# Diel pattern of migration in a poisonous toad from Brazil and the evolution of chemical defenses in diurnal amphibians

**Raquel Rocha Santos · Taran Grant**

**Abstract** Most amphibians with biphasic life cycles have aquatic eggs and larvae and terrestrial adults that migrate between terrestrial habitats and aquatic breeding sites. Migration usually occurs at night in order to avoid desiccation and predation. However, some amphibians also migrate during the day, and it has been proposed that this may have evolved as a result of poisonous skin secretions and aposematic coloration that release individuals from visually oriented diurnal predators. Based on this hypothesis and recent observations of 24 h breeding activity in the poisonous, aposematic toad *Melanophryniscus cambaraensis*, we predicted that migration in this species would occur equally during the day and night. To test our prediction we documented the diel pattern of migratory activity over multiple explosive breeding events from October 2008 to February 2009 (127 nights) at a temporary stream in Rio Grande do Sul state, southern Brazil. We also obtained environmental data to determine if diel activity could be attributed to variation in rainfall, barometric pressure, temperature, and/or relative air humidity. Contrary to our prediction, migratory activity in *M. cambaraensis* is strongly diurnal. Although temperature and humidity varied significantly between day and night intervals, this variation does not account for the diurnal-only migratory activity of *M. cambaraensis*. We suggest that the diurnal-only migratory activity of *M. cambaraensis* is best explained by phylogeny, not contemporary functions or selective pressures. Diurnal activity is primitive for this species and evolved in the common ancestor of Agastorophrynia, prior to the chemical defenses found in toads (Bufonidae) and poison frogs (Dendrobatidae). This suggests that chemical defenses in these groups may have evolved as a result of the diurnal activity that brought them into contact with visually oriented diurnal predators, and not the other way around.

**Keywords** Movement · Toxicity · Diel activity · Breeding · Reproduction · Environmental variables · Phylogeny · Amphibia · Anura · Bufonidae · *Melanophryniscus cambaraensis*

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## Introduction

Most species of amphibians with biphasic life cycles have terrestrial adults and aquatic eggs and larvae (Altig and McDiarmid 1999). To complete their life cycle, adults of these species must risk predation and desiccation to migrate between terrestrial foraging or over-wintering habitats and aquatic breeding sites (Russell et al. 2005; Semlitsch 2008). Amphibian migration usually occurs at night when temperatures are low and humidity is high (Noble 1931; Semlitsch 1985; Sinsch 1988; Todd and Winne 2006), which reduces desiccation risk and contributes to predator avoidance (Semlitsch and Pechmann 1985). Nevertheless, Semlitsch and Pechmann (1985) found that migratory activity in the salamander *Notophthalmus viridiscens* (Rafinesque 1820) did not differ between day and night intervals. This species has poisonous skin secretions (tetrodotoxin; Daly 2004; Hanifin 2010) and aposematic coloration, and Semlitsch and Pechmann (1985) hypothesized that chemical defenses coupled with warning coloration enable diurnal migratory activity by eliminating the need to restrict activity to periods of darkness to avoid detection by visually oriented diurnal predators, i.e., by shifting from a predator avoidance mechanism (nocturnal migration) to an antipredator mechanism (poisonous skin secretions and aposematic coloration; Brodie et al. 1991). Many species of poisonous, brightly colored frogs and toads also exhibit diurnal activity, and Semlitsch and Pechmann's (1985) hypothesis provides a plausible explanation for the evolution of diurnality in these taxa as well.

Like *Notophthalmus viridiscens*, the red-bellied toads of southern South America (*Melanophryniscus*, Bufonidae) secrete defensive chemicals and exhibit aposematic coloration. Specifically, they secrete lipophilic alkaloids and bufadienolides (Daly et al. 2008) and employ the unken reflex to display bright red or orange ventral surfaces when disturbed (Fig. 1). Until recently it was believed that all activity in *Melanophryniscus* was diurnal or at most crepuscular (Langone 1994; Kwet and Di-Bernardo 1999; Baldo and Basso 2004; Kwet et al. 2005). However, *M. simplex* Caramaschi and Cruz 2002 was recently observed calling during both daytime and nighttime (Colombo et al. 2007; P. Colombo personnel communication), and the number of calling male *M. cambaraensis* Braun and Braun 1979 does not differ significantly between day and night (V. Z. Caorsi, R. R. Santos and T. Grant, unpublished data).

Reproduction in *Melanophryniscus cambaraensis* is explosive (sensu Wells 1977), being concentrated in bursts of breeding activity over a few days when many individuals simultaneously migrate to temporary pools or streams during and immediately following intense rains (Santos et al. 2010). Unlike most species that form explosive breeding aggregations, *M. cambaraensis* breeds aseasonally. That is, despite inhabiting a subtropical region characterized by well-defined seasons, explosive breeding events occur repeatedly at irregular intervals throughout the year. Like other explosive breeders, males of *M. cambaraensis* exhibit many of the typical strategies of scramble competition (Wells 2007), including intense male-male combat and struggles to possess females and displace amplectant males (V. Z. Caorsi, R. R. Santos and T. Grant, unpublished data).

Based on Semlitsch and Pechmann's (1985) hypothesis and the defensive chemicals (confirmed in this species by gas chromatography/mass spectrometry analysis; P. Colombo & T. Grant, unpublished data), aposematic coloration, and recent observations of continuous 24 h breeding activity of *Melanophryniscus cambaraensis*, we predicted that migratory activity in this species would occur equally during day and night intervals, as in *Notophthalmus viridiscens*. To test our prediction we documented the diel pattern of migratory activity in *M. cambaraensis* over multiple breeding events. We also obtained
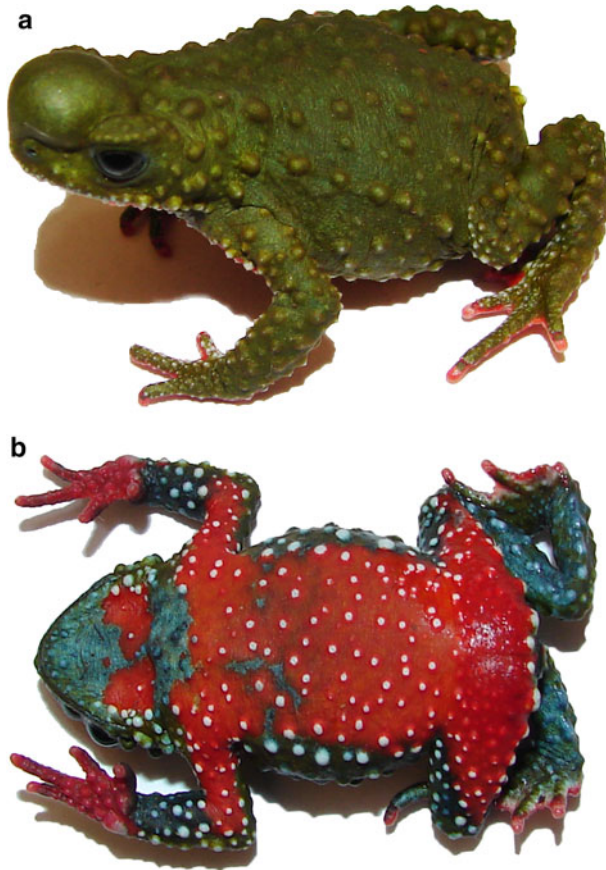
**Fig. 1** *Melanophryniscus cambaraensis* showing **a** green dorsal and **b** contrasting bright red ventral coloration. Defensive chemicals are secreted form skin glands, including the massive frontal gland on the dorsal surface of the head, visible in **a**

environmental data to determine if diel activity in this species could be attributed to variation in rainfall, barometric pressure, temperature, and/or relative air humidity.

## Materials and methods

Study site and data collection

*Melanophryniscus cambaraensis* is endemic to the subtropical southeastern Araucaria Plateau in the Campos de Cima da Serra micro-region of Rio Grande do Sul state, Brazil. Only two isolated populations of this vulnerable species have been discovered, each at single localities separated by approximately 50 km in the municipalities of Cambará do Sul and São Francisco de Paula in Rio Grande do Sul state, Brazil (Garcia and Vinciprova 2003). We studied the São Francisco de Paula population, which is restricted to the Floresta Nacional de São Francisco de Paula (FLONA). The native Mixed Ombrophilous Forest is dominated by the Parana pine *Araucaria angustifolia* (Bertol.) Kuntz (Sonego

et al. 2007) and covers about 56% of the 1606.70 ha of the FLONA, the remainder being composed of planted *A. angustifolia*, *Pinus* spp. and *Eucalyptus* spp. (Bonatti et al. 2006). The climate is classified as Temperate Superhumid (Maluf 2000), with temperatures ranging from −3 to 18°C in winter months and 18.3–27°C in the summer months, and an annual average of 14.5°C. The region has high levels of rainfall in all months, with average monthly precipitation of more than 200 mm for all months of the year (Buriol et al. 2009) and average annual precipitation of more than 2,200 mm (Backes et al. 2005).

We collected field data from October 2008 to February 2009 (127 nights) at a temporary stream that forms following heavy rainfall (29°25′41.3″S 50°23′44.5″W, 866 m above sea level) and the adjacent forest. The breeding site is approximately 20 m long and 4 m wide and is located at the edge of a small, unpaved road on a rocky outcrop partially covered by a thin layer of topsoil and vegetation dominated by grasses (Poaceae) and herbs (*Eryngium* sp.). Except for the small road, which is used very rarely, the area around the stream is composed of forest (planted *Araucaria angustifolia*), with shrubs and saw-grass (Cyperaceae) between the temporary stream and the forest.

We captured migrating individuals using two methods. First, we installed three arrays of pitfall traps at 7.5, 15, 30, 60, and 120 m on each side of the breeding site, totaling 30 arrays, with another three arrays installed during the course of the study (December) at 240 m on one side of the stream. Each array was composed of three plastic drift fences (3 m long, 40 cm high, buried 5 cm into the ground) arranged in a "Y" with a 4 L bucket buried at the vertex and tips of the "Y" (Corn 1994). We drilled small holes in the bottom of each bucket to allow rainwater to escape. Second, we completely encircled the breeding site with drift fences (approximately 51 m long and 40 cm high) and installed 34 funnel traps to capture individuals in pre- and post-reproductive migration (Santos et al. 2010).

We checked traps twice daily, just before sunrise and sunset. All captured individuals were photographed and marked by toe-clipping, the most common technique to mark anurans (Ferner 2007). After processing, individuals captured in funnel traps were released on the opposite side of the fence and individuals captured in pitfall traps were released next to the traps in which they was captured.

We obtained data for barometric pressure, temperature, and relative air humidity from the Instituto Nacional de Meteorologia's nearby automatic meteorological station in the municipality of Canela, 29°22′S, 50°49′W, 830 m above sea level. Immediately before checking traps we used a rain gauge to measure the rainfall that accumulated over the preceding day or night.

Statistical analyses

To avoid non-independence of pseudoreplicates we excluded all recaptures from analyses by randomly selecting a single capture datum per individual. Because we conducted our study during the spring and summer, day and night lengths were not equal (day:night ratio approximately 1.4:1), which could bias cumulative parameters. To correct for this, we multiplied nighttime captures and rainfall by 1.4 and used the corrected values for all analyses.

We performed a $\chi^2$-test to determine if the observed day and night captures differed significantly from a 50:50 expectation. We used a multiresponse permutation procedure in the BLOSSOM software package (version W2008.04.02; Cade and Richards 2005) to determine if the environmental variables differed significantly between day and night intervals, using a Monte Carlo approximation with 4,999 replications to obtain the $P$ values. We performed a partial Mantel test (Smouse et al. 1986) in the program NTSYSpc

(Rohlf 2000) to evaluate the correlation of captures and diel interval after controlling for the effect of the environmental variables (i.e., after accounting for the correlation between captures and environmental variables and between diel interval and environmental variables); we assessed significance by generating a Monte Carlo null distribution using 5,000 replications. For all tests we considered $P < 0.05$ to indicate significant departure from null hypotheses.

## Results

We captured 147 individuals (51 males and 96 females) 502 times, 439 (87%) during the day and 63 (13%) at night. All captures were associated with explosive breeding events. Excluding pseudoreplicates, our dataset included 127 individuals (86%; 82% following correction for unequal day length) captured during the day and 20 individuals (14%; 28 and 18% following correction) captured at night (Fig. 2). These values for diurnal and nocturnal captures were significantly different from a 50:50 expectation ($\chi^2 = 63.24$; $P < 0.001$).

Environmental data are summarized in Table 1. Among the environmental variables we tested, only temperature ($P = 0.0002$) and relative air humidity ($P = 0.0002$) varied
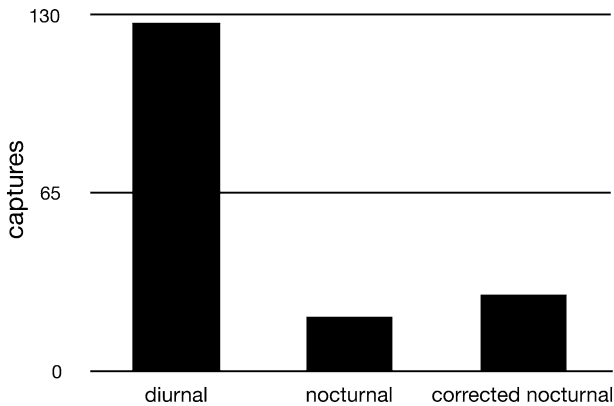


**Fig. 2** Numbers of individuals captured migrating during day and night intervals, excluding recaptures. To account for greater day length we multiplied nocturnal captures by 1.4 (corrected nocturnal) and used the corrected value in statistical analyses

**Table 1** Summary of environmental data for days and nights from 11 October 2008 to 26 February 2009

|  | N | Day vs. night $P$ | Maximum | | Minimum | | Mean | | Standard deviation | |
|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  | Day | Night | Day | Night | Day | Night | Day | Night |
| T (°C) | 139 | 0.0002 | 26.4 | 21.7 | 10.3 | 9.8 | 19.6 | 15.9 | 3.4 | 2.6 |
| RH (%) | 139 | 0.0002 | 99.0 | 99.0 | 46.9 | 74.3 | 77.7 | 90.2 | 12.2 | 5.4 |
| Rain (mm/m²) | 125 | 0.259 | 61.5 | 44.8 | 0.0 | 0.0 | 3.2 | 2.5 | 8.3 | 7.1 |
| BP (hPa) | 139 | 0.561 | 927.0 | 925.4 | 913.0 | 912.7 | 919.9 | 919.6 | 2.9 | 2.7 |

We corrected nighttime rainfall for unequal day and night lengths. We derived $P$ values from a multiresponse permutation procedure using 4,999 replications to generate the null distribution

*T* temperature, *RH* relative air humidity, *BP* barometric pressure

significantly between day and night. Rainfall ($P = 0.259$) and barometric pressure ($P = 0.561$) were not significantly different between day and night.

Consistent with the result of the $\chi^2$-test, the Mantel correlation of captures and diel interval was highly significant ($r = -0.17740$, $P < 0.0001$). This correlation remained highly significant following removal of the combined effect of the environmental variables in the partial Mantel test ($r = -0.13016$, $P < 0.0001$).

## Discussion

Our results clearly show that migration in the Brazilian red-bellied toad *Melanophryniscus cambaraensis* is strongly diurnal. Following Semlitsch and Pechmann (1985), we predicted that migratory activity would be equally likely to occur during day and night intervals because individuals are protected from visually oriented diurnal predators by toxic skin secretions and aposematic coloration. Semlitsch and Pechmann's (1985) hypothesis could explain the diurnal migratory activity of this species, but it cannot explain the lack of migratory activity at night.

Several other factors could explain the diurnal-only migratory activity of *Melanophryniscus cambaraensis*. For amphibians that migrate over terrestrial habitats, migratory activity may be highly dependent on adequate climatic factors to mitigate risk of desiccation (Todd and Winne 2006). Among the environmental variables we examined, temperature and relative air humidity differed significantly between day and night intervals. However, these do not appear to be causal factors in explaining diurnal-only migration. First, despite the significant diel variation in temperature and humidity, the correlation of captures and diel activity remained highly significant following removal of the combined effect of the environmental variables, which showed that the correlation between captures and diel interval cannot be attributed to environmental variables. Further, temperature was higher and humidity lower during the day than at night, indicating increased desiccation risk during the day. Therefore, in terms of desiccation risk, strongly diurnal migratory activity occurred in spite of significant differences in temperature and humidity, not because of them.

Thermal dependence of locomotor performance could explain the association of greater migratory activity with higher (diurnal) temperatures (Bennett 1990). Although data on locomotor performance are lacking for *Melanophryniscus cambaraensis*, two considerations suggest that this is unlikely to be a causal factor. First, during our study migratory activity was recorded over a broad range of temperatures covering almost 15°C, with considerable variation during both day (11.9–25.2°C) and night (11.3–20.1°C). Second, although our study was confined to spring and summer months, explosive breeding events occur on cold days in winter as well. For example, we observed males calling at air temperatures as low as 7.4°C during a mid-winter breeding event on 11 August 2009 (V. Z. Caorsi, R. R. Santos and T. Grant, unpublished data). The temperatures at which anurans achieve maximum locomotor performance vary considerably across species (Navas et al. 2008), and our observations of migratory and breeding activity suggest that *M. cambaraensis* is adapted for locomotor activity over a broad range of temperatures. This hypothesis is also consistent with the results of the partial Mantel test that showed that environmental variables do not account for the diurnal-only migratory activity.

Some species of anurans that are typically nocturnal also exhibit occasional diurnal activity. Heliothermic basking by otherwise nocturnal anurans may increase digestive efficiency by elevating body temperature (Brattstrom 1979), but this involves immobility

and exposure to solar energy, not continuous migratory activity through leaf-litter and vegetation. In numerous species of bufonid toads adults are nocturnal while post-metamorphic juveniles are diurnal, which may maximize growth rate and shorten the time to reach adult size by increasing body temperature (Lillywhite et al. 1973). Alternatively, Pizzatto et al. (2008) proposed that diurnal activity enables post-metamorphic juveniles of *Rhinella marina* (Linnaeus 1758) to avoid predation by larger nocturnal conspecifics. Neither of these explanations can account for the diurnal activity of migrating adults.

Given the inability of ecological variables to account for the diurnal-only migratory activity of *Melanophryniscus cambaraensis*, we suggest that the explanation lies not in current functions and selective pressures but rather in phylogenetic history. Although most bufonids are characteristically nocturnal, "basal" bufonid genera are diurnal, including *Atelopus*, *Dendrophryniscus*, and *Melanophryniscus* (McDiarmid 1971; Lötters 1996). Further, Grant et al. (2006) found bufonids to be imbedded within Agastorophrynia as the sister group of Nobleobatia, a clade formed by the diurnal families Aromobatidae, Dendrobatidae, and Hylodidae (Fig. 3). Parsimonious optimization of diel activity indicates that the shift from nocturnal to diurnal behavior occurred in the common ancestor of Agastorophrynia, prior to the origin of *Melanophryniscus* and other bufonids. As such, diurnal activity is primitive in *Melanophryniscus* and the nocturnal activity of most bufonids is due to a subsequent reversal in diel activity.

Given that species of *Melanophryniscus* are primitively diurnal, what demands a proximate evolutionary explanation is not the diurnal migratory activity of *M. cambaraensis* but rather the nocturnal breeding activity. We hypothesize that nocturnal breeding activity resulted from the extremely short duration of the explosive reproductive events of
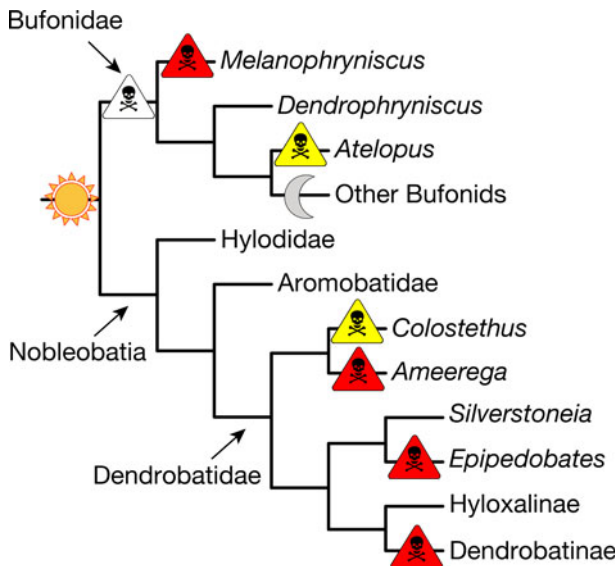


**Fig. 3** Phylogenetic relationships of Agastorophrynia, adapted from Grant et al. (2006), showing the origin of diurnal activity (*sun*) in the ancestor of Agastorophrynia and the return to nocturnality within Bufonidae (*moon*), as well as the evolution of noxious or toxic chemical defenses (*skull and crossbones*), including bufadienolides (*white*), tetrodotoxin and analogous compounds (*yellow*), and lipophilic alkaloids (*red*). The successive sister groups (not shown) are Leiuperidae, Cycloramphidae, Ceratophryidae, Leptodactylidae, Centrolenidae, and Hylidae, all of which are primitively nocturnal

in this species. Over such short intervals, individuals capable of extending their reproductive activity beyond daylight hours would greatly increase their fitness relative to those that are restricted to diurnal activity. The fitness advantage from continuous breeding would provide a strong selective pressure to shift to 24 h activity.

Phylogenetic evidence also challenges the causal relationship between the evolution of diurnal activity and chemical defenses. According to Semlitsch and Pechmann's (1985) hypothesis, diurnal activity is enabled by the evolution of chemical defenses and aposematic coloration that provide protection from visually oriented diurnal predators. This theory entails that chemical defenses either precede or evolve contemporaneously with diurnal activity. Although several of the diurnal groups within Agastorophynia are also poisonous, including the true toads (Bufonidae) and Neotropical poison frogs (Dendrobatidae), others are not. The occurrence of bufadienolides is a synapomorphy of Bufonidae (Daly et al. 2008), the occurrence of lipophilic alkaloids is independently derived in *Melanophryniscus*, *Ameerega*, *Epipedobates*, and Dendrobatinae, and the occurrence of tetrodotoxin and related compounds is independently derived in *Atelopus* and *Colostethus* (Grant et al. 2006; Grant 2007), whereas Aromobatidae, Hylodidae, and the dendrobatid clades Hyloxalinae and *Silverstoneia* are not poisonous. As such, the shift to diurnal activity occurred prior to the evolution of chemical defenses in Bufonidae and Dendrobatidae (Fig. 3). This phylogenetic sequence suggests that chemical defenses in these taxa may have evolved as a result of the diurnal activity that brought them into contact with visually oriented diurnal predators, and not the other way around.

Our hypothesis complements Darst et al's (2005) proposal that the shift to diurnal activity may have enabled the evolution of chemical defenses in dendrobatid poison frogs by bringing individuals into contact with new food sources from which they could sequester defensive alkaloids. Together, these two hypotheses suggest a complex interaction of pressures and opportunities that originated with diurnal activity and resulted in a shift from predator avoidance mechanisms to anti-predator mechanisms in multiple lineages of diurnal amphibians.

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