Rhinella schneideri*; (F) *Pseudopaludicola* aff. *saltica* in amplexus; (G) *Pseudopaludicola* aff. *saltica* in vocalization activity and (H) juvenile of *Leptodactylus labyrinthicus*.

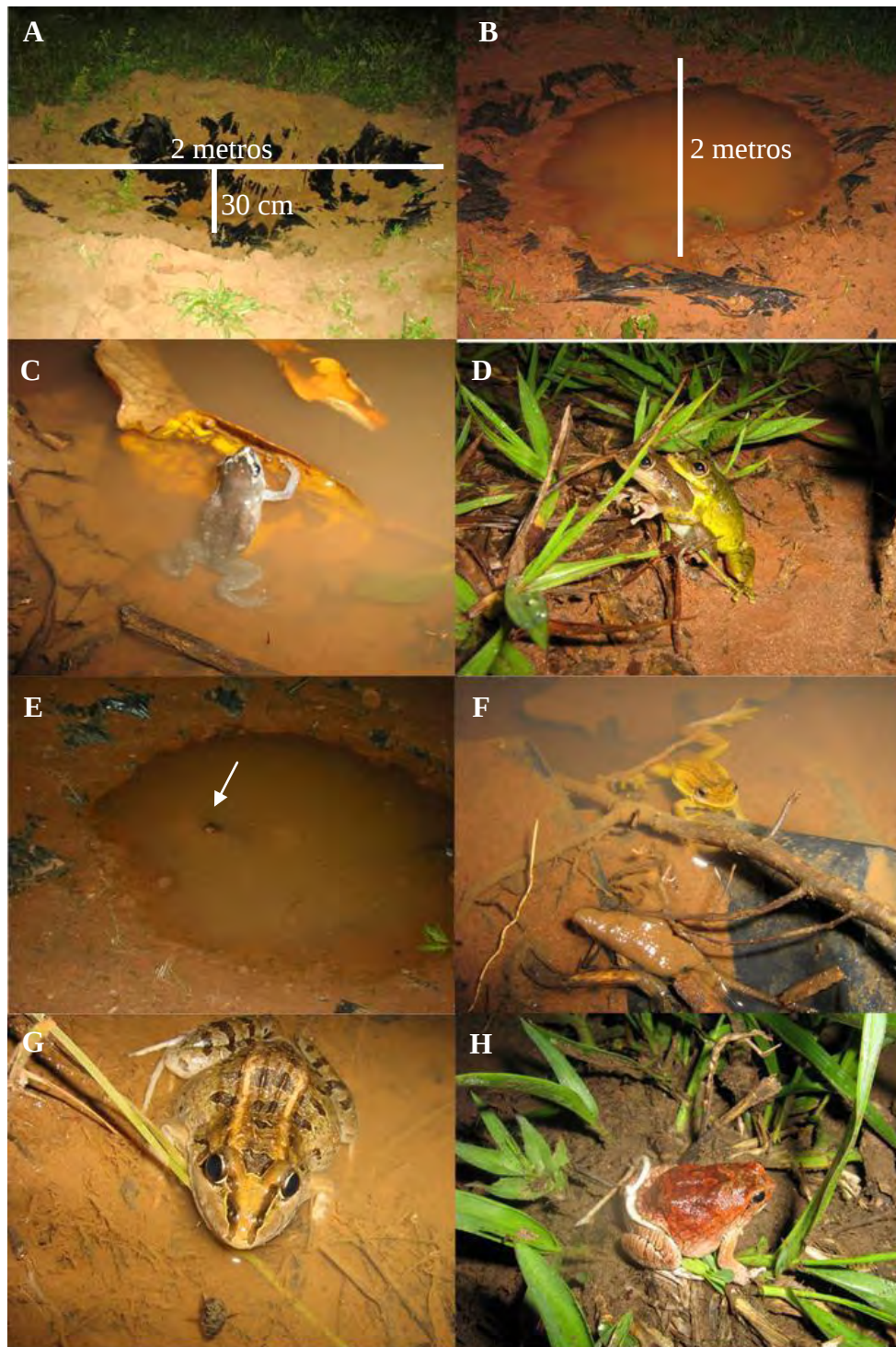


Figure 3 – Artificial ponds in the rainy season 2008/2009 (Experiment II): (A) after installation; (B) filled with rainwater; (C) *Chiasmocleis albopunctata* in vocalization activity; (D) *Scinax fuscovarius* in amplexus; (E) *Rhinella schneideri*; (F) *Dendropsophus minutus* in vocalization activity; (G) *Leptodactylus gr. ocellatus*; e (H) *Eupemphix nattereri* marked.

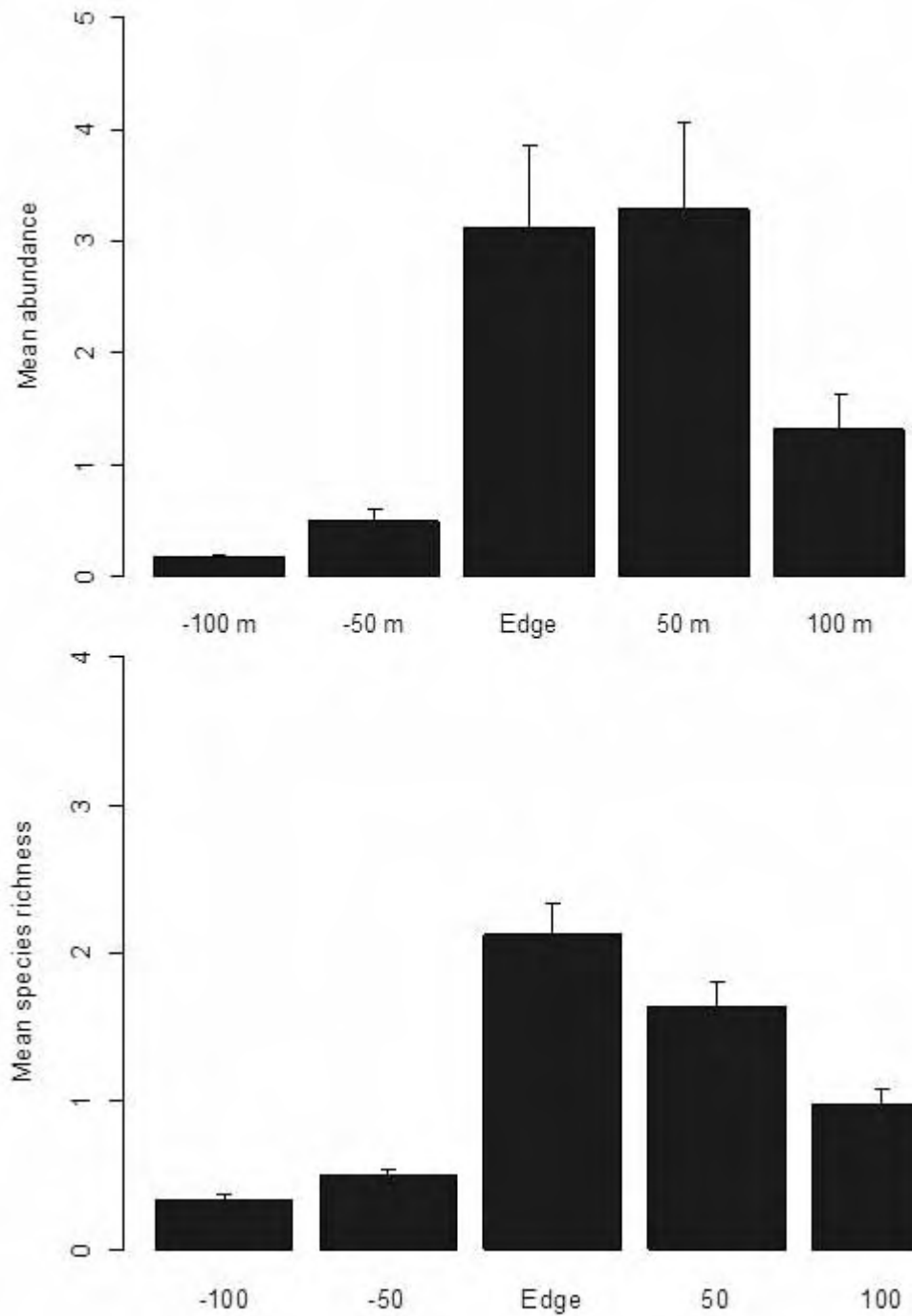


Figure 4 - (A) Mean abundance of frogs and toads (± 1 SE) and (B) mean species richness (± 1 SE) predicted by generalized linear mixed model in the artificial ponds installed at different distance from six forest fragments (Experiment I). Distances: Edge, 50 to 100 m from the edge toward the matrix (pasture) and -50 and -100 m from the edge toward the interior of the forest fragments.

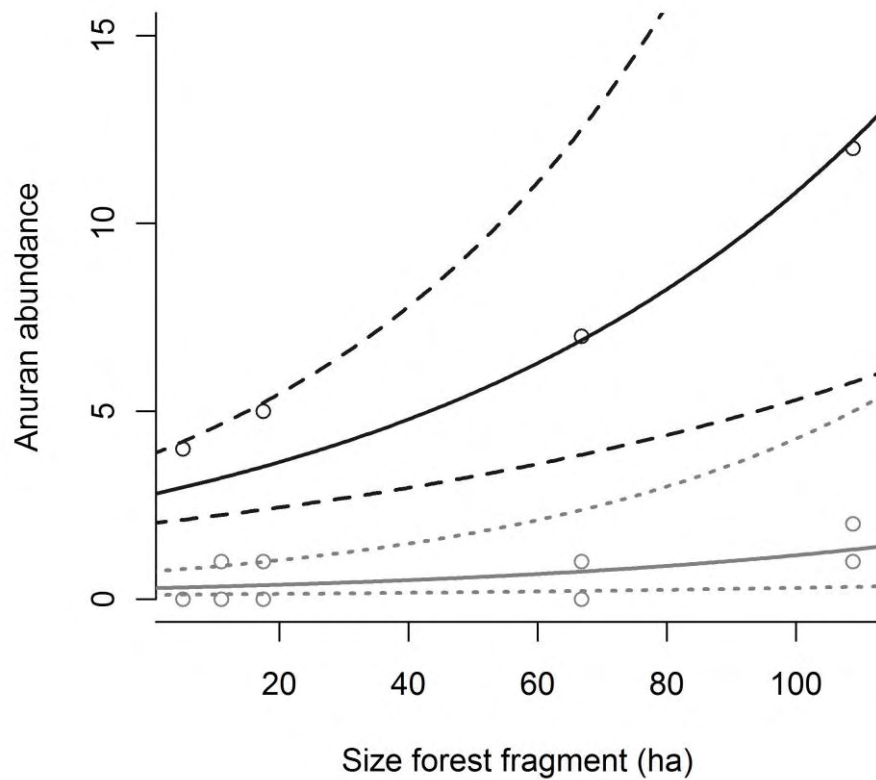


Figure 5 – Frog and toad abundance predicted by generalized linear mixed model (GLMM) in artificial ponds installed at different distance from five forest fragments (Experiment II). The lines represent the GLMM fit to the data with 95% confidence interval. Black circles = abundance recorded in artificial ponds installed at edge of forest fragments; Gray circle = abundance recorded in artificial ponds installed at 100 and 200 m from the edge toward the matrix (pasture).

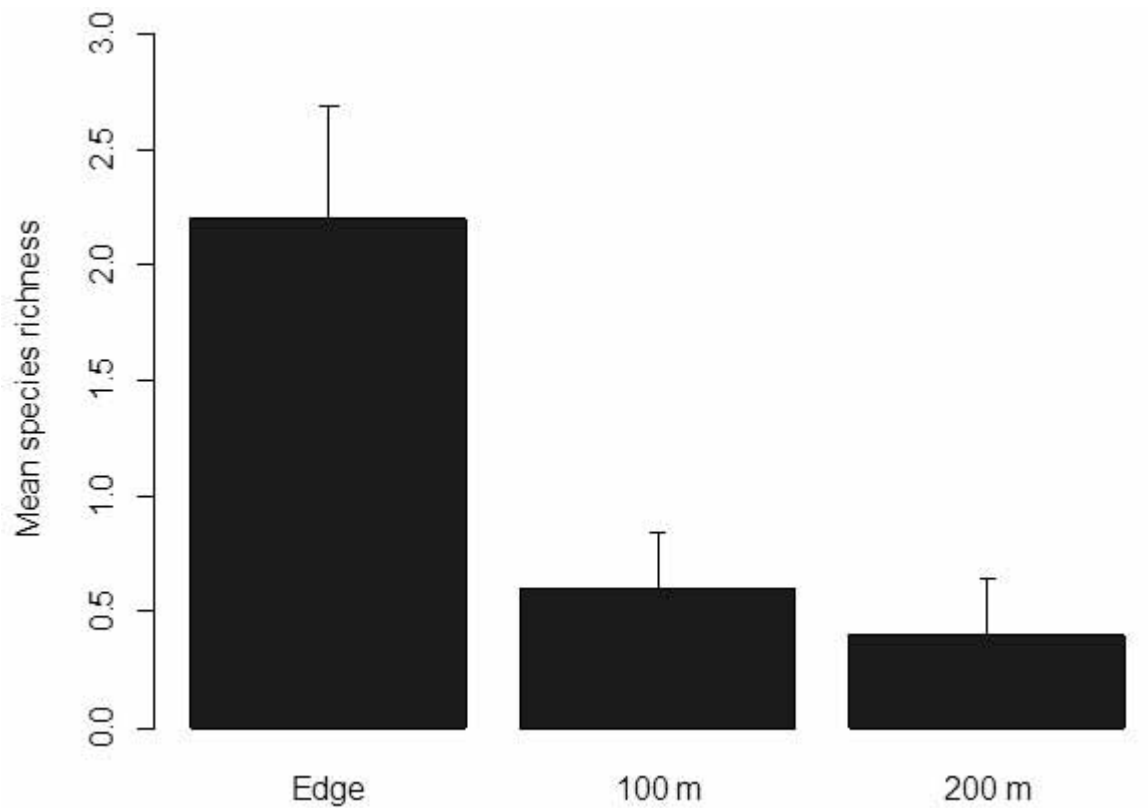


Figure 6 – Mean frog and toad species richness ($\pm 1SE$) predicted by generalized linear mixed model in artificial ponds installed at different distance from five forest fragments (Experiment II). Distances: Edge versus 100 to 200 m from the edge toward the matrix (pasture).

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Capítulo 2

Breeding habitat characteristics and landscape configuration explain frog diversity and abundance in tropical agricultural landscapes of southeastern Brazil

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Manuscript accepted to publication with major revision in Wetlands

ABSTRACT

Amphibians require multiple habitats to complete their life cycles, including aquatic habitat for breeding and larval growth and terrestrial habitat for adult growth, foraging, hibernation, and dispersal. We sought to evaluate the effect of environmental variables at local scale (breeding pools) and regional scale (landscape configuration) in determining species richness and abundance of altered landscapes anurans in a region in southeastern Brazil. Anuran communities of 18 temporary breeding pools were sampled every two weeks from October 2008 to March 2009. Two variables — hydroperiod and percentage of vegetation in the interior of the pools — explained approximately 62% of species richness variation. Three other variables — distance of pools to forest fragments, distance of pools to road and pool area — explained much of variation in anuran abundance. Our results indicate that both the local- and regional-scale variables, and most importantly their interaction, are important drivers of the structure of anuran communities in these agricultural landscapes. To facilitate amphibian conservation we suggest that cattle ranchers create and maintain heavily vegetated temporary pools located near legally protected forest reserves. These pools could be used by livestock as a water source and also would be used by anuran species that depend on breeding habitat outside of forest fragments. These science-based guidelines represent the first for preservation and conservation of breeding habitats for amphibians in this extensive region.

Keywords: amphibians; conservation; generalized linear model; breeding habitat; hierarchical partitioning; spatial scale; temporary pools.

1. INTRODUCTION

Understanding the processes that regulate distribution patterns of species and, consequently, that regulate the structure of communities has been a long-standing objective in ecology. The challenge facing community ecologists is to understand the interaction of processes operating at local (breeding habitats) and regional (landscape configuration) scales (Ricklefs 1987; Cornell and Lawton 1992; Caley and Schluter 1997). Amphibians have been a popular focus for ecologists to evaluate questions regarding the effects of local and regional variables on community structure (e.g., Babbitt et al. 2009; Werner et al. 2009; Karraker and Gibbs 2009; Bickford et al. 2010; Shulze et al. 2010; Wassens et al. 2010) because amphibians' complex life cycles are affected by habitat changes operating at different spatial scales. Local factors are known to affect tadpole survival and vocalization and oviposition sites for adults. Regional factors affect the dispersal capabilities of adults as well as juveniles which determines population connectivity and ultimately regional persistence of populations (Cushman 2006).

Local-scale variables have been identified as particularly important predictors of amphibian species richness, with hydroperiod predominant (Snodgrass et al. 2000; Otto et al. 2007; Babbitt et al. 2009; Karraker and Gibbs 2009). Additionally composition and quantity aquatic vegetation also influence species richness (Burne and Griffin 2005; Shulze et al. 2010). Recently increasing attention is being devoted to understanding the influence of landscape scale habitat components on the abundance distribution patterns of amphibians. These studies have revealed as important drivers of amphibian occurrence variables such as distance from breeding pools to forest fragments (Laan and Verboom 1990; Findlay and Houlihan 1997; Otto et al. 2007), native forest extent (Hecnar and M'Closkey 1998; Vallan 2000; Guerry and Hunter Jr. 2002) and road density (Fahrig et al. 1995; Gibbs 1998;

Eigenbrod et al. 2008; Sutherland et al. 2010).

Despite many evaluations of the influence of environmental variables on anuran occurrence, conclusions have been divergent, which may indicate that a single environmental descriptor has different effects in different ecological systems. For example, size of breeding pools can have a positive correlation (Burne and Griffin 2005; Werner et al. 2007), a negative correlation (Scheffer et al. 2006; Afonso and Eterovick 2007), or it can have no influence on species richness. Furthermore, most studies of environmental drivers of amphibian communities have been focused on temperate zone forests (Burne and Griffin 2005; Van Buskirk 2005; Karraker and Gibbs 2009; Werner et al. 2009; Shulze et al. 2010; Wassens et al. 2010), which represent an interesting yet distinct and relatively small component of the World's amphibian fauna. Species richness and abundance of anurans seem to be regulated by different factors, in tropical regions are related to rainfall, whereas in temperate regions are commonly correlated with temperature (Duellman and Trueb 1994; Pough et al. 2001). Therefore, it is impossible to assume that the dynamic evidenced in the temperate zones is directed applied to tropical zones without testing.

Amphibians of Brazil comprise the greatest component of frog and toad diversity in the World (AmphibiaWeb 2011). Despite this there are very few studies focusing on environmental drivers of amphibian communities in the Brazilian Atlantic Forest, considered a global biodiversity hotspot (Myers et al. 2000). There have been no studies of habitat relationships of amphibians in Mesophytic Semideciduous Forest, a subdivision of the Brazilian Atlantic Forest (Oliveira-Filho and Fontes 2000). The Mesophytic Semideciduous Forest is one of the most threatened types of tropical forest in the world (Jansen 1997), with only 4% of original cover remaining in north-western region of São Paulo state (SMA/IF 2005) now largely replaced by a matrix of pasture with sparse trees converted for cattle

ranching systems and, recently, sugar cane cultivation (Rodrigues et al. 2008).

Agricultural ecosystems of north-western region of São Paulo state, Brazil, host 37 anuran species adapted to anthropogenic habitats (Vasconcelos and Rossa-Feres 2005; Santos et al. 2007; Prado et al. 2008; Silva et al. 2009; Silva et al. 2010). Temporary ponds of this region provide breeding habitat for most anuran species (Santos et al. 2007) and are under threat particularly from sugarcane expansion for the production of ethanol. Although these ephemeral waters are highly vulnerable to human activities and threatened in many regions (Beja and Alcazar 2003), their biodiversity value is frequently overlooked (Snodgrass et al. 2000; Beja and Alcazar 2003), contributing to their neglect and inadequate management. Currently within the Brazilian government there is no conservation strategy currently employed to protect temporary or permanent pools from sugarcane expansion or other human activities. Identification of habitat features that are strongly associated with the occurrence of anuran species is therefore important for predicting impacts of habitat change and identifying key habitats for conservation (Wassens et al. 2010).

To address this knowledge gap, we evaluated the effect of environmental variables measured at local and regional scales in determining species richness and abundance of open-area anurans in 18 temporary pools located in grassland areas. Developing better understanding of the habitat and landscape-scale characteristics that are most important to pool-breeding amphibian communities will facilitate the development of conservation goals and priorities that will increase the effectiveness of conservation initiatives. We asked the following questions: (1) Which are the most important variables (local or regional) explaining the distribution pattern of open-area anuran in temporary breeding pools?; and (2) How do different species respond to these variables? By assuming that vegetation in the temporary pools increases the number of microhabitats available for reproduction and juvenile

protection (Egan and Paton 2004) and forest fragments are used as shelter during the dry season (Silva and Rossa-Feres 2007); we hypothesize that temporary pools with long hydroperiod, heavily vegetated and located near forest fragments will harbor both a greater species diversity and greater abundance of anurans.

2. MATERIAL AND METHODS

2.1. Study Area

This study focused on northwestern São Paulo State, Brazil (20° 33' S/50° 13' W to 21° 35' S, 48° 29' W, Fig. S1), which occupies an area of approximately 39 000 km² with 64 inhabitants/km² (Nalon 2008), where currently 37 anuran (frog and toad) species have been recorded, corresponding to almost 15% of the State's species and 5% of Brazil's anuran richness (Rossa-Feres et al. 2008). Most of the anuran species of this region are considered habitat generalists and are not listed as threatened species; lack of knowledge about species diversity in the region is of concern (Rodrigues et al. 2008). From the 37 anuran species recorded to the region, four have been recorded in the last three years (Prado et al. 2008; Silva et al. 2009; Silva et al. 2010).

The original vegetation of the region has been devastated by agricultural activities (Rodrigues et al. 2008). This impact puts the region as the most deforested and fragmented of the State, with the lowest concentration of conservation areas (Rodrigues et al. 2008). At present the region is characterized by an agricultural landscape which is dominated by small forest fragments embedded in grassland, sugarcane and rubber plantations (Rodrigues et al. 2008). Grassland system is still prevalent in the region; however, the recent sugarcane expansion in the region (Rodrigues et al. 2008; Joly et al. 2010) is of concern because temporary pools tend to be eliminated from sugarcane production areas.

The climate is characterized as seasonal tropical with annual mean temperature between 22°C

and 23°C with two well-defined climatic seasons: rainy season generally between October and March, and a pronounced dry season, between April and September, which receives only 15% of the total annual rainfall of 1100 mm ($\pm$ 225 mm) (Barcha and Arid 1971). The first month of the rainy season is unpredictable and the volume of rain is unstable, so temporary pools dry once or twice after the first rains (Santos et al. 2007). Most temporary pools in the region are used as cattle tank or irrigation channels by farmers. The temporary pools become dry between April and September. Despite agricultural pressure and high instability, temporary pools retain considerable conservation value for anurans (Beja and Alcazar 2003; Santos et al. 2007).

2.2. Methodology

Prior to fieldwork we selected 18 temporary pools (constructed pools, with dams and swamps excluded) in the pasture matrix not in contact with permanent flooded habitats. Sites were located by field reconnaissance because most pools used by anurans in the region are small ($< 400 \text{ m}^2$) and difficult to detect by satellite images. All pools selected were located in pasture matrix and at least 600 m from sugarcane, orange and rubber plantations. Population surveys of pools were conducted during rainy season, October 2008 to March 2009, every two weeks, for 12 surveys per pool, when most anuran species in the region are active (e.g., Vasconcelos and Rossa-Feres 2005; Santos et al. 2007). Fieldwork was conducted between 1900 to 2400 h. Site visits lasted 30 minutes/survey. Visual and acoustic surveys involved walking the perimeter of each pool upon arrival and recording the number of each species observed or heard calling. Abundance of each species was recorded by listening for calling males. Therefore, individuals not recorded while calling were left out of the abundance analysis but included in the species richness analysis. All voucher specimens collected were

deposited in the amphibian collection DZSJRP, of the Departamento de Zoologia e Botânica of UNESP, *campus* of São José do Rio Preto, SP.

2.3. Environmental variables

We used nine local- and regional-scale variables affecting the structure of anuran communities in various regions with different physiognomy and climate. All local variables were measured through field inspection between December 2007 and January 2008 when rainfall volume and anuran diversity was highest in the region. Local-scale variables included (Table 1): i) **temporary pool size (AREA)**: calculated in the field using a 200 m tape to measure length and width and extrapolated to pool size based on the formula of an ellipse given a = pool length, b = pool width, such that pool area = $a \times b \times \pi$ because all pools had an ellipse form,; ii) **hydroperiod (HDP)**: during the study pools accumulated water between one and six months, based on observations of pools in the field, hydroperiod classes were defined as short (≤ 3 months with water) or long (> 3 months with water); iii) **percentage of vegetation in the interior of the temporary pool (PVI)**: (1) 0 – 20%, (2) 21 – 50%, (3) 51 – 80% e (4) 81 – 100%; iv) **percentage of vegetation in the margins (PVM)**: (1) 0 – 20%, (2) 21 – 50%, (3) 51 – 80% e (4) 81 – 100%.; v) **heterogeneity of vegetation in the interior of the temporary pool (HVI)**: (1) prevalence of one type of vegetation, (2) emergent vegetation herbaceous, possibly including floating aquatic pteridophytes (*Salvinia* sp.) and/or umbellifers (*Eringyum* sp.), (3) herbaceous and shrubs vegetation and (4) herbaceous and shrubs vegetation and sparse trees; vi) **heterogeneity of vegetation in the margin (HVM)**: (1) prevalence of grassland, (2) grassland and other herbaceous vegetation, (3) herbaceous and shrubs vegetation and (4) herbaceous and shrubs vegetation and sparse trees. For each pool, PVI, PVM, HVI and HVM were visually estimated.

Landscape-scale variables included (Table 1): i) **distance from the pool to the nearest forest fragment (DFR)**: the area of forest fragments near focal pools which was highly variable (2 to 2189 ha); ii) **distance to the nearest breeding pool (DBA)**: because the number other pools around our focal pool was low we considered only distance to nearest other pools; iii) **distance to the nearest road (DR)**: only paved roads were considered because unpaved roads had very low traffic volume. All landscape-level variables were determined from recent high-resolution aerial photographs (Google Earth: <http://earth.google.com/>) of the region combined with field inspection.

2.4. Statistical Analysis

To evaluate if all pools were satisfactorily sampled in order to have an adequate representation of their species richness, we compare species richness recorded in all pools with two quality estimators (Jackknife I and II), using EstimateS 8.20 program (Colwell 2004). Estimators were generated as a function of accumulated number of samples in 500 randomizations (Colwell 2004).

Because ecological variables often do not constitute spatially independent observations due to the spatial structure of abiotic factors operating at different scales (Legendre 1993), we inspected all variables for spatial autocorrelation (through Moran's I test) using the package spdep (Bivand et al. 2009) and found none showed any evidence of spatial structure. Multi-collinearity among variables was examined with the variance inflation factor (VIF) using the package AED (Zuur et al. 2009), which identified HVM with a high VIF value (> 7.0) and therefore it was excluded from the analysis. After this procedure all VIF values were lower than four.

To investigate which variables explain the values species richness (number of species) of open-area anurans in the 18 temporary pools, we fitted generalized linear models (GLMs) to the data using the `glm` function implemented in the `nlme` package (Pinheiro et al. 2009), with Poisson distribution and log link function. To determine the optimal model, we started with a model in which the fixed component contained all explanatory variables. We used Akaike's information criterion, corrected for small sample sizes (AICc, Burnham and Anderson 1998), to select explanatory variables that were driving total species richness. We used Akaike weights to evaluate model-selection uncertainty. We calculated the percentage deviance explained (%DE) to model selected as a measure of goodness-of-fit.

The relative importance of local and landscape variables in the abundance for each anuran species was examined by use of hierarchical partitioning (Mac Nally 2000, 2002), using the `hier.part` package (Walsh and Mac Nally 2008). Because the data for species abundance were overdispersed, the analysis was realized with a quasi-Poisson distribution. Hierarchical partitioning compares all possible models in a multiple regression setting and determines the independent capacities of the predictive variables to explain the patterns of variability in the corresponding response variable (Chevan and Sutherland 1991). For each predictor, its independent explanatory power on the dependent variable is characterized with an index "I," which reflects the independent contribution of the predictor to the variance explained by the models. A second parameter "J" measures the interaction between each predictor and the others. Variables that independently explained a larger proportion of variance than by chance were identified using randomization tests (Mac Nally 2002). For each predictor, the observed contribution to the explained variance (I) was compared to the distribution of a population of I's of 1000 randomizations of the data matrix. Significance was accepted at the upper 95% confidence limit (Z score 1.65) (Mac Nally 2002). For this

analysis, the species abundance was considered the greatest abundance registered among the 12 samples realized in each pool. This approach was adopted because, according to Vasconcelos and Rossa-Feres (2005), it avoids both sub-estimates of population abundance, due to the use of the average of the samples, and super-estimates, due to the recounting of individuals in successive samples. Moreover, only species that occurred in at least six of the 18 temporary pools studied were considered in this analysis to diminish the noise caused by the presence of rare species. All statistical analyses were tested at 5% level and conducted using R Ver. 2.10.1 (R Development Core Team 2005).

3. RESULTS

We recorded 27 anuran species distributed among five families (Frost 2010): Bufonidae (1), Hylidae (12), Leiuperidae (5), Leptodactylidae (6) and Microhylidae (3) (Table S1). *Physalaemus cuvieri* Fitzinger 1826 and *Leptodactylus fuscus* (Schneider 1799) were recorded in all the pools whereas *Dendropsophus melanargyreus* (Cope 1887), *Hypsiboas faber* (Wied-Neuwied 1821), *Pseudopaludicola mystacalis* (Cope 1887), *Trachycephalus venulosus* (Linnaeus 1758) and *Elachistocleis cesarii* (Miranda-Ribeiro 1920) were in only one pool. The richness estimators produced stable estimates, close to the observed richness (Table S2), indicating that pools were adequately sampled.

Pools with a longer hydroperiod and pools with higher percentage of vegetation in the interior presented higher species richness than habitats with a short hydroperiod and lower PVI (Fig. 1). The model with hydroperiod and PVI was chosen as the most parsimonious (AICc = 91.81) with deviance explaining 61.14 % of variation of species richness (Fig. 1; Table 2).

From the 27 anuran species recorded in the pools only 11 species were recorded in at

least six pools and used in the hierarchical partitioning analysis (Table S3). Abundance of *Leptodactylus fuscus*, *Scinax fuscovarius* (Lutz 1925), *Physalaemus cuvieri* and *P. centralis* Bokermann 1962 were not influenced by any explanatory variable (Table S3) but among remaining species five environmental descriptors were important variables explaining their patterns of abundance. Heterogeneity of vegetation in the interior was the most important variable explaining the abundance distribution for *D. nanus* (Boulenger 1889) ($R^2 = 35.45\%$) and *Scinax fuscomarginatus* (Lutz 1925) ($R^2 = 33.04\%$) (Fig. 2). Distance from the pool to the nearest forest remnants was the most important variable explaining the abundance distribution for *Elachistocleis bicolor* (Guérin-Méneville 1838) ($R^2 = 39.04\%$), while Distance from the pool to the nearest forest remnants and hydroperiod were important variables for *Eupemphix nattereri* Steindachner 1863 ($R^2 = 23.67$ and $R^2 = 22.73\%$, respectively) (Fig. 2). Distance from pool to the nearest road was the most important variable explaining the abundance distribution for *D. minutus* (Peters 1872) ($R^2 = 45.98\%$), while DR and area were important variables for *Leptodactylus podicipinus* (Cope 1862) ($R^2 = 26.17$ and $R^2 = 24.14\%$, respectively) (Table S3, Fig. 2).

4. DISCUSSION

Our results showed that both local and regional variables are important drivers of the structure of open-area anuran communities in southeastern Brazil. Species richness was directly related to the hydroperiod and percentage of vegetation in the interior of the temporary pool. Species-specific responses of abundance to explanatory variables were observed, with percentage of vegetation in the interior of the temporary pools, distance from the pool until the nearest forest fragment, distance until the nearest road and hydroperiod being the primary variables explaining their distribution. Our studies emphasize that species diversity cannot be explained only by factors in local scale or of regional, but between factors

of both scales (see also Burne and Griffin 2005; Van Buskirk 2005; Karraker and Gibbs 2009; Bickford et al. 2010; Shulse et al. 2010; Wassens et al. 2010).

In many studies on amphibian communities reported to date, species richness of amphibians was correlated to temporary pools that presented intermediate to long hydroperiod (Semlitsch and Bodie 1998; Snodgrass et al. 2000; Egan and Paton 2004; Lichtenberg et al. 2006; Otto et al. 2007; Karraker and Gibbs 2009). The most ephemeral pools may retain only early breeders, whereas longer duration pools may hold both early and late breeders (Beja and Alcazar 2003), since hydroperiod influences the period during which a pool is available for colonization. This may contribute to species richness increasing with hydroperiod via the addition of species that breed progressively later in the season (Hecnar and M'Closkey 1998; Beja and Alcazar 2003). The climate severity of the sampled region (long and pronounced dry season, unpredictability and inconstancy of the rains in the beginning of the rainy season) and predator pressure are likely the primary factors that lead to the use of long-term temporary habitats for reproduction. For example, a study of Santa Fé do Sul municipality, in the northwestern São Paulo state, verified that short-term temporary breeding pools dried more than once during the rainy season, causing the death of thousands of tadpoles and the loss of tens of clutches (Santos et al. 2007). Therefore, for the majority of the species, it seems more advantageous to occupy long-term temporary breeding pools, where there are few predators, than to occupy breeding pools free from predators, but with high risk of desiccation (short-term temporaries) or even permanent breeding pools, but with great predator abundance (Wellborn et al. 1996).

In addition to hydroperiod, other variables were important drivers for some species: vegetation in the interior of the temporary pool, and the distance from pools to roads and forest fragments. The requirement of many amphibians for multiple habitats throughout their

life (Marsh and Trenham 2001; Gibbons 2003; Cushman 2006), including aquatic habitat for breeding and larval growth and terrestrial habitat for adult growth, foraging, hibernation, and dispersal, reinforces the importance of environmental variables operating at local and regional scales. The highest abundances of *S. fuscomarginatus*, and *D. nanus*, two small hyliid species that vocalize mainly perched in the vegetation, were recorded in pools with high heterogeneity of vegetation in the interior. Pools with high structural complexity may make the environment more appropriate to the anuran reproduction because it offers vocalization, amplexus and oviposition sites, as well as shelters from predators for recently metamorphosed tadpoles and adults (Egan and Paton 2004; Burne and Griffin 2005; Shulse et al. 2010; Wassens et al. 2010).

Notably, the highest abundances of *D. minutus* and *L. podicipinus* were recorded in pools distant from roads. Many studies suggest that roads present negative effects for many amphibians (Fahrig et al. 1995; Gibbs 1998; Cushman 2006; Eigenbrod et al. 2008; Sutherland et al. 2010). Habitat fragmentation by roads or other barriers diminishes dispersal (Gibbs 1998), increases mortality (Fahrig et al. 1995; Sutherland et al. 2010) and reduces genetic diversity (Reh and Seitz 1990). Distance from forests was also an important variable for some species. More specifically, pools near forest fragments presented higher abundance of *E. bicolor* and *E. nattereri*, two small burrowing frogs with probably low capacity of displacement, than pools distant from fragments, corroborating results of other studies (e.g., Laan and Verboom 1990; Findlay and Houlahan 1997; Herrmann et al. 2005; Otto et al. 2007). Furthermore, in a study with experimental pools installed in different distances from forest fragments in the same region verified that pools installed at edge of forest fragments presented higher species abundance of *E. nattereri* than those installed at 100 and 200 m far from the forest fragments (F.R. Silva, unpubl. data). The apparent vulnerability of many

amphibians due to physiological limitations (Duellman and Trueb 1986), low mobility (Gibbs 1998), and high mortality rate during displacement through roads or inhospitable areas (Fahrig et al. 1995; Becker et al. 2007) are factors that limit the colonization and favor the extinction of species in pools more isolated, maintaining metapopulation dynamics (Marsh and Trenham 2001; Cushman 2006). Therefore, populations in temporary pools with longer hydroperiod and higher vegetation may function as sources of colonists to temporary pools more isolated that experience localized extinctions, following the dynamic of source-sink populations (Pulliam 1988).

The fact that *Dendropsophus elianeae* (Napoli and Caramaschi 2000), *Leptodactylus fuscus*, *Physalaemus cuvieri* and *P. centralis* did not present any relationship with environmental descriptors can be explained by the spatial distribution of these species. *Leptodactylus fuscus* and *P. cuvieri* were registered on 18, and 17 temporary pools respectively with very similar abundances among pools (Table S1). On the other hand, *P. centralis* was registered on six pools and in five of them with just one individual (Table S1). Therefore, we failed to associate the distribution of the abundance of these species with any environmental variable because they were generalist species on the use of pools or rare species with low abundance among pools.

5. CONCLUSION

Despite our focal species suite being comprised of generalist species, our results show that contribution of local and regional features has important implications for understanding anuran distributions in an agricultural landscape. Descriptors related with complexity of the habitat and isolation of temporary pools from roads and forests are important variables

explaining the abundance and species richness of anuran. Temporary ponds of this region provide breeding habitat for most anuran species (Santos et al. 2007) and are under threat from sugarcane expansion for the production of ethanol (Rodrigues et al. 2008). There is no conservation strategy currently employed by the Brazilian government to protect temporary or permanent pools from sugarcane expansion or other human activities. However, the Brazilian government requires the creation of a legal reserve or forest area within every rural property, equivalent to at least 20% of the total area of the property (laws nº 4.771/65; 7.803/89). We suggest that preservation of pools could occur through collaborative initiatives with farm owners. Cattle ranchers could maintain and even create temporary pools heavily vegetated and located near their legal reserve. These pools could be used by livestock as a drinking water source and would be useful targets for conservation and management of anuran species that depend on breeding pools outside of forest fragments. Our study presents the first science-based guidelines for preservation and conservation of temporary pools for anurans in this vast and biodiversity rich regions heavily altered by human activities.

6. ACKNOWLEDGMENTS

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7. TABLE

TABLE 1 – Summary of local- and regional-scale environmental variables from 18 temporary breeding pools in the northwestern São Paulo state, Brazil. See text for details.

	Area (m ²)	Hydroperiod	% Vegetation Interior	% Vegetation Margin	Heterogeneity Vegetation Interior	Heterogeneity Vegetation Margin	Distance nearest fragment (m)	Distance nearest pool (m)	Distance nearest road (m)
P1	775.11	2	2	3	1	1	5	82	215
P2	37.5	1	2	3	1	1	306	115	196
P3	102.3	1	2	2	1	1	163	22	140
P4	339.1	2	3	4	3	4	0	102	20
P5	256.3	2	2	2	2	3	133	800	15
P6	676	2	2	4	1	4	0	290	10
P7	348.5	2	1	3	1	1	800	50	5
P8	231.6	2	3	4	3	4	45	485	89
P9	400.3	1	1	2	1	2	855	55	20
P10	618.4	2	3	4	2	4	45	80	85
P11	123.4	1	1	2	1	1	710	103	120
P12	152	1	4	4	1	1	550	245	25
P13	337.4	2	2	4	3	4	0	80	258
P14	300.7	2	2	3	2	2	210	50	118
P15	384.6	1	3	2	1	1	70	391	40
P16	73.4	1	2	3	1	2	10	175	0
P17	539	2	2	4	2	3	77	300	25
P18	86.3	2	2	3	2	2	60	250	5

TABLE 2 –The a priori model set used to examine the relationship between anuran species richness (response variables) with environmental variables, in 18 temporary breeding pools in the northwestern São Paulo state, Brazil, in the rainy season 2008/2009. $\Delta AICc$ = difference in Akaike's Information Criterion for each model from the most parsimonious model; k = number of parameters; wAICc = AICc weight; and %DE = percent deviance explained in the response variable by the model under consideration.

Models	$\Delta AICc$	k	weight	%DE
richness ~ HDP + PVI	0	3	0.31	61.14
richness ~ HDP	0.1	2	0.29	50.72
richness ~ HDP + PVI + PVM	0.2	4	0.28	71.94
richness ~ HDP+ PVI + PVM+ DFR	2.6	5	0.08	77.06
richness ~ HDP+ PVI + PVM+ DFR + DBA	6.3	6	0.01	80.41
richness ~ HDP+ PVI + PVM+ DFR + DBA + HVI	11.5	7	0.001	81.47
richness ~ 1	12.5	1	<0.001	0
richness ~ HDP+ PVI + PVM+ DFR + DBA + HVI + AREA	18.2	8	<0.001	82.02
richness ~ HDP+ PVI + PVM+ DFR + DBA + HVI + AREA + DR	26.6	9	<0.001	82.11

8. FIGURES

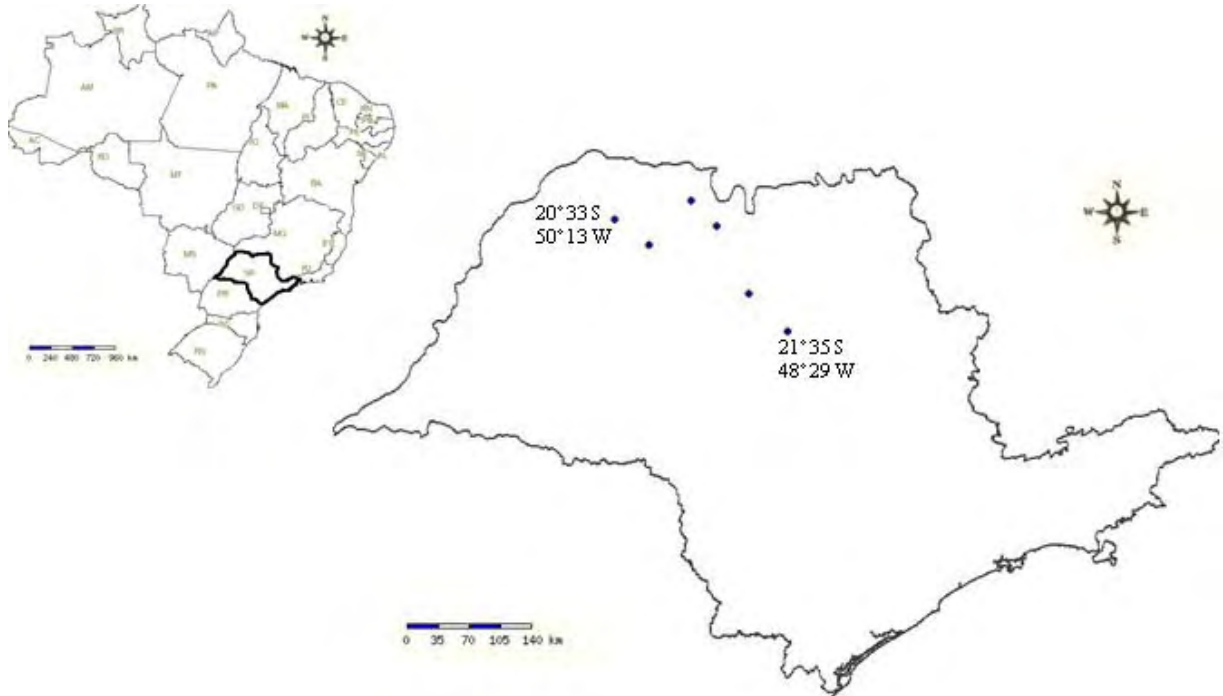


Figure 1 – Location of study area within Brazil, with São Paulo state highlighted, showing the limits of the northwestern region of the state and the location of the six municipalities where the 18 temporary ponds were sampled in this study in 2008 and 2009 (blue circles).



Figure 2 – Breeding areas sampled in the municipalities of Pindorama (A – C) e Palestina (D – F).



Figure 3 – Breeding areas sampled in the municipalities of Onda Verde (A – C), Matão (D – E) e Macaubal (F).



Figure 4 – Breeding areas sampled in the municipalities of Macaubal(A – B) e Magda(C – F).

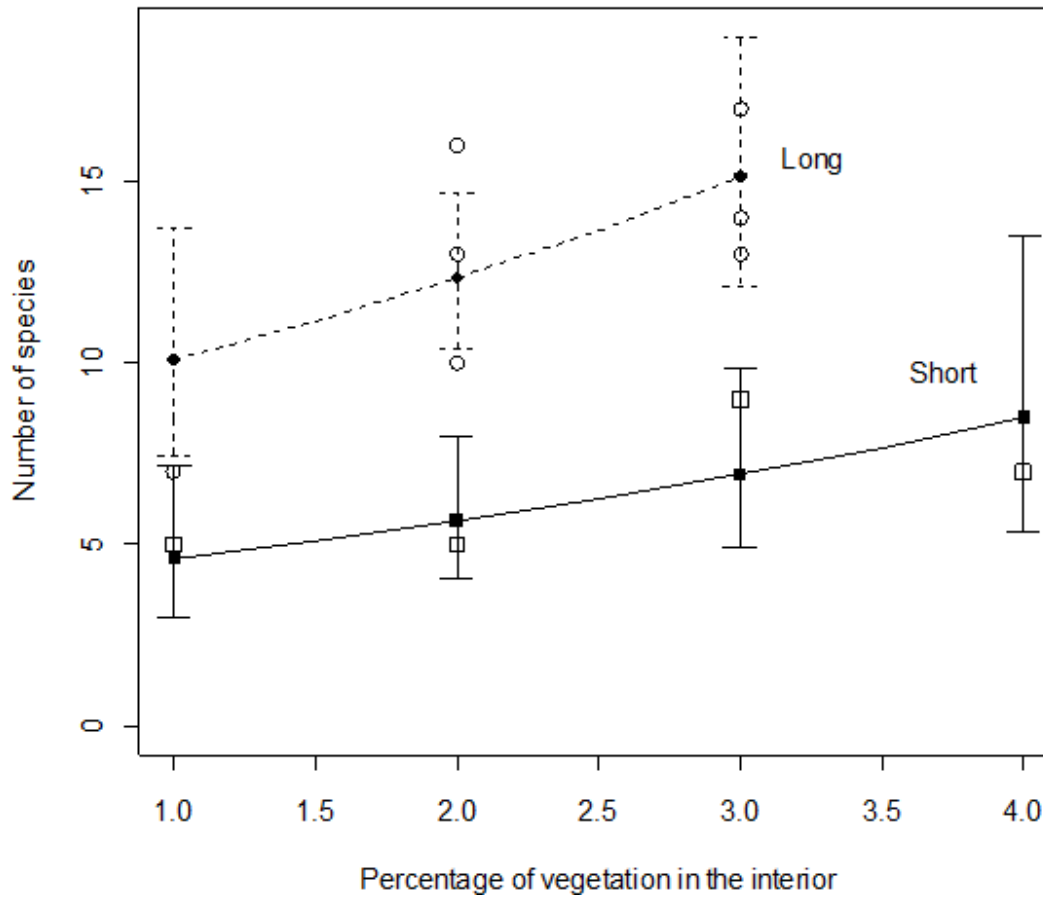


Figure 5 – Predicted number of anuran species in 18 breeding pools of different hydroperiod and percentage of vegetation in the interior (PVI), sampled in northwestern São Paulo state Brazil in the rainy season 2008/2009. The lines represent the GLM fit to the data, with 95% confidence intervals. Open circles: observed species richness in relation to long hydroperiod; open square: observed species richness in relation to short hydroperiod; black circles: estimated species richness in relation to long hydroperiod; black square: short hydroperiod. GLM (number of species) = $0.9354 + 0.6158 * \text{hydroperiod} + 0.1768 * \text{PVI}$.

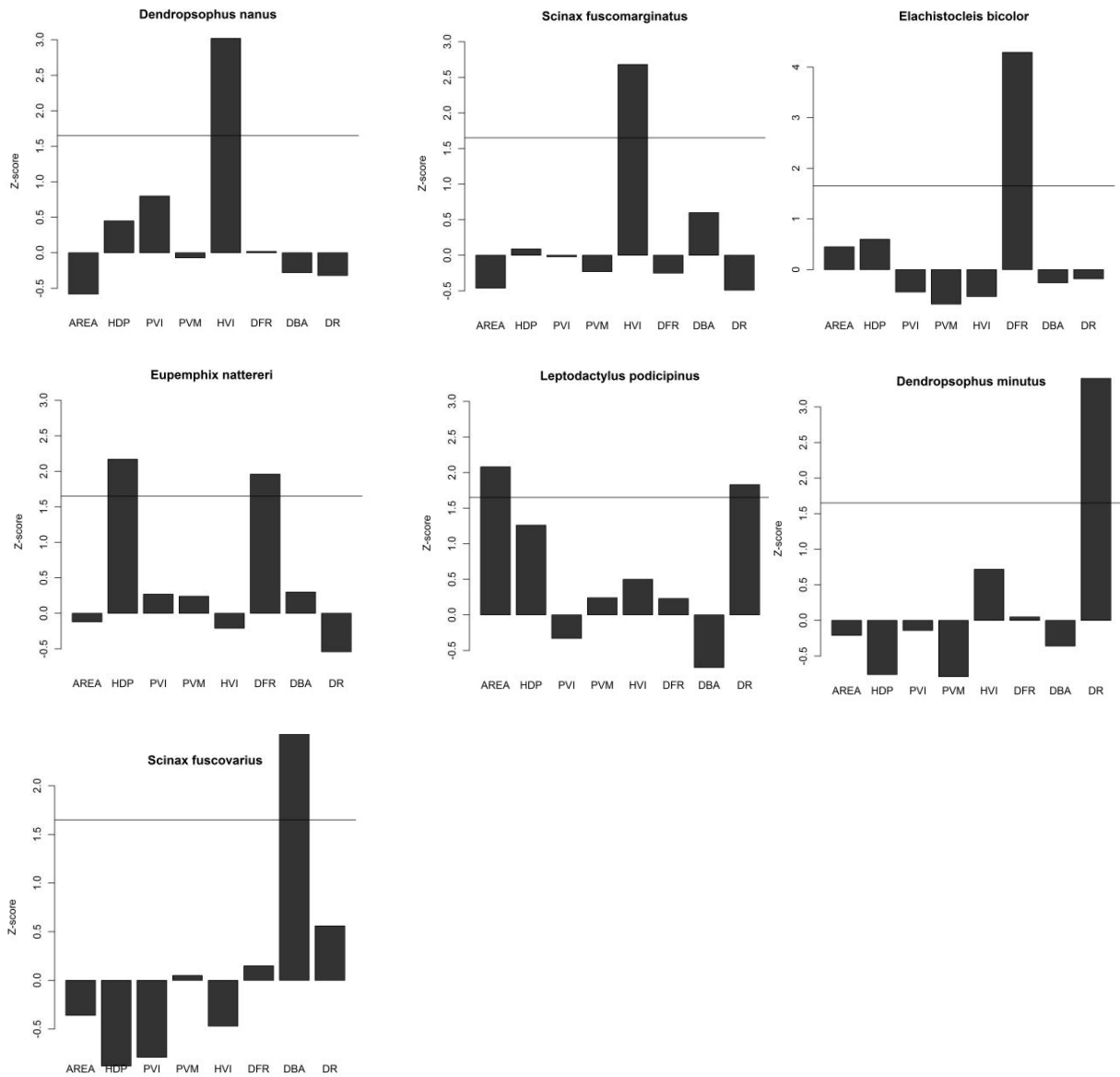


Figure 6 – Independent contributions of environmental drivers to abundance of 11 anurans species sampled in northwestern São Paulo state Brazil in the rainy season 2008 - 2009. Plots are Z-scores for independent contributions, I , from randomizations of data matrices for potential explanatory predictor variables for abundance of each species. The horizontal line represents upper 95% confidence value for the Z-scores. AREA = breeding pool size; HDP = hydroperiod; PVI = percentage vegetation in the interior; PVM = percentage of vegetation in the margin; HVI

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= heterogeneity of vegetation in the interior; DFR = distance from the nearest forest fragment;

DBA = distance from the nearest breeding pool, DR = distant from the nearest road.

9. APPENDIX 1 – Anuran species recorded in 18 temporary ponds distributed in six municipalities in the northwestern São Paulo state, Brazil, from October 2008 to March 2009.

	Macaubal			Magda			Matão		Pindorama			Onda Verde			Palestina			
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18
<i>Dendropsophus elianeae</i>			V	V	V	V					V	V	V	V	V	V	V	V
<i>Dendropsophus melanargyreus*</i>					V													
<i>Dendropsophus minutus</i>		V	V	V	V	V		V	V	V	V	V	V	V	V		V	V
<i>Dendropsophus nanus</i>	V		V	V	V	V		V		V	V	V	V	V	V	V	V	V
<i>Dermatonotus mulleri</i>			V	V	V		V											
<i>Elachistocleis bicolor</i>	V		V	V	V	V	V	V		V	V	V	V	V		V		V
<i>Elachistocleis sp.</i>		V	V															
<i>Eupemphix nattereri</i>		V	V	F	V		V		V			V		V			V	
<i>Hypsiboas albopunctatus</i>	V					V		V					V			V		
<i>Hypsiboas faber</i>								V										
<i>Hypsiboas raniceps</i>	V			V		V							V			V		
<i>Leptodactylus chaquensis</i>	F			F	V	F	V				F		F			F	F	F
<i>Leptodactylus fuscus</i>	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
<i>Leptodactylus labyrinthicus</i>								V			F		V			V		
<i>Leptodactylus mystacinus</i>					V			V			V	V						
<i>Leptodactylus ocellatus</i>	F			F													F	
<i>Leptodactylus podicipinus</i>	V			V	V	V		V					V			V	V	
<i>Physalaemus centralis</i>			V	V	V	V						V					V	
<i>Physalaemus cuvieri</i>	V	V	V	V	V	V		V	V	V	V	V	V	V	V	V	V	V
<i>Pseudopaludicola falcipes</i>	V		V			V										V	F	
<i>Pseudopaludicola mystacalis</i>													V					
<i>Pseudis paradoxo</i>	V			V	V												V	
<i>Rhinella schneideri</i>	V	F	F	V	F					V			V			F	V	
<i>Scinax fuscomarginatus</i>	V		V	V		V		V					V		V	V	V	
<i>Scinax fuscovarius</i>		V	V	V	V	V	F	V	F	V	V	V	V	V	V		V	V
<i>Scinax similis</i>								V									V	V
<i>Trachycephalus venulosus</i>							V											
Number of species	13	6	13	17	16	13	7	13	5	5	10	10	14	5	7	13	16	9

V = species registered in vocalization activity; F = species registered foraging in the breeding area, but not registered in vocalization activity.

* First register of the specie to São Paulo state (Silva et al., 2010 – Chapter 3).

Capítulo 2 - Appendix

Appendix 2 – Anuran species richness recorded and two quality estimators (Jackknife I and II) generated by 500 randomized in 18 temporary breeding pools in the northwestern São Paulo state, Brazil, from October 2008 to March 2009.

	Species richness	Jackknife I (mean $\pm$ SD)	Jackknife II (mean $\pm$ SD)
P1	11	13.79 $\pm$ 0	15.3 $\pm$ 0
P2	5	6.86 $\pm$ 1.86	6.99 $\pm$ 0
P3	12	17.57 $\pm$ 5.57	14.44 $\pm$ 0
P4	14	15.86 $\pm$ 1.26	14.41 $\pm$ 0
P5	15	17.79 $\pm$ 1.48	14.03 $\pm$ 0
P6	12	16.64 $\pm$ 2.2	18.55 $\pm$ 0
P7	6	10.64 $\pm$ 3.76	14.93 $\pm$ 0
P8	13	13.93 $\pm$ 0.93	13.99 $\pm$ 0
P9	5	5.93 $\pm$ 0.93	4.41 $\pm$ 0
P10	12	13.86 $\pm$ 1.26	13.99 $\pm$ 0
P11	5	9.64 $\pm$ 2.93	13.93 $\pm$ 0
P12	7	7.93 $\pm$ 0.93	4.83 $\pm$ 0
P13	13	17.64 $\pm$ 3.23	20.35 $\pm$ 0
P14	11	12.86 $\pm$ 1.26	12.99 $\pm$ 0
P15	8	11.71 $\pm$ 2.87	13.56 $\pm$ 0
P16	5	5.93 $\pm$ 0.93	4.41 $\pm$ 0
P17	10	13.71 $\pm$ 2.87	17.14 $\pm$ 0
P18	8	8.93 $\pm$ 0.93	9.79 $\pm$ 0

Appendix 3 – Hierarchical partition explaining the abundance of 11 anuran species in 18 temporary breeding pools distributed in six municipalities in the northwestern São Paulo state, Brazil, from October 2008 to March 2009. I = reflects the independent contribution of the predictor; J = measures the interaction between each predictor and the others predictors; and R^2 = percentage of total explained variance. * Significance values from 1000 randomizations of data. AREA = breeding pool size; HDP = hydroperiod; PVI = percentage vegetation in the interior; PVM = percentage of vegetation in the margin; HVI = heterogeneity of vegetation in the interior; DFR = distance from the nearest forest fragment; DBA = distance from the nearest breeding pool, DR = distant from the nearest road.

	I	J	R ²	I	J	R ²	I	J	R ²		
<i>Dendropsophus minutus</i>											
AREA	0.04	-0.001	7.2	AREA*	0.19	0.14	24.14	AREA	0.02	0.03	4.86
HDP	0.01	-0.003	2.55	HDP	0.12	0.22	15.53	HDP	0.14	0.1	26.55
PVI	0.05	0.06	9.28	PVI	0.02	0.03	3.72	PVI	0.06	0.02	12.29
PVM	0.01	0.002	2.54	PVM	0.06	0.17	8.56	PVM	0.05	-0.03	10.13
HVI	0.09	0.03	16.46	HVI	0.09	0.16	11.64	HVI	0.05	0.11	10.61
DFR	0.06	0.02	10.87	DFR	0.07	0.17	8.94	DFR	0.15	0.17	27.75
DBA	0.02	0.03	5.07	DBA	0.009	-0.009	1.26	DBA	0.02	0.04	5.01
DR*	0.25	-0.02	45.98	DR*	0.2	0.04	26.17	DR	0.01	0.001	2.76
<i>Dendropsophus nanus</i>											
AREA	0.01	-0.01	2.09	AREA	0.08	0.03	14.68	AREA	0.03	0.01	7.34
HDP	0.08	0.09	12.72	HDP	0.09	0.04	16.02	HDP	0.01	-0.007	2
PVI	0.11	0.08	18.45	PVI	0.03	-0.02	5.5	PVI	0.01	0.0007	2.25
PVM	0.05	0.11	8.56	PVM	0.02	0.02	5.04	PVM	0.06	-0.02	12.14
HVI*	0.22	0.18	35.45	HVI	0.02	0.01	5.06	HVI	0.02	0.005	5
DFR	0.06	0.11	9.77	DFR*	0.22	-0.17	39.04	DFR	0.06	0.001	12.37
DBA	0.03	-0.03	6.15	DBA	0.04	-0.02	7.45	DBA*	0.2	0.14	39.36
DR	0.04	-0.02	6.77	DR	0.04	-0.03	7.16	DR	0.1	0.03	19.51
<i>Leptodactylus podicipinus</i>											
AREA*	0.19	0.14	24.14	AREA	0.02	0.03	4.86				
HDP	0.12	0.22	15.53	HDP	0.14	0.1	26.55				
PVI	0.02	0.03	3.72	PVI	0.06	0.02	12.29				
PVM	0.06	0.17	8.56	PVM	0.05	-0.03	10.13				
HVI	0.09	0.16	11.64	HVI	0.05	0.11	10.61				
DFR	0.07	0.17	8.94	DFR	0.15	0.17	27.75				
DBA	0.009	-0.009	1.26	DBA	0.02	0.04	5.01				
DR*	0.2	0.04	26.17	DR	0.01	0.001	2.76				
<i>Elachistocleis bicolor</i>											
AREA	0.08	0.03	14.68	AREA	0.03	0.01	7.34				
HDP	0.09	0.04	16.02	HDP	0.01	-0.007	2				
PVI	0.03	-0.02	5.5	PVI	0.01	0.0007	2.25				
PVM	0.02	0.02	5.04	PVM	0.06	-0.02	12.14				
HVI	0.02	0.01	5.06	HVI	0.02	0.005	5				
DFR*	0.22	-0.17	39.04	DFR	0.06	0.001	12.37				
DBA	0.04	-0.02	7.45	DBA*	0.2	0.14	39.36				
DR	0.04	-0.03	7.16	DR	0.1	0.03	19.51				
<i>Scinax fuscovarius</i>											
AREA	0.03	0.01	7.34	AREA	0.03	0.01	7.34				
HDP	0.01	-0.007	2	HDP	0.01	-0.007	2				
PVI	0.01	0.0007	2.25	PVI	0.01	0.0007	2.25				
PVM	0.06	-0.02	12.14	PVM	0.06	-0.02	12.14				
HVI	0.02	0.005	5	HVI	0.02	0.005	5				
DFR	0.06	0.001	12.37	DFR	0.06	0.001	12.37				
DBA*	0.2	0.14	39.36	DBA*	0.2	0.14	39.36				
DR	0.1	0.03	19.51	DR	0.1	0.03	19.51				

TABLE S3 continued

	I	J	R ²	I	J	R ²	
<i>Scinax fuscomarginatus</i>							
AREA	0.03	0.02	5.85	<i>Physalaemus centralis</i>			
HDP	0.06	0.09	10.93	AREA	0.04	0.05	12.46
PVI	0.05	0.03	9.94	HDP	0.04	0.02	11.23
PVM	0.04	0.1	8.67	PVI	0.02	-0.02	7.48
HVI*	0.18	0.12	33.04	PVM	0.04	-0.003	11.18
DFR	0.04	0.09	8.73	HVI	0.07	-0.06	19.35
DBA	0.1	-0.05	18.89	DFR	0.07	-0.009	19.4
DR	0.02	0.02	3.91	DBA	0.01	0.003	4.53
<i>Eupemphix nattereri</i>							
AREA	0.04	-0.03	6.89	DR	0.05	0.003	14.33
HDP*	0.16	-0.13	22.73	<i>Physalaemus cuvieri</i>			
PVI	0.09	0.08	12.94	AREA	0.01	-0.01	3.92
PVM	0.06	0.04	9.68	HDP	0.1	0.08	31.99
HVI	0.04	-0.02	6.26	PVI	0.01	0.01	4.94
DFR*	0.16	0.01	23.67	PVM	0.02	0.02	6.39
DBA	0.1	-0.01	14.4	HVI	0.11	0.12	33.67
DR	0.02	0.02	3.4	DFR	0.03	0.07	11.43
<i>Dendropsophus elianae</i>							
AREA	0.01	-0.006	2.81	DBA	0.09	-0.07	19.51
HDP	0.01	-0.007	4.16	DR	0.02	-0.02	4.31
PVI	0.11	0.04	25.28				
PVM	0.03	0.04	7.2				
HVI	0.15	0.03	31.93				
DFR	0.02	0.03	4.77				
DBA	0.09	-0.07	19.51				
DR	0.02	-0.02	4.31				

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CONCLUSÕES FINAIS

- Our results identify the importance of proximity between aquatic and terrestrial habitats in determining local patterns of abundance and species richness. Preservation of forest fragments larger than 70 ha in agricultural areas will also generally enhance local population sizes and thereby overall population viability.
- Descriptors related with complexity of the habitat and isolation of temporary pools from roads and forests are important variables explaining the abundance and species richness of anuran. Temporary pools with long hydroperiod and high percentage of vegetation located near forest fragments and distant from roads will harbor a greater number of species and abundance of anuran and hence are useful targets for conservation and management of these amphibian communities.
- Despite our focal species suite being generalists, our results showed that factors affecting anuran abundance varied greatly among species. These inter-species differences likely emphasize the importance of conservation and species-specific management strategies considering.
- The record of two new species (*C. albopunctata* and *D. melanargyreus*) to the region highlights the need for inventories of anurans even in a region well studied as the northwestern state of São Paulo.

ANEXO 5

NOTES ON GEOGRAPHIC DISTRIBUTION

Amphibia, Anura, Microhylidae, *Chiasmocleis albopunctata*: Filling gap and geographic distribution map

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Chiasmocleis albopunctata (Figure 1) is a small microhylid frog recognized by the white or whitish bars on the region of snout, canthus rostralis, and upper eyelids; large whitish blotches over a gray background in ventral surfaces; and fingers and toes only slightly fringed (Caramaschi and Cruz 1997). According to Caramaschi and Cruz (1997) and Frost (2008), the geographic distribution of *C. albopunctata* is eastern Bolivia, Paraguay, and Brazil (Federal District and the states of Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais and São Paulo). The geographic distribution presented by Caramaschi and Cruz (1997), Brasileiro et al. (2005) and Bertoluci et al. (2007) shows a gap of approximately 375 Km between the Itirapina Ecological Station (state of São Paulo) and Uberlândia (state of Minas Gerais), and another gap of approximately 503 Km between the Assis Ecological Station (state of São Paulo) and Maracajú (state of Mato Grosso do Sul) (Figure 2).

Recently, specimens of *C. albopunctata* were collected in five municipalities of northwestern and one municipality in the westernmost region of the state of São Paulo, filling the distribution gaps in this state (Figure 2). Specimens were identified according to the diagnosis presented by Caramaschi and Cruz (1997) and deposited in the *Coleção do Departamento de Zoologia e Botânica* (DZSJRP) housed at UNESP, São José do Rio Preto, state of São Paulo, and *Coleção Célio F. B. Haddad* (CFBH) housed at UNESP, Rio Claro, state of São Paulo.

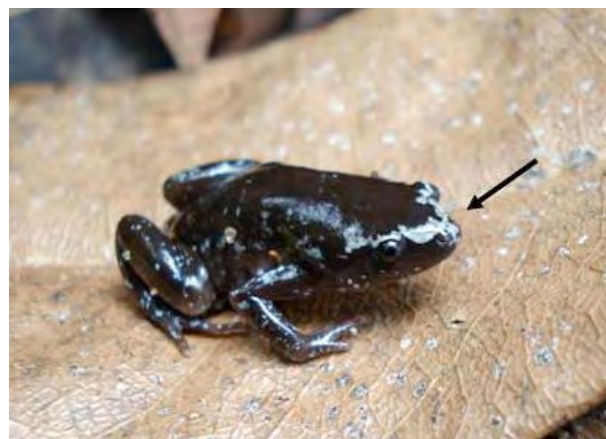


Figure 1. *Chiasmocleis albopunctata* from the municipality of Matão, São Paulo, Brazil. The arrow shows a white or whitish bar on the snout region, canthus rostralis, and upper eyelid, a diagnostic character of this species according to Caramaschi & Cruz (1997). Photo by V. H. M. Prado.

Five specimens of *C. albopunctata* (DZSJRP 11746-1750) were collected in January 2008 by pitfall traps installed into Mesophytic Semideciduous Forest fragments in the municipalities of Novo Horizonte (21°31'33" S, 49°18'08" W), Macaúbal (20°44'34" S, 49°55'42" W), and Matão (21°36'50" S, 48°32'03" W). One adult (DZSJRP 11384) and one lot of tadpoles (DZSJRP 1347-1) were collected in January 2008 in artificial ponds (1,5 x 1,0 x 0,3 m) installed in the edge and into Mesophytic Semideciduous Forest fragments in the municipality of Onda Verde (20°32'58" S, 49°14'59" W) and at the

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Pindorama Biological Reserve, municipality of Pindorama (21°13'20" S, 48°55'07" W), respectively. Eight adults (CFBH 10292, 18356, 18371, 18402; DZSJRP 11431, 11456-58) and two lots of tadpoles (CFBH 12084 and 14473) were collected between October 2005 and December 2007 in temporary ponds in the Morro do Diabo State Park, municipality of Teodoro Sampaio (22°31'38" S, 52°17'49" W).

Native vegetation at the northwestern region of the state of São Paulo, characterized by Mesophytic Semideciduous Forest and patches of *Cerrado* (South-American Savanna), have been replaced by pasture, plantations or urban areas, which reduced its original area to 4% (SMA/IF 2005). Nowadays, this region is considered the most deforested and fragmented region of the state (Kronka et al. 1993). According to Aquino et al. (2004), *C. albopunctata* is well adapted to anthropogenic disturbance and is

normally associated with temporary water bodies and flooded areas in forests, grasslands, and fields, where it breeds. It is important to emphasize that all specimens cited in this study were recorded in conservation units in the state of São Paulo (Itirapina Ecological Station, Assis Ecological Station, Morro do Diabo State Park, and Pindorama Biological Reserve), as well as in fragments of Mesophytic Semideciduous Forest (municipalities of Matão, Macaúbal, and Novo Horizonte). Although *C. albopunctata* breeds in ponds associated to pasture or agriculture areas, these new records indicate that habitat selection seems to be associated to the presence of forest fragments. Furthermore, the discovery of *C. albopunctata* in the northwestern region of the state of São Paulo shows that new records of Brazilian frogs may occur even on well sampled places, as already highlighted by Prado et al. (2008).

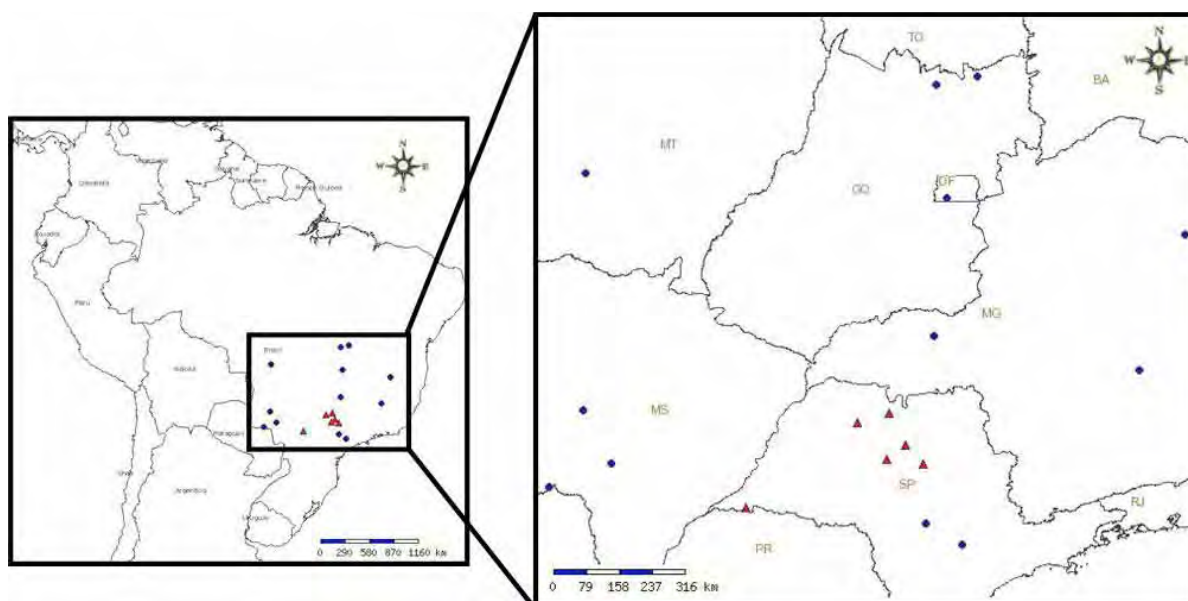


Figure 2. Geographic distribution map of *Chiasmocleis albopunctata*: blue circles represent known records from Caramaschi and Cruz (1997), Brasileiro et al. (2005), and Bertoluci et al. (2007); red triangles represent new records in São Paulo, Brazil.

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Amphibia, Anura, Hylidae, *Dendropsophus melanargyreus* (Cope, 1887): Distribution extension, new state record and geographic distribution map

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ABSTRACT: We present the first record of *Dendropsophus melanargyreus* for the state of São Paulo and a distribution map for this species. This new record represents the southeastern limit of distribution, which is 106 Km from the nearest locality previously recorded for this species and is the fourth new register of anuran to northwestern region of São Paulo in the last two years, increasing species list of the region from 33 to 36 species. This results evidence the importance of this region as priority area for inventory.

Dendropsophus melanargyreus (Cope, 1887) (Figure 1) is a hylid frog included in the *D. marmoratus* group (*sensu* Gomes and Peixoto 1996; Faivovich *et al.* 2005). According to these authors there are eight species in this group, and they are diagnosed by the warty skin around the margin of the lower lip, the crenulated margin of limbs, large vocal sacs, and the dorsal marbled pattern (Cochran 1955; Bokermann 1964). The *D. marmoratus* group is widespread in the South America occurring in Amazon, Atlantic Rain Forest, and Savannah formations, and the species can habit forested and open areas, reproducing as in permanent as in temporary ponds (Azevedo-Ramos *et al.* 2004a, b; Carvalho-e-Silva and Bertoluci 2004; Garcia and Silvano 2004; Peixoto and Pimenta 2004; Peixoto and Bastos 2004; Silvano *et al.* 2004; Silvano and Peixoto 2004). *Dendropsophus melanargyreus* is characterized

by dorsum brownish to dark yellow with dark brown blotches, limbs with transversal dark bands, hidden part of the thighs dark brown, males with vocal sac usually blackish, presence of small fringes on the external part of the members, and venter cream with dark edges (Cope 1887; Uetanabaro *et al.* 2008). According to Frost (2009) and Silvano *et al.* (2004) this species occurs in interior basins of Goiás and Mato Grosso to northeastern Brazil, Surinam and French Guiana; have been reported in the Departamento de Santa Cruz in Bolivia and the Pantanal of northeastern Paraguay (Alto Paraguay province). Herein, we present the first record of *D. melanargyreus* for the state of São Paulo, Brazil.

Two calling males were heard and collected in the municipality of Magda (20°33'08" S, 50°12'42" W), state of São Paulo, by F. R. Silva and V. H. M. Prado in 28 January

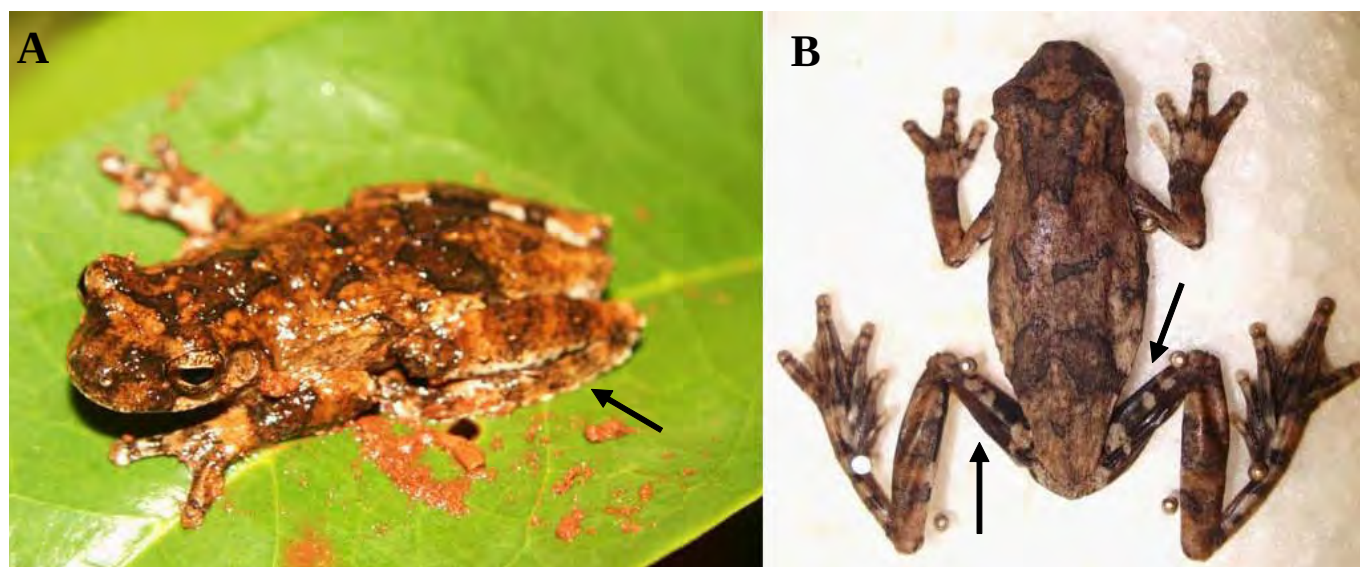


FIGURE 1. *Dendropsophus melanargyreus* from municipality of Magda, state of São Paulo. (A) Color in life: dorsum brownish to dark yellow with dark brown blotches, and the arrow shows the presence of small fringes on the external part of the members (Photo by Diogo Borges Provete); (B) fixed specimen (DZSJRP 11755; snout-vent length = 35.0 mm), the arrows show limbs with transversal dark bands and hidden part of the thighs dark brown (Photo by Vitor H. M. do Prado).

2009 (license number 18163-1/IBAMA). The specimens were found perching on shrubs on the bank of temporary pond in a pasture area, ca. 148 m far from a Mesophytic Semideciduous Forest fragment (*sensu* Kronka *et al.* 1993). The pond has 256.4 m² of total area, 50 cm of maximum depth, and 10 % of plant cover (Poaceae). The specimens were identified according to the diagnosis presented by Cope (1887), Caramaschi and Jim (1983) and Uetanabaro *et al.* (2008), and deposited in the Coleção do Departamento de Zoologia e Botânica (DZSJRP-11754-11755) housed at UNESP, São José do Rio Preto, São Paulo, Brazil.

This record now represents the southeastern limit of the distribution of *D. melanargyreus* (Figure 2), which is ca. 106 km from the nearest locality previously recorded for this species (Aparecida do Taboado, in the state of Mato Grosso do Sul, Brazil, 20°05'28" S, 51°06'09" W), according to Proyecto BOLFOR (1995), Cacciali and Scott (2004), Gottsberger and Gruber (2004), Coleção do Departamento de Zoologia e Botânica (DZSJRP-AMPHIBIA), Coleção Célio F. B. Haddad (CFBH) and Coleção de Anfíbios do Museu de Zoologia da UNICAMP (ZUEC-AMP), which data are available on http://splink.cria.org.br/centralized_search?criaLANG=pt, Coleção Herpetológica da Universidade de Brasília (CHUNB), Museu Nacional do Rio de Janeiro (MNRJ), Coleção da Universidade Federal do Mato Grosso (UFMT) and Coleção da Universidade

de Uberlândia (UFU), which data were provided by the curators of the collections, and Museu de Zoologia da Universidade de São Paulo (MZUSP), which data were obtained looking at the register book.

The record of *D. melanargyreus* is the fourth new register of amphibian species to northwestern region of São Paulo in the last two years (see Prado *et al.* 2008; Silva *et al.* 2009; Prado, V. H. M. unpublished data). According to Duellman (1999) and Santos *et al.* (2009) the anuran composition in the Mesophytic Semideciduous Forest is similar to those anuran assemblages recorded for Cerrado biome. Therefore, finding such species in this region of São Paulo state should be expected. This result confirms the prediction of Duellman (1999) and Santos *et al.* (2009) and also the indication in the "Diretrizes para conservação e restauração da Biodiversidade no Estado de São Paulo" (Rodrigues *et al.* 2008) that the northwestern region of the state is a priority area for inventory, as it represents a gap of knowledge for different taxonomic groups. Although anurans were well sampled in this region, the development of the project "Fauna e flora de fragmentos florestais remanescentes no noroeste paulista: base para estudos de conservação da biodiversidade" (BIOTA/FAPESP-Proc. 04/04820-3) in areas not yet sampled contributed to the increasing of the species list of the region from 33 (Candeira, C.P. unpublished data) to 36 species.

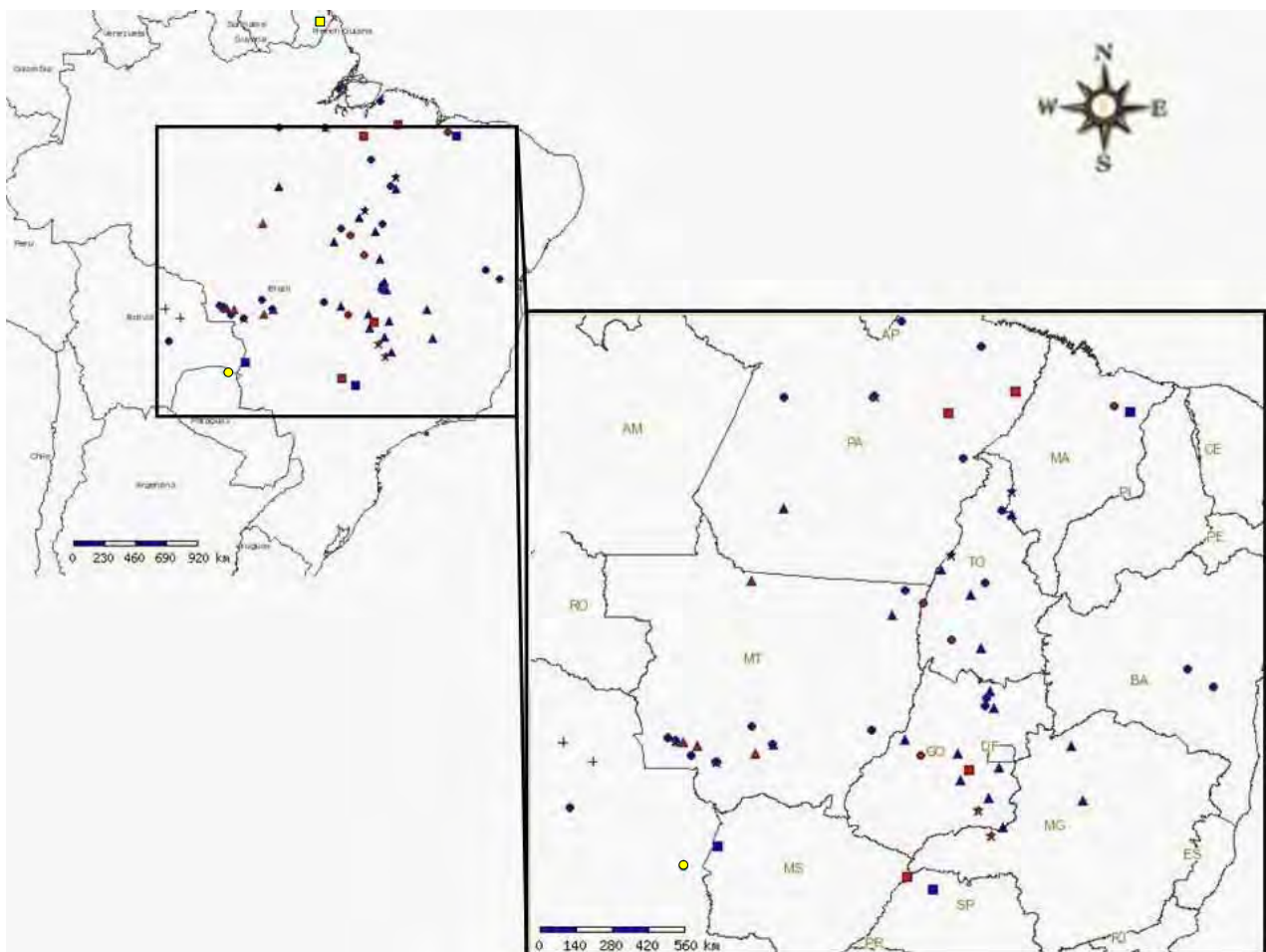


FIGURE 2. Geographic distribution map of *Dendropsophus melanargyreus*: according to Coleção Herpetológica da Universidade de Brasília (blue triangle), Coleção da Universidade Federal do Mato Grosso (red triangle), Museu de Zoologia da Universidade de São Paulo (blue circle), Coleção de Anfíbios do Museu de Zoologia da Universidade de Campinas (red circle), Coleção de Anfíbios do Departamento de Zoologia e Botânica de São José do Rio Preto (blue square, including the new record for state of São Paulo), Museu Nacional do Rio de Janeiro (red square), Coleção Célio F. B. Haddad (blue star), Coleção da Universidade Federal de Uberlândia (red star), Proyecto BOLFOR (1995, blue cross), Cacciali and Scott (2004, yellow circle) and Gottsberger and Gruber (2004, yellow square).

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