

**INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA – INPA  
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**COMPORTAMENTO REPRODUTIVO DO ANURO AMAZÔNICO  
*Allobates paleovarzensis* (AROMOBATIDAE) E O PAPEL DOS  
PARENTAIS NA SOBREVIVÊNCIA DA PROLE**

**SULAMITA MARQUES CORREIA DA ROCHA**

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**PARENTAIS NA SOBREVIVÊNCIA DA PROLE**

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### **Sinopse:**

Estudou-se a territorialidade, comportamento de corte, acasalamento e cuidado parental do anfíbio amazônico *Allobates paleovarzensis*. Avaliou-se o papel de características intrínsecas e extrínsecas do macho sobre o sucesso de acasalamento e o efeito da remoção de machos parentais na sobrevivência da prole.

**Palavras-Chave:** Corte, sucesso de acasalamento, território, cuidado paternal.

Dedico o esforço e os produtos finais do meu mestrado a todos os machos, fêmeas, ovos, embriões e girinos de *Allobates paleovarzensis* que conheci e também aos próximos indivíduos que viverão nas paleo-várzeas da Amazônia. Muita sobrevivência e reprodução bem-sucedida para vocês, meus sapinhos.

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“Coro de cor  
sombra de som  
de cor  
de mal me quer  
de mal me quer  
de bem  
de bem me diz  
de me dizendo assim serei feliz  
serei feliz de flor  
de flor em flor  
de samba em samba em som de vai e vem  
de verde  
verde ver  
pé de capim  
bico de pena  
pio de bem-te-vi  
amanhecendo sim perto de mim  
perto da claridade da manhã  
a grama, a lama tudo  
é minha irmã  
a rama,  
o sapo,  
o salto de uma rã.”

Composição: Caetano Veloso (letra) e João Donato (melodia)

## Resumo

O estudo da biologia reprodutiva dos anfíbios permite delinear padrões entre os diferentes grupos taxonômicos e testar diversas hipóteses evolutivas e comportamentais. Nesse estudo utilizamos a espécie *Allobates paleovarzensis* (Anura: Aromobatidae) como modelo, uma vez que esta exhibe cuidado paternal e territorialidade masculina. Realizamos observações comportamentais e experimentos em campo nos períodos de atividade reprodutiva em duas localidades, onde descrevemos o comportamento de corte, acasalamento, cuidado parental e territorialidade (Capítulo 1); testamos o papel de características intrínsecas e extrínsecas do macho sobre o sucesso de acasalamento (Capítulo 2); e testamos o efeito da remoção de machos parentais na sobrevivência da prole e no comportamento parental da fêmea (Capítulo 3). A maior parte dos estudos sobre esses tópicos em anuros foram conduzidos em cativeiro, e experimentos de campo são importantes para testar as relações comportamentais e a sobrevivência sob condições bióticas e abióticas naturais. Nossas observações indicam que *A. paleovarzensis* é uma espécie poligâmica de cuidado uniparental realizado pelo macho, territorialidade relacionada à competição masculina por espaço e por fêmeas, alto investimento em número de ovos e com evidência de transporte simultâneo do maior número de girinos no gênero. O tamanho do território foi o principal determinante do sucesso de acasalamento dos machos e este fator não teve relação com os demais atributos testados. As fêmeas parecem acessar o tamanho do território do macho por meio do processo de corte que antecede a oviposição, momento em que o macho conduz a fêmea pelo território. O cuidado parental em *Allobates paleovarzensis* é realizado exclusivamente pelo pai, sem nenhuma evidência de participação materna. O macho cuida da prole durante todo o período de desenvolvimento em que esta se encontra associada ao seu território, de ovo até a fase de girino transportado para um corpo d'água. Na ausência do pai, a mortalidade da prole é elevada em todas as fases de desenvolvimento.



# **Reproductive behavior of the Amazonian frog *Allobates paleovarzensis* (Aromobatidae) and the role of the parents on offspring survival**

## **Abstract**

The study of amphibians' reproductive biology allows to outline patterns among different taxonomical groups and to test many evolutionary and behavioural hypotheses. In this study, we used the species *Allobates paleovarzensis* (Anura: Aromobatidae) as a model due to its parental care and male territoriality. We conducted behavioural observations and field experiments during reproductive seasons in two localities, where we described courtship behaviour, mating, parental care and territoriality (Chapter 1); tested the role of intrinsic and extrinsic characteristics of the males in mating success (Chapter 2); and tested the effect of males' removal in the survival rate of the offspring and in the parental behaviour of females (Chapter 3). Most of the studies concerning these topics in anurans were conducted in captivity, and field experiments are important for testing behavioural relations and survival rates under natural biotic and abiotic conditions. Our observations show that *A. paleovarzensis* is a polygamical species with uniparental care by the males, territoriality related to male competition for space and females, high investment on the number of eggs with evidence of the simultaneous transport of the highest number of tadpoles for the genus. Territory size is the main driver for males' mating success, and this determinant was uncorrelated with other tested attributes. Females appear to access males' territorial size during courtship, before mating, when males conduct females around the territory. Parental care in *A. paleovarzensis* is exclusive to males, with no evidence for maternal care. Males nurse the offspring throughout the whole developmental process that occur inside their territories. Without a male providing parental care, offspring mortality is very high in every developmental stage.

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## Introdução Geral

A reprodução é um período determinante na vida de todo ser vivo e diferentes estratégias são adotadas pelos organismos, evolutivamente e por plasticidade ambiental, a fim de elevar o sucesso da reprodução individual (Klug *et al.*, 2012). Dentre as estratégias encontradas entre os animais, comumente observamos o investimento em uma corte elaborada, modelada pelas pressões da escolha sexual e da competição intrasexual, a defesa de um espaço para reproduzir, o ajuste do período reprodutivo em relação ao clima ou a determinadas variações ambientais e a realização de cuidado com os filhotes nos períodos mais vulneráveis da vida, por um ou por ambos os pais (Trivers, 1972). Dessa forma, a reprodução se traduz no período mais custoso da vida dos organismos e qualquer investimento que eleve a aquisição de parceiros, maximize a produção de filhotes e reduza a mortalidade destes pode se estabilizar na população por favorecer o *fitness* individual do investidor (Summers & Tumulty, 2013).

A reprodução é o aspecto mais característico dos anfíbios anuros, visto que muitas espécies são conhecidas, visualizadas ou suas vocalizações identificadas apenas no período reprodutivo (Wells, 2007). Mesmo pessoas não especialistas nessa área de estudo podem se recordar de experiências auditivas de sapos cantando na lagoa para atrair fêmeas ou sobre as distintas fases de vida dos anuros: ovos, girinos, imagos e adultos (Wells, 2007). *Allobates paleovarzensis* (Lima *et al.*, 2010) é um sapo da superfamília Dendrobatoidea que, assim como muitas espécies do grupo, apresenta promiscuidade, territorialidade relacionada à reprodução e cuidado parental por parte do macho. É uma espécie diurna que habita a serapilheira de remanescentes de florestas de paleo-várzeas, sazonalmente alagáveis, da Amazônia Central (Lima *et al.*, 2010). Investigamos aspectos da biologia reprodutiva da espécie, abrangendo a descrição das unidades comportamentais relacionadas à corte, à defesa do território, ao acasalamento, ao cuidado parental e ao ciclo de atividade vocal anual e diária (Capítulo 1); testamos quais características do macho apresentam maior influência no sucesso de acasalamento e na escolha da fêmea (Capítulo 2); e testamos o efeito da remoção de machos cuidadores na sobrevivência da prole e no comportamento das fêmeas parentais (Capítulo 3). Dessa forma, traçamos o conhecimento do comportamento reprodutivo da espécie desde a corte entre os parentais até a sobrevivência dos filhotes sob influência do cuidado paternal, testando hipóteses sustentadas em teorias comportamentais e em padrões encontrados em outros táxons. Adicionalmente, nossos resultados estão contextualizados com

as pressões sociais e ambientais reais em ambiente natural, onde machos e fêmeas possuem uma gama de interesses e decisões relacionadas com o investimento em reprodução, com a competição intrasexual e com os custos e benefícios de realizar cuidado parental.

## **Objetivos**

Geral: Conhecer o comportamento reprodutivo de *Allobates paleovarzensis*, testando o papel de características do macho sobre o sucesso de acasalamento e o efeito da remoção de machos parentais na sobrevivência da prole.

Capítulo 1: Descrever o comportamento de corte, acasalamento, cuidado parental e territorialidade da espécie.

Capítulo 2: Testar o papel de características intrínsecas e extrínsecas do macho sobre o sucesso de acasalamento.

Capítulo 3: Testar o efeito da remoção de machos parentais na sobrevivência da prole, bem como no comportamento parental da fêmea.

## Capítulo 1

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Rocha, S.M.C; Lima, A.P. & Kaefer, I.L. Reproductive behaviour of the Amazonian nurse frog *Allobates paleovarzensis* (Aromobatidae). Manuscrito em preparação para *South American Journal of Herpetology*.

**Reproductive behaviour of the Amazonian nurse-frog *Allobates paleovarzensis*  
(Dendrobatoidea, Aromobatidae)**

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**Abstract.** *Allobates paleovarzensis* is a diurnal leaf-litter anuran of the Dendrobatoidea superfamily inhabiting Amazonian forests. Based on behavioral field observations, conducted between 2008 and 2017 at three localities, we describe the courtship, mating, parental care behavior and territoriality of the species. Vocal activity is more intense in the morning and during the rainy season. Resident males respond aggressively to other males invading their territories, while females are not territorial. Females are attracted by the advertisement call of males and enter their territories to breed. The male courtship call has a lower frequency than the advertisement call, indicating that males can modulate the frequency characteristics of their vocalizations. Amplexus stimulates the release of oocytes by females. Males tend for multiple egg clutches within their territories and transport developed tadpoles on their backs to an aquatic environment. Males may carry up to 60 tadpoles on a single transport event, and this is the largest number ever recorded for *Allobates*. We did not register neither male nor female cannibalism of tadpoles. In summary, we found that *Allobates paleovarzensis* is a polygamous species with uniparental care performed by the male, complex courtship behavior, high fecundity, and territoriality related to male competition for space and females.

**Keywords.** Courtship; mating; parental care; territoriality.

## INTRODUCTION

The neotropical Dendrobatoidea superfamily, composed of 307 species in the families Dendrobatidae and Aromobatidae (Grant et al., 2006; Frost, 2017), includes species with complex reproductive behavior, territoriality, multimodal signaling, prolonged reproductive periods, costly courtship behaviors and a variety of forms of parental care (e.g., Weygoldt, 1987; Lotters et al., 2007; Wells, 2007). Several reproductive modes are present in the group, with different forest strata being used for growth and development of the young, with or without associated development within water bodies (Haddad and Prado, 2005; Wells, 2007). Parental care may involve one or both parents at different stages of development (Lotters et al., 2007).

Among the genera of the Dendrobatoidea superfamily, *Allobates* Zimmermann and Zimmermann, 1988 is the second most species-rich, with 51 described taxa (Grant et al.,



2006; Frost, 2017). However, the reproductive biology of most species never been described. Reproductive traits are useful and often used in determining species limits within the genus, since the morphological similarity between many of them contrasts with the high interspecific variability in advertisement and courtship calls, as well as spawning and parental care (Grant et al., 2006; Tsuji-Nishikido et al., 2012). An exception is *A. femoralis* (Boulenger, 1884), for which several aspects of natural history have been investigated, including courtship behavior (e.g., Montanarin et al., 2011), vocalization activity (e.g., Kaefer et al., 2012), territoriality (e.g., Ringler et al., 2011) and communication (e.g., Narins et al., 2003; Hodl et al., 2004). This accumulation of knowledge about the species has allowed hypotheses-testing about broader issues such as cognition (e.g., Pašukonis et al., 2013, 2014), sexual selection (e.g., Roithmair, 1992), and the evolution of parental care (e.g., McKeon and Summers, 2013; Ringler et al., 2013, 2015, 2016, 2017).

The dendrobatoid frog *Allobates paleovarzensis* Lima, Caldwell, Biavati, and Montanarin, 2010 is a recently described species. While it has already been studied for its genetic, morphological and acoustic traits (Kaefer and Lima, 2012; Kaefer et al., 2013), the reproductive behavior is unknown. Thus, this study aimed to describe vocalization, courtship and mating behavior, parental care and territoriality of *A. paleovarzensis*. This knowledge may aid future studies on the systematics and evolution of reproductive behavioral traits in the genus *Allobates*, and well as in the Dendrobatoidea superfamily in general.

## MATERIALS AND METHODS

Field work was conducted at three localities (WSG84; EPSG: 4623), all in the state of Amazonas, Brazil: municipality of Careiro da Várzea (03°22'26.3"S, 59°52'06.4"W), type-locality of *A. paleovarzensis* (Lima et al., 2010); Hiléia (03°11'52.80"S, 60°26'33.00"W) in the municipality of Manacapuru; municipality of Barcelos (00°54'57.70"S, 62°58'14.30"W). The mean annual precipitation varies from 2000 to 2400 mm (Sombroek, 2001). The rainy season occurs from November to April, and the average annual temperature is about 26°C. *Allobates paleovarzensis* inhabits the litter of Amazonian paleo-várzea forest, which grows on alluvial deposits of Andean origin (Assis et al., 2014). Paleo-várzeas are considered transitions between *terra firme* forests (which are never flooded), and várzea forests (which

are flooded seasonally). The region's floristics contains both várzea and igapó species (Assis et al., 2014).

Data collection was carried out on two sample track grids subdivided into 12 quadrants, each of 10 m<sup>2</sup>. These were visited for six days per week between 04:30h and 18:30h (GMT-04:00). These field visits occurred between November 2008 to April 2011, December 2015 to April 2016 and December 2016 to February 2017 (in Careiro, 402 days), from June 2009 to April 2011 (in Hileia, 131 days) and January to February 2009 (in Barcelos, 10 days). Captured individuals were measured for snout-vent length (SVL in mm), and adults were tagged via toe ablation (Phillott et al., 2007) to allow individual monitoring over time. Ablation occurred on up to two digits on each individual, a procedure that did not interfere with the recapture rates of *A. femoralis* (Ursprung et al., 2011a), and hence is considered non-prejudicial. Individuals were sexed by color: yellow throat in females, and violet throat in males (Lima et al., 2010).

Territories were defined based on the locations of actively vocalizing males, which were monitored from 05:00h to 18:00h on 12 sampling days at each study locality. Females were located and georeferenced at the time of courting or in movement, and these points were used to measure the extent of the areas they inhabited. Ambient air temperature was measured with a digital thermometer each time the number of males vocalizing within the grid was estimated. Such censuses occurred for 10 minutes every hour, from 05:00h to 18:00h, once a week. During these surveys a researcher walked along a standardized track, counting actively vocalizing individuals. Records of daily pattern of vocalization activity was based on 226 hourly censuses, conducted from November to January, the period of most intense vocal activity. Data for monthly total precipitation were obtained using measurements from Pluviometric Station No. 360000 (Hydrological Information System - HidroWeb), located in Manaus, distant about 30 km from Careiro and 110 km from Hileia.

The number of spawns within each territory was estimated by daily censuses, during which surface leaves of the leaf-litter were examined for evidence of spawning. Each spawn was marked with a numbered plastic flag and the developmental stage, following Gosner (1960), was visually estimated every two days. All male individuals carrying tadpoles to bodies of water were tracked and the number of tadpoles attached to their backs was counted.

Behavioral data were obtained by the *ad libitum* and focal animal methods (Altmann, 1974). The number of observations for each behavior ( $n$ ) refers to distinct individuals, thus constituting independent observations. Some behavioral units were observed in all individuals and in these cases the number of samples was omitted. Visual signals were described according to the classification proposed by Hödl and Amézquita (2001), Hartmann et al. (2005) and Montanarin et al. (2011).

We also recorded courtship calls of two males. Each male had his calls recorded for about three minutes with a Marantz PMD660 digital recorder (44.1 kHz sampling rate; 16-bit resolution) and a Sennheiser K6/ME66 directional microphone positioned approx. 1 m from the calling site. After each recording, we measured environmental (air) temperature with a digital thermometer.

Call measurements were obtained from recordings in Raven Pro 1.3 software (Charif et al., 2008). The call of *A. paleovarzensis* consists of groups of single notes separated by silent intervals (Lima et al., 2010). For each recording, we chose ten calls with less background noise for detailed analyses. Note measurements, including the spectral traits, were taken from the middle or next note of each call. Spectral measurements were taken after a fast Fourier transform with frequency resolution of 82 Hz and 2048 points. We considered ten call traits: Note rate (NR, in notes/s); Note duration (ND, in s); Internote interval, as the silent interval between two consecutive notes of a call (InI, in s); Call rate (CR, in calls/s); Call duration (CD, in s); Intercall interval, as the silent interval between two consecutive calls (IcI, in s); Maximum frequency (MF, in Hz); Lowest frequency (LF, in Hz); Highest frequency (HF, in Hz); Note modulation, as the difference between the highest and the lowest frequencies of the call (NM, in Hz).

### **Data analysis**

Student t test were used to determine the difference between male and female body size and between the size of their territories and living areas. To test the relationship between total monthly precipitation and reproductive activity (presence of spawn) a binary linear logistic regression model was used. The relationship between the number of vocalizing males each hour and the air temperature was tested using linear regression. All analyzes were conducted

in the R (R Core Team, 2016) computer environment. The size of male and female home ranges was estimated using the Minimum Convex Polygon (MCP) method (Kenward and Hodder, 1995), with all individual location points sampled via the Ranges program, version 9 (Kenward et al., 1996).

## RESULTS

### Body size and individual spatial distributions

Body size varied between 19.6 mm and 22.4 mm in males ( $X = 20.95$ ;  $SD = 0.67$ ;  $n = 60$ ), and 18.4 mm to 22.0 mm in females ( $X = 20.62$ ;  $SD = 0.87$ ;  $n = 33$ ) and showed no significant difference between the sexes ( $t = 0.34$ ;  $p = 0.73$ ). The male:female sex ratio at Careiro was 1:1 ( $n = 14:14$ ) and at Hiléia was 1.2:1 ( $n = 17:14$ ). Male territory size varied between 0.31 a 44.62 m<sup>2</sup> ( $X = 12.19$ ;  $SD = 16.40$ ;  $n = 9$ ) at Careiro and from 0.57 to 13.88 m<sup>2</sup> ( $X = 8.17$ ;  $SD = 5.66$ ;  $n = 6$ ) at Hiléia. Female home range size varied from 5.14 to 15.31 m<sup>2</sup> ( $X = 10.59$ ;  $SD = 5.12$ ;  $n = 3$ ) at Careiro and from 2.99 to 22.10 m<sup>2</sup> ( $X = 12.55$ ;  $SD = 13.51$ ;  $n = 2$ ) at Hiléia. There was no difference in male territory size between Careiro and Hiléia ( $t = 0.67$ ;  $p = 0.51$ ), nor for the female home ranges at these localities ( $t = -0.19$ ;  $p = 0.87$ ). There was no significant difference in the size of male territories and female home ranges (Careiro:  $t = 0.25$ ;  $p = 0.80$ ; Hileia:  $t = -0.44$ ;  $p = 0.72$ ).

### The relationship of annual reproductive activity to precipitation

The reproductive activity of *A. paleovarzensis*, as indicated by presence/absence egg clutches, was related to total monthly precipitation ( $z = 0.59$ ,  $p = 0.03$ ,  $n = 31$  months, Fig. 1), with oviposition occurring during the rainy season. Reproductive activity ceased in the driest months, when total rainfall fell below 100 mm (June to November). All oviposition and observed tadpole-transportation events occurred on rainy days.

### Daily patterns of vocalization

Males were recorded vocalizing exclusively during the day. Call activity was most intense during the morning, with a peak occurring around 06:00 (GMT-04:00) (Fig. 2), and was negatively related to the ambient temperature ( $r^2 = 0.24$ ;  $p < 0.01$ ;  $n = 226$  hours).

Male *A. paleovarzensis* show four types of vocalization: advertisement call (described in Lima et al., 2010), territorial call, agonistic call and courtship call (described below). Territorial calls (not recorded) were emitted by a male when an intruder is inside its territory, and apparently showed a lower frequency than the advertisement call. Agonistic calls (not recorded) constitute the emission of a single note, in the form of an agonistic scream, during physical combats between males.

### **Courtship call**

Courtship calls were recorded from two males at Hiléia and Barcelos. These calls were emitted while mating pairs were close together, early in the morning (06:35 at 24.2 °C and 06:52 h at 25.3 °C, respectively), after a courtship march and before a cephalic amplexus. The courtship call of *A. paleovarzensis* showed a similar temporal structure when compared to the advertisement call (Lima et al., 2010; Kaefer & Lima, 2012), consisting of the continuous emission of groups of notes separated by silent intervals (Fig. 3). However, spectral properties were clearly distinct from advertisement calls: courtship calls had a lower lowest frequency ( $3,260.3 \pm 131.43$ ,  $n = 10$  calls, and  $3,446.0 \pm 107.51$  Hz,  $n = 10$  calls, respectively) and a higher note modulation ( $1,356.2 \pm 214.6$ ,  $n = 10$  calls, and  $1,639.4 \pm 196.4$  Hz,  $n = 10$  calls, respectively).

### **Courtship, mating and oviposition**

Reproductive behavior in *Allobates paleovarzensis* includes acoustic, tactile and visual signals. We observed 39 successful courting events, all followed by amplexus and oviposition. Pair-formation begun at dawn the day before amplexus ( $n = 6$ ), when the male emitted an advertising call from a raised area of leaves, branches, trunks or roots to attract the female to his territory (Fig. 4A). Early the following morning, after having seen a female in his territory, the male started emitting the courtship vocalization ( $n = 18$ ). The male then

moved through the territory (running-jumping display), while vocalizing and the female followed (Fig. 4B). Males and females performed rapid up and down movements involving lifting one limb and often standing on wet leaves ( $n = 22$ ). The male moved onto a leaf while continuing to emit courtship calls. The female followed the male and moved onto the leaf. Some females remain on the first leaf the male showed them ( $n = 12$ ), while others continued to visit other leaves that were indicated by the male. After female chose a nest leaf, the male moved to the back of the female and grasped her head with his arms (Fig. 4C). The pair remained in this cephalic amplexus for 80 seconds on average (33-165;  $SD = 32$ ;  $n = 25$ ). After an interval, the male moved one of his hands vertically, sliding it in front of the female's mouth ( $n = 11$ ). The male then moved to the side of the female and returned to his calling platform to utter advertisement calls. The female rotated around on her own axis (circling) while contracting her abdomen and releasing oocytes onto the leaf, taking 89 seconds on average to release the oocytes (50-140;  $SD = 33$ ;  $n = 12$ ). The cluster of oocytes deposited by a female on a single leaf was considered as a single spawning (nest). The female remained on the spawn for on average for 169 seconds (70-285;  $SD = 101$ ;  $n = 9$ ), and then left the nest. Some females remained in the male territory from oviposition until the next day ( $n = 8$ ). Following oviposition, all males sat on moist substrates, such as wet leaves, before returning to the oviposition site. The male sat on the oocyte mass, pressing the eggs against the leaf with his belly and moving over the spawn, spinning around on his own axis (circling). In all observations, the gelatinous covering of the spawn increased in size following the male's departure. Four spawnings were completely unviable from oviposition onwards, and in these cases the males did not return to the oviposition site after amplexus. We observed 23 courting attempts that were unsuccessful due to female rejection. In these cases, the female entered the territory of the male, but left before amplexus. In almost all cases, the leaves used as nests were used for only one spawning. We recorded a single case of two spawnings deposited on a single leaf. As these were of the same age, they were probably laid by different females.

### **Clutch size and parental care**

Mean clutch size was 29 eggs (15-50;  $SD = 7.81$ ;  $n = 79$ ). Recently deposited eggs were usually found in the central part of the leaf, surrounded by a gray and opaque gelatinous substance (Fig. 4D). The mean development time from egg phase to the free tadpole phase (approximately stage 20-25, Gosner, 1960) was 21 days (17-29;  $SD = 3.59$ ;  $n = 17$ ). Initially

all fertile eggs had a uniform milky-gray pigmentation. After 48 hours it was possible to distinguish two distinct poles within the eggs. After fertilization, the eggs developed to embryos within gelatinous capsules (stages 1 to 25; Gosner, 1960). During this period, the gelatin absorbs a large amount of water (Gosner, 1960). Embryos develop until the encapsulated tadpole stage, with presence of tail, operculum, as well as developed oral and digestive structures. Encapsulated tadpoles actively wriggled until their bodies were outside the capsule (Fig. 4E), and could be transported to a body of water. Successful males continued to vocalize and mate after an oviposition event, and at the same time simultaneously caring for multiple egg clutches in their territory. Clutches were usually comprised of young at the same stage of development, except for any infertile eggs, or those that died during development. Summing all the observations, an average of 44.12% of laid eggs did not develop (0-100%;  $SD = 44.01$ ). We recorded only one case of two spawnings deposited on a single leaf (30 eggs and 27 eggs). All the tadpoles of the double spawning survived and were transported by the male in two transport events.

Nine tadpole transport events were monitored, all performed by parent males. When transporting the tadpoles to a water body, the male moved over the spawning, in the center of the gelatin and moved its feet vertically while the tadpoles actively ascended onto his dorsum. The male remained on the spawn for 480 seconds on average (300-7,200;  $SD = 216$ ;  $n = 3$ ). After the tadpoles were attached, the male left the nest (Fig. 4F). During transport, the mean number of tadpoles adhering to a male's back was 25 ( $SD = 17.67$ ;  $n = 7$ ), ranging from 3 to 60 tadpoles in a single transport event. Males moved through the study area, crossing the territory of other males and even puddles, but only released tadpoles from their backs when they reached the edge of a body of flowing water. Here they adopted a partially submerged position, with their heads out of the water. Then the male moved his legs vertically and the tadpoles swam away from him. The male then left the water and returned to its territory. In some cases, males submerged several times in different bodies of water until all tadpoles were released. All observed males returned to their territories after the release of the tadpoles in the waterbody (bodies).

### **Mating system and confrontation between males**

Males courted different females simultaneously and mated with up to three females consecutively within a single territory, with a minimum interval of less than 20 minutes between two amplexi. Polygyny was common, with males having spawned from up to 5 different females in simultaneous development. Females were also observed in amplexus with different partners, with average interval of 23 days between each amplexus (19-27;  $SD = 4.04$ ;  $n = 3$ ), characterizing polyandry.

We recorded fifteen events of aggressive interactions between males. These occurred when two males attempted to attract the same female ( $n = 5$ ), or when one male entered the territory of another ( $n = 10$ ). Conflict began with one or both males displaying their vocal sac, either in silence or accompanied by vocalizations. The territorial call had a lower frequency than the advertisement call and was issued simultaneously by competitors during a confrontation. Each male kept his body erect and moved in small jumps next to his opponent. After such displays, one of the opponents walked away and was followed by the winner. Disputes lasted 1,260 seconds on average (240-2,700;  $SD = 889$ ;  $n = 10$ ). At the end of this time, one male left the territory as a result of the directed jumping behavior of the other male. The loser male left the territory, closely followed by the winning male. In one of the observed conflicts, which resulted in territory loss by the owner, this tracking continued until the two animals were some 12 meters away from the territory boundary. In this case, the nest of the loser male remained in the territory and the embryos died by desiccation.

We recorded only one confrontational event with physical contact. In this case, the pursued male did not move and was caught by the other male. The territory defending male jumped over the other and held onto its head, leaning over him and pressing him toward the ground. The male retained this position for 205 seconds, until the issuing of an agonistic call by the other male. The winning male jumped to the side of the losing male, who remained motionless for 4 minutes. This male then left the territory and concealed itself in the leaf-litter. The winning male emitted advertisement territorial calls from the start of the contest until 7 minutes after releasing the losing male. In all interactions between observed males, the winning male differs from the losing male in the uninterrupted persistence of his vocalization activity. We did not register aggression among females.

## DISCUSSION



*Allobates paleovarzensis* is a polygamous species with uniparental care carried out by the male, territoriality is related to male:male competition for space and for females. There is high investment in eggs number, and evidence of transport of the largest number of tadpoles yet-known in the genus *Allobates*. The behavioral patterns shown by *A. paleovarzensis* are consistent with the range of behaviors known for other species of the Dendrobatoidea superfamily. Despite the sparse knowledge of the reproductive biology of species among dendrobatoids, studies show that the conquest of the terrestrial environment result in behavioral and physiological strategies that increase species reproductive success (Wells, 2007), such as polygamy and strong intra-sexual competition (Ursprung et al., 2011b), reproduction-associated territoriality (Prohl, 2005), high investment in offspring (e.g., Lüddecke, 1999; Downie et al., 2005; Quiguango-Ubillús and Coloma, 2008), and parental care during the most vulnerable phases of offspring development (e.g., Caldwell and Oliveira, 1999; Killius and Dugas, 2014).

The lack of body size sexual dimorphism recorded here for *A. paleovarzensis* has also been recorded for *A. marchesianus* (Melin, 1941; Lima and Keller, 2003) and *A. bacurau* (Simões, 2016). In addition, *A. paleovarzensis* has a large body size compared to other species of the genus, although *A. alessandroi* and *A. melanolaemus* (Grant and Rodriguez, 2001), *A. femoralis* (Montanarin et al., 2011), *A. hodli* (Simões et al., 2010) and *A. undulatus* (Myers and Donnelly, 2001) are nearly as large. Body size is highly correlated with reproductive investment and, for females, a large body size influences clutch size, egg size and the amount of stored energy associated with each oocyte (Summers et al., 2006; Wells, 2007). Thus, females with large body sizes can develop and carry a larger number of eggs, with more space available for egg development and possibly more yolk allocated with each egg. For males, large body size facilitates performance during confrontations with other males and the better vocal performance during territorial, courtship and territorial vocalization bouts, influencing mating (Wells, 2007). Males of some frogs may use vocalization characteristics of other males to evaluate the body size of potential opponents in agonistic encounters (e.g., Davies and Halliday, 1978; Arak, 1983; Robertson, 1984; Wagner, 1989). Ramer et al. (1983) showed that male frog *Lithobates clamitans* (Latreille, 1801) showed different aggressive response levels depending on the size of the potential competitor male. For *A. femoralis*, Luna et al. (2010) showed experimentally that males attacked smaller models more frequently than large ones.

During the reproductive period male *A. paleovarzensis* establish territories and defend them from invading males. The territory is that part of the male's home range that is delimited and defended during the reproductive period. Territories include vocalization platforms, courtship and oviposition sites, where the embryos develop to the tadpole stage (Prohl, 2005). Exclusive courting areas may allow females to be courted without interference from other males, which is particularly important in species such as *A. paleovarzensis* where courting is prolonged and complex. Female *A. paleovarzensis* move within their home ranges accessing males that vocalize to attract them. The home range of a female includes the territories of several different males, so that the females access different partners during the reproductive period. In *A. femoralis*, the number of effective partners per female is significantly related to the number of male candidates within an area of 20 m (Ringler et al., 2012). Polygynandry in *A. femoralis* can therefore act as a mechanism that counteracts the risk of negative effects associated with single mating decisions, especially with regard to paternal care, since females do not exercise parental care and so cannot influence reproductive success after oviposition (Ursprung et al., 2011b). Studies involving the reproductive biology of several species of dendrobatoids show that polygamous mating is common and is an evolutionarily stable strategy for the group (Summers, 1989, 1990; Prohl, 2002).

In *A. paleovarzensis* reproductive activity is restricted to the regional rainy season. During the mating season, males vocalize throughout the day with a drop in activity around mid-day, when the air temperature is highest. Vocalization, courting, egg deposition, and tadpole transport all took place only in the mornings of cloudy, rainy days when the local temperature was lowest. Similar patterns of seasonal and daily cycles in reproductive activity have been reported for *A. femoralis* (Kaefer et al., 2012) and *A. subfolionidificans* (Lima et al., 2007; Souza et al., 2017). Thus, water availability seems to be the determining factor in *Allobates* reproductive seasonality, whereas ambient temperature, which affects evapotranspiration and neuromuscular activity in ectotherms (Prestwitch, 1994), appears to be the main modulator of daily vocalization patterns.

Courtship in *Allobates paleovarzensis* involves both visual and acoustic signals. Females appeared to signal visually in response to males' courtship calls, following them and engaging in a limb lifting display. Males appeared to display visual cues as they guided the females to the leaves and also into the nest before amplexus. In addition, an acoustic signal for reproduction was emitted by the male in the form of a courtship call, which in many

species of anurans occurs as a modification of the advertisement call (Wells, 2007; Kollarits et al., 2017). However, the lack of detailed observations of the courting behavior for many species means patterns of variability in this behavior cannot currently be evaluated for the group. The overall structure of the acoustic signal of *A. paleovazensis* did not change in courtship calls. Nonetheless, these calls could be clearly distinguished by a static (low frequency) and a dynamic (frequency modulation) property. Future studies involving playback experiments, as well as the description of the mating call for additional species, should clarify the evolutionary implications of spectral changes in frog calls during mating interactions.

The cephalic amplexus exhibited by *A. paleovarzensis* is also found in other species of the superfamily (Grant et al., 2006), while the absence of amplexus in some dendrobatoids is considered a derived loss (e.g., Duellman and Trueb, 1986; Haas, 2003). The absence of amplexus can also be explained by the variety of pseudoamplectic positions employed in aggressive behavior and during courting (Grant et al., 2006). Females of *A. paleovarzensis* showed partner choice on the day preceding amplexus, as also recorded for *A. femoralis*, where the female remains within the male territory for up to two days before oviposition and is courted daily by the resident male (Roithmair, 1994). Some *A. paleovarzensis* females left the territory before the amplexus, indicating that female choice is also influenced by courtship signals after attraction to a territory. In addition, females showed acceptance or rejection of the nest site prior to oviposition, actively selecting an appropriate leaf to host the egg mass. This behavior has been discussed, by many authors and for different species, as a form of pre-zygotic parental care (Kirkpatrick, 1985; Shuster and Wade, 2003; Royle et al., 2012).

*Allobates paleovarzensis* has the largest known number of eggs per reproductive event of any species in the genus, as *A. caeruleodactylus* (10-30 eggs; Lima and Caldwell, 2001; Lima et al., 2002), *A. magnussoni* (13-18 eggs; Lima et al., 2014), *A. tapajos* (23-27 eggs; Lima et al., 2015), and *A. subfolionidificans* (5-15 eggs; Souza et al., 2017). *Allobates paleovarzensis* is also the species of the genus *Allobates* that carries the largest number of tadpoles in a transport event (although knowledge of this aspect of the reproductive biology is currently sparse for *Allobates* species). The number of tadpoles transported varies between *Allobates*. So that, while some species are reported to carry small numbers of tadpoles, as *A. magnussoni* (Lima et al., 2014) and *A. flaviventris* (Melo-Sampaio et al., 2013) with 7 tadpoles per transport, others are known to carry large numbers as *A. subfolionidificans* (24

tadpoles; Souza et al., 2017), *A. marchesianus* (37 tadpoles; Lima and Keller, 2003), *A. caeruleodactylus* (48 tadpoles; Lima et al., 2002). The transport of tadpoles to aquatic environments is a synapomorphy of the Dendrobatoidea (Grant et al., 2006). As in other species of this taxa (e.g., Lima and Keller, 2003, Castillo-Trenn and Coloma, 2008; Souza et al., 2017), tadpole transport in *A. paleovarzensis* is performed by the male. When transporting, male *A. paleovarzensis* gather tadpoles from different nests and thus can maximize transport to a single event, potentially reducing the total transport costs per reproductive season. During transport, the male is exposed to the risk of predation and loss of its territory, which may be occupied by another male, resulting in transporting male losing any remaining spawn currently developing to the transportable tadpole stage (e.g., Downie et al., 2005; Ringler et al., 2013; Rojas, 2014; Trenkwalder et al., 2016).

The disputes between *A. paleovarzensis* males can be considered as progressive stages in a combat ritual, with displays, persecution and physical confrontation. This is also known for *A. femoralis* (Luna et al., 2010). Maynard Smith and Price (1973; Maynard Smith, 1974) concluded that ritualized fighting may be an evolutionarily stable strategy to avoid injury. To minimize the risk of physical damage resulting from combat, territorial frogs exhibit a graded series of behaviors prior to physical combat (Narins et al., 2003). Results from speaker experiments conducted with robotic frogs suggested that vocal sac pulsation, combined with the emission of territorial calls and possession of a male body size were multimodal requirements for triggering male *Allobates femoralis* attacks on simulated intruders (Narins et al., 2003; Luna et al., 2010). Thus, if the individuals involved in a confrontation adopt the ritualized display strategy without initial injury, as observed in most *A. paleovarzensis* bouts, disputes can be resolved without bodily harm to the participants.

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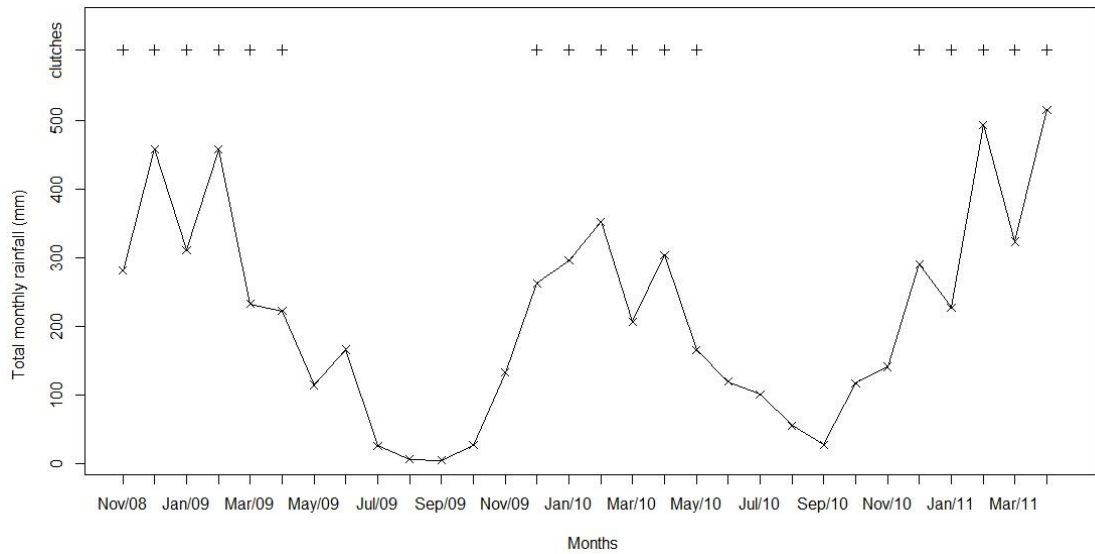
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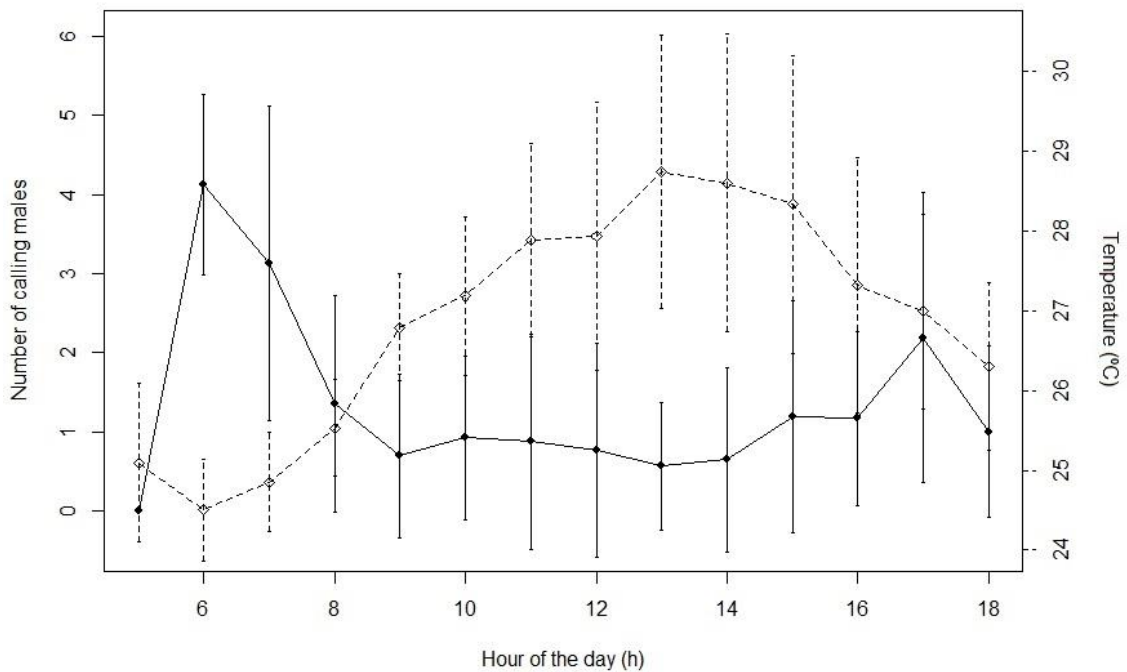
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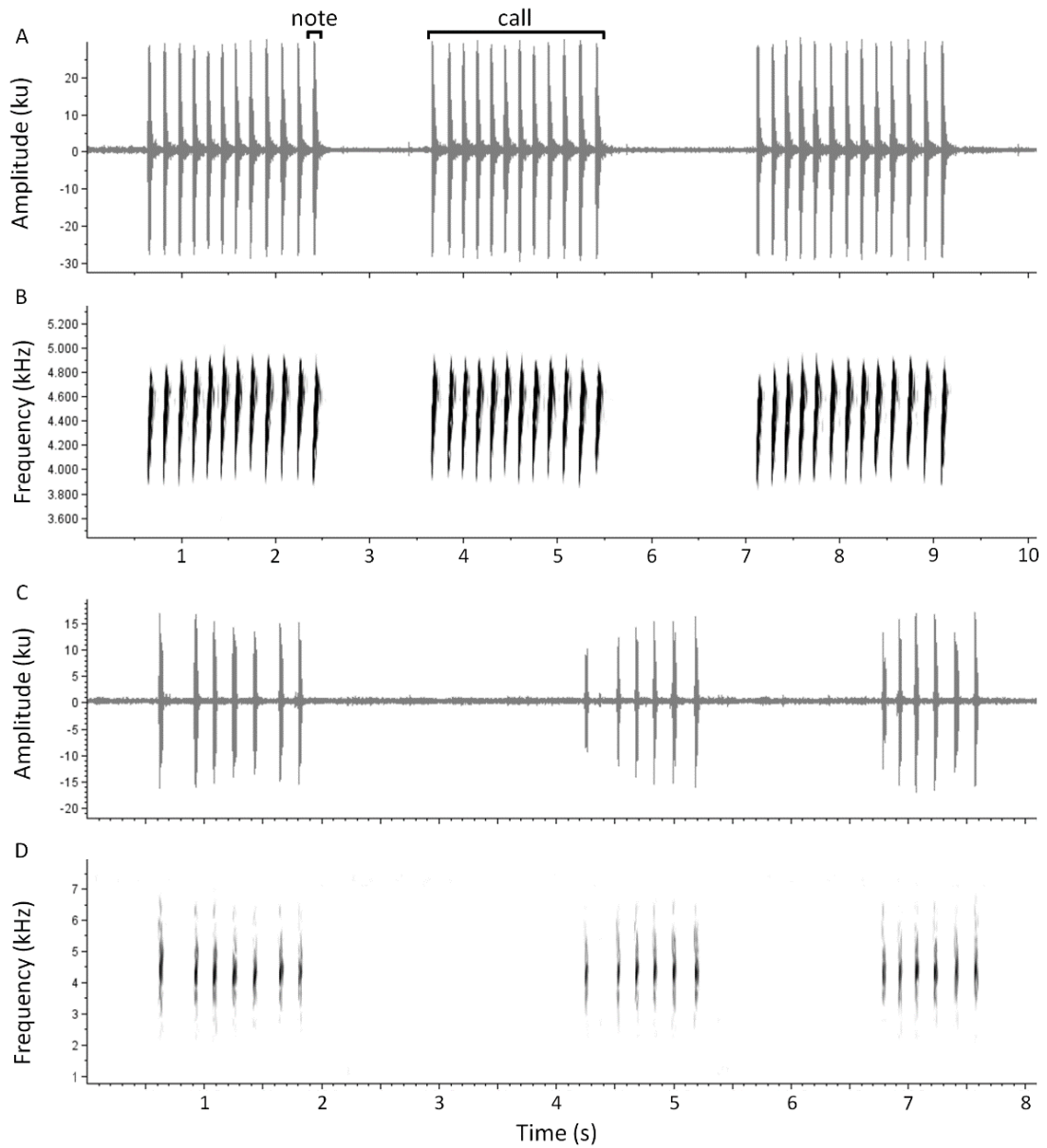
### Figure captions



**Figure 1.** Reproductive activity (presence of egg clutches, + symbol) of *Allobates paleovarzensis* in relation to total monthly rainfall in the study region (x symbol).



**Figure 2.** Daily cycle of *Allobates paleovarzensis* calling activity at Careiro and Hileia, Amazonas, Brazil. Black dots represent the mean number of calling males recorded in each interval at the study site. Empty diamonds represent the average air temperature in each interval at the study site. Vertical bars correspond to standard deviations.



**Figure 3.** (A) Oscillogram and (B) spectrogram of three multinote advertisement calls of male *Allobates paleovarzensis* at its type-locality in Careiro da Várzea, Brazil. (C) Oscillogram and (D) spectrogram of three multinote courtship calls of male *Allobates paleovarzensis* at Barcelos, Brazil.



**Figure 4.** A) Male of *Allobates paleovarzensis* vocalizing on a leaf; B) male vocalizing and moving across territory, followed by female; C) cephalic amplexus; D) young spawn after oviposition, in a leaf nest; E) spawning at the free tadpole stage; (F) male leaving the leaf, transporting the tadpoles to a water body. Photos: S.M.C.R.

## Capítulo 2

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Rocha, S.M.C; Lima, A.P. & Kaefer, I.L. Territory size as a main driver of male mating success in an Amazonian nurse frog (*Allobates paleovarzensis*, Dendrobatoidea). Submetido à *Acta Ethologica*.



**Territory size as a main driver of male mating success in an Amazonian nurse frog  
(*Allobates paleovarzensis*, Dendrobatoidea)**

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**Abstract**

In polygamous mating systems, it is most often males that compete for the opposite sex, using strategies to provide access to as many females as possible. Females, on the other hand, constitute the sex that exerts the choice, and so require a means of accessing the quality of a potential partner to in comparison to its competitors. A common challenge in sexual selection studies is to identify the most relevant trait for mating success, since many are correlated with each other. In addition, little is known about how the female accesses the aspects related to male quality. In this context, we tested the role of different male characteristics on mating success in a natural environment using the Amazonian frog *Allobates paleovarzensis* as a model. A multiple linear regression model showed a positive relationship between territory size and number male matings, while calling persistence was slightly related to the mating success. We did not detect a relation of the number of matings with the distance to the nearest body of water nor with male body size. Additionally, we observed that territory size was not related to calling persistence, but had a positive relation with the duration of the couple's courting process. Thus, we conclude that: 1) territory size is the main determinant of male mating success, and this is not correlated with the other attributes tested, and 2) females access the size of the males' territory through the courting process that precedes oviposition.

**Keywords:** Anura, Aromobatidae, behavior, courtship, sexual selection

## **Introduction**

Within a species interindividual variability in mating success determines the intensity of sexual selection: the greater the uncertainty about reproductive success, the more intense competition for the opposite sex tends to be (Trivers 1972). However, in many species little is known about the determinants of individual reproductive success, since a number of intrinsic and extrinsic factors, acting alone or in combination, may affect the fitness of a given individual (Sirkiä and Laaksonen 2009). Intrinsic factors include the morphological, behavioral and/or physiological characteristics of the individual that act as a reproductive or attractive resource for reproduction. Such factors include facial features (Batres and Perrett 2016), courtship displays (Haddad and Giaretta 1999) and pheromones (Houck and Reagan 1990; Rollmann et al. 1999). Extrinsic factors refer to attributes external to the individual, but which are also used as a reproductive or attractive resource for reproduction, such as nest quality (Sargent 1982), bridal gifts (Thornhill 1980), and territory quality (Wells 1977). Variability in mating success, and consequently the strength of intrasexual selection, is usually more pronounced in males because females can be seen as a valuable and scarce resource, a pattern explained by anisogamy (Parker et al. 1972), mating investment, differential parental investment between the sexes (Trivers 1972), and the sex ratio in a population (Fisher 1930).

Thus, in polygynous and polyandry mating systems, males are usually the sex that competes for mating opportunities, using strategies that facilitate access to the greatest possible number of females (Emlen and Oring 1977). Females, on the other hand, exert mate choice, and this requires a means of assessing the quality of a potential partner, and a means of comparing him against competing males (Sargent 1982; Bart and Earnst 1999; Sirkiä and Laaksonen 2009). Anuran amphibians are organisms that have evolved diverse reproductive strategies that are evolutionarily influenced by strong intra- and inter-sexual selection (e.g. Prado and Haddad 2003; Izzo et al. 2012). In addition, anurans make good models for behavioral studies because they are easy to observe and manipulate in the field. Anurans of the Dendrobatoidea superfamily are especially interesting because they frequently show terrestrial reproduction, territorial behavior and elaborate strategies of partner attraction, courtship and parental care (Pröhl 2005; Wells 2007). For this superfamily, the small number of existing studies suggest that male reproductive success is related to the defense of a reproductive territory and to the persistence of vocalization, which simultaneously repulses competing males and attract females (Roithmair 1992; Pröhl 2003; Wells 2007).

Most species in the Dendrobatoidea superfamily reproduce terrestrially, with oviposition occurring on leaves in the litter and part of the development occurring in the aquatic environment (Pröhl 2005). From a certain stage onwards, the offspring require water for development and there are many different strategies to achieve this while maximize reproductive success; including the use of water in bromeliad leaf bases (Young 1979), ponds (Eterovick and Fernandes 2002), seed capsules (Moravec et al. 2009) and small streams (Montanarin et al. 2011). Because dendrobatoid anurans are territorial in the reproductive season, the developmental environments of the offspring usually lie within the territorial area of the parents. Females and males may exhibit territorial behavior, depending on which sex is under selective intra-sexual pressure and the parental effort of each sex during the development of the young. The most commonly found pattern in the superfamily is for males be territorial and act as caregivers. The most studied aspects of male intrinsic reproductive attributes of the superfamily are the spectral and temporal parameters of adult vocalization, persistence of calling activity, coloration and male size, and residency duration of males on territories (Bourne et al. 2001; Pröhl 2003; Maan and Cummings 2009; Ursprung et al. 2011). The study of extrinsic attributes is usually limited to territory size and those characteristics related to territory quality and the reproductive resources contained therein, such as sites for tadpole growth (Pröhl et al. 2001; Pröhl 2002, 2003). Another feature of potential relevance for many species - but never yet investigated - is the distance from the territories to bodies of water, since in most species the young are transported by their parents to aquatic environments, where they complete their development.

A common challenge in sexual selection studies is to separate and identify the feature(s) most closely causally-linked to mating success, since many are correlated with each other (e.g. Yasukawa 1981; Bart and Earnst 1999; Candolin and Voigt 2001). Moreover, little is known about the perspectives of the females engaged in such processes, and how they assess attributes related to male quality. In this context, we aim in the current study to test the role of intrinsic and extrinsic male characteristics on the male mating success in a natural environment. We used the anuran species *Allobates paleovarzensis* Lima et al. (2010) as a model, a species that inhabits seasonally floodable environments (paleo-várzeas) in central Amazonia. Based on previous studies with similar species (Roithmair 1992, 1994), our hypothesis is that both intrinsic and extrinsic factors of the male are related to male mating success. In this study we also test a mechanism through which an anuran female assesses an extrinsic attribute relevant to the male's reproductive success.

## Methods

*Allobates paleovarzensis* is a day-active anuran inhabiting the forest litter in Amazonian seasonally-flooded forests (Lima et al. 2010). The mating system is polygamous, with defense of reproductive territories by males (Lima et al. 2010). Courtship begins with the attraction of females by the male advertisement call, which is issued continuously throughout the day. Males call to attract females and repel invading males from their territories. As the female approaches, the male moves across the territory emitting the mating call, and the female follows. Male and female perform a courtship march within the territory, during which the female chooses a leaf for oviposition and decides whether to accept or reject the male. Females may desist before or during the courtship march process and may also visit more than one leaf before choosing on that is a suitable oviposition site. Once the pair are on the selected leaf, cephalic amplexus occurs, which stimulates the female to release of her oocytes. In *A. paleovarzensis*, the eggs are deposited in nests of dry leaves in the leaf litter, and they develop there, within the male's territory and under his care, until tadpole phase is reached. The female apparently plays no part in the parental care of the eggs or tadpoles. When tadpoles are close to stage 25 (Gosner 1960), the tadpoles adhere to the male parent's back, in which they are transported to a water body. This is generally as a small permanent or semi-permanent lotic water body, connected to other higher order streams. Reproductive activity occurs during the rainy season, which usually begins around mid-November and ends in mid-April.

The study was carried out at Careiro da Várzea (03°22'26.3"S; 59°52'06.4"W) in state of Amazonas, Brazil. Average annual precipitation varies from 2000 to 2400 mm (Sombroek 2001). The locality has remnants of paleo-várzea forests, which are located in plains flooded periodically after the rainy season by freshwater systems rich in Andean sediments (Irion et al. 1995; Assis et al. 2014). The study area has periods of total seasonal inundation (Cintra et al. 2013), beginning in mid-March and April with flooding and overflowing of the region's water bodies.

Data were obtained between December 22, 2016 and February 22, 2017 in a forest fragment containing a network of *igarapés* (small streams), and where males of *A. paleovarzensis* occupy territories during the breeding season. Field observations were performed daily for six days per week between 06:00 and 18:00 hours (GMT-04:00). Individuals resident in territories were identified by means of photographs of their color

patterns, which varies individually, and sexed according to the color of the belly, which is dark in males and yellowish in females (Lima et al. 2010).

We considered both intrinsic and extrinsic variables as potential predictors of male mating success for this species. For intrinsic variables, we assessed body size (snout-vent length, SVL, in mm) and calling persistence, which was defined as the percentage of censuses in which the individual was recorded vocalizing during his territorial residency. Censuses ( $n = 114$ ) were performed hourly (between 06:00 and 12:00) for 10 minutes over 26 days ( $mean = 101.31$  censuses,  $sd = 18.44$ ,  $n = 16$  males). As an extrinsic factor, the following variables were measured for the territory defended by the male: distance from the territory to the nearest permanent water body (in straight-line meters), and territory size (measurement via sides of the polygon, in  $m^2$ ). Territory size was measured considering each vocalization platform and spawning sites as vertice of the polygon. The minimum convex polygon (Kenward and Hodder 1995) was used to calculate the area of the territories, since it is a widely-used method for dendrobatoid territories measurement (e.g. Brown et al. 2009; Kaefer et al. 2012; Valenzuela-Sánchez et al. 2014). The number of spawns in which each male was involved during the sampling period was considered as the measure of mating success.

To test the determinants of mating success (number of spawnings), we used a multiple linear regression model with the following predictor variables: nearest distance to a water body, territory size, male body size and calling persistence. Statistics and graphs were generated using the R (R Core Team 2016) computer environment.

## Results

Males had 0 to 8 successful matings. The model had a high adjustment in relation to the observed data (adjusted  $r^2 = 0.82$ ,  $p < 0.01$ ). Territory size was positively related to the number of spawnings by the resident male (adjusted  $r^2 = 0.57$ ,  $p < 0.01$ ,  $n = 16$  males; Fig.1), while calling persistence was marginally related to the number of spawnings by the resident male ( $p = 0.05$ ), and contributed little in the regression model (adjusted  $r^2 = 0.05$ ). No relationship was detected with number of spawnings and water body distance ( $r^2 = -0.003$ ,  $p = 0.64$ ) or male body size ( $r^2 = -0.22$ ,  $p = 0.69$ ). There was no correlation between any of the predictor variables tested in the model.

Since size of the territory was the only relevant variable and highly correlated with mating success, we tested the relationship of this predictor with vocalization persistence and the courtship march duration, hypothesizing that both can be used by a female as indicators of

territory size. Courtship march duration was determined by the analysis of ethograms (Focal Animal; Altmann 1974) recorded for each successful mating event. We recorded the time elapsed (in seconds) from the beginning of the movement of the male and the female within the territory up until a leaf was selected for oviposition. Territory size was not related to calling persistence ( $r^2 = 0.008$ ,  $p = 0.74$ ,  $n = 16$  males), but it is positively related to courtship march duration ( $r^2 = 0.73$ ,  $p = 0.002$ ,  $n = 9$  males; Fig. 2).

## Discussion

The results of this study did not corroborate the hypothesis that both intrinsic and extrinsic factors of the male influence mating success in *Allobates paleovarzensis*, since only one extrinsic factor was responsible for males' success: the size of the territory. Possibly territory size represents an extended male phenotype and remains under the influence of sexual selection across generations, a pattern also found in other animal species that defend reproductive territories (e.g. Reid and Weatherhead 1990; Dale and Slagsvold 1990; Seddon et al. 2004; Vanpé et al. 2009). All known members of the Dendrobatoidea superfamily show territoriality, and the territory is always a defended resource that is occupied for reproduction (Pröhl 2005). The paramount influence of the territory in dendrobatoid courtship, mating and parental care strategies supports the results of our study, in which the territory was the principle indicator of male quality.

Pröhl and Hödl (1999) and Roithmair (1992, 1994) demonstrated that male mating success is positively correlated with territory size in *Oophaga pumilio*, *Allobates femoralis* and *Ameerega trivittata* and suggested that females use the size of a territory as an indicator of male quality. However, it was not known how the female accessed the size of the territory, and related this information to owner quality. Some type of land quality assessment is certainly important for any species in which males defend oviposition sites because the quality of these sites may have an immediate effect on a female's reproductive success (Wells 2007). If males defend territories centered on oviposition sites and these locations vary in quality, then females are expected to choose mates based on this attribute. There is evidence for female choice based on territory size in *Rana clamitans* and *Lithobates catesbeianus* (Wells 1977; Howard 1978). In both species, physical characteristics of the territories were correlated with mating success of the territory owners. Although large males tended to acquire the best territories, small males with good territories were more likely to mate than large males with poor territories. In our study we obtained evidence that the period when the male

leads the female through the territory on the mating march provides an opportunity for the female to access the information about the size of the territory defended by that male. In this way, the female can know if the male is a good competitor for space and how much space he can maintain and defend for reproduction. We consider that at that moment the female makes the choice between continuing or desisting courtship with the male under evaluation. To date, the function of the courtship march was unknown, generally being interpreted simply as a movement of the female following the male to the nest site within the territory (e.g. Limerick 1980; Lima et al. 2002; Montanarin et al. 2011; Forti et al. 2013). We consider that the choice of a nest site by the female is of great importance at this point in the courtship, but that the courtship march also has the additional function of assessing male territory size.

Individual male body size is a factor positively related to mating success in various animal groups (e.g. Partridge et al. 1987; Shine et al. 2000; Bourne et al. 2001; McElligott et al. 2001). This factor provides clues to the individual quality, since large size in a male reflects good nutrition throughout life and large females can nurture more young and possibly provide better nutrition for them (Wells 2007). However, in our study, male body size was not a determinant in mating success, and the ability to defend a territory and succeed in attracting and mating with a female was not determined by the size of the male. As there was no significant relationship between male size and mating success and considering the negative correlation between male size and call spectral characters in *A. paleovarzensis* (Kaefer and Lima 2012), we suggest that spectral characteristics of the advertisement call are not relevant to females in the context of intrasexual competition in this species.

In *A. paleovarzensis*, mating success was marginally related to calling persistence. Studies with other anuran species indicate the importance of this variable in courting and mating success (e.g. Roithmair 1992; Bourne et al. 2001; Pröhl 2003). The persistence of vocalization activity can provide information on energy, endurance and competitive potential of males, but in the present study this variable was of little importance as a determinant in male mating success.

Considering the reproductive biology and habitat of the species, we suggest the distance to a body of water as a variable determinant of mating success. In *A. paleovarzensis* transport of the tadpoles by the male from the nest to a body of water where they will continue their development is the main form of parental care. Thus, the distance from the territory held by the male to the nearest body of water could influence cost and risk of tadpole transport. Studies have reported, among transport costs, energy expended in locomotion,

predation risk, and risk of loss of territory to another male (e.g. Townsend 1986; Downie et al. 2005; Ringler et al. 2013; Trenkwalder et al. 2016). Males generally transport tadpoles to permanent bodies of water and, therefore, the proximity to these bodies of water could indicate the quality of the male in question. However, the distribution of the species is restricted to paleo-várzea regions (Kaefer et al. 2012), and these are subject to periodic flooding during the rainy season (Cintra et al. 2013; Schietti et al. 2016). Males that establish territories closer to large *igarapés* can reduce the cost of tadpole transport, but increase the risk of their territories being flooded, and consequently have their fathered clutches drowned or inundated by streams.

In summary, this study clarifies two important topics for the understanding of mating success in *A. paleovarzensis* and potentially in other species of territorial anurans: 1) territory size constitutes the main determinant of male mating success via positive selection by females, and this is not correlated with other extrinsic or intrinsic attributes, and 2) females access the size of the male territory during the courtship march that precedes oviposition. Attempts to integrate male and female perspectives is a growing trend in behavioral studies of anurans (e.g. Ursprung et al. 2011; Ringler et al. 2012, 2017), and should also allow an understanding of additional factors related to the emergence and maintenance of reproductive strategies in other animal groups.

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### **Ethical approval**

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in



accordance with the ethical standards of the institution or practice at which the studies were conducted.

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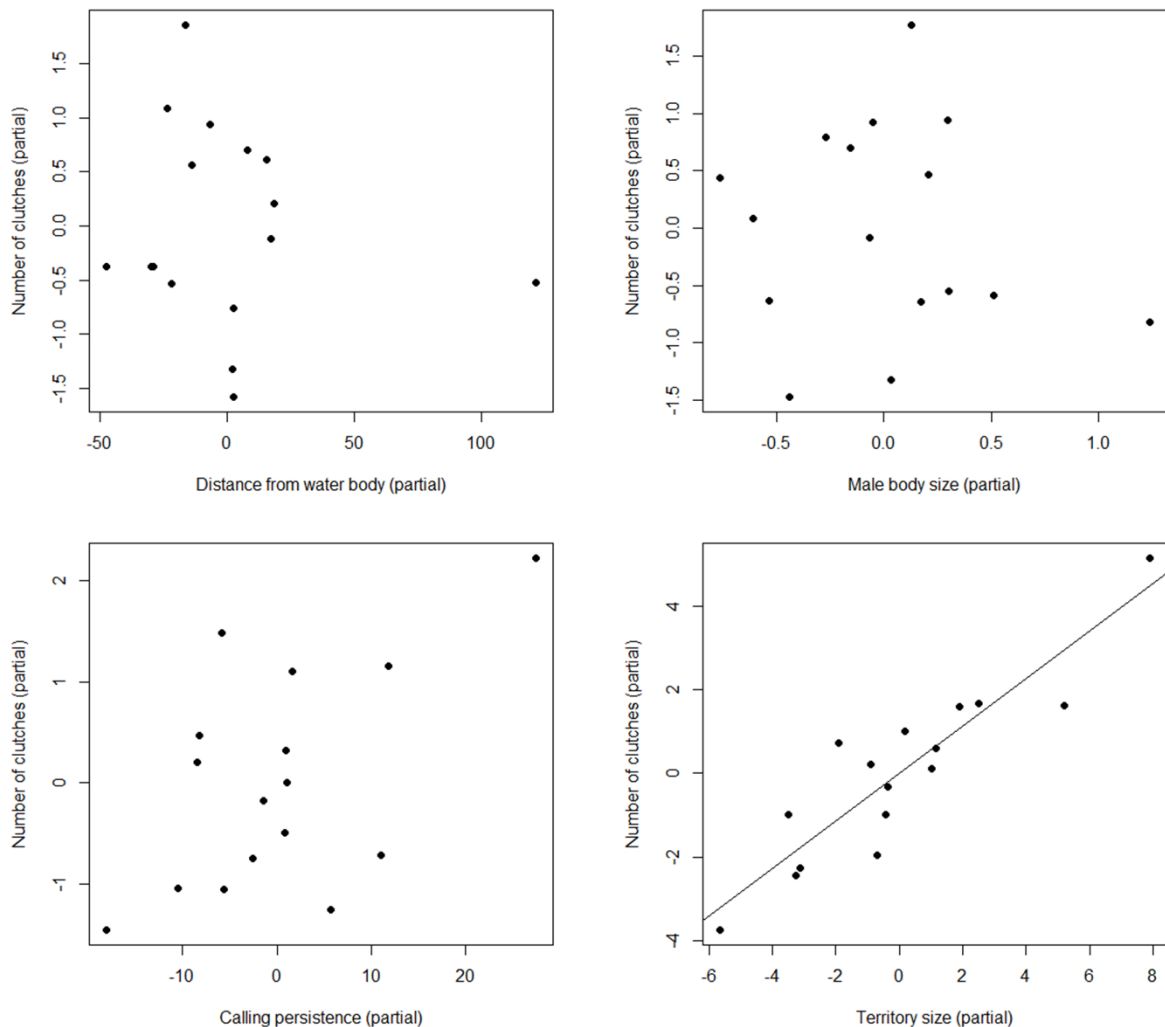
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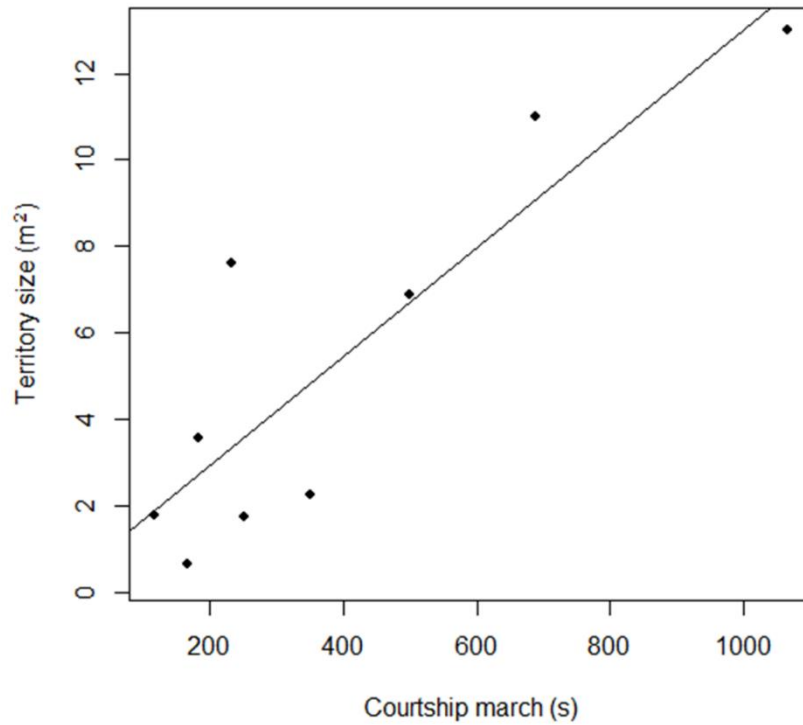
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## Figures



**Fig. 1** Relation between the number of spawnings for each male ( $n = 16$ ) and the predicted variables distance from the body of water (meters), size of the territory (square meters), snout-vent length (millimeters) and calling persistence (hours) in *Allobates paleovarzensis* from Central Amazonia



**Fig. 2** Relation between courtship march duration and male territory size ( $n = 9$ ) in *Allobates paleovarzensis* from Central Amazonia

## Capítulo 3

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Rocha, S.M.C; Lima, A.P. & Kaefer, I.L. A field experiment on parental care and offspring survival of a Neotropical nurse-frog (*Allobates paleovarzensis*). Manuscrito em preparação para *Behaviour*.

# A field experiment on parental care and offspring survival of a Neotropical nurse-frog (*Allobates paleovarzensis*)

## Offspring survival of a Neotropical nurse-frog

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### Abstract

Parental care may be undertaken by one or both parents, a pattern that is determined by the physiology and anatomy of each sex and by environmental and behavioral conditions. *Allobates paleovarzensis* (Dendrobatoidea) is a diurnal terrestrial anuran species in which males hold reproductive territories and take care of the young until the tadpole stage. Females have no territory and their role in offspring care is unknown. In an experimental field study with the species, we tested the following hypotheses: (H1) Offspring mortality is higher when males are absent than when they are present; (H2) Male care of offspring occurs throughout their development; (H3) In the absence of the male, the female takes care of the offspring. The experiment consisted of two treatments: (i) no manipulation, and (ii) removal of parental males. Tadpole survival percentage was higher in the group where the male was present: transport by the male parent was the most common fate of tadpoles with parental care, while submersion or being washed away by rainwater was the most common fate of tadpoles lacking parental care. All tadpole transportation was performed by the male caretakers. Females were not recorded giving care in the absence of the male. Our study revealed that parental care in *Allobates paleovarzensis* is performed mostly (if not exclusively) by the male



parent, and this seems to impact offspring survivorship during the developmental period until the tadpole phase, when larvae are transported to a body of water. Compensatory female, recorded in congenics, has little or no effect on the survival of *A. paleovarzensis* offspring, and the male's performance is decisive in this process.

Keywords: Anura, Aromobatidae, Dendrobatoidea, paternal care, poison frog.

## 1. Introduction

Parental care probably originated and continued to increase offspring survival in settings where early mortality is a frequent event (Klug et al., 2012). Ecological and stochastic factors can affect offspring survival, and the presence of parental care can increase the chances that a greater number of offspring will survive to the next developmental stage (Klug & Bonsall, 2014). In consequence, the form, intensity and duration of parental care can differently affect pup survival (Trivers, 1972). As a result, during that portion of development when offspring are associated with the parental caregiver, continuity of offspring care may be decisive (Klug & Bonsall, 2014).

Parental care can lead to costs that may include: reduced parental survival due to predation exposure, limited access to food resources, increased energy and time expenditures, as well as the loss of additional mating opportunities (Klug et al., 2012; Royle et al., 2014). Such costs are generally offset by increases in offspring survival, growth and quality. This benefit occurs during the stage of life in which care-giving parents and the offspring are physically associated, the main forms of care being: guarding, protection against predators, provision of food resources, regulation of humidity and/or temperature, removal of parasites, residues and dead individuals, and transport to suitable environments (e.g., Trivers, 1972; Klug et al., 2012, Schulte & Lötters, 2013; McKeon & Summers, 2013).

Parental care can be given by one or both parents, a pattern that is determined by the physiology and anatomy of each sex and by environmental and behavioral conditions occurring over evolutionary and ecological time which, together, define which sex is able to provide care to offspring (Webb et al., 1999; Klug et al., 2012, Royle et al., 2014). In addition, patterns of parental care are related to parental investment costs for each parent, where differences in functions occur when relative fitness advantages and disadvantages vary

between sexes (Kokko & Jennions, 2012). As a result, since the behavior of each parent can affect the fitness of both, a change in intensity or frequency of care by one partner should lead to modifications in the behavior of the other (Lessels, 2012).

When, in biparental care species, one of the parental caregivers abandons the offspring, the remaining partner may either increase care activity in its current form or assume the behaviours and duties of the absentee in order to ensure offspring survival (e.g., Jones et al. 1998; Suzuki & Nagano, 2009; McNamara et al., 2003; Johnstone et al., 2014). In species where care is uniparental, caregiver desertion or death can lead to high or complete mortality of a group of offspring (e.g., Juncá 1996; Vockenhuber et al., 2009; Lehtinen et al., 2014). Thus, successful parental care can be a very strong determinant of reproductive success of uniparental care species. Consequently, to maximize the probability of offspring survival, it is likely that the other sex will provide compensatory offspring care in response to the loss of a parental caregiving partner.

For species of uniparental care, there are examples of compensation in birds and mammals (Kappeler & Kraus, 2010), fish (Itzkowitz et al., 2001), reptiles (Wilkinson & Huber, 2012), amphibians (Ringler et al., 2015), insects (Suzuki & Nagano, 2009) and molluscs (Finn et al., 2009). Such studies support the hypothesis that, although they do not show such behavior at any one time, both sexes may have the capacity for care, and the parent that has assumed the caregiver role has done so due to the current optimality of conflicts of costs and benefits (Klug et al., 2012; Lessels, 2012; Royle et al., 2014). In addition to clarifying questions relating to the capacity of each sex for parental care, such studies provide additional knowledge regarding the duration and forms of care performed, and the quantitative relevance of parental care for the reproductive success of the species involved.

An experimental laboratory study with the Amazonian frog *Allobates femoralis*, considered a species with single male parent caretaking, showed that females can exhibit flexible offspring caring behavior to compensate for male loss (Ringler et al., 2015). It is expected that species taxonomically close, and with similar natural history to *A. femoralis*, would also show such behavior. Of the few studies that have been carried on this topic in anurans, the majority have occurred in captivity. Yet field research is important in order to test the behavioral and survival relations under the influence of real biotic and abiotic conditions.

In the current study we used as our model the dendrobatoid frog *Allobates paleovarzensis*, which shows paternal care and male territoriality. The species inhabits Central Amazonian paleo-várzea environments, and is behaviourally similar to *A. femoralis*, a widely studied pan-Amazonian species. In an experimental field study, we tested the following hypotheses: (H1) Offspring mortality is higher when males are absent than when they are present; (H2) Male care of offspring occurs throughout their development; (H3) In the absence of the male, the female will take care of the offspring. This meant it was possible to discover the causes of tadpole death when the male parent was present or absent, quantify the relevance of the presence of the male at different stages of the tadpole development, and assay whether females commonly exhibit compensatory offspring care. This is the first experimental study of parental care in the field involving a taxon of the genus *Allobates*.

## 2. Material and Methods

### 2.1. Aspects of the reproductive biology of the species

*Allobates paleovarzensis* (Lima et al., 2010) is an anuran of the Dendrobatoidea superfamily with male territorial behavior and parental care. It is a diurnal species that inhabits leaf litter of seasonally flooding forests (Lima et al., 2010). Mating is polygamous and courting begins with male attraction of females, principally via an advertisement call which is issued continuously throughout the day. Males call to attract females and avoid deter other males from entering their territories. Attracted females select a partner, choose a dry leaf on the forest floor as a nest and enter into amplexus with the male, an action which stimulates oocytes release. Courtship and oviposition occur within male territories and eggs remain in the leaf nests until the tadpole phase, when they must reach an aquatic environment to continue development. The male cares for the young and continues vocal activity to attract females and repel invading males. As males mate with various females, male territories may have the results of multiple spawns developing simultaneously, at different stages of development. When tadpoles are close to stage 25 (*sensu* Gosner, 1960), the male transports them to the nearest body of water by adhering the tadpoles to his back. The place to which the male transports the tadpoles and deposits them is generally characterized as a small permanent or semi-permanent lotic water body, connected to other bodies of water of higher order. Male call activity is most intense during the rainy season, which usually occurs between November and April.

## 2.2. Study area

The study was carried out in the *Allobates paleovarzensis* type locality, located in the municipality of Careiro da Várzea (03°22'26.3"S, 59°52'06.4"W), Amazonas State, Brazil. Average annual rainfall varies between 2000 and 2400 mm (Sombroek, 2001). The area has annual periods of seasonal flooding (Cintra et al., 2013), beginning in mid-March and April when region's water bodies overflow and mix. Since the reproductive activity of the species occurs during the rainy season, the study occurred in two consecutive rainy seasons (December 2015 to April 2016, and December 2016 to February 2017). The first (2015/2016) sampling period was impacted by the ENSO (El Niño Southern Oscillation) climatic anomaly (Jiménez-Muñoz et al., 2016; Kogan & Guo, 2017). Data from previous years (2008-2015, season 360000; Hydrological Information System - HidroWeb) shows October as the first month of the rainy season (*average* = 44.5 mm in September, *average* = 147.7 mm in October). In 2015, rainfall increased only in December (monthly total = 15.8 mm in September, 31.3 mm in October, 90.7 mm in November, 126.4 mm in December), and daily rainfall patterns stabilized only in February 2016 (Monthly total = 235.3 mm).

## 2.3. Experimental and observational sampling

Field sampling occurred in a paleo-várzea fragment with a 19% canopy openness (*range* = 11.44-25.22; *sd* = 4.74: measured at 16 widely-spaced points within the fragment using a spherodensimeter (Lemmon, 1957)). Sampling occurred daily between 05:30 and 18:00 (GMT-04: 00), the period of reproductive activity for the species. Male and female individuals were captured to measure body size (SVL, in mm) and, to facilitate later identification during behavioral sampling, were individually marked via toe ablation (Phillott et al., 2007). A maximum of 2 articulations were removed from each individual and tissues stored in absolute ethanol for future work on molecular kinship (Gonser & Collura, 1996). The ablation method was used due to a lack of alternative marking for small animals such as *A. paleovarzensis* (males: 20.1 mm, females: 19.8 mm; Lima et al., 2010). Based on studies with *A. femoralis*, ablation of up to two toes has no detrimental effect on the rate of recapture of this species (Ursprung et al., 2011), and therefore survivorship is unimpeded by this action. Individuals were sexed based on coloration: yellow throat in females, throat violet in males (Lima et al., 2010).

The experiment consisted of two treatments: non-manipulation of parental, territory-owning, males (fathered, control), and removal of parental males and from their territory (non-fathered). Study area males were selected for inclusion in the experiment on the criterion of having spawn in development. We did not remove females, since our aim was to know whether they would care for the offspring after oviposition. The experiment involved 16 unremoved males and 11 males removed from their territories: seven males removed in the first sampling period ( $n = 385$  eggs) and four males removed in the second period ( $n = 187$  eggs). Among the eleven males removed, four were removed from their territories after oviposition ( $n = 304$  eggs, 11 spawns, oviposition day), four were removed when the young were in the embryo stage ( $n = 180$  embryos, 6 spawns; Seven days after oviposition), and three males removed when the young were in the tadpole phase at Gosner (1960) stages 20-25 ( $n = 88$  tadpoles, 4 spawns, 15 days after oviposition). We removed males at different stages of offspring development to test the effect of male parent absence on this process. Removed males were released at least 400 meters away from their territories so that they were unlikely to return to their points of origin (Pašukonis et al., 2013, Pašukonis et al., 2014). Nests (and the spawn therein) were identified and monitored daily to quantify the daily mortality of offspring, and to record their development stages (done according to Gosner, 1960). The visual appearance of the eggs, presence of fungi and signs of predation were recorded. We monitored tadpole transport, timing it from the time just before transport, with tadpoles still in the nest, to their arrival at the water body release site. To avoid an interference or alteration of behavior, the observer maintained a minimum distance of two meters. The study was approved by the Ethics Committee on the Use of Animals (CEUA) of the National Institute of Amazonian Research (INPA, process 0142016), and by the Biodiversity Information and Authorization System (SISBio) of the Chico Mendes Institute for Biodiversity Conservation, process 51412).

#### 2.4. Analysis

Independent of its developmental stage, we considered each individual offspring as a unit of survival in terms of its relation to the presence/absence of care, since each individual (egg to tadpole) in the same nest has, potentially, a distinct fate. The gross offspring survival values for each parent were transformed into a percentage, since the various males had different numbers of spawnings and, in these, there were different numbers of eggs. The difference between the means in the number of eggs in the nest in the different treatments was tested with a Student  $t$  test. The difference between the means of offspring survival for each

parental male (territory) in both treatments was tested with a Mann-Whitney-Wilcoxon U test. Analyses were performed in the R computational environment (R Core Team, 2016).

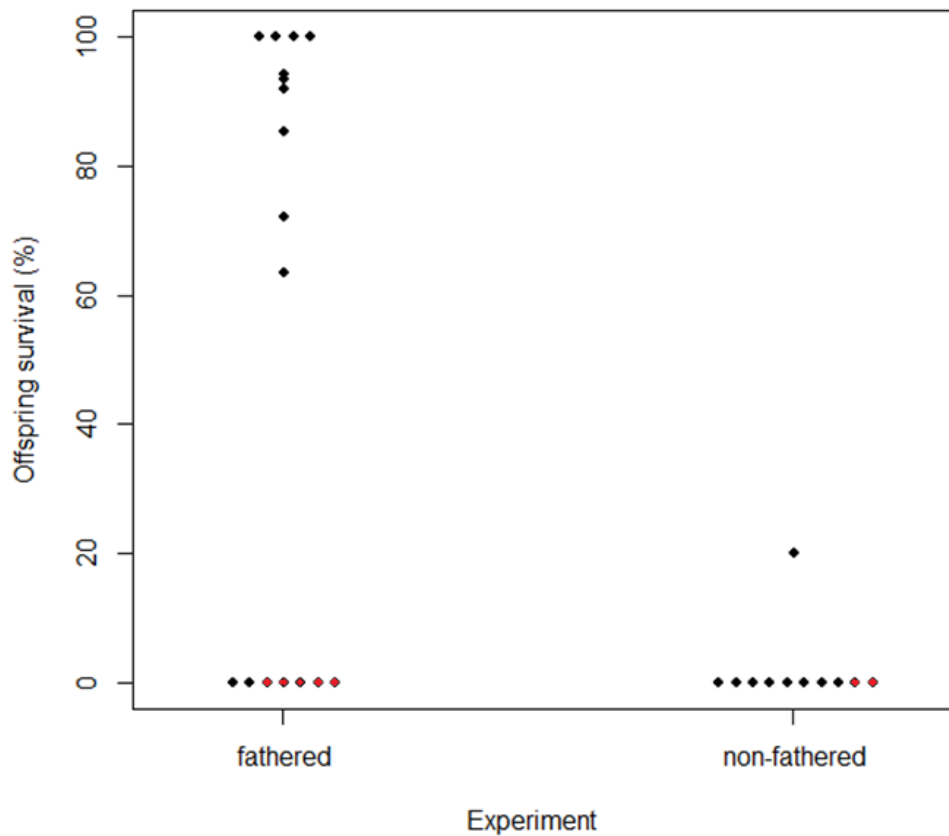
### 3. Results

Initial clutch size per spawn varied between 11 and 43 eggs ( $mean = 27.24$ ,  $sd = 8.81$ ,  $n = 21$  spawns, 572 eggs) in the group without parental care, and 12 to 41 eggs ( $mean = 25.78$ ,  $sd = 8.95$ ,  $n = 32$  spawns, 789 eggs) in the parental care group. The mean initial clutch size per spawn did not differ significantly between the two groups ( $p = 0.55$ ;  $t = 0.59$ ). Males had between one and five spawn clutches developing simultaneously in their territories. All spawnings were deposited and developed within the territory of the parental males. Percentage of offspring survival differed significantly ( $p < 0.01$ ,  $W = 48$ ,  $n = 27$  territories, Figure 1) between the groups with ( $mean = 52.95\%$ ,  $sd = 46.67$ ,  $n = 16$  territories) and without care ( $mean = 1.82\%$ ,  $sd = 6.03$ ,  $n = 11$  territories). Survivorship of young in the two experimental groups was strongly related to the presence or absence of care, and also to stochastic factors (Table 1); transport by paternal male was the commonest fate for young with parental care (60.7%), and removal by submersion or rainwater the most common fate of offspring without parental care (38.1%). Additionally, 21.2% of the eggs under male care died from desiccation. However, all these mortality events occurred during the first sampling period, during the 2015/2016 ENSO. Egg desiccation in the experimental group without a care-giving male occurred both in the period under ENSO influence and in the second sampling period, when this climatic anomaly was not in operation.

Submersion by free water and battering by rain affected both groups. As all territories near creeks, they were rapidly flooded with increasing rainfall. Strong rains also carried leaf nests into creeks, which could not be prevented by the male caregivers. However, nests without parental care were submerged or carried away more often than nests with parent care (Table 1). Predation was mainly due to ants, which consumed and removed the protective gelatinous covering to reach the young. A grey-necked wood rail (*Aramides cajaneus*, Rallidae) was recorded preying on two spawnings of the same male from the parental care group. The male was not visible or active at the time of predation. Three predation events were observed; two by ants on two spawnings of different males in the no-parental care group and rail-predation event described above, where two spawns were predated even though the parental care was present.

In the absence of transport, many young died in the nest (28.7% of the deaths in the no-parental care class). In the group with parental care, four tadpoles were not transported by their parent males and remained in the nest and dried out. Fungi infected both experimental groups, but in different ways; in the absence of care, fungi would establish on one egg and quickly occupy the entire clutch, until all eggs decomposed. In the control group receiving parental care group, fungi appeared on undeveloped or infertile eggs, but did not affect the development of the others. A single, purely stochastic event, occurred when spawn of an unmanipulated male were crushed by a branch that fell over the territory. Unlike the offspring without parental care, young receiving parental care were carried by the male to a nearby body of water. In the absence of a male caregiver only five individuals survived to Gosner stage 25. It was not recorded how, as tadpoles, they left the nest.

All observed incidences of tadpole transport were performed by male caretakers. We recorded no examples of females carrying tadpoles or having any kind of post-oviposition contact with them. Young in both care classes suffered mortality during development (Figure 2a). However, the percentage curve of surviving offspring in the class without parental care had a sharper declination than the presence of care class. Figure 2b shows longitudinal survival data, illustrating the effect when the parental caregiver was removed at three different stages of offspring development (the egg, embryo and tadpole stages). Offspring without male care died at all stages of the developmental process, so that the survivorship curve reached zero due to the high mortality following male removal. In the single exception, five tadpoles survived, when the male caregiver was removed when the young were at the embryo stage.

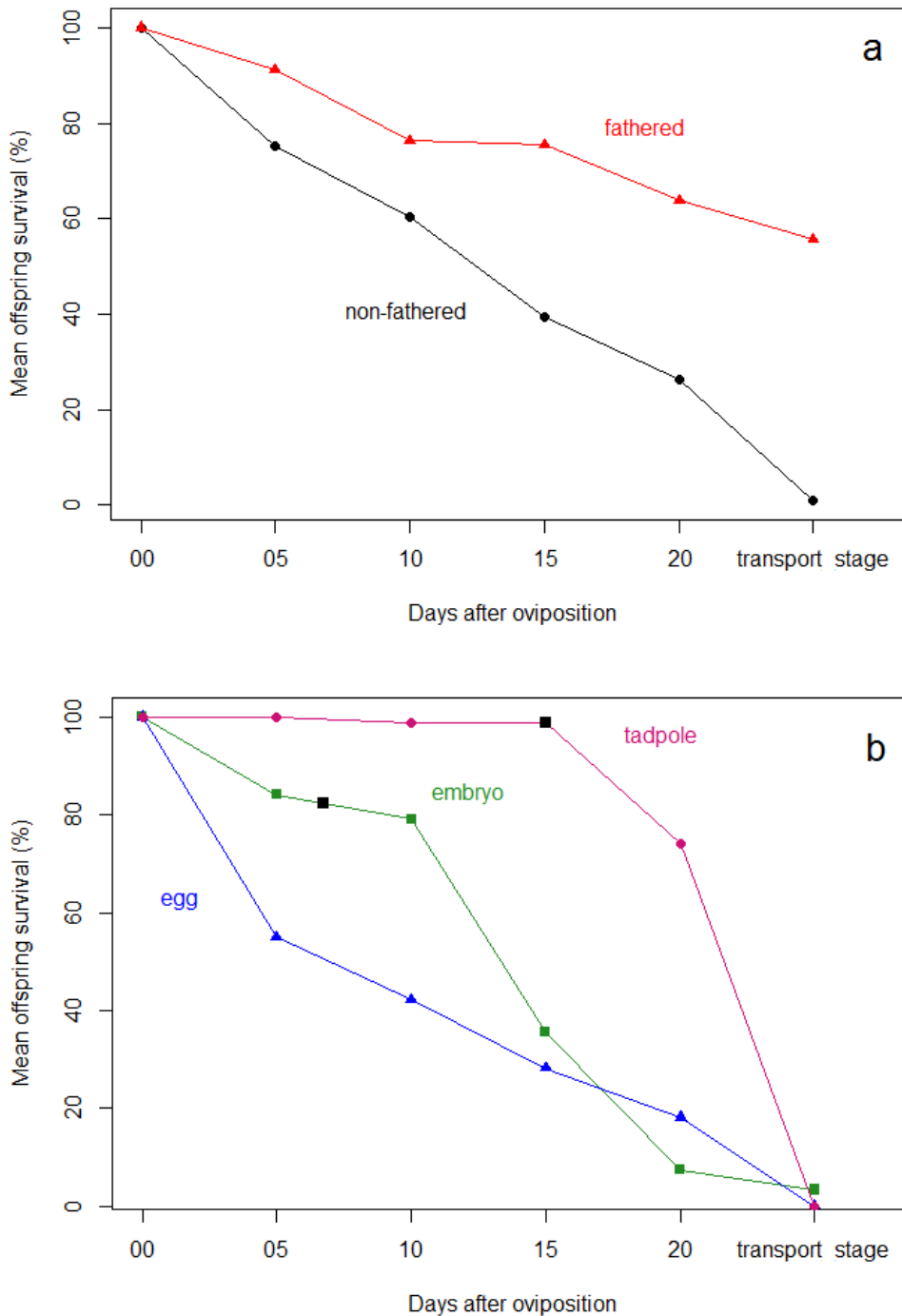


**Figure 1.** Percentage of young surviving until transport to a water body for the male care retained (fathered) ( $n = 16$  territories), and male care removal (non-fathered) ( $n = 11$  territories) groups for *Allobates paleovarzensis* in Central Amazonia. The red dots represent experimental spawns corresponding to the sampling period under the influence of the 2015/2016 ENSO.

**Table 1.** Offspring fate (in percentage) with ( $n = 32$  spawning, total of 789 eggs) and without ( $n = 21$  spawning, total of 572 eggs) parental care for *Allobates paleovarzensis* in Central Amazonia.

Fate		Fathered	Non-fathered
Died	Dried-out	21.2 %	12.8 %
	Submerged/washed-away	2.8 %	38.1 %
	Predated	9.0 %	8.2 %
	Absence of transport/inaction	0.8 %	28.7 %
	Fungal infestation	2.4 %	11.4 %
	Accidental crushing	3.2 %	0.0 %
Survived	Parental transport	60.7 %	0.9 %





**Figure 2.** (a) Survival of *Allobates paleovarzensis* young as a function of time of presence (red,  $n = 32$  spawns, 789 eggs) and post experimental removal of the male caretaker (black,  $n = 21$  spawns, 572 eggs). Circles represent mean survival percentages as a function of time. (b) Graph of percentage of young surviving at each stage of male caregiver removal. For young lacking parental care from the egg stage onwards, the male parent was removed on the day of oviposition (in blue,  $n = 11$  spawns, 304 eggs); for those lacking care from the embryo stage onwards, the male caregiver was removed at seven days following oviposition (green,  $n = 6$  spawns, 180 embryos); In the tadpole phase the male caregiver was removed 15 days following oviposition (pink,  $n = 4$  spawnings, 88 tadpoles). The colored dots represent

averages for each corresponding category; Black squares represent the date after the oviposition on which the male caregiver was removed.

#### 4. Discussion

Our study revealed that parental care in *Allobates paleovarzensis* is performed mostly (if not exclusively) by the male, and this seems to exert influence on offspring survival throughout the developmental period until, in the tadpole phase, the young are transported to a body of water. Mortality may impact offspring in both the absence and presence of parental care, however, mortality is highest at all stages of development, when a male caregiver is absent. We recorded the common and occasional biotic and abiotic factors responsible for offspring mortality in a natural environment. Our work under field conditions also allowed analysis of realistic ecological context under which males and females make reproductive investment-based decisions involving the costs and benefits of parental care.

Embryonic survival was higher in the parental care group than in the removed male group. However, there was great variation in the survival of the young in the group where the males were not removed (the control group). In the group without parental care, only 0.9% of the tadpoles survived to leave their natal nests (albeit in some way that we did not observe). All other offspring of the group without care died, as did 39.3% of the offspring of the group with paternal care. Anuran embryo mortality may be caused by a variety of biotic and abiotic factors (Vockenhuber et al., 2009; Lehtinen et al., 2014), many of which were observed in this study.

In *A. paleovarzensis* clutch dehydration appeared to be strongly aggravated by climatic variations influenced by El Niño (Jiménez-Muñoz et al., 2016). Recent studies have addressed the consequences of the climatic anomalies on a variety of wildlife, including the effect of the 2015/16 ENSO on the abundance of sea lions and seals (*Zalophus californianus* and *Arctocephalus philippii townsendi*, Elorriaga-Verplancken et al., 2016), and that of the influence of the 2010/11 El Niño on reduction of parental care, total copulatory frequency, probability of extrapair mating, and of oviposition in female blue-footed boobies (*Sula nebouxii*; Kiere & Drummond, 2016). El Niño events result in reduced food availability, especially for birds and marine predators, so favoring strategies that prioritize individual short-term self-maintenance, at the expense of immediate reproductive success (Schreiber, 2002; Kiere & Drummond, 2016). For *A. paleovarzensis*, the delay in the onset of the rainy season caused by ENSO 2015/16 led to the death by dehydration of most of the egg clutches

being cared for by males. A combination of a lack of water available for hydration, high temperatures throughout the day, and strong solar incidence may have caused the observed high offspring mortality, even in the presence of the parental caretaker. A study of the tree frog *Hyalinobatrachium fleischmanni* found that male care varied in response to the fluctuating nature of the climate and the hydration requirements of the embryos; with males increasing levels of hydration-related care in hot and dry periods (Delia et al., 2013). In our study, males were unable to protect their offspring from dehydration, nor were they able to protect them from the heavy rains and floods that naturally occur once the breeding season had begun. Offspring deposited in territories under open canopy, near creeks, in areas of low relief or on steep slopes were generally washed into waterbodies by rain or were submerged when the region flooded.

Predation of offspring occurred in both experimental groups. In the group with parental care a single predation event occurred, in which two clutches were eaten by a bird (*Aramides cajaneus*) that forages in litter near water bodies and flooded environments (Sick, 1997). The male was not present during predation and, in any case, would not have been able to prevent the event without also being at risk of predation itself. The other observed predation events were carried out by ants. All occurred in nests of the group lacking parental care, and the dehydration of the gelatinaceous sheath that otherwise protects the developing eggs had facilitated the access of the ants. A parental caregiver can directly or indirectly prevent offspring predation by ants, by keeping them away from the young and/or by keeping them hydrated during development. For the tree frogs *Hyalinobatrachium valerioi* and *H. orientale* most offspring mortality was caused by predation by invertebrates and snakes, with dehydration being of lesser importance (Vockenhuber et al., 2009; Lehtinen et al., 2014). For *Anomaloglossus stepheni*, a species phylogenetically close to *A. paleovarzensis*, predation by invertebrates and a gekkonid lizard caused most deaths, with fungal infestation being the second most important factor (Juncá, 1996). Fungal infestation of *A. paleovarzensis* eggs occurred frequently, possibly of those that had died due to developmental problems. However, with the presence of a male caregiver the fungal infection was restricted to single eggs. This contrasted with the situation for clutches receiving no parental care, in which all eggs were eventually infected by fungus. Apparently the male provides some kind of care that prevents the development of fungi on the clutches under his care (Juncá, 1996).

Our results suggest that transport to a body of water is the key factor for tadpole survival, and that this depends directly on the presence of the male caregiver, corroborating

other studies that indicate the importance of a parental caregiver at this crucial moment in offspring development (Downie et al., 2005; Ringler et al., 2013, 2015; Killius & Dugas, 2014). In the group with parental care, some tadpoles were left in their nests without transport, probably because they were not able to adhere to the back of the male. In addition to the importance of tadpole transport, our results suggest that the male performs some kind of care during the different stages of offspring development; we suggest that males may hydrate clutches by positioning themselves above them as suggested for other anuran species (Vockenhuber et al., 2009; Lehtinen et al., 2014), including dendrobatoids (Juncá, 1996). This hydration might well prevent both drying-out and fungal proliferation, the two factors most responsible for offspring mortality in *A. paleovarzensis*.

In *A. femoralis*, females can replace paternal care in the transport of tadpoles in case of male absence. Such compensatory care by a female was recorded in 7.8% of transport events in a natural population, and most cases of female flexibility occurred when the male had not been recorded as present for several days prior to transport by the female (Ringler et al., 2013). Captive studies confirmed this behavior (Ringler et al., 2015). In the current field-based study, no *A. paleovarzensis* female was recorded transporting tadpoles to water bodies, and all transports observed were performed by males. Although *A. paleovarzensis* and *A. femoralis* are similar models of study, other investigations have demonstrated *A. femoralis* behaviors in captivity, such as clutch cannibalism by males (Ringler et al., 2017), that have not been observed in natural populations of *A. paleovarzensis*.

It is probable that females of *A. paleovarzensis* gain greatest benefits with a strategy involving complete desertion of offspring and, after oviposition, investment in selection of new partners. Such a strategy in females would allow the greatest number of descendants within a reproductive life-span, and avoids all costs associated with parental care (Downie et al., 2005). The females may be physiologically and behaviorally capable of parental care but, in *A. paleovarzensis*, the male assumes the role of caregiver due to a conflict of costs and benefits (Klug et al., 2012; Lessels, 2012; Royle et al., 2014). Probably these costs and benefits are associated with the presence of a reproductive territory defended by the male where spawn is deposited, allowing the female to desert first and leave the clutch and its care as the responsibility of the male. We suggest that male desertion is not common enough for females to return to nests to verify male efficiency in providing offspring care. It is likely that there are occasional cases of tadpole transport by females, and this being this may have been the fate of the surviving tadpoles in the experimental male-less nest, in the current study. In

this case, we suggest that transport may have been carried out by the female, by a male adjacent to the experimental territory. Studies with a greater number of removed males, and with daily monitoring of females could clarify the role of female parents in this system.

The developmental stages between the egg and tadpole represent the particularly vulnerable stages of the anuran life cycle (Gosner, 1960). The egg and embryo are immobile units and under threat of moisture loss (e.g., Taigen et al., 1984; Townsend et al., 1984; Juncá, 1996; Lehtinen et al., 2014), predation (e.g., Juncá, 1996; Burrowes, 2000; Lehtinen et al., 2014) and infestation by parasites and diseases (e.g., Juncá, 1996; Bourne, 1998; Green, 1999). The tadpole phase is mobile and, in addition to the threats mentioned in the previous stages, requires an aquatic environment for breathing, feeding, locomotion and for the continuity of development (Gosner, 1960; Weygoldt, 1987). Any behavior that leads to reduced offspring mortality in any of these stages has a strong potential to increase the reproductive success of the parental caregiver involved (Vockenhuber et al., 2009). In our study, male removal directly affected offspring survival, with the main drop in the percentage survivorship occurring soon after the removal of the male care-giver, irrespective of the phase in development at which this was operationally manipulated. This result shows how much the young are vulnerable to the loss of paternal care in these phases and how much the promotion of paternal care is determinant in the survival of young *Allobates paleovarzensis*.

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## Síntese

O comportamento reprodutivo em *Allobates paleovarzensis* é determinado pela conquista e defesa de um território pelo macho, o qual pode abranger áreas de vida de diferentes fêmeas. Os machos vocalizam no período diurno, com maior atividade pela manhã, e a atividade reprodutiva da espécie está estritamente relacionada com os períodos de maior pluviosidade do ano. A corte é composta pela atividade de chamada dos machos, pela escolha das fêmeas por um ninho de folha na serapilheira e termina com amplexo cefálico. Os ovos são depositados em uma folha sobre o solo, dentro do território dos machos. Dessa forma, o macho provê cuidado a todos os filhotes presentes em seu território, da fase de ovo até a de girino, quando o macho os transporta para um corpo d'água onde os filhotes se desenvolvem até a metamorfose. Machos realizam defesa de seus territórios com vocalizações direcionadas a invasores e disputas agressivas, que abrangem exibição dos competidores, perseguição e confrontos físicos.

O tamanho do território constitui o determinante principal do sucesso de acasalamento dos machos por meio da seleção positiva das fêmeas, não sendo este correlacionado com seus outros atributos extrínsecos ou intrínsecos. As fêmeas possivelmente acessam o tamanho do território dos machos por meio do processo de corte que antecede a oviposição, momento em que o macho conduz a fêmea pelo território e ela pode saber se o macho é um bom competidor e o quanto ele consegue manter e defender de espaço para reprodução. Consideramos que nesse momento a fêmea faz a escolha entre prosseguir com a corte ou desistir de acasalar com o macho em avaliação.

O cuidado parental em *Allobates paleovarzensis* é realizado exclusivamente pelo pai, sem nenhuma participação materna no desenvolvimento dos filhotes. O macho cuida da prole durante todo o período de desenvolvimento associado ao território do pai, de ovo até a fase de girino transportado para um corpo d'água. A mortalidade pode afetar tanto filhotes com cuidado parental quanto sem cuidado. Contudo, sem a presença do pai a mortalidade é elevada e um maior número de filhotes morre durante cada fase do desenvolvimento. O transporte para o corpo d'água é determinante para a sobrevivência dos girinos e depende diretamente da presença do pai cuidador.

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