

## ORIGINAL ARTICLE

# Aspects of the reproductive ecology of *Trachycephalus cunauaru* (Anura: Hylidae) in the southern Amazon

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## ABSTRACT

*Trachycephalus cunauaru* is an Amazonian hylid that uses phytotelmata to reproduce. There is relatively little information about the species, mainly due to the difficulty of accessing their reproductive sites. In this study, we gathered data on the ecology and natural history of *T. cunauaru* in the southern Amazon, in the state of Mato Grosso, Brazil. In addition to natural phytotelmata, we used buckets installed at a height of 10 m as artificial phytotelmata. We compared physical and chemical characteristics, as well as the presence of tadpoles between natural and artificial phytotelmata. We also collected data on the reproductive behavior of the species through the use of camera traps. We recorded a density of 14.1 reproductive sites per km<sup>2</sup>. Environmental parameters differed significantly between artificial and natural phytotelmata. In artificial sites, the presence of tadpoles was directly related to trees with a larger diameter. We registered oophagy for the first time for the species and observed that males can use more than one phytotelm. We also recorded the presence of snakes within the reproductive sites. We determined that artificial sites and digital camera traps are a satisfactory alternative for behavioral observations of *T. cunauaru* and possibly for other species with a similar habit.

**KEYWORDS:** phytotelmata, artificial reproductive sites, canopy sampling, oophagy, amphibians

## Aspectos da ecologia reprodutiva de *Trachycephalus cunauaru* (Anura: Hylidae) na Amazônia meridional

### RESUMO

*Trachycephalus cunauaru* é um hílideo amazônico que utiliza fitotelmatas para se reproduzir. Existem relativamente poucas informações sobre a espécie, principalmente devido à dificuldade de acesso aos seus sítios reprodutivos. Nesse trabalho, reunimos dados de ecologia e história natural de *T. cunauaru* em uma área da Amazônia meridional, no estado de Mato Grosso, Brasil. Além de fitotelmatas naturais, utilizamos baldes instalados a uma altura de 10 m como fitotelmatas artificiais. Comparamos características físicas e químicas, bem como a presença e ausência de girinos, entre fitotelmatas naturais e artificiais. Também coletamos dados sobre o comportamento reprodutivo da espécie por meio de armadilhas fotográficas. Registramos uma densidade de 14,1 sítios reprodutivos por km<sup>2</sup>. Os parâmetros ambientais diferiram significativamente entre fitotelmatas artificiais e naturais. Em sítios artificiais a presença de girinos esteve diretamente relacionada à árvores com maior diâmetro. Registramos, pela primeira vez, oofagia para a espécie e observamos que machos podem utilizar mais de um fitotelmata. Também registramos a presença de cobras nos sítios reprodutivos. Constatamos que sítios artificiais e armadilhas fotográficas representam uma alternativa satisfatória para registros comportamentais para *T. cunauaru* e, possivelmente, para outras espécies com hábitos similares.

**PALAVRAS-CHAVE:** fitotelmatas, sítios reprodutivos artificiais, amostragem de dossel, oofagia, anfíbios

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## INTRODUCTION

More than 250 species of anuran amphibians use water bodies contained in plants as reproductive sites (Lehtinen 2020). These structures, known as phytotelmata, can be tree holes, bamboo hollows, bromeliad axils or fruit capsules (Lehtinen *et al.* 2004; Rödel *et al.* 2004). These environments can represent safer places for eggs and larvae, due to the lower incidence of predators and competitors, but, at the same time, they can be challenging habitats for the offspring due to low dissolved oxygen concentrations, high risk of desiccation and unpredictable food availability (Grillitsch 1992; Jungfer and Weygoldt 1999; Rödel *et al.* 2004). Important environmental factors such as risk of desiccation and predation can determine population recruitment, parental fitness and offspring survival in this type of reproductive site (von May *et al.* 2009; Lantyer-Silva *et al.* 2018).

Phytotelmata are often located in the canopy and sub-canopy of the forest, but in tropical forests, where trees can reach more than 30 m in height, the difficulty to reach the sites and apply sampling protocols lead to a lack of scientific data on species that use these environments (McCracken and Forstner 2008). Consequently there is little information about behavior and ecological parameters that determine habitat selection by phytotelmata breeders in Amazon rainforests (*e.g.* Jungfer and Weygoldt 1999; Gordo *et al.* 2013). The use of artificial reproductive sites is an effective alternative for the collection of ecological data, mainly for invertebrates (*e.g.* Petermann *et al.* 2016; Yoshida *et al.* 2017). For anurans, the use of this methodology has been more restricted to species that reproduce either at ground level or up to 2 m in height (*e.g.* von May *et al.* 2009; Khazan *et al.* 2019).

The difficulty of access to phytotelmata resulted in the hylid *Trachycephalus cunauaru* Gordo *et al.* 2013, having been mistakenly identified as *Trachycephalus resinifictrix* (Goeldi 1907) for a long time (Gordo *et al.* 2013). Similarly to *T. resinifictrix* (Lima *et al.* 2006), *T. cunauaru* uses holes in trees as reproductive sites. Considering the lack of information available on the natural history and the absence of sampling alternatives for the species, this study aimed to: 1) test whether *T. cunauaru* would use artificial phytotelmata as breeding sites; 2) determine whether chemical and physical parameters of natural phytotelmata are sufficiently similar to those of artificial phytotelmata for the latter to be used as reliable proxies for sampling of ecological and behavioral data on phytotelmata breeders; 3) analyze the relation of physicochemical parameters of phytotelmata with the presence/absence of tadpoles; and 4) report behavioral data of the species through the use of digital camera traps.

## MATERIAL AND METHODS

### Study area

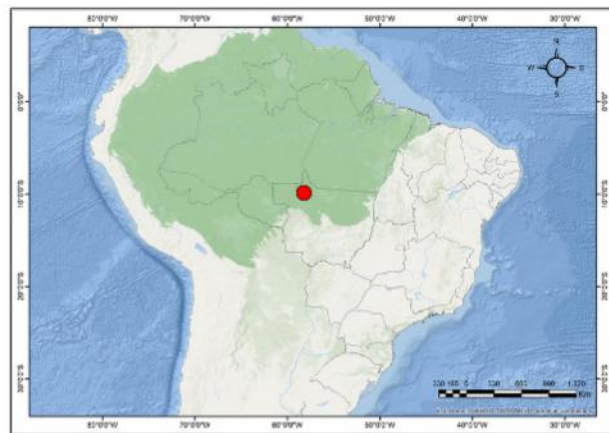
The study was carried out at Fazenda São Nicolau (9°51'S; 58°14'W), located in the city of Cotriguaçu, Mato Grosso state, Brazil, in the southern Amazon (Figure 1). The region's vegetation is characterized as open and dense rainforest (Velooso *et al.* 1991). The climate is humid tropical (Am, according to the Köppen classification) with average annual temperature of 25 °C, and average annual rainfall of 2000 mm (Camargo *et al.* 2010), with two well-defined seasons: a dry season between April and September and a rainy one from October to March.

Surveys were carried out in a standardized RAPELD sampling module of the Biodiversity Research Program - PPBio (Magnusson *et al.* 2005). The 5-km<sup>2</sup> module consists of two parallel 5-km trails connected by six perpendicular 1-km trails. We surveyed the two 5-km trails and two of the 1-km trails, as well as a 700-m trail that gives access to the module.

The identification, marking and monitoring of phytotelmata was carried out during the rainy seasons of 2014, 2015 and 2016, at nighttime, between 6 pm and 4 am. We walked along the trails as we marked and georeferenced phytotelmata in which males were calling. This search methodology was possible because *T. cunauaru* has a very characteristic vocalization that is audible at distances up to 1,000 m (Gordo *et al.* 2013). We delimited a sampling strip of 200 m to the right and left of the trail, resulting in a total search area of 5.08 km<sup>2</sup>. Males that were outside this area were not counted.

### Characterization of phytotelmata

We georeferenced each phytotelm located with calling males within it. With the aid of a diametric tape, we obtained the diameter at breast height (DBH) of the trees. Behavioral data and physicochemical characteristics of the water could only be obtained in natural phytotelmata with heights lower



**Figure 1.** Location of the study area of reproductive sites of *Trachycephalus cunauaru* in northern Mato Grosso state, southern Brazilian Amazon region. This figure is in color in the electronic version.



than 10 m, since these could be reached with a ladder. The height of phytotelmata above 10 m was estimated using a laser measure (GLM 40 Professional, Bosh, Luxemburg, Germany). We visited all accessible sites in December 2015, January, February and March 2016, to take measurements and to log the presence/absence of tadpoles. We measured the depth and the radius of each phytotelm to estimate its volume using the cylinder volume formula (Schiesari *et al.* 2003). We also measured dissolved oxygen and water pH using a multiparameter (Oakton PCD-650, Oakton Instruments, Vernon Hills, USA). The canopy opening was measured with a concave spherodensimeter (Robert and Lemmon Forest Densimeter, model C). When possible, the males were identified by photo-identification using the dorsal patterns.

### Artificial reproductive sites

We installed 15 artificial reproductive sites in November 2014, which consisted of plastic buckets with different water volumes attached to the trees. Five buckets with 795 ml, five with 8064 ml and five with 19935 ml were installed at a height of 10 m. They covered an area of 0.005 km<sup>2</sup>, with a minimum spacing of 10 m between buckets, in trees with different DBHs (average  $\pm$  SD = 27.6  $\pm$  8.8 cm). There was no relationship between bucket volume and DBHs ( $r = 0.08$ ,  $F_{1,13} = 0.15$ ,  $p = 0.30$ ). Behavioral data were sampled over a period of 12 days in November 2016 using nine Bushnell digital camera traps (Trophy Cam HD Brown Model 119676, USA), five installed at locations of natural phytotelmata and four at locations of artificial phytotelmata. The cameras were fixed approximately 60 cm from hollow openings and were programmed to record for 40 seconds when they detected motion or heat, during the day and night.

### Statistical analysis

With the set of physicochemical variables of phytotelmata we built two axes of NMDS, which were later tested using MANOVA to check if there were differences in these parameters between natural and artificial sites. Logistic regressions were performed to analyze whether abiotic factors influenced the presence/absence of tadpoles in natural and artificial reproductive sites. The variables were log-transformed when they did not meet the assumptions of normality and homoscedasticity. The analyses were performed using the *vegan* (Oksanen *et al.* 2020) and *ggplot2* packages (Wickham 2016) in R software (R Development Core Team 2020).

## RESULTS

### Characterization of reproductive sites

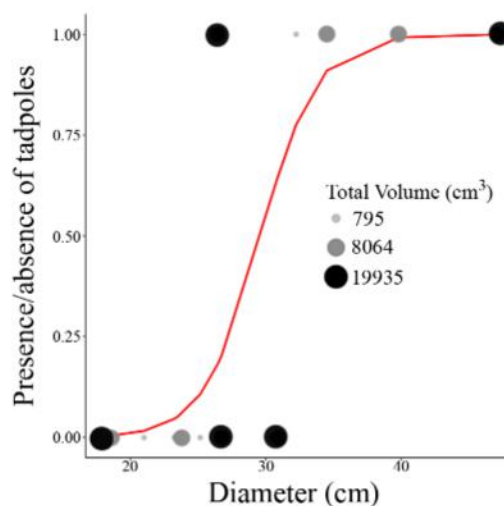
We identified 72 natural reproductive sites, resulting in a density of 14.1 sites per km<sup>2</sup>. The average distance between phytotelmata was 188.4  $\pm$  193.6 m (mean  $\pm$  SD) (Table 1). The lowest measured DBH was 13 cm and the highest 138.2

cm (35.9  $\pm$  20.5 cm). The lowest recorded height was of a male found calling at 30 cm in a buttress, while the highest was 30 m (13.5  $\pm$  7.1 m). Due to the height, only 22 natural phytotelmata could be measured and monitored monthly. The phytotelmata had an average total volume of 1535 ml, and, throughout the four months of the rainy season, they remained filled with water at 80% of their capacity (1231 ml).

We found no substantial difference in the physicochemical parameters of natural phytotelmata over the four months studied (Pillai-Trace = 0.02;  $F_{6,168} = 0.34$ ;  $p = 0.91$ ). Likewise, there was no change in the physicochemical parameters at the artificial sites over the months (Pillai-Trace = 0.13;  $F_{6,120} = 1.47$ ;  $p = 0.19$ ). Due to the absence of monthly variation of the parameters, we used the average variable values for each phytotelm in subsequent analyses. The physicochemical parameters differed between natural and artificial sites (Pillai-Trace = 0.45;  $F_{2,34} = 14.11$ ;  $p < 0.01$ ). None of the evaluated environmental variables had an influence on presence or absence of tadpoles in natural sites, while in artificial reproductive sites there was an influence of DBH (McFadden's  $Rho^2 = 0.61$ ;  $Z = 1.90$ ;  $p = 0.05$ , Figure 2). Tadpoles were found in five of the 15 artificial sites, two with 8064 ml, two with 19935 ml and one with 795 ml. A sixth bucket of 795 ml was used by a male to vocalize, but no tadpole was found.

### Behavioral data

A total of 124 hours of footage were recorded, distributed among 186 recordings, with an average of 20 hours per camera trap. According to the recordings of the camera traps and inspections that were carried out, the males never remained inside or visibly close to the reproductive sites during the



**Figure 2.** Influence of tree diameter at breast height (DBH) on the presence/absence of tadpoles of *Trachycephalus cunauaru* in artificial sites in Cotriguaçu, Mato Grosso, Brazil. This figure is in color in the electronic version.

**Table 1.** Characteristics of the reproductive sites (natural and artificial) of *Trachycephalus cunauaru* in Cotriguaçu, Mato Grosso, Brazil. Values indicate the mean  $\pm$  standard deviation. n = samples size.

Phytotelm parameter	Natural sites		Artificial sites (n = 15)	
Distance between occupied phytotelmata (m)	188.4 $\pm$ 193.6 (n = 72)		14.5 $\pm$ 6.8	
Phytotelm height (m)	13.6 $\pm$ 7.0 (n = 72)		10	
Tree diameter (cm)	35.9 $\pm$ 20.5 (n = 72)		27.6 $\pm$ 8.8	
Total volume (cm <sup>3</sup> )	1535 $\pm$ 1237 (n = 22)	795	8064	19935
Monthly volume (cm <sup>3</sup> )	1231 $\pm$ 1003 (n = 22)	642 $\pm$ 207	5960 $\pm$ 2626	17268 $\pm$ 5446
Water depth (cm)	13.1 $\pm$ 10.4 (n = 22)	11.3 $\pm$ 3.6	22.1 $\pm$ 9.7	38.9 $\pm$ 12.2
Canopy opening (%)	9.7 $\pm$ 4.9 (n = 22)	11.4 $\pm$ 6.4	13.7 $\pm$ 4.6	11.3 $\pm$ 4.0
Water pH	7.1 $\pm$ 0.6 (n = 22)	6.9 $\pm$ 0.4	6.9 $\pm$ 0.5	7.0 $\pm$ 0.6
Dissolved oxygen (mg/L)	1.9 $\pm$ 1.2 (n = 22)	2.66 $\pm$ 1.7	2.4 $\pm$ 1.2	2.5 $\pm$ 1.2

day. At nighttime, however, they remained both in the phytotelmata water (Figure 3a) and at the edge of the hollows (Figure 3b). Males started calling around 07:15 pm, and ended at around 03:00 am (Figure 3c). We observed in the images that a single male used two nearby artificial reproductive sites, approximately 10 m apart.

We recorded nine observations of amplexus, all of them at artificial sites (Figure 3d). However, only two were recorded from the beginning and the females were already inside the reproductive site. In one of the recordings (see video included in the html version) the female shows up approximately 10 cm from the male that continued calling. Then, the female



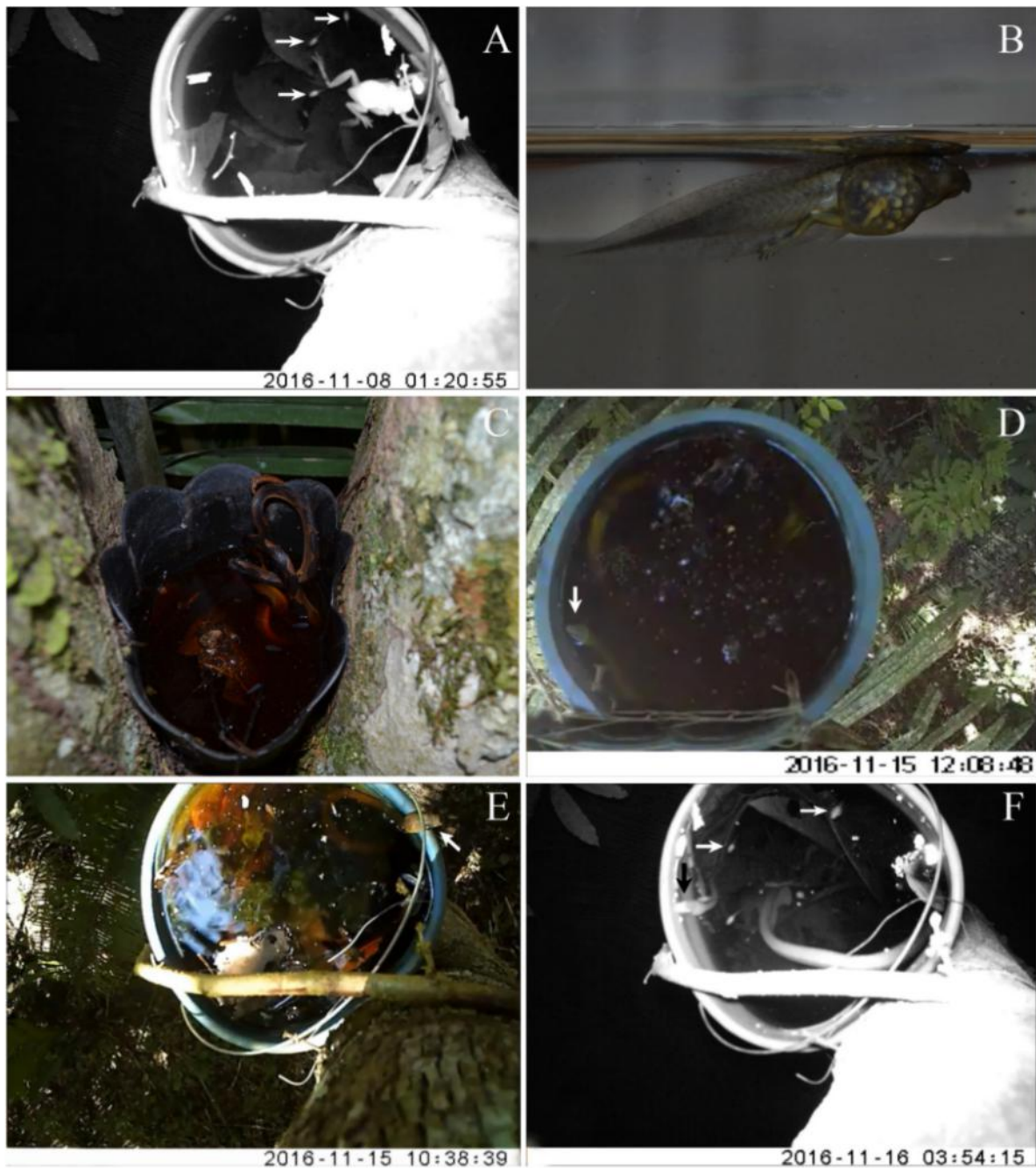
**Figure 3.** A-B – Males of *Trachycephalus cunauaru* in natural phytotelmata; C – Male calling in artificial reproductive site; D – Pair in amplexus with tadpole in an artificial site in Cotriguaçu, Mato Grosso, Brazil. This figure is in color in the electronic version.



approached the male swimming and touched him several times. Afterwards the pair engaged in the amplexus. This process started at 07:36 pm and lasted about 35 seconds. The exact time of oviposition has not been determined, but the last recording of the pair while still clasped was 10:36 pm.

The second registered pair (Figure 4a) came to the site swimming and soon engaged in amplexus, at around 08:12

pm. During the time in which the pairs remained clasped, the male would casually perform rhythmic movements from side to side, possibly to stimulate oviposition by the female. We observed that tadpoles of the species nibble the males and females when they are present in the reproductive site, possibly to stimulate the release of eggs so they can feed. We recorded oophagy for the first time for the species (Figure



**Figure 4.** A – Pair of *Trachycephalus cunauaru* in amplexus, white arrows indicate tadpoles; B – Oophagous tadpole; C – *Leptodeira annulata*; D – *Philodryas olfersii*, the white arrow indicates the head of the snake; E – *Chironius multiventris* resting inside the bucket during the day, the white arrow indicates the head of the snake; F – *Chironius multiventris* (the same individual) at night, the black arrow indicates the head of the snake and the white arrows indicate tadpoles. This figure is in color in the electronic version.



4b). In one of the registered amplexus, we could see that the tadpoles already present at the site entered a state of feeding frenzy when the female released the eggs (see video included in the html version).

We recorded the presence of three species of snakes inside artificial reproductive sites: *Leptodeira annulata* (Linnaeus 1758), (Figure 4c); *Philodryas olfersii* (Liechtenstein 1823), (Figure 4d) and *Chironius multiventris* Schmidt and Walker 1943 (Figure 4e-f). During a recording, an individual of *Philodryas olfersii* appears to be actively moving around in the bucket, suggesting that it was feeding on the tadpoles present in the site (see video included in the html version). The individual of *Chironius multiventris* was also in a bucket with the presence of tadpoles, but the footage did not reveal whether it was eating. It stayed there for about 41 hours with the body submerged and the head resting on the edge of the bucket.

## DISCUSSION

Pairs of *Trachycephalus cunauaru* used artificial phytotelmata as reproductive sites, therefore we confirmed the hypothesis that this methodology can be a good tool for the study of this species. However, we found significant differences between natural and artificial sites, regarding their physicochemical parameters. In a study comparing natural and artificial sites (tires) with *Aedes triseriatus* (Say 1823) mosquito larvae, the highest concentration of oxygen was in artificial sites (Walker 2016). As with tires, the buckets used in this study had a larger surface area than natural phytotelmata, allowing a greater exchange of oxygen between the water and the external environment. However, artificial phytotelmata offer several advantages over natural tree holes for observational or manipulative experiments: they are portable, replicable, and have standardized ecological histories (Yanoviak and Fincke 2005; Yanoviak *et al.* 2006). With a colonization rate of 40% (records of males and tadpoles) we believe that artificial phytotelmata can be widely used for behavioral studies of *T. cunauaru* and possibly for other species with a similar natural history in the Amazon. However, we suggest parsimony in the use of ecological results, since physicochemical parameters were different from natural sites.

The factors that most affect the survival of tadpoles in phytotelmata are desiccation, predation, cannibalism and competition with heterospecifics (Heying 2004). We found that in natural phytotelmata none of the variables evaluated influenced the presence of tadpoles. We had expected that some factor related to a larger amount of water within the phytotelmata would influence the presence of tadpoles. The use of smaller phytotelmata may decrease the risk of predation (Brown *et al.* 2008), since in larger sites there would be a greater probability of the existence of predator occurrence (Rödel *et al.* 2004). In fact, all records of snakes, which are potential predators, were in artificial reproductive sites, where

the size of the opening and the volume of water were larger than in natural phytotelmata. The recorded behavior and fast movements of the *Philodryas olfersii* individual inside the artificial phytotelm suggested that they would be more difficult to execute in a smaller natural phytotelm, nor would the snake be able to access the natural phytotelm with its entire body. Thus, we suppose there may be a complex balance in the choice of natural phytotelmata, in which the risk of predation and the risk of desiccation are considered.

Oophagy and/or cannibalism were recorded for the first time for the species. This behavior is common for species that use phytotelmata as a reproductive site, such as *Osteocephalus oophagus* Jungfer and Schiesari 1995 and *T. resinifictrix* (Jungfer and Weygoldt 1999; Lima *et al.* 2006). Most of the time, tadpoles in this type of environment are subject to rainfall unpredictability, food scarcity and high densities of conspecifics. Cannibalistic behavior includes advantages related to the acquisition of energy-rich food source and elimination of potential competitor and/or potential predator (Lehtinen 2004). We observed that a single male can occupy different reproductive sites. By siring offspring in different phytotelmata, the male can increase the number of reproductive events, consequently reducing the interval between successive mating attempts and mitigating the effects of cannibalism/oophagy and competition between tadpoles genetically related (Ling *et al.* 2008).

The use of camera traps for recording behavior presented some limitations, since the cameras used were activated by a combination of movement and temperature (the difference between the animal's body temperature and the environment). Because the species is a small ectothermic animal in water, the camera was often not activated. Even so, important behavioral aspects could be registered, which would not be possible with *in situ* observations only. The scarcity of studies on amphibians and reptiles that inhabit the canopy is alarming (Kays and Allison 2001) and the current protocol for most research on anuran amphibians covers a vertical sampling range of approximately two meters (Guayasamin *et al.* 2006). We believe that many species and ecological processes that occur in the Amazon canopy are still unknown. Thus, we recommend the use of artificial sites and camera traps in a combined manner as an alternative for monitoring canopy and sub-canopy species. New methodologies are essential and urgent, both due to the lack of information available and the high rates of habitat loss in the region.

Larger diameter trees have a higher number of microhabitats, including phytotelmata (Blakely *et al.* 2008; Vuidot *et al.* 2011). We observed that the mean DBH of trees used as a reproductive site by *Trachycephalus cunauaru* was 35 cm, and that the presence of tadpoles was related to artificial reproductive sites installed in trees with larger DBH. According to the practices that regulate logging in natural forests in the Amazon, in the absence of



technical studies that define the minimum cut diameter of each species, trees with a DBH equal to or greater than 50 cm can be cut (Brasil 2009). Coincidentally, Kitching (1971) found that in trees with DBH over 50 cm the occurrence of phytotelmata was higher in the Wytham Woods in the United Kingdom. In our study, 15% of the trees used as reproductive sites would be in this cut range. There are more phytotelmata in natural forests compared to managed forests (Vuidot *et al.* 2011) and the composition of macroinvertebrate species changes significantly in phytotelmata where the environment has been subjected to management (Yanoviak *et al.* 2006). Therefore, even sustainable management can affect the hundreds of species of animals that use phytotelmata as a resource (Fish 1983), especially those with reproductive habitats as specific as *T. cunauaru*. Natural history studies on forest species are crucial not only to broaden our knowledge, but also to provide important information for management and conservation actions.

## CONCLUSIONS

We presented unprecedented aspects of the ecology and behavior of *T. cunauaru*. The presence of tadpoles was directly related to trees with a larger diameter in artificial sites, an important information due the current practices that regulate logging in natural forests in the Brazilian Amazon. We registered oophagy for the first time for the species and observed that males can use more than one phytotelm. We determined that artificial sites and digital camera traps are a satisfactory alternative for behavioral observations of *Trachycephalus cunauaru* and possibly for other species with a similar habit in the Amazon.

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