Short notes

Pipe refuge occupancy by herpetofauna in the Amazonia/Cerrado ecotone

Eduardo Ferreira[1,2], Rita Gomes Rocha[1], Adriana Malvasio[2] & Carlos Fonseca[1]

[1]Departamento de Biologia & CESAM, Universidade de Aveiro, Portugal

[2]Laboratório de Ecologia e Zoologia, Universidade Federal do Tocantins, Brazil

We evaluated the usefulness of arboreal pipe refuges for studying Neotropical herpetofauna, by quantifying the effects of microhabitat variables and pipe colouration on pipe occupancy rates. We used fifty five sets of refuges that each comprised three pipes with different colours (white, grey and black). We recorded 122 occupancy events by four hylid and one scincid species. Refuge colour did not significantly affect occupancy rates. Environmental data explained a significant portion (10.6%) of the total variance of occupancy, with vegetation type and height of opening being most important.

Key words: height, Hylidae, PVC refugia, Scincidae, vegetation type

Tree frogs can be easily observed during the mating season at breeding sites, but due to their elusive behaviour are difficult to find outside these areas and periods (Pittman et al., 2008). Consequently, knowledge about tree frog habitat use away from breeding sites is limited (Lemckert, 2004). This lack of knowledge is striking, because terrestrial areas surrounding breeding habitats are crucial for the survival of amphibian populations (Marsh & Trenham, 2000; Semlitsch & Bodie, 2003). Even species with prolonged reproductive seasons spend the majority of their lifetime in terrestrial habitats (Wilbur, 1984). In the case of some Neotropical hylids, reproductive cycles are related to arboreal microhabitats such as tree holes and bromeliads (Haddad & Sawaya, 2000; Haddad & Prado, 2005). For such species, additional information on microhabitat use outside the breeding season is largely lacking.

Artificial pipe refuges have been suggested as a useful sampling method for hylids (Boughton et al., 2000; Johnson, 2005; Myers et al., 2007). Pipes can be installed on trees or on the ground, facilitating their use in forest habitats and reproductive areas. This methodology was successfully used in ecological (Mahan & Johnson, 2007; Liner et al., 2008; Pittman et al., 2008) and conservation studies (Schurbon & Fauth, 2003; Wyatt & Forys, 2004). Colonization success of pipe refuges can be influenced by pipe diameter, placement of pipes, sex and size of tree frogs, tree species and tree frog species under study (Myers et al., 2007; Bartareau, 2004; Johnson et al., 2008; Hoffman et al., 2009). The effect of refuge colour on the colonization success of pipe refuges has not been examined, although Crawford and Kurta (2000) found that anurans were more efficiently captured in black pitfall traps, suggesting that light-coloured traps are perceived as different from the substrate and avoided.

Despite the high tree frog diversity in the Neotropics, pipe refuges are not commonly used in this region (but see Laurencio & Malone, 2009; Silva & Rossa-Feres, 2007). Our aim is to assess the usefulness of pipe refuges for biodiversity surveys and ecological studies in Neotropical habitats, through documenting pipe refuge use by different species, and through correlating environmental data with occupancy rates. We also aim to assess whether pipe colour influences occupancy rates.

We conducted this study in two areas: at the gallery and alluvial forests of the mid-Araguaia River in Parque Estadual do Cantão (PEC, state of Tocantins, east bank) and Fazenda Santa Fé (FSF, state of Pará, western bank), and at Fazenda Lago Verde (FLV, state of Tocantins). PEC is a 90,000 ha state conservation unit, mainly composed of seasonally flooded forests, and FSF is a 65,000 ha private ranch, with 65% of its area covered by well-preserved semi-deciduous tropical forest. Forest cover is dense on both sides of the river (percentage of canopy cover: 78–98%). FLV is an 8,000 ha private ranch with 70% of the area constituted by pristine Cerrado physiognomies. Natural forest fragments (ipucas; percentage of canopy cover: 74–98%) occur within the agricultural and Cerrado matrices.

Three sampling locations were chosen in the alluvial and gallery forests of PEC, two in FSF and three in the ipucas in FLV. We used 55 sets of three arboreal pipe refuges in white, grey and black, respectively. Five sets of pipes were installed at each sampling point in PEC and FSF, and ten at each sampling point in FLV. Pipes were tightly attached to branches at different angles from the upright position (average 12.1º; range 1–50º). They were installed above head height (average 205.9 cm; range 173–237 cm) and at waist level (average 130.0 cm; range 90–142 cm). Pipe refuges were adapted from Johnson (2005), with a length of 40 cm, an inner diameter of 4 cm, a 5 cm diameter of the bottom cap, and a bottom cap length (maximum water level) of 8 cm.

Each area was sampled over three periods of seven days each: during the end of the rainy season (April–May 2008), during the dry season (June–September 2008) and the beginning of the next rainy season (October–November 2008). Refuges were visited every second day, totalling twelve visits per sampling point. Captured individuals were individually marked with visible implant elastomer technology (VIE – Northwest Marine Technology, Inc.) and released about 30 m away from the capture location. The first three individuals of each species were collected as voucher specimens and deposited at Coleção Herpetológica da Universidade de Brasília (CHUNB).

The snout-vent length (SVL) of all individuals was measured prior to release.

For each arboreal pipe refuge, we measured nine microhabitat variables: height of opening; circumference of installation branch; circumference of widest branch on installation tree/shrub; distance to widest branch; number of branches; angle of installation; percentage of canopy cover; vegetation type (tree or shrub) and bark rugosity [from smooth (0) to very rugose (3)]. Damages to pipe refuges included marks from mammal teeth and claws, which in some cases caused deformation of the shape of the opening, extensive proliferation of galleries built by insects and rupture of the attachment device, causing a few refuges to fall from trees. Damage to pipes was classified according to its intensity: no damage (0), minor (1), moderate (2) or severe damage (3). Refuges were repaired or replaced whenever needed.

Individuals were released 30 to 50 m away from the capture location, thus recaptures were considered independent occupancy events and included in the analyses. Our data did not conform to the assumptions of homocedasticity and normality of distribution. Therefore, comparisons were performed using nonparametric analysis of variance (Kruskal-Wallis test) implemented in STATSDIRECT. Redundancy analysis (RDA) was performed using CANOCO® for Windows to assess correlations between environmental data and occupancy. Sampling point and refuge colour were included as covariates in RDA. The null hypothesis of independence between species and environmental data sets was tested using a Monte Carlo test with 1000 permutations. The significance test (p=0.05) was run for the first canonical axis and for all axes together.
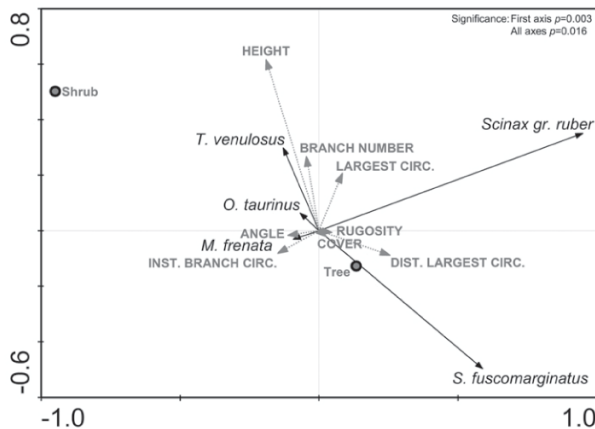

Fig. 1. RDA correlation biplot of species and microhabitat variables. Species are represented by black solid lines, microhabitat variables (MH) are represented by dotted grey lines and capitalized letters. Centroids from the variables "tree" and "shrub" of nominal MH "vegetation type" are represented as grey, filled circles.

We registered 114 occupancy events by hylids (including 4 recaptures) and eight by the skink *Mabuya frenata*. Up to three *Trachycephalus venulosus* individuals were found simultaneously occupying the same pipe; simultaneous occupancy by two individuals was found in *Scinax*

gr. *ruber* and *S. fuscomarginatus*. Average SVL for captured species (Table 1) varied between 16.7±1.71 mm (*Scinax fuscomarginatus*) and 53.2±16.24 mm (*Osteocephalus taurinus*). The smallest individual occupying the pipes was a *S. fuscomarginatus* (SVL=14.2 mm), and the largest was an *O. taurinus* (SVL=74.5 mm). We found no significant effect of colour on the average number of individuals per refuge (white, 0.84±1.014; gray, 0.54±0.741; black, 0.84±1.102; df=2, total observations=165; T=2.2403; p=0.326). Differences in occupancy success were also non-significant in single-species comparisons (0.1659>T>3.8801; 0.144>p>0.920). Several refuges were damaged during the study and sometimes they were found lying at the base of installation trees. Simultaneous comparison of all samples revealed a significant effect of colour on the average damage level of the arboreal refuges (white, 0.58±0.956; gray, 0.11±0.369; black, 0.13±0.388; T=14.5037; p=0.001). Pairwise comparisons (critical q=3.3145; df=162) revealed that average damage level for white refuges was significantly greater than for grey (p=0.004) and black (p=0.010) refuges, whereas there was no significant difference between the latter two colours (p=0.948).

The two first canonical axes of the RDA (Fig.1) explained 9.9% of the cumulative percentage of variance in the species data. The sum of all canonical eigenvalues was 0.086, accounting for 10.6% of the total variance in the species data. The proportion of variance explained by the first and by all canonical axes was significantly higher than expected by chance (p=0.003 and p=0.016, respectively). Vegetation type was the variable most correlated with the first environmental axis (tree=0.380; and shrub=-0.550) and the first species axis (tree=0.178; and shrub=-0.258). Height of opening was the variable most correlated with the second environmental (0.752) and the second species (0.201) axes. Relatively to the vegetation type, the score of the variable "tree" was higher than for "shrub" for both *Scinax* species, with an inverse relationship for *T. venulosus* and *O. taurinus*. The presence of the latter two species was positively correlated with the height of the opening (*T. venulosus*=174.4 cm; median=195 cm; range=125–226 cm; *O. taurinus*=178.0 cm; median=199 cm; range=117–237 cm). The presence of *S. fuscomarginatus* was negatively correlated with opening height (=167.5 cm, median=147 cm; range=126–218 cm), and *Scinax* gr. *ruber* and *M. frenata* were indifferent with regard to opening height.

Our study showed that microhabitat variables influence the occupancy of artificial refuges by hylids, and that these refuges are also used by skinks. A pilot survey revealed that four other hylids (*Dendropsophus minutus, D. nanus, D. rubicundulus* and *Hypsiboas raniceps*) occupied pipes placed around ponds. Six other hylids – *Dendropsophus melanargyreus, Hypsiboas fasciatus, H. punctatus, H. albopunctatus, Phyllomedusa azurea* and *Scinax nebulosus* are known from the region (as documented by voucher specimens CHUNB44914–46212 at Colecção Herpetológica da Universidade de Brasília), but were not captured in our pipe refuges. Hylid species that occupied the refuges are typical of forested or border areas (e.g. *O. taurinus*) and/or disturbed areas (e.g. *S.*

Table 1. Snout-vent length and number of individuals occupying arboreal pipe refuges with different colours.

Species	N	Snout-vent length (mm)		Refuge colour		
		Mean±SD	Min–max	White	Grey	Black
Hylidae						
Osteocephalus taurinus	5	53.2±16.24	37.7–74.5	2	3	0
Scinax fuscomarginatus	39	16.7±1.71	14.2–20.4	14	13	12
Scinax gr. *ruber*	39	30.6±4.82	22.4–42.5	21	2	16
Trachycephalus venulosus	31	50.5±7.49	37.0–69.3	8	10	13
Scincidae						
Mabuya frenata	8	58.8±6.14	52.0–64.0	1	2	5
Total	122			46	30	46

gr. *ruber*, Lutz, 1973; Lima et al., 2006). *Trachycephalus venulosus* is frequently found in hollow metal tubes containing water (Lutz, 1973). All other species that were found in ground pipes in the pilot study or that did not occupy the arboreal pipes are more frequent in open areas (Lutz, 1973; Brasileiro et al., 2008). Some species, like the small *Dendropsophus*, are more frequently found in herbaceous vegetation at the margin of water bodies (Lutz, 1973). Some of the smallest and largest species occurring in this region occupied our refuges. Therefore, the size and diameter of pipes do not appear to have limited the size of species using the refuges. However, arboreal refuges were placed at the core or edge of forested patches, which might have restricted the range of occupying species. We believe that if arboreal pipes are placed in more open habitats, then more species will be able to occupy the artificial refuges. The skink *Mabuya frenata* also occupied the arboreal refuges. Colonization of pipe refuges by lizards had already been reported by Johnson (2005). These findings suggest that artificial refuges might also be useful for studying other groups of herpetofauna.

Our results reveal that pipe refuges are more useful to survey Neotropical herpetofauna than reported in previous studies (Silva & Rossa-Feres, 2007; Laurencio & Malone, 2009). Laurencio & Malone (2009) captured seven individuals from three species, but none were captured in pipes placed in the canopy. Our results suggest that refuge colour did not affect occupancy success, contrarily to our initial hypothesis based on Crawford & Kurta (2000). However, darker refuges were less prone to damage, and thus their use might be preferable. Despite the rather small sample size it was possible to explain a significant proportion of the total variance of species data. Boughton et al. (2000) also found evidence for the influence of height on colonization success by *Hyla* species. In their study, refuges placed higher in trees were more efficient than those at ground level. In our study, occupancy by *T. venulosus* and *O. taurinus* was positively correlated with the height of opening, while occupancy by *S. fuscomarginatus* was negatively correlated with height.

Apart from determining which factors influence occupancy rates, analysing microhabitat characteristics of artificial refuges can also help us to understand the ecological preferences of hylids (Johnson & Semlitsch, 2003; Pittman et al., 2008). Considering the number of species and the ecological and reproductive diversity of tree frogs in the Neotropics, we believe that pipe refuges can become an important methodology in biodiversity surveys and ecological studies in this region.

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