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Are breeding sites a limiting factor for the Tandilean red-belly toad (Bufonidae) in pampean highland grasslands?

Locais de reprodução são fator limitante para o sapo-de-barriga-vermelha de Tandil (Bufonidae) nas serras dos Pampas?

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Abstract

The selection of breeding sites in response to temporary pond characteristics has been frequently documented in several anuran species. Small and shallow temporary ponds are usually preferred. The Tandilean red-belly toad, *Melanophryniscus* aff. *montevidensis*, is an endemic species that breeds in temporary ponds of remnants of highland grasslands in Tandilia mountain system, Buenos Aires Province, Argentina. The goals of this work are to characterize breeding sites of Tandilean red-belly toad, and to assess the availability of potential breeding sites at remnants of highland grasslands. Tandilean red-belly toad was observed using ponds and temporary creeks in valleys and slopes of mountains. The median of dimensions for the breeding sites was 8m length, 4m wide and 10cm depth (*n* = 40). The majority of the breeding sites were circular and of natural origin. The dominant breeding site substrates were mud and vegetation. We found 115 potential breeding sites in grassland remnants of surroundings of Tandil city, giving a density of 7.1 potential breeding sites/km^2. The density of potential breeding sites in highland grasslands of Tandilia Mountains was similar in most surveyed remnants. Therefore, our results suggest that size of remnant is not affecting the breeding habitat availability.

Keywords: pampas, temporary ponds, reproduction, conservation, Tandil.

Resumo

A seleção dos locais de reprodução em resposta a características de áreas úmidas temporárias tem sido frequentemente documentada para várias espécies de anuros, sendo as poças temporárias pequenas e rasas geralmente preferidas. *Melanophryniscus* aff. *montevidensis* é uma espécie endêmica que se reproduz em poças temporárias em remanescentes de pastagens da serra da Tandilia, Buenos Aires, Argentina. Os objetivos deste trabalho são caracterizar locais de reprodução de *Melanophryniscus* aff. *montevidensis*, e avaliar a disponibilidade de potenciais locais de reprodução em remanescentes de pastagens das serras. *Melanophryniscus* aff. *montevidensis* foi observado usando poças e riachos temporários em vales e encostas de serras. A mediana das dimensões dos locais de reprodução foi de 8m de comprimento, 4m de largura e 10cm de profundidade (n = 40), sendo a maioria circular e de origem natural. Os substratos desses locais eram principalmente lama e vegetação. Encontramos 115 poças temporárias potenciais em remanescentes de pastagem de arredores da cidade de Tandilia, perfazendo densidade de 7,1 potenciais poças por km^2. A densidade de poças potenciais em pastagens dos planaltos de Tandilia foi semelhante entre os remanescentes pesquisados. Portanto, os resultados sugerem que o tamanho do remanescente não está afetando a disponibilidade de locais de reprodução.

Palavras-chave: Pampas, áreas úmidas temporárias, reprodução, conservação, Tandilia.

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Amphibians have the highest proportion of species threatened with extinction among vertebrates, with 32.5% of species in decline (Stuart *et al*., 2004) and 41% of species listed inside a threatened category (Pimm *et al.,* 2014). Habitat fragmentation, degradation and alteration are defined as one of the most important drivers of amphibians decline (Lips *et al*., 2008). Understanding the relationships between the characteristics of habitats and their use by amphibians is imperative due to the rapid environmental changes.

Temporary ponds act as breeding habitats for several amphibian species (Williams *et al*., 2003; Scheffer *et al*., 2006). An analysis of the diversity of temporary ponds used by anurans suggests that not all ponds are equally suitable (Resetarits Jr., 1996). The selection of breeding sites in response to temporary pond characteristics has been frequently documented in several species (Vos and Chardon, 1998; Hazell *et al*., 2003; Goldberg *et al*., 2006). Best breeding sites will be those that keep enough water to complete the metamorphosis, and provide resources for tadpoles, i.e. food and shelter from predators (Skelly, 2001). An advantage of shallow temporary ponds is that they restrain the establishment of potential large-sized predators, such as fishes or other anuran species (Skelly *et al*., 1999; Blaustein *et al*., 2001). Furthermore, the presence of aquatic vegetation usually provides food, shelter from predators and sites to fix eggs (Crump, 1991).

Due to habitat loss and replacement for agriculture, the southernmost populations of the Tandilean red-belly toad are restricted to remnants of highland grasslands in the south of Buenos Aires Province, Argentina (Cei, 1980). Over the last century, the habitat of this toad species was dramatically reduced and currently less than 5% of these grasslands remain as isolated remnants (Bilenca and Miñarro, 2004). Taxonomy of the species belonging to the *M. stelzneri* group has not yet been adequately resolved (Kwet *et al*., 2005). Consequently, we refer to the population that inhabits mountains of Tandilia as *Melanophryniscus* aff. *montevidensis* (Vaira *et al*., 2012), or Tandilean red-belly toad (Figure 1). A recent study recognizes these populations as a valid taxonomic unit with a high priority of conservation (Zank *et al.,* 2015). The Tandilean red-belly toad is restricted to only two small and fragmented highland grassland areas (lesser than 10 km^2) of Tandilean Mountain System (Vega and Bellagamba, 1990; Cortelezzi, 2015). A mosaic of highland grassland fragments characterizes one of these areas, the surroundings of Tandil city, Buenos Aires Province, in Argentina. In this area, most records of the presence of Tandilean red-belly toad are concentrated in largest remnants. Some authors suggest that the lack of breeding sites could be explaining the absence or low density of Tandilean red-belly toad in small fragments (Cortelezzi, 2015).

In order to test if breeding sites availability is a limiting factor on the distribution of Tandilean red-belly toad, we first characterized breeding sites of Tandilean red-belly toad, and, second, we assessed the availability of potential breeding sites at remnants of highland grasslands of surroundings of Tandil city. The knowledge on the habitat requirements of Tandilean red-belly toad will help develop better conservation strategies.

We explored highland grassland remnants of the Protected Area “La Poligonal” (141.6 Km^2; 37°19′S, 59°08′W, Tandil, Argentina), which currently lacks of effective management. Some of these patches of grasslands are immersed in the urban landscape, and the grassland remnants have undergone several human activities, as stone quarry, cattle ranching, introduction of invasive species, and touristic development (Bilenca and Miñarro, 2004). This highland grassland holds temporary sites that are commonly used as breeding site by several species of anurans (Soler *et al.*, 2014).

During an explosive breeding event occurred on October 8th 2012, we surveyed 40 breeding sites that were being used by Tandilean red-belly toad. Red-belly toads shows explosive breeding events (usually 24 to 48h), usually associated with heavy rainfall (Cairo *et al*., 2008). We applied a visual encounter survey (Crump and Scott, 1994) for detecting ponds and toads,

Figure 1. Tandilean red-belly toad, *Melanophryniscus* aff. *montevidensis* (2.5cm length).

and we complemented it by considering male vocalizations. We conducted a daily survey between 10 to 17h. For each breeding site, we recorded: location (geographic coordinates), dimensions (length, width, and depth), shape (strip or circular), origin (natural or artificial, e.g. those site generated by human activity, as road sides, quarries, etc.), and dominant substrate in the bottom (vegetation, rock or mud).

After this explosive breeding event, we completely scanned five grassland remnants in two days of survey: Las Animas (15.3 km^2; 36°46′S, 57°69′W), El Calvario (1.9 km^2; 34°67′S, 54°93′W), La Movediza (0.8 km^2; 37°31′S, 59°17′W), Las Tunitas (0.4 km^2; 37°34′S, 59°15′W) and Parque Independencia (0.1 km^2; 37°34′S, 59°12′W, Figure 2); looking for all potential breeding sites and recording the same variables previously described for breeding sites. As expected, during this exploratory survey, we have not detected any breeding activity from toads. We considered potential breeding site all ponds or creeks. We used descriptive statistics to characterize the breeding sites. Dimensions of sites were shown as mean ± SE. We explored continuous variables of sites with a Principal Component Analysis (PCA). We used a t-test to analyze differences between means of dimensions of sites.

Tandilean red-belly toad was observed using ponds and temporary creeks in valleys and slopes of Tandilean mountains. The typical breeding site was a temporary grassy shallow pond (Figure 3A). The median of dimensions for the breeding sites was 8m length, 4m wide and 10cm depth (n = 40). Similar values were reported for other red-belly toads of mountain areas as *Melanophryniscus* sp. and *Melanophryniscus stelzneri* (Cairo *et al*., 2008; Pereyra *et al*., 2011). Our observations agree with the generalized idea that toads would use shallow temporary ponds. Shallow temporary ponds usually have good thermal properties, high level of productivity and lack of large-sized predators (Wilbur, 1997; Gunzburger, 2005; Richter-Boix *et al*., 2007). The majority of the breeding sites (71%) were circular (i.e. pond) and of natural origin (58%). The dominant pond substrates were mud (46%) and vegetation (44%, Figure 3B). Vegetation within the ponds may generate complex microhabitats that could provide shelter from potential predators (Egan and Paton, 2004), and provide substrate of fixing the eggs to submerged plants near shallow coasts (Cairo *et al*., 2008). A dominance of mud as main substrate in the bottom would indicate that even ponds with few vegetation are used.

In the whole region, we found 115 potential breeding sites. This value includes the 41 breeding sites described above. The remnant "Las Animas", with 15.3km^2, concentrated 73% of ponds (Figure 2). Remaining ponds were distributed in four remnants: El Calvario (12%), La Movediza (7%), Parque Independencia (6%), and Las Tunitas (2%). The average density of ponds among these remnants was 7.14 ± 2.1 ponds/km^2 (n = 4 remnants). The Parque Independencia was excluded from this average because it showed an extreme value of density (69 ponds/km^2; only 7 ponds in a small area). The density of potential breeding sites in highland grasslands of Tandilia mountains was similar in

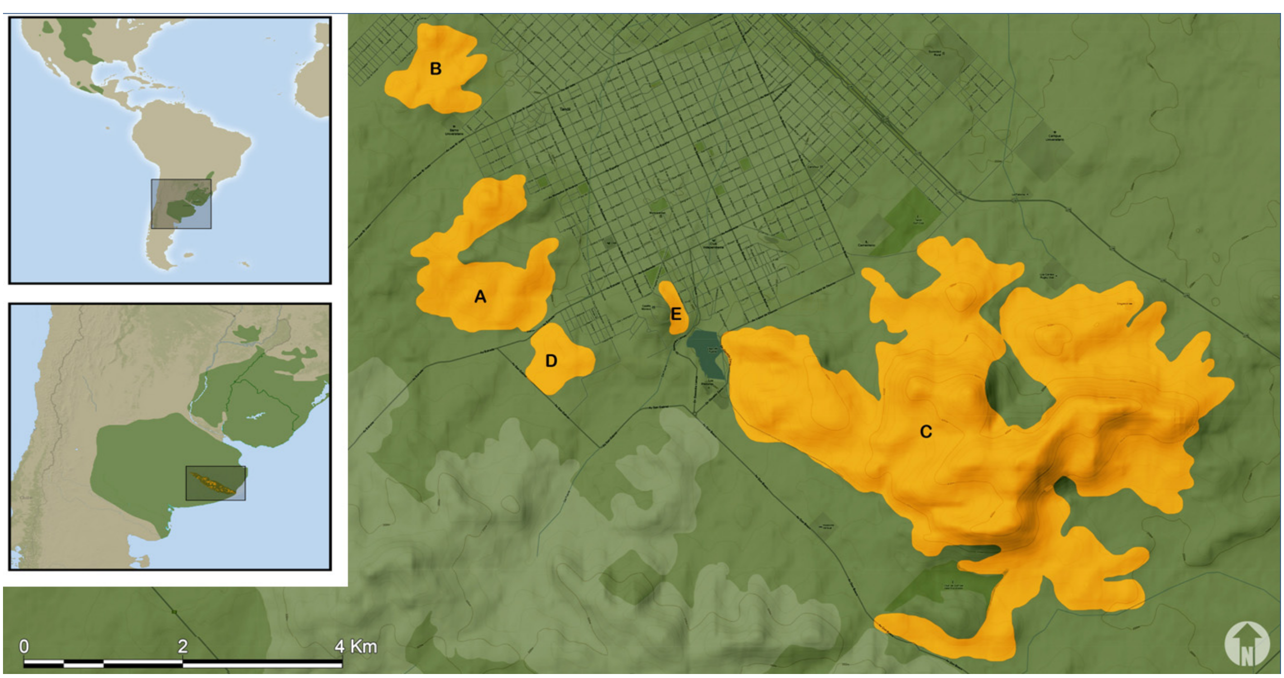

Figure 2. Location of surveyed remnants of highland grasslands (orange) at the La Poligonal protected area, Tandil, Buenos Aires Province, Argentina: El Calvario (A), La Movediza (B), Las Animas (C), Las Tunitas (D), and Parque Independencia (E).

Figure 3. Breeding sites of *Melanophryniscus aff. montevidensis* at remnants of pampean highland grasslands, municipality Tandil, Buenos Aires Province, Argentina.

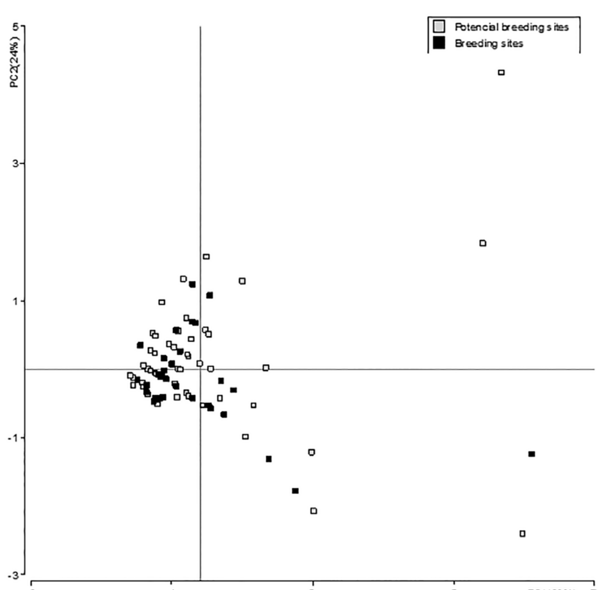

Figure 4. Results of Principal Components Analysis of potential breeding sites and used breeding sites by *Melanophryniscus aff. montevidensis* on the first main plane.

most surveyed remnants, suggesting that breeding habitat exists in all remnants. Even the smallest remnants maintain potential breeding sites, being suitable sites to promote area-based conservation approaches.

Potential breeding sites were relatively small sized and shallow, mostly associated with vegetation and mud as substrates. Rocky substrates were uncommon. Half of potential breeding sites have artificial origin. This could be a consequence of quarry activities during the recent past and the expansion of the city and touristic activities over the remnants. The average dimensions of potential and confirmed breeding ponds were similar: length ($t_{102} = 0.39$, $p > 0.05$), wide ($t_{101} = -0.65$, $p > 0.05$), and depth ($t_{99} = 1.90$, $p > 0.05$). The PCA shows no clustering of potential and used sites (Figure 4).

Even when breeding and potential breeding sites had similar characteristics, probably, toads would not be using all potential breeding ponds. As occurs with *Melanophryniscus stelzneri* (Weyenbergh, 1875), the toads could be using the habitat in a non-random pattern at the spatial scale (Pereyra *et al*., 2011). However, our results suggest that the availability of breeding sites would not be limiting the population of Tandilean red-belly toad in highland grassland remnants near to Tandil city. The presence of potential breeding sites in all surveyed remnants encourages us to develop management actions on these sites (e.g. fencing). Future studies should search for spatial preferences in this species, by assessing the effect of several external covariates related to the breeding sites, like the distance to roads, the size of the relicts and/or the presence of cattle, among others.

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