Calling site selection by male *Boana punctata* (Anura: Hylidae)

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Abstract. Amphibian species require specific conditions for reproduction, such as cover structures and shelters, in both aquatic and terrestrial habitats, which directly influence reproductive success. A careful selection of calling sites is an important process, driving life-history strategies and tactics in amphibians. Despite this, a significant knowledge gap on how different variables modulate amphibian microhabitat selection processes is noted. Thus, we aimed to: (1) describing the microhabitat use of adult neotropical *Boana punctata* males during the breeding season; and (2) quantitatively analyzing the selection process of five resource variables (i.e., vegetation cover, vegetation density, vegetation height, water depth, and distance to the water shore). We then compared the microhabitat selection of males that achieved spawning and that of males that did not achieve spawning. To quantify selection, we used a resource selection function approach, applying a case/control design where the calling site used by each male was paired to eight surrounding, unused locations. We found that males selected microhabitats with higher vegetation than surrounding areas for reproduction, which suggests territorialism, and selected microhabitats slightly ($\sim$ 30 cm) inside the assessed ponds. Males also acted randomly with regard to the other variables, not influencing the males' microhabitat selection. Microhabitat use of males successful in obtaining females to spawn was similar to that of males exhibiting mating failure. In addition to being of ecological interest, our study highlights that preserving tall vegetation and the ponds' shores is important for the conservation of existing *B. punctata* urban populations.

Keywords: frog, reproductive success, resource selection function, spatial ecology, spawning site, vegetation height.

Introduction

Unravelling the role of different variables in the process of habitat selection provides a mechanistic way to understand how animals use available space (Duchesne, Fortin and Courbin, 2010; Ortega et al., 2019). The use of favorable calling sites is a key process of anuran life histories (Hödl, 1977; Jiménez-Robles et al., 2017; Cicchino et al., 2020), strongly influencing reproductive success (Bell, 1991; Refsnider and Janzen, 2010). Most anurans require very specific reproductive resources, which led them to the selection of microhabitats consisting of specific vegetation structure, water depth, and/or substrate composition traits (Martins, 1993; Goldberg, Quinzio and Vaira, 2006; Afonso and Eterovick, 2007; Refsnider and Janzen, 2010). Thus, understanding how anurans make their calling site decisions and which resources or environmental traits guide their choices allow us to explain species distribution and implement conservation measures (Spieler and Linsenmair, 1997; Cayuela et al., 2017). This is particularly important for urban fauna, since microhabitat selection results of key species are used to directly inform urban planners on the management of green areas (Baldwin, Calhoun and deMaynadier, 2006; Kumar et al., 2018).

Selecting a suitable reproductive site is predicted to provide certain advantages to males with regard to obtaining a mate (Wells, 1977). Some studies have indicated that vegetation structure interferes in the propagation of sounds emitted by males aiming to attract females or

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defend their territories (Wells and Schwartz, 1982; Lopez et al., 1988; Forrest, 1994; Schwartz et al., 2016). Thus, choosing microhabitats located in high vegetation can improve male sound propagation, increasing the chances of successfully obtaining a reproductive partner (Wells, 1977; Wells and Schwartz, 2006; Narins et al., 2018). For territorial anurans, selecting a calling site also implies selecting a spawning site, since the female will spawn there (Wells, 2007). In these cases, both biotic (e.g., risk of predation) and abiotic (e.g., the pond's water holding capacity) calling site factors may also influence offspring development and survival (Spieler and Linsenmair, 1997). For some species, limitation in the availability of microhabitats with suitable properties is known to restrict individual fitness and population density (Alonso-Alvarez and Velado, 2012).

Both extrinsic and intrinsic traits, including physiological, morphological, and behavioral constraints, modulate reproductive microhabitat choices of anurans (Crump, 1971; Wells, 2007; Refsnider and Janzen, 2010). An example of an intrinsic factor, morphology, affecting microhabitat choices can be found on hylid frogs, that are adapted to move vertically in high vegetation, due to their adhesive discs (Duellman and Trueb, 1994). However, the perch height of hylid frogs varies with abiotic factors, such as air temperature and wind speed, as these frogs seek to avoid dehydration and heat loss (Pough Stewart and Thomas, 1977; Melo et al., 2014). Among extrinsic factors, predation pressure, temperature, and humidity are the most noteworthiness. For example, it is known that many frog species avoid sites of high predation pressure (Rudolf and Rödel, 2005; Kopp, Wachlevski and Eterovick, 2006; Indermaur et al., 2010; Buxton, Ward and Sperry, 2017), being shallow water usually safer than deep water for oviposition (Rudolf and Rödel, 2005).

The calling sites can be considered a reproductive resource and thus can be studied following a resource selection approach. Here, we defined 'resource use' as the description of the resources used by animals (e.g., microhabitats, feeding items, perching sites) and defined 'resource selection' as the analysis of resource use in relation to resource availability (Manly et al., 2002). Despite the importance of microhabitat selection to amphibian ecology and evolution, previous studies have mainly focused on microhabitat use. Describing where amphibians are, without a quantitative comparison to the available choices that the animals have, precludes the understanding of the role of the environment on their spatial distribution within their home ranges (Manly et al., 2002; Beyer et al., 2010). To fill this gap, microhabitat use research should be complemented with assessments of microhabitat selection processes and their ecological drivers. In the present study, we aim to evaluate the calling site selection of the hylid frog *Boana punctata* (Schneider, 1799). Our three aims were to: (1) characterize the calling site used by adult males; (2) investigate how vegetation and water traits modulate the calling site choice; and (3) analyze whether microhabitat selection varies between successful and nonsuccessful males in obtaining females to spawn. We set up two sets of hypotheses: whether vegetation cover (H1), vegetation density (H2), vegetation height (H3), shore distance (H4), and water depth (H5) modulate the microhabitat selection of *B. punctata* males, and whether these five variables differentially condition the microhabitat selection by males successful and non-successful in obtaining females to spawn (H6-H10; fig. 1). Results from hypotheses H1-H5 will allow us to fulfill the second aim of the study; and results from H6-H10, the third.

Material and methods

Study area

We studied reproductive characteristics of *Boana punctata* males at the Anhanduí (20°30′27.0″S, 54°38′31.2″W; 10.8 ha) and Imbirussu (20°26′52.8″S, 54°41′34.8″W; 13.2 ha) ecological parks, both located in the urban perimeter of the city of Campo Grande (Mato Grosso do Sul, Brazil). Both parks comprise savanna habitats subjected to
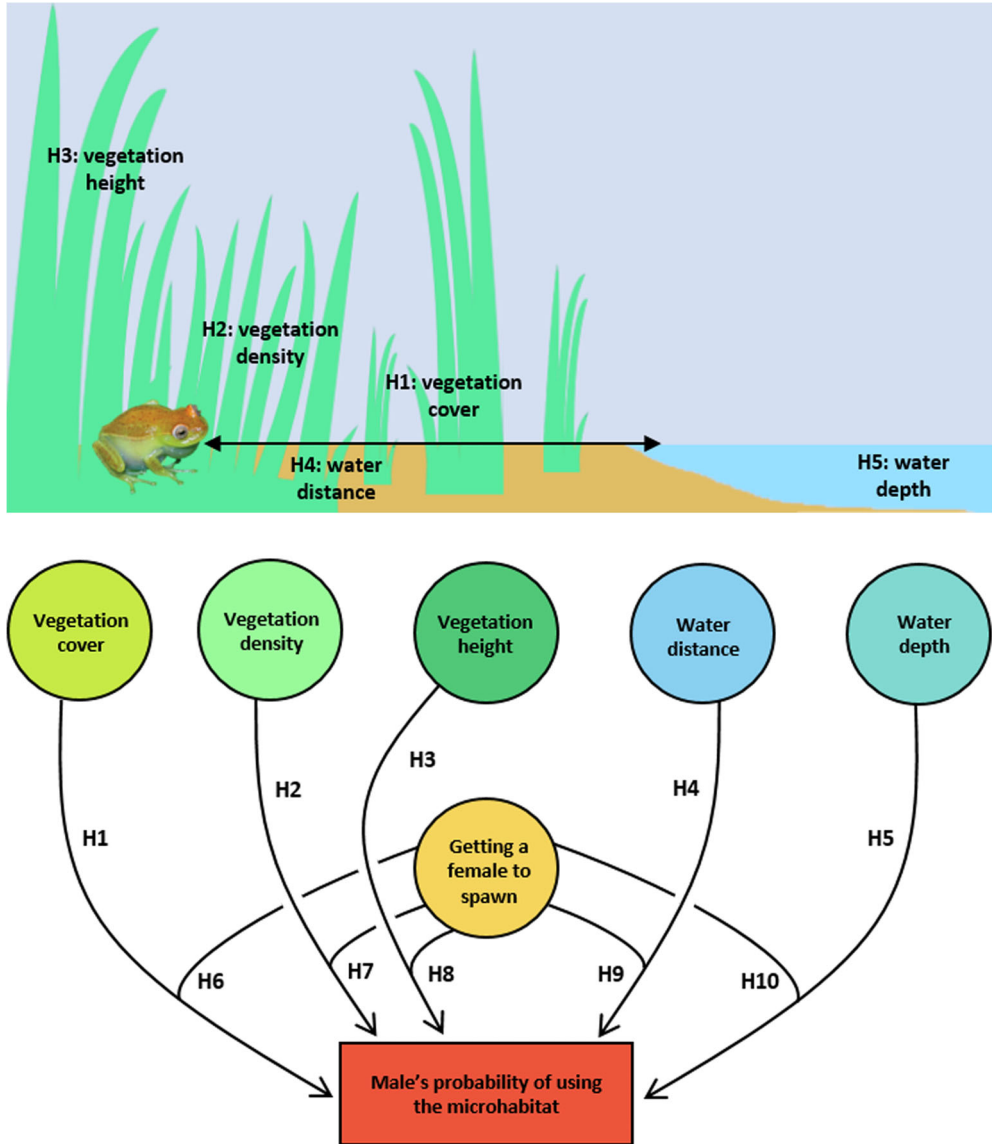
Figure 1. Role of five environmental traits in the selection of calling sites by individual males of *Boana punctata* (H1-H5) and different selection processes for successful and non-successful males in obtaining females to spawn (H6-H10). The top diagram depicts a scheme of the variables which affect habitat selection we aimed to unravel, and the lower diagram shows the structure of our hypotheses in the statistical model used for the analyses. The hypotheses are as follows: vegetation cover (H1), vegetation density (H2), vegetation height (H3), shore distance (H4), and/or water depth (H5) significantly influence the probability of a reproductive male occupying a certain microhabitat; occurrence of a significant interation between spawning (1 = obtaining a female to spawn / 0 = not obtaining a female to spawn) and the effect of the vegetation cover (H6), vegetation density (H7), vegetation height (H8), water depth (H9), and/or distance to the water shore (H10) on the probability of the presence of a *B. puntata* male in a certain microhabitat.

anthropogenic impacts. The study region exhibits a tropical climate, with average annual temperature of 23°C and annual rainfall between 1000 and 2000 mm, distributed in two seasons, rainy, from December to February, and dry, from June to August (Alvares et al., 2013; Instituto Nacional de Meteorologia, 2017).

Study species

Boana punctata (fig. 2) occurs throughout the Amazon basin, South of the Paraguayan Chaco and along the basins of the Paraguay and Paraná rivers, in Argentina (Manzano, 1992; Bernarde, 1998; Barrio, Rivero and Manrique, 2000;

Figure 2. Territorial males (Campo Grande, Brazil) during the late evening (left) and night (right). Nocturnal individuals were identified by the use of an ultraviolet lantern.

Chacón-Ortiz, Pascual and Godoy, 2002). Frogs of this species generally live in grassy and shrubby areas, either seasonally or permanently flooded (Hödl, 1977; Rodríguez and Duellman, 1994). The species does not present significant sexual size dimorphism, with mean snout-vent length of approximately 36 mm (Crump, 1974). This species exhibits a prolonged reproductive season (Duellman, 1978; Prado, Uetanabaro and Haddad, 2005) and eggs and tadpoles develop in the water (reproductive mode 1, as described by Haddad and Prado, 2005).

Boana punctata males exhibit territorial behavior, and physical combat is predicted to occur if one male invades the calling site of another. These males emit well-diversified and context-specific acoustic signals and vocalize on the water surface to attract potential mates. Males also climb periodically from the water surface into the vegetation and emit territorial calls while patrolling their territory to keep other males away (Brunetti, Taboada and Faivovich, 2015). The amplexus and spawning take place within the male's territory (Brunetti, Taboada and Faivovich, 2014). Males show site fidelity and usually use a breeding site for more than one night (Brunetti, Taboada and Faivovich, 2014). Females seem to account for male calls, male territory, and perhaps chemical signals when choosing their mates. After choosing a male, the females deposit a floating spawn above the water surface and within the vegetation (see fig. 4 of Brunetti, Taboada and Faivovich, 2014). A peculiar feature of this species is its recently discovered fluorescence (Taboada et al., 2017a) (fig. 2), function of which remains unknown, but may be related to reproductive or territorial behaviors (Taboada et al., 2017b).

Data collection

Beginning on September 4th 2017, we visited two study areas once a week at night and actively searched for animals and their spawn, using an UV lantern to aid in visualizing the fluorescent males (fig. 2). We recorded the first spawns on January 8th 2018. Once the reproductive season began, we visited each study area three times per week, both during the day and at night, to study the reproductive

males' microhabitat use and selection until May15th, when we observed the last spawning. Therefore, we assessed microhabitat selection during the entire reproductive season. During the nocturnal surveys, we marked the area from which each male was vocalizing. During the day following the night survey, we scored both the environmental resource variables of the marked microhabitats used by vocalizing males and the presence or absence of spawning.

For each microhabitat used by a vocalizing male, we scored the following variables: (1) vegetation cover, (2) vegetation density, (3) vegetation height, (4) shore distance, and (5) water depth. We scored vegetation cover, vegetation density, and water depth, using a 1×1 m grid subdivided into 100 plots measuring 10×10 cm. We estimated vegetation cover as a percentage by counting the number of plots (0 to 100) of the grid occupied by vegetation. We counted numbers of leaves and/or stems in previously established 5-plots for each grid and then averaged the numbers to calculate vegetation density, as expressed by the number of leaves and/or stems per 100 cm^2. We scored vegetation height by measuring the tallest plant in a grid (to within 1 cm). We established water depths in the 5-plots using a grid (to within 1 cm) and averaged. We determined shore distance from the border of the pond to the center of the grid (to within 1 cm). We set positive shore distance values when the microhabitat was located inside the pond, negative values when the microhabitat was located outside the pond (on land), and a value of zero when the microhabitat was located right on the pond shore. We measured height, depth, and shore distance (to within 1 cm) with a measuring tape.

For each point where we observed the presence of a vocalizing male (used microhabitat), we assessed eight related points of availability (unused microhabitats). To achieve a representative number of available microhabitats, locations of which were systematized, we selected 8-points at 5 m from the center of a used microhabitat, angled at 45 degrees each (i.e., north, northeast, east, southeast, south, southwest, west, and northwest). Thus, the used point is located in the center of eight surrounding, unused points located 5 m away in the eight equidistant cardinal directions, forming an asterisk. Since males are highly territorial

and are known to defend a territory of approximately 1 m (Brunetti, Taboada and Faivovich, 2014), we considered a 5 m distance from the microhabitat selected by a territorial male to be enough for an available, unused microhabitat (Brunetti, Taboada and Faivovich, 2014). We scored the same five variables at each unused microhabitat.

This case/control method seeks to compare the used microhabitat with those available to each male at that time, constituting true availabilities (e.g., Benício et al., 2019; Ortega et al., 2019). To avoid pseudoreplication, we marked all the microhabitats sampled and subsequently searched for males, always focusing on a new potential microhabitat in each study area. *Boana punctata* males are highly territorial in the reproductive season and are expected to use the same microhabitat for many days (Brunetti, Taboada and Faivovich, 2014; Martins, unpublished data).

Data analysis

We graphically explored the characteristics of the used and available microhabitats through a Principal Component Analysis (PCA) biplot. The PCA does not consider the paired condition of a used microhabitat with its respective eight available microhabitats. We thus used the PCA for both characterizing microhabitat use or availability of the five variables of interest and visualizing a scenario generalized for the microhabitat use or availability, but not for assessing microhabitat selection.

Habitat selection is traditionally divided into three scales: (1) population distribution, (2) home range selection within the distributional range, and (3) within home ranges, considered as microhabitat selection (Johnson, 1980). We analyzed microhabitat selection (i.e., the third-order scale established by Johnson, 1980 or the local scale set by Mayor et al., 2009) using a resource selection function (RSF) approach (Manly et al., 2002). The RSF analyzes the probability of an individual to use a given resource (in this case, a microhabitat) based on its availability. In addition, the RSF allows for the quantification of the importance of each explanatory variable in the same probabilistic selection process (Liedke et al., 2017), as well as the specification of the availability of environmental conditions for each individual at the exact sampling moment (Liedke et al., 2017; Ortega et al., 2019).

We used a Conditional Logistic Regression (CLR) to solve this RSF, since we obtained the data through direct observations of the covariates for each registered individual (scored 1) and of the covariates of the microhabitats available to each one (scored 0). We conditioned the he CLR model by individual observations, to ensure that each covariate value of each used microhabitat was related to their corresponding available and unused microhabitats (Duchesne, Fortin and Courbin, 2010; Liedke et al., 2017). Thus, the environmental variable values of the microhabitat where a vocalizing male was observed were compared to the values at the respective eight surrounding and unused microhabitats, guaranteeing that the unused points are true absences (Duchesne, Fortin and Courbin, 2010). The CLR displays the advantage of allowing for more than one unused point paired to each used point, so that the habitat availability of each individual is better represented that through a classical paired logistic regression. We used a multi-model inference (Burnham and Anderson, 2004) to assess the effects of the five environmental covariates in the selection process and their potential changes on successful female attraction and subsequent spawning. In order to assess whether microhabitat selection was different for successfully breeding males compared to those that did not achieve a spawning, we included the interaction between the variable spawn (binomial) and each studied environmental variable. From the full model that tested our ecological hypotheses of interest (fig.1):

(full model) Presence of the frog = vegetation density*spawning + vegetation cover*spawning + water depth*spawning + vegetation height*spawning + shore distance*spawning + strata (ID),

where ID is the individual identification number of the frog (the same for the microhabitat of male presence and the eight related and available microhabitats for each individual frog) and was included as a random factor in the model. We conducted a model selection to find the best fit for the data. We considered models with delta AICc < 2 to be similarly adequate as the best model, and models with accumulated Akaike weight up to 0.95 to have a high probability (95%) of containing the best model. As the set of best models (AICc < 2 and Akaike weight > 0.95) contained many models (i.e., exhibiting model uncertainty), we averaged the coefficients to obtain a robust result (Burnham and Anderson, 2004; Johnson and Omland, 2004). We conducted all analyses in R environment (R Core Team, 2019) using the survival (Therneau and Lumley, 2015), FactorMineR (Lê, Josse and Husson, 2008), factoextra (Kassamvara and Mundt, 2017), and MuMIn (Barton, 2016) packages.

Results

We detected the first male vocalizations on November 24th 2017. However, we only found the first spawning on January 8th 2018. The reproductive activity of these animals ceased completely on May 15th 2018, when we did not observe any vocalizing male and neither found any spawning thereafter. We assessed 67 microhabitats used vocalizing males (associated with 536 surrounding, unused microhabitats). Males got a spawn in 36 of the 67 microhabitats (associated with 288 surrounding, unused microhabitats) but not in the other 31 ones (associated with 248 surrounding, unused microhabitats).

Descriptive values of used microhabitats are presented in table 1. The PCA captured a moderate portion of the observed data variance,

Table 1. Descriptive statistics of *Boana punctata* male microhabitat use in Campo Grande (Brazil) for the studied variables. Water depths, measured to within 1 cm in the 5-plots established in a grid, were averaged for the analysis.

	Used microhabitats means ± SD (min, max) ($n = 67$)	Surrounding, unused microhabitats means ± SD (min, max) ($n = 536$)
Vegetation density	41.5 ± 30.8 (0, 221)	39.7 ± 48.0 (0, 298)
Vegetation cover (%)	47.7 ± 28.0 (0, 100)	44.3 ± 34.8 (0, 100)
Vegetation height (cm)	138.6 ± 72.3 (25, 350)	102.8 ± 84.9 (0, 450)
Shore distance (cm)	27.0 ± 74.7 (−40, 500)	−43.3 ± 152.9 (−600, 400)
Water depth (cm)	7.5 ± 5.5 (0, 23.4)	8.2 ± 15.4 (0, 150)

with the first two axes representing 68.56% of the total variability of the microhabitat traits (48.74% in PC1 and 19.82% in PC2). The variables that contributed the most to PC1 were "vegetation density" (0.530) and "vegetation cover" (0.475), while "water depth" (0.677) and "shore distance" (0.485) contributed the most to PC2. The biplot indicated that the values of vegetation and water conditions were more dispersed in available, unused microhabitats than in used microhabitats (fig. 3). Thus, used microhabitats seemed less variable than available microhabitats for the studied variables, suggesting that males selected their calling sites among available areas.

Regarding the multi-model inference CLR approach, four models exhibited delta $AIC_c < 2$ and 70 models were included within an accumulative Akaike weight of 0.95. Since model uncertainty was evident, we conducted model averaging. Results from averaging the sets of models conformed by the two selection criteria (delta $AIC_c < 2$ and Akaike weight up to 0.95) were similar. Thus, for the sake of simplicity, we reported herein the model averaging results of the four top models conformed by the first selection criteria (table 2). These results revealed that (1) habitat selection by reproductive *B. punctata* males was only modulated by vegetation height (animals selected higher vegetation) and distance to the shore (they selected microhabitats slightly within the ponds), (2) vegetation density, vegetation cover, and water depth had no effect on the selection process, and (3) microhabitat selection was similar for males both successful and non-successful in obtaining females to spawn (table 2, fig. 4). Positive coefficients

clearly indicated that males actively selected both higher vegetation than would be expected by chance and microhabitats further from the shore of the pond than would be expected by chance. Specifically, the odds of a male selecting a calling site within the ranges of environmental variables increased both by 0.08% with the height (cm) of the tallest vegetation and by 0.07% with the distance (cm) from the shore of the pond (table 2 and fig. 4).

Discussion

It is well-known in many ectotherms, containing amphibians, that various environmental factors, such as the warm or cool environmental temperatures during winter and the delay of the rainy season, generate plasticity in the within-year timing of oviposition or breeding phenology shifts through the use of a reaction norm (e.g., Scheiner, 1993; Schlichting and Pigliucci, 1998; Kakegawa and Hasumi, 2017). In the urban areas studied herein, a reproductive period of *B. punctata* began late due to the delay of the rainy season (January-May). This corroborates a previous report from the Pantanal Wetland (Prado, Uetanabaro and Haddad, 2005).

Vegetation height is an important influencing factor of calling site selection for several hylid species (Wells and Schwartz, 1982; Bastos and Haddad, 1999; Eterovick and Ferreira, 2008; Santos et al., 2016). Male perch height can improve visual communication between individuals (Abrunhosa and Wogel, 2004). Thus, a well-positioned male obtains advantages over
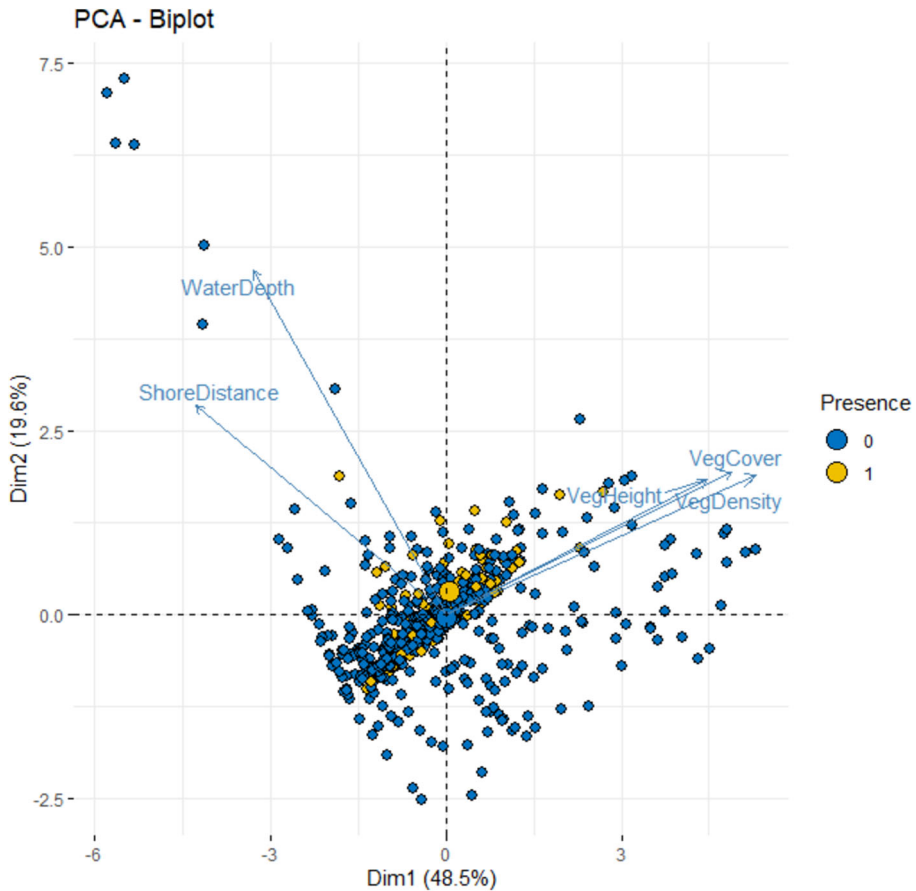
Figure 3. Principal Component Analysis (PCA) biplot displaying availability areas (blue circle), microhabitat areas used by males (yellow circle), and five environmental variables (VegDensity = vegetation density, VegCover = vegetation cover, VegHeight = vegetation height, WaterDepth = water depth, and ShoreDistance = distance from the shore of the pond: refer to the text for details).

other individuals, male or female, for its visualization (Hödl and Amézquita, 2001). Therefore, in territorial species, such as *B. punctata* (Brunetti, Taboada and Faivovich, 2014), perching in tall vegetation can facilitate territory defense and avoidance of physical combat, which requires more energy expenditure and entails risks (Hartmann et al., 2005; Giasson and Haddad, 2006). Auditive communication, the main type of anuran communication, can also be influenced by vegetation structure (Greer and Wells, 1980; Narins and Hurley, 1982; Dyson and Passmore, 1988; Lopez et al., 1988; Gerhardt, 1994; Schwartz et al., 2016). Sound is better propagated from elevated vocalization sites, mainly because they are located in

open areas, avoiding physical obstacles (Wells and Schwartz, 1982; Townsend, 1989; Forrest, 1994; Bastos and Haddad, 1999; Schwartz et al., 2016). Selecting microhabitats with high vegetation allows males to reach higher perches. This selection improves the vocalizing propagation-efficiency (rather than the territorial or reproductive efficiency) and facilitates the visual encounters with other individuals (Chinchilla-Lemus, Serrano-Cardozo and Ramírez-Pinilla, 2020). Hence, selecting higher vegetation may be important to enhance *B. punctata* male communication.

Boana punctata emits different types of calls in different situations, i.e., territorial, courtship, aggressive, fighting, and release calls (Brunetti,

Table 2. Multi-model inference results from the Conditional Logistic Regression model for the effect of five environmental covariates (vegetation cover, vegetation density, vegetation height, water depth, and shore distance) and their interaction terms between successful and non-successful males in obtaining females to spawn on the probability of male microhabitat use, including the identity of individual males as a random factor. The top portion of the table displays the averaged parameters of the models with delta < 2 AIC_c (significant results are marked in bold) and the lower portion of the table provides information about the four top models and their weight in the averaged parameters.

	Estimate	SE	Adjusted SE	z	Probability (P)
Intercept	−2.828	0.307	0.308	9.183	**<0.001**
Shore distance	0.007	0.001	0.001	4.642	**<0.001**
Vegetation height	0.008	0.002	0.002	4.355	**<0.001**
Water depth	−0.019	0.014	0.014	1.348	0.178
Vegetation density	−0.003	0.004	0.004	0.771	0.440
Spawning	−0.117	0.272	0.273	0.429	0.668

Model	Df	logLik*	AIC_c	$\Delta\,AIC_c$	Akaike weight
145	5	−189.59	389.28	0.00	0.39
14	4	−190.97	390.00	0.72	0.27
1345	6	−189.28	390.69	1.41	0.19
1245	6	−189.50	391.14	1.85	0.15

*Maximum likelihood of the model. Coefficients are averaged based on their Akaike weights for each of the four top models.
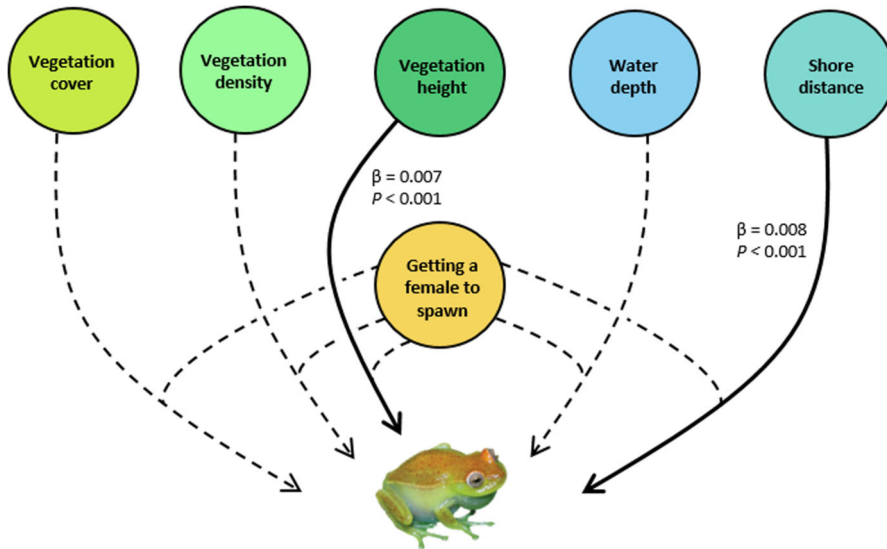

Figure 4. Results of the averaged coefficients of the best Conditional Logistic Regression (CLR) models fitting the effect of the five environmental covariates (vegetation cover, vegetation density, vegetation height, water depth, and shore distance) in the probability of the presence of reproductive *Boana puntacta* males, depending on whether they obtained a female to spawn or not, and model conditioning by the ID of each frog (with one used vs eight unused microhabitats per individual, in a case/control design; refer to the text for more details). Dashed lines represent non-significant relationships, and solid lines represent signticant effects. Refer to the text for more details on how models were fitted and averaged.

Taboada and Faivovich, 2015), as shown in *Boana atlanticus* (Hylidae) (Camurugi, Röhr and Juncá, 2015). Male advertisement and territoriality calls are performed at different vegetation heights; territorial calls are emitted from high vegetation locations, while advertisement calls are emitted at the water level (Brunetti, Taboada and Faivovich, 2014). In our study, both spawning males and non-spawning males selected for tall vegetation. This fact suggests that male-male communication in territorial defense and delimitation (that happens in tall

vegetation) plays an important role in calling site selection in *B. punctata*.

For some anurans, vegetation density is an important factor in choosing spawning microhabitats, providing spawning fixation locations and defense against predation (Wells, 1977; Given, 1988). Vegetation, as well as the substrate, can also be a source of food for future tadpoles (Kopp, Wachlevski and Eterovick, 2006). *Melanophryniscus stelzneri* (Bufonidae) bases microhabitat decisions on the density of emerging vegetation that is located around the pond margins on the water surface; fixing the spawn to the emergent vegetation can be important for this species (Pereyra, Lescano and Leynaud, 2011). In addition, predation rates by aquatic insects on *Dendropsophus minutus* (Hylidae) and *Scinax curicica* (Hylidae) tadpoles from the Serra do Caraça (Minas Gerais, Brazil) are higher in microhabitats without aquatic vegetation, demonstrating the importance of vegetation in microhabitat selection and tadpole survival (Kopp, Wachevski and Eterovick, 2006). Considering these examples and the fact that *B. punctata* also fixes its spawn in emergent vegetation, we expected that microhabitat vegetation density would modulate site selection. Why vegetation density did not play a significant role in calling site selection for this species is still unknown, and future comparative studies are required.

Predation has been shown as a very powerful factor in calling site selection. Several studies have demonstrated that individuals from different species are able to detect the presence of predators in water bodies and avoid those location (Spieler and Linsenmair, 1997; Eterovick and Sazima, 2000; Rudolf and Rödel, 2005; Buxton and Sperry, 2017). Cayuela et al. (2017) also demonstrated that reproductive partner selection by *Bombina variegata* (Bombinatoridae) females can be influenced by water characteristics of the pond chosen by males, so the presence of predators in the pond is a relevant factor for this choice, surpassing the importance of male calls. Some environmental factors are important for the selection of spawning microhabitats in other species, including the depth of the body of water (Goldberg, Quinzio and Vaira, 2006). Many studies suggest that the depth of the body of water is important as an environmental factor and, in fact, there is a lower predation rate in a shallower water that makes animals safe in a spawning area (e.g., Rudolf and Rödel, 2005; Pereyra, Lescano and Leynaud, 2011). Despite the importance of water depth for microhabitat selection of some species, and contrary to what was expected in the present study, this variable was not associated to spawning microhabitat selection by *B. punctata* males. However, the fact that these individuals selected microhabitats slightly inside the assessed ponds suggests that this could be enough to effectively avoid predation in these urban populations. This would also explain the lack of selection of sites presenting denser vegetation.

We did not expect the similarity of microhabitat selection between successful and nonsuccessful males in obtaining females to spawn, where both selected calling sites located on higher vegetation and at greater distances from the pond shore (inside the pond) than expected by chance. This may be due to territoriality, since male-male defense calls are emitted from high vegetation perches in this species. The observed distance to the water could be related to humidity conditions for egg development, although further studies are required. Other environmental factors not determined herein, such as predation pressure and intraspecific competition for spawning sites, are known and frequently reported as important for site selection in anurans (Given, 1988; Eterovick and Sazima, 2000; Rudolf and Rödel, 2005; Goldberg, Quinzio and Vaira, 2006). Therefore, these factors should be assessed in future research on spawning microhabitat selection in *B. punctata*. Notwithstanding, our results provide for urban planners an important piece of information about conservation of this species in urban

ponds. It is essential to preserve an adequately large pond having an adequate shore distance surrounded by adequately tall vegetation up to 140 cm. In such a pond, frogs can select suitable microhabitats for reproduction. Assessing amphibian habitat selection through a resource selection approach is a non-invasive and powerful tool that goes beyond classic habitat use studies to provide more specific information for the urgent conservation measures that must be implemented (both in the wild and in urban areas) in the following decades.

Conclusion

This is the first study to report the microhabitat selection of *B. punctata*. Our results indicated that vegetation structure was important for males in the selection of calling sites, as expected. Males chose higher microhabitats significantly further from the water shore (inside the pond) than expected by chance regarding available urban pond habitat. Nonetheless, contrary to our expectations, microhabitat selection was similar for males successful and non-successful in obtaining females to spawn.

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