Reproductive biology of *Ameerega trivittata* (Anura: Dendrobatidae) in an area of terra firme forest in eastern Amazonia

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**ABSTRACT**

The reproductive success of tropical amphibians is influenced by factors such as body size and the characteristics of breeding sites. Data on reproductive biology are important for the understanding of population dynamics and the maintenance of species. The objectives of the present study were to examine the abundance of *Ameerega trivittata*, analyze the use of microhabitats by calling males and the snout-vent length (SVL) of breeding males and females, the number of tadpoles carried by the males and mature oocytes in the females, as well as the relationship between the SVL of the female and both the number and mean size of the mature oocytes found in the ovaries. Three field trips were conducted between January and September, 2009. A total of 31 plots, with a mean area of 2.3 ha, were surveyed, resulting in records of 235 individuals, with a mean density of 3.26 individuals per hectare. Overall, 66.1% of the individuals sighted were located in the leaf litter, while 17.4% were perched on decaying tree trunks on the forest floor, 15.7% on the aerial roots of *Cecropia* trees, and 0.8% on lianas. Males were observed transporting a mean of 10.8 tadpoles on their backs. A significant correlation was found between the size of the females and the mean diameter of the oocytes. New data were collected on the size of oocytes and no pattern was found in the type of perches used by calling males of the different *Ameerega* species.

**KEYWORDS:** Calling site, oocyte size, sexual dimorphism, tadpole transportation.

Biologia reprodutiva de *Ameerega trivittata* (Anura: Dendrobatidae) em uma área de terra firme na Amazônia Oriental

**RESUMO**

O sucesso reprodutivo das espécies de anfíbios tropicais sofre influência de fatores como local de reprodução e tamanho do corpo dos reprodutores. Informações sobre a biologia reprodutiva são importantes para entender a dinâmica populacional e a manutenção das espécies. Os objetivos deste estudo foram de verificar a abundância, o uso de microhabitats por machos, o comprimento rostro-cloacal (CRC) de machos e fêmeas em atividade reprodutiva, a quantidade de girinos transportados por machos e o número de oócitos maduros por fêmea, e relacionar o número de oócitos maduros e o tamanho médio dos oócitos maduros com o CRC da fêmea. Um total de 31 parcelas foi vistoriado, tamanho médio de 2,3 ha, e um registro de 235 indivíduos, média de 3,26 por hectare. Sessenta e seis por cento dos indivíduos avistados estavam na serapilheira, 17,4% sobre troncos em decomposição no chão da floresta, 15,7% sobre raízes aéreas de *Cecropia* sp. e 0,8% sobre cipó. Foram observados machos transportando girinos no dorso, a média de girinos transportados foi de 10,8 por macho. A quantidade média de oócitos maduros no ovário das fêmeas foi de 20,17. Houve correlação significativa entre o diâmetro médio dos oócitos e CRC. Neste estudo obtivemos dados novos relacionados ao tamanho médio de oócitos em fêmeas ativamente reprodutivas e observamos que o tipo de sítio de vocalização usado por machos varia entre as espécies do gênero *Ameerega*.

**PALAVRAS-CHAVE:** Local de vocalização, tamanho dos oócitos, dimorfismo sexual, transporte de girinos.
INTRODUCTION

The reproductive success of tropical amphibians may vary in relation to the body size of the species and the characteristics of its breeding sites (Fellers 1979; Nascimento et al. 2001). Crump (1974) and Halliday and Tejedo (1995) observed a significant relationship between the body size of the female and the size of the spawn, indicating that larger animals make a relatively greater contribution to the subsequent generation. Data on reproductive biology are fundamentally important for the understanding of the population dynamics and conservation of species.

The dendrobatid amphibians are widely distributed in the Neotropics. These frogs lay their eggs in terrestrial nests, such as leaf litter, or arboreal phytotelmata (Duellman 1992; Grant et al. 2006). The parents typically take care of the eggs and once they hatch, the male, female or both parents transport the tadpoles on their backs to an appropriate location in which metamorphosis can be completed (Rico et al. 2004; Grant et al. 2006). In Brazil, this family is represented by 26 species distributed in five genera, of which Ameerega is the second most diverse, with nine species (Segalla et al. 2012; Frost 2013).

The triple-striped poison-dart frog, Ameerega trivittata, is one of the most widely distributed species of the genus, occurring practically throughout the Amazon basin, including Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Paraguay, Peru, Suriname, and Venezuela (Silverstone 1976; Grant et al. 2006; Frost 2013). In Peru, Roithmair (1994a) observed that the reproductive success of A. trivittata was related to the size of the territory defended by the male. Differences in the size of the territories of male A. trivittata may be related to structural features of the habitat, such as the types of substrate used for vocalization, which have implications for the reproductive success of the population. However, no published data are available on the use of microhabitats by the males or the reproductive characteristics of the females of this species.

The present study aimed to: (1) verify the density of Ameerega trivittata in an area of terra firme forest in the eastern Amazon basin, and (2) characterize the use of microhabitats by calling males, as well as recording (3) the body size (snout-vent length, SVL) of breeding males and females, (4) the number of mature oocytes carried by the females and the number of tadpoles carried by the males, and analyzing (5) the relationship between the SVL of the female and the number and mean size of the mature oocytes found in the ovaries.

MATERIALS AND METHODS

Study site

The present study was conducted in a 50,000 ha tract of terra firme rainforest in the municipality of Juruti, in the west of the Brazilian state of Pará (02°09´08”S, 56°05´32”W; Figure 1). This area is characterized by the presence of relatively large trees, which may reach 50 m in height, such as the Brazil-nut (Bertholletia excelsa), macáranduba (Manilkara huberi), and pekea (Caryocar villosum), with an understory dominated by palms (Ribeiro et al. 2002). Clearings and forest edges are dominated by pioneer tree species, such as those of the genus Cecropia (Santos 2000).

Mean annual temperature is approximately 26°C, while relative humidity is normally over 80% throughout the year. Mean annual rainfall is approximately 2200 mm. The rainy season coincides with the period between December and May, with the drier season lasting from June to November (Moraes et al. 2005).

Sampling and population density

We collected samples during three field trips to the study area in 2009 (January 19-February 13; April 25-June 18; July 20-September 1). During the first two expeditions, we surveyed 31 transects, which were separated by a distance of at least 200 m, in order to guarantee the independence of the samples. The sampled area in each transect varied from 1.2 ha to 3.7 ha (x̄ = 2.3; ha ± 0.7) with a total area of 72 ha. We considered the population density, as the total number of recorded individuals, divided by the total sampled area.

We visited each transect once, with six transects being visited during the first expedition, and 25 during the second. As both expeditions took place during the same rainy season, we considered the transects to be spatial, but not temporal replicates.

The sampling procedure consisted of active searching for individuals of the species A. trivittata by two collectors during a period of 1 hour and 30 minutes (Martins and Oliveira 1998). We conducted the surveys between 06:00 - 18:00 h, and recorded all the animals heard or sighted within the transect during the period of search. At the end of each search, we recorded the total length covered in each transect, in order to calculate the sampled area.

Activity patterns and male calling sites

For each individual sampled, the following data were recorded: (I) sex, (II) behavior of the individual when first sighted, (III) the substrate being used at first sighting, (IV) presence of temporary pools in the immediate vicinity, and (V) the height above the soil at which the individual was located. All the individuals found vocalizing were considered male, while the sex of the specimens not calling could not be determined in the field. When individuals were sighted, they were allocated to one of three activity categories: (I) motionless and silent; (II) motionless and calling, and (III) moving. The substrates were classified as leaf litter, decomposing tree trunk, aerial Cecropia roots, and liana.
Female reproductive parameters and sexual dimorphism

We collected a total of 60 Ameerega trivittata specimens, which we weighed using a Pesola® spring balance (0.1 g precision, Baar, Switzerland) and measured (SVL) with digital calipers (0.5 mm precision, Mitutoyo®, Suzano, São Paulo, Brazil). We then euthanized the specimens in 5% Lidocaine for analysis in the laboratory. Two of the specimens were considered to be juveniles and were not included in the analysis of reproductive status. Of the others, 28 were female, and 30, male. We analyzed all the specimens for the presence of longitudinal gular folds, which are typical of the males, but absent in the females (Roithmair 1994a). The males considered to be reproductively active presented two longitudinal gular folds, while females were registered as mature when they had grayish oocytes in the active vitellogenesis class and/or well-defined black and yellow poles, consistent with the post-vitellogenesis class (Díaz-Páez and Ortiz 2001). We used the t test to evaluate the difference in body size between males and females.

We dissected the female specimens and removed their ovaries for the measurement of the number of mature oocytes and their mean diameter. We measured the diameter of the mature oocytes from the animal pole to the vegetal pole with digital calipers (0.1 mm precision, Mitutoyo®, Suzano, São Paulo, Brazil). We examined the relationship between the quantity of mature oocytes and the SVL of the females only for the individuals with post-vitellogenesis class oocytes. The atrophied unspawned post-vitellogenesis class oocytes were not quantified or measured, and were thus excluded from the analysis. We used a simple linear regression to verify the possible relationship between body size (SVL) and the number and mean diameter of the oocytes observed in each female with a 5% significance level.

Figure 1. Location of Juruti in the Brazilian state of Pará (map, right) and the study area, demarcated by a dotted line (satellite image on the left).
Additional observations on resting sites and the number of tadpoles transported

We dedicated the third expedition to nocturnal (18:00 - 22:00 h) and diurnal searches (06:00 - 18:00 h). The nocturnal searches were conducted for the collection of data on resting sites and the diurnal searches for the determination of the numbers of tadpoles being carried by the males. Resting individuals were detected with a flashlight along pre-existing trails. Each night, a trail of approximately 2 km was inspected. The resting site and height above the ground of each individual encountered was recorded. These data were not included in the census analysis.

During the diurnal searches, we collected data along five dirt roads that traverse the study area, varying in length from 1.4 km to 10 km. The data were collected by two researchers over a one hour period, using a vehicle running at a constant speed of 5 km per hour. All individuals sighted carrying tadpoles were photographed before being captured in order to count the number of tadpoles being carried. We fixed all the specimens euthanized during the study in 10% formalin and then preserved them in 70% ethanol prior to depositing them in the herpetological collection of the Goeldi Museum (Museu Paraense Emílio Goeldi) in Belém, Pará state, Brazil.

RESULTS

Population density, activity patterns, and male calling sites

We recorded 235 individuals in the 31 transects, with a mean of 3.26 individuals per hectare, of which 118 were only heard vocalizing, and registered as males. The remaining 117 individuals were sighted, of which 36 were vocalizing and could thus also be recorded as males, while the sex of the other 81 was undetermined. Temporary ponds were observed in only two of the 31 transects. In one of these, we observed nine temporary ponds, while we found six in the other plot. The plot with nine ponds presented the highest abundance of *A. trivittata* (*n* = 31), whereas the plot with six ponds presented the third highest abundance (*n* = 16). *Ameerega trivittata* tadpoles were found in 86.76% of the ponds. The surface area of the ponds varied from 0.01 m² to 24.63 m² (*x̄* = 1.71 m² ± 6.33).

Overall, 66.6% of the individuals sighted were in the leaf litter when observed, 17.1% were located in decomposing tree trunks on the forest floor, 15.5% were observed on aerial *Cecropia* roots, and 0.8% on lianas. With regard to activity patterns, almost half (44.4%) of the individuals were resting in silence, 29.1% were calling, and 26.5% were moving (Table 1). The males were calling on perches, at heights of between 10 cm and 110 cm (*x̄* = 44.60 cm ± 24.60). Half of the perches used by these males were *Cecropia* roots, 46.9% were decaying trunks on the forest floor, and 3.1% were lianas (Table 2).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Number (%) of <em>Ameerega trivittata</em> individuals sighted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf litter</td>
<td>78 (66.6)</td>
</tr>
<tr>
<td>Decomposing tree trunk</td>
<td>20 (17.1)</td>
</tr>
<tr>
<td>Aerial <em>Cecropia</em> roots</td>
<td>18 (15.5)</td>
</tr>
<tr>
<td>Liana</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Total</td>
<td>117 (100)</td>
</tr>
</tbody>
</table>

Table 1. Substrate used by *Ameerega trivittata* in an area of terra firme forest in eastern Amazonia and the activity of the individuals at the moment of sighting.

<table>
<thead>
<tr>
<th>Perch</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cecropia</em> roots</td>
<td>16 (50.0)</td>
</tr>
<tr>
<td>Decaying trunk tree</td>
<td>15 (46.9)</td>
</tr>
<tr>
<td>Liana</td>
<td>1 (3.1)</td>
</tr>
<tr>
<td>Total</td>
<td>32 (100)</td>
</tr>
</tbody>
</table>

Table 2. Calling perches used by male *Ameerega trivittata* when sighted in an area of terra firme forest in eastern Amazonia.

Female reproductive parameters and sexual dimorphism

Of the 28 females collected, 89.3% had AV and/or PV type oocytes, and were thus considered to be reproducitively active. The number of non-atrophied PV oocytes recorded per female ranged from 3 to 39 (*x̄* = 20.17 ± 8.80; *n* = 17), although no significant relationship was found between female SVL and the number of mature oocytes (*r*² = 0.04; *P* = 0.42; Figure 2).

The diameter of mature oocytes varied from 1.69 to 3.88 mm (*x̄* = 2.51 mm ± 0.42; *n* = 17), and the mean diameter was correlated significantly with female size, i.e., SVL (*r*² = 0.58; *P* = 0.01; Figure 3). Twenty-eight of the 30 male specimens were mature. The SVL of the males ranged from 32.02 to 43.21 mm (*x̄* = 38.19 mm ± 2.46; *n* = 28) and that of the mature females from 39.88 to 46.37 mm (*x̄* = 42.87 mm ± 1.66; *n* = 25). On average, then, females were 4.68 mm longer than males (*t* = 8.00; *P* < 0.01; Figure 4).

Additional observations on resting sites and the number of tadpoles transported

We encountered a total of 43 *A. trivittata* along the road transects, of which, 10 were males transporting tadpoles on their backs. These males were carrying between two and 23 tadpoles (*x̄* = 10.8 ± 8.8; *n* = 10).

We encountered a further 10 individuals during informal nocturnal surveys, sleeping at heights of between zero and
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60 cm above the ground ($\bar{x} = 30.00 \text{ cm} \pm 21.08$). Eight of these individuals were resting on the leaves of shrubs (one of which had seven tadpoles on its back), one was sleeping on a palm leaf, and the other in the leaf litter. No *A. trivittata* were observed calling or moving after 19:00 h. All individuals observed after this time were registered as sleeping.

**DISCUSSION**

Interspecific differences in anuran calling sites may be related primarily to the size and morphology of the animal (Bertoluci and Rodrigues 2002) and the local availability of perches. *Cecropia* roots and decaying trunks were probably used frequently as perches by calling males in the present study due to local availability. The use of perches raised up off the ground by calling *A. trivittata* males is not a typical behavioral pattern in this amphibian family. In Cerrado savanna, for example, Forti *et al.* (2010) observed that most *Ameerega braccata* called from the leaves of bushes and herbaceous plants. Haddad and Martins (1994) observed that *Ameerega flavopicta* usually call from rocks near streams, *Ameerega hahneli* from the leaf litter, and *A. picta* from the branches of dead trees on the leaf litter. Whilst, Brown and Towmey (2009) in a research conducted in north-central Peru recorded that *Ameerega yoshina* males appear to call from the leaf litter and *Ameerega pepperi* males call from elevated positions on boulders.

In the present study, no significant relationship was found between female SVL and the number of mature oocytes. This is consistent with the results of Roithmair (1994b), but not with other studies (e.g. Halliday and Tejedo 1995). Reproductive success in female amphibians is generally related to their SVL, with larger females producing more offspring than smaller ones. However, intraspecific variation in reproductive output of anurans may be related to variation in female properties and environmental conditions (Lüddecke 2002). Another point is that some comparative studies have indicated that egg size increases in the presence of parental care (e.g. Crump 1995; Crump 1996). The positive relationship observed in the present study between female size and the size of the eggs indicates that *A. trivittata* females may invest more in the size of the yolk than the number of oocytes.

Most species of dendrobatids transport their larvae or tadpoles to different locations to prevent cannibalism, while also enhancing dispersal. The mean number of tadpoles being carried by *A. trivittata* males was equivalent to half the mean number of mature oocytes found in the ovaries of the females. The deposited eggs of frogs are exposed to multiple factors such as predation by invertebrates, desiccation, and fungal attack. Assuming that all oocytes hatch, a larger number of tadpoles would be expected to be transported by the males, as observed in *A. trivittata* by Roithmair (1994b). This
suggests that the males either do not remove all the tadpoles all at once or deposit them in different pools. In this case, a male may be found carrying different numbers of tadpoles at any given moment, depending on the stage of the transfer process. Alternatively, some of the tadpoles may be lost during transportation, or not all oocytes hatch.

In some species, the males may carry the tadpoles for a number of days before finding a temporary pool (Wells 1980), which would account for the individuals found sleeping at night with tadpoles on their backs, as observed in the present study, in which one male was observed sleeping with 10 tadpoles on its back on a fallen tree trunk 30 cm above the ground. However Roithmair (1994b) recorded a different pattern – in all but one case, all the tadpoles of a given clutch were transported together. In the one exception, the male transported 24 tadpoles on the first day and returned on the following day to retrieve the remaining eight tadpoles. The transportation of varying numbers of tadpoles has also been observed in the congeneric species, Ameerega flavopicta by Toledo et al. (2004), and Ameerega bahneli by Haddad and Martins (1994).

CONCLUSION

This study presents new data on the size of the oocytes of Ameerega trivittata, as well as the relationship between oocyte size and SVL. The sexual dimorphism observed in the study was typical of the dendrobatids in general. The use of perches above the ground for calling is also typical of the genus Ameerega, although the type of substrate used – Cecropia roots and fallen trunks – are different from those used by other species. More detailed data on the other species that inhabit the same biome, such as Ameerega bahneli, A. peteri, and A. picta, would provide a more reliable basis for the understanding of phylogenetic patterns in this group.

ACKNOWLEDGMENTS

We thank João Carlos, Fabrício Correa, Heriberto Figueira, and Marcelo Viana for their assistance in both the field and the laboratory. We would also like to thank Taran Grant, Marcelo Menin, Ulisses Gallati, Gleomar Maschio, Maria C. Santos-Costa, Paulo Bernardes, Felipe Toledo, and Domingos J. Rodrigues for their suggestions. We are also grateful to Rafael Salomão, coordinator of the fauna monitoring project, for making fieldwork possible, Alcoa World Alumina for financial support, and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing a scholarship. Specimen collection was authorized by Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA), special license number 02018/001126/07.

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Reproductive biology of *Ameerega trivittata* (Anura: Dendrobatidae) in an area of terra firme forest in eastern Amazonia


**Recebido em 07/11/2013**

**Aceito em 08/05/2014**