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Source: Folia Zoologica, 59(2) : 122-128

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: https://doi.org/10.25225/fozo.v59.i2.a7.2010

Folia Zool. – 59 (2): 122– 128 (2010)

Terrestrial habitat use by the burrowing toad, *Rhinella fernandezae* (Anura: Bufonidae)

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Received 13 January 2009; Accepted 2 June 2009

Abstract. We studied the association between environmental variables and the terrestrial habitat use by adults of the burrowing toad *Rhinella fernandezae* in Buenos Aires, Argentina. We assessed the cover type, height of vegetation, soil moisture and hardness. We compared these habitat variables in quadrats where toads were present along a transect (n = 30 quadrats, 19 with toad burrows) with respect to randomly chosen quadrats (n = 17). We found that *R. fernandezae* does not use the habitat at random, and that habitat variables that mostly influenced its habitat use were soil hardness after raining, probably because it facilitates the construction of burrows, and broad-leaved plant cover, which may be affecting toads indirectly through other habitat variables, such as shadowing, air moisture and refuge from predators.

Key words: microhabitat, distribution of toad burrows, vegetation, soil moisture, soil hardness

Introduction

Amphibian habitat use depends on many environmental factors and is influenced by the behavioral and physiological requirements of each species (Thompon & Gates 1982). The complexity of amphibian habitat requirements results, in part, from their need of adequate conditions for the specific requirements of each life stage, since most of them breed in aquatic habitats but spend most of their lives in terrestrial habitats (Trenham & Shaffer 2005, Hartel et al. 2008). However, for many amphibian taxa, biological information concerning the use of terrestrial habitats adjacent to aquatic breeding sites remains sparse (Miaud et al. 2000, Rothermel & Semlitsch 2002). This research inequality between aquatic and terrestrial amphibian habitat is probably due to the relative difficulty of terrestrial studies, because of their secretive life style (Trenham & Shaffer 2005).

In this study, we examined the association between environmental variables and the terrestrial habitat use by adults of a burrowing toad. The burrowing toad, *Rhinella fernandezae* (Gallardo, 1957), is found in Uruguay, southern Paraguay, southern Brazil and northeastern Argentina (IUCN et al. 2004). Adult size ranges from 56 to 80 mm (Gallardo 1987). Both sexes use individual burrows where they spend most of the day. *R. fernandezae* digs with its hind feet in moist soil, and sometimes uses natural cavities or burrows left by other animals (Gallardo 1957, 1969). The size of the burrow made by the toad is approximately three times its size, varying in adults from 17 to 28 cm (Gallardo 1957, 1969). This species reproduces from October to March (Gallardo, 1957) in ponds, ditches, flooded areas, temporary swamps, and occasionally in the periphery of permanent lakes. Although *R. fernandezae* is a very common species in its area of distribution, little is known about its

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ecology. Rosset & Alcalde (2004) described its presence in areas with abundant cover of grasses, cyperaceas, and legumes. Yanosky et al. (1997) observed that *R. fernandezae* uses grassland more frequently than forested areas. The foraging strategy of this species is to wait in the burrow and prey upon many types of invertebrates that pass near the entrance (Lajmanovich 1995). They prey mainly on Formicidae, and to a lesser extent on Coleoptera and Araneae (Basso 1990). Its predation strategy implies that they travel small distances around the burrow, although they sometimes change burrows (Sanchez & Busch 2008) or move larger distances, especially during the reproductive period (Gallardo 1969). The aim of this study is to test the hypothesis that toad burrows are localized nonrandomly. Specifically, we assess the relation between the distribution of toad burrows and various biotic (total plant cover, broad-leaved plant cover, grass cover, shrubs and trees cover, herbs height, and shrubs height) and abiotic factors (soil moisture and hardness).

Material and Methods

Study area

The study was conducted at Escobar (34º21´S, 58º48´W), Buenos Aires province, Argentina (Fig. 1), in a riparian environment within the Pampean Region, undulated subdivision (Soriano 1992). The climate is subhumid with an annual average precipitation of 950 mm and without a clear dry season (Viglizzo et al. 2001). The soil of the area is sedimentary, with a high proportion of clay, and it is saline. The study site was a footpath (approximately four meters wide and 400 m long) surrounded on both sides by low frequently flooded areas, which are connected to the Luján River, where we observed *R. fernandezae* reproducing and where we collected eggs. The margins of the footpath were covered by natural vegetation (e.g. grasses, cyperaceas, broad-leaved plants, shrubs and trees). The dominant plant species were *Baccharis pingraea*, *B. leptophyllum*, *Beta vulgaris*, *Bromus* sp., *Celtis tala*, *Centaurium* sp., *Cortaderia selloana*, *Dipsacus* sp., *Distichlis* sp., *Eryngium* sp., *Hydrocotyle* sp., *Juncus* sp., *Lantana* sp., *Lepidium* sp., *Malva parviflora*, *Phyla sp.*, *Solidago chilensis*, *Sporobolus* sp., and *Stipa* sp.

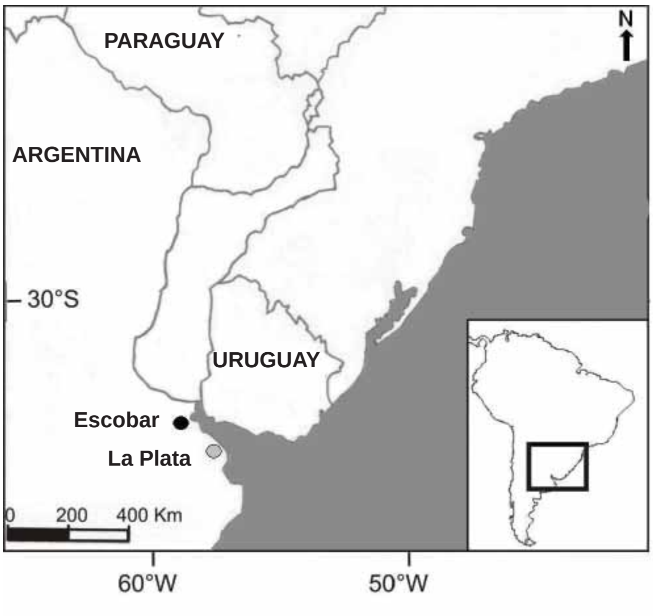

Fig. 1. *Location of the study area at Escobar, Buenos Aires province, Argentina, and the Rosset & Alcalde (2004) study area in La Plata.*

Microhabitat use

To study the pattern of habitat use we followed the Design I and protocol A (Manly et al. 1993), in which used and available resource units are sampled at the population level. According to protocol A, available resource units were randomly sampled and a random sample of used resource units was taken.

We conducted samplings between 6 December 2002 and 7 January 2003. In order to asses habitat use we sampled for location of toad burrows along a transect located on a footpath. The initial point at the transect was randomly selected, and then we sampled every 10 meters covering a total distance of 300 m (n = 30 quadrats). At each point we look for toad burrows within 9 m^2 quadrats centered in the footpath but covering the natural vegetation of the surroundings, because in a preliminary sampling we found toad burrows in both types of places. The size of the quadrat (9 m^2) was chosen taking in account the probability of the detection of burrows, and the size of the surrounding area whose characteristics may influence their location (e.g. providing shadow). These sampling units (quadrats) were considered “used” if we found at least one occupied toad burrow in them (n = 19). We considered a burrow occupied (and named it “toad burrow”) when we saw a toad inside, or when the toad put its head out of the burrow when it was filled by water. To estimate habitat availability, we randomly selected 17 quadrats along the 300 m of the footpath (independently of the quadrats previously examined for toad burrows). These random quadrats included both occupied and unoccupied quadrats. Because of logistic problems, we could study habitat characteristics in only 17 random quadrats, instead of 19, which would have been better in order to compare with used quadrats (equal sample size).

In each quadrat we registered: cover and height of vegetation, soil hardness, and relative soil moisture. These variables were selected based on previous investigations about amphibians habitat use, because they were previously found significant indicators of habitat suitability (Wyman 1988, Ildos & Ancona 1994, Hamer et al. 2002, Beard et al. 2003, Crawford & Semlitsch 2008). We also registered soil hardness because this factor could potentially influence habitat use of burrowing amphibians (Carson et al. 2002).

Plant cover was estimated as the proportion of the quadrat covered by vegetation, classified in categories as follow: 0%, 1-20%, 21-40%, 41-60%, 61-80%, and > 80%. Coverage was estimated for the following variables: total plant cover, broad-leaved plant cover, grass cover, shrub cover, and tree cover. For trees and shrubs we considered the projection of the canopy. We measured plant height with a tape measure for herbs and shrubs, whereas taller plants were only recorded as > 200 cm. For herbs, we took the mean and maximal height. Similarly, we recorded any heights less than 5 cm as < 5 cm.

Both soil hardness and relative soil moisture were taken at a depth of about 20 cm, accounting for the size of the toad burrows (Gallardo 1957, 1969). Soil hardness was measured by a Lang penetrometer with a scale that ranges from 0 (soft) to 20 (hard). According to the instructions of the manufacturers of the penetrometer, we multiplied these values by 3587.03 to obtain hardness in g/cm^2. Relative soil moisture was estimated weighing soil samples in electronic scales to the nearest 0.0001 g before and after being dried in a stove during 72 h at 90ºC: Relative moisture = [(weight of moist soil – weight of dry soil) / weight of dry soil] × 100. For both soil hardness and moisture, we took three measures in each quadrat. We computed the mean of these measurements to use in the statistical analyses to have one datum per quadrat. We assessed soil characteristics at least three days after precipitation (variables: soil moisture and soil hardness), and we also sampled immediately (one day) following rainfall, in 28 different quadrats (14 with toad and 14 randomly chosen quadrats), along the same footpath (variables: soil moisture after rain and soil hardness after rain).

Data analysis

In order to assess if the characteristics of the habitat where toad burrows were placed were not a random sample from the available range of habitat variables, we firstly conducted a Principal Components Analysis (PCA) to reduce the number of habitat characteristics to two principal components, which explained the most variation of original variables. We then located both used quadrats and available quadrats within the space generated by these components and performed a spatial analysis under the hypothesis that used quadrats will not be a random sample of available sites, therefore, distances in the multidimensional space would be smaller between pairs of used quadrats than between pairs of used-available quadrats. We computed the values of used quadrat (scores) on each axis starting from the eigenvalues and eigenvectors matrix. Differences between availability and use were assessed by a multidimensional spatial segregation test based on nearest neighbour distances (Dixon 1994). Each point was classified as "random" or "used", and expected distances according to random distribution were estimated according to the frequencies of the two types of points. The global spatial segregation (deviation of "used points" from "random points") was tested by means of the analysis of a 2 × 2 contingency table according to the calculation of expected frequencies, and with the C statistic which has an asymptotic Chi-square distribution with two degrees of freedom, as proposed by Dixon (1994). In case of having an evidence of global segregation, it was tested which category was more or less grouped than that expected by random, with the Z statistic, with asymptotic normal distribution (Dixon 1994).

The data matrix consisted of 9 rows (variables) and 17 columns (quadrats where we measured availability), and both were normalized for their processing (Ter Braak & Smilauer 1998). We did not include in this analysis soil hardness and moisture after rain because they were not measured at the same sites as the other variables.

In order to compare individual habitat variables between used and available quadrats we conducted univariate Mann-Whitney U-tests. A significance level of $P = 0.05$ was used for all statistical tests. The analyses were performed with the program Statistica, version 6.0 (Statsoft 2001).

Results

The range of variation of habitat variables in used sites was equal or smaller than the availability range (Fig. 2) but, only in broad-leaved plants cover and soil hardness after rain, a great percentage of the values showed at used sites were not contained in the

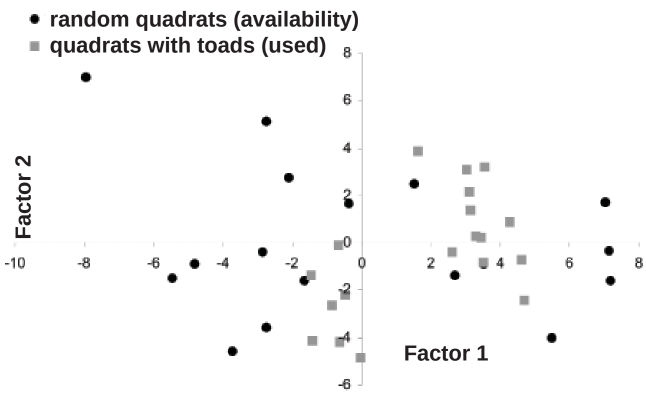

Fig. 2. *Location of the points corresponding to random quadrats (black circles) and quadrats with toads (grey squares) in the space of the first two PCA axes obtained using the 17 random quadrats.*

values recorded at availability sites (Table 1).

The first two PCA axes explained 61.96% of the availability matrix variability (Table 2). The first component (Factor 1) was related to broad-leaved plants cover, grass cover, shrub cover and height of shrubs. The second component (Factor 2) was related to height of shrubs, soil relative moisture, and soil hardness. Random and used quadrats were spatially segregated in the bidimensional space determined by the first two PCA axes (Fig. 2; *C* statistic = 7.7199, df = 2, 0.01 < *P* < 0.025). According to the *Z* statistics, there was a higher than expected frequency of nearest neighbours of the same type (used-used or random-random; Z_{used} = 2.699, df = 1, *P* = 0.0035, Z_{random} = 2.114, df = 1, *P* = 0.0174).

According to the previous analysis, toad habitat use was different than random, and according to univariate test broad-leaved plant cover and soil hardness after rain were the variables that affected selection (Table 3). Broad-leaved plant cover was higher in quadrats with toads than in random quadrats

Table 2. *PCA results with the loading of the variables for the first two axes (factors), the eigenvalues, and the percentage of total variance (* – the most important correlations).*

	Factor 1	Factor 2
Broad-leaved plants cover	-1.0899*	0.7460
Grass cover	1.7435*	-0.2866
Shrub cover	-1.2072*	-0.6773
Tree cover	0.4139	0.4286
Maximal herb height	0.6338	0.8586
Mean herb height	-0.2658	0.5897
Height of shrubs	-1.0539*	-1.0823*
Soil relative moisture	0.7445	-1.7541*
Soil hardness	0.0810	1.1773*
Eigenvalues	7.3464	3.1862
% Total Variance	43.2140	18.7422

Table 1. *Variability in the habitat variables in the randomly chosen quadrats (availability, n = 17) and the quadrats with toads (used, n = 19).*

Variable	QUADRAT					
	RANDOM			USED		
	Median	Lower Quartile	Upper Quartile	Median	Lower Quartile	Upper Quartile
Total plant cover (%)	90.5	70.5	90.5	70.5	70.5	90.5
Broad-leaved plants cover (%)	30.5	10.5	50.5	50.5	50.5	70.5
Grass cover (%)	50.5	30.5	70.5	50.5	10.5	50.5
Shrub cover (%)	30.5	0.0	50.5	0.0	0.0	50.5
Tree cover (%)	0.0	0.0	0.0	0.0	0.0	0.0
Maximal herb height (cm)	130.0	100.0	180.0	110.0	100.0	140.0
Mean herb height (cm)	65.0	60.0	80.0	60.0	55.0	70.0
Height of shrubs (cm)	150.0	0.0	197.0	0.0	0.0	200.0
Soil relative moisture (%)	13.91	11.51	16.42	13.78	9.46	17.42
Soil relative moisture after rain (%)	35.10	33.61	37.01	33.06	32.09	40.15
Soil hardness	9.83	9.00	11.33	10.67	9.00	12.83
Soil hardness after rain	4.99	4.67	5.50	3.50	2.63	4.67

Table 3. *Mann-Whitney U-test for environmental variables in quadrats with toad (use, n = 19) and randomly chosen quadrats (availability, n = 17) (Z – Mann-Whitney U-test statistic;* P *– statistical probability; * – statistically significant values).*

	Mann-Whitney *U*-tests	
Variable	*Z*	*P*
Total plant cover	118.5	0.173
Broad-leaved plants cover	82.5	0.012*
Grass cover	122.0	0.211
Shrub cover	132.5	0.358
Tree cover	144.5	0.590
Maximal herb height	112.5	0.121
Mean herb height	143.5	0.568
Height of shrubs	137.5	0.447
Soil relative moisture	153.0	0.788
Soil relative moisture after rain	76.0	0.312
Soil hardness	160.5	0.975
Soil hardness after rain	51.0	0.031*

(Mann-Whitney U-test, Z = 82.5, n1 = 19, n2 = 17, *P* = 0.012), while soil hardness after rain was lower in used than in random quadrats (Mann-Whitney U-test, Z = 51.0, n1 = 14, n2 = 14, *P* = 0.031).

Discussion

Our results suggest that sites used by toads are not a random sample of those available with respect to measured environmental characteristics. According to the univariate test, the two environmental variables that were related to habitat use by *R. fernandezae* were soil hardness after rain and broad-leaved plant cover. The estimation of the effect of environmental variables on habitat use is influenced both by their variability and the interval of variation: variables which show little variation among quadrats or whose values are all included within the zone of tolerance of the studied animal will not be useful for habitat characterization. Rosset & Alcalde (2004), in their study realized in La Plata (34º53’S, 58º05’W), Buenos Aires province (Fig. 1), found a lack of association between toad burrow density and vegetation cover, probably because this variable was homogeneous in their study area, which was a pasture (1 ha) of annual herbs. But, in contrast with our results, they did not find a relation with soil hardness after rain, probably because the values (which varied between 6 and 18.5 cm) were all included in the range of tolerance of the species. In our study, soil hardness after rain was higher, varying from one to seven in the scale of the Lang penetrometer (equivalent to 0.6 to 4.2 cm). These values probably included both favourable and unfavourable sites, and allowed the expression of selection by toads. Soil hardness may affect burrowing, and toads probably dig their burrows after rain when the soil is soft (Gallardo 1957, 1969). The comparison between these two studies is limited because we sampled different areas (154 m^2 in the study of Rosset & Alcalde, 9 m^2 in our study), and used different methods for estimating hardness.

Broad-leaved plant cover may be indirectly influencing habitat characteristics by providing shelter from the sun and predators or increasing air moisture. Similarly, Crawford & Semlitsch (2008) found that canopy cover, leaf litter depth, and soil moisture, were all higher in plots where salamanders were encountered as compared to plots where salamanders were not encountered. Although we did not find an effect of soil moisture on habitat use, air moisture above surface may affect toads when they leave the burrows. Smith et al. (2003) reported an effect of shade conditions on amphibian habitat use. However, we observed *R. fernandezae* burrows located in sites without shade, and thus this variable may not be as important for species protected in burrows as it is for species on the soil surface. Burrows provide a microclimate that may make toads independent of some surface habitat variables that are important for other amphibians (Gallardo 1969, 1974).

Among the dominant broad-leaved plant species were *Baccharis pingraea* and *B. leptophyllum*, which maintain an associated insect fauna (Kincaid et al. 1983, Boldt 1989) that may provide increased food resources for the toads. Since *R. fernandezae* use a “sit-and-wait” foraging strategy (Lajmanovich 1995), it can be expected that burrows may be placed close to habitats with high abundance of prey or in prey path. Invertebrate mass within a habitat has been linked to anuran mass, indicating that prey availability can affect habitat quality (Sztatecsny & Schabetsberger 2005). Therefore, microhabitat use may be the result of a compromise between the need to maintain hydration levels and also obtaining foraging opportunities (Rittenhouse & Semlitsch 2007).

Although soil moisture has been reported to be an important factor influencing amphibian occurrence and distribution for the anuran *Lithobates sylvaticus* and the caudates *Eurycea bislineata, Desmognathus* *fuscus, Notophthalmus viridescens* and *Ambystoma maculatum* (Wyman 1988), the habitat use of *R. fernandezae* was not influenced by this variable. The climate of the study area, subhumid with an annual average precipitation of 950 mm and without a clear dry season (Viglizzo et al. 2001), probably does not offer restrictions for this species, especially in areas located near temporary flooded areas, as the study site.

In summary, we found that *R. fernandezae* does not use the habitat at random, and that habitat variables that mostly influenced its habitat use are soil hardness after raining, probably because it facilitates the construction of burrows, and broad-leaved plant cover, which may be affecting toads indirectly through other habitat variables. More detailed studies may help to understand the causes of these relations.

Acknowledgements

Thanks to our colleagues from the laboratory of rodent ecology (FCEN, UBA) for their help in early stages of this work. G. Carrizo and J. Faivovich provide valuable literature. A. Ambas, B. Blotto, C. Díminich and P. Bellini help us in the field. The Lang penetrometer was provided by the laboratory of environmental ecology (FCEN, UBA). The botanists R. Palacios and P. Picca helped in the identification of plant species. We thank P. Peltzer and J. Faivovich for their valuable comments on this manuscript.

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