Foam nest construction and first report of agonistic behaviour in *Pleurodema tucumanum* (Anura, Leptodactylidae)

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**Abstract**

Reproductive strategies are the combination of physiological, morphological, and behavioural traits interacting to increase species reproductive success within a set of environmental conditions. While the reproductive strategies of Leiuperinae are known, few studies have been conducted regarding the reproductive behaviour that underlies them. The aim of this study was to document the structural characteristics of nesting microsites, to describe the process of foam nest construction, and to explore the presence of male agonistic and chorus behaviour in *Pleurodema tucumanum*. Nests were found close to the edge of a temporary pond and the mean temperature of the foam nests was always close to the mean temperature of the pond water. Our observations corroborate that the foam nest construction phases for *P. tucumanum* are similar to those described for other Leiuperinae, including dispersion and the beating of the foam, realized by male hind limbs. We also recorded the first scientific observations of male agonistic encounter in *Pleurodema tucumanum*.

**Keywords**

Amphibians, Dry Chaco, male-male interaction, oviposition site, reproductive mode

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Introduction

Reproductive modes can substantially vary among anuran species (Haddad and Prado, 2005) and are therefore considered of taxonomic value (Duellman and Trueb, 1986). Foam nest reproductive modes in anurans are a convergent behaviour that evolved independently in various frog lineages (Duellman and Trueb, 1986; Seymour and Loveridge, 1994; Faivovich et al. 2012). The ancestral oviposition mode for the Neotropical anuran clade Leiuperinae, composed by the genera *Edalorhina*, *Engystomops*, *Physalaemus*, and *Pleurodema*, is the floating foam nest (Grant et al. 2006; Pyron and Wiens, 2011; Faivovich et al. 2012).

The genus *Pleurodema* is notable for its particularly large variety of oviposition modes (Duellman and Veloso, 1977; Faivovich et al. 2012; Valetti et al. 2014). Faivovich et al. (2012) reported four egg-clutch structures proposed by different authors. However, recently Ferraro et al. (2016) rejects the egg-clutch structure proposed by Martori et al. (1994). Thus, three reproductive modes are known for *Pleurodema*: (1) eggs laid in subspherical gelatinous masses in groups of 1–6 eggs (Barrio, 1977; Valetti et al. 2009), (2) eggs laid in gelatinous strings (Fernández, 1927; Weigandt et al. 2004), and (3) eggs laid in a floating foam nest (Hödl, 1992; Cardoso and Arzabe 1993).

In arid environments, under temporarily harsh, unpredictable and variable conditions, species should possess special mechanisms for tolerating or avoiding stressful situations. During the aquatic phase, larvae and nests are often at risk of drying in ephemeral ponds (Spieler and Linsenmair, 1997). The foam nest structure protects eggs and tadpoles from dehydration, and is a beneficial reproductive strategy for species that inhabit arid environments (Prado et al. 2002; Zina, 2006). Also, choice of oviposition site plays an important role in the reproductive success of temporary pond amphibians (Resetarits and Wilbur, 1989). Various studies have suggested that female anurans select oviposition sites based on factors such as water depth (Caldwell, 1986), absence of predators (Howard, 1978; Resetarits and Wilbur, 1989), or water temperature (Howard, 1978). The suitability of a breeding site directly affects hatching success, larval performance, and, thereby, parental fitness (Resetarits and Wilbur, 1989). Thus, oviposition site-selection can have a strong impact on the spatial distribution of a species and thereby affect population dynamics (Hassel, 1987; Pearman and Wilbur, 1990) and the structure of communities (Morris, 2003). However, the mechanisms for oviposition site-selection in spatially and temporally variable environments are still poorly understood (Pearman and Wilbur, 1990).

Most *Pleurodema* species inhabit arid environments prone to strong seasonal fluctuations in temperature and precipitation, and are explosive breeders (Cei, 1980; Hödl, 1992; Cardoso and Arzabe, 1993). This temporal reproductive pattern is usually associated with marked spatial clumping around suitable oviposition sites (Wells, 1977). The explosive breeders form dense aggregations in which the males scramble for females. In this kind of breeders, struggles between males are common (Arak, 1983). For many anurans, male-male interactions consist of exclusively ter-
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K01; 1.62 mm diameter). In addition, we counted the number of amplectant pairs and foam nests located in this pond. In order to describe the foam nest temperatures and compare these temperatures with those of the amplectant pair and the air, we measured foam nest temperatures introducing a thermocouple (TES TP-K01; 1.62 mm diameter) 1 cm into the center of each foam nest. Air temperature was measured 1 cm above the foam using the same thermocouple, and body temperatures of the amplectant pair were measured using an infrared thermometer (GM550 Digital Laser Infrared Thermometer Temperature Measurement Electronic Point Gun) at a distance of 10 cm. In order to describe the process of construction of the foam nest, we recorded the whole process of construction using a digital camera (Nikon Coolpix S210V1.0). The time lapses for construction were obtained from the recorded video and were measured in minutes. While we were recording the foam nest construction, we also observed the behaviour of the remaining individuals in the reproductive pond. In addition to the nests recorded in December, during a subsequent sampling carried out in December 2015, we found new foam nests in other small bodies of water. For all observed foam nests (both found in December 2013 and in December 2015) we measured the following variables: depth of water under the nest, distance from the foam nest to the nearest shore, and maximum diameter of the foam nests, using a tape measure (nearest 0.1 cm).

Fifteen males and five females were observed in the pond. The dimensions of the pond were 435 cm by 86 cm, with a maximum depth of 7 cm and a minimum of 3.5 cm. The mean temperature of the pond water (N=5) was 20.7 °C (± 0.07 °C). From the total number of individuals, four of them were paired in amplexus. The remaining males were observed singing in chorus. In this reproductive pond, we observed three foam nests, one already constructed, and the others were seen since the beginning of the process of construction till the end (Table 1). We also observed the presence of agonistic male behaviour: we saw a combat between three males that involved frontal charging (Figure 1e) and included one climbing on and submerging another (Figure 1f), then, the third male attacked an amplexus pair. The aggression was repeated several times until the individual seemed to achieve his objective, interrupting the formation of the nest.

The foam nest construction process involved alternating phases of rest and activity (sensu Hödl, 1990). During the rest phases, the amplectant pair floated immobile. The male was in a position of axillary amplexus (Figure 1a–c), with his feet retracted and his cloaca upon that of the female. The active phase can be divided into three stages. The first stage began when the male slipped his feet between the thighs of the female and the cloacae of both were juxtaposed. The female laid her eggs while the male pushed his tarsal tubercles against the upward tilted cloacal region of the female and then rapidly brought his legs back to the initial position. In the second stage, the male beat the foam using alternating leg kicks. The movement involved the rotation of the femur and tibia perpendicular to the body axis, with the feet almost parallel to the body. The third and final stage began once a substantial amount of foam has been produced. Following the last kick, the male distributed the
foam by spreading his legs outward over the mass. The male then returned to the rest position. This behaviour was repeated until the foam nest was completed and egg deposition has finished.

In a subsequent sampling another 15 foam nest were recorded in other small bodies of water. The mean diameter of foam nests (N=18) was 104 mm ± 32.5 mm (mean ± SE), mean distance to the shore was 806.7 mm ± 63.4 mm (mean ± SE), and the mean water depth beneath the nests was 118 mm ± 63.3 mm (mean ± SE).

The mean foam nest temperature was similar to the mean water temperature, but always lower than the air temperature. Among temperate zone amphibians, aquatic egg masses are known to maintain temperatures slightly above the water surrounding them (Salthe and Mecham, 1974). All of the foam nests were located close to

Figure 1. Oviposition and reproductive behaviour of Pleurodema tucumanum in a temporary pond near La Majadita, Valle Fértil department, San Juan province, Argentina. a–b Amplexus and construction of foam nest and finalized nest corresponding to nest 1 (Table 1). c–d Amplexus and foam nest corresponding to nest 2 (Table 1). e–f Agonistic encounter between males. g Axillary amplexus.
the edges of the pond, in superficial water. Egg deposition and larval development in small temporary ponds markedly reduces vulnerability of eggs and tadpoles to aquatic predators (Duellman and Trueb, 1986; Hödl, 1986; Heyer et al. 1990). However, the use of those habitats as oviposition sites increases the vulnerability of developmental stages to desiccation (Zina, 2006). Eggs and larvae within foam nests placed in microsites that provide thermally favorable microclimate should develop somewhat faster than eggs and larvae within nests placed in cooler microsites (Dobkin and Gettinger, 1985), increasing the probability of tadpole survival.

Recorded construction times were very different between the two nests. The amplexant pair that dedicated more time to the foam nest construction produced a larger nest with more consistent foam (Figure 1a–b) than those that spent less time (Figure 1c–d). Such difference in foam nest size could be explained because of foam needs mechanical processing for the incorporation of air bubbles (Dalgetty and Kennedy, 2010). Thus nest size may depend on the time spent on construction. All males found in this study were singing in chorus, despite the fact that Martori et al. (1994) did not record the chorus behaviour in \textit{P. tucumanum} in Córdoba Province. This behaviour constitutes a vocalized agonistic interaction with other males of the species with the objective of territorial establishment and female attraction (Wells, 1977; Penna et al. 2008; Velásquez et al. 2014). The agonistic behaviour found in this study has been observed in several pipids, leptodactylids, dendrobatids, hylids and ranids (e.g. Brattstrom and Yarnell, 1968; Rabb and Rabb, 1965; Wells, 1977; Halliday and Tejedo, 1995; Abrunhosa and Vogel, 2004), but there are no previous records of aggressive/agonistic combat behaviour in \textit{Pleurodema} species.

Foam nest construction processes employed by \textit{Pleurodema tucumanum} were analogous to those described for \textit{P. diplolister} (Hödl, 1990) and \textit{P. guayapae} (Valetti et al. 2014) and other Leiuperinae (e.g. Heyer and Rand, 1977; Dalgetty and Kennedy, 2010; Schaefer and Kehr, 2010). Male of \textit{P. tucumanum} were observed utilizing their hind limbs to beat the foam, similar behaviour was found in \textit{P. diplolister} (Hödl, 1992), \textit{P. borellii} (Halloy and Fiaño, 2000), and \textit{P. guayapae} (Valetti et al. 2014) and other Leiuperinae (e.g. Heyer and Rand, 1977; Dalgetty and Kennedy, 2010; Schaefer and Kehr, 2010). Similarly to our observations in \textit{P. tucumanum}, in which during the rest phases the amplexant pair floated immobile and the male was in a position of axillary amplexus, axillary amplexus was observed in several Leiuperinae species (Barrio, 1964; Duellman and Veloso, 1977; Giaretta and Facure, 2009; Valetti et al. 2009; 2014; Velasco et al. 2017). The active phase can be divided into three stages that have been previously described for \textit{Physalaemus ephippifer} (Hödl, 1990), \textit{Pleurodema diplolister} (Hödl, 1992), and \textit{P. guayapae} (Valetti et al. 2014).

The data presented in this study verify the existence of \textit{P. tucumanum} foam nest construction and confirms observations made by Ferraro et al. (2016). Surveys conducted days after oviposition led to determine that the ovoid plate nest proposed by
Martori et al., (1994) is the result of the dilution and collapse of the foam nest after several hours (Figure 2). Similar results were found by Ferraro et al. (2016) in the field and laboratory.

*Pleurodema tucumanum* is able to live in deserts and salty habitats (Cei, 1980). The foam nests contain substances that delay larval development (Pisano, 1963), while protecting the eggs and early-stage embryo from dehydration (Prado et al. 2002; Zina, 2006). The *Pleurodema* genus appears to be a promising clade for evolutionary studies of physiological responses to extreme environmental conditions (Faivovich et al. 2012).

Our observations highlight the importance of studying reproductive behaviour systems. In addition, this study offers the first report of this type of reproductive behaviour strategies in Chacoan species, though further studies are needed to provide more detail for *P. tucumanum* reproductive behaviour such as the influence of both biotic and abiotic variables on oviposition site selection, timing of embryonic and larval development, the reproductive cycle, mating selection, among others.
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Resumo

Estratégias reprodutivas são combinações de características fisiológicas, morfológicas e comportamentais que interagem para aumentar o sucesso reprodutivo em certas condições ambientais. Embora as estratégias reprodutivas do Leiuperinae sejam conhecidas, poucos estudos foram realizados sobre os comportamentos reprodutivos que as sustentam. O objetivo deste trabalho foi documentar as características estruturais dos microsítios de oviposição, descrever o processo de construção do ninho de espuma e registrar a presença de comportamento agonístico e de coro em machos de *Pleurodema tucumanum*. Os ninhos foram encontrados próximos às margens de uma poça temporária e a temperatura média dos ninhos de espuma esteve sempre próxima da temperatura média da água. Nossas observações corroboram que as fases de construção do ninho de espuma para *P. tucumanum* são similares às aquelas descritas para outros Leiuperinae, incluindo a dispersão e o batimento da espuma realizada pelos membros posteriores do macho. Também registramos pela primeira vez a observação de encontros agonísticos entre machos de *Pleurodema tucumanum*.

Palavras-chave

Anfíbios, Chaco Seco, Interações entre machos, modo reprodutivo, sítio de oviposição.