NRC Research Press

ARTICLE

Diurnal? Calling activity patterns reveal nocturnal habits in the aposematic toad *Melanophryniscus rubriventris*

L.C. Pereyra, M.S. Akmentins, E.A. Sanabria, and M. Vaira

Abstract: In diurnal species with short breeding seasons, an extension of diel activity to the night hours could be favoured to maximize mating opportunities, but individuals must deal with physiological and behavioural constraints. We tested this hypothesis in the Yungas Red-belly Toad (*Melanophryniscus rubriventris* (Vellard, 1947)). We registered the diel pattern of male calling activity in two localities using automated recording systems, and related it to abiotic factors such as temperature, relative air humidity, and precipitation. The diel pattern of vocalization was mainly diurnal. Interestingly though, nocturnal calling activity was a common feature, representing between 40% and 43% of call records in both localities. Vocal activity was significantly influenced by time of the day and presence of rainfall. Calling males showed high plasticity, with activity in the entire environmental range of relative air humidity and temperature. Nocturnal calling seems to play an important role in the mating strategy of males, and it is probably more frequent in the genus *Melanophryniscus* than currently assumed. We discuss the implications of our findings in relation to different aspects of ecology of the species and suggest that bright colouration in *M. rubriventris* might result from a compromise between several nonconflicting functions (e.g., aposematism and thermoregulation).

Key words: Melanophryniscus rubriventris, Yungas Red-belly Toad, breeding behaviour, advertisement call, diel pattern, nocturnality, aposematism.

Résumé : Chez les espèces diurnes à courte période de reproduction, le prolongement de l'activité circadienne durant la nuit pourrait être privilégié pour maximiser les possibilités d'accouplement, mais les individus sont aux prises avec des contraintes physiologiques et comportementales. Nous avons vérifié cette hypothèse chez le crapaud *Melanophryniscus rubriventris* (Vellard, 1947). Nous avons enregistré le cycle circadien d'activité de chant des mâles en deux endroits à l'aide de systèmes d'enregistrement automatisés et l'avons relié à des facteurs abiotiques, dont la température, l'humidité relative de l'air et les précipitations. Le cycle circadien de vocalisation était principalement diurne. Il est toutefois intéressant de noter que l'activité de chant nocturne était répandue, représentant de 40 % à 43 % des chants enregistrés aux deux emplacements. L'heure du jour et la présence de pluie exerçaient une influence significative sur l'activité vocale. Les mâles chanteurs présentaient une plasticité élevée, leur activité couvrant toute la gamme ambiante d'humidité relative et de températures. Le chant nocturne semble jouer un rôle important dans la stratégie d'accouplement des mâles et il est probablement plus fréquent chez le genre *Melanophryniscus* que ce qui est actuellement présumé. Nous discutons de la portée de nos résultats en ce qui concerne différents aspects de l'écologie de l'espèce et proposons que la coloration très marquée de *M. rubriventris* pourrait découler d'un compromis entre plusieurs fonctions n'étant pas en conflit (p. ex. l'aposématisme et la thermorégulation). [Traduit par la Rédaction]

Mots-clés : Melanophryniscus rubriventris, comportement reproducteur, chant d'appel, cycle circadien, nocturnalité, aposématisme.

Introduction

In most anuran amphibians, interactions between members of a population are exhibited only during the reproductive season (Wells 2007). Most of them are related to inter- and intra-sexual competition for access to mates, and although different among species, the intensity of competition is a consequence of variations in length of the breeding season, local population densities, sex ratio, and mechanisms of mate choice (Wells 1977). Two contrasting patterns of breeding behaviour related with the duration of the breeding season were described in anurans. The prolonged breeders, who breed over periods of weeks to months, and the explosive breeders, with one and (or) several short breeding events lasting hours to days (Wells 1977). In explosive breeders, males greatly outnumber females in every explosive reproductive event, and alongside the synchronous arrivals of the sexes, strongly influence male mating opportunities. Male mating tactics in such species may be shaped by time constraints (Wells 2007).

Given the high level of competition limiting mating opportunities during short reproductive events, shifts in temporal activity patterns could be a mechanism to maximize the fitness of males by avoiding or reducing direct male–male confrontation and exploiting underutilized temporal resources (e.g., extending periods of daily activity). However, individuals extending or switching the periods of activity over multiple breeding periods must deal with new physiological and behavioural constraints related to different thermal and light environments differing significantly between day and night intervals (e.g., Gamble et al. 2015). Those pressures should be more pronounced in anurans occupying hab-

Received 3 November 2015. Accepted 22 April 2016.

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 Published at www.nrcresearchpress.com/cjz on 7 May 2016.

itats with considerable thermal fluctuations between day and night intervals (Navas et al. 2008). A common thermoregalulatory strategy in anurans is to restrict calling activity to a temporal period with compatible environmental conditions (Navas 1996).

Explosive breeding spans over several short reproductive pulses among species of the bufonid genus *Melanophryniscus* Gallardo, 1961, particularly for species that use ephemeral water bodies for reproduction (Kwet et al. 2005; Goldberg et al. 2006; Cairo et al. 2008; Pereyra et al. 2011; Santos et al. 2010; Caldart et al. 2013; Caorsi et al. 2014). The Yungas Red-belly Toad (*Melanophryniscus rubriventris* (Vellard, 1947)) is an endemic species of the humid montane forests of northwestern Argentina and southern Bolivia in the ecoregion of Southern Andean Yungas (Vaira 2002). Breeding behaviour in this species has been defined as highly opportunistic, with an explosive reproductive mode characterized by multiple short reproductive events of intense breeding activity after heavy rainfalls in spring and summer where mating success is often not distributed homogeneously among males. Operational sex ratios are generally male-biased and only occasionally males advertise from stationary positions to attract females (Vaira 2005). Previous observations reveal that males move intensively and physically clasp each other. Physical contact, however, is brief after the clasped males produce a release call (Goldberg et al. 2006). Observation of females entering a breeding arena showed that they first remain undetected. Amplexus is initiated when reaching a distance less than 10 cm to a male. We suggest the capacity to find a mate could be related to male mobility (L.C. Pereyra, M.S. Akmentins, E.A. Sanabria, and M. Vaira, personal observation). Even though *M. rubriventris* is considered a diurnal species (Vaira 2002), nocturnal reproductive activity has also been reported (Garraffo et al. 2012; Sanabria et al. 2014). Nocturnal activity in most bufonids is considered a reversal of the diel activity from diurnality to nocturnality. Consequently, diurnal activity is a primitive condition in the “basal” genus *Melanophryniscus*, reinforced with the posterior acquisition of chemical defences and aposematic colouration (Santos and Grant 2011). These characters have been well documented in *M. rubriventris* (Daly et al. 2007; Garraffo et al. 2012; Bonansea and Vaira 2012).

Under a scenario of multiple explosive reproductive events of short duration and a strong diel weather fluctuation on the humid montane forests where these toads breed, we hypothesized that extended nocturnal breeding activity is part of a mating strategy of males of *M. rubriventris* constrained to particular weather conditions. We predict that individuals are capable of extending their reproductive activity beyond daylight hours but solely during nights with mild temperatures, high percentages of relative air humidity, and presence of rainfall. To test our prediction, we registered the diel calling activity pattern of males of *M. rubriventris* in two different localities of the subtropical montane forest of northwestern Argentina and investigated calling activity in relation to environmental cues.

Materials and methods

Study sites

The study was carried out in two localities of Jujuy province, northwestern Argentina. The locality of Potrero de Yala (24°08′49.2″S, 65°28′08.5″W; datum WGS 84; 2107 m above sea level) and the locality of Angosto de Jaire (24°01′24.4″S, 65°23′24.1″W; datum WGS 84; 1745 m above sea level). The climate in the Yungas forest is highly seasonal, with rainfalls concentrated during the warmest months, from October to May, and a dry season during the coldest months, from June to September (Akmentins et al. 2015). Even during the warmest months, temperatures and humidity highly fluctuate on a daily basis (more than 11 °C and 98% of relative air humidity between maximum and minimum values, respectively; M.S. Akmentins, unpublished data).

Permits for fieldwork were provided by Secretaría de Gestión Ambiental, Dirección Provincial de Biodiversidad, Jujuy, Argentina.

Data collection

In each study site, we employed an automated recording device or “frog-logger” to register the daily pattern of advertisement call activity of males of *M. rubriventris* (for frog-logger specifications see Akmentins et al. 2015). The frog-logger recorded for 1-min every hour and a data logger (HOBO U10-003 Temp/RH Data Logger; Onset, Bourne, Massachusetts, USA) was programmed to simultaneously register the weather variables of air temperature (°C) and relative air humidity (% RH) at hourly intervals. For each 1-min interval recording, the intensity of the advertisement call activity was quantified according to the numerical classification scheme used by Bridges and Dorcas (2000) as follows: 0 = no vocalization; 1 = only one male vocalizing; 2 = multiple males vocalizing with the possibility of distinguishing individual calling males; 3 = many males vocalizing without the possibility of distinguishing between individual calls (hereafter full vocal activity).

We also noted the presence or absence of rainfall from the sound of raindrops on the plastic hood of the microphone (only heavy and moderate rainfall was detected with this method). Data of daily cumulative precipitation was obtained from the Augusto M. Romain weather station (Universidad Nacional de Jujuy), located 15 km from both study-site localities.

Statistical analysis

We compared the weather conditions (temperature, relative air humidity, and occurrence of rainfall) between registries with and without calling activity of male *M. rubriventris* during daylight and night hours for each surveyed locality separately. Because the data were not normally distributed, we used the nonparametric Kruskal–Wallis one-way analysis of variance (ANOVA) by ranks for the continuous variables and a Pearson χ^2 test using 2 × 2 tables for the discrete variable.

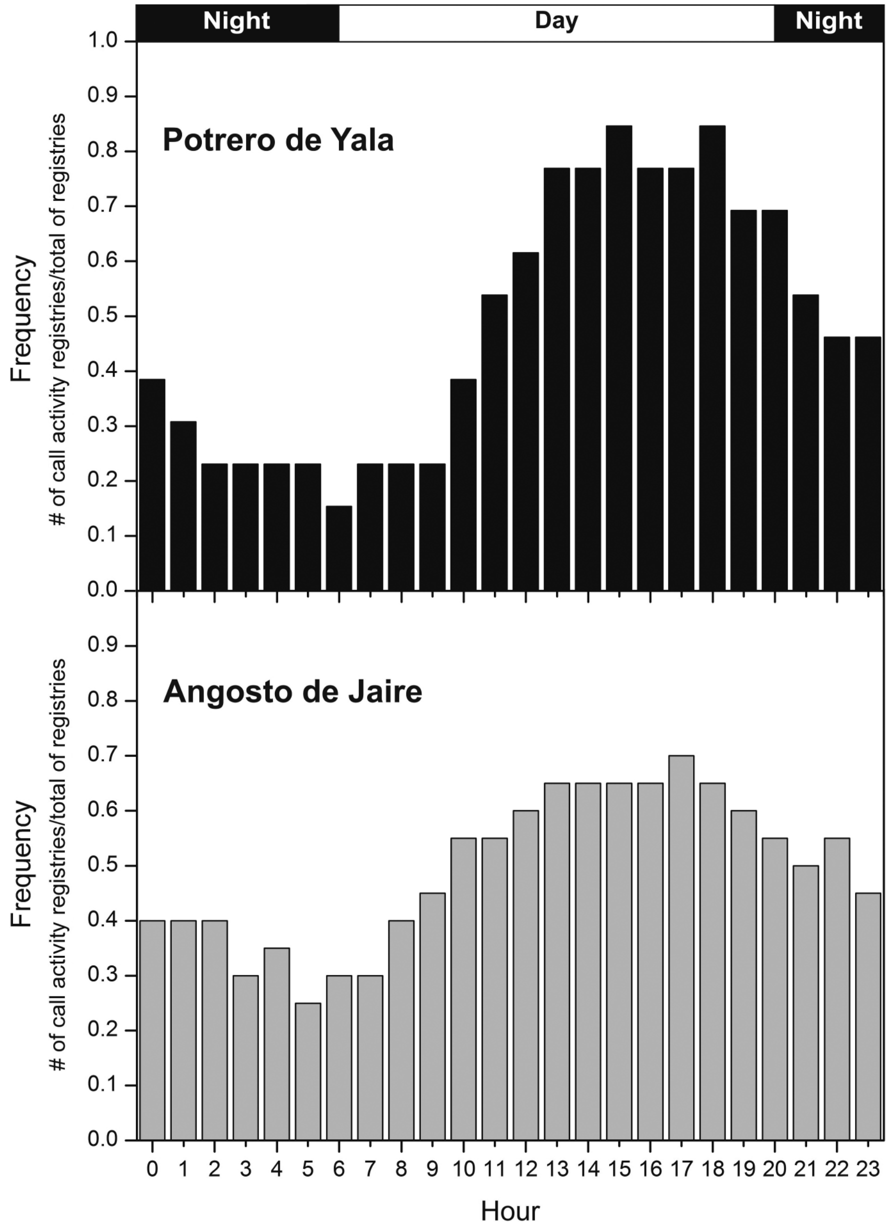
We used generalized linear mixed models (GLMM) assuming binomial distribution and a logit-link function to relate the advertisement call activity of *M. rubriventris* to the abiotic environmental variables. We employed the total of 1-min interval records to run the modes. Calling activity was coded as a binary variable, with presence (1) or absence (0) of calling activity. We included time of each 1-min record (h), relative air humidity (% RH), air temperature (°C), and presence or absence of rainfall as fixed effects; the two surveyed localities were treated as random effects. Variables with a strong correlation (Spearman’s rank correlation ≥ 0.7) were not included in the same model (Fielding and Haworth 1995). Because correlation coefficients ranged below the suggested cutoff, all independent variables were included in the analysis. We tested all possible combinations of variables included in the global model and used an information–theoretical approach based on Akaike’s information criterion (AIC) to select the best model given the data. The model with the lowest AIC value was considered the one best supported by the data (Burnham and Anderson 2002). We calculated the ΔAIC; models of less than two AIC units from the best model were considered good candidates (Burnham and Anderson 2002). We performed all statistical analyses using software R version 3.2 (R Core Team 2015).

To calculate frequency of occurrence of vocalization activity at each daily hour during the diel cycle of vocal activity of male *M. rubriventris* and percentage of diurnal or nocturnal advertisement call activity, we only considered days with 24 h of continuous records and with at least one 1-min period with registered calling activity.

Results

We obtained a total of 850 1-min interval records in the locality of Potrero de Yala, beginning on 25 October 2014 and extended until 1 December 2014; a subset of 744 of these records were em-

Fig. 1. Frequencies of occurrence of full vocalization activity during the daily cycle of vocal activity of male Yungas Red-belly Toads (*Melanophryniscus rubriventris*). Frequencies were calculated with the number registries at each daily hour over all days with 24 h of continuous registries and at least 1 h with call activity. Black-and-white bar at the top indicates day and night hours.



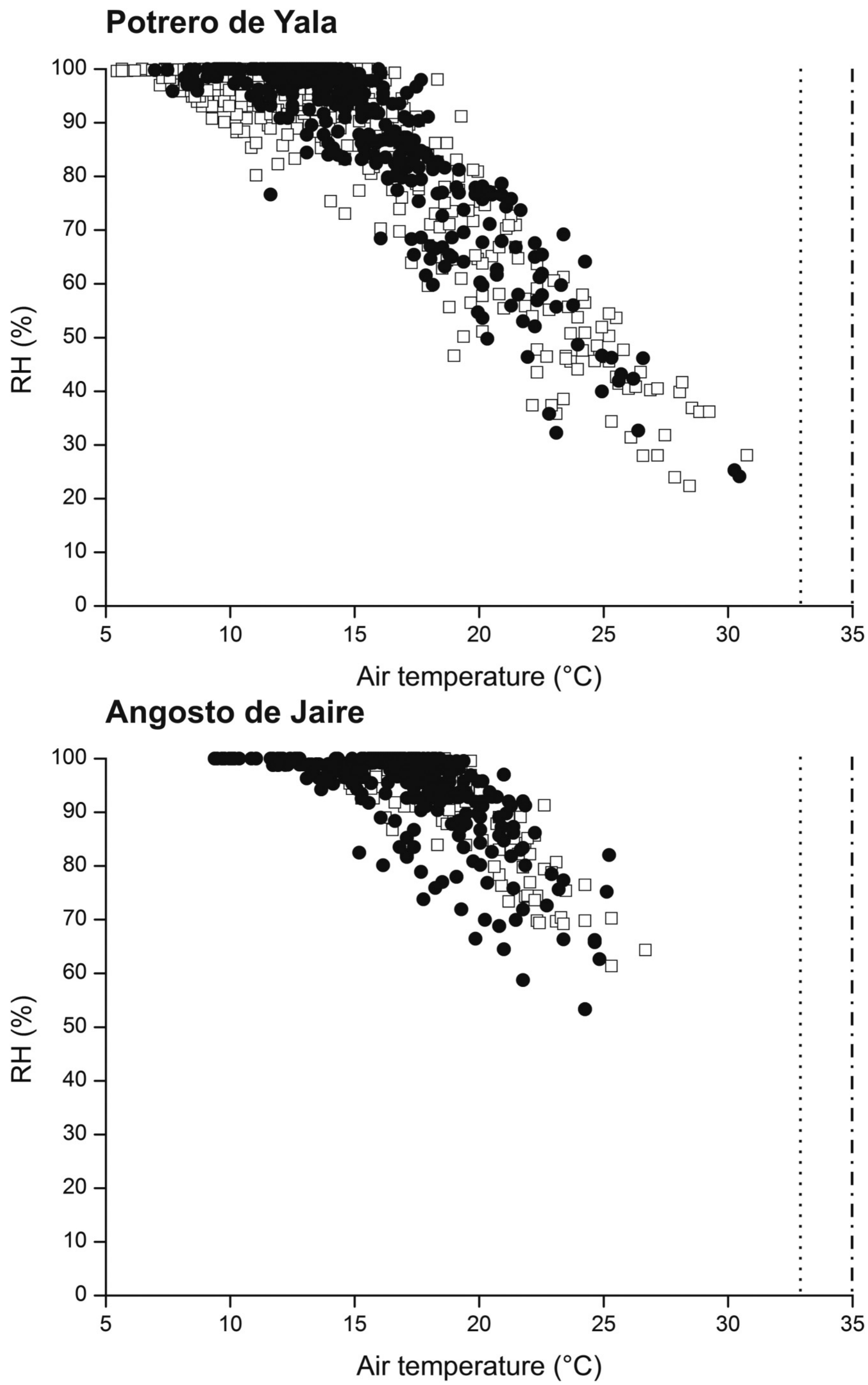
ployed to describe the diel pattern of vocal activity in this locality. At Angosto de Jaire, we obtained a total of 570 1-min interval records, beginning on 20 December 2014 and extended until 31 January 2015; a subset of 480 of these records were employed to describe the diel pattern of vocal activity in this locality.

The daily pattern of vocalization of *M. rubriventris* in both localities, particularly the "full vocal activity", was mainly diurnal, as expected, with a peak between the hours of 1100 and 1900 (Fig. 1). Nocturnal calling activity (between the hours of 2000 and 0500) was common in males of *M. rubriventris*, representing 40.1% of call records at Potrero de Yala (146 of 364 records with registered calling activity) and 42.7% of call records at Angosto de Jaire (143 of 335 records with registered calling activity). Considering only the "full vocal activity", the percentage of nocturnal calling resulted in 32.5% of the records at Potrero de Yala and 35% of the records at Angosto de Jaire.

In both localities, calling behaviour was associated with short reproductive events, constituted by periods of at least 24 h of continuous and intense calling activity (calling activity intensity 2 or 3), within a span of 1–3 days. We registered three reproductive pulses in the locality of Potrero de Yala, which occurred after moderate rainfall of 9.5–36.5 mm (cumulative precipitation in the previous 24–48 h). In the case of Angosto de Jaire, we registered four of these events after heavy rains of 25–49 mm.

Calling activity was recorded almost at the entire registered range of air temperature and relative air humidity in both localities (Fig. 2). Ranges of temperature and relative air humidity of calling activity in the locality of Potrero de Yala varied from 6.9 to 30. 4 °C and the minimum percentage of relative air humidity was 24.1%. In Angosto de Jaire, males called in a range of air temperatures between 9.3 and 25.2 °C and the minimum percentage of relative air humidity was 53.3%.

Fig. 2. Registries of air temperature (°C) and relative air humidity (% RH) during the 1-min records of male Yungas Red-belly Toads (*Melanophryniscus rubriventris*) with advertisement call activity (solid circles) and hourly registries without calling activity (open squares). Lines indicate the critical thermal maximum temperatures registered for the two extreme colour phenotypes of the species, where the vertical dotted line is for the low dorsal melanization morph and the vertical dashed–dotted line is for the high dorsal melanization morph (Sanabria et al. 2014).



Relative air humidity values during daylight and night hours in the locality of Potrero de Yala presented significant statistical differences between registries of calling activity of males and registries with no calling activity; calling activity mostly occurred in conditions of high relative air humidity values (up to 80%). Air temperature and calling or no calling hourly registries showed no differences during daylight hours, but did vary at night, with calling activity occurring at warmer temperatures than no calling times (Table 1). In Angosto de Jaire, relative air humidity did not vary significantly between calling and no calling activity registries during daylight hours, but calling activity occurred at higher relative air humidity values during the night. Considering air temperatures, males were calling at lower temperatures during daylight hours and there were no differences between calling and no calling activities at night (Table 1). Registries of calling activity were associated with the presence of rainfall in both localities (Table 2).

Considering the best model selected on the basis of AIC values, advertisement call activity was influenced by time of the day and presence of rainfall (Table 3) According to AIC weights, the probability that this was the best model, based on the data, was 0.92.

Table 1. Kruskal–Wallis test results comparing relative air humidity and air temperature values between hourly registries with and without calling activities of male Yungas Red-belly Toads (*Melanophryniscus rubriventris*) during daylight (0600–1900) and night (2000–0500) periods for the two surveyed localities.

Variable	Activity	Mean ± 1 SD	Kruskal–Wallis *H*	*p*
Relative air humidity (% RH)				
Potrero de Yala				
Daylight	Calling	84.2±18.3B	72.12	<0.001
	No calling	78.9±22.4A		
Night	Calling	95.2±6.1D		
	No calling	94.1±5.7C		
Angosto de Jaire				
Daylight	Calling	92.6±9.7A	35.41	<0.001
	No calling	90.8±10.7A		
Night	Calling	97.8±3.4B		
	No calling	96.4±3.3A		
Temperature (°C)				
Potrero de Yala				
Daylight	Calling	16.2±4.4C	160.34	<0.001
	No calling	16.6±5.9C		
Night	Calling	13.1±2.1B		
	No calling	11.6±2.8A		
Angosto de Jaire				
Daylight	Calling	17.5±3.2B	70.27	<0.001
	No calling	19.0±2.8C		
Night	Calling	16.1±1.8A		
	No calling	16.8±1.3A		

Note: A posteriori test results are included and different letters indicate significant statistical differences between groups.

Table 2. Pearson χ^2 test results relating registries with frequencies of calling and no calling activities of male Yungas Red-belly Toads (*Melanophryniscus rubriventris*) with presence or absence of rainfall during daylight (0600–1900) and night (2000–0500) periods for the two surveyed localities.

	Activity	Frequencies	Pearson χ^2	*p*
Potrero de Yala				
Daylight				
With rain	Calling	0.66	14.31	<0.001
	No calling	0.34		
Without rain	Calling	0.43		
	No calling	0.57		
Night				
With rain	Calling	0.55	9.89	0.002
	No calling	0.45		
Without rain	Calling	0.37		
	No calling	0.63		
Angosto de Jaire				
Daylight				
With rain	Calling	0.93	22.53	<0.001
	No calling	0.07		
Without rain	Calling	0.6		
	No calling	0.4		
Night				
With rain	Calling	0.96	20.91	<0.001
	No calling	0.04		
Without rain	Calling	0.62		
	No calling	0.38		

Both variables showed a positive relationship with the probability of calling activity (presence of rainfall: coefficient = 0.24, SE = 0.03, t = 7.69; time of the day: coefficient = 0.008, SE = 0.002, t = 4.64).

Discussion

As predicted, although both populations of *M. rubriventris* showed calling activity peaks between midday and noon, calling males remained highly active until first night hours. Our data suggests that nocturnal calling activity could play an important role in the mating strategy of males of *M. rubriventris*, accounting for more than one-third of the invested time in diel vocal activity. Most of the species of the genus *Melanophryniscus* have been previously defined as strictly diurnal (Kwet et al. 2005; Van Sluys and Guido-Castro 2011; Dalmolin et al. 2012; Duré et al. 2015). This constitutes the first report of extended diel calling pattern for a species of the *Melanophryniscus stelzneri* (Weyenbergh, 1875) group (the account of species in Baldo et al. 2014). Nocturnal breeding activity seems to be more common in the *Melanophryniscus tumifrons* (Boulenger, 1905) group, because a similar diel pattern was observed in *Melanophryniscus cambaraensis* Braun and Braun, 1979 and *Melanophryniscus macrogranulosus* Braun, 1973 (Santos and Grant 2011; Caorsi et al. 2014), and mainly nocturnal activity in *Melanophryniscus pachyrhynus* (Miranda-Ribeiro, 1920) and *Melanophryniscus simplex* Caramaschi and Cruz, 2002 (Colombo et al. 2007; Caldart et al. 2013). Nocturnal calling activity was also reported in the recently described *Melanophryniscus biancae* Bornschein, Firkowski, Baldo, Ribeiro, Belmonte-Lopes, Corrêa, Morato, and Pie, 2015 (Bornschein et al. 2015), but more information about natural history is needed to determine the diel calling pattern of this phytotelm-breeding species.

Diurnal species of the Neotropical clades Aromobatidae, Brachycephalidae, Bufonidae, Dendrobatidae, and Hylodidae rely on acoustic and (or) visual cues for mate recognition, generally within a context of high male territoriality (Cocroft et al. 1990; Pombal et al. 1994; Haddad and Giaretta 1999; Hödl and Amézquita 2001; Gardner and Graves 2005; Almeida-Gomes et al. 2007; Kaefer et al. 2012). Both advertisement call and male territoriality do not constitute effective strategies in the explosive breeder mating system of *Melanophryniscus* toads, where males display active searches for females in high-density aggregations (Goldberg et al. 2006). In this context of trial-and-error process of mate search, prolonged diel breeding activity should be a valuable strategy to maximize male mating success and probably is more spread in the genus *Melanophryniscus* than currently assumed. Similar considerations were made by Santos and Grant (2011) in a population of *M. cambaraensis* breeding in a mixed ombrophilous forest at the subtropical southeastern Araucaria Plateau in Brazil. Comparative studies will be useful for interpreting or reinterpreting the role of extended reproductive activity and to examine the evolution of temporal activity patterns in these bufonids.

Prolonged diel reproductive patterns could be advantageous by extending the breeding period and maximizing reproductive fitness, but also implicate physiological adjustments or increased energetic expenditures for calling males (McCauley et al. 2000; Riddell and Sears 2015). Many amphibians should limit the duration of activity to avoid overheating, freezing, or dehydration,

Table 3. First five generalized linear mixed models (GLMMs) and the null model investigating the relationship between environmental variables and probability of calling activity of Yungas Red-belly Toads (*Melanophryniscus rubriventris*).

Rank	Model	*k*	AIC	ΔAIC	w_i
1	~ hour + rain	5	1937.86	0	0.92
2	~ hour + rain + temperature + humidity	7	1943.8	5.94	0.05
3	~ hour + rain + humidity	6	1946.41	8.55	0.01
4	~ rain	4	1946.42	8.57	0.01
5	~ hour + rain + temperature	6	1949.46	11.61	0
8	~ 1 (null model)	3	2001.16	63.35	0

Note: The models are ranked according to Akaike's information criterion (AIC). Predictor terms are "hour", referring to time of each 1-min record; "rain", referring to presence or absence of rainfall; "temperature", referring to air temperature (°C); "humidity", referring to percentage of relative air humidity (% RH). *k* is the number of parameters in the model; ΔAIC is the difference between the AIC value of each model and the AIC value of the best model; w_i is Akaike weights representing the probability of each model being the best.

illustrating how physiological constraints influence the possibility of extend diel activity (Navas 1996; Navas et al. 2013).

Melanophryniscus rubriventris is an aposematic toad (i.e., chemically defended prey carrying warning signals to advertise unprofitability to predators) that exhibits remarkable variation in skin colouration differing in the size and shape of black and bright patches (Bonansea and Vaira 2012). Our findings of extended reproductive activity until first night hours seem to challenge the expectations for an aposematic toad whose colour warning signals should be selectively advantageous during daylight hours, when visually oriented predators like birds are active (Saporito et al. 2007; Paluh et al. 2014, 2015). At night, these toads could be exposed to other potential predators (such as arthropods, snakes, and mammals) that rely mostly on chemical cues and can counteract their ability to signal distastefulness through vivid colours. Therefore, nocturnal activity could increase chances of predation. On the other hand, the extension of bright and dark skin colouration in the species may be the result of mechanisms to improve thermoregulation where bright colours reduce heat absorption and avoid overheating when exposed to sunlight, but black patches may help nocturnal individuals to warm up faster in the evening (Sanabria et al. 2014), allowing the activity of *M. rubriventris* in a wide range of environmental temperatures. Our study adds the aspect of nocturnal activity to the role of colouration in the species and may allow a better understanding on the theory of aposematic signal evolution (Stevens and Ruxton 2012). Our results might also stimulate further research to better interpret the relationships of colour patterns of aposematic species with differences in thermal and light conditions.

Rainfalls were the trigger for reproductive pulses of *M. rubriventris* in our study sites, and as previously described, an intense breeding activity occurred over the next 1–3 days (Vaira 2005; Goldberg et al. 2006). Our data indicates that male calling activity was continuous throughout days during these reproductive events. The synchronicity between reproductive pulses and moderate to heavy rainfalls remarks the opportunistic and explosive reproductive behaviour of this toad species (Vaira 2005; Goldberg et al. 2006). The onset of breeding season of some Nearctic and Neotropical bufonid species are influenced by distinct abiotic cues related to the season in which breeding period occurs (winter, spring, summer) and phenology of the water bodies (ephemeral, temporal, permanent) used to spawn (Saenz et al. 2006; Cook et al. 2011; Klaus and Lougheed 2013; Schalk and Saenz 2016). For species using ephemeral water bodies to breed with hydroperiods that fluctuate largely due to patterns of precipitation, the reproductive activity may be timed to coincide with rainfall episodes that ensure the presence of water within breeding sites (Bustos Singer and Gutiérrez 1997; Vaira 2005; Saenz et al. 2006; Van Sluys and Guido-Castro 2011).

The presence of precipitation was the most influential abiotic cue related to the diel calling activity pattern of males of *M. rubriventris*. Males of *M. rubriventris* showed high plasticity in relation to abiotic cues and called in almost the entire registered range of temperature and relative air humidity. The registered maximum temperature values with vocal activity in the locality of Potrero de Yala were surprisingly close to the critical thermal maximum registered for the species (Sanabria et al. 2014). This tolerance to environmental extremes, particularly to conditions of low air humidity and high temperatures, could be attributed to observed behaviour of males, which call partially submerged in the same water bodies used later to breed (Goldberg et al. 2006; Ferrari and Vaira 2008). Acclimatization and changes in thermoregulatory behaviour during the reproductive season was reported in other Neotropical bufonid species, to better exploit the entire temperature environmental range (Sanabria and Quiroga 2011; Sanabria et al. 2012). The current study shows a preference for more warm and humid conditions during nocturnal calling activities in both studied populations of *M. rubriventris.* The close relationship between reproductive activity of *M. rubriventris* and local weather conditions, particularly reflected in the onset and extent of annual breeding seasons (Vaira 2005), should be considered when evaluating possible effects of climate change on this endemic toad beyond the projected distributional area reduction as consequence of the future scenarios of climate change (Zank et al. 2014).

Despite the utility of passive monitoring techniques to study the reproductive activity of anuran amphibians (Ospina et al. 2013; Williams et al. 2013; Akmentins et al. 2015; Willacy et al. 2015), this methodology does not provide an accurate assessment of densities and does not allow individual recognition; moreover, they are biased toward males. Consequently, our study leaves some unanswered questions: What is the density of individuals during nocturnal breeding events? What is the sex ratio during these nocturnal reproductive events? Are nocturnal individuals the same diurnal males that extend their diel calling activity? Does nocturnality increase male mating success? Determining the relative importance of shifts in temporal reproductive activity to both male–male competition and male mating strategies, also in regard to body condition (Humfeld 2013), will require further research.

Acknowledgements

We thank Cátedra de Agroclimatología FCA–UNJu for the climate data from the Augusto M. Romain weather station. We thank G. Galvani, C. García, L. Hernández, N. Gonzáles, and M.F. Quiroga for their assistance during fieldwork. We specially thank the insightful comments of B. Rojas that greatly improved an early version of the manuscript. We are also grateful to the anonymous reviewers for comments that greatly improved the manuscript.

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