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Influence of microhabitat on the richness of anuran species: a case study of different landscapes in the Atlantic Forest of southern Brazil

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Abstract: Environmental heterogeneity is a factor which can help explain the higher local species richness. The objective of this study was to test if richness and composition of anurans species are related to available microhabitats and landscape type of sampled sites. We assume that a higher number of microhabitats increase environmental heterogeneity and this, in turn, affects species richness of amphibians. We performed the study in the Mesophytic Semideciduous Forest, a vegetation type within Atlantic Forest Domain. Between October 2010 and February 2011, we sampled 23 water bodies located in the agricultural, forest, and urban landscapes. The species richness was determined using survey at breeding sites methodology, and the availability of microhabitats was estimated visually. Thirty-four anuran species belonging to 12 families were recorded. The species richness in water bodies ranged from two to 13 species. The highest species richness was recorded in environments with a higher number of microhabitats, while the species composition in water bodies was partially grouped according to the predominant landscape type that is agricultural, forest, forest edge or urban. Our results suggest that species use specific environments (e.g. landscapes, habitat and microhabitat) for their reproductive activities.

Key words: Amphibians, Anthropic actions, Biodiversity, Fragmentation.

INTRODUCTION

Environmental heterogeneity is one of the leading factors that contribute to higher species richness in an environment in a variety of organisms (e.g. Tews et al. 2004, Townsend et al. 2009), including anurans (Keller et al. 2009, Vasconcelos et al. 2009). Higher local richness can be achieved through species specialization, which drives the occupancy of specific microhabitats of a given area (Santos et

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al. 2007, Vasconcelos et al. 2009, Silva et al. 2012, Santos and Conte 2014), promoting high functional and phylogenetic diversity (Campos et al. 2017).

The advance of urbanization and agricultural frontiers has adverse effects on the occurrence of species due to the habitat loss, fragmentation and degradation, which results in a dramatic decrease in the availability of microhabitats (Knutson et al. 1999, Cushman 2006, Cruz-Elizalde et al. 2016, Garey and Provete 2016, Lourenço-de-Moraes et al. 2018). The Atlantic Forest Domain (AFD), for example, is one of the most threatened tropical forests because of fragmentation (Ribeiro et al. 2009). The AFD also harbors the highest rates of endemism and species diversity of anurans, with 529 species reported (Haddad et al. 2013). However, owing to the annual addition of new species descriptions (e.g. Malagoli et al. 2017, Monteiro et al. 2018), richness is expected to increase in the AFD. Mesophytic semideciduous forest (MSF), for example, a vegetation type within the AFD (Oliveira-Filho and Fontes 2000) and the focus of this study, has a considerable richness with 111 species of amphibians, five of which are endemic (Garcia et al. 2007).

Amphibians anurans require specific habitats throughout their life cycle, such as water bodies (e.g. ponds, dams, swamps, marshes, and streams) suitable for breeding and development of tadpoles (Duellman and Trueb 1994, Wells 2007, Provete et al. 2014). Soon after, they depend upon terrestrial environments for the growth and dispersal of juveniles (Knutson et al. 1999, Price et al. 2004, Wells 2007). In fact, this dependence of both aquatic and terrestrial environments, their sensitive skins, and eggs (due to the absence of a shell) have led those organisms to be considered as excellent bioindicators of environmental change (Blaustein and Wake 1990, Wells 2007, Toledo 2009). Indeed, many microhabitats are used by anurans, especially during the breeding season, including marginal vegetation (e.g. grasses, herbaceous vegetation and tree vegetation) and inside of aquatic environments (e.g. shrubs and aquatic plants) (Bernarde and Anjos 1999, Bertoluci and Rodrigues 2002, Conte and Machado 2005, Conte and Rossa-Feres 2006, 2007, Santos et al. 2007, Vasconcelos et al. 2009, Santos and Conte 2014).

Although several studies have shown that a relationship exists between anuran species richness and environmental heterogeneity (Parris 2004, Menin et al. 2005, Vasconcelos et al. 2009, Keller et al. 2009, Silva et al. 2011c, 2012, Santos and Conte 2014), responses to this association are related to the spatial scale. On a local scale, species occurrence in water bodies is driven, for example, by the duration of water availability (Vasconcelos et al. 2009), by the water depth (Burne and Griffin 2005, Gonçalves et al. 2015) and by the vegetation type within and around the water body (Keller et al. 2009, Gonçalves et al. 2015). On the regional scale, factors driving species occurrence are, for example, distance to forest fragments (Silva et al. 2011a, b, Gonçalves et al. 2015) and the geographical distance between water bodies (Burne and Griffin 2005, Santos and Conte 2016).

Because of the differences between the spatial scales, studies on amphibians that combine both of those approaches, local environmental heterogeneity, and landscape type, seem relevant (Knutson et al. 1999, Vasconcelos et al. 2009, Silva et al. 2012, Oda et al. 2017). For instance, such studies provide better information for selecting priorities for conservation and management (Santos et al. 2012, Campos et al. 2017) and particularly to the MSF, the most threatened and fragmented ecosystem of the AFD (Viana and Tabanez 1996). In this study, we investigated the variations in species richness, abundance, and composition of aquatic-breeding anurans in water bodies with a different number of microhabitats within urban, agricultural and forested landscapes. We controlled our samplings through the available microhabitats in each landscape type and tested the following hypotheses: (1) urban and agricultural landscapes have less species richness than forest and forest edge landscapes; (2) species richness of anurans is positively related to the number of microhabitats available in water bodies; (3) the species composition varies according to the landscape type.

MATERIALS AND METHODS

STUDY REGION

Assemblages of anurans were studied in the region encompassed by the northern part of the state of Paraná and the southeast part of the state of São Paulo (Figure 1), a region dominated initially by MSF vegetation type within the AFD (Maack 1981). The MSF is a seasonal forest with a dry period and lower temperatures, and another period with higher rainfall and highest temperatures (Veloso et al. 1991). The climate of the region is classified according to Köppen-Geiger's as a humid subtropical climate (Cfa) (Peel et al. 2007), with average annual temperature ranging between 22 ºC - 25 ºC and an annual rainfall of 1,612.5 mm (INPE/CPTEC 2015).

STUDY SITES

Water bodies were studied within the urban, agricultural, forest and forest edge landscapes (Table I). In the northern area of the state of Paraná, sampling took place in the municipality of Londrina located in the Tibagi river basin at an average elevation of 700 meters a.s.l. The Parque Estadual Mata dos Godoy (PEMG ~ 675 ha), a protected area in that municipality, which was also included in the study given that the PEMG and other fragments connected to it form the largest forested area in northern Paraná (Anjos 1998). Also in northern Paraná, another protected area was sampled, the Parque Estadual Mata São Francisco (PEMSF ~ 832,5 ha) which is located between municipalities of Cornélio Procópio and Santa Mariana in the Rio das Cinzas river basin at an average elevation of 543 meters a.s.l. In the state of São Paulo only the municipality of Ourinhos, located in the Paranapanema river basin at elevation 492 meters a.s.l. was sampled.

DATA COLLECTION

Anuran surveys

For each water body, the samplings were carried out during the hottest and wettest seasons of the year, which coincide with the breeding season of the anurans species. In this period (October to March) species detection is increased due to abiotic factors such as precipitation and temperature, which are relevant for breeding of most species (e.g. Bernarde and Anjos 1999, Eterovick and Sazima 2000, Conte and Rossa-Feres 2007, Garey and Silva 2010). A total of 23 water bodies were sampled: eleven in Londrina; four in the PEMSF; and eight in Ourinhos. These samples represented the different landscapes as follows: agricultural landscape with nine samples; urban landscape with six samples; forest landscape with four samples; and forest edge landscape with four samples (see Figure 1; Table I).

Water bodies were sampled between October 2010 and February 2011 to determine the species richness and abundance of anurans. For this, a breeding site survey methodology was used (Scott and Woodward 1994). Each water body within both open and forested areas had covered environments, where all visual and acoustic contacts of the species were recorded. Each environment was sampled on a single day, during a six-hour period from 18:00h to 00:00h and with the same sampling effort (hours/water body), totaling 120 field hours. Between 2010 and 2017, we performed occasional field visits to the study region and used data from the PEMSF species list (Storti 2012), which were added to the list of species richness overall (see Table II).

One individual of each species was manually collected when possible, euthanatized with xylocaine 5%, fixed with formalin 10%, and preserved in

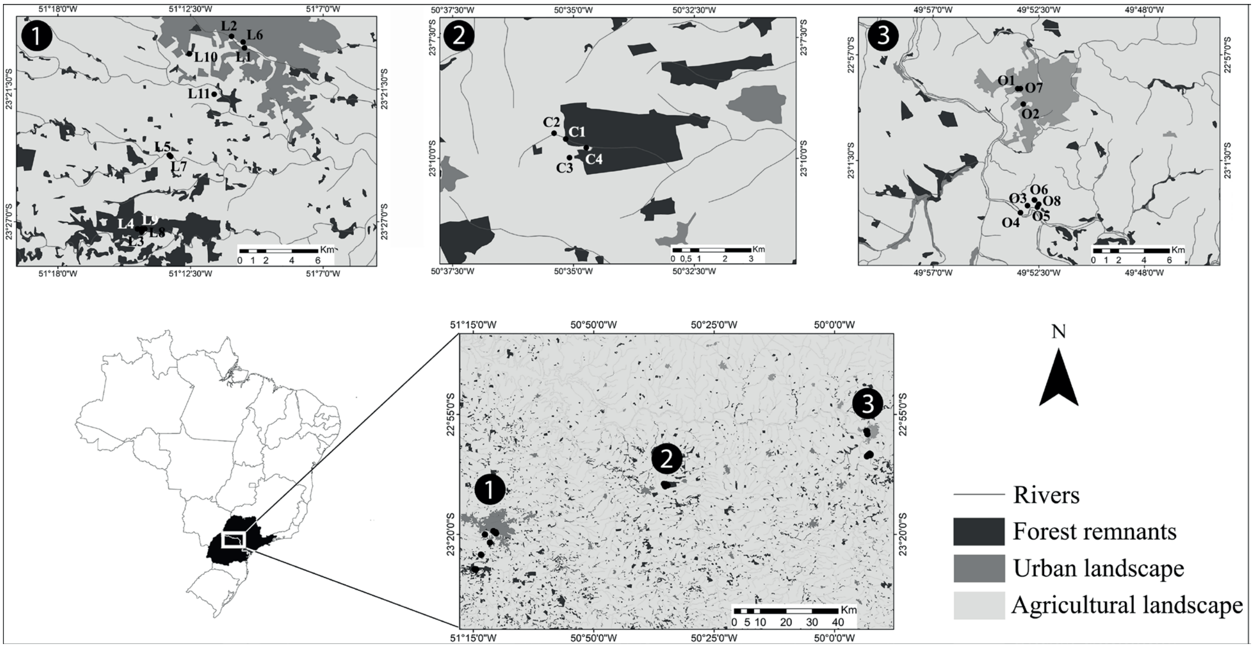

Figure 1 - Sampled water bodies (black dots) located in the Mesophytic Semideciduous Forest within Atlantic Forest Domain, between October 2010 and February 2011 in northern of the state of Paraná and southeast of the state of São Paulo, southern Brazil. 1 = Londrina (L); 2 = Cornélio Procópio (C); 3 = Ourinhos (O). Codes of water bodies as presented in Table I.

ethanol 70%. All the animal handling and collecting procedures follow resolution 301 of the Federal Council of Biology. Collecting permits were provided by Ministry of the Environment, Chico Mendes Institute for Biodiversity Conservation (SISBIO 2920-1 and 12120-1). Specimens were deposited in the Museum of the State University of Londrina (MZUEL) (Appendix I).

Landscape and microhabitat description

The landscapes were classified as follows: agricultural landscape (AL: areas at a distance of more than 500 m from forest remnants, with the size of these above 200 ha); forest edge landscape (FEL: less than 50 m from the interior of the remnants, with the size of these above 200 ha and less 500 m from the edge of the remnant; or riparian forest with 200 ha of area overall); forest landscape (FL: located more than 50 m from the edge to the interior of the remnants, with the size of these above 200 ha); and urban landscape (UL: area with houses, buildings, people transit and vehicles). Characterization of the environments involved collecting data on the vegetation on the banks of water bodies and in the water, taking into consideration the microhabitats reported in Conte and Machado (2005), Conte and Rossa-Feres (2007), Santos et al. (2007), plus new microhabitats that were added in the present study (Table III). For the soil and marginal vegetation, we recorded the following: arboreal vegetation (AV), bulrushes (BR), grasses (GR), non-vegetation (NV), shrub vegetation (SV) and trunks (TR). For the type of soil and vegetation in the water, we recorded the following: arboreal vegetation (AV), bulrushes (BR), grasses (GR), non-vegetation (NV), shrub vegetation (SV), and water hyacinths (WH).

DATA ANALYSIS

Species richness vs microhabitat

We investigated the relationship between species richness and the availability of microhabitats in the environment with simple linear regression analysis and, subsequently, a correlation graph was constructed. Through the characterization of the

TABLE I
Municipalities, water body code, landscape types, and coordinates of the studied water bodies between October 2010 and February 2011 in northern of the state of Paraná, southern Brazil. The output format of coordinates is in Geographic Coordinate System and datum WGS84.

Municipality	Water body code	Landscape type	Coordinates	
	O1	Urban landscape	22°58'26.40"S	49°53'16.80"W
	O2	Agricultural landscape	22°59'06.00"S	49°53'09.60"W
	O3	Agricultural landscape	23°03'10.80"S	49°52'40.80"W
Ourinhos	O4	Agricultural landscape	23°03'43.20"S	49°53'16.80"W
	O5	Agricultural landscape	23°03'21.60"S	49°52'30.00"W
	O6	Agricultural landscape	23°03'25.20"S	49°52'58.80"W
	O7	Urban landscape	22°58'26.40"S	49°53'24.00"W
	O8	Agricultural landscape	23°03'28.80"S	49°52'33.60"W
	L1	Urban landscape	23°19'33.60"S	51°10'19.20"W
	L2	Urban landscape	23°19'19.20"S	51°10'48.00"W
	L3	Forest landscape	23°27'18.00"S	51°14'31.20"W
	L4	Forest landscape	23°27'25.20"S	51°14'31.20"W
	L5	Agricultural landscape	23°24'14.40"S	51°13'22.80"W
Londrina	L6	Urban landscape	23°19'48.00"S	51°10'15.60"W
	L7	Agricultural landscape	23°24'18.00"S	51°13'19.20"W
	L8	Forest landscape	23°27'18.00"S	51°14'24.00"W
	L9	Forest landscape	23°27'18.00"S	51°14'42.00"W
	L10	Urban landscape	23°20'02.40"S	51°12'32.40"W
	L11	Agricultural landscape	23°21'43.20"S	51°11'31.20"W
	C1	Forest edge landscape	23°09'36.00"S	50°35'09.60"W
Cornélio	C2	Forest edge landscape	23°09'28.80"S	50°35'24.00"W
Procópio	C3	Forest edge landscape	23°09'46.79"S	50°34'44.10"W
	C4	Forest edge landscape	23°09'59.14"S	50°35'04.85"W

environments, it was possible to quantify the number of available microhabitats, making it a predictive variable, and to consider the anuran species richness in each sampled environment as a response variable. To achieve the assumption of data normality, the response variable was $\log_{10}$ transformed. Afterward, we perform a Shapiro-Wilk test to check if the data had a normal distribution indeed. Significant values were considered when $p < 0.05$. The analysis was performed using the software R statistic version 3.4.2 (R core team 2017).

Species composition vs breeding environment

To identify patterns in the composition of anuran communities in the breeding environments concerning the landscape type where water bodies were located (agricultural, forest, forest edge and urban), a cluster analysis (UPGMA) was performed with the index of Bray-Curtis similarity (Clarke 1993), in which the abundance of each species was considered in each water body. Subsequently, to test the significance of the generated groups, we used

TABLE II
Species recorded in the Mesophytic Semideciduous Forest in the Ourinhos (ORS), Cornélio Procópio (CP) and Londrina (LDN) municipalities in 23 water bodies, southern Brazil. *Species which were found in the study region but were not recorded in water bodies between October 2010 and February 2011.

Family/Species	Sampled sites			Landscape			
	ORS	**CP**	**LDN**	**UL**	**AL**	**FEL**	**FL**
Brachycephalidae							
Ischnocnema cf. *henselii*		●*	●				●
Bufonidae							
Rhinella ornata (Spix, 1824)		●	●			●	●
Rhinella schneideri (Werner, 1894)	●	●	●	●	●	●	●
Centrolenidae							
Vitreorana uranoscopa (Müller, 1924)		●*	●				●
Craugastoridae							
Haddadus binotatus (Spix, 1824)			●				●
Cycloramphidae							
Proceratophrys avelinoi Mercadal de Barrio & Barrio, 1993			●			●	●
Hylidae							
Aplastodiscus perviridis Lutz, 1950			●			●	●
Boana albopunctata (Spix, 1824)	●	●	●		●	●	
Boana faber (Wied-Neuwied, 1821)	●	●	●		●	●	
Boana prasina (Burmeister, 1856)		●*	●			●	●
Boana raniceps Cope, 1862	●	●	●		●	●	
Dendropsophus anceps (Lutz, 1929)	●*				●	●	
Dendropsophus minutus (Peters, 1872)	●	●	●	●	●	●	
Dendropsophus nanus (Boulenger, 1889)	●	●	●	●	●	●	
Dendropsophus sanborni (Schmidt, 1944)	●*				●	●	
Ololygon berthae (Barrio, 1972)		●*	●*		●	●	
Ololygon rizibilis (Bokermann, 1964)		●	●*			●	●
Scinax fuscomarginatus (Lutz, 1925)	●				●	●	
Scinax fuscovarius (Lutz, 1925)	●	●	●	●	●	●	
Scinax perereca Pombal, Haddad & Kasahara, 1995		●	●			●	●
Trachycephalus typhonius (Linnaeus, 1758)		●*	●*			●	●
Hylodidae							
Crossodactylus cf. *schmidti* Gallardo, 1961		●*	●*				●
Leptodactylidae							
Leptodactylus fuscus (Schneider, 1799)	●	●	●	●	●	●	
Leptodactylus latrans (Steffen, 1815)	●	●	●	●	●	●	
Leptodactylus mystacinus (Burmeister, 1861)	●*	●*	●	●	●	●	
Leptodactylus notoaktites Heyer, 1978		●*				●	●

Family/Species	Sampled sites			Landscape			
	ORS	**CP**	**LDN**	**UL**	**AL**	**FEL**	**FL**
Leptodactylus podicipinus (Cope, 1862)	●	●	●		●	●	
Leptodactylus labyrinthicus (Spix, 1824)	●*	●*	●*		●	●	●
Physalaemus cuvieri Fitzinger, 1826	●	●	●	●	●	●	
Physalaemus nattereri (Steindachner, 1863)	●				●		
Microhylidae							
Elachistocleis bicolor (Guérin-Menéville, 1838)	●*	●	●	●	●	●	
Odontophrynidae							
Odontophrynus americanus (Duméril & Bibron, 1841)	●*	●	●*		●	●	
Phyllomedusidae							
Phyllomedusa tetraploidea Pombal & Haddad, 1992	●*	●*	●			●	●
Ranidae							
Lithobates catesbeianus (Shaw, 1802)			●*	●	●		
Total richness	20	26	29	10	23	28	14

a multivariate ANOSIM similarity analysis with 999 permutations. ANOSIM is a robust analysis when comparing two or more groups based on distance matrices, which are converted into ranks and compared within and between groups (Clarke 1993). To visualize the pairwise test resultant from the ANOSIM, we generated a second hierarchical cluster analysis (UPGMA, Euclidean distance) which ranges from a distance of zero (highest similarity) to one (lowest similarity). To estimate the contribution of each species to the dissimilarity of the observed groups, a percentage similarity analysis (SIMPER) with a cumulative contribution of 90% was performed (Clarke and Warwick 1994). The analyses were performed using the software Primer-E version 6 (Clarke and Gorley 2006).

RESULTS

We recorded 2.695 individuals, 12 families and 34 anuran species (Table II): Brachycephalidae (1), Bufonidae (2), Centrolenidae (1), Craugastoridae (1), Cycloramphidae (1), Hylidae (15), Hylodidae (1), Leptodactylidae (8), Microhylidae (1), Odontophrynidae (1), Phyllomedusidae (1), and Ranidae (1). All species recorded are classified by the IUCN Red List of Threatened Species (IUCN 2016) as Least Concern (LC), with the exception of *Crossodactylus* cf. *schmidti*, wich is classified as Near Threatened (NT). The municipality of Ourinhos (ORS) presented the lowest species richness with 20 species, followed by the municipality of Cornélio Procópio (CP) with 26 species and municipality of Londrina (LDN), the highest, with 29 species. Richness in water bodies ranged from 2 – 13 species. The range of species richness, however, varied according to the landscape type: the highest species richness was recorded in the FEL, between 8 – 13 species, 2 – 9 species in the AL, 4 – 8 species in the FL, and 3 – 5 in the UL (see Table II and Figure 3).

Our fitted model of the linear regression (Figure 2) was normally distributed with $\log_{10}$-transformation (Shapiro-Wilk W test $W = 0.954$, p = 0.3612). The species richness of all water bodies studied can be partially explained by the variability of microhabitats (df = 21, F = 57.42, p < 0.0001, $R^2_{adj} = 0.7194$).

The composition of the anuran communities grouped partially according to the landscape types

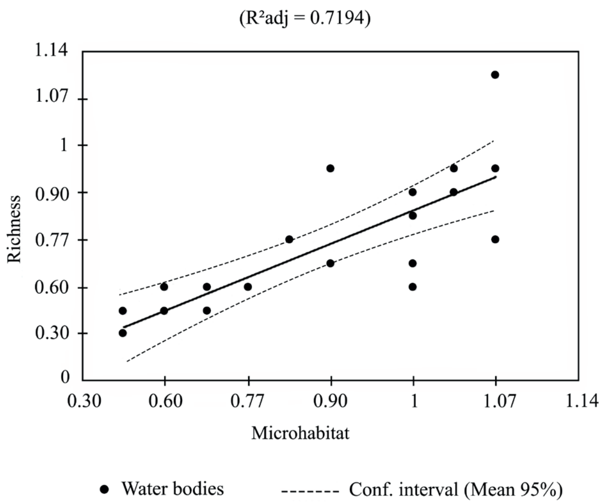

Figure 2 - Linear regression between microhabitats and richness of anuran species in the 23 water bodies located in the agricultural landscape, forest landscape, forest edge landscape, and urban landscape.

(ANOSIM: R = 0.451; p <0.001) (see Figure 3). Thus, based on Figure 3 (left side), we can highlight a trend in the cluster arrangement, which is associated with the composition of anurans species in water bodies related to the type of landscape: (group 1) water bodies in agricultural landscape (e.g. L7, L5, L11, and O3); (group 2) water bodies in forest landscape (e.g. L3, L4, and L9); (group 3) water bodies in urban landscape (e.g. L1, L10, and O7); and (group 4) water bodies in forest edge landscape (e.g. C1, C2, and C3).

Species which had occurrence exclusively in the AL and FEL were: *Boana albopunctata*, *Boana faber*, *Boana raniceps*, *Dendropsophus anceps, Dendropsophus sanborni*, *Ololygon berthae, Scinax fuscomarginatus*, *Leptodactylus podicipinus*, and *Odontophrynus americanus*. Species which had occurrence exclusively in the FL were: *Crossodactylus* cf. *schmidti, Haddadus binotatus*, *Ischnocnema* cf. *henselii*, and *Vitreorana uranoscopa*. Species which, had occurrence exclusively in the FEL and FL were: *Aplastodiscus perviridis, Boana prasina*, *Leptodactylus notoaktites, Ololygon rizibilis*, *Phyllomedusa tetraploidea, Proceratophrys*

TABLE III
Microhabitats of the studied water bodies in southern Brazil. Microhabitats were: arboreal vegetation (AV), bulrushes (BR), grasses (GR), no vegetation (NV), shrub vegetation (SV), trunks (TR), and water hyacinths (WH). L = Londrina; O = Ourinhos; C = Cornélio Procópio.

Water body	Microhabitats Availability		Total
	Inside	Edge	
O1	NV, GR	NV, GR	4
O2	NV, GR	NV	3
O3	NV, GR	NV, GR	4
O4	GR, SV	NV	3
O5	NV, GR, BR, SV, AV, TR	NV, GR, BR, SV, AV, WH	12
O6	NV, GR, SV	GR, SV	5
O7	NV, GR, AV	NV, GR	5
O8	NV, GR, BR, SV, AV, TR	NV, GR, BR, SV, AV, WH	12
L1	NV, GR, SV, AV	NV, GR, SV, AV	8
L2	NV, GR, AV, TR	NV, GR	6
L3	GR, SV, AV, TR	NV, GR, BR, SV, AV	10
L4	GR, SV, AV, TR	NV, GR, BR, SV, AV	10
L5	GR, SV, AV, TR	NV, GR, BR, SV, AV	10
L6	NV, GR, TR	NV, GR	5
L7	GR, SV, AV	GR, SV, AV, TR	7
L8	GR, SV, AV, TR	NV, GR, BR, SV, AV	10
L9	GR, SV, AV, TR	NV, GR, BR, SV, AV	10
L10	NV, GR, SV, TR	NV, GR	6
L11	NV, GR, TR	NV, GR	5
C1	NV, GR, BR, SV, AV, TR	NV, GR, BR, SV, AV	11
C2	NV, GR, BR, SV, AV, TR	NV, GR, BR, SV, AV	11
C3	GR, BR, SV, AV, TR	GR, BR, AV	8
C4	NV, GR, BR, SV, AV, TR	NV, GR, BR, SV, AV, WH	12

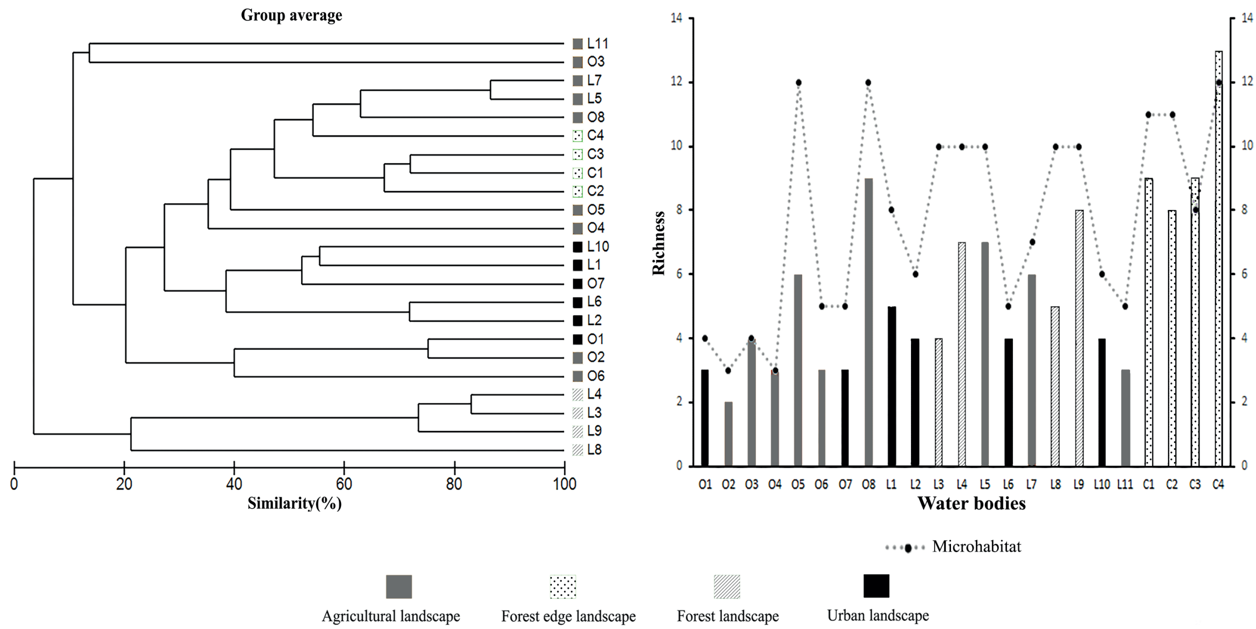

Figure 3 - On the left side, dendrogram generated using the Bray-Curtis Similarity Index among water bodies sampled across the landscapes types relating to the abundance of anuran species in Mesophytic semideciduous forest areas between October 2010 and February 2011. On the right side, numbers of species (bars)/microhabitats (interrupted line) in each water body. Patterns of the bars represent the landscape types: agricultural landscape, forest edge landscape, forest landscape, and urban landscape. Codes of water bodies as presented in Table I.

avelinoi, *Rhinella ornata, Scinax perereca*, and *Trachycephalus typhonius*. The only species which occurred in AL, FEL, and FL but did not found in UL was *Leptodactylus labyrinthicus*. The species of anurans recorded in UL occurring in three or more landscapes, were: *Rhinella schneideri, Dendropsophus minutus, Dendropsophus nanus, Scinax fuscovarius*, *Physalaemus cuvieri*, *Leptodactylus fuscus*, *Leptodactylus latrans*, *Leptodactylus mystacinus*, and *Elachistocleis bicolor* with the exception of *Lithobates catesbeianus*, which was the only species exclusive to AL and UL.

The cluster generated by the pairwise test from ANOSIM (Figure 4) demonstrates that the FL was the most distinctive landscape in species composition, FEL and AL were the most similar landscapes and UL was intermediary between these last ones. The most distinct landscapes were UL × FL. The pairwise test from SIMPER for all groups of landscape types compared with each other had an average dissimilarity of approximately 85% (85.3 ± 11.9). Twenty species contributed to this dissimilarity in the composition of the species among the four landscapes where water bodies were located (Appendix II).

DISCUSSION

We found that (1) the highest species richness was in the FEL and not in the FL, (2) the number of microhabitats is positively correlated with species richness, and (3) the species composition is strongly affected by the landscape type.

The anurans richness recorded in this study represented 30% of the known species for the MSF ecosystem (Garcia et al. 2007). In the region of Londrina 27 species were recorded (Bernarde and Anjos 1999, Machado et al. 1999, Machado and Bernarde 2002), all of which were also recorded in the present study. We included two new records for the region, *Ololygon berthae* and *Ololygon rizibilis* (see distribution map in Nascimento et al. 2016 and Figueiredo et al. 2014 respectively). Compared with other studies performed in the MSF, our study demonstrated higher local species richness than others (see Table IV), except municipalities of Gália and Avilândia in São Paulo (34 species; Brassaloti et al. 2010) that had the same species richness.

Our results corroborate studies in which environmental heterogeneity partly explains variations in the richness and composition of the anuran communities (Parris 2004, Vasconcelos and Rossa-Feres 2005, 2008, Vasconcelos et al. 2009, Oda et al. 2016, 2017). In fact, the number of microhabitats used by males as vocalization sites tends to influence the anurans species richness and composition (Afonso and Eterovick 2007, Vasconcelos and Rossa-Feres 2008, Pirani et al. 2013).

Water bodies with higher microhabitat numbers encompass habitat heterogeneity: water bodies with a higher number of microhabitats available (e.g. presence of arboreal vegetation, shrub vegetation, and bulrushes) provide calling sites for arboreal species (e.g. *Boana* spp., *Phyllomedusa* spp.), as well as shelter for larvae and adults from predators, which facilitate the increase of species richness (Vasconcelos et al. 2009, Oda et al. 2016). Otherwise, water bodies with lower microhabitats number (e.g. only soil and grasses) in the surroundings reduces the species richness since they offer calling sites only for terrestrial species (e.g. *Leptodactylus* spp.) and those species which reproduce in herbaceous vegetation (e.g. *Dendropsophus nanus*) (Santos et al. 2007, Vasconcelos and Rossa-Feres 2008, Vasconcelos et al. 2009, Silva et al. 2011a). Indeed, the decrease in microhabitat availability limits the possibility of spatial partitioning (Cardoso et al. 1989).

We did not find the highest species richness of anurans in the FL but in the FEL probably related to the reproductive mode of species. The specific ecological characteristics related to their reproductive modes (open or forested areas) provide the establishment of species (Haddad and Prado 2005, Cruz-Elizalde et al. 2016, Oda et al. 2016). Amphibians anurans associated with forest use water bodies connected to the forest edge for their reproduction (Silva et al. 2011b). However, species related to open areas also access the forest edge for breeding (Ferreira et al. 2016, Ferrante et al. 2017). Thus, the edge of forest remnants in the MSF maintain higher species richness, since those associated to the forest and those associated to the open areas use the FEL to breeding (Becker et al. 2007, Ferreira et al. 2016, Ferrante et al. 2017; present study).

In fact, MSF exhibits just a few species restricted to the forest interior (Bernarde and Anjos 1999, Santos et al. 2009, Garey and Silva 2010, Santos and Conte 2016, Lourenço-de-Moraes et al. 2018). Most of the species exclusive to forest interior have specialized reproductive mode, such as leaf-litter breeders with direct development (mode 23: Haddad and Prado 2005) (e.g. *Ischnocnema* cf. *henselii* and *Haddadus binotatus*), and others, dependent on streams inside the forest for breeding (e.g. *Vitreorana uranoscopa*; *Crossodactylus* cf. *schmidti*) (mode 23 and mode 3 respectively: Haddad and Prado 2005). Probably, because of the specialized breeding of anurans, the FL species composition was the most distinct among the studied landscapes (see Figures 3 and 4). Moreover, the restrict species of FL (Bernarde and Anjos 1999, Machado and Bernarde 2002) could be considered the most sensitive to forest fragmentation in the present study.

Water bodies that were constructed for use in crop irrigation or fish production (Hartel and Wehrden 2013) allow some anurans species to use these artificial habitats for their reproduction (Babitt and Tanner 2000, Vasconcelos and Rossa-Feres 2005, Colombo et al. 2008). Most of the recent studies using anurans as a model in the agricultural landscapes suggest that the species richness is

TABLE IV
List of municipalities that had the anurofauna studied in the Mesophytic semideciduous forest in the states of São Paulo (SP) and Paraná (PR) considered in this study.

Municipality	State	Richness	Reference
Avilândia and Gália	SP	34	Brassaloti et al. 2010
Araçatuba	SP	23	Oda et al. 2017
Guararapes	SP	26	Bernarde and Kokubum 1999
Icém	SP	12	Silva and Rossa-Feres 2007
Nova Itapirema	SP	27	Vasconcelos and Rossa-Feres 2005
Pontal do Paranapanema	SP	21	Vasconcelos et al. 2009
Rio Claro	SP	24	Zina et al. 2007
Rio Claro	SP	21	Toledo and Haddad 2003
Santa Fé do Sul	SP	20	Santos et al. 2007
Teodoro Sampaio	SP	28	Santos et al. 2009
Diamante do Norte	PR	19	Oda et al. 2016
Maringá	PR	21	Affonso et al. 2014
Porto Rico	PR	18	Affonso et al. 2013
São Pedro do Ivaí	PR	14	Santos and Conte 2016
Fênix	PR	15	Santos and Conte 2016
Três Barras do Paraná	PR	23	Bernarde and Machado 2001/2000
Tuneiras do Oeste and Cianorte	PR	22	Affonso and Gomes 2013
Londrina	PR	24	Bernarde and Anjos 1999
Londrina	PR	24	Machado et al. 1999
Londrina	PR	27	Machado and Bernarde 2000
Ourinhos/Londrina and Cornélio Procópio	SP/PR	34	Present study
Chavantes/Ribeirão Claro and Jacarezinho	SP/PR	25	Nazaretti and Conte 2015

strongly associated with forest cover (Ferreira et al. 2016, Collins and Fahrig 2017, Ferrante et al. 2017, Gangenova et al. 2018) and negatively influenced by the mean crop field size (Collins and Fahrig 2017). In this way, farmland with smaller mean field sizes should benefit all tolerant anurans species, due to available food sources and for providing more effortless movement between the refuge habitats and breeding environments (Collins and Fahrig 2017). Furthermore, in the agricultural landscape, water bodies configuration related to the microhabitat availability changes according to the matrix type where it is located, which has a bearing on species richness and composition (Ferrante et al. 2017).

Among the studied water bodies, those in the agricultural landscapes of Ourinhos are located in the ecotone region between the Cerrado and the MSF and this explains the recording of typical Cerrado species, such as *Physalaemus nattereri* (Aquino et al. 2004, Santos et al. 2009). These species are opportunistic and are benefited by some anthropic activities, which could explain their extended geographical distributions (Haddad and Sazima 1992).

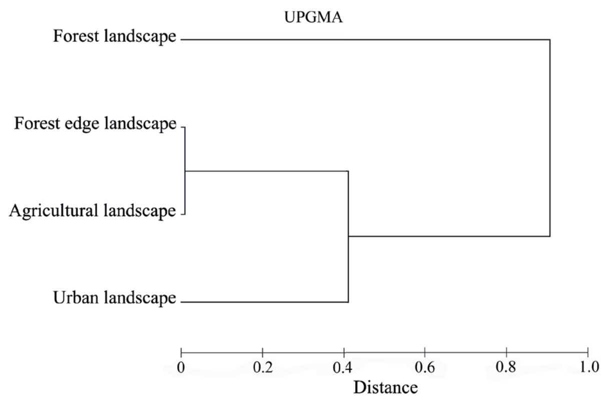

Figure 4 - Hierarchical cluster analysis (UPGMA, Euclidean distance) resulting from the pairwise test of ANOSIM comparing the four landscapes types: agricultural landscape, forest landscape, forest edge landscape and urban landscape.

We highlight the record of the invasive species *Lithobates catesbeianus* native to North America which has been introduced to the region of Londrina for commercial purposes (Machado and Bernarde 2002). In occasional visits to areas of Londrina, we recorded a water body with more than 25 individuals of *L. catesbeianus* breeding. This is a concerning situation because this species is a generalist predator (Toledo et al. 2007) which compete for prey (Leivas et al. 2012), transmit pathogens (Schloegel et al. 2010), and have several negative impacts on native species of anurans (Both et al. 2011).

As several studies have shown, our results suggest that some species of amphibians tolerate environments altered by anthropic actions, such as urban areas (e.g. *Dendropsophus nanus*, *Elachistocleis bicolor*, *Leptodactylus latrans*, *Physalaemus cuvieri*, and *Scinax fuscovarius*), while others mentioned above are dependent on microhabitats and/or environmental conditions only found in forested areas (Moraes et al. 2007, Haddad et al. 2013) and some are sensitive to the edge effect (Lourenço-de-Moraes et al. 2014, Ferreira et al. 2016). Anurans are negatively influenced by the use of habitats in urban areas as a result of a variety of factors, such as pollution (air, water, and noise), fragmentation, loss and isolation of habitat (see review in Hammer and McDonnell 2008), artificial lighting (Perry et al. 2008) and roads and traffic (Rytwinski and Fahrig 2015).

The availability of microenvironments to anurans in these urban areas is also another factor which negatively affects species richness (Gagné and Fahrig 2007). In this way, in the UL, we commonly found water bodies with the lowest vegetation structure available and could record only species with reproductive mode 1 (eggs and exotrophic tadpoles in still water) and those modes resistant to desiccation, such as modes 11 and 30 (eggs embedded in foam nest; sensu Haddad and Prado 2005). However, forest remnants near or inserted in urban areas, even with a variety of microenvironments available, are negatively affected as to richness and composition of amphibian species (Lourenço-de-Moraes et al. 2018).

The physiological dependence of anurans of both water and terrestrial habitats have highlighted their importance as good bioindicators (Blaustein and Wake 1990, Wells 2007, Toledo 2009). However, we found only four species which could be considered as a true indicator, in the studied case indicator of the FL, which are: *Crossodactylus* cf. *schmidti, Haddadus binotatus*, *Ischnocnema* cf. *henselii*, and *Vitreorana uranoscopa*. Others nine species could be regarded as moderate indicators of FL since they can use both FL and FEL as breeding habitat: *Aplastodiscus perviridis, Boana prasina*, *Leptodactylus notoaktites, Ololygon rizibilis*, *Phyllomedusa tetraploidea, Proceratophrys avelinoi*, *Rhinella ornata, Scinax perereca*, and *Trachycephalus typhonius*. So, the use of anurans as indicators of the forest should be restricted to a relatively small proportion of the recorded species (38%). The other species are mainly tolerant to different levels of environmental disturbances.

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AUTHOR CONTRIBUTIONS

GdTF and LFS idealized the project, proposed the environmental characters, performed the fieldwork, interpreted and analyzed the data, wrote and reviewed the manuscript. OAS, LDA, and RLdM, interpreted and analyzed the data, wrote and reviewed the manuscript.

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APPENDIX I

Voucher specimens collected during the study in the Mesophytic Semideciduous Forest, southern Brazil.

Aplastodiscus perviridis – MZUEL1426; *Boana albopunctata* – MZUEL1428; *Boana faber* – MZUEL 1419; *Boana prasina* – MZUEL1793; *Boana raniceps* – MZUEL1793; *Crossodactylus* cf. *schmidti* – MZUEL1801; *Dendropsophus minutus* – MZUEL; *Dendropsophus nanus* – MZUEL 1425; *Elachistocleis bicolor* – MZUEL1502; *Haddadus binotatus* – MZUEL1800; *Ischnocnema* cf. *henselii* – MZUEL1583; *Leptodactylus fuscus* – MZUEL1456; *Leptodactylus labyrinthicus* – MZUEL 1802; *Leptodactylus latrans* – MZUEL1450; *Leptodactylus mystacinus* – MZUEL 1691; *Leptodactylus notoaktites* – MZUEL1575; *Leptodactylus podicipinus* – MZUEL1511; *Odontophrynus americanus* – MZUEL1544; *Ololygon berthae* – MZUEL1552; *Ololygon rizibilis* – MZUEL1523; *Phyllomedusa tetraploidea* – MZUEL1423; *Physalaemus cuvieri* – MZUEL1461; *Physalaemus nattereri* – MZUEL1442; *Proceratophrys avelinoi* – MZUEL1421; *Rhinella ornata* – MZUEL1790; *Rhinella schneideri* – MZUEL1688; *Scinax fuscovarius* – MZUEL1446; *Scinax perereca* – MZUEL1565; *Trachycephalus typhonius* – MZUEL1385; *Vitreorana uranoscopa* – MZUEL1420.

APPENDIX II

Anuran species contribution to the average dissimilarity between the four landscapes sampled: **AL**: agricultural landscape; **FL**: forest landscape; **FEL**: forest edge landscape; **UL**: urban landscape. **Av. Diss**: Average Dissimilarity; **Contrib.%**: Contribution (%); **Cum.%**: Cumulative Percentage.

	Species	Av.Diss	Contrib%	Cum.%		Species	Av.Diss	Contrib%	Cum.%
UL x AL	*Dendropsophus nanus*	12.07	15.89	15.89	**UL x FEL**	*Dendropsophus nanus*	14.8	19.02	19.02
	Boana albopunctata	9.75	12.84	28.73		*Leptodactylus podicipinus*	13.29	17.08	36.1
	Leptodactylus fuscus	8.88	11.7	40.43		*Boana faber*	8.52	10.95	47.05
	Physalaemus cuvieri	8.09	10.65	51.08		*Boana albopunctata*	7.27	9.34	56.39
	Dendropsophus minutus	6.83	9	60.08		*Scinax perereca*	6.23	8	64.39
	Boana raniceps	4.79	6.31	66.39		*Physalaemus cuvieri*	4.66	5.99	70.38
	Leptodactylus mystacinus	4.34	5.72	72.11		*Dendropsophus minutus*	3.82	4.91	75.29
	Boana faber	3.65	4.81	76.92		*Leptodactylus fuscus*	2.83	3.64	78.93
	Scinax fuscovarius	3.59	4.72	81.64		*Scinax fuscovarius*	2.74	3.53	82.45
	Rhinella schneideri	3.16	4.17	85.81		*Ololygon rizibilis*	2.53	3.25	85.71
	Elachistocleis bicolor	3	3.95	89.76		*Elachistocleis bicolor*	2.43	3.12	88.82
	Leptodactylus latrans	2.76	3.63	93.39		*Boana raniceps*	2.34	3	91.83

APPENDIX II (continuation)

	Species	Av.Diss	Contrib%	Cum.%		Species	Av.Diss	Contrib%	Cum.%
UL x FL	*Dendropsophus nanus*	15.32	15.75	15.75	**AL x FEL**	*Dendropsophus nanus*	15.24	21.64	21.64
	Proceratoprhys avelinoi	10.26	10.55	26.3		*Leptodactylus podicipinus*	11.96	16.99	38.63
	Leptodactylus fuscus	8.91	9.16	35.46		*Boana faber*	6.11	8.68	47.3
	Vitreorana uranoscopa	7.19	7.4	42.86		*Scinax perereca*	5.67	8.05	55.35
	Boana prasina	6.84	7.04	49.9		*Boana albopunctata*	5.45	7.73	63.08
	Physalaemus cuvieri	6.67	6.86	56.76		*Dendropsophus minutus*	4.09	5.8	68.89
	Aplastodiscus perviridis	5.76	5.92	62.68		*Physalaemus cuvieri*	3.84	5.45	74.34
	Ischnocnema cf. *henselii*	5.48	5.64	68.32		*Boana raniceps*	3.41	4.85	79.19
	Haddadus binotatus	4.76	4.9	73.21		*Leptodactylus fuscus*	2.72	3.86	83.05
	Scinax perereca	4.76	4.89	78.1		*Scinax fuscovarius*	2.48	3.53	86.57
	Leptodactylus mystacinus	4.67	4.8	82.91		*Ololygon rizibilis*	2.29	3.26	89.83
	Rhinella schneideri	3.05	3.13	86.04		*Elachistocleis bicolor*	2.27	3.22	93.05
ALxFL	*Boana albopunctata*	9.31	9.55	9.55	**FLxFEL**	*Dendropsophus nanus*	20.8	22.4	22.4
	Proceratoprhys avelinoi	8.95	9.18	18.73		*Leptodactylus podicipinus*	12.91	13.9	36.3
	Dendropsophus nanus	8.34	8.55	27.28		*Boana faber*	7.17	7.72	44.02
	Physalaemus cuvieri	6.56	6.73	34.01		*Boana albopunctata*	7.05	7.59	51.61
	Dendropsophus minutus	6.51	6.68	40.68		*Physalaemus cuvieri*	6.4	6.89	58.5
	Vitreorana uranoscopa	6.28	6.44	47.12		*Scinax perereca*	4.98	5.36	63.86
	Boana prasina	6	6.16	53.27		*Proceratoprhys avelinoi*	4.54	4.89	68.75
	Leptodactylus fuscus	5.11	5.24	58.51		*Boana prasina*	2.9	3.13	71.87
	Aplastodiscus perviridis	5.02	5.15	63.66		*Vitreorana uranoscopa*	2.89	3.11	74.98
	Ischnocnema cf. *henselii*	4.78	4.9	68.56		*Dendropsophus minutus*	2.75	2.96	77.94
	Boana raniceps	4.54	4.66	73.22		*Aplastodiscus perviridis*	2.55	2.75	80.69
	Haddadus binotatus	4.16	4.27	77.49		*Ololygon rizibilis*	2.45	2.64	83.33
	Scinax perereca	4.15	4.25	81.74		*Ischnocnema* cf. *henselii*	2.35	2.53	85.86
	Boana faber	3.9	4	85.74		*Boana raniceps*	2.28	2.45	88.31