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Article

Self-made home: how and where does the anuran *Rhinella dorbignyi* build its retreat sites

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ABSTRACT. In this study, we observed that burrows of *Rhinella dorbignyi* (Duméril & Bibron, 1841) are distributed in a non-random manner in the habitat, suggesting a microhabitat selection for digging. This conclusion was based on a characterization of 36 burrows and surrounding micro-habitat. We established a 1 m x 1 m quadrat with the burrow in its central point (n=36) to measure the percentage (density) and the average heights of grasses, herbs, and shrubs. All measurements were repeated in two unused quadrats (without burrows) to evaluate the available microhabitat (n=72). The burrows are built in specific areas of the habitat with a higher percentage of grass, taller herbs, lower density of shrubs and low shaded sites than the founded at control sites. Based on three-dimensional models of the interior of the burrow (n=15), we observed that all of them were constructed with an elliptical opening that opens into a narrow channel perpendicular to the ground. Channels had a mean maximum diameter of 38 mm and a mean minimum diameter of 18 mm. The mean length of the burrows is 182 mm, and the mean volume is 95 mL.

KEYWORDS. Amphibians; shelter; site selection; thermoregulation; burrowing; Bufonidae.

RESUMO. Com as próprias patas: Como e onde o Sapinho-de-Jardim *Rhinella dorbignyi* constrói seus abrigos. Neste estudo, observamos que tocas de *Rhinella dorbignyi* (Duméril & Bibron, 1841) são distribuídas de uma forma não-aleatória no habitat, sugerindo que esta espécie seleciona sítios para cavá-las. Esta conclusão foi baseada em uma caracterização de 36 tocas e do micro-habitat que as cerca. Estabelecemos um quadrante de 1 m x 1 m com a toca como seu ponto central (n=36) para medir a porcentagem (densidade) e a altura média de gramíneas, plantas herbáceas e arbustos. Todas as medidas foram repetidas em dois quadrantes não utilizados (sem tocas), para avaliar o micro-habitat disponível ao anfíbio (n=72). As tocas são construídas em áreas específicas do habitat com maior porcentagem de gramíneas, ervas mais altas, menos arbustos presentes e pouca área sombreada. Baseado em modelos tridimensionais do interior das tocas (n=15), observamos que todas elas são construídas com uma abertura elíptica que se abre para um canal estreito, perpendicular ao chão. Os canais têm diâmetro máximo médio de 38 mm e diâmetro mínimo médio de 18 mm. O comprimento médio das tocas é de 182 mm, e o volume médio é de 95 mL.

PALAVRAS-CHAVE. Anfíbios; abrigo; seleção de sítio; termorregulação; escavação; Bufonidae.

Amphibians are strongly affected by fluctuations in humidity and air temperature (Duellman & Trueb, 1994; Navas *et al*., 2008) because, besides being ectotherms, they have poorly keratinized skin. Ectothermy demands specific behaviours that help them maintain operative body temperature and reduce susceptibility to desiccation (Wygoda, 1984; Navas, 1996; Young *et al*., 2006). Selecting retreat sites is a common strategy to deal with adverse conditions (Tozetti & Toledo, 2005; Wells, 2007; do Vale *et al*., 2018).

Retreat sites must provide more stable temperature and humidity conditions than more exposed locations (Denton & Beebee, 1993) and increase survival in dry weather (Schwarzkopf & Alford 1996; Seebacher & Alford, 2002). The environment offers a series of available natural retreat sites, e.g., under the leaf litter, under rocks or in tree hollows (Gudynas & Gehrau, 1981; Peixoto, 1995; Kwet *et al*., 2010; Maneyro *et al*., 2017; Moser *et al*., 2017). On the other hand, many anurans "build" their retreat sites by burying themselves into the ground. Most burrowing anurans use the burrows temporarily, for example, during the hottest and driest periods of the day to decrease the rate of water evaporation through the skin, lose heat or rest outside the reach of predators (Wells, 2007).

Some leptodactylids, for example, can also use burrows as reproductive sites (Prado *et al*., 2002). This behavior was observed in several species of *Leptodactylus* belonging to *Leptodactylus fuscus* group, as is the case of the whistling frog (Martins, 1988; Freitas *et al*., 2001). In this species, the burrow is used for egg laying and its

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construction is limited to reproductive behavior (Haddad & Prado, 2005). The South American Dorbigny's toad, *Rhinella dorbignyi* (Duméril & Bibron, 1841), uses its hind limbs to dig burrows (Langone, 1994; Wells, 2007; Maneyro *et al*., 2017) which, different from other species, it uses for several consecutive days, alternating its use with foraging periods outside (Sanchez & Busch, 2008; Maneyro *et al*., 2017, appendix, video A1).

However, details on the construction or selection of sites for use remain unknown (Achaval & Olmos, 1997). As digging is a time and energy-consuming behavior (Wells, 2007), it is plausible to consider that *R. dorbignyi* selects specific microhabitats to build them. In this study, we describe the shape of the burrows of *R. dorbignyi* and evaluate the microhabitat selection for their construction.

MATERIALS AND METHODS

Between April 2 and 6, 2016, we studied some burrows of the Dorbigny's Toad, *Rhinella dorbignyi* (Duméril & Bibron, 1841) in the Taim Ecological Station (ESEC Taim), a federal protected area and Ramsar site in Brazil's extreme south (32°20' – 33°00'S, 52°20' – 52°45'W). The area consists of wetlands associated with natural grasslands with rare tree formations with shrub areas (Fig. 1; Wollmann & Simioni, 2013; Josende *et al*., 2015).

We studied the toads in a plain terrain of a coastal area of many small temporary ponds. In terms of soil classification, the substrate did not vary along the ESEC Taim (Cunha *et al*., 2018). According to the federal land survey service (available at https://dados.gov.br/dataset/cren_solos_5000/resource/4b1042a4-b88e-470d-8b2a-e716714fb3e5), all Taim's area is over a hydromorphic vertosol (Santos *et al*., 2006; EMBRAPA, 2013). Due to its high concentration of organic matter and silt, the Taim's soil is little permeable, favoring flooding and the formation of temporary ponds. Usually, the silt layer reaches up to 2 m in depth (Cunha *et al*., 2018). Because of flooding and drying events, the substrate is, in general, highly compacted (Cunha *et al*., 2018).

Four people searched for the toads' burrows during daytime via visual search. We only sampled burrows with indications that they were being used until recently by the toads (without signs of dryness or spiders inside). When a burrow was detected, its interior was inspected with the aid of a flashlight to check for the presence of the "resident" amphibian. Recently abandoned burrows that were not currently occupied by a toad were used to build three-dimensional casts to characterize the internal design. To minimize the stress of animals, only burrows without toads were modeled. To create the models, we diluted Plaster of Paris in water until it acquired a pasty consistency. Afterward, the mixture was poured into the opening of the hole until filling it. After 20 minutes, the model was hard enough to be removed (Fig. 2). To describe the internal design of the burrows, we performed the following measurements from models: chamber length, straight from the top to the bottom of the cast; maximum channel diameter, and minimum channel diameter, measured in the burrow opening. The

Fig. 1. Representation of the vegetation cover from Taim Ecological Station (ESEC Taim), a federal protected area and Ramsar site in Brazil's extreme south. In the first plain, a grassy microhabitat (where we can find *Rhinella dorbigbyi*'s burrows), and a shrub area in the back (Photo: A. M. Tozetti).

Fig. 2. Characterization of the shape of the *Rhinella dorbignyi* (Duméril & Bibron, 1841) burrow opening, three-dimensional model in plaster of the burrow and the representation of a longitudinal view of the excavation that forms the burrow (Photo: L. K. Schuck).

linear measurements were made by an electronic caliper with a precision of 0.01 mm. To obtain the total volume of the model, we submerged it into a graduated container filled with water and measured the displacement of the liquid.

To characterize the micro-habitat where each in-use burrow was built, we established a 1 m x 1 m quadrat with the in-use burrow in its central point. Within this quadrat, the percentage (density) of each vegetation type and exposed soil was visually estimated. For each site, we estimated the percentage of the quadrat that would be shaded at midday. The height of grasses, herbs, shrubs was measured from the highest plant origin in soil to its highest branch. All described measurements were repeated in two additional quadrats, with no burrow, situated one quadrat in the north and the other in the south direction, with its nearest border 2 m from the burrow. We chose this distance because distances smaller than 2 m include very spatially dependent areas, having a similar vegetation cover. At the same time, greater distances include very different points in terms of water accumulation, prey availability, and other elements that we did not assess. This decision is somewhat arbitrary but, according to literature, the toads are not expected to move very much (Oliveira *et al*., 2017; Moser *et al*., 2019). Therefore, supported by previous data about this species, we believe that the chosen distance is compatible with the area that could be chosen by the toads at the time of site selection for the construction of the burrow. The measured characteristics of the used micro-habitat were compared to the micro-habitat available around each burrow.

To test whether the sites where in-use burrows were found were selected by anurans considering the measured habitat characteristics, we performed a Permutation Multivariate Analyses of Variance (PermANOVA) with Randomization Tests based on a matrix of Euclidian distances between sites described by the habitat variables, previously centered and normalized within variables (Pillar & Orlóci, 1996). The test statistic for main effects was the sum of squares between groups (Qb), while the pseudo-F ratio was the test statistic for the interaction term (Pillar, 2013). This numerical analysis was run in MULTIV version 3.55b (Pillar, 2006). We then performed a Principal Component Analysis (PCA) based on a correlation matrix between the microhabitat variables using PAST3 (Hammer *et al*., 2001), transforming percentage values of vegetation cover in its arcsin for normalization. We selected the ordination axes that explained 75% of the variance in the site's characteristics for interpretation. Using a standard linear (least-squares) regression, we tested the proportionality between the average length and maximum diameter of the burrow and the length of the burrow. For this analysis, we used PAST3 (Hammer *et al*., 2001).

RESULTS

We evaluated the location of 36 in-use burrows of *Rhinella dorbignyi* and built 15 models of recently abandoned burrows in plaster to describe their shape. All burrows had a single entrance (opening) that is slightly elliptical and connects to a channel. The channel is perpendicular to the ground and ends in a single blind bottom. The mean length of the burrows was 182 mm. The average maximum diameter of the channel was 38 mm, and the average minimum diameter was 18 mm. Both diameters (maximum and minimum) showed a small range of variation, the average total volume of burrows was 95 ml (Tab. I; appendix, table A1).

We observed a positive relationship between the maximum diameter and the length of the channels (linear regression; $r = 0.590$; $t = 2.638$; $p = 0.021$; appendix, fig. A1). The microhabitat where toads built their burrows was significantly different from the available microhabitat in terms of vegetation cover ($Q = 0.21853$; $P = 0.024$). The two first PCA axes explained 76% of the variance in the micro-habitat characteristics on each site. The first axis loadings (56% variance explained) were positively related to shrubs height, shrubs percentage and shaded area, and negatively related to the grass percentage and herbaceous height. The second axis (22% variance explained) loadings were positively related to the percentage of herbs and shaded areas and negatively related to the percentage of grass (Tab. II; Fig. 3).

Tab. I. Means and standard deviation for cast measurements of *Rhinella dorbignyi* toad's burrows.

	Length (mm)	Maximum Diameter (mm)	Minimum Diameter (mm)	Volume (mL)
Mean	182.73	38.44	17.52	95.04
Standard deviation	84.94	12.11	5.36	52.99

Tab. II. PCA's first two principal components (PCs): eigenvalues, percentage of variance and variable loadings for *Rhinella dorbignyi* (Duméril & Bibron, 1841) burrow site's microhabitat characteristics.

PC	1	2
Eigenvalue	3166.7	1245.51
% variance	55.499	21.829
Herbaceaous height	**-0.12603**	0.4222
Herbaceous percentage	-0.077098	**0.5258**
Shrub height	**0.82765**	-0.21668
Shrub percentage	**0.30358**	-0.10104
Grass height	-0.049938	-0.073513
Grass percentage	**-0.218**	**-0.45913**
Percentage of shadowed area	**0.38545**	**0.52114**
Percentage of exposed substrate Area	-0.049152	0.01782

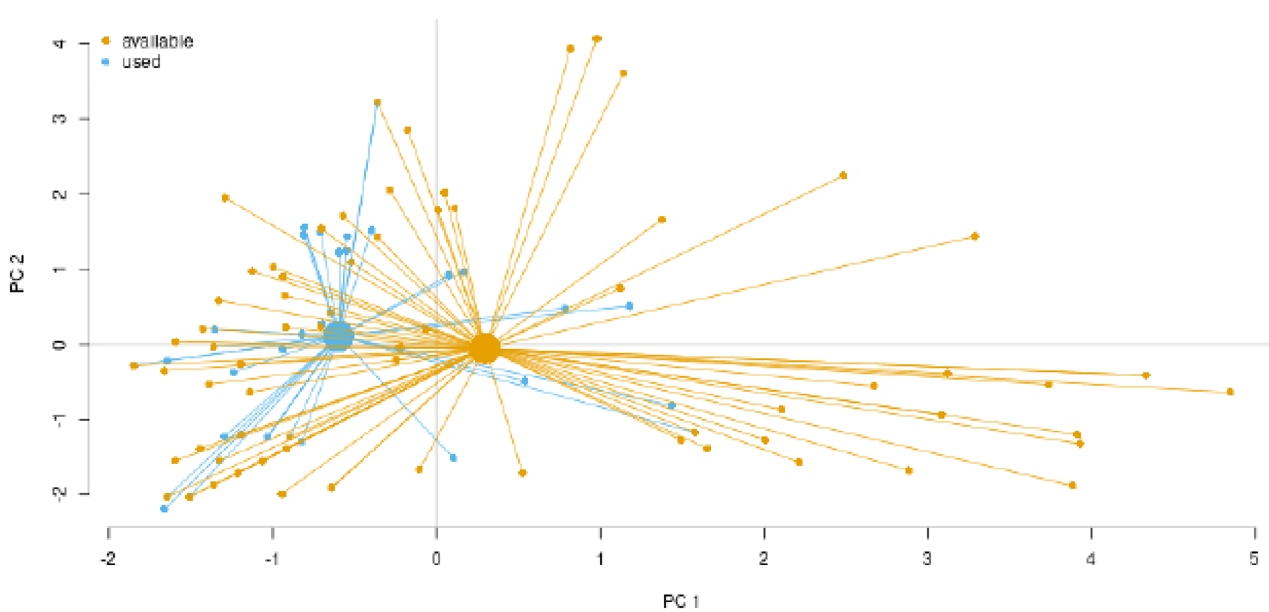

Fig. 3. Microhabitat ordination calculated using principal component analysis based on Euclidean distances. Colors define the use of the site to the construction of *Rhinella dorbignyi*'s burrows. Used sites are in blue (n = 36) and available sites in orange (n = 72). Large circles indicate centroids for each group. The points represent each evaluated site, and the lines connect each sample to the group centroid.

DISCUSSION

We found a consistent pattern in the construction of all the observed burrows, as they all had a well-defined single entrance connected to a channel perpendicular to the surface. There was less variation in the height and width of the burrows' opening than the variation found in burrow length (Tab. I), which could suggest a tendency of individuals to build burrows adjusted to their size, which does not show much variation for adults in this species (Sanchez & Busch, 2008). Constructing a smaller burrow, adjusted to the animal's body size, could minimize the energetic cost of excavation and reduce the thermal exchange, with surface thermal stability (Schwarzkopf & Alford, 1996). Adjusted burrows may also be more efficient in preventing predators from entering since only animals with the size equal to or smaller than the resident of the burrow will be able to access the shelter. Unfortunately, we were unable to crossmatch measurement data from toads and their burrow since we sampled abandoned burrows exclusively. We assumed this weakness in our sampling (modelling only recently abandoned burrows) not to affect toad survival since our burrow modelling process implicated in the destruction of the burrows (see methods for more details).

The channel's length was the linear component with the greatest coefficient of variation (CV% = 46.48) in comparison with the maximum diameter (CV% = 31.48) and minimum diameter (CV% = 30.59). As the channel is arranged perpendicular to the ground, we presume that the longer the channel, the greater the thermal gradient between the opening (surface) and the bottom. By adjusting their position along the channel, the toads possibly could obtain the appropriate conditions of temperature and humidity, even when external conditions are harsh (Székely *et al*., 2018). In addition, considering the correlation between the maximum diameter and length of the burrows, it is possible that larger individuals, with greater energy capacity, can build deeper burrows. Studies in other locations with *R. dorbignyi* (Pereyra *et al*., 2021) found that the length of the burrows corresponds to about three times the size (SVL) of the inhabiting toad (Gallardo, 1957; 1969). As amphibians can rehydrate by absorbing water from the soil (Booth, 2006), the level of soil moisture may influence the extent of the dug channels. Thus, it is plausible to think that, during hot sunny days, a long channel would offer a wide range of conditions, from hot/dry to cold/moist as the toads move from the entrance to the bottom of the burrows.

In addition to the design of the burrows, the choice of the location for their construction also proved to be an important factor. Burrow sites were not randomly distributed between the nearby available microhabitats. As showed in our results, the toads selected sites that were less shadowed than the average burrow surrounding microhabitats (see our PCA). They also selected sites with a higher percentage of herbaceous vegetation and a smaller shrub extent. It seems plausible to suppose that the configuration of the selected sites helps toads improve the control of body temperature. When selecting little shaded and more "open" microhabitats, the toads enable solar irradiance over the burrow opening. This would be an important strategy considering the harsh winter in the study area compared to the tropical pattern.

We emphasize that our study sheds light on the possibility of the burying behaviour contributing to active thermoregulation in toads. This increases the relevance of the data we present and is also important for observing behavioural aspects of ectotherms.

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APPENDIX

Tab. A1. Values of burrows' measurements taken from the plaster models of *Rhinella dorbignyi* burrows, each model represent a burrow.

Models	Length (mm)	Maximum diameter (mm)	Minimum diameter (mm)	Volum (mL)
Model 1	200.93	33.28	14.78	68.35
Model 2	85.81	35.63	15.78	28.00
Model 3	93.92	35.84	14.08	34.50
Model 4	342.95	46.52	14.01	150.50
Model 5	212.35	51.99	17.17	125.34
Model 6	171.08	39.77	21.62	109.00
Model 7	89.27	34.52	18.61	26.50
Model 8	155.49	36.20	14.82	44.00
Model 9	221.93	38.91	18.67	104.50
Model 10	146.20	33.69	18.71	55.34
Model 11	231.13	50.92	25.47	122.01
Model 12	92.74	21.36	14.94	104.00
Model 13	242.91	42.80	17.75	121.00
Model 14	260.88	39.79	20.37	164.50
Model 15	193.32	35.45	16.03	168.00
Mean	182.73	38.44	17.52	95.04
Standard deviation	84.94	12.11	5.36	52.99

Video A1. *Rhinela dorbignyi* using its burrow at the study site. Link: https://youtu.be/iBmci3LLCXE

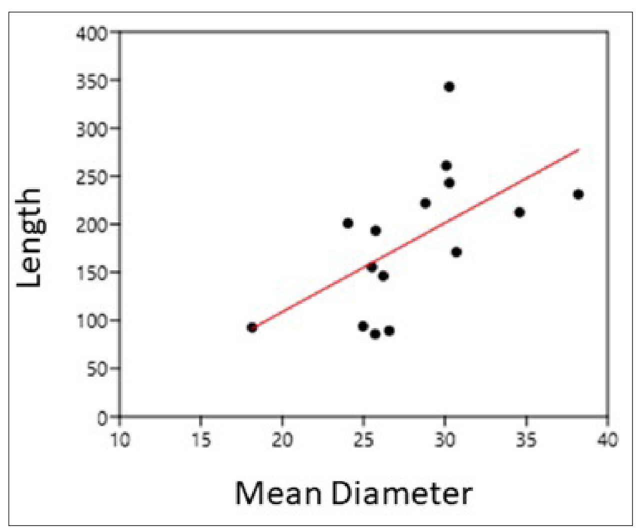

Fig. A1. Linear regression plot between mean diameter of the channel's opening and channel length