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Use of Reproductive Microhabitat by *Melanophryniscus montevidensis* (Anura: Bufonidae) from Uruguay

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This study describes the reproductive microhabitat of *Melanophryniscus montevidensis* and its use in two water bodies (WBs) in Barra de la Laguna de Rocha, Uruguay. Monthly field trips were performed between March 2012 and February 2013. Variables related to the WBs and vegetation, as well as parameters linked to the usage the amphibians make of the site (e.g: distance to the border of the pond, water depth and the vegetation use) were recorded. The behavior shown by the individuals during the breeding activity was recorded. This activity occurs in shallow temporary WBs with abundant hydrophilic vegetation. The individuals were found more frequently in areas near the edge of the pond, which has denser vegetation. The calling males were found closer to the border of the pond, and they showed better body condition than the non-calling males. In addition to calling activities, males used alternative tactics to find couples, such as active search of females, and aggressive behaviors, such as male displacing and physical combat. Such behaviors are common in anurans with explosive reproductive dynamics. The characterization of the reproductive microhabitats permits the proposal of strategies for the conservation of the species in Uruguay, given that the loss and fragmentation of habitats is one of the main causes considered for the decrease in their populations.

Key words: reproductive microhabitat, behavior, Bufonidae, *Melanophryniscus montevidensis*, Uruguay

INTRODUCTION

A significant worldwide decrease in amphibian populations has been reported in recent decades, and habitat destruction or modification have been proposed as the main causes (Stuart et al., 2008; Collins and Crump, 2009). Studies related to the determinants of the use of the habitat and the understanding of the reason for using specific places gain relevance when predicting how the loss or modification of the habitat may affect the populations' survival (Rittenhouse and Semlitsch, 2007; Johnson et al., 2008). The information available on habitat-use patterns in amphibian populations is scarce (Lemckert and Brassil, 2000; Krishnamurthy, 2003), which hinders the development of strategies for the conservation of the species through protection or management of the habitat.

The presence and abundance of anurans in breeding sites depends on a series of environmental and biotic factors (Lemckert and Mahony, 2010). Within the first category are the depth, temperature and quality of the water, the hydroperiod, and the size of the water bodies (WBs) (Stumpel and Van Der Hoet, 1998; Watson et al., 2003; Beja and Alcazar, 2003; Afonso and Eterovick, 2007). Other environmental factors are the amount of superficial vegetation and the kind and degree of vegetal coverage on the land around (Pavignano et al., 1990; Munger et al., 1998; Bosch and Martinez-Solano, 2003). The amount of vegetation may generate complex microhabitats within the ponds, which may provide shelter for larvae and froglets against potential predators (Kopp et al., 2006), food for the larvae (Lane et al., 2007), or an increase in the offer of environments favorable for the spawning (Pereyra et al., 2011). Among the main biotic influential factors, the presence of predator and competitors stands out (Lane et al., 2007; Pereyra et al., 2011).

Most of the studies related to the use of breeding sites by amphibians are focused on anurans assemblages. In these assemblages, the activity is characterized by the aggregation of various species in the reproductive WBs, thus the potential of interspecific interactions is very high (Bertoluci and Rodriguez, 2002; Vasconcelos and Rossa-Feres, 2008), and biotic interactions are important mechanisms in the structure of anuran communities (Daza-vaca and Castro-Herrera, 1999; Boelter, 2004; Muñoz-Guerrero et al., 2007). Studies at the level of populations and microhabitats however are very scarce (Rorabaugh et al., 2004; Miaud and Sanuy, 2005; Guimarães et al., 2011), but nonetheless demonstrate the important roles that abiotic factors (such as pond characteristics) can play in the use of the site by anurans.

Melanophryniscus montevidensis (Philippi, 1902) is distributed along the Atlantic coastline of Uruguay and the Brazilian south-east edge (Toranza and Maneyro, 2013), and is locally (Carreira and Maneyro, 2015) and globally threatened (Langone, 2004) by the destruction and degra-

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dation of its habitat. The species exhibits explosive breeding behavior, with reproductive activity associated to heavy rainfalls throughout all the year (Alonzo et al., 2002). It is characterized for having a short active period (of hours or days), and for making dense aggregations in breeding sites, where competition among males for access to females is more important than selection by the females (Cairo et al., 2013). Males are more abundant during the entire breeding event and combat among them have been recorded (Prigioni and Garrido, 1989). Males thus need to alternate between calling activity and other tactics, such as the active search of gravid females and male displacing strategies, so as to increase their fitness (Wells, 1977; Pombal & Haddad, 2005). These toads use temporary ponds where males call mainly during light hours (morning to sunset), and hold on to emerging vegetation (Maneyro and Carreira, 2012). Amplectant pairs make several immersions to deposit small egg masses (Alonzo et al., 2002).

The aims of this study were to characterize the breeding microhabitat and its use by *M. montevidensis* in Barra de la Laguna de Rocha, and to describe some aspects of the reproductive behaviour of the individuals of this species during breeding events.

MATERIALS AND METHODS

Study area

The study was developed in Barra de la Laguna de Rocha (BLR) (34°40′02″S; 54°14′00″W), Rocha, Uruguay (Fig. 1). This area is part of the Paisaje Protegido Laguna de Rocha del Sistema Nacional de Áreas Protegidas (MVOTMA, 2015). The area presents coast sand dune systems and associated meadows prone to floods, with temporary ponds used by *M. montevidensis.* The predominant vegetation is psammophilic.

Field work and data collection

Monthly three-day field trips were carried out by four people between March 2012 and February 2013 in two temporary water bodies where the species reproduces: C1 (34°39′48.6″S) (54°13′14.3″W) and C2t (34°39′49.7″S) (54°13′33.4″W), which are separated by 215.78 m.

Several variables related to the WBs and the local vegetation were registered for the characterization of the breeding microhabitat. The type of vegetation and dominance, as the different strata and the percentage of vegetation cover were determined within the pond and in its nearest surroundings using the Relevé method (Mueller-Dombois and Ellenberg, 1974). The kind of substratum, the size (m^2) and the depth in centimeters (measured through usage of a 1mm precision plastic folding rule) (Pereyra et al., 2011), were recorded for each pond. In order to know the size of the pond, its length, width and diagonal length were measured with measuring tape (10 mm precision). The location was then identified in Google Earth and digitalized according to the measures taken in the field. Finally, the data was registered in a GIS through which the area and perimeter of each pond were calculated.

Sampling was performed in each pond *per* reproductive event, along the day (between 11 and 20–30 hrs, see Table 1 for details about date and hours), and in a standardized way (the ponds were looked through from their edges to their centers following a spiral, for at least three hours). The water temperature and pH were measured at the beginning of each sampling session (Table 1). All the individuals found in the ponds during the reproductive event were captured, sexed (the males were identified by presence of developed vocal sacs) and put in individually labeled jars, with a small amount of local water. Amplectant pairs found were captured as well, and put in plastic containers with water and a bit of vegetation from the pond. The depth (cm) and temperature of the water (°C), and the distance in meters to the nearest edge of the pond (ED) was measured in each spot of capture. In order to mitigate disturbance, we sought to minimize the permanence of the observers inside the ponds (for example handling the toads outside the pond).

The use of vegetation and the behavior shown by the individuals at the moment of their capture were also registered. The calling behavior and use of the vegetation shown by the individuals within the breeding ponds were categorized. The categories for the calling males were: 1. calling while floating on water surface (not attached or supported by any structure or plant); 2. calling on an emerging structure ("little islands" inside the pond) and 3. calling while holding on to emerging vegetation. For the non-calling males the categories were: 1. moving (swimming or walking in the pond) and 2. still (floating in the water surface or attached to vegetation). For females were considered the same categories than for the non calling males. Finally, the behavior shown by amplectant pairs was categorized in: 1. still among emerging vegetation, 2. swimming within the pond and 3. still in the water mirror. We also measured the distances between those calling males that were close located. Every individual was measured (snout-vent length = SVL) with a digital caliper, and their weight (M in g) was determined with a dynamom-

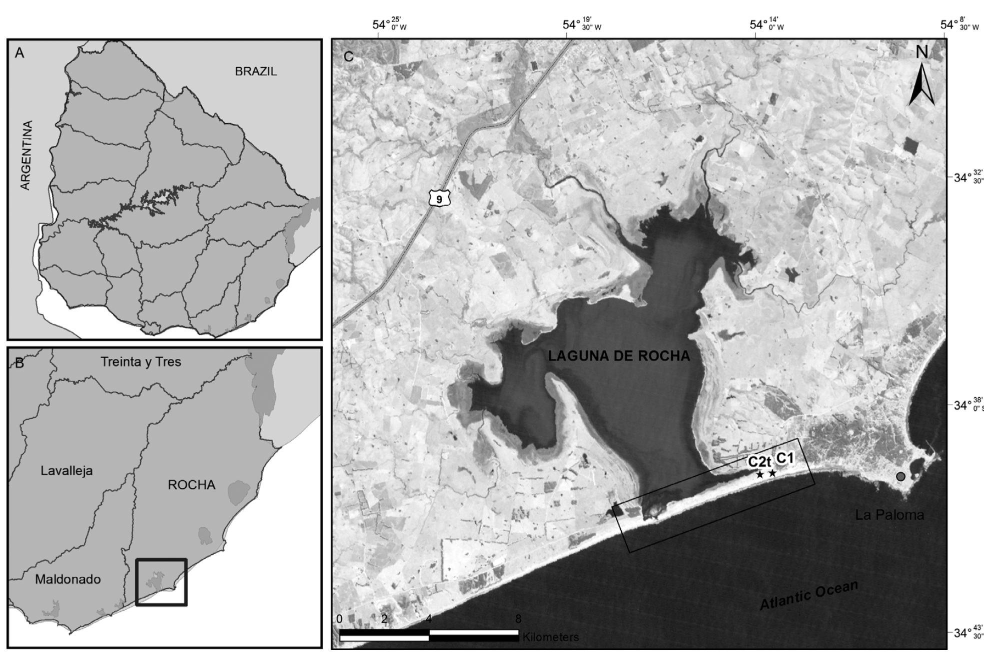

Fig. 1. Study sites. **(A)** Uruguay with departments. **(B)** Southeastern Uruguay showing Rocha department. **(C)** Zoom in the square of **(B)**, showing "Laguna de Rocha". The rectangle indicates the "Barra de la Laguna de Rocha" zone. The black stars indicate the specific sampling sites: C1 (34°39′48.6″S, 54°13′14.3″W) and C2t (34°39′49.7″S, 54°13′33.4″W).

Table 1. Sampling data and descriptive variables of water bodies used by *Melanophryniscus montevidensis* during the breeding activity events in the BLR. REFERENCES: AWT: average water temperature (°C); SD: standard deviation; min: minimum value; max: maximum value; AWD (cm): average water depth.

Sampling day	2012/8/22	2012/8/22	2012/10/30	2012/10/31	2012/12/20	2013/2/22
Pond	1	2t	2t	1	2t	2t
Time start/final	11:00/14:10	15:03/18:10	12:00/15:15	17:10/20:20	12:50/16:00	13:20/16:40
AWT (°C) ± SD (min-max)	24.38 ± 1.56 (21.2–27.4)	22.66 ± 1.12 (21.2–27.4)	20.42 ± 0.50 (19.2–21.7)	30.23 ± 1.78 (25.6–32.3)	27.35 ± 1.24 (24.3–29.4)	29.11 ± 2.25 (18.3–31.5)
Water pH	6.96	6.72	7.24	6.48	7.27	6.74
AWD (cm) ± SD (min-max)	4.22 ± 2.35 (1–10)	6.61 ± 2.03 (0.8–10.9)	6.81 ± 2.50 (1–13.5)	6.79 ± 3.63 (1.5–12.2)	15.54 ± 5.50 (2.2–23.8)	13.39 ± 2.29 (6.9–22)

eter (Pesola®) of 0.01 g of precision. Finally, they were released back in the place of capture. Additionally, we recorded other behaviors as male-male combats and the male displacing strategy, and we evaluate how size (SVL) and distance among males affect such combats.

Data analysis and processing

The variation of temperature of the water (WT) throughout the reproductive events was evaluated through a Kruskal-Wallis test using each of the four months as independent factor. Calling activity was treated as a binary variable (presence-absence) and its relation with the ED was explored by logistic regression analysis. The U Mann-Whitney test was used in order to determine the existence of significant differences in the positions in the breeding ponds between the calling and the non-calling males.

Finally, we calculated the body condition index (BCI) for all males captured. The BCI is a measure of energetic condition of an individual (Schulte-Hostedde et al., 2005), and is calculated from the residue of the regression between $\log_{10}$ of the weight and the $\log_{10}$ of the SVL (Reading, 2007). The BCI was compared between calling and non-calling males with a U Mann-Whitney test. All analyses were performed using PAST (PAleontological STatistics) v1.94b and Statistica v6.1.

RESULTS

Four reproductive events were recorded in August, October and December 2012 and in February 2013. Reproductive activity was found in C2t during the four events, meanwhile in C1, it was observed only in August and October. A total of 413 individuals were found in reproductive activity (81.8% were males and 18.2% were females) and 59 amplectant pairs were identified.

Description of the breeding microhabitat

Both of the ponds under observation correspond to shallow temporary WB (under 30 cm deep), which had water throughout most of the months in study. The area of C1 was of around 338.83 m^2, and its perimeter was 77.63 m; meanwhile the area of C2t was 797 m^2, and its perimeter was 109.1 m. The changes in the parameters of the ponds in the different breeding events are shown in the Table 1.

In the system of study (breeding WBs and their surroundings), the vegetation was composed of hydrophilic, mesophilic and xerophilic grasslands. The predominant vegetation formations in the hydrophilic grassland were: 1) *caraguatal* (composed by species of the *Eryngium* genus); 2) reed (composed by *Juncus macrocephalus* y *Schoenoplectus californicus*; 3) hydrophilic grass (dense grassland of up to 50 cm long represented by *Ischaemum minus*, *Eleocharis* sp., *Panicum gouinii*, *Leersia hexandra*, and *Hydrocotyle* sp., among others); and 4) *hunquillar* (mainly *Juncus acutus* of up to 1 m long). The principal vegetation formation in the mesophilic grassland was psammophilic grass, which was represented by *Panicum sabulorum*, *Baccharis spicata*, *Androtrichum trigynum*, *Achyrocline satureioides*, and *Pluchea sagittalis*, among others. Finally, the principal vegetation formation in the xerophilic grassland was psammophilic steppe, a wide grassland dominated by *Panicum racemosum*, *Margyricarpus pinnatus*, and *Hydrocotyle* sp. In addition, the presence of *Acacia longifolia* was registered in the area (Fig. 2).

Fig. 2. System of study. The psammophilic and hydrophilic vegetation is clearly distinguishable, as well as the presence of *Acacia longifolia*.

The psammophilic vegetation laid on the sand hills whose vegetation cover was approximately 75%. Two strata can be distinguished: the first one, of up to 15 cm long, was mainly dominated by *Panicum sabulorum*; in the second one *Baccharis spicata* and *Androtrichum trigynum*, were mainly found with a length of 30–50 cm. On the other hand, the hydrophilic vegetation was the predominant one in the system, with ground coverage of approximately 80–90%. It presents two main strata; the first one includes hydrophilic 25–50 cm long grass (Fig. 3), meanwhile the second one (of up to a meter in length) is mainly formed by *Schoenoplectus californicus*, *Juncus acutus*, and *Eryngium paniculatum*. The percentage of emerging vegetation inside the WB varied along the different seasons, and it was 60% in autumn, 50–60% in spring, 20–30% or less in winter, and 75% or

Fig. 3. Reproductive site of *M. montevidensis* in BLR. The hydrophilic vegetation, represented mainly by the hydrophilic grass (height between 25 and 50 cm) is distinguishable.

Table 2. Data on water temperature (°C) and ED (m) for males, females and amplectant pairs of *M. montevidensis* in reproductive water bodies in BLR. REFERENCES: WT: Water temperature (°C); ED: Distance to the edge of the nearest pond (m); SD: Standard deviation.

	Males	Females	Amplectant pairs
WT ± SD (Min-Max) (°C)	25.62±3.71 (19.2–32.3)	24.09±4.13 (17.6–31.5)	22.43±2.86 (18.3–29.9)
ED ± SD (Min-Max) (m)	3.89±2.95 (0.28–11.37)	1.88±1.96 (0.3–8.3)	3.35±2.35 (0.24–10.7)

higher in summer.

Use of the breeding microhabitat

The water temperature (WT) presented a significant variation throughout the different breeding events ($H = 171.20$; $P < 0.05$) and an average of 24.90 ± 3.99 (17.6–32.3) °C. The average distance to the nearest pond edge (ED) was 3.87 ± 2.91 (0.24–11.37) m.

The data on WT and ED for males, females and amplectant pairs is summarized in Table 2. Most males were found between 0 and 3 m away to the nearest edge of the pond (Fig. 4A) at temperatures between 25 and 29°C (Fig. 4B). Moreover, the females and amplectant pairs predominated in the areas near to the edge of the pond (between 0–3 m) at temperatures between 17 and 21°C and between 17 and 25°C, respectively (Table 3). A total of 27% of the males found in the WBs were calling. The average ED for the callers was 2.92 ± 2.47 (0.28–10.0) m; while for the rest of the males, the distance was 4.27 ± 3.03 (0.3–11.37) m. An association was found between the calling activity and the ED ($Chi^2 = 12.41$; $P < 0.05$). The calling males used positions that were closer to the edge of the pond, in comparison to the non-callers ($U = 5440$; $P < 0.05$). Finally, significant differences in the body condition index (BCI) were found between calling and non-calling males; the calling males showed a higher BCI, therefore better energetic state, independently from the size (SVL) of the individuals ($U = 6202$; $P < 0.05$).

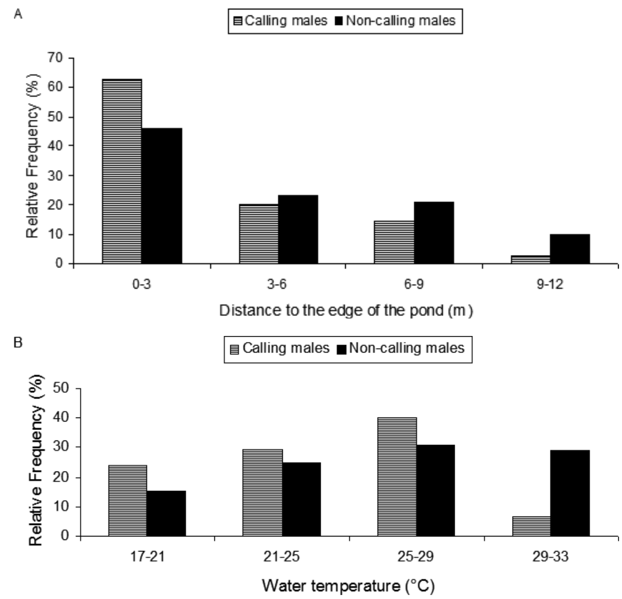

Fig. 4. Distribution of frequency of calling and non-calling males of *M. montevidensis* during the period of study, according to: A) Distance to the edge of the pond (m); B) Water temperature (°C).

Table 3. Data on distribution of females and amplectant pairs of *M. montevidensis* according to: Distance to the edge of the pond (m); Water temperature (°C). REFERENCES: WTR: Water temperature range (°C); RFF: Relative frequency of females (%); RFAP: Relative frequency of amplectant pairs (%). EDR: Distance to the edge of the pond range (m).

WTR (°C)	17–21	21–25	25–29	29–33
RFF (%)	31	25	25	19
RFAP (%)	42	42	12	3
EDR (m)	0–3	3–6	6–9	9–12
RFF (%)	88	6	6	–
RFAP (%)	58	31	8	5

Within the areas around the breeding WBs, individuals of *M. montevidensis* have been repeatedly found using *Schoenoplectus californicus*, *Juncus acutus*, and *Eryngium paniculatum* as shelter, and animals have been observed in active forage in the contiguous areas.

Behavioral displays

A total of 92% of calling males were holding on to emerging vegetation. These males showed a posture typical of calling, which consists in inflating their vocal sac, spreading arms, holding both hand on to the vegetation and split their legs (Fig. 5). However, most of the males that were not calling (72.5%) were seen moving actively, mainly within the pond. The same behavior was observed in 87.5% of the females. Finally, the amplectant pairs showed the three behaviors observed in similar proportions (still among emerging vegetation (37.3%), swimming within the pond (30.5%) and still in the water mirror (32.2%)). Only two clutches were found in situ fixed to the vegetation which was very close to the edge of the pond at 2 cm from the surface of the water mirror.

Fig. 5. Male of *M. montevidensis* showing a typical calling posture during an event of breeding activity in August 2012.

Table 4. Data on snout-vent-length (SVL mm) and weight (M g) in males of *M. montevidensis* observed in combat during the study in the BLR.

COMBAT 1	SVL (mm)	M (g)	RESULTS
Male a	22.07	1.2	remain
Male b	20.15	0.9	move
COMBAT 2	SVL (mm)	M (g)	RESULTS
Male a	21.55	1.15	remain
Male b	20.49	0.85	move

Four combats among males were observed; these consisted of direct body-to-body wrestling. Such behavior was observed when males were calling within a distance shorter than 10 cm from one another. The males which were calling nearby, but within longer distances, did not show such behavior. The smaller males were confirmed to having withdrawn from two of the combats, resulting in the bigger ones being the defeaters (Table 4). In another combat between individuals of similar sizes (male 1: SVL = 22.19 mm; M = 1.15 g; male 2: SVL = 22.38 mm; M = 0.95 g), the males (both of them with vocal sacs inflated) separated after a few minutes of wrestling, and the swam in opposite directions. In October, a male (SVL = 21.33 mm; M = 0.85 g) was observed trying to violently push away another male (SVL = 21.38 mm; M = 1.05 g) which was in amplexus. The male displacing was not successful, and the amplectant pair left the place a few minutes later.

DISCUSSION

Characterization and use of the breeding microhabitat

Melanophryniscus montevidensis used shallow temporary ponds (less than 30 cm deep) of variable sizes and sandy substrata as breeding sites in Barra de la Laguna de Rocha. The water looked clear in all the stages of the activity, and the pH was around 7. The hydrophilic vegetation predominated the system, and was very abundant both inside and outside the pond, becoming noticeably denser on the edges. The use of shallow ponds of under-50-cm depth has been previously reported for other populations of the species in Uruguay (Kolenc, 1988; Prigioni and Garrido, 1989; Alonzo et al., 2002; Maneyro and Achaval, 2004). In addition to this, the preference for ephemeral, superficial habitats with dense vegetation both inside and outside the WB, was previously displayed by other species of the genus, such as *Melanophryniscus rubriventris* and *M. stelzneri* in Argentina (Bustos-Singer and Gutiérrez, 1997; Vaira, 2005; Pereyra et al., 2011). The role of the "complementary habitat" has recently been recognized in the determination of the use of the breeding sites by the anurans, as it has been demonstrated that species will only reproduce if such habitats feature contiguous specific habitats that provide shelter and food (Pope et al., 2000; Blomquist and Hunter Jr., 2009; Lemckert and Mahony, 2010).

The average temperature of the water varied among the reproductive events, and was higher in December and February. The water was from mild to warm (above 20°C on average) on each reproductive instance, and very few individuals were found in the areas of the ponds with temperatures of 30°C or more. This was expected given the ectothermic condition in these animals (along with thin, defenseless and highly permeable skin) (Wells, 2007). Moreover, *M. montevidensis* feature a fast process of metamorphosis (a month, approximately, according to Maneyro and Carreira, 2012), and so do other species of the genus (Langone, 2002). This rapid metamorphosis represents an advantage for the species that breed in temporary habitats, and may be related to the higher temperatures in shallow waters (Griffiths, 1997). The temperature influences the growth and the rate of development of the anuran larvae (Harkey and Semlitsch, 1988); therefore, warmer waters might accelerate the larva development in unpredictable, transitory water bodies.

Males, females and amplectant pairs were mainly found in shallower waters near to the edge of the pond, where both immerse and surface vegetation was noticeably denser. There are two possible, non-exclusive explanations proposed so as to understand the pattern observed. On the one hand, the vegetation might provide heterogeneity to the habitat through its providing of shelter from potential predators (Kopp et al., 2006; Pereyra et al., 2011). Consistent with this, it has been found that the large number of anuran species is positively associated with the extent of surface vegetation on the edges of the pond (Lane et al., 2007). Another possible explanation is the fact that dense vegetation may cause lower temperature gradients, which would discourage a quick increase in the temperature of the water, and thus, reduce the risk of desiccation (Pereyra et al., 2011). Such factors might have an influence on the preference shown by *M. montevidensis* individuals for certain locations within the breeding ponds.

Compared to the non-calling ones, calling males were found in areas closer to the edge of the pond. Kolenc (1988) had reported that males of the species called from the shores. Also was observed in the species *Melanophryniscus stelzneri stelzneri* from Argentina, that the males would call from the edges, leant against or hidden in the vegetation (Bustos-Singer and Gutiérrez, 1997). The dense vegetation present in these areas may be used for shelter, which would be advantageous given that the males will generally become more conspicuous for predators while calling (Wells, 2007). Additionally, the hydrophilic vegetation (grass, mainly) is used as support or fastener while calling. In the breeding congregations of *M. montevidensis* the males outnumber the females widely, thus, their reproductive success will depend on their ability to locate the females (Wells, 1977). As a consequence, taking positions near the edge of the pond would be a good strategy for calling males, as they could intercept the gravid females approaching in search of amplex, first. Finally, it was we found that, independently from body size, calling males featured higher body condition index than non-calling ones, and thus, a better energetic state. Such result was expected *a priori* given that the calling activity is highly energy-consuming, and it requires great distribution of resources from the males (Leary et al., 2004), which must keep good energy reserves. For that purpose, during the periods of inactivity, these males will probably devote a great portion of energy to the improvement of their physical fitness instead of investing it all in their growth (Reading, 2007).

The suitability that a breeding site presents for a species may be evaluated according the presence of such species in the area (Goldberg et al., 2006). The WBs identified in this study as *M. montevidensis* breeding sites were used in several reproductive events, which indicates that oviposition in these ponds is not occasional. To reinforce the idea that these ponds are adequate to breeding requirements of *M. montevidensis*, the same individuals were identified through photo identification using the same specific pond in different months of their reproductive activity (Pers. obs). A similar pattern was found in the *M. stelzneri* species, in Córdoba, Argentina (Pereyra et al., 2011).

Behavioral displays

Calling males used the predominant vegetation as their main support while calling. The use of the vegetation during calling activity was previously observed in *M. montevidensis* (Alonzo et al., 2002; Maneyro and Carreira, 2012); in other species of the genus (Bustos-Singer and Gutiérrez, 1997; Vaira, 2005); and in other anuran species (Ávila et al., 2004; Zank et al., 2008). Non-calling males were found on or among the vegetation, as well, but the vast majority (72.5%) was observed moving actively along the water body. Wells (1977) proposes that when the density of males within a congregation of explosive breeders is high, such males have to alternate their calling activity with other behavior strategies to obtain mates. Dense congregations of individuals were observed in the breeding sites, and males were always more abundant than females. Consequently, those found moving along the pond may have been searching for gravid females. Alternative behaviors have been observed in plenty of other species of the genus, such us *M. stelzneri*, *M. rubriventris*, *M. cambaraensis* and *M. montevidensis* from Argentina; being active search for females the most frequent strategy used by males (Bustos-Singer and Gutiérrez, 1997; Vaira, 2005; Goldberg et al., 2006; Caorsi, 2011; Cairo et al., 2008).

Most females were found moving actively along the WB, as well. Nonetheless, throughout the reproductive events, the percentage of gravid females observed was very low (18.2%), which would make it necessary to perform further studies in order to understand the behavior of such females. On the other hand, 59 amplectant pairs were found. Some authors have reported that *M. montevidensis* couples float on the water during amplexus, using vegetation as an aid, and then dive at regular intervals to the bottom of the pond to put a group of eggs on the submerged vegetation (Kolenc, 1988; Alonzo et al., 2002). In this study, it was not possible to observe the complete in-amplexus behavior, until the moment of "in situ" spawning. The couples were found swimming or still on the vegetation or water surface, probably while going through one of the stages of the behavior described above. Neither amplexus between males, nor multiple amplexus, which might be frequent in the congregations of explosive breeders (Wells, 1977), were observed. Only once was it possible to observe a male trying to displace another male which was in amplexus. Such aggressive behavior is usual in explosive breeding males, and has been previously reported in other species of the genus (Vaira, 2005; Goldberg et al., 2006; Caorsi, 2011).

Apart from the aggressive behavior of male displacing, we observed four body-to-body wrestling encounters between *M. montevidensis* males. Physical interactions among males of the species have been observed in other Uruguayan populations (Prigioni and Garrido, 1989; Alonzo et al., 2002). Similar results were found in a population of the species from the East of Uruguay, as their aggressive demonstrations took place when the males overstepped the 100 mm separating area (Alonzo et al., 2002). Zank et al. (2010) proposed that the keeping of distances among calling males is important when the density of males is high, as it provides a reduction in the number of aggressive interactions. In two of the combats observed in this study, it was possible to confirm that the smaller males withdrew defeated after having wrestled with males of larger sizes. When the males presented similar body sizes, it was not possible to determine the victor, as the anurans would separate and swim in opposite directions after some minutes of wrestling. A population of *Pseudis minutus* in Brazil, showed no aggressive behavior, only acoustic interactions between males, when the difference in size was considerable. However, when the males featured similar sizes, there was both acoustic and physical interaction, resulting in a defeater (Zank et al., 2008). Unlike *M. montevidensis*, *P. minutus* present prolonged, seasonal reproduction. Therefore, the behavior observed in *M. montevidensis* may be related to their explosive breeding dynamics, since given the narrow breeding span, the males invest all their resources in that event, and do not need to "save" energy avoiding wrestling against larger males, where they hold little chance of winning. On the contrary, prolonged breeding species avoid such confrontations, as they need to focus their energy on the continuing of their calling activity, which is held for longer periods, as their reproductive success depends on their persistence in the breeding pond (Wells, 1977). So, we could confirm that apart from the calling strategy, the males of *M. montevidensis* in BLR use alternative behavior strategies, active female search mainly, so as to attract co-specific females when the male density is high.

Conclusions

Based on the results obtained, the study site seems to be an important area for the conservation of wild populations of *Melanophryniscus montevidensis*. Additionally, our results can be an intake for the management plan of the BLR and other preservation areas along the Uruguayan shores. The data obtained will be useful to determinate the adequacy of coastal areas for the preservation of the species, keeping in mind that such areas must include shallow temporary ponds of variable sizes, with warm water, sandy substrata and emerging hydrophilic vegetation among other features. For example, the use of vegetation for holding during both the amplexus and the calling activities, highlights its importance in the breeding ponds used by *M. montevidensis*.

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