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Selection of calling sites by the Neotropical treefrog *Hypsiboas crepitans*

Selección de sitios de llamado de la rana Neotropical *Hypsiboas crepitans*

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Abstract

We studied the selection of calling sites by the emerald-eyed treefrog (*Hypsiboas crepitans*) based on microhabitat factors and body size using a resource selection probability function, determined by a multiple logistic regression model. Distance from the pond edge was the most reliable predictor of frog calling site. A significant difference in body size was found between frogs calling: in the pond, at the edge, far from the pond, on plants, and on rocks. Frogs at the edge of the pond were larger than frogs calling far from the edge.

Keywords: Amphibian, Microhabitat use, Sexual selection.

Resumen

Se estudió la selección de sitio de llamada por la rana platanera de ojos verdes (*Hypsiboas crepitans*) basados en factores del microhábitat y tamaño corporal usando una función de probabilidad de selección de recursos, determinada por un modelo de regresión logística múltiple. Se encontró que la distancia desde el borde del cuerpo de agua fue la variable que predijo de forma más confiable el sitio de llamada. Una diferencia estadísticamente significativa entre los tamaños corporales fue encontrada para las ranas llamando desde el cuerpo de agua, el borde, lejos del cuerpo de agua y sobre las plantas y rocas. Las ranas en el borde del cuerpo de agua fueron consistentemente más grandes que las ubicadas lejos del borde.

Palabras clave: Anfibio, Selección sexual, Uso de microhábitat.

Introduction

Advertisement calls in frogs are important for both reproductive and social behaviours, allowing males to attract females and defend their territories from other males (Gerhardt 1994). Nevertheless, it also may attract predators (Bernal *et al*. 2009, Ryan *et al*. 2007). Acoustic signal information may be altered by the surrounding habitat (Ziegler *et al*. 2011), and by simultaneous calls of other species (Amézquita *et al*. 2011, Vélez *et al*. 2012, Vargas-Salinas *et al*. 2014). On the other hand, well-transmitted call signals when combined with the fulfilment of other reproductive needs (i.e. nest building, proximity to adequate egg-laying areas) enhance mating success of an individual (Martin *et al*. 2011). Therefore, the effective selection of the calling site is critical for males to secure reproductive success and survival.

The emerald-eyed treefrog (*Hypsiboas crepitans*) has a wide distribution that extends from eastern Brazil northwards through Venezuela, the Guianas, Surinam and Colombia to Panama, and some Caribbean islands (AmphibiaWeb 2014, La Marca *et al*. 2010). It has been reported at elevations from 0 to 2300 m through its range (La Marca *et al*. 2010).

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In Colombia, *H. crepitans* is a common nocturnal frog in lowland forest up to 1700 m (Lynch *et al.* 1997, Ruiz *et al.* 1996); it has a pulsing advertisement call (Bernal *et al.* 2004) and it is generally found calling from the ground (Lynch 2006), especially during the breeding season, when it tends to form aggregations around temporary pools or water bodies (La Marca *et al.* 2010). *Hypsiboas crepitans* is a species of least concern for conservation due to its' high tolerance to human disturbance (La Marca *et al.* 2012). Despite its wide distribution, little is known about its ecology and behaviour. Here we provide information on the microhabitat use patterns, particularly on factors influencing the selection of calling sites by *H. crepitans*. We hypothesize that in *H. crepitans* the selection of calling site is determined by characteristics of the microhabitat and influenced by frogs' body size.

Methods

This study was conducted around two man made ponds at the Villa Marina experimental farm (07°32'N, 72°38'W; elevation of 1100 m), 25 km from Pamplona, on the road Pamplona-Cúcuta, in the department of Norte de Santander, Colombia. The two ponds were 18 m long, 6 m wide, 1.5 m deep, and separated by 10 m from each other. They were dig holes in the ground to provide water for the cattle and are permanently kept filled with water. Vegetation around both ponds is similar and dominated by shrubs of the families Asteraceae, Fabaceae, Malvaceae, Myrtaceae and Rubiaceae, and aquatic plants in the genera *Ludwigia, Cyperus,* and *Eleocharis*. The mean annual temperature of the study area was 26°C. Precipitation is seasonal, rainfall peaks by August (~270 mm in 2005), and there is a less rainy season from December to February.

We surveyed around the two ponds, covering a distance of 10 m around each pond, to search for frogs between 18:00 and 01:00 hours, four days per month (once per week) from July 2005 to January 2006. Individuals of *H. crepitans* were located by acoustic search. For each located frog, we recorded snout-vent length (SVL) and the distance from the frog to: pond edge, nearest shrub, nearest tree (>2 m tall), and nearest rock (>20 cm diameter) as potential hiding places during day light hours. Location of each frog was also categorized as being in the water, on land, on a rock, or on a plant. Additionally, each sampled frog was kept in an individual bag to the end of the sampling day, and then released in the same place of capture, avoiding sampling the same individual twice. Each sampling day we also selected random areas were frogs where not present and recorded the same variables equalizing the numbers of frogs found. Percentages of substrates around the ponds were 10% rocky outcrops, 70% grass, 12% shrubs and 8% trees. The distinction between *H. crepitans* and *H. pugnax* was confirmed by measurements of body size and the distinctive call of *H. crepitans*.

We calculated a resource selection probability function (RSF) in a separate sample of available and used units design (Manly *et al.* 2002). Selection probability function describes how different types of resource units are chosen to be used by individuals, with different probabilities for the resulting final distribution of used units in the habitat. Moreover, the probability of an individual being recorded at a given site may vary with the habitat type, which so far has been dealt with the use of occupancy models (Mackenzie 2006). In this study, we did not consider occupancy, given the small spatial scale of our sampling. We used a multiple logistic regression analysis to find the set of values that were the strongest predictors for the calling site of the treefrogs. Logistic regression is a common and widely used technique for wildlife habitat-selection studies (Keating and Cherry 2004). In our analyses we used the presence of treefrogs as used units and the random points as available units. A p-value of less than 0.05 was used to denote statistical significance.

Differences in body size were evaluated with respect to the calling position using a Kruskal-Wallis test, followed by a pairwise multiple comparison using Dunn's Method. We tested for correlations between body size and distance from the pond edge to assess the importance of body size in calling position. All statistical analyses were conducted in SigmaStat v.3.1 and results were plotted in SigmaPlot v.10 (Hilbe 2003).

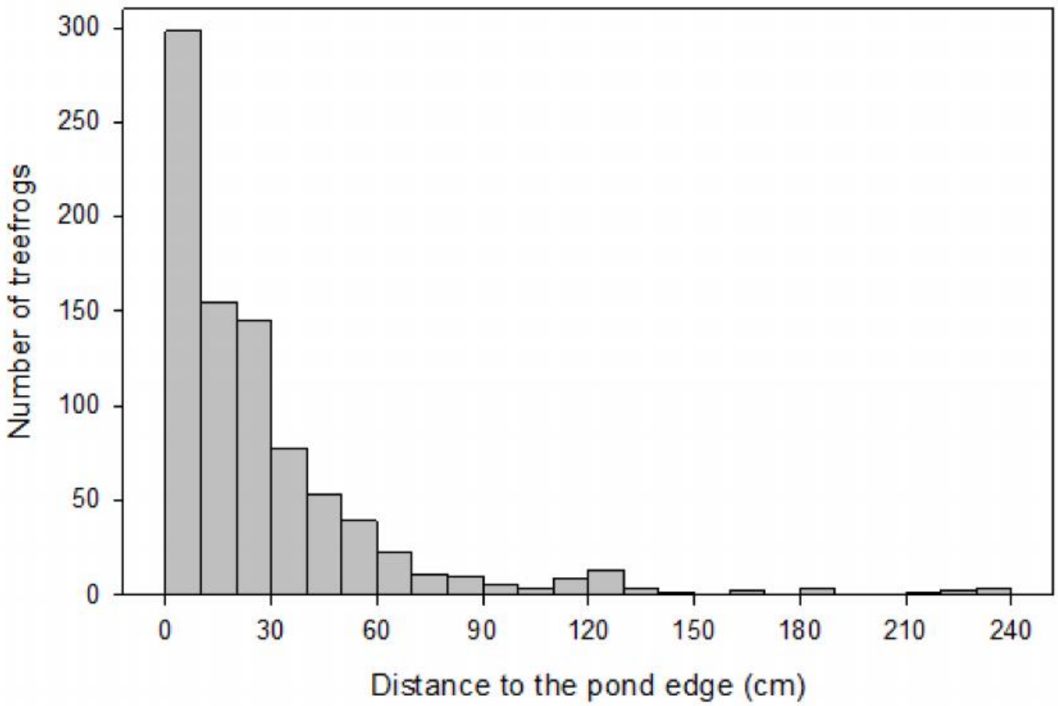

Figure 1. Distribution of emerald-eyed treefrogs (*Hypsiboas crepitans*) in relation to the distance from the edge of the pond.

Results and discussion

After sampling 875 frogs and equal number of random points, we found that the best variable predictor for calling site was the distance to the edge of the pond (Wald Statistic 18.438, $p<0.001$). Our model was based on the logistic equation:

$$p=\exp \{0.550 - (0.0292 * \text{distance from the edge}) + (0.00493 * \text{distance to nearest rock}) + (0.0000927 * \text{distance to nearest tree}) + (0.00320 * \text{distance to nearest shrub})\}$$

The distance from the pond edge can be described by an exponential decay function of the form $f=1/a^{bx}$ where a=104.112, b=0.044. The majority of the frogs (76.5%) were found between 0 and 20 cm away from the pond (Figure 1). Average distance to pond edge selected by frogs was 15±32.1 cm.

A statistically significant difference in body size ($H=20.992$, $df=5$, $p<0.001$) was found between frogs calling in the pond, at the edge, far from the edge (>20 cm), on plants, and on rocks. When multiple comparisons were performed, calling frogs at the pond edge were found to be significantly larger ($p<0.05$) than the others. However, for frogs located more than one meter away from the pond edge, body size was not correlated with distance from the edge of the pond. Seasonal differences in calling sites were not detected.

The importance of effectively choosing the site to call during courtship has been clearly identified in anurans (Byrne and Dale 2004, Martin *et al.* 2011). In frogs that breed in lentic water bodies, like most of tropical treefrogs, effectiveness has been associated with the distance to the water (Byrne and Dale 2004). We found that for *H. crepitans*, a treefrog that breeds in ponds, the distance to the edge of the pond was the most important factor when choosing the calling site, similarly to what occurs with the Australian frog *Crinia georgiana*, where females select the males based on their distance from the water (Byrne and Dale 2004). Calling on top of rocks or plants was not an explanatory variable for calling site selection in our study, contrasting with other tree frogs that select specific perches that facilitate sound propagation (Fellers 1979a). Moreover, water temperature has been suggested to influence the breeding site selection in temperate areas for frogs like *Rana lessonae*, which forms choruses in the warmest parts of ponds (Sjogren *et al.* 1988);

however, in tropical regions such as our study site, the temperature does not change significantly around the pond, so we did not consider it as a variable factor that could help explaining the selection of calling sites.

One factor that appears to increase the mating probability, in addition to the selection of calling sites, is the capacity to meet other reproductive requirements, which also has been documented in the Túngara frog, *Physalaemus pustulosus*, where females tend to choose males located near adequate foam nests (Martin *et al.* 2011). In our study, frogs were generally found along the edge of the ponds, typically less than 20 cm away from the water (Figure 1). In this case the preference for positions near the water may also ensure a higher chance of males being selected by gravid females, as after choosing their male, females need to look for a suitable site for oviposition; this highlights the importance of distance from the pond edge to both males and females. Similarly, most male and female *Acris crepitans* frogs in Missouri (USA) were found between 10 and 35 cm from the water during the breeding season (Smith *et al.* 2003). However, in ranids such as *Rana sylvatica*, the number of females increases with the distance to ponds while the number of males remains similar irrespective of the distance to water (Regosin *et al.* 2003).

H. crepitans is a nocturnal species that in our study did not showed a preference for shaded or sheltered places, or near trees or rocks, even though such microhabitats could help reduce desiccation during day hours, so we hypothesise that *H. crepitans* frogs reduce or avoid exposure to sunlight by moving away from the pond edge, that is in the open, and hiding in sheltered places nearby. It could be expected that frogs moving too far from the pond edge before sunshine may arrive later at calling sites in the evening and be excluded from the best calling sites. Males of *H. crepitans* located at the pond edge were overall larger than those located on plants, rocks, in the pond, or far from the edge. However, there was no significant correlation between body size (SVL) and the distance to the water, either within those frogs located between 0 and 20 cm away from the pond edge, nor in the frogs calling far from the edge (more than 20 cm away). Larger frogs may be more effective at defending their calling sites from other males but also have better chances of escaping from predators. Predation by giant water bugs (*Lethocerus americanus*) on hylid frogs has been well documented (Bernard 2007, Hirai and Hidaka 2002, Toledo 2003). We regularly observed *Lethocerus* sp. in both ponds in our study area, and in spite of the lack of observations of any predation event, we hypothesized that smaller individuals of *H. crepitans* may be easily predated by giant water bugs, and this may be a reason explaining why larger frogs were calling at the pond edge but primarily out of the water. This supports the observation of Toledo (2003) who found that hylid frogs reduce the predation risk by calling at dry sites, which is also true for lowland leptodactylid frogs that select their calling sites based on reducing juvenile and adult predation risk by insects (Murphy 2003).

Social interactions are also important when selecting reproductive sites. Factors like tadpole presence in ponds as well as environmental factors, simultaneously impact reproductive site choice in leptodactylid frogs (Murphy 2003); while territoriality has proven to be influential for temperate hylid species where males defend calling positions and subordinate males waiting for available calling sites (Fellers 1979b). In fact, we observed several agonistic interaction between males around the ponds. Even though we did not quantify agonistic behaviours and territoriality, most of large frogs used better calling sites (near the edge) which could also suggest they have higher chance of winning when fighting for such places. Future long term monitoring and ethological records for *H. crepitans* may reveal the influence of social factors in calling position choice. Further habitat use studies in *H. crepitans* will be useful to clarify the relationship between body size, territoriality, predation risk and calling site selection.

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