

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE BIOCÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA

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

RAQUEL ROCHA SANTOS

PORTO ALEGRE
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GRANDE DO SUL, BRASIL**

Raquel Rocha Santos

Orientador: Dr. Taran Grant

DISSERTAÇÃO DE MESTRADO

PORTO ALEGRE - RS - 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 Agastrophrynia, 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 significant 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 Agastrophrynia, 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 as 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; Szatecsny 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 aposemática. 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 significantivo para os movimentos de anfíbios com reprodução explosiva. Em alguns casos, com na 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

(2005) sugere que reprodutor oportunista é o termo que melhor se ajusta aos padrões de reprodução de *M. rubiventris*, devido à sincronicidade da atividade reprodutiva com fortes chuvas (Crump 1974). Até recentemente, acreditava-se que a atividade de vocalização de *Melanophryniscus* era diurna ou, no máximo, crepuscular (Langone 1994; Kwet e Di-Bernardo 1999; Baldo e Basso 2004; Kwet et al. 2005). Entretanto foi observado que *M. simplex* vocaliza tanto durante o dia como à noite (Colombo et al. 2007; P. Colombo, comunicação pessoal), e que em *M. cambaraensis* o número de machos em atividade de vocalização não difere significativamente entre o dia e a noite (V. Z. Caorsi, R. R. Santos e T. Grant, dados não publicados). Aspectos da migração de bufonídeos de reprodução explosiva foram estudados em poucas espécies (ex., Sinsch 1988), sendo que não há estudos sobre os processos migratórios das espécies do gênero *Melanophryniscus*. O sapinho-verde-de-barriga-vermelha *Melanophryniscus cambaraensis* é uma espécie endêmica do sudeste do Planalto das Araucárias do Rio Grande do Sul (micro-região dos Campos de Cima da Serra) (Kwet e Di-Bernardo 1999). É um sapo pequeno (cerca de 30 mm de comprimento rostro-cloacal), que, assim como outras espécies do gênero, secreta alcalóides lipofílicos tóxicos (P. Colombo e T. Grant, dados não publicados). Está classificado como vulnerável na lista de espécies ameaçadas do Rio Grande do Sul, onde são conhecidas apenas duas populações, separadas por aproximadamente 50 km, nos municípios de Cambará do Sul e São Francisco de Paula (Braun e Braun 1979; Garcia e Vinciprova 2003).

Diferentemente das outras espécies de anfíbios estudadas, a reprodução de *Melanophryniscus cambaraensis* é sazonal, 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*)

1 Directional Orientation of Migration in an Aseasonal Explosive Breeding Toad from
2 Brazil

3

4 Running title: Orientation of Migration in a Brazilian Toad

5

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

8

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18

19 Abstract

20 The directional orientation of pre- and post-reproductive migration was studied in the
21 Brazilian red bellied toad *Melanophryniscus cambaraensis*, a species that forms
22 explosive breeding aggregations at irregular intervals throughout the entire year.
23 Migrating toads were captured by enclosing the breeding site in dual drift fences with
24 inward- and outward-facing funnel traps. Data were collected over 5 mo and totalled
25 333 captures. The observed directional orientation was significantly different from
26 expected under a uniform distribution for both pre- and post-reproductive migration,
27 regardless of gender or treatment of pseudoreplicate captures. Furthermore, males and
28 females did not differ significantly from each other in the orientation of entry or exit.
29 Collectively, these findings indicate that this is a population-level characteristic. Herein,
30 we suggest that this directional bias may be due to the presence of a dirt road next to the
31 breeding site. When pseudoreplicate captures (*i.e.*, repeated captures of the same
32 individual migrating in the same direction) were included in analyses, the directional
33 orientation of pre-reproductive migration was significantly different from post-
34 reproductive migration; however, there was no difference in the directional orientation
35 of pre- and post-reproductive migration when pseudoreplicate captures were excluded,
36 illustrating the importance of considering the potential influence of pseudoreplicate
37 captures when designing studies of migration orientation.

INTRODUCTION

38

39 Most species of amphibians with biphasic life cycles have terrestrial adults and aquatic
40 eggs and larvae (Altig & McDiarmid 1999). The persistence of local populations of
41 these species requires adults to migrate, potentially multiple times and considerable
42 distances, between terrestrial foraging or over-wintering habitats and aquatic breeding
43 sites to complete their life cycle (Russell *et al.* 2005, Semlitsch 2008, Todd *et al.* 2009).
44 Knowledge of migration between habitats is necessary to understand ecological
45 interactions, population dynamics, and the selective pressures that affect local
46 populations, as well as to develop effective conservation and management strategies
47 (Semlitsch 2008). Habitat use by frogs in general is poorly understood (Lemckert 2004),
48 and habitat split, defined as anthropogenic disconnection of foraging and breeding
49 habitats, has been implicated as a major cause of amphibian declines (Becker *et al.*
50 2007).

51 Previous studies have found that amphibian migration usually occurs in a non-
52 random manner, with individuals entering and exiting breeding sites at the same place
53 and using certain habitats and migratory routes (Marty *et al.* 2005, Rittenhouse &
54 Semlitsch 2006, Wells 2007). However, studies of migration orientation in amphibians
55 have focused on annual breeders with prolonged breeding (*sensu* Wells 1977) extended
56 over more than one month and several months (or more) between breeding events. In
57 contrast, reproduction in the red bellied toads of southern South America
58 (*Melanophryniscus*, Bufonidae) is explosive (*sensu* Wells 1977), being concentrated in
59 bursts of breeding activity over a few days when many individuals simultaneously
60 migrate to temporary pools or streams during and immediately following intense rains
61 (Achaval & Olmos 2007, Garcia & Vinciprova 2003, Kwet & Di-Bernardo 1999).
62 Aspects of the migration of a few other species with explosive breeding have been

63 studied (Sinsch 1988, Todd *et al.* 2009), but the ecology of species of
64 *Melanophryniscus* is poorly known and there are no studies of their migration.

65 The Brazilian red bellied toad *Melanophryniscus cambaraensis* (Fig. 1) differs
66 from all previously studied species in breeding aseasonally. That is, despite inhabiting a
67 subtropical region characterized by well defined seasons, explosive breeding events
68 occur repeatedly at irregular intervals over the course of the entire year. Other species in
69 this region breed seasonally, with seasonal reproductive activity explained by
70 photoperiod and not variation in temperature, rainfall, or air humidity (Both *et al.* 2008).
71 We have observed explosive breeding events in *M. cambaraensis* in spring (October),
72 summer (December, January, and February), and winter (August), and breeding has
73 been recorded in all remaining months by numerous workers (Garcia & Vinciprova
74 2003, P. Garcia, unpublished data, P. Colombo, pers. comm., A. Samir, pers. comm.).
75 As in other species that form explosive breeding aggregations, males of *M.*
76 *cambaraensis* exhibit many of the typical strategies of scramble competition (Wells
77 2007), including intense male combats and struggles to possess females and displace
78 amplexant males (V. Z. Caorsi, R. R. Santos & T. Grant, unpublished data).

79 In light of the differences between the breeding biology of *Melanophryniscus*
80 *cambaraensis* and previously studied species, we undertook a study of the migration of
81 one of the two known populations of this species. The aim of this paper is to document
82 the directional orientation of pre- and post-reproductive migration. Specifically, we
83 tested (1) if the direction of migration occurred in a random or nonrandom manner and
84 if certain angles were used more frequently than others and (2) whether there were
85 differences in entry and exit points between pre- and post-reproductive migrations and
86 between males and females. Studies of migration often sample the same individuals in
87 multiple migration events. Although these pseudoreplicate captures may bias results to

88 reflect individual propensities for particular orientations (Dodd & Cade 1998), they are
89 often not excluded from analyses (e.g., Malmgren 2002). We therefore tested for the
90 effect of pseudoreplicates by running all analyses with and without pseudoreplicate
91 captures.

92

93

METHODS

94 **Study site**

95 *Melanophryniscus cambaraensis* is a small (ca. 35 mm snout-vent length), vulnerable
96 (Garcia & Vinciprova 2003) species endemic to the subtropical southeastern Araucaria
97 Plateau in the Campos de Cima da Serra micro-region of Rio Grande do Sul state,
98 Brazil. Only two isolated populations of this species have been discovered, each known
99 from single localities separated by approximately 50 km in the municipalities of
100 Cambará do Sul and São Francisco de Paula in Rio Grande do Sul state, Brazil (Garcia
101 & Vinciprova 2003). We studied the São Francisco de Paula population, which is
102 restricted to the Floresta Nacional de São Francisco de Paula (FLONA). The native
103 mixed ombrophilous (high-rainfall) forest is dominated by *Araucaria angustifolia*
104 (Sonego *et al.* 2007) and covers about 56% of the 1607-ha area of the FLONA, the
105 remainder being composed of planted *Araucaria angustifolia*, *Pinus* spp. and
106 *Eucalyptus* spp. (Bonatti *et al.* 2006). Temperatures range from -3–18°C in winter
107 months and 18.3–27°C in the summer months, and an annual average of 14.5°C. The
108 region has high levels of rainfall in all months, with average monthly precipitation of
109 more than 200 mm for all months of the year (Buriol *et al.* 2009) and average annual
110 precipitation of more than 2200 mm (Backes *et al.* 2005).

111 We collected data from October 2008 to February 2009 at a temporary stream
112 formed by heavy rainfalls (29°25'41.3" S 50°23'44.5" W, 866 m asl). The breeding site

113 (Fig. 2A) is approximately 20 m long and 4 m wide and is located at the edge of a small
114 dirt road on a rocky outcrop partially covered by a thin layer of topsoil and vegetation
115 dominated grasses (Poaceae) and herbs (*Eryngium* sp.). Except for the dirt road, the area
116 around the stream is composed of forest (planted *Araucaria angustifolia*), with shrubs
117 and sedges (Cyperaceae) covering the few meters between the temporary stream and the
118 forest proper. This is the only locality in the municipality of São Francisco de Paula
119 where *Melanophryniscus cambaraensis* is known to breed, and this is the only species
120 of amphibian that reproduces in this temporary stream, although *Aplastodiscus*
121 *perviridis* (Hylidae), *Ischnocnema henselii* (Brachycephalidae), *Hypsiboas marginatus*
122 (Hylidae), *Leptodactylus araucaria* (Leptodactylidae), and *Rhinella icterica*
123 (Bufonidae) also inhabit the adjacent forest.

124

125 **Data collection**

126 We completely enclosed the breeding site in inner and outer drift fences composed of
127 durable plastic sheets approximately 51 m long and 40 cm high (Figures 2a, 3). The
128 solid rock substrate of the breeding site made it impossible to bury the base of the
129 fences or dig holes for pitfall traps. Instead, we held the fences flush to the ground by
130 folding over and stapling the base of the plastic sheet to form a pocket that we filled
131 with soil and small rocks to weight down the base of the fence (Figure 2b). This allowed
132 the fence to be flexible enough to follow the contour of the surface while also
133 maintaining tight contact with the ground. To hold the fence upright, we built U-shaped
134 supports from wooden stakes that we held in place with heavy rocks and bags of soil.
135 We constructed funnel traps from 5-L plastic bottles by cutting off the top third of each
136 bottle and inserting it, inverted, into the base of the bottle (Figure 2b). To provide
137 protection from sun and rain, we fixed black plastic sheets over each pair of funnels.

138 We installed pairs of funnel traps (one facing inwards, the other outwards) at 3
139 m intervals around the entire circumference of the fences, totaling 34 funnels (17 facing
140 each way). We assumed that all individuals caught in the outward-facing traps were in
141 pre-reproductive migration and all individuals caught in the inward-facing traps were in
142 post-reproductive migration. To determine the angular position of the traps, we
143 measured the angle of each pair of traps from the centre of the reproductive site with a
144 compass.

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

154

155 **Statistical analysis**

156 We used RAO's spacing test (Rao 1976) to determine if the distributions of entry and
157 exit angles were significantly different from a uniform distribution. This test has greater
158 power to detect departures from uniformity for data with multiple modes and wide
159 angular dispersion than Rayleigh's or Watson's U2 tests (Bergin 1991). We grouped
160 data by sex and direction of migration (pre-reproduction and post-reproduction). To
161 compare distributions between sexes and between pre- and post-reproductive
162 migrations, we used a multi-response permutation procedure (MRPP) for circular

163 distributions. This procedure compares the treatment groups, similar to analysis of
164 variance, based on distance functions. To evaluate the possible effect of
165 pseudoreplicates, we performed tests both including all capture data and including only
166 a single, randomly selected capture datum per individual per direction (i.e., each
167 individual could have at most two data points, one entering and another one exiting the
168 breeding site). We considered $P < 0.05$ to indicate of significant departure of the
169 observed data from the null distribution. We performed all analyses using the
170 BLOSSOM software package (version W2008.04.02, Cade & Richards 2005), as in
171 previous studies of amphibian orientation (Dodd & Cade 1998, Marty *et al.* 2005). We
172 visualized circular distributions using the Oriana 3.0 software package (version 3.0,
173 Kovach Computing Services, Pentraeth, Isle of Anglesey, Wales, U.K.).

174

175

RESULTS

176 Summary of capture data

177 In 5 mo of field work we observed explosive breeding events in all months but
178 November, which was exceptionally dry and had only two captures. In total, we
179 obtained 333 captures (181 male, 152 female), corresponding to 126 individuals (48
180 males, 78 females; Table 1). Of the 333 captures, 139 were pseudoreplicate captures
181 (i.e., repeated captures of the same individual migrating in the same direction).
182 Individual pseudoreplicate capture rates were much greater for males than females. We
183 captured 75% of males two or more times in pre- and/or post-reproductive migration
184 (pseudoreplicate captures per individual: maximum = 9, mean = 2.0, mode = 1),
185 compared to only 33% of females (pseudoreplicate captures per individual: maximum =
186 4, mean = 0.54, mode = 0).

187 We did not capture any juveniles of *Melanophryniscus cambaraensis* entering or
188 exiting the breeding site. However, occasionally we captured six other species of
189 anurans in funnel traps, including *Elachistocleis bicolor* (Microhylidae, one
190 individual), *Ischnocnema henselii* (Brachycephalidae, three individuals), *Leptodactylus*
191 *gracilis* and/or *L. plaumanni* (Leptodactylidae, five individuals), *Physalaemus cuvieri*
192 and *P. lisei* (Leiuperidae, three and 19 individuals, respectively), and *Rhinella icterica*
193 (Bufonidae, 65 individuals). Most of the captured individuals were juveniles, and we
194 observed no breeding activity in any of these species in or near the temporary stream.
195 We speculate that these individuals entered the breeding area in search moisture and
196 may have used the funnel traps as refuges.

197

198 **Analyses including all captures**

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

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

210 In general, migrating toads entered and exited the breeding site through different
211 routes (MRPP, $P = 0.004$), and this pattern was observed in both sexes (MRPP, males P

212 = 0.049, females $P = 0.025$) (Table 3). Males and females did not differ significantly
213 from each other in the orientation of entry and exit ($P = 0.115$ and $P = 0.504$,
214 respectively).

215

216 **Analyses excluding pseudoreplicate captures**

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

224 As shown in Table 2, the exclusion of pseudoreplicate captures resulted in only
225 minor changes in the frequencies of entry and exit angles. Over half of the entries and
226 nearly half of the exits were made through the southeast (126° and 152°) and west
227 (250° , 262° and 266°), and approximately 9% of entries and 8% of exits were made
228 through the northwest (298° and 322°). The percentage of entries through the southwest
229 (222°) decreased from nearly 25% to approximately 17%, and the percentage of
230 departures to the west (12.6%, 280°) and east (9.5%, 108° and 94°) differed only
231 slightly.

232 Despite only small changes in the relative frequencies of orientations of entries
233 and exits, none of the tests comparing pre- and post-reproductive migrants and between
234 sexes were significant when pseudoreplicate captures were excluded (all $P > 0.05$;
235 Table 3).

236

DISCUSSION

237

238 Although the aim of the present study is only to address the orientation of migration, the
239 results reported above indicate clear differences between the reproductive dynamics of
240 males and females in this population of *Melanophryniscus cambaraensis*. As indicated
241 by the different rates at which individual males and females were repeatedly captured
242 migrating in the same direction (i.e., individual pseudoreplicate capture rates), males
243 participate in more explosive breeding events than females. Most males were recaptured
244 migrating in the same direction at least twice, whereas most females were never
245 recaptured migrating in the same direction. These and additional considerations will be
246 addressed in greater detail elsewhere.

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

260 Several studies have related non-random orientation of migration with habitat
261 characteristics, finding that individuals preferentially migrate between breeding sites

262 and forested areas (Jehle & Arntzen 2000, Malmgren 2002, Marty *et al.* 2005,
263 Rittenhouse & Semlitsch 2006, Todd *et al.* 2009, Vasconcelos & Calhoun 2004,
264 Waltson & Mullin 2008). This does not appear to be a determining factor for this
265 population of *Melanophryniscus cambaraensis* because the breeding site is completely
266 surrounded by homogeneous planted *Araucaria angustifolia* forest that is occupied by
267 this species.

268 We did not explicitly test explanations for the non-random orientation. However,
269 as part of our larger study of migration in this species we placed drift fences and pitfall
270 traps in the forest surrounding the breeding site, and we found 85% of male individuals
271 and 58% of female individuals exclusively in the forest southeast of the temporary
272 stream, adjacent to the points with the greatest frequency of captures. Given the
273 apparent homogeneity of the surrounding forest, we suggest that this may be due to the
274 road that separates the breeding site from the forest to the north-west. In light of the
275 explosive breeding of this species, it is unlikely that the road represents a significant
276 barrier to adult movement (Todd *et al.* 2009). We also captured five individuals (1 male,
277 4 females) in the forest on both sides, which demonstrates that adults are capable of
278 crossing the road. Instead, we suggest the road may affect juvenile dispersal from the
279 breeding site by either increasing the mortality of juveniles that attempt to cross the road
280 through greater desiccation and/or predation or acting as a deterrent to dispersal (i.e.
281 juveniles avoid crossing the road), with adults migrating to and from the forest they
282 dispersed into as juveniles. This hypothesis is consistent with the findings of Walston &
283 Mullin (2008) that juveniles of several amphibian species rely on direct environmental
284 cues to guide non-random orientation when dispersing from breeding ponds to forest.

285 Analyses including pseudoreplicate captures found the orientation of pre- and
286 post-reproductive migration to be significantly different, both when data were pooled

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

302 Our study was affected to an unknown degree by three problems that should be
303 considered when interpreting our results and designing future studies. First, although
304 our field work extended over 5 mo and multiple explosive breeding events, it may have
305 been too short to detect long-term trends that emerge over multiple seasons or years. For
306 example, although this species differs from most other amphibians in this region by
307 breeding in all months of the year, it is possible that breeding is less frequent in winter
308 months with shorter days and colder temperatures than summer months with longer
309 days and higher temperatures. Similarly, migration orientation might vary over time in
310 response to landscape changes or population turnover. Second, our results are based on
311 only a single breeding population of *Melanophryniscus cambaraensis*. Overcoming this

312 problem may be difficult, as breeding activity at the only other known locality has not
313 been observed in over a decade, and searches for additional populations have, so far,
314 been fruitless. Third, trap efficiency was not 100% (see below). The breeding site is
315 located on a rocky outcrop, which prevented us from burying the drift fences and
316 required us to invent a novel method to fix them to the substrate (Fig. 2). We also
317 attempted to use soil and other loose substrate to bury the base of the fence, but it was
318 quickly washed away by the frequent heavy rains. Stronger methods, such as cementing
319 the fence in place, were not feasible, as they would permanently alter the breeding site,
320 which is located inside a conservation area. When designing the capture method, we
321 assumed that a double-fence design would help prevent trap avoidance, as individuals
322 would have to overcome two barriers. However, we made only 10 captures between the
323 two fences, so it appears that increasing the number of fences is not effective.

324 To estimate trap efficiency, we examined the number of complete captures (i.e.,
325 individuals captured entering and then exiting the breeding site in a given explosive
326 breeding event) in relation to the total number of captures. According to this estimate,
327 overall trap efficiency was only 44%. This is probably an underestimate, as it does not
328 include individuals that were not detected entering or exiting the breeding site. Monthly
329 trap efficiency data are suggestive of a trend to decrease (complete captures as a
330 percentage of total captures: October = 60%, November = 0% (dry month, only two
331 captures), December = 21%, January = 46%, February = 32%), but there is little
332 indication that individuals learned to consistently avoid traps. For individuals captured
333 in multiple events, we determined the number of individuals that had complete captures
334 (1) always ($N = 4$), (2) after partial captures ($N = 18$), and (3) exclusively prior to partial
335 captures ($N = 17$). Consequently, 17 individuals may have learned to consistently avoid
336 traps, whereas 22 did not. We did not detect any holes or breaks in the fence or find

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368

369

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454

455

TABLES

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

| Gender | Number of individuals | All captures | | | Excluding pseudoreplicate captures | | |
|--------|--------------------------|--------------|------|-------|---------------------------------------|------|-------|
| | | Entry | Exit | Total | Entry | Exit | Total |
| Male | 48 | 91 | 90 | 181 | 40 | 44 | 84 |
| Female | 78 | 78 | 74 | 152 | 57 | 51 | 108 |
| Total | 126 | 169 | 164 | 333 | 97 | 95 | 192 |

460

461

462 Table 2. Summary of the directions/angles used by migrating *Melanophryniscus*
 463 *cambaraensis*. Direction refers to the approximate compass orientation. Entry and exit
 464 refer to pre- and post-reproductive captures, respectively. Pseudoreplicate captures are
 465 repeated captures of the same individual migrating in the same direction.

| Direction | Angles (degrees) | All captures (%) | | Excluding pseudoreplicate captures (%) | |
|-----------|---------------------|------------------|------|---|------|
| | | Entry | Exit | Entry | Exit |
| SW | 222 | 24.3 | 4.3 | 17.5 | 4.2 |
| SE | 152 | 12.4 | 11.6 | 12.4 | 10.5 |
| SE | 126 | 4.1 | 9.1 | 5.2 | 9.5 |
| E | 114 | 2.4 | 1.8 | 2.1 | 1.1 |
| E | 108 | 0.0 | 7.3 | 0.0 | 8.4 |
| E | 94 | 2.4 | 4.9 | 3.1 | 1.1 |
| E | 82 | 3.0 | 1.8 | 1.0 | 3.2 |
| E | 76 | 1.8 | 1.2 | 2.1 | 2.1 |
| NE | 64 | 1.8 | 1.8 | 3.1 | 1.1 |
| NE | 26 | 1.8 | 3.7 | 3.1 | 5.3 |
| NW | 322 | 5.9 | 6.1 | 6.2 | 5.3 |
| NW | 298 | 4.1 | 3.7 | 3.1 | 3.2 |
| W | 288 | 0.0 | 1.8 | 0.0 | 3.2 |
| W | 280 | 1.8 | 11.0 | 1.0 | 12.6 |
| W | 266 | 12.4 | 11.6 | 16.5 | 12.6 |
| W | 262 | 11.2 | 9.1 | 12.4 | 8.4 |
| W | 250 | 10.7 | 9.1 | 11.3 | 8.4 |

466

467 Table 3. Comparison of the directional orientation of male and female
 468 *Melanophryniscus cambaraensis* in pre- and post-reproductive migration using a multi-
 469 response permutation procedure (MRPP) for circular distributions. Entry and exit refer
 470 to pre- and post-reproductive captures, respectively. Pseudoreplicate captures are
 471 repeated captures of the same individual migrating in the same direction.

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

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.

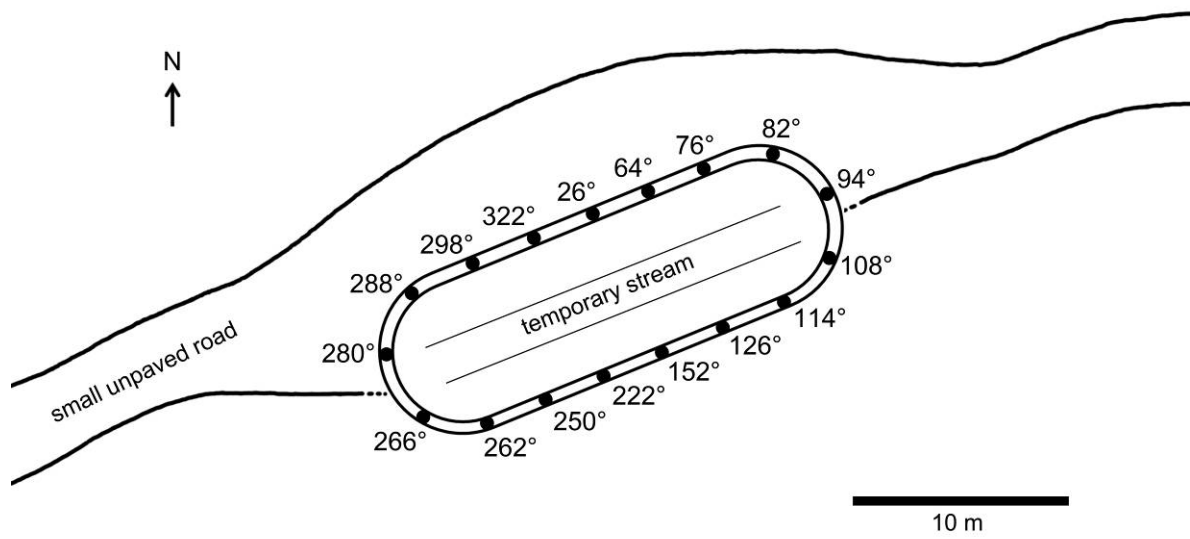


Figure 3. Schematic map showing arrangement of drift fences and funnel traps around the temporary stream where *Melanophryniscus cambaraensis* form explosive breeding aggregations. The areas north and south of the road are composed of planted Paraná Pine (*Araucaria angustifolia*) forest.

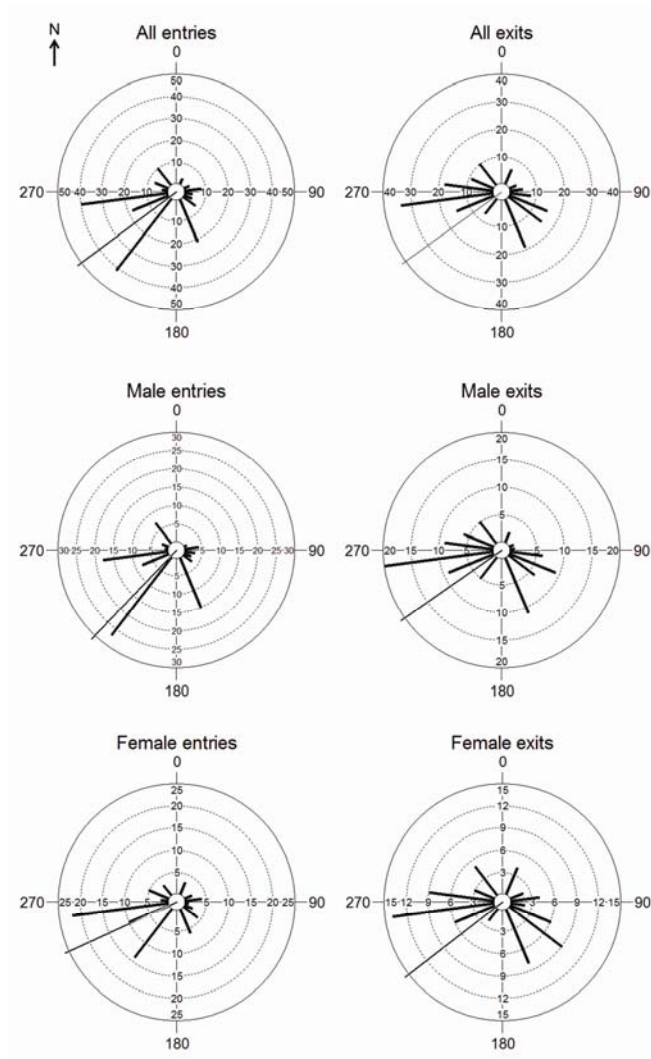


Figure 4. Circular histograms showing the frequency, and mean vector for the directional orientation of pre- and post-reproductive migration in *Melanophryniscus cambaraensis*, including all capture data. The observed data were significantly different from expected under a uniform distribution for both pre- and post-reproductive migration, regardless of gender (Rao's spacing test, all $P < 0.001$).

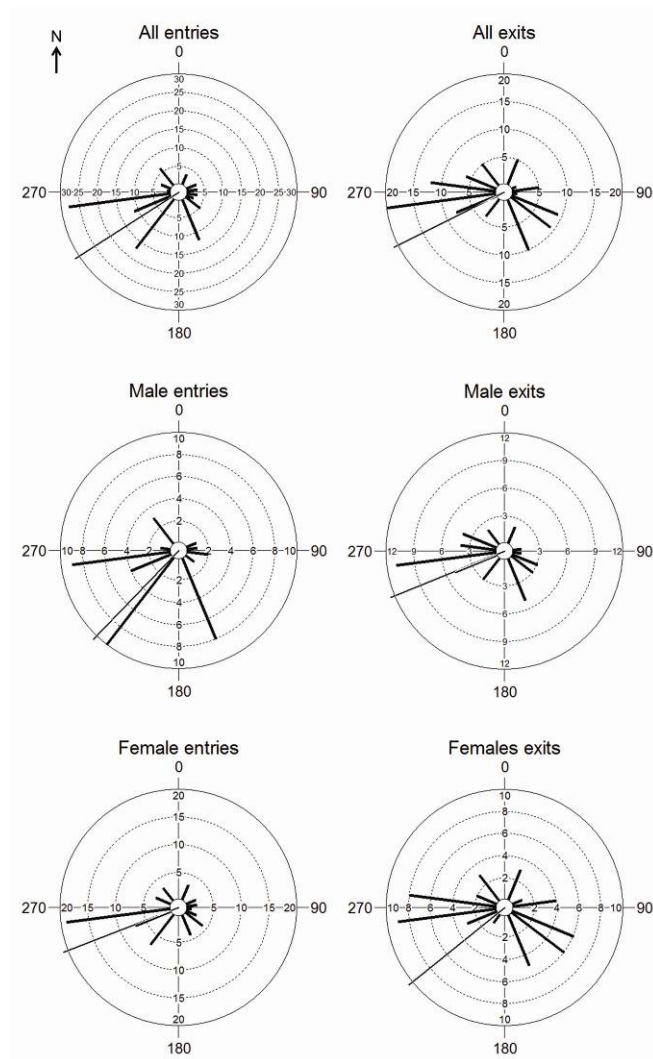


Figure 5. Circular histograms showing the frequency, and mean vector for the directional orientation of pre- and post-reproductive migration in *Melanophryniscus cambaraensis*, excluding pseudoreplicate captures. The observed data were significantly different from expected under a uniform distribution 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*)

1 Diel Activity of Migration in a Poisonous Toad from Brazil and the Evolution of
2 Chemical Defenses in Diurnal Amphibians

3

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8

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25

26 **Abstract**

27 Most amphibians with biphasic life cycles have aquatic eggs and larvae and terrestrial
28 adults that migrate between terrestrial habitats and aquatic breeding sites. Migration
29 usually occurs at night in order to avoid desiccation and predation. However, some
30 amphibians also migrate during the day, and it has been proposed that this evolved as a
31 result of poisonous skin secretions and aposematic coloration that release individuals
32 from visually oriented diurnal predators. Based on this hypothesis and recent
33 observations of 24 hour breeding activity in the poisonous toad *Melanophryniscus*
34 *cambaraensis*, we predicted that migration in this species would occur equally during
35 the day and night. To test our prediction we documented the diel activity of migratory
36 movements over multiple explosive breeding events from October 2008 to February
37 2009 (127 nights) at a temporary stream in Rio Grande do Sul state, southern Brazil. We
38 also obtained environmental data to determine if diel activity could be attributed to
39 variation in rainfall, barometric pressure, temperature, and/or relative air humidity.
40 Contrary to our prediction, migratory activity in *M. cambaraensis* is strongly diurnal.
41 Although temperature and humidity varied significantly between day and night
42 intervals, this variation does not account for the diurnal-only migratory activity of *M.*
43 *cambaraensis*. We suggest that the diurnal-only migratory activity of *M. cambaraensis*
44 is best explained by phylogeny, not contemporary functions or selective pressures.
45 Diurnal activity is primitive for this species and evolved in the common ancestor of
46 Agastrophrynia, prior to the chemical defenses found in toads (Bufonidae) and poison
47 frogs (Dendrobatidae). This suggests that chemical defenses in these groups may have
48 evolved as a result of the diurnal activity that brought them into contact with visually
49 oriented diurnal predators, and not the other way around.

50

51 **Introduction**

52

53 Most species of amphibians with biphasic life cycles have terrestrial adults and aquatic
54 eggs and larvae (Altig and McDiarmid 1999). Adults of these species must risk
55 predation and desiccation to migrate between terrestrial foraging or over-wintering
56 habitats and aquatic breeding sites to complete their life cycle (Russell et al. 2005;
57 Semlitsch 2008). Amphibian migration usually occurs at night when temperatures are
58 low and humidity is high (Semlitsch 1985; Sinsch 1988; Todd and Winne 2006; Wells
59 2007), which reduces desiccation risk and contributes to predator avoidance (Semlitsch
60 and Pechmann 1985). Nevertheless, Semlitsch and Pechmann (1985) found that
61 migratory activity in the salamander *Notophthalmus viridiscens* did not differ between
62 day and night intervals. This species has poisonous skin secretions (tetrodotoxin; Daly
63 2004) and aposematic coloration, and Semlitsch and Pechmann (1985) hypothesized
64 that chemical defenses coupled with warning coloration enable diurnal migratory
65 activity by eliminating the need to restrict activity to periods of darkness to avoid
66 detection by visually oriented diurnal predators, i.e. by shifting from a predator
67 avoidance mechanism (nocturnal migration) to an antipredator mechanism (poisonous
68 skin secretions and aposematic coloration; Brodie et al. 1991). Many species of
69 poisonous, brightly colored frogs and toads also exhibit diurnal activity, and Semlitsch
70 and Pechmann's (1985) hypothesis provides a plausible explanation for the evolution of
71 diurnality in these taxa as well.

72 Like *Notophthalmus viridiscens*, the red-bellied toads of southern South
73 America (*Melanophryniscus*, Bufonidae) secrete defensive chemicals and exhibit
74 aposematic coloration. Specifically, they secrete lipophilic alkaloids and bufadienolides
75 (Daly et al. 2008) and employ the unken reflex to display bright red or orange ventral

76 surfaces when disturbed (Fig. 1). Until recently it was believed that all activity in
77 *Melanophryniscus* was diurnal or at most crepuscular (Langone 1994; Kwet and Di-
78 Bernardo 1999; Baldo and Basso 2004; Kwet et al. 2005). However, *M. simplex* was
79 recently observed calling during both daytime and nighttime (Colombo et al. 2007; P.
80 Colombo pers. comm.), and the number of calling male *M. cambaraensis* does not differ
81 significantly between day and night (V. Z. Caorsi, R. R. Santos and T. Grant,
82 unpublished data).

83 Reproduction in *Melanophryniscus cambaraensis* is explosive (sensu Wells
84 1977), being concentrated in bursts of breeding activity over a few days when many
85 individuals simultaneously migrate to temporary pools or streams during and
86 immediately following intense rains (Santos et al. 2010). Unlike most species that form
87 explosive breeding aggregations, *M. cambaraensis* breeds aseasonally. That is, despite
88 inhabiting a subtropical region characterized by well-defined seasons, explosive
89 breeding events occur repeatedly at irregular intervals throughout the year. Like other
90 explosive breeders, males of *M. cambaraensis* exhibit many of the typical strategies of
91 scramble competition (Wells 2007), including intense male-male combat and struggles
92 to possess females and displace amplectant males (V. Z. Caorsi, R. R. Santos and T.
93 Grant, unpublished data).

94 Based on Semlitsch and Pechmann's (1985) hypothesis and the toxicity
95 (confirmed in this species by gas chromatography/mass spectrometry analysis; P.
96 Colombo & T. Grant, unpublished data), aposematic coloration, and recent observations
97 of continuous 24 hour breeding activity of *Melanophryniscus cambaraensis*, we
98 predicted that migratory activity in this species would occur equally during day and
99 night intervals, as in *Notophthalmus viridiscens*. To test our prediction we documented
100 the diel activity of migratory movements over multiple breeding events. We also

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

104

105 **Materials and methods**

106

107 Study site and data collection

108

109 *Melanophryniscus cambaraensis* is endemic to the subtropical southeastern Araucaria
110 Plateau in the Campos de Cima da Serra micro-region of Rio Grande do Sul state,
111 Brazil. Only two isolated populations of this vulnerable species have been discovered,
112 each at single localities separated by approximately 50 km in the municipalities of
113 Cambará do Sul and São Francisco de Paula in Rio Grande do Sul state, Brazil (Garcia
114 and Vinciprova 2003). We studied the São Francisco de Paula population, which is
115 restricted to the Floresta Nacional de São Francisco de Paula (FLONA). The native
116 Mixed Ombrophilous Forest is dominated by the Parana pine *Araucaria angustifolia*
117 (Sonego et al. 2007) and covers about 56% of the 1606.70 ha area of the FLONA, the
118 remainder being composed of planted *A. angustifolia*, *Pinus* spp. and *Eucalyptus* spp.
119 (Bonatti et al. 2006). The climate is classified as Temperate Superhumid (Maluf 2000),
120 with temperatures ranging from -3–18°C in winter months and 18.3–27°C in the
121 summer months, and an annual average of 14.5°C. The region has high levels of rainfall
122 in all months, with average monthly precipitation of more than 200 mm for all months
123 of the year (Buriol et al. 2009) and average annual precipitation of more than 2,200 mm
124 (Backes et al. 2005).

125 We collected field data from October 2008 to February 2009 (127 nights) at a
126 temporary stream that forms following heavy rainfall (29°25'41.3" S 50°23'44.5" W,
127 866 m above sea level) and the adjacent forest. The breeding site is approximately 20 m
128 long and 4 m wide and is located at the edge of a small, unpaved road on a rocky
129 outcrop partially covered by a thin layer of topsoil and vegetation dominated by grasses
130 (Poaceae) and herbs (*Eryngium* sp.). Except for the small road, which is used very
131 rarely, the area around the stream is composed of forest (planted *Araucaria*
132 *angustifolia*), with shrubs and saw-grass (Cyperaceae) between the temporary stream
133 and the forest. This is the only locality in the municipality of São Francisco de Paula
134 where *Melanophryniscus cambaraensis* is known to breed, and this is the only species
135 of amphibian known to reproduce in this temporary stream.

136 We captured migrating individuals using two methods. First, we installed three
137 sets of pitfall traps at 7.5 m, 15 m, 30 m, 60 m, and 120 m on each side of the breeding
138 site, totaling 30 sets of traps. Each set of pitfall traps consisted of four buckets buried in
139 the ground and connected by drift-fences that intersected individuals in movement
140 (Corn 1994). Each trap was composed of three plastic fences (3 m long, 40 cm high,
141 buried 5 cm into the ground) arranged in a “Y” with a 4 L bucket buried at the vertex
142 and tips of the “Y”. We drilled small holes in the bottom of each bucket to allow water
143 to escape. During the course of the study (in December 2008), we installed another three
144 sets of pitfall traps 240 m from breeding site on one side of the stream. Second, we
145 completely encircled the breeding site with drift fences (approximately 51 m long and
146 40 cm high) and installed 34 funnel traps to capture individuals in pre- and post-
147 reproductive migration (Santos et al. 2010).

148 We checked traps twice daily, just before sunrise and sunset. All captured
149 individuals were photographed and marked by toe-clipping, the most common

150 technique to mark anurans (Ferner 2007). After processing, individuals captured in
151 funnel traps were released on the opposite side of the fence and individuals captured in
152 pitfall traps were released next to the traps in which they was captured.

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

158

159 Statistical analyses

160

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

167 We performed a χ^2 -test to determine if the observed day and night captures
168 differed significantly from a 50:50 expectation. We used a multiresponse permutation
169 procedure in the BLOSSOM software package (version W2008.04.02; Cade and
170 Richards 2005) to determine if the environmental variables differed significantly
171 between day and night intervals, using a Monte Carlo approximation with 4,999
172 replications to obtain the *P*-values. To determine if diel activity could be attributed to
173 variation in environmental variables, we performed a partial Mantel test (Smouse et al.
174 1986) in the program NTSYSpC (Rohlf 2000) to evaluate the correlation of captures and

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

180

181 **Results**

182

183 We captured 147 individuals (51 males and 96 females) 502 times, 439 (87%) during
184 the day and 63 (13%) at night. All captures were associated with explosive breeding
185 events. Excluding pseudoreplicates, our dataset included 127 individuals (86%; 82%
186 following correction for unequal day length) captured during the day and 20 individuals
187 (14%; 28 and 18% following correction) captured at night (Fig. 2). These values for
188 diurnal and nocturnal captures were significantly different from a 50:50 expectation (χ^2
189 = 63.24; $P < 0.001$).

190 Environmental data are summarized in Table 1. Among the environmental
191 variables we tested, only temperature ($P = 0.0002$) and relative air humidity ($P =$
192 0.0002) varied significantly between day and night. Rainfall ($P = 0.259$) and barometric
193 pressure ($P = 0.561$) were not significantly different between day and night.

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

198

199 **Discussion**

200

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

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

222 Thermal dependence of locomotor performance could explain the association of
223 greater migratory activity with higher (diurnal) temperatures. Although data on
224 locomotor performance are lacking for *Melanophryniscus cambaraensis*, two

225 considerations suggest that this is unlikely to be a causal factor. First, during our study
226 migratory activity was recorded over a broad range of temperatures covering almost 15
227 °C, with considerable variation during both day (11.9–25.2 °C) and night (11.3–20.1
228 °C). Second, although our study was confined to spring and summer months, explosive
229 breeding events occur on cold days in winter as well. For example, we observed males
230 calling at air temperatures as low as 7.4 °C during a mid-winter breeding event on 11
231 August 2009 (V. Z. Caorsi, R. R. Santos and T. Grant, unpublished data). The
232 temperatures at which anurans achieve maximum locomotor performance vary
233 considerably across species (e.g. Knowles and Wiegand 1990; Navas 1996), and our
234 observations of migration and breeding activity suggest that *M. cambaraensis* is adapted
235 for locomotor activity over a broad range of temperatures. This hypothesis is also
236 consistent with the results of the partial Mantel test that showed that environmental
237 variables do not account for the diurnal-only migratory activity.

238 Given the inability of environmental variables to account for the diurnal-only
239 migratory activity of *Melanophryniscus cambaraensis*, we suggest that the explanation
240 lies not in current functions and selective pressures but rather in phylogenetic history.
241 Although most bufonids are nocturnal, “basal” bufonid genera are characteristically
242 diurnal, including *Atelopus*, *Dendrophryniscus*, and *Melanophryniscus* (McDiarmid
243 1971; Lötters 1996). Further, Grant et al. (2006) found bufonids to be imbedded within
244 Agastrophrynia as the sister group of Nobleobatia, a clade formed by the diurnal
245 families Aromobatidae, Dendrobatidae, and Hylodidae (Fig. 3). Parsimonious
246 optimization of diel activity indicates that the shift from nocturnal to diurnal behavior
247 occurred in the common ancestor of Agastrophrynia. As such, diurnal activity is
248 primitive in *Melanophryniscus* and the nocturnal activity of most bufonids is due to a
249 subsequent reversal in diel activity.

250 Given that species of *Melanophryniscus* are primitively diurnal, what demands a
251 proximate evolutionary explanation is not the diurnal migratory activity of *M.*
252 *cambaraensis* but rather the nocturnal breeding activity. We hypothesize that nocturnal
253 breeding activity resulted from the extremely short duration of the explosive
254 reproductive events of in this species. Over such short intervals, individuals capable of
255 extending their reproductive activity beyond daylight hours would greatly increase their
256 fitness relative to those that are restricted to diurnal activity. The fitness advantage from
257 continuous breeding would provide a strong selective pressure to shift to 24 hour
258 activity.

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

275 as a result of the diurnal activity that brought them into contact with visually oriented
276 diurnal predators, and not the other way around.

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

284

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299

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Tables

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 \pm 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.

| | N | Diurnal vs. nocturnal <i>P</i> | Maximum | | Minimum | | Mean | | Standard Deviation | |
|--------------------------|-----|---|---------|-----------|---------|-----------|---------|-----------|--------------------|-----------|
| | | | Diurnal | Nocturnal | Diurnal | Nocturnal | Diurnal | Nocturnal | Diurnal | Nocturnal |
| Temperature | 139 | 0.0002 | 26.4 | 21.7 | 10.3 | 9.8 | 19.6 | 15.9 | 3.4 | 2.6 |
| Relative air humidity | 139 | 0.0002 | 99.0 | 99.0 | 46.9 | 74.3 | 77.7 | 90.2 | 12.2 | 5.4 |
| Rainfall | 125 | 0.259 | 61.5 | 44.8 | 0.0 | 0.0 | 3.2 | 2.5 | 8.3 | 7.1 |
| Barometric pressure | 139 | 0.561 | 927.0 | 925.4 | 913.0 | 912.7 | 919.9 | 919.6 | 2.9 | 2.7 |

Figures

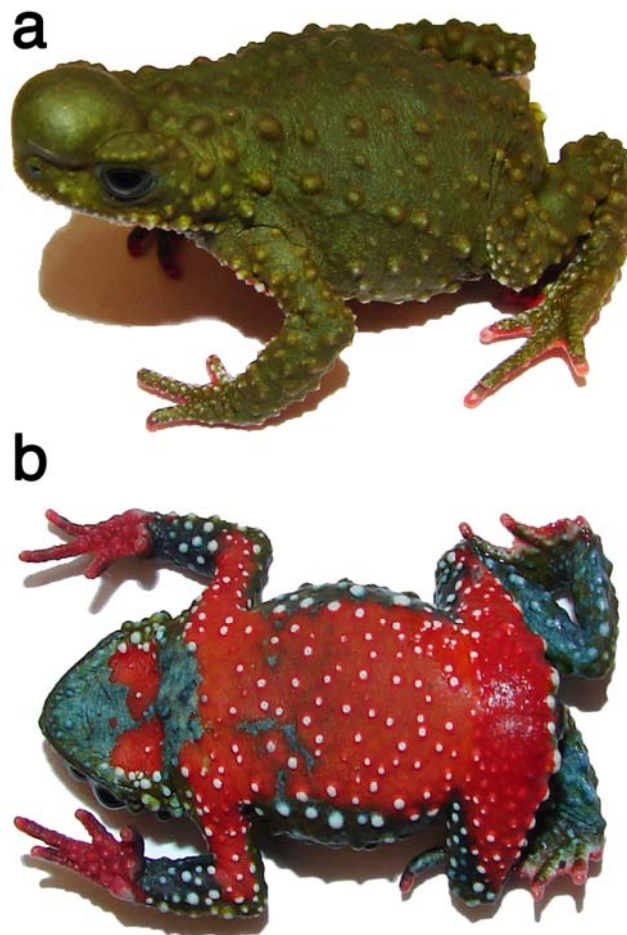


Figure 1. *Melanophryniscus cambaraensis* showing (a) green dorsal and (b) contrasting bright red ventral coloration. Defensive chemicals are secreted from skin glands, including the massive frontal gland on the dorsal surface of the head, visible in (a).

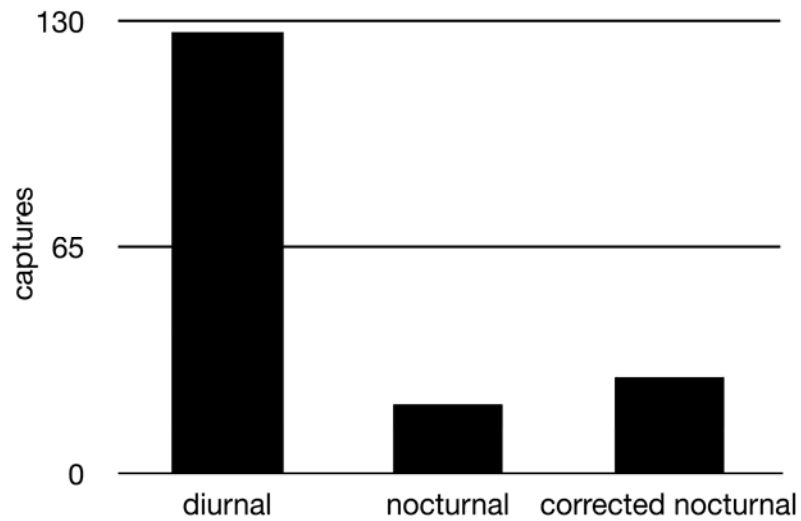


Figure 2. Numbers of individuals captured migrating during day and night intervals, excluding recaptures. Absolute numbers including recaptures are much greater, but proportions are almost identical (see text). To account for greater day length we multiplied nocturnal captures by 1.4 (corrected nocturnal) and used the corrected value in statistical analyses.

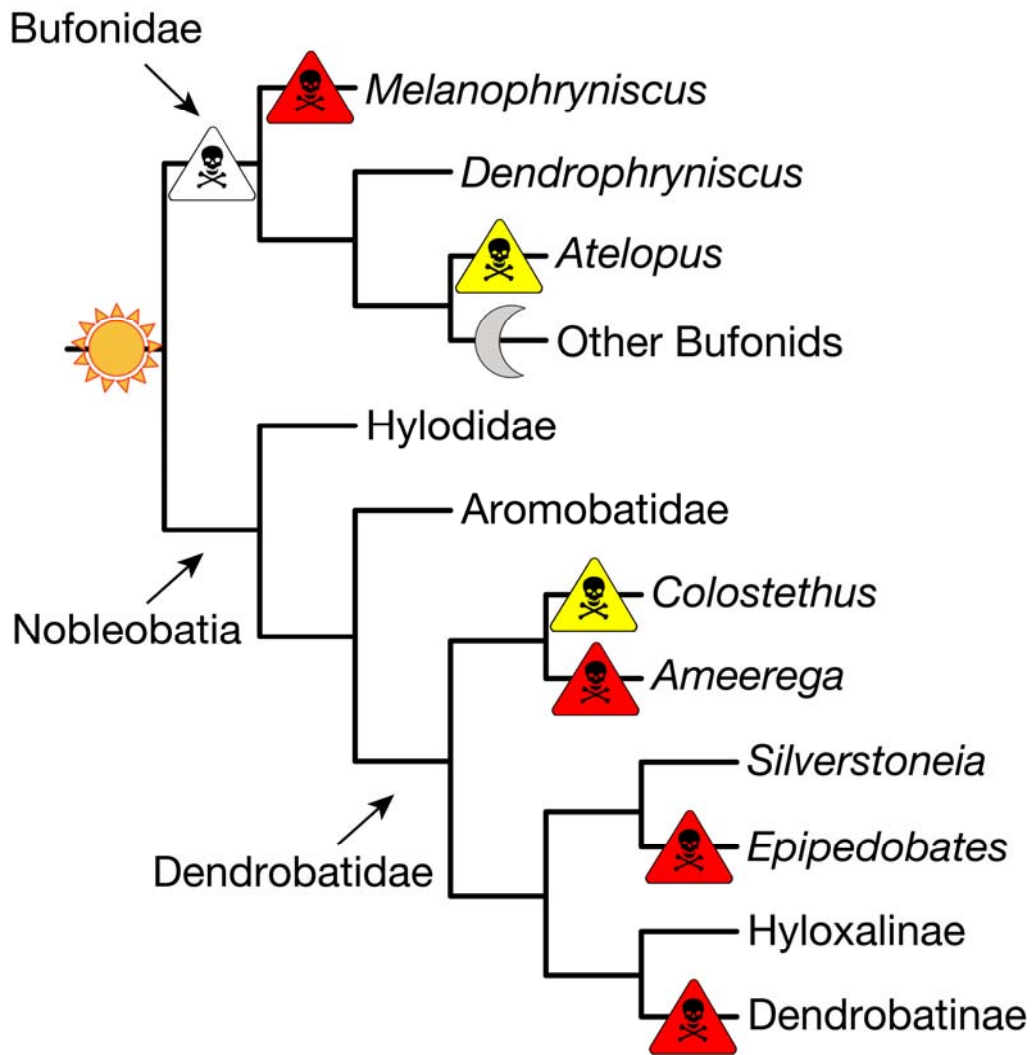


Figure 3. Phylogenetic relationships of Agastorophrynia, adapted from Grant et al. (2006), showing the origin of diurnal activity (sun) in the ancestor of Agastorophrynia and the return to nocturnality within Bufonidae (moon), as well as the evolution of noxious or toxic chemical defenses (skull and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

1 Temporal Migration Patterns in an Aseasonal Explosive Breeding Toad from Brazil

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15

16 Temporal Migration Patterns in an Aseasonal Explosive Breeding Toad from Brazil

17

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

19

20 **Abstract**

21 The temporal patterns of migration of the aseasonal explosive breeding toad

22 *Melanophryniscus cambaraensis* were studied from October 2008 to February 2009 in

23 São Francisco de Paula, Brazil. Migrating toads were captured with sets of drift fences

24 and pitfall traps in the forest and drift fences completely enclosing the breeding site with

25 inward and outward-facing funnel traps. Spectral analysis was used to explore the

26 cyclical patterns of data. Cross-correlations were used to select the best potential

27 predictors on the appropriate time lags. Stepwise backward multiple regression analysis

28 was used to construct a linear model to predict of migratory activity. Five major and

29 three minor peaks of migratory activity were identified, with no differences between

30 males and females. The periodogram identified two main migratory cycles: one lasting

31 23 days and another 13.8 days. The oscillations of 23 days correspond with fluctuations

32 of close rainfalls, and based on the strong association of migratory activity with rainfall,

33 we propose that the analysis get near migratory events together, considering as only one

34 event. Therefore we suggest that the duration cycle that best applies to *M. cambaraensis*

35 is 13.8 days. The highest correlation values as predictors of migration activity were

36 mean relative air humidity (0.337) and accumulated rainfall of 72 h (0.856), both at lag

37 zero. The accumulated rainfall of the previous 72 h was the only predictor of number of

38 captures, accounting for 73% of variation.

39 **Introduction**

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

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

57 There are several other studies that correlate the timing and magnitude of migration
58 with selected environmental variables (e.g. date, temperature, precipitation) (Semlitsch
59 1985; Sinsch 1988; Greenberg and Tanner 2005; Timm et al. 2007), however the
60 Brazilian red bellied toad *Melanophryniscus cambaraensis* differs from all previously
61 studied species in breeding aseasonally. That is, despite inhabiting a subtropical region
62 characterized by well defined seasons, explosive breeding events occur repeatedly at
63 irregular intervals over the course of the entire year. Other species in this region breed

64 seasonally, with reproductive activity explained by photoperiod and not variation in
65 temperature, rainfall, or air humidity (Both et al. 2008). We have observed explosive
66 breeding events in *M. cambaraensis* in October, December, January, February, and
67 August, and breeding has been recorded in all remaining months by numerous workers
68 (Garcia and Vinciprova 2003; P. Garcia, unpublished data; P. Colombo, pers. comm.;
69 A. Samir, pers. comm.). As in other species that form explosive breeding aggregations,
70 males of *M. cambaraensis* exhibit many of the typical strategies of scramble
71 competition (Wells 2007), including intense male combats and struggles to possess
72 females and displace amplexant males (V. Z. Caorsi, R. R. Santos and T. Grant,
73 unpublished data).

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

86

87 **Materials and methods**

88 **Study site**

89 *Melanophryniscus cambaraensis* is a vulnerable (Garcia and Vinciprova 2003)
90 species endemic to the subtropical southeastern Araucaria Plateau in the Campos de
91 Cima da Serra micro-region of Rio Grande do Sul state, Brazil. Only two isolated
92 populations of this species have been discovered, each known from single localities
93 separated by approximately 50 km in the municipalities of Cambará do Sul and São
94 Francisco de Paula in Rio Grande do Sul state, Brazil (Garcia and Vinciprova 2003).
95 We studied the São Francisco de Paula population, which is restricted to the Floresta
96 Nacional de São Francisco de Paula (FLONA). The native Mixed Ombrophilous Forest
97 is dominated by the Parana pine *Araucaria angustifolia* (Sonego et al. 2007) and covers
98 about 56% of the 1606.70 ha area of the FLONA, the remainder being composed of
99 planted *A. angustifolia*, *Pinus* spp. and *Eucalyptus* spp. (Bonatti et al. 2006). The
100 climate is temperate, with temperatures ranging from -3–18°C in winter months and
101 18.3–27°C in the summer months, and an annual average of 14.5°C. The region has high
102 levels of rainfall in all months, with average monthly precipitation of more than 200
103 mm for all months of the year (Buriol et al. 2009) and average annual precipitation of
104 more than 2,200 mm (Backes et al. 2005).

105 Field data were collected from October 2008 to February 2009 at a temporary stream
106 formed by heavy rainfalls (29°25'41.3" S 50°23'44.5" W, 866 m above sea level) and
107 adjacent forest. The breeding site is approximately 20 m long and 4 m wide and is
108 located at the edge of a small unpaved road on a rocky outcrop partially covered by a
109 thin layer of topsoil and vegetation dominated Poaceae and Apiaceae. Except for the
110 small road, which is used very rarely, the area around the stream is composed of forest
111 (planted *Araucaria angustifolia*), with shrubs and saw-grass (Cyperaceae) covering the
112 few meters between the temporary stream and the forest proper. This is the only locality
113 in the municipality of São Francisco de Paula where *Melanophryniscus cambaraensis* is

114 known to breed, and this is the only species of amphibian that reproduces in this
115 temporary stream, although *Aplastodiscus perviridis* (Hylidae), *Ischnocnema henselii*
116 (Brachycephalidae), *Hypsiboas marginatus* (Hylidae), *Leptodactylus araucaria*
117 (Leptodactylidae), and *Rhinella icterica* (Bufonidae) also inhabit the adjacent forest.
118

119 **Sampling procedure**

120 Migrating individuals were captured using two methods. We installed three sets of
121 pitfall traps at 7.5 m, 15 m, 30 m, 60 m, and 120 m on each side of the breeding site,
122 totaling 30 sets of traps. Each set of pitfall traps consisted of four buckets buried in the
123 ground and connected by drift-fences that intersected individuals in movement (Corn
124 1994). Each trap was composed of three plastic fences (3 m long, 40 cm high, buried 5
125 cm into the ground) arranged in a “Y” with a 4 L bucket buried at the vertex and tips of
126 the “Y”. We drilled small holes in the bottom of each bucket to allow water to escape.
127 During the course of the study (in December 2008), we installed another three sets of
128 pitfall traps 240 m away from breeding site on one side of the stream. In addition to
129 pitfall trap arrays in the forest, we completely encircled the breeding site with drift
130 fences (approximately 51 m in long and 40 cm high) and installed 34 funnel traps (5 L
131 plastic bottles with the top third cut, inverted and inserted into the base of the bottle)
132 outward and inward-facing to capture individuals in pre- and post-reproductive
133 migration (Santos et al. in review).

134 We checked traps twice daily, just before sunrise and sunset, from October 2008 to
135 February 2009, totaling 139 days, from first to last sampling day. All captured
136 individuals were photographed and marked by phalangeal amputation, the most
137 common technique to mark anurans (Ferner 2007). After processing, individuals

138 captured in funnel traps were released on the opposite side of the fence and individuals
139 captured in pitfall traps were released next to the trap in which it was captured.

140

141 **Environmental variables**

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

148

149 **Statistical analysis**

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

156 We used spectral analysis to explore the cyclical patterns of the data. The purpose of
157 the analysis is to decompose a complex time series with cyclical components into a few
158 underlying sine and cosine functions of different frequencies, in order to identify those
159 that are particularly strong (Legendre and Legendre 1998). The periodogram quantifies
160 this strength. Spectral analysis requires temporal contiguity, so the few missing values
161 were completed by interpolation from adjacent points. We believe that the missing
162 values did not have a negative effect on the analysis, since they were a small number

163 (only six missing days, corresponding to 4.3% of the total). To compare the periodicity
164 of males and females, we applied cross spectral analyses on ln-transformed data. This is
165 an extension of single spectral analysis to the simultaneous analysis of two series
166 (Legendre and Legendre 1998). All spectral analyses were performed using a Hamming
167 smoothing window with a width of 5.

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

179

180 **Results**

181 **Summary of capture data**

182 In 5 mo of sampling we obtained 498 captures (237 males, 257 females and 4
183 juveniles). The total number of individuals captured was 154 (53 males, 97 females and
184 4 juveniles).

185

186 **Autocorrelation**

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

191

192 **Spectral analysis**

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

201 Periodogram values at different frequencies are shown in Fig. 3. We found two high
202 periodogram values, the largest one (670.7) was at a frequency of 0.043, corresponding
203 to a period of 23 days, which is the number of days necessary to complete one full
204 cycle. The other one was 608.5 at the frequencies 0.072, corresponding to a period of
205 13.8 days. Males and females showed no difference; the peaks of migration activity
206 were the same (Fig. 4), and the frequencies were approximately the same when
207 considering the total number of captures (Fig. 5).

208

209 **Cross-correlations and multiple regression**

210 Mean temporal patterns in environmental variables and the number of captures
211 through time are shown in Fig. 6. The significant environmental variables and their

212 respective lags are shown in Table 3. Among the sampled variables, only relative air
213 humidity (mean and minimum) and rainfall (accumulated 24 h, 48 h, 72 h, 7 and 30
214 days) had a correlation value above 0.3. Considering the correlation between these
215 variables, we chose only those with highest correlation values as predictors of migration
216 activity: mean relative air humidity (0.337) and accumulated rainfall of the previous 72
217 h (0.856), both at lag zero. The accumulated rainfall of the previous 72 h was the only
218 predictor of number of captures, accounting for 73% of their variation ($R^2 = 0.733$,
219 $F_{2,139} = 359.437$, $P > 0.001$; Fig. 7).

220

221 **Discussion**

222 Studies involving migration patterns are normally carried out with seasonal species
223 that reproduce once per year (e.g., Semlitsch 1985; Sinsch 1988; Greenberg and Tunner
224 2005), and migrations are limited to a rather short time of the annual activity period
225 (Sinsch 1990). To obtain replicates of migratory events in such species it is necessary
226 that the study last several years. Migration of *Melanophryniscus cambaraensis* is a good
227 object of study because multiple migration events occur in a few months. Our data
228 indicate that over a five month interval there were five major and three minor migratory
229 events.

230 The periodogram identified two main migratory cycles: one lasting 23 days and the
231 other 13.8 days, included in these time pre- and post-reproductive migrations and the
232 reproduction activity. The analysis probably get two near migratory events together,
233 considering as only one event, in a cycle of migration of 23 days. For example,
234 migration peaks 1 and 2 (Table 1; Fig. 2) occurred in October were possibly considered
235 as one event because of the temporal dependence of the data. The strong association of
236 migratory events of *Melanophryniscus cambaraensis* with rainfall may explain this

237 cycle of 23 days, since there were at least two events of consecutive rainfalls
238 accompanied by close spaced migration, then the analysis identified as a single cycle.
239 The oscillations of 23 days correspond with fluctuations in close rainfalls (Fig. 6e).
240 Therefore we propose that the duration cycle that best applies to *M. cambaraensis* is
241 13.8 days (the second highest value of periodogram), although consecutive cycles are
242 common given the rainfall in the region. In the example cited above, the peaks 1 and 2
243 were considered as different events in the cycle of 13.8 days. We confirmed the absence
244 of migration and reproduction on dates between the identified peaks, which supports
245 this cycle. The smaller peaks of migratory activity in October and January (6 and 8,
246 Table 2) are probably related to the close proximity of major peaks events in those
247 months, as the number of captures between the peaks did not decline completely (Fig.
248 2). However, the minor peak that occurred in December (peak 7) is certainly a different
249 event since it is widely separated from other peaks of migration.

250 Among amphibians, variation between the migratory patterns of males and females
251 of a species is common and has evolved to maximize reproductive success (Russel et al.
252 2005; Semlitsch 2008). Males tend to arrive earlier than females, which maximizes the
253 chances of breeding, while females often come later, which maximizes the mate choice
254 (Douglas 1979). This happens, for example, in the salamanders *Ambystoma*
255 *jeffersonianum* and *A. maculatum*, in which males tend to migrate earlier than females,
256 since males seem to have a lower threshold for environmental cues, and will migrate as
257 soon as the minimal conditions for migration are met (Douglas 1979; Sexton et al.
258 1990). However, in this study, males and females showed no differences in the peaks of
259 migration. We suppose that the females do not wait because they have only a few days
260 to reproduce.

261 The multiple regression analysis showed that only rainfall accumulated in 72 h was a
262 significant predictor of migratory activity, accounting for 73% of variance. Many
263 studies have correlated amphibian migrations with heavy rains (e.g., Semlitsch 1985;
264 Greenberg and Tanner 2005). Total daily rainfall is often the most important climatic
265 factor influencing the magnitude of amphibian movements (Tood and Winne 2006;
266 Timm et al. 2007). For example, Greenberg and Tunner (2004) found that heavy rains
267 influence the temporal patterns of *Scaphiopus holbrookii* breeding biology by
268 potentially eliciting an explosive breeding response. Spieler and Linsenmair (1998) also
269 found that rainfall was the best predictor, explaining 26% of migration activity of
270 *Hoplobatrachus occipitalis*.

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

280

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292

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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.

| Date | Migration peak | Time (days) | Number of captures | Number of individuals |
|-------------------|----------------|-------------|--------------------|-----------------------|
| October 15, 2008 | 1 | 5 | 18 | 17 |
| October 16, 2008 | 1 | 6 | 12 | 10 |
| October 26, 2008 | 2 | 16 | 27 | 25 |
| October 27, 2008 | 2 | 17 | 17 | 16 |
| October 28, 2008 | 2 | 18 | 10 | 10 |
| January 3, 2009 | 3 | 85 | 20 | 20 |
| January 4, 2009 | 3 | 86 | 22 | 19 |
| January 5, 2009 | 3 | 87 | 25 | 24 |
| January 6, 2009 | 3 | 88 | 12 | 12 |
| January 19, 2009 | 4 | 101 | 13 | 13 |
| January 20, 2009 | 4 | 102 | 24 | 22 |
| January 21, 2009 | 4 | 103 | 23 | 23 |
| February 16, 2009 | 5 | 129 | 17 | 17 |
| February 17, 2009 | 5 | 130 | 34 | 30 |
| February 18, 2009 | 5 | 131 | 32 | 28 |
| February 19, 2009 | 5 | 132 | 20 | 20 |
| February 20, 2009 | 5 | 133 | 11 | 10 |

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.

| Date | Migration peak | Time (days) | Number of captures | Number of individuals |
|-------------------|----------------|-------------|--------------------|-----------------------|
| October 22, 2008 | 6 | 12 | 11 | 10 |
| December 15, 2008 | 7 | 66 | 10 | 10 |
| January 9, 2009 | 8 | 91 | 11 | 11 |

Table 3. Cross-correlation coefficients between number of captures and environmental variables and their respective lags. Only the significant environmental variables are shown ($P < 0.001$).

| Variable | Lag | Cross-correlation | Std Error | Variable | Lag | Cross-correlation | Std Error |
|-------------------------------------|-----|-------------------|-----------|---------------------|-------|-------------------|-----------|
| Photoperiod | -8 | -0.199 | 0.087 | Rainfall 48 h | -1 | 0.444 | 0.085 |
| | -7 | -0.225 | 0.087 | | 0 | 0.790 | 0.085 |
| | -6 | -0.233 | 0.087 | | 1 | 0.695 | 0.085 |
| | -5 | -0.224 | 0.086 | | 2 | 0.370 | 0.085 |
| | -4 | -0.212 | 0.086 | Rainfall 72 h | -2 | 0.323 | 0.085 |
| | -3 | -0.200 | 0.086 | | -1 | 0.686 | 0.085 |
| | -2 | -0.187 | 0.085 | | 0 | 0.856 | 0.085 |
| | -1 | -0.174 | 0.085 | | 1 | 0.628 | 0.085 |
| Mean temperature | 13 | 0.247 | 0.089 | 2 | 0.312 | 0.085 | |
| | 14 | 0.248 | 0.089 | Rainfall 7 days | -5 | 0.364 | 0.086 |
| Minumum temperature | -8 | 0.194 | 0.087 | | -4 | 0.547 | 0.086 |
| | -7 | 0.185 | 0.087 | | -3 | 0.607 | 0.086 |
| | 13 | 0.203 | 0.089 | | -2 | 0.634 | 0.085 |
| Maximum temperature | 0 | -0.193 | 0.085 | | -1 | 0.633 | 0.085 |
| | 13 | 0.223 | 0.089 | | 0 | 0.586 | 0.085 |
| | 14 | 0.260 | 0.089 | 1 | 0.368 | 0.085 | |
| Mean relative air humidity | -8 | 0.225 | 0.087 | Rainfall 30 days | -9 | 0.184 | 0.088 |
| | -7 | 0.192 | 0.087 | | -8 | 0.236 | 0.087 |
| | 0 | 0.337 | 0.085 | | -7 | 0.288 | 0.087 |
| | 1 | 0.280 | 0.085 | | -6 | 0.313 | 0.087 |
| | 13 | -0.180 | 0.089 | | -5 | 0.312 | 0.086 |
| | 14 | -0.240 | 0.089 | | -4 | 0.335 | 0.086 |
| Minimum relative air humidity | -8 | 0.190 | 0.087 | | -3 | 0.345 | 0.086 |
| | -7 | 0.189 | 0.087 | | -2 | 0.347 | 0.085 |
| | 0 | 0.331 | 0.085 | | -1 | 0.350 | 0.085 |
| | 1 | 0.250 | 0.085 | | 0 | 0.303 | 0.085 |

| | | | | | | | |
|-------------------------------|-----|--------|-------|--|----|--------|-------|
| | 14 | -0.202 | 0.089 | | 3 | -0.256 | 0.086 |
| Maximum relative air humidity | -10 | 0.198 | 0.088 | | 4 | -0.266 | 0.086 |
| | -9 | 0.187 | 0.088 | | 5 | -0.255 | 0.086 |
| | -8 | 0.231 | 0.087 | | 6 | -0.230 | 0.087 |
| | -7 | 0.197 | 0.087 | | 7 | -0.224 | 0.087 |
| | 0 | 0.261 | 0.085 | | 8 | -0.223 | 0.087 |
| | 1 | 0.202 | 0.085 | | 9 | -0.216 | 0.088 |
| | 11 | 0.205 | 0.088 | | 10 | -0.189 | 0.088 |
| Mean barometric pressure | -11 | -0.251 | 0.088 | | 11 | -0.184 | 0.088 |
| | -10 | -0.263 | 0.088 | | 12 | -0.194 | 0.089 |
| Minimum barometric pressure | -11 | -0.263 | 0.088 | | 13 | -0.215 | 0.089 |
| | -10 | -0.272 | 0.088 | | 14 | -0.199 | 0.089 |
| Maximum barometric pressure | -11 | -0.239 | 0.088 | | | | |
| | -10 | -0.239 | 0.088 | | | | |
| Rainfall 24 h | 0 | 0.601 | 0.085 | | | | |
| | 1 | 0.665 | 0.085 | | | | |
| | 2 | 0.448 | 0.085 | | | | |

Figures

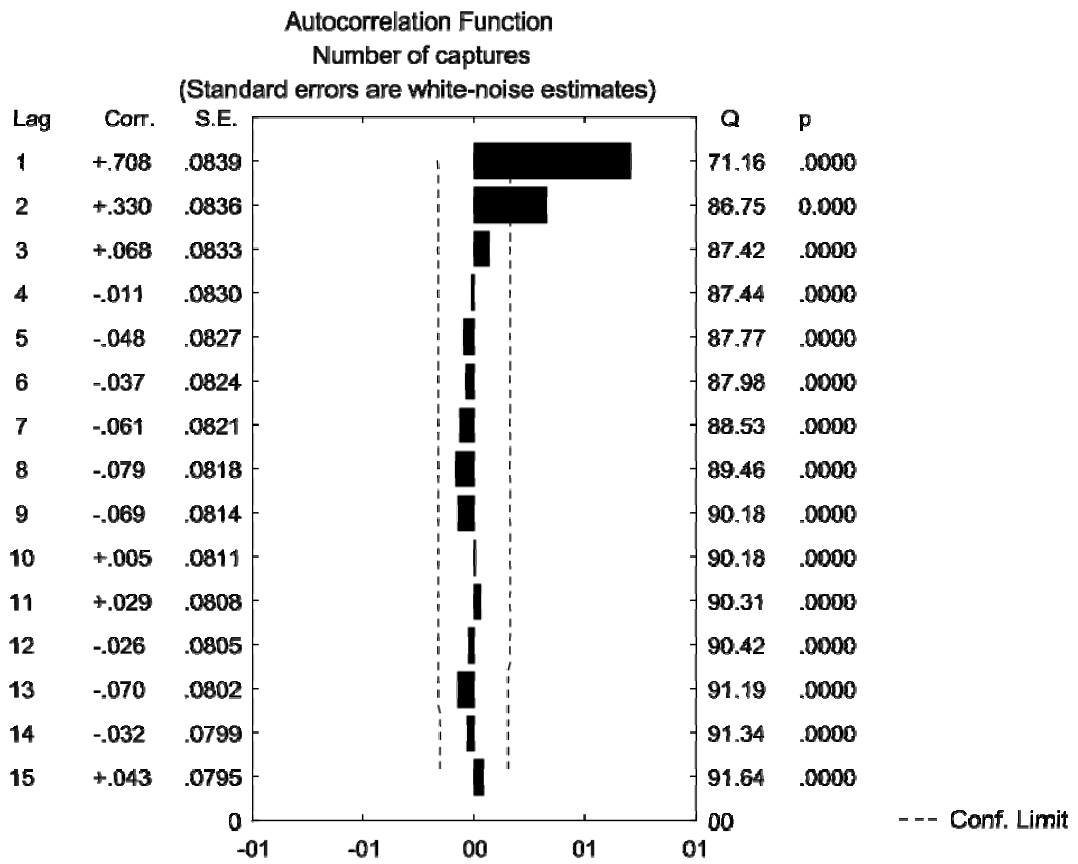


Fig. 1. The autocorrelation values for each lag. They were significant at all lags ($P < 0.001$).

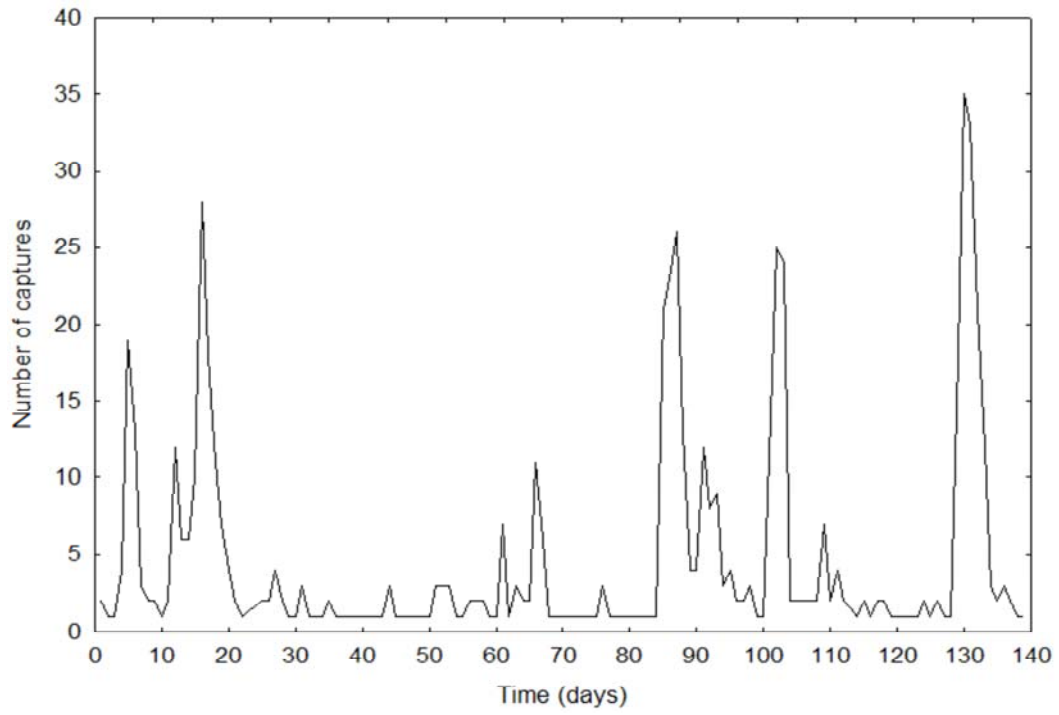


Fig. 2. Number of captures through time. There are five main peaks of migration activity and other three smaller, with around 10 captures.

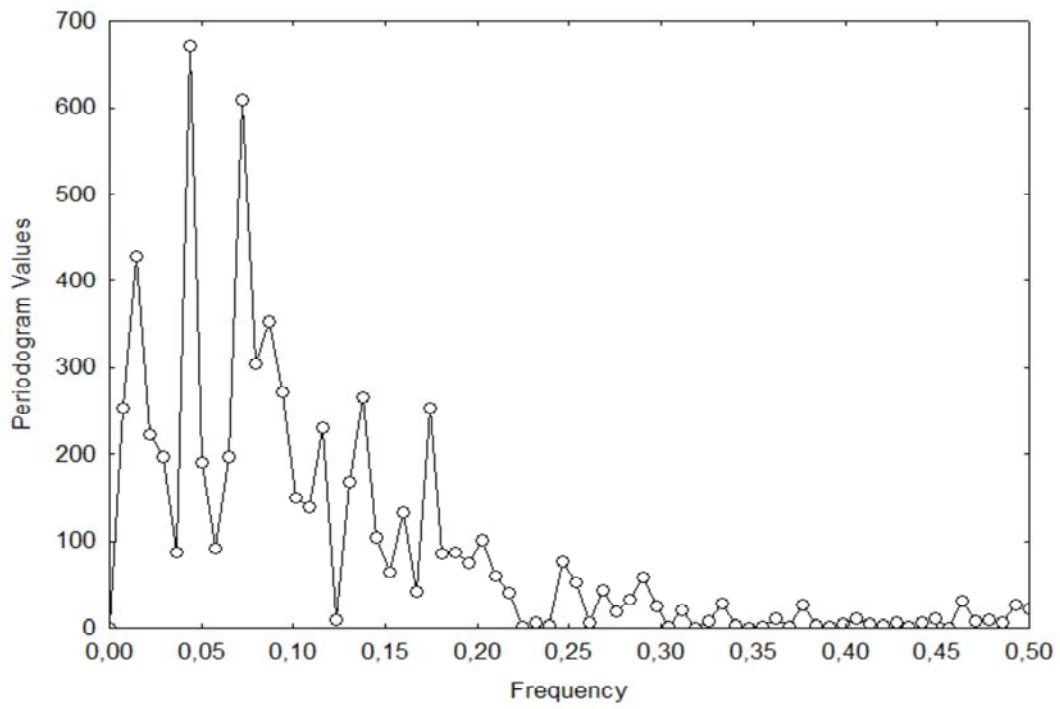


Fig. 3. Periodogram values at different frequencies. Highest periodogram values are 670.7, 608.5 and 428.7, at 0.043, 0.072 and 0.014 frequencies, respectively.

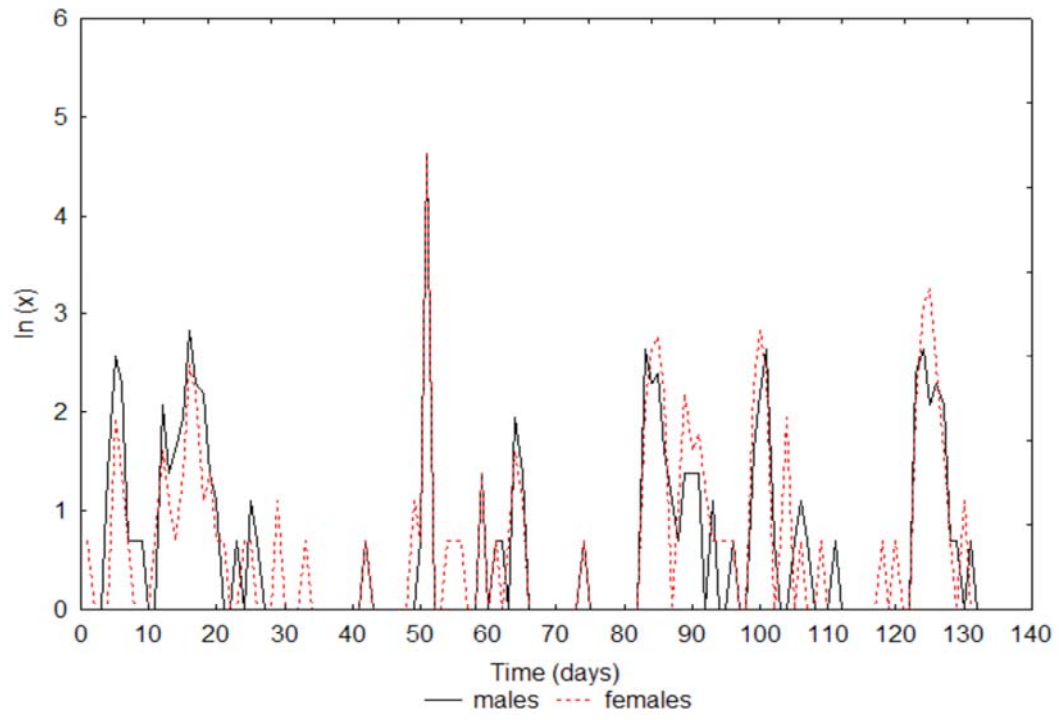


Fig. 4. Numbers (ln-transformed) of males and females captured through time. The peaks of migration activity are the same in both sexes.

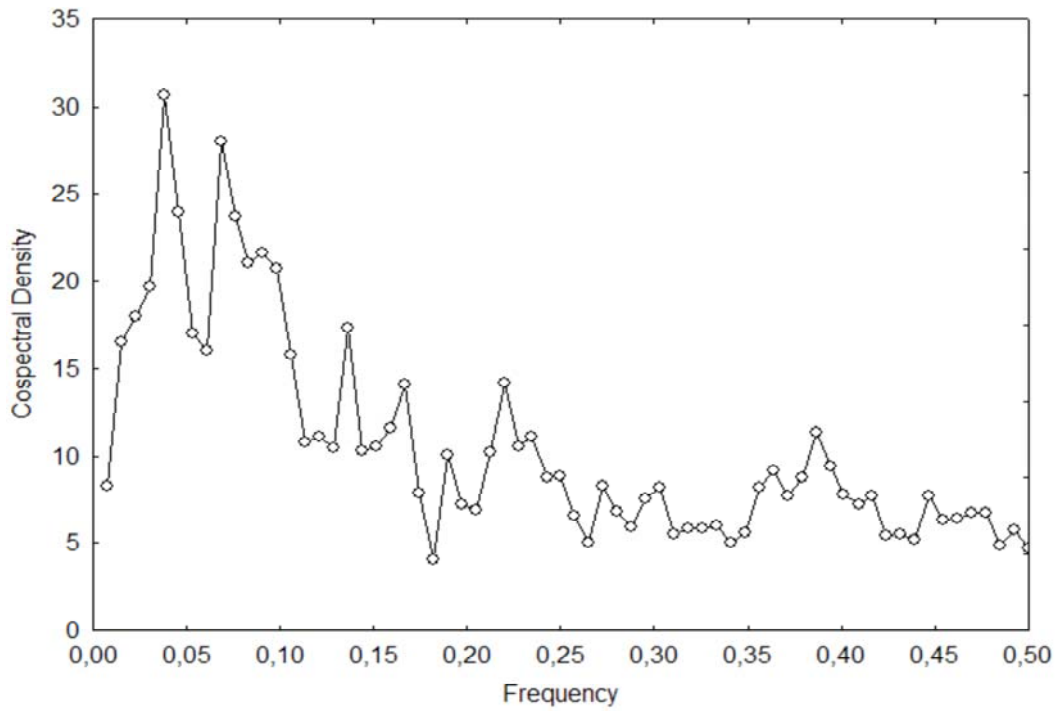


Fig. 5. Cospectral densities of males and females and the frequencies that contribute most to the overall periodic behavior of the series. These frequencies were approximately the same when considering the total number of captures.

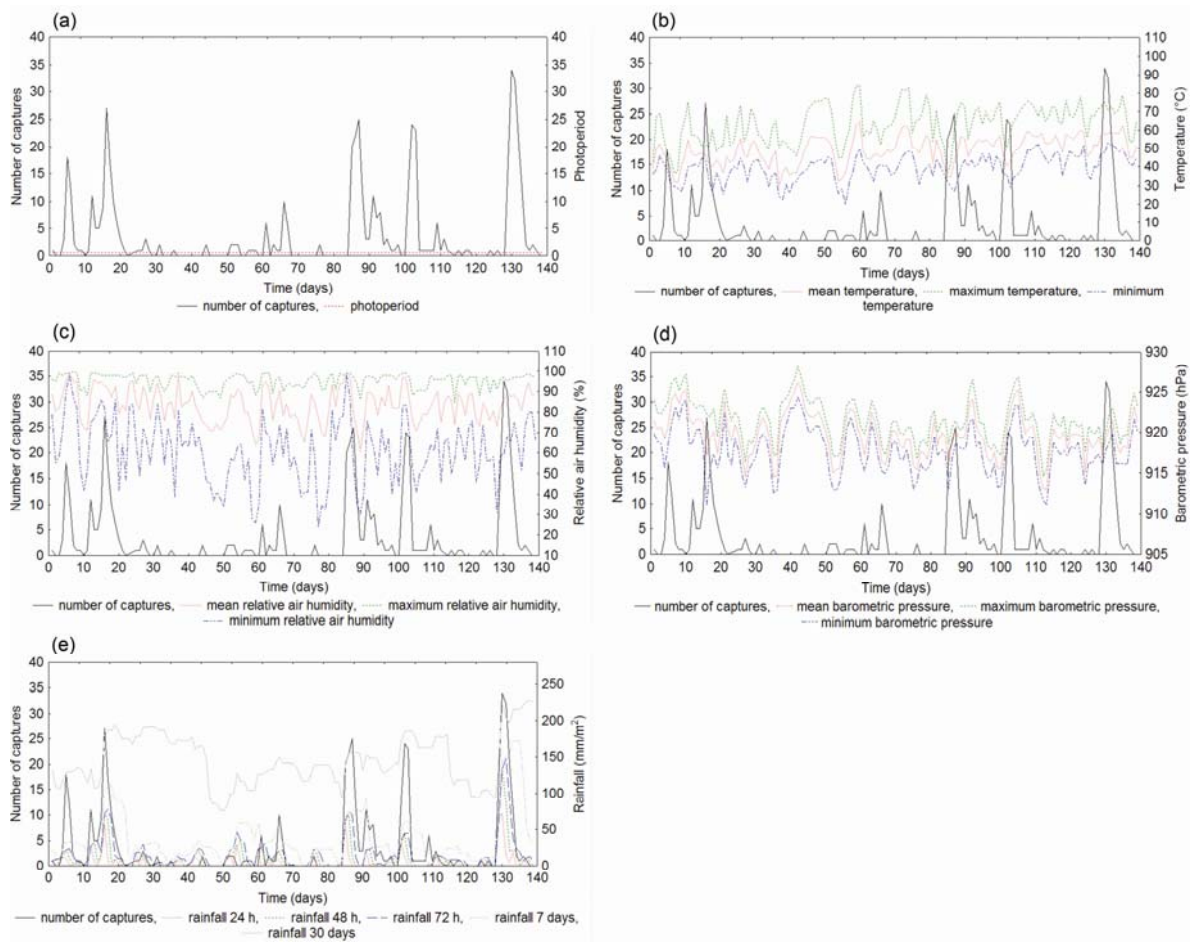


Fig. 6. Mean temporal patterns in environmental variables and the number of captures through time. (a) Photoperiod. (b) Temperature (°C). (c) Relative air humidity (%). (d) Barometric pressure (hPa). (e) Rainfall (mm/m²).

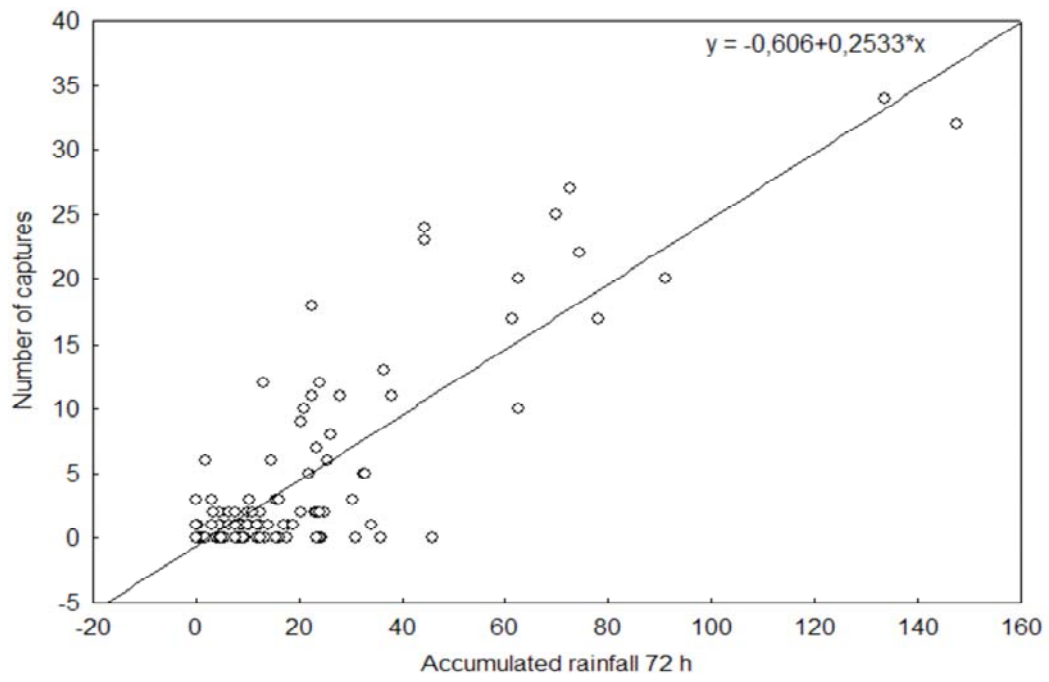


Fig. 7. Relationship between number of captures and rainfall accumulated in 72 h.

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 pseudorélicas (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 pseudorélicas. Quando excluímos as pseudorélicas das análises, os resultados não foram significativos. Isso ilustra a importância de avaliar o potencial efeito da inclusão de pseudorélicas 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 pseudorélicas 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 nessa 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 Agastrophrynia, 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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