Migração de *Melanophryniscus cambaraensis* (Anura, Bufonidae) no Município de São Francisco de Paula, Rio Grande do Sul, Brasil

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MIGRAÇÃO DE *MELANOPHRYNISCUS CAMBARAENSIS* (ANURA, BUFONIDAE) NO MUNICÍPIO DE SÃO FRANCISCO DE PAULA, RIO GRANDE DO SUL, BRASIL

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J.C. Michaels (Firebelly)
RESUMO

Foram estudados alguns padrões gerais da migração do sapinho-de-barriga-vermelha *Melanophryniscus cambaraensis*, uma espécie que forma agregações de reprodução explosiva em intervalos irregulares ao longo de todo o ano. Os indivíduos em atividade de migração foram capturados através de dois métodos: conjuntos de armadilhas de interceptação e queda (*pitfall traps*) instalados na floresta, e cercas-guia fechando completamente o sítio reprodutivo com armadilhas em forma de funil (aberturas voltadas para o interior e exterior). A orientação direcional das migrações pré- e pós-reprodutivas foi não aleatória, independente do sexo ou do tratamento das pseudoréplicas, indicando que o padrão observado foi a nível populacional. A orientação direcional das migrações pré-reprodutivas foi significativamente diferente das pós-reprodutivas quando as pseudoréplicas foram incluídas nas análises. Entretanto, não foi encontrada diferença quando as pseudoréplicas foram excluídas, indicando a importância de considerar sua potencial influência no desenho de estudos sobre a orientação da migração. Ao contrário de nossa hipótese, a atividade migratória de *M. cambaraensis* foi fortemente diurna, e não ocorreu devido a mudanças nas variáveis ambientais. Nós sugerimos que a migração diurna é melhor explicada pela filogenia e não por pressões contemporâneas. A atividade diurna é primitiva em *M. cambaraensis* e evoluiu no ancestral comum Agastorophrynia, antes da evolução das defesas químicas encontradas nos sapos (Bufonidae) e nas rãs venenosas (Dendrobatidae). Isso sugere que as defesas químicas nesses grupos podem ter evoluído como resultado da atividade diurna, que os colocou em contato com predadores diurnos orientados pela visão, e não o contrário. Sobre os padrões temporais, nós identificamos cinco picos principais de atividade migratória e três menores. Machos e fêmeas não apresentaram diferenças temporais nos picos de migração. O periodograma identificou dois ciclos migratórios principais: um com duração de 23 dias e outro com duração de 13.8 dias. Nós sugerimos que o ciclo que melhor se aplica ao *M. cambaraensis* é o com duração de 13.8 dias. Os maiores valores de correlação dos preditores da atividade migratória foram umidade relativa do ar e chuva acumulada nas 72 h anteriores, ambas no lag zero. A chuva acumulada em 72 h foi o único preditor do número de capturas, representando 73% de sua variação.
ABSTRACT

Some general patterns of migration were studied in the Brazilian red bellied toad *Melanophryniscus cambaraensis*, a species that forms explosive breeding aggregations at irregular intervals throughout the entire year. Migrating toads were captured by two methods: sets of drift fences with pitfall traps in the forest, and drift fences completely enclosing the breeding site with inward and outward-facing funnel traps. The directional orientation was non-random for both pre- and post-reproductive migration, regardless of gender or treatment of pseudoreplicate captures, indicating that this is a population-level characteristic. The directional orientation of pre-reproductive migration was significantly different from post-reproductive migration when pseudoreplicate captures were included in analyses. However, none of the differences were significantly when pseudoreplicate captures were excluded, illustrating the importance of considering their potential influence when designing studies of migration orientation. Contrary to our hypothesis, we found that migratory activity is strongly diurnal in *M. Cambaraensis*, and that this does not owe to environmental variables. We suggest that this is best explained by phylogeny, not contemporary pressures. Diurnality is primitive for *M. cambaraensis* and evolved in the common ancestor of Agastorophrynia, prior to the chemical defences found in toads (Bufonidae) and poison frogs (Dendrobatidae). This suggests that chemical defences 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. On the temporal patterns, five high and three smaller peaks of migratory activity were identified. Males and females showed no difference. The periodogram identified two main migratory cycles: one lasting 23 days and another with a length of 13.8 days. We suggest that the duration cycle that best applies to *M. cambaraensis* is 13.8 days. The highest correlation values as predictors of migration activity was mean relative air humidity and accumulated rainfall of 72 h, both at lag zero. The accumulated rainfall of the previous 72 h was the only predictor of number of captures, accounting for 73% of their variation.
APRESENTAÇÃO

A migração é um processo indispensável no ciclo de vida de muitas espécies de animais (Semlitsch 1985). A maioria das migrações resulta da necessidade dos indivíduos seguirem recursos efêmeros (ex., alimento, refúgios, locais para reprodução), que flutuam através do tempo e do espaço (Semlitsch 1985; Dingle e Drake 2007). Por exemplo, muitas aves efetuam migrações sazonais que coincidem com mudanças latitudinais na disponibilidade de alimento (Alerstam 1990), e gnus e zebras migram milhares de quilômetros conforme os recursos hídricos se tornam escassos (Wolanski e Gereta 2001). Muitas vezes essas migrações são preventivas, nas quais os habitats são abandonados antes de diminuir muito sua qualidade (Dingle e Drake 2007).

No caso dos anfíbios, o conceito de migração é definido como movimentos de aproximação e afastamento do sítio reprodutivo, executados, principalmente, pelos indivíduos adultos (Semlitsch 2008). Durante a época reprodutiva, os anfíbios adultos migram pelo ambiente terrestre, saindo de seus refúgios em direção a corpos d’água para reproduzir (migração pré-reprodutiva); após esta época, machos e fêmeas retornam ao habitat terrestre (migração pós-reprodutiva) (Semlitsch 2008). Ainda existem as chamadas migrações secundárias, que são aquelas que ocorrem entre habitats de forrageio e refúgios utilizados durante a época não reprodutiva (Lamoureux e Madison 1999; Lamoureux et al. 2002).

Muitas espécies de anfíbios possuem um ciclo de vida bifásico, com ovos e girinos aquáticos e jovens e adultos terrestres (Altig e McDiarmid 1999). A persistência das populações locais dessas espécies requer que os adultos migrem, às vezes por distâncias consideráveis e com grande frequência, entre os habitats terrestres e os sítios reprodutivos aquáticos para completar seu ciclo de vida (Semlitsch 2008). Entretanto, o
uso de habitats pelos anfíbios, bem como os processos migratórios de muitas espécies, em geral, é pouco entendido (Lemckert 2004). Um dos fatores que tem sido apontado como a maior causa de declínio dos anfíbios é a desconexão provocada pelo homem entre os habitats de forrageio e reprodução (habitat split) (Becker et al. 2007) quebrando ou interrompendo uma importante fase no ciclo de vida desses organismos. O conhecimento dos processos e padrões de migração é uma importante ferramenta para a compreensão das interações ecológicas, dinâmicas populacionais e pressões seletivas que afetam as populações locais, bem como para desenvolver estratégias efetivas de manejo e conservação (Semlitsch 2008).

Os padrões de migração variam de acordo com as espécies de anfíbios. A maioria dos estudos sobre orientação direcional encontrou que, normalmente, a migração em anfíbios adultos ocorre de uma maneira não aleatória, com os animais entrando e saindo do sítio reprodutivo pelos mesmos lugares, e preferencialmente utilizando determinados habitats como rotas migratórias (Marty et al. 2005; Sztatecsny e Schabetsberger, 2005, Rittenhouse e Semlitsch 2006; Wells 2007). Em alguns desses trabalhos foi encontrada relação da migração com o habitat circundante, na qual foi notada uma preferência para o movimento em direção a áreas florestadas (Jehle e Arntzen 2000; Malmgren 2002; Vasconcelos e Calhoun 2004; Marty et al. 2005; Waltson e Mullin 2008).

A maioria dos anfíbios migra durante os períodos noturnos, quando as temperaturas são baixas e a umidade do ar é alta (Semlitsch 1985; Sinsch 1988; Todd e Winne 2006; Wells 2007), o que reduz o risco de dessecação e predação (Semlitsch e Pechmann 1985). Entretanto, Semlitsch e Pechmann (1985) encontraram que a atividade migratória da salamandra *Notophthalmus viridiscens* não foi diferente entre o dia e a noite. Estes autores hipotetizaram que a atividade diurna desta salamandra é possível
porque ela possui secreções tóxicas na pele (tetrodoxina; Daly 2004) e coloração apossemática. Poranto a predação por predadores diurnos orientados pela visão é evitada eliminando a necessidade de restringir os movimentos aos períodos escuros.

As migrações reprodutivas parecem ser desencadeadas por certos fatores ambientais, e pode haver considerável variação entre as espécies e populações nas suas respostas migratórias a esses fatores (Sinsch 1990; Russel et al. 2005; Todd e Winne 2006). A precipitação é o principal fator ambiental que desencadeia a migração de muitas espécies de anfíbios, porém a queda na pressão barométrica, na ausência de precipitação, pode ser um gatilho para algumas espécies (Russel et al. 2005). Greenberg e Tanner (2004) observaram que a interação entre a chuva e a máxima mudança na pressão barométrica é o preditor mais significativo para os movimentos de anfíbios com reprodução explosiva. Em alguns casos, com a salamandra *Ambystoma maculatum*, a chuva tem apenas uma função limiar, sendo que o fator que melhor explica a migração é a media da temperatura do ar dos últimos três dias (Sexton et al. 1990).

A maioria dos estudos de migração dos anfíbios foi realizada com espécies da América do Norte e Europa, principalmente naquelas que geralmente se deslocam para os locais de reprodução uma vez por ano e que apresentam reprodução prolongada (sensu Wells 1977). Os períodos reprodutivos nestes anfíbios podem durar até vários meses. Em contraste, a reprodução dos sapinhos-de-barriga-vermelha da América do Sul (*Melanophryniscus*, Bufonidae) é do tipo explosiva (sensu Wells 1977), caracterizada por ocorrer em um curto período de tempo (geralmente apenas alguns dias). Neste período, vários indivíduos deixam seus abrigos e, simultaneamente, migram em direção a corpos d’água temporários durante e imediatamente após intensas chuvas (Kwet e Di-Bernardo 1999; Garcia e Vinciprova 2003; Vaira 2005; Achaval e Olmos 2007). Vaira

Diferentemente das outras espécies de anfíbios estudadas, a reprodução de *Melanophryniscus cambaraensis* é asazonal, ou seja, apesar de habitar uma região subtropical caracterizada por apresentar estações bem definidas, os eventos de reprodução explosiva ocorrem repetidamente, em intervalos irregulares, ao longo de todo o ano. Outras espécies presentes em uma localidade nesta região possuem
reprodução sazonal, com a atividade reprodutiva explicada pelo fotoperíodo e não pela variação de temperatura, chuva ou umidade do ar (Both et al. 2008). Nós observamos eventos de reprodução explosiva (presença de machos vocalizando, casais em amplexo e posturas) de *M. cambaraensis* em meses de primavera (outubro), verão (dezembro, janeiro e fevereiro) e inverno (agosto). Nos meses restantes, a reprodução foi registrada por Garcia e Vinciprova (2003), P. Garcia (dados não publicados), P. Colombo (comunicação pessoal), A. Samir (comunicação pessoal).

Além de representar o primeiro trabalho de migração em um anfíbio de reprodução explosiva asazonal, este é o primeiro estudo detalhado de migração propriamente dita no Brasil. O objetivo geral foi estudar a migração de uma população de *Melanophryniscus cambaraensis* na Floresta Nacional de São Francisco de Paula, município de São Francisco de Paula, Rio Grande do Sul, Brasil. A dissertação está dividida em três capítulos. O primeiro, “*Directional Orientation of Migration in an Aseasonal Explosive Breeding Toad from Brazil*”, é um manuscrito que foi submetido para a revista *Journal of Tropical Ecology*, na forma de *Full Paper*. O objetivo principal deste capítulo foi documentar a orientação direcional das migrações pré- e pós-reprodutivas. Especificamente, nós testamos (1) se as direções de entrada e saída do sítio reprodutivo ocorreram de uma maneira aleatória ou não e se certos ângulos (em relação ao centro do sítio reprodutivo) foram mais utilizados que outros, e (2) se existiam diferenças nos pontos de entrada e saída entre as migrações pré- e pós-reprodutivas e entre machos e fêmeas. Considerando que em alguns estudos sobre migração um mesmo indivíduo é registrado em múltiplos eventos reprodutivos, originando pseudoréplicas, e que essas pseudoréplicas podem ter efeito nos resultados, nós avaliamos a possível influência de sua inclusão, realizando as análises com e sem as pseudoréplicas.
No segundo capítulo desta dissertação, nós documentamos a atividade diária de migração ao longo de múltiplos eventos reprodutivos, testando se esta foi diurna ou noturna. Nossa hipótese foi que a migração ocorreu igualmente, tanto durante o dia quanto à noite, devido à toxicidade, à coloração aposemática e às recentes observações de atividade reprodutiva contínua durante 24h em *Melanophryniscus cambaraensis*. Nós também testamos se o turno de atividade de migração foi atribuído a uma variação de chuva, pressão barométrica, temperatura e umidade relativa do ar. A partir dos resultados do segundo capítulo foi redigido o manuscrito “Diel Activity of Migration in a Poisonous Toad from Brazil and the Evolution of Chemical Defenses in Diurnal Amphibians” submetido para o periódico *Evolutionary Ecology*, sob a forma de Research Article.

O terceiro capítulo, “Temporal Migration Patterns in an Aseasonal Explosive Breeding Toad from Brazil”, será submetido para a revista *Canadian Journal of Zoology*, sob a forma de Article. Neste trabalho nós caracterizamos alguns padrões temporais da migração de *Melanophryniscus cambaraensis*. Especificamente, nós respondemos às seguintes perguntas: (1) quantos eventos de migração reprodutiva houveram ao longo do período de estudo? (2) Quando (quais) foram esses eventos? (3) Quanto tempo durou um evento? (4) Houve diferença na atividade migratória de machos e fêmeas? (5) Quais variáveis ambientais, entre temperatura, precipitação, umidade relativa do ar e pressão barométrica, poderiam explicar os ciclos migratórios?
CAPÍTULO 1: DIRECTIONAL ORIENTATION OF MIGRATION IN AN ASEASONAL EXPLOSIVE BREEDING TOAD FROM BRAZIL

(Artigo submetido ao periódico Journal of Tropical Ecology)
Directional Orientation of Migration in an Aseasonal Explosive Breeding Toad from Brazil

Running title: Orientation of Migration in a Brazilian Toad

Key words: Amphibia; Anura; Bufonidae; ecology; Melanophryniscus cambaraensis; movement; South America.

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Abstract

The directional orientation of pre- and post-reproductive migration was studied in the Brazilian red bellied toad *Melanophryniscus cambaraensis*, a species that forms explosive breeding aggregations at irregular intervals throughout the entire year. Migrating toads were captured by enclosing the breeding site in dual drift fences with inward- and outward-facing funnel traps. Data were collected over 5 mo and totalled 333 captures. The observed directional orientation was significantly different from expected under a uniform distribution for both pre- and post-reproductive migration, regardless of gender or treatment of pseudoreplicate captures. Furthermore, males and females did not differ significantly from each other in the orientation of entry or exit. Collectively, these findings indicate that this is a population-level characteristic. Herein, we suggest that this directional bias may be due to the presence of a dirt road next to the breeding site. When pseudoreplicate captures (*i.e.*, repeated captures of the same individual migrating in the same direction) were included in analyses, the directional orientation of pre-reproductive migration was significantly different from post-reproductive migration; however, there was no difference in the directional orientation of pre- and post-reproductive migration when pseudoreplicate captures were excluded, illustrating the importance of considering the potential influence of pseudoreplicate captures when designing studies of migration orientation.
INTRODUCTION

Most species of amphibians with biphasic life cycles have terrestrial adults and aquatic eggs and larvae (Altig & McDiarmid 1999). The persistence of local populations of these species requires adults to migrate, potentially multiple times and considerable distances, between terrestrial foraging or over-wintering habitats and aquatic breeding sites to complete their life cycle (Russell et al. 2005, Semlitsch 2008, Todd et al. 2009).

Knowledge of migration between habitats is necessary to understand ecological interactions, population dynamics, and the selective pressures that affect local populations, as well as to develop effective conservation and management strategies (Semlitsch 2008). Habitat use by frogs in general is poorly understood (Lemckert 2004), and habitat split, defined as anthropogenic disconnection of foraging and breeding habitats, has been implicated as a major cause of amphibian declines (Becker et al. 2007).

Previous studies have found that amphibian migration usually occurs in a non-random manner, with individuals entering and exiting breeding sites at the same place and using certain habitats and migratory routes (Marty et al. 2005, Rittenhouse & Semlitsch 2006, Wells 2007). However, studies of migration orientation in amphibians have focused on annual breeders with prolonged breeding (sensu Wells 1977) extended over more than one month and several months (or more) between breeding events. In contrast, reproduction in the red bellied toads of southern South America (Melanophryniscus, Bufonidae) 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 (Achaval & Olmos 2007, Garcia & Vinciprova 2003, Kwet & Di-Bernardo 1999).

Aspects of the migration of a few other species with explosive breeding have been
studied (Sinsch 1988, Todd et al. 2009), but the ecology of species of *Melanophryniscus* is poorly known and there are no studies of their migration.

The Brazilian red bellied toad *Melanophryniscus cambaraensis* (Fig. 1) differs from all previously studied species in breeding aseasonally. That is, despite inhabiting a subtropical region characterized by well defined seasons, explosive breeding events occur repeatedly at irregular intervals over the course of the entire year. Other species in this region breed seasonally, with seasonal reproductive activity explained by photoperiod and not variation in temperature, rainfall, or air humidity (Both et al. 2008).

We have observed explosive breeding events in *M. cambaraensis* in spring (October), summer (December, January, and February), and winter (August), and breeding has been recorded in all remaining months by numerous workers (Garcia & Vinciprova 2003, P. Garcia, unpublished data, P. Colombo, pers. comm., A. Samir, pers. comm.).

As in other species that form explosive breeding aggregations, males of *M. cambaraensis* exhibit many of the typical strategies of scramble competition (Wells 2007), including intense male combats and struggles to possess females and displace amplexant males (V. Z. Caorsi, R. R. Santos & T. Grant, unpublished data).

In light of the differences between the breeding biology of *Melanophryniscus cambaraensis* and previously studied species, we undertook a study of the migration of one of the two known populations of this species. The aim of this paper is to document the directional orientation of pre- and post-reproductive migration. Specifically, we tested (1) if the direction of migration occurred in a random or nonrandom manner and if certain angles were used more frequently than others and (2) whether there were differences in entry and exit points between pre- and post-reproductive migrations and between males and females. Studies of migration often sample the same individuals in multiple migration events. Although these pseudoreplicate captures may bias results to
reflect individual propensities for particular orientations (Dodd & Cade 1998), they are often not excluded from analyses (e.g., Malmgren 2002). We therefore tested for the effect of pseudoreplicates by running all analyses with and without pseudoreplicate captures.

**METHODS**

**Study site**

*Melanophryniscus cambaraensis* is a small (ca. 35 mm snout-vent length), vulnerable (Garcia & Vinciprova 2003) species 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 species have been discovered, each known from 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 & 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 (high-rainfall) forest is dominated by *Araucaria angustifolia* (Sonego *et al.* 2007) and covers about 56% of the 1607-ha area of the FLONA, the remainder being composed of planted *Araucaria angustifolia, Pinus* spp. and *Eucalyptus* spp. (Bonatti *et al.* 2006). Temperatures range from -3–18°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 2200 mm (Backes *et al.* 2005).

We collected data from October 2008 to February 2009 at a temporary stream formed by heavy rainfalls (29°25'41.3" S 50°23'44.5" W, 866 m asl). The breeding site
(Fig. 2A) is approximately 20 m long and 4 m wide and is located at the edge of a small

dirt road on a rocky outcrop partially covered by a thin layer of topsoil and vegetation
dominated grasses (Poaceae) and herbs (Eryngium sp.). Except for the dirt road, the area
around the stream is composed of forest (planted Araucaria angustifolia), with shrubs
and sedges (Cyperaceae) covering the few meters between the temporary stream and the
forest proper. This is the only locality in the municipality of São Francisco de Paula
where Melanophryniscus cambaraensis is known to breed, and this is the only species
of amphibian that reproduces in this temporary stream, although Aplastodiscus
perviridis (Hylidae), Ischnocnema henselii (Brachycephalidae), Hypsiboas marginatus
(Hylidae), Leptodactylus araucaria (Leptodactylidae), and Rhinella icterica
(Bufonidae) also inhabit the adjacent forest.

Data collection

We completely enclosed the breeding site in inner and outer drift fences composed of
durable plastic sheets approximately 51 m long and 40 cm high (Figures 2a, 3). The
solid rock substrate of the breeding site made it impossible to bury the base of the
fences or dig holes for pitfall traps. Instead, we held the fences flush to the ground by
folding over and stapling the base of the plastic sheet to form a pocket that we filled
with soil and small rocks to weight down the base of the fence (Figure 2b). This allowed
the fence to be flexible enough to follow the contour of the surface while also
maintaining tight contact with the ground. To hold the fence upright, we built U-shaped
supports from wooden stakes that we held in place with heavy rocks and bags of soil.
We constructed funnel traps from 5-L plastic bottles by cutting off the top third of each
bottle and inserting it, inverted, into the base of the bottle (Figure 2b). To provide
protection from sun and rain, we fixed black plastic sheets over each pair of funnels.
We installed pairs of funnel traps (one facing inwards, the other outwards) at 3 m intervals around the entire circumference of the fences, totaling 34 funnels (17 facing each way). We assumed that all individuals caught in the outward-facing traps were in pre-reproductive migration and all individuals caught in the inward-facing traps were in post-reproductive migration. To determine the angular position of the traps, we measured the angle of each pair of traps from the centre of the reproductive site with a compass.

We monitored traps continuously from October 2008 to February 2009 (127 nights), checking them at least twice per day. All captured individuals were photographed and marked by phalangeal amputation, the most common technique for marking anurans (Ferner 2007). We determined sex by examining secondary sex characteristics. Adult males possess a conspicuous brown nuptial pad on fingers I and II, which is absent in females. Also, males occasionally emitted a release call when handled, which was never emitted by females, and females tend to be slightly larger than males. After processing, individuals were released on the opposite side of the fence.

**Statistical analysis**

We used RAO’s spacing test (Rao 1976) to determine if the distributions of entry and exit angles were significantly different from a uniform distribution. This test has greater power to detect departures from uniformity for data with multiple modes and wide angular dispersion than Rayleigh’s or Watson’s U2 tests (Bergin 1991). We grouped data by sex and direction of migration (pre-reproduction and post-reproduction). To compare distributions between sexes and between pre- and post-reproductive migrations, we used a multi-response permutation procedure (MRPP) for circular
distributions. This procedure compares the treatment groups, similar to analysis of variance, based on distance functions. To evaluate the possible effect of pseudoreplicates, we performed tests both including all capture data and including only a single, randomly selected capture datum per individual per direction (i.e., each individual could have at most two data points, one entering and another one exiting the breeding site). We considered $P < 0.5$ to indicate of significant departure of the observed data from the null distribution. We performed all analyses using the BLOSSOM software package (version W2008.04.02, Cade & Richards 2005), as in previous studies of amphibian orientation (Dodd & Cade 1998, Marty et al. 2005). We visualized circular distributions using the Oriana 3.0 software package (version 3.0, Kovach Computing Services, Pentraeth, Isle of Anglesey, Wales, U.K.).

RESULTS

Summary of capture data

In 5 mo of field work we observed explosive breeding events in all months but November, which was exceptionally dry and had only two captures. In total, we obtained 333 captures (181 male, 152 female), corresponding to 126 individuals (48 males, 78 females; Table 1). Of the 333 captures, 139 were pseudoreplicate captures (i.e., repeated captures of the same individual migrating in the same direction). Individual pseudoreplicate capture rates were much greater for males than females. We captured 75% of males two or more times in pre- and/or post-reproductive migration (pseudoreplicate captures per individual: maximum = 9, mean = 2.0, mode = 1), compared to only 33% of females (pseudoreplicate captures per individual: maximum = 4, mean = 0.54, mode = 0).
We did not capture any juveniles of *Melanophryniscus cambaraensis* entering or exiting the breeding site. However, occasionally we captured six other species of anurans in funnel traps, including *Elachistocleis bicolor* (Microhylidae, one individual), *Ischnocnema henselii* (Brachycephalidae, three individuals), *Leptodactylus gracilis* and/or *L. plaumanni* (Leptodactylidae, five individuals), *Physalaemus cuvieri* and *P. lisei* (Leiuperidae, three and 19 individuals, respectively), and *Rhinella icterica* (Bufonidae, 65 individuals). Most of the captured individuals were juveniles, and we observed no breeding activity in any of these species in or near the temporary stream. We speculate that these individuals entered the breeding area in search moisture and may have used the funnel traps as refuges.

**Analyses including all captures**

The 333 total captures included 169 captures (91 male, 78 female) in pre-reproductive migration, and 164 captures (90 male, 74 female) in post-reproductive migration (Table 1). The observed directional orientation was significantly different from expected under a uniform distribution for both pre- and post-reproductive migration, regardless of gender (*P* < 0.001 for all tests; Fig. 4).

Males and females entered and exited the breeding site through all angles (Table 2), but approximately half of all entries and exits were made through traps in the southeast (126° and 152°) and west (250°, 262° and 266°). Approximately 10% of entries and exits were made in the northwest (298° and 322°), and nearly 25% of all entries were from the southwest (222°). We also observed the departure of some individuals to the west (11%, 280°) and east (12.2%, 108° and 94°).

In general, migrating toads entered and exited the breeding site through different routes (MRPP, *P* = 0.004), and this pattern was observed in both sexes (MRPP, males *P*...
Males and females did not differ significantly from each other in the orientation of entry and exit ($P = 0.115$ and $P = 0.504$, respectively).

**Analyses excluding pseudoreplicate captures**

Including no more than a single randomly selected pre- and post-reproduction capture datum per individual, our dataset was reduced to 192 captures corresponding to 97 (40 male, 57 female) in pre-reproductive migration and 95 (44 male, 51 female) in post-reproductive migration (Table 1). A different random sample of captures did not affect our findings. The observed directional orientation of migration was significantly different from expected under a uniform distribution, regardless of gender ($P < 0.001$ for all tests; Fig. 5).

As shown in Table 2, the exclusion of pseudoreplicate captures resulted in only minor changes in the frequencies of entry and exit angles. Over half of the entries and nearly half of the exits were made through the southeast ($126^\circ$ and $152^\circ$) and west ($250^\circ$, $262^\circ$ and $266^\circ$), and approximately 9% of entries and 8% of exits were made through the northwest ($298^\circ$ and $322^\circ$). The percentage of entries through the southwest ($222^\circ$) decreased from nearly 25% to approximately 17%, and the percentage of departures to the west ($12.6\%$, $280^\circ$) and east ($9.5\%$, $108^\circ$ and $94^\circ$) differed only slightly.

Despite only small changes in the relative frequencies of orientations of entries and exits, none of the tests comparing pre- and post-reproductive migrants and between sexes were significant when pseudoreplicate captures were excluded (all $P > 0.05$; Table 3).
Although the aim of the present study is only to address the orientation of migration, the results reported above indicate clear differences between the reproductive dynamics of males and females in this population of *Melanophryniscus cambaraensis*. As indicated by the different rates at which individual males and females were repeatedly captured migrating in the same direction (i.e., individual pseudoreplicate capture rates), males participate in more explosive breeding events than females. Most males were recaptured migrating in the same direction at least twice, whereas most females were never recaptured migrating in the same direction. These and additional considerations will be addressed in greater detail elsewhere.

Todd *et al.* (2009) found that *Scaphiopus holbrookii* migrated indiscriminately through forested and clearcut habitats, which suggests uniform directional orientation. They explained this by noting that *S. holbrookii* breeds for only a few days in torrential rains when physiological risks of moving through clearcuts would be minimal. In contrast, for *Melanophryniscus cambaraensis*, the directional orientation of both males and females in pre- and post-reproductive migration was significantly different from expected under a uniform distribution, despite exhibiting the same reproductive characteristics as *S. holbrookii*. This result was upheld when pseudoreplicate captures were excluded, which suggests that this is a population-level pattern and not the result of bias introduced by a subset of individuals that migrated more frequently than others. There were no significant differences between male and female entry or exit points, which, combined with the non-random orientation detected for both sexes, suggests that both sexes follow the same routes of entry and exit.

Several studies have related non-random orientation of migration with habitat characteristics, finding that individuals preferentially migrate between breeding sites...
and forested areas (Jehle & Arntzen 2000, Malmgren 2002, Marty et al. 2005, Rittenhouse & Semlitsch 2006, Todd et al. 2009, Vasconcelos & Calhoun 2004, Waltson & Mullin 2008). This does not appear to be a determining factor for this population of *Melanophryniscus cambaraensis* because the breeding site is completely surrounded by homogeneous planted *Araucaria angustifolia* forest that is occupied by this species.

We did not explicitly test explanations for the non-random orientation. However, as part of our larger study of migration in this species we placed drift fences and pitfall traps in the forest surrounding the breeding site, and we found 85% of male individuals and 58% of female individuals exclusively in the forest southeast of the temporary stream, adjacent to the points with the greatest frequency of captures. Given the apparent homogeneity of the surrounding forest, we suggest that this may be due to the road that separates the breeding site from the forest to the north-west. In light of the explosive breeding of this species, it is unlikely that the road represents a significant barrier to adult movement (Todd et al. 2009). We also captured five individuals (1 male, 4 females) in the forest on both sides, which demonstrates that adults are capable of crossing the road. Instead, we suggest the road may affect juvenile dispersal from the breeding site by either increasing the mortality of juveniles that attempt to cross the road through greater desiccation and/or predation or acting as a deterrent to dispersal (i.e. juveniles avoid crossing the road), with adults migrating to and from the forest they dispersed into as juveniles. This hypothesis is consistent with the findings of Walston & Mullin (2008) that juveniles of several amphibian species rely on direct environmental cues to guide non-random orientation when dispersing from breeding ponds to forest.

Analyses including pseudoreplicate captures found the orientation of pre- and post-reproductive migration to be significantly different, both when data were pooled...
and when each sex was analyzed separately. However, elimination of pseudoreplicate
captures greatly reduced the structure in the data. Given the large number of male
pseudoreplicate captures and the variation of individual pseudoreplicate capture rates
among males (0–9 pseudoreplicate captures per male), we expected the exclusion of
pseudoreplicate captures to have a large effect on both the pooled (all data: $P = 0.004$;
excluding pseudoreplicates: $P = 0.129$) and male-only (all data: $P = 0.049$; excluding
pseudoreplicates: $P = 0.273$) results. However, despite the low female pseudoreplicate
capture rate, exclusion of pseudoreplicate captures greatly decreased the structure in the
data (all data: $P = 0.025$, excluding pseudoreplicates $P = 0.095$). This shows that even a
low frequency of pseudoreplicate captures can have a large effect on results. It is
therefore advisable to evaluate the potential influence of pseudoreplicate captures when
designing studies of migration orientation, considering both the scope of the study (e.g.,
single or multiple breeding periods) and the reproductive dynamics of the target species
(e.g., even in seasonal breeders with prolonged breeding seasons, individual males may
enter and leave repeatedly whereas females may migrate only once).

Our study was affected to an unknown degree by three problems that should be
considered when interpreting our results and designing future studies. First, although
our field work extended over 5 mo and multiple explosive breeding events, it may have
been too short to detect long-term trends that emerge over multiple seasons or years. For
example, although this species differs from most other amphibians in this region by
breeding in all months of the year, it is possible that breeding is less frequent in winter
months with shorter days and colder temperatures than summer months with longer
days and higher temperatures. Similarly, migration orientation might vary over time in
response to landscape changes or population turnover. Second, our results are based on
only a single breeding population of *Melanophryniscus cambaraensis*. Overcoming this
problem may be difficult, as breeding activity at the only other known locality has not
been observed in over a decade, and searches for additional populations have, so far,
been fruitless. Third, trap efficiency was not 100% (see below). The breeding site is
located on a rocky outcrop, which prevented us from burying the drift fences and
required us to invent a novel method to fix them to the substrate (Fig. 2). We also
attempted to use soil and other loose substrate to bury the base of the fence, but it was
quickly washed away by the frequent heavy rains. Stronger methods, such as cementing
the fence in place, were not feasible, as they would permanently alter the breeding site,
which is located inside a conservation area. When designing the capture method, we
assumed that a double-fence design would help prevent trap avoidance, as individuals
would have to overcome two barriers. However, we made only 10 captures between the
two fences, so it appears that increasing the number of fences is not effective.

To estimate trap efficiency, we examined the number of complete captures (i.e.,
individuals captured entering and then exiting the breeding site in a given explosive
breeding event) in relation to the total number of captures. According to this estimate,
overall trap efficiency was only 44%. This is probably an underestimate, as it does not
include individuals that were not detected entering or exiting the breeding site. Monthly
trap efficiency data are suggestive of a trend to decrease (complete captures as a
percentage of total captures: October = 60%, November = 0% (dry month, only two
captures), December = 21%, January = 46%, February = 32%), but there is little
indication that individuals learned to consistently avoid traps. For individuals captured
in multiple events, we determined the number of individuals that had complete captures
(1) always ($N = 4$), (2) after partial captures ($N = 18$), and (3) exclusively prior to partial
captures ($N = 17$). Consequently, 17 individuals may have learned to consistently avoid
traps, whereas 22 did not. We did not detect any holes or breaks in the fence or find
evidence that toads passed beneath it, nor did we find any evidence that certain parts of
the fence were more vulnerable than others. Indeed, the area we assumed would be most
vulnerable due to the occurrence of vegetation between the fence and the substrate had
among the highest of capture frequencies (222°, 250°, 262° and 266°; Fig. 2A, Fig. 3).
Although increased trap efficiency would be ideal, these considerations suggest that trap
avoidance was probably random and therefore did not bias our results.

In this paper we presented the results of the first study of migration orientation
in an aseasonal, explosive breeding amphibian. This is also the first detailed study of the
migration of any Brazilian species of amphibian. Brazil has the richest amphibian fauna
in the world, with species occurring in both strongly seasonal subtropical environments
and tropical environments with minimal climatic variation. Brazilian amphibians also
exhibit a vast diversity of reproductive modes and mating strategies (Haddad and Prado
2005), and we expect that the patterns of migration will vary considerably across
lineages and habitats. Given the importance of migration in amphibian ecology and
evolution (Semlitsch 2008) and the recent suggestion that the anthropogenic disruption
of amphibian migration may be a major cause of amphibian declines (Becker et al.
2007), detailed studies of migration are a promising and important area of research on
Brazilian amphibians.

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Patrick Colombo and Ralph Saporito for advice on the design and implementation of this study, and to Camila Both for assistance and guidance on data analysis. Edenice Brandão Ávila de Souza and the personnel at the Floresta Nacional de São Francisco de Paula supported all aspects of our field work. Finally, we express our gratitude to the many individuals who helped with trap installation and data collection. Ralph Saporito provided helpful suggestions that improved the manuscript.

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Table 1. Summary of *Melanophryniscus cambaraensis* capture data from October 2008 to February 2009. Entry and exit refer to pre- and post-reproductive captures, respectively. Pseudoreplicate captures are repeated captures of the same individual migrating in the same direction.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number of individuals</th>
<th>All captures</th>
<th>Excluding pseudoreplicate captures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Entry</td>
<td>Exit</td>
</tr>
<tr>
<td>Male</td>
<td>48</td>
<td>91</td>
<td>90</td>
</tr>
<tr>
<td>Female</td>
<td>78</td>
<td>78</td>
<td>74</td>
</tr>
<tr>
<td>Total</td>
<td>126</td>
<td>169</td>
<td>164</td>
</tr>
</tbody>
</table>
Table 2. Summary of the directions/angles used by migrating *Melanophryniscus cambaraensis*. Direction refers to the approximate compass orientation. Entry and exit refer to pre- and post-reproductive captures, respectively. Pseudoreplicate captures are repeated captures of the same individual migrating in the same direction.

<table>
<thead>
<tr>
<th>Direction (degrees)</th>
<th>All captures (%)</th>
<th>Excluding pseudoreplicate captures (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Entry</td>
<td>Exit</td>
</tr>
<tr>
<td>SW</td>
<td>222</td>
<td>24.3</td>
</tr>
<tr>
<td>SE</td>
<td>152</td>
<td>12.4</td>
</tr>
<tr>
<td>SE</td>
<td>126</td>
<td>4.1</td>
</tr>
<tr>
<td>E</td>
<td>114</td>
<td>2.4</td>
</tr>
<tr>
<td>E</td>
<td>108</td>
<td>0.0</td>
</tr>
<tr>
<td>E</td>
<td>94</td>
<td>2.4</td>
</tr>
<tr>
<td>E</td>
<td>82</td>
<td>3.0</td>
</tr>
<tr>
<td>E</td>
<td>76</td>
<td>1.8</td>
</tr>
<tr>
<td>NE</td>
<td>64</td>
<td>1.8</td>
</tr>
<tr>
<td>NE</td>
<td>26</td>
<td>1.8</td>
</tr>
<tr>
<td>NW</td>
<td>322</td>
<td>5.9</td>
</tr>
<tr>
<td>NW</td>
<td>298</td>
<td>4.1</td>
</tr>
<tr>
<td>W</td>
<td>288</td>
<td>0.0</td>
</tr>
<tr>
<td>W</td>
<td>280</td>
<td>1.8</td>
</tr>
<tr>
<td>W</td>
<td>266</td>
<td>12.4</td>
</tr>
<tr>
<td>W</td>
<td>262</td>
<td>11.2</td>
</tr>
<tr>
<td>W</td>
<td>250</td>
<td>10.7</td>
</tr>
</tbody>
</table>
Table 3. Comparison of the directional orientation of male and female *Melanophryniscus cambaraensis* in pre- and post-reproductive migration using a multi-response permutation procedure (MRPP) for circular distributions. Entry and exit refer to pre- and post-reproductive captures, respectively. Pseudoreplicate captures are repeated captures of the same individual migrating in the same direction.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>N</th>
<th>Standardized test statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All captures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All entries vs. exits</td>
<td>333</td>
<td>-4.686</td>
<td>0.004</td>
</tr>
<tr>
<td>Male entries vs. exits</td>
<td>181</td>
<td>-2.014</td>
<td>0.050</td>
</tr>
<tr>
<td>Female entries vs. exits</td>
<td>152</td>
<td>-2.759</td>
<td>0.025</td>
</tr>
<tr>
<td>Male entries vs. female entries</td>
<td>169</td>
<td>-1.145</td>
<td>0.115</td>
</tr>
<tr>
<td>Male exits vs. female exits</td>
<td>164</td>
<td>0.366</td>
<td>0.504</td>
</tr>
<tr>
<td><strong>Excluding pseudoreplicate captures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All entries vs. exits</td>
<td>192</td>
<td>-1.005</td>
<td>0.129</td>
</tr>
<tr>
<td>Male entries vs. exits</td>
<td>84</td>
<td>-0.250</td>
<td>0.273</td>
</tr>
<tr>
<td>Female entries vs. exits</td>
<td>108</td>
<td>-1.322</td>
<td>0.095</td>
</tr>
<tr>
<td>Male entries vs. female entries</td>
<td>97</td>
<td>-0.362</td>
<td>0.248</td>
</tr>
<tr>
<td>Male exits vs. female exits</td>
<td>95</td>
<td>-0.701</td>
<td>0.169</td>
</tr>
</tbody>
</table>
FIGURES

Figure 1. A breeding pair of *Melanophryniscus cambaraensis* (snout-vent length approximately 35 mm) at the study site.
Figure 2. Method of data collection at the *Melanophryniscus cambaraensis* breeding site. (a) Layout of drift fences and funnel traps. The breeding site was completely enclosed with two plastic sheets and funnel traps facing inwards and outwards were distributed around the fences. (b) Close-up showing fence and funnel trap design. To hold the fence tightly to the rocky substrate, we weighted the base of the fence by folding over and stapling the lower edge of the plastic sheets and filling the resulting pocket with soil and small rocks. We inserted soil and rocks next to the funnel traps to seal spaces in the fence and placed soil on the floor of the funnel to facilitate toad entry.
Figure 3. Schematic map showing arrangement of drift fences and funnel traps around the temporary stream where Melanophryniscus cambaraensis form explosive breeding aggregations. The areas north and south of the road are composed of planted Paraná Pine (Araucaria angustifolia) forest.
Figure 4. Circular histograms showing the frequency, and mean vector for the directional orientation of pre- and post-reproductive migration in *Melanophryniscus cambaraensis*, including all capture data. The observed data were significantly different from expected under a uniform distribution for both pre- and post-reproductive migration, regardless of gender (Rao’s spacing test, all $P < 0.001$).
Figure 5. Circular histograms showing the frequency, and mean vector for the directional orientation of pre- and post-reproductive migration in *Melanophryniscus cambaraensis*, excluding pseudoreplicate captures. The observed data were significantly different from expected under a uniform distribution for both pre- and post-reproductive migration, regardless of gender (Rao’s spacing test, all $P < 0.001$).
CAPÍTULO 2: DIEL ACTIVITY OF MIGRATION IN A POISONOUS TOAD FROM BRAZIL AND THE EVOLUTION OF CHEMICAL DEFENSES IN DIURNAL AMPHIBIANS

(Artigo submetido ao periódico *Evolutionary Ecology*)
Diel Activity of Migration in a Poisonous Toad from Brazil and the Evolution of Chemical Defenses in Diurnal Amphibians

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Running Title: Diurnal Migration and Chemical Defense

Keywords Movement, Anura, toxicity, breeding, reproduction, environmental variables, Mantel, phylogeny

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Results = 180 words
Discussion = 910 words
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Number of cited references = 36
Number of figures = 3
Number of tables = 1
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 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 hour breeding activity in the poisonous 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 activity of migratory
movements 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.
Introduction

Most species of amphibians with biphasic life cycles have terrestrial adults and aquatic eggs and larvae (Altig and McDiarmid 1999). Adults of these species must risk predation and desiccation to migrate between terrestrial foraging or over-wintering habitats and aquatic breeding sites to complete their life cycle (Russell et al. 2005; Semlitsch 2008). Amphibian migration usually occurs at night when temperatures are low and humidity is high (Semlitsch 1985; Sinsch 1988; Todd and Winne 2006; Wells 2007), 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 did not differ between day and night intervals. This species has poisonous skin secretions (tetrodotoxin; Daly 2004) 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* was recently observed calling during both daytime and nighttime (Colombo et al. 2007; P. Colombo pers. comm.), and the number of calling male *M. cambaraensis* 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 amplexant males (V. Z. Caorsi, R. R. Santos and T. Grant, unpublished data).

Based on Semlitsch and Pechmann’s (1985) hypothesis and the toxicity (confirmed in this species by gas chromatography/mass spectrometry analysis; P. Colombo & T. Grant, unpublished data), aposematic coloration, and recent observations of continuous 24 hour 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 activity of migratory movements over multiple breeding events. We also
obtained 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* (Sonego et al. 2007) and covers about 56% of the 1606.70 ha area 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 -3–18°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. This is the only locality in the municipality of São Francisco de Paula where *Melanophryniscus cambaraensis* is known to breed, and this is the only species of amphibian known to reproduce in this temporary stream.

We captured migrating individuals using two methods. First, we installed three sets of pitfall traps at 7.5 m, 15 m, 30 m, 60 m, and 120 m on each side of the breeding site, totaling 30 sets of traps. Each set of pitfall traps consisted of four buckets buried in the ground and connected by drift-fences that intersected individuals in movement (Corn 1994). Each trap was composed of three plastic 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”. We drilled small holes in the bottom of each bucket to allow water to escape. During the course of the study (in December 2008), we installed another three sets of pitfall traps 240 m from breeding site on one side of the stream. 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. To determine if diel activity could be attributed to
variation in environmental variables, 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 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 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 a strongly diurnal activity. 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 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. 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 (e.g. Knowles and Wiegl 1990; Navas 1996), and our observations of migration 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.

Given the inability of environmental 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 nocturnal, “basal” bufonid genera are characteristically 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 Hylidae (Fig. 3). Parsimonious optimization of diel activity indicates that the shift from nocturnal to diurnal behavior occurred in the common ancestor of Agastorophrynia. 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 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 hour 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), but Aromobatidae, Hylodidae, and the dendrobatid clades Hylocladiinae and *Silverstonea* 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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Santos RR, Leonardi SB, Caorsi VZ, Grant T. 2010. Directional orientation of migration in an aseasonal explosive-breeding toad from Brazil. J Trop Ecol IN PRESS.


### Table 1. Summary of environmental data for days and nights from 11 October 2008 to 26 February 2009. Values are reported as maximum–minimum followed by the mean ± standard deviation. Nighttime rainfall is corrected for unequal day and night lengths. P-values are derived from a multiresponse permutation procedure using 4,999 replications to generate the null distribution.

<table>
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<th></th>
<th>Diurnal vs. nocturnal</th>
<th>Maximum</th>
<th>Minimum</th>
<th>Mean</th>
<th>Standard Deviation</th>
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<td>N</td>
<td>Diurnal</td>
<td>Nocturnal</td>
<td>Diurnal</td>
<td>Nocturnal</td>
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<td>99.0</td>
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<td>44.8</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Barometric pressure</strong></td>
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<td>0.561</td>
<td>927.0</td>
<td>925.4</td>
<td>913.0</td>
</tr>
</tbody>
</table>
Figure 1. *Melanophryniscus cambaraensis* showing (a) green dorsal and (b) contrasting bright red ventral coloration. Defensive chemicals are secreted from skin glands, including the massive frontal gland on the dorsal surface of the head, visible in (a).
Figure 2. Numbers of individuals captured migrating during day and night intervals, excluding recaptures. Absolute numbers including recaptures are much greater, but proportions are almost identical (see text). To account for greater day length we multiplied nocturnal captures by 1.4 (corrected nocturnal) and used the corrected value in statistical analyses.
Figure 3. Phylogenetic relationships of Agastorophryinia, adapted from Grant et al. (2006), showing the origin of diurnal activity (sun) in the ancestor of Agastorophryinia 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 related compounds (yellow), and lipophilic alkaloids (red). The successive sister groups (not shown) are Leiuperidae, Cycloraphidae, Ceratophryidae, Leptodactylidae, Centrolenidae, and Hylidae, all of which are nocturnal.
CAPÍTULO 3: TEMPORAL MIGRATION PATTERNS IN AN ASEASONAL EXPLOSIVE BREEDING TOAD FROM BRAZIL

(Artigo a ser submetido ao periódico Canadian Journal of Zoology)
Temporal Migration Patterns in an Aseasonal Explosive Breeding Toad from Brazil

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Temporal Migration Patterns in an Aseasonal Explosive Breeding Toad from Brazil

Raquel Rocha Santos (R. R. Santos), Camila Both (C. Both) and Taran Grant (T. Grant)

Abstract

The temporal patterns of migration of the aseasonal explosive breeding toad *Melanophryniscus cambaraensis* were studied from October 2008 to February 2009 in São Francisco de Paula, Brazil. Migrating toads were captured with sets of drift fences and pitfall traps in the forest and drift fences completely enclosing the breeding site with inward and outward-facing funnel traps. Spectral analysis was used to explore the cyclical patterns of data. Cross-correlations were used to select the best potential predictors on the appropriate time lags. Stepwise backward multiple regression analysis was used to construct a linear model to predict migratory activity. Five major and three minor peaks of migratory activity were identified, with no differences between males and females. The periodogram identified two main migratory cycles: one lasting 23 days and another 13.8 days. The oscillations of 23 days correspond with fluctuations of close rainfalls, and based on the strong association of migratory activity with rainfall, we propose that the analysis get near migratory events together, considering as only one event. Therefore we suggest that the duration cycle that best applies to *M. cambaraensis* is 13.8 days. The highest correlation values as predictors of migration activity were mean relative air humidity (0.337) and accumulated rainfall of 72 h (0.856), both at lag zero. The accumulated rainfall of the previous 72 h was the only predictor of number of captures, accounting for 73% of variation.
Introduction

Most species of amphibians with biphasic life cycles have terrestrial adults and aquatic eggs and larvae (Altig and McDiarmid 1999). The persistence of local populations of these species requires adults to migrate, potentially multiple times and considerable distances, between terrestrial foraging or over-wintering habitats and aquatic breeding sites to complete their life cycle (Semlitsch 2008).

The reproductive migrations appear to be triggered by certain environmental conditions, and there may be considerable variation among species and populations in their migratory responses to these factors (Sinsch 1990; Russel et al. 2005; Todd and Winne 2006). Precipitation is the main environmental factor that triggers the migration of many amphibians, although the drop in barometric pressure in the absence of precipitation can be a trigger for some (Russel et al. 2005). Greenberg & Tanner (2004) report that at the landscape-level, the interaction between rainfall and the maximum change in barometric pressure is the most significant predictor for the movement of explosive breeding amphibians. In some cases, as in the salamander Ambystoma maculatum, rainfall has only a threshold function, whereas the average of air temperature over the last three days is the factor that best explains the migration (Sexton et al. 1990).

There are several other studies that correlate the timing and magnitude of migration with selected environmental variables (e.g. date, temperature, precipitation) (Semlitsch 1985; Sinsch 1988; Greenberg and Tanner 2005; Timm et al. 2007), however the Brazilian red bellied toad Melanophryniscus cambaraensis differs from all previously studied species in breeding aseasonally. That is, despite inhabiting a subtropical region characterized by well defined seasons, explosive breeding events occur repeatedly at irregular intervals over the course of the entire year. Other species in this region breed
seasonally, with reproductive activity explained by photoperiod and not variation in
temperature, rainfall, or air humidity (Both et al. 2008). We have observed explosive
breeding events in *M. cambaraensis* in October, December, January, February, and
August, and breeding has been recorded in all remaining months by numerous workers
(Garcia and Vinciprova 2003; P. Garcia, unpublished data; P. Colombo, pers. comm.;
A. Samir, pers. comm.). As in other species that form explosive breeding aggregations,
males of *M. cambaraensis* exhibit many of the typical strategies of scramble
competition (Wells 2007), including intense male combats and struggles to possess
females and displace amplexant males (V. Z. Caorsi, R. R. Santos and T. Grant,
unpublished data).

Previous studies on the movement of *Melanophryniscus cambaraensis* indicated that
migration is overwhelmingly diurnal (Santos and Grant, in review) and that the
orientation of migratory activity occurs in a nonrandom manner with no difference
between sexes (Santos et al. in review). Nevertheless there are still many questions
about the migration of this species, especially when considering its reproductive
characteristics. The aim of this study was to characterize the general temporal patterns
of migration of *M. cambaraensis*. Specifically, we addressed the following questions:
(1) How many migration events occurred during the study period? (2) When did these
events occur? (3) How long did events last? (4) Are there differences between migration
activity of males and females? (5) Which environmental variables (temperature,
precipitation, relative air humidity and barometric pressure) could explain the migration
cycles?

**Materials and methods**

**Study site**
Melanophryniscus cambaraensis is a vulnerable (Garcia and Vinciprova 2003) species 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 species have been discovered, each known from 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 (Sonego et al. 2007) and covers about 56% of the 1606.70 ha area of the FLONA, the remainder being composed of planted A. angustifolia, Pinus spp. and Eucalyptus spp. (Bonatti et al. 2006). The climate is temperate, with temperatures ranging from -3–18°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).

Field data were collected from October 2008 to February 2009 at a temporary stream formed by heavy rainfalls (29°25'41.3" S 50°23'44.5" W, 866 m above sea level) and 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 Poaceae and Apiaceae. 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) covering the few meters between the temporary stream and the forest proper. This is the only locality in the municipality of São Francisco de Paula where Melanophryniscus cambaraensis is
known to breed, and this is the only species of amphibian that reproduces in this temporary stream, although *Aplastodiscus perviridis* (Hylidae), *Ischnocnema henselii* (Brachycephalidae), *Hypsiboas marginatus* (Hylidae), *Leptodactylus araucaria* (Leptodactylidae), and *Rhinella icterica* (Bufonidae) also inhabit the adjacent forest.

**Sampling procedure**

Migrating individuals were captured using two methods. We installed three sets of pitfall traps at 7.5 m, 15 m, 30 m, 60 m, and 120 m on each side of the breeding site, totaling 30 sets of traps. Each set of pitfall traps consisted of four buckets buried in the ground and connected by drift-fences that intersected individuals in movement (Corn 1994). Each trap was composed of three plastic 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”. We drilled small holes in the bottom of each bucket to allow water to escape. During the course of the study (in December 2008), we installed another three sets of pitfall traps 240 m away from breeding site on one side of the stream. In addition to pitfall trap arrays in the forest, we completely encircled the breeding site with drift fences (approximately 51 m in long and 40 cm high) and installed 34 funnel traps (5 L plastic bottles with the top third cut, inverted and inserted into the base of the bottle) outward and inward-facing to capture individuals in pre- and post-reproductive migration (Santos et al. in review).

We checked traps twice daily, just before sunrise and sunset, from October 2008 to February 2009, totaling 139 days, from first to last sampling day. All captured individuals were photographed and marked by phalangeal amputation, 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 trap in which it was captured.

Environmental variables

We obtained mean, maximum and minimum data for barometric pressure, temperature, and relative air humidity from the Instituto Nacional de Meteorologia’s automatic meteorological station in the municipality of Canela, 29°22’ S, 50°49’ W, 830 m above sea level. We used a rain gauge installed in the study area to measure rainfall. Photoperiod data were obtained from http://euler.on.br/ephemeris/index.php, Observatório Nacional Brasileiro.

Statistical analysis

In order to characterize the general temporal patterns of migration we used a time series analysis. A time series is a set of ordered observations, each with an observation time associated (Manly 2007). We calculate the autocorrelation function, which is used to characterize the temporal dependence of the series. The autocorrelation function measures the correlational dependency of order k between each i’th element of the series and the (ik)’th element; k is called the lag (Legendre and Legendre 1998).

We used spectral analysis to explore the cyclical patterns of the data. The purpose of the analysis is to decompose a complex time series with cyclical components into a few underlying sine and cosine functions of different frequencies, in order to identify those that are particularly strong (Legendre and Legendre 1998). The periodogram quantifies this strength. Spectral analysis requires temporal contiguity, so the few missing values were completed by interpolation from adjacent points. We believe that the missing values did not have a negative effect on the analysis, since they were a small number.
(only six missing days, corresponding to 4.3% of the total). To compare the periodicity of males and females, we applied cross spectral analyses on ln-transformed data. This is an extension of single spectral analysis to the simultaneous analysis of two series (Legendre and Legendre 1998). All spectral analyses were performed using a Hamming smoothing window with a width of 5.

We applied cross-correlation to investigate the relationship between temporal fluctuations in the number of captures and each environmental variable. This method determines the extent to which two data series exhibit concordant periodic variations and is used to identify the time lag that maximizes the correlation between the explanatory and target variables (Legendre and Legendre 1998). Based on cross-correlation results we selected the best potential predictors of migration activity to build a linear model on the appropriate time lags. The variables identified in this way were submitted to a stepwise multiple regression analysis (procedure: backward selection of variables) to construct a quantitative model for the prediction of the migration activity (Sokal and Rohlf 1995). We performed all analyses using Statistica version 6.0 (StatSoft Inc., Tulsa, Oklahoma, USA).

**Results**

**Summary of capture data**

In 5 mo of sampling we obtained 498 captures (237 males, 257 females and 4 juveniles). The total number of individuals captured was 154 (53 males, 97 females and 4 juveniles).

**Autocorrelation**
The autocorrelation values were significant at all lags (P < 0.001; Fig. 1). However, there is a strong serial dependency for lags one and two, with the highest value of autocorrelation for a lag of one (interval of one day), meaning that each observation is mostly similar to the previous observation.

**Spectral analysis**

Mean temporal patterns in number of captures are shown in Fig. 2. We could identify eight peaks of migratory activity over the five months of sampling. The five major peaks occurred on October, January and February, and the highest numbers of captures of different events were between 18 and 34 catches (including recaptures; Table 1). The other three peaks were smaller, with 10 and 11 captures, in October, December and January (Table 2). We identified these smaller peaks as migratory events because we observed reproductive activity of *Melanophryniscus cambaraensis* in the corresponding dates.

Periodogram values at different frequencies are shown in Fig. 3. We found two high periodogram values, the largest one (670.7) was at a frequency of 0.043, corresponding to a period of 23 days, which is the number of days necessary to complete one full cycle. The other one was 608.5 at the frequencies 0.072, corresponding to a period of 13.8 days. Males and females showed no difference; the peaks of migration activity were the same (Fig. 4), and the frequencies were approximately the same when considering the total number of captures (Fig. 5).

**Cross-correlations and multiple regression**

Mean temporal patterns in environmental variables and the number of captures through time are shown in Fig. 6. The significant environmental variables and their
respective lags are shown in Table 3. Among the sampled variables, only relative air humidity (mean and minimum) and rainfall (accumulated 24 h, 48 h, 72 h, 7 and 30 days) had a correlation value above 0.3. Considering the correlation between these variables, we chose only those with highest correlation values as predictors of migration activity: mean relative air humidity (0.337) and accumulated rainfall of the previous 72 h (0.856), both at lag zero. The accumulated rainfall of the previous 72 h was the only predictor of number of captures, accounting for 73% of their variation ($R^2 = 0.733$, $F_{2,139} = 359.437$, $P > 0.001$; Fig. 7).

Discussion

Studies involving migration patterns are normally carried out with seasonal species that reproduce once per year (e.g., Semlitsch 1985; Sinsch 1988; Greenberg and Tunner 2005), and migrations are limited to a rather short time of the annual activity period (Sinsch 1990). To obtain replicates of migratory events in such species it is necessary that the study last several years. Migration of *Melanophryniscus cambaraensis* is a good object of study because multiple migration events occur in a few months. Our data indicate that over a five month interval there were five major and three minor migratory events.

The periodogram identified two main migratory cycles: one lasting 23 days and the other 13.8 days, included in these time pre- and post-reproductive migrations and the reproduction activity. The analysis probably get two near migratory events together, considering as only one event, in a cycle of migration of 23 days. For example, migration peaks 1 and 2 (Table 1; Fig. 2) occurred in October were possibly considered as one event because of the temporal dependence of the data. The strong association of migratory events of *Melanophryniscus cambaraensis* with rainfall may explain this
cycle of 23 days, since there were at least two events of consecutive rainfalls accompanied by close spaced migration, then the analysis identified as a single cycle. The oscillations of 23 days correspond with fluctuations in close rainfalls (Fig. 6e). Therefore we propose that the duration cycle that best applies to *M. cambaraensis* is 13.8 days (the second highest value of periodogram), although consecutive cycles are common given the rainfall in the region. In the example cited above, the peaks 1 and 2 were considered as different events in the cycle of 13.8 days. We confirmed the absence of migration and reproduction on dates between the identified peaks, which supports this cycle. The smaller peaks of migratory activity in October and January (6 and 8, Table 2) are probably related to the close proximity of major peaks events in those months, as the number of captures between the peaks did not decline completely (Fig. 2). However, the minor peak that occurred in December (peak 7) is certainly a different event since it is widely separated from other peaks of migration.

Among amphibians, variation between the migratory patterns of males and females of a species is common and has evolved to maximize reproductive success (Russel et al. 2005; Semlitsch 2008). Males tend to arrive earlier than females, which maximizes the chances of breeding, while females often come later, which maximizes the mate choice (Douglas 1979). This happens, for example, in the salamanders *Ambystoma jeffersonianum* and *A. maculatum*, in which males tend to migrate earlier than females, since males seem to have a lower threshold for environmental cues, and will migrate as soon as the minimal conditions for migration are met (Douglas 1979; Sexton et al. 1990). However, in this study, males and females showed no differences in the peaks of migration. We suppose that the females do not wait because they have only a few days to reproduce.
The multiple regression analysis showed that only rainfall accumulated in 72 h was a significant predictor of migratory activity, accounting for 73% of variance. Many studies have correlated amphibian migrations with heavy rains (e.g., Semlitsch 1985; Greenberg and Tanner 2005). Total daily rainfall is often the most important climatic factor influencing the magnitude of amphibian movements (Tood and Winne 2006; Timm et al. 2007). For example, Greenberg and Tunner (2004) found that heavy rains influence the temporal patterns of *Scaphiopus holbrookii* breeding biology by potentially eliciting an explosive breeding response. Spieler and Linsenmair (1998) also found that rainfall was the best predictor, explaining 26% of migration activity of *Hoplobatrachus occipitalis.*

In this study we report for the first time temporal patterns of migratory cycles of *Melanophryniscus cambaraensis*, and its association with rainfalls. The strong association between migration and rainfall is not surprising given that the temporary stream where *M. cambaraensis* reproduces only fills with heavy rainfall. This result also supports field observations noted by several natural historians (Braun and Braun 1979; Kwet and Di-Bernardo 1999; Garcia and Vinciprova 2003). Moreover, considering that *M. cambaraensis* is a threatened species, additional studies in this area and with other species in this genus are extremely important to help guide future strategies of management and conservation.

**Acknowledgements**

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Fundação de Amparo à Pesquisa do Estado de São Paulo (Proc. 2008/50928-1). We are grateful to Patrick Colombo and Ralph Saporito for advice on the design and implementation of this study. Edenice Brandão Ávila de Souza and the personnel at the Floresta Nacional de São Francisco de Paula supported all aspects of our field work. Finally, we express our gratitude to the many individuals who helped with trap installation and data collection.

References


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Tables

**Table 1.** The five main peaks of migration occurred from October 2008 to February 2009, and respective numbers of captures and individuals. The peaks were identified by spectral analysis. The time represents the number of day since the experiment began, being time one the first day of captures.

<table>
<thead>
<tr>
<th>Date</th>
<th>Migration peak</th>
<th>Time (days)</th>
<th>Number of captures</th>
<th>Number of individuals</th>
</tr>
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<tr>
<td>October 15, 2008</td>
<td>1</td>
<td>5</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
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<td>1</td>
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<td>12</td>
<td>10</td>
</tr>
<tr>
<td>October 26, 2008</td>
<td>2</td>
<td>16</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>October 27, 2008</td>
<td>2</td>
<td>17</td>
<td>17</td>
<td>16</td>
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<tr>
<td>October 28, 2008</td>
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<td>10</td>
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<td>3</td>
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<tr>
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<td>87</td>
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<td>88</td>
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<td>12</td>
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<td>13</td>
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<td>January 20, 2009</td>
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<td>February 18, 2009</td>
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Table 2. The three smaller peaks of migration occurred from October 2008 to February 2009, and respective numbers of captures and individuals. The peaks were identified by spectral analysis, and were considered migration because we observed reproductive activity on these days.

<table>
<thead>
<tr>
<th>Date</th>
<th>Migration peak</th>
<th>Time (days)</th>
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<th>Number of individuals</th>
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<td>December 15, 2008</td>
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<td>January 9, 2009</td>
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<td>91</td>
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Table 3. Cross-correlation coefficients between number of captures and environmental variables and their respective lags. Only the significant environmental variables are shown (P < 0.001).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lag</th>
<th>Cross-correlation</th>
<th>Std Error</th>
<th>Variable</th>
<th>Lag</th>
<th>Cross-correlation</th>
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Fig. 1. The autocorrelation values for each lag. They were significant at all lags (P < 0.001).
Fig. 2. Number of captures through time. There are five main peaks of migration activity and other three smaller, with around 10 captures.
Fig. 3. Periodogram values at different frequencies. Highest periodogram values are 670.7, 608.5 and 428.7, at 0.043, 0.072 and 0.014 frequencies, respectively.
Fig. 4. Numbers (ln-transformed) of males and females captured through time. The peaks of migration activity are the same in both sexes.
Fig. 5. Cospectral densities of males and females and the frequencies that contribute most to the overall periodic behavior of the series. These frequencies were approximately the same when considering the total number of captures.
Fig. 6. Mean temporal patterns in environmental variables and the number of captures through time. (a) Photoperiod. (b) Temperature (°C). (c) Relative air humidity (%). (d) Barometric pressure (hPa). (e) Rainfall (mm/m²).
Fig. 7. Relationship between number of captures and rainfall accumulated in 72 h.
CONCLUSÕES GERAIS

Neste estudo nós descrevemos alguns padrões da migração de uma das duas populações conhecidas do sapinho-verde-de-barriga-vermelha *Melanophryniscus cambaraensis* (Anura, Bufonidae), no município de São Francisco de Paula, Rio Grande do Sul, Brasil. Sobre a orientação da migração, nós encontramos que, tanto para machos quanto para fêmeas, as direções de entrada e saída do sítio reprodutivo foram significativamente diferentes de aleatório. Este resultado foi encontrado mesmo quando excluímos pseudoréplicas (i.e. indivíduos capturados mais de uma vez entrando ou saindo do sítio reprodutivo), indicando que o padrão não-aleatório foi em nível populacional. Nós encontramos diferenças significativas entre os ângulos de entrada e saída do sítio reprodutivo (tanto analisando os dados agrupados quanto cada um dos sexos separadamente) somente quando consideramos as pseudoréplicas. Quando excluímos as pseudoréplicas das análises, os resultados não foram significativos. Isso ilustra a importância de avaliar o potencial efeito da inclusão de pseudoréplicas no delineamento de estudos sobre a orientação da migração, considerando os objetivos do estudo e as dinâmicas reprodutivas das espécies de interesse. No nosso caso, o ideal foi excluir as pseudoréplicas das análises. Apesar disso, devido a essas recapturas, foi possível identificar diferenças na dinâmica reprodutiva dos machos e fêmeas desta população de *M. cambaraensis*, resultando que os machos participaram de mais eventos de reprodução explosiva do que as fêmeas.

Nós também concluímos que a atividade migratória de *Melanophryniscus cambaraensis* é diurna. Na maioria dos anfíbios, a migração normalmente ocorre durante a noite, o que reduz o risco de dessecação e contribui para evitar predadores. Entretanto, alguns anfíbios também migram durante o dia. Semlitsch e Pechmann
(1985) propõem que este aspecto evoluiu como um resultado da presença de secreções tóxicas na pele e de coloração aposemática, liberando os indivíduos que possuem tais características de predadores diurnos orientados pela visão. Baseado nesta hipótese e nas recentes observações de atividade reprodutiva contínua durante 24 h em *M. cambaraensis*, nós hipotetizamos que a migração dessa espécie ocorreria igualmente durante o dia e a noite. Contrariamente às nossas previsões, a atividade migratória foi diurna, e este resultado não ocorreu em função de mudanças nas variáveis ambientais (temperatura, umidade do ar, pressão barométrica e precipitação). Nós sugerimos que a migração diurna é melhor explicada pela filogenia e não por pressões contemporâneas. A atividade diurna é primitiva em *M. cambaraensis* e evoluiu no ancestral comum Agastorophrynia, antes da evolução da defesa química encontrada nos sapos (Bufonidae) e nas rãs venenosas (Dendrobatidae). Isso sugere que a defesa química nesses grupos podem ter evoluído como resultado da atividade diurna, que os colocou em contato com predadores diurnos orientados pela visão, e não o contrário.

Sobre os padrões temporais da migração, nós identificamos oito picos de atividade migratória ao longo do período de estudo: cinco principais, envolvendo mais capturas, e três menores, com um menor número de capturas. Machos e fêmeas não apresentaram diferenças temporais nos picos de migração. Através da realização de uma análise espectral identificamos dois ciclos migratórios principais: um com duração de 23 dias e outro com duração de 13.8 dias, incluídas nesses dias as migrações pré e pós-reprodutivas e a atividade de reprodução. O ciclo de 23 dias pode ser explicado pela forte associação da atividade migratória com a chuva, como houve pelo menos dois eventos de chuvas consecutivas acompanhadas por migrações próximas, a análise identificou dois eventos como um único. Portanto, nós sugerimos que o ciclo que melhor se aplica ao *M. cambaraensis* é o com duração de 13.8 dias. Isto também é
suportado pela ausência de migração e reprodução entre os principais picos migratórios identificados. Os maiores valores de correlação dos preditores da atividade migratória foram os da umidade relativa do ar e da chuva acumulada nas 72 h anteriores, ambas no lag zero. A chuva acumulada em 72 h foi o único preditor do número de capturas, representando 73% de sua variação.

Nesta dissertação, nós apresentamos os resultados do primeiro estudo de migração de um anfíbio asazonal, de reprodução explosiva. Este também é o primeiro estudo detalhado sobre migração de anfíbios no Brasil. Ainda existem muitas questões a serem respondidas sobre a migração de *Melanophryniscus cambaraensis*, principalmente na escala individual, já que neste estudo os objetivos consideravam a população. Então um próximo passo é realizar estudos mais específicos, utilizando outros recursos como, por exemplo, radiotelemetria. Desta maneira poderemos descobrir as distâncias que os indivíduos migram, os microhabitats terrestres ocupados, a periodicidade na qual os indivíduos participam dos eventos reprodutivos, entre outros. Além disso, seria relevante observar os padrões migratórios ao longo de um ano completo comparando diferentes anos, populações e espécies de *Melanophryniscus*.

O Brasil tem a fauna de anfíbios mais rica do mundo, com espécies ocorrendo tanto em ambientes subtropicais fortemente sazonais quanto em ambientes tropicais com mínima variação climática. Além disso, os anfíbios brasileiros apresentam uma vasta diversidade de modos reprodutivos e estratégias de acasalamento (Haddad e Prado 2005), e nós acreditamos que os padrões de migração irão variar consideravelmente entre os diferentes habitats e linhagens. Devido à importância da migração na ecologia e evolução dos anfíbios (Semlitsch 2008) e a recente sugestão de que perturbações antrópicas na migração dos anfíbios pode ser a maior causa de declínios desses animais.
(Becker et al. 2007), estudos detalhados sobre a migração são áreas de pesquisa promissoras e importantíssimas para os anfíbios brasileiros.
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