Reproductive biology of Pleurodema guayapae (Anura: Leptodactylidae: Leiuperinae)

Reproductive biology of *Pleurodema guayapae* (Anura: Leptodactylidae: Leiuperinae)

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**Abstract:** *Pleurodema guayapae* is a species that inhabits saline environments and semidesert zones from central Argentina. To date, the knowledge about the reproductive biology of this species is very poor, and our aim is to contribute to its knowledge with the description of some important reproductive aspects. For this, field work was undertaken in an area near to Patquía, La Rioja province. Sampling was undertaken during three summer periods (2006-2007; 2007-2008; 2008-2009) in Chamical-Patquía area, where we could find reproductively active populations. We observed and described breeding sites, type of clutch, process of foam nest construction, clutch and egg number and sizes, and hatching time and stage. Behaviour observations were performed from the time that males began to call until the pairs ended up the foam nests building, and layed the eggs. Additionally, one amplexed pair was observed and filmed in the process of foam nest construction, and four amplexant pairs were collected and separately placed in plastic containers, for nests observations in the laboratory. Hatching time was based on three different foam nests of known age. We found that *P. guayapae* populations were acoustically active only after a rainfall. Its breeding sites were represented by ephemeral ponds of fresh water, product of rains. The males emitted their calls inside or outside these ponds. A detailed description of the foam nest construction process by both females and males was made. The clutches were in dome-shaped foam nest type of 6-9cm in diameter and 1-3cm in height, some of which were in communal nests. The nests had an average of 1137 pigmented eggs. This species showed a short hatching time. Our results allow us to conclude that this species should be considered an extreme explosive breeder. Our results are discussed with others obtained for related species. Rev. Biol. Trop. 62 (1): 173-181. Epub 2014 March 01.

**Key words:** communal nest, ephemeral environment, explosive breeder, foam nest construction.

The reproductive mode in amphibians was defined by Salthe & Duellman (1973) as “a combination of traits that includes oviposition site, ovum and clutch characteristics, rate and duration of development, stage and size of hatchling, and type of parental care, if any”. Thirty-nine reproductive modes have been recorded for anurans around the world (Haddad & Prado, 2005). Altig & McDiarmind (2007) provide a generalized framework for diversity of amphibian eggs and ovipositional modes and introduce a standardized terminology for clutch structures.

The genus *Pleurodema* Tschudi (Leptodactylidae: Leiuperinae) is distributed from Panama throughout South America to Southern Chile and Argentina, and currently is represented by 15 species (Cei, 1980; Valetti, Salas & Martino, 2009; Maciel & Nunez, 2010; Kolenc et al., 2011; Faivovich et al., 2012). This genus has very variable ovipositional modes. The egg-clutch structure in the Southern Chilean populations of *P. thaul* and *P. buforinum* is strings in water (Weigandt, Úbeda & Díaz, 2004). This egg-laying mode is accompanied by inguinal amplexus (Duellman & Veloso, 1977). *P. thaul*
of central and Northern Chile, *P. bibroni*, *P. kriegi* and *P. cordobae* lay eggs in semisubmerged gelatinous eggs-masses (Valetti et al. 2009; Valetti, Otero, Grenat & Martino, 2011; Faivovich et al., 2012). *P. tucumanum* lays eggs in plate-like floating masses (Martori, Aún & Vignolo, 1994). All other known *Pleurodema* species lay eggs in aquatic foam nest (Barrio, 1964; Hulse, 1979; Cei, 1980; Peixoto, 1982; Hödl, 1992; Faivovich et al., 2012).

*Pleurodema guayapae* inhabits saline and dry Chacoan environments from Central Argentina (Barrio, 1964; Cei, 1980; Ferraro & Casagrande, 2009). In addition, De la Riva and Gonzales (1998) reported a population tentatively assigned to *P. guayapae* from the dry Chacoan areas of Santa Cruz, Bolivia. To date, the knowledge about the reproductive biology of *P. guayapae* was only briefly described by Barrio (1964). Consequently, the aims of this investigation are: (1) to identify the breeding sites of the species; (2) to describe the process of foam nest construction; (3) to determine clutch and egg sizes, and (4) to identify the time and stage of hatching.

**MATERIALS AND METHODS**

Field work was undertaken in an area near to Patquía, La Rioja province, Argentina (23°26'S - 45°04'W) at 434m in elevation. This arid environment was well-described by Barrio (1964). After three summers of sampling (2006-2007; 2007-2008; 2008-2009) in Chamical-Patquía area (La Rioja province), we could find reproductively active populations of *Pleurodema guayapae*. Field observations of breeding aggregations were made at dawn of 18 January 2009 in two ephemeral ponds of 60m² and 160m², respectively. Behaviour observations were performed from the time that male individuals began to emit the advertisement calls until the pairs ended up building the foam nests and lay the eggs. One amplexed pair was observed and filmed in the process of the foam nest construction with a Canon Powershot S5 IS camera (at 30 frames per second), and this behaviour is described following Hödl (1990, 1992).

To determine the number of eggs per foam nest, four amplexant pairs were collected and each pair was put into a plastic container so that nests could be obtained in the laboratory. All eggs of these foam nests were counted. Hatching time was based on three different foam nests of known age. The tadpoles were staged according to Gosner (1960).

Additionally, on the morning of 18 January 2009, data on foam nests were collected in the field to describe the general aspect of the nests and eggs. The number of foam nests in each pond was registered and height and diameter of the nests were measured. One foam nest was placed in 10% formalin solution for morphometric description of its eggs. Egg diameters without the gelatinous capsule were measured using a Zeiss SR stereomicroscope with a micrometer to nearest 0.01mm. Eggs, tadpoles and adult specimens are housed at the herpetological collection of the Department of Natural Sciences, National University of Río Cuarto, Argentina.

**RESULTS**

**Breeding sites of *P. guayapae***: Calling and spawning activities were limited only to one night after ca. 30mm of rainfall during a very short time. The calling activity began at 4.00am (immediately after the rain when the ponds were formed) and ended at 7.00am (sunrise). Two ponds were formed in sites where water was stored after the rain. The sizes of the two rain ponds were 15x4 and 40x4 meters (Fig. 1A) and a depth less than 25cm, with approximately 50 males calling in each pond. The males emitted their calls on stones in the pond, inside the pond near the edge or outside the pond (Fig. 1B). The amplexus in *P. guayapae* is axillary and the pairs were observed out of the water, near the edge of the pond or floating on the surface of the water (Fig. 2A). Once the amplexus is formed and the pair is into the pond, starts the construction of the foam nest.
Process of foam nest construction: The construction of the foam nest has alternating phases of activity and rest (sensu Hödl, 1990). In the rest phase, the pair floats immobile. The male is in axillary amplexus on the female with his feet retracted and the cloaca above that of the female (Fig. 2A). These rest phases are alternated by activity phases that can be described in three stages. The first stage is when the male immerses the feet in the water between the thighs of the female and then the cloacae of both individuals are juxtaposed...
(Fig. 2B). In this moment, the female spawns and the male lifts the eggs with his tarsal tubercles by retracting the feet above his cloaca returning to the starting position (1 260ms). In the second stage, the male begins a rotational movement of the legs beating the foam. The femur and the tibia rotate perpendicular to the longitudinal axis of the body and the foot is almost parallel to it. After 12 of these cyclical movements (1 500ms, Fig. 2C), the third stage starts and the male opens the legs and his open cloaca can be observed (see Fig. 2D). The sperm release is probably produced here, but this event could not be appreciated clearly. Immediately, the male performs approximately six lateral movements of his extended legs pushing the eggs toward the centre of the foam (430ms, Fig. 2D). Once these movements have been completed, the pair begins another rest phase. This behaviour is repeated until the construction of the foam nest and the simultaneous egg-laying have been completed.

**Clutch and egg sizes. Time and stage of hatching:** Eleven foam nests were counted in the smaller pond and 25 in the bigger one. The dome-shaped foam nests have diameter and height that varied from 6 to 9cm and 1 to 3cm, respectively. Most of the foam nests were floating alone, although some of them were attached to vegetation. A small number of foam nests were also observed in groups of 2 to 5 interconnected nests (Communal nests; Fig. 3).

The pigmented eggs (see Fig. 3) have the animal pole darker than the vegetal one (Fig. 4). All eggs are covered by one gelatinous capsule, and the average egg diameter (stage 6-7; Gosner, 1960) without the gelatinous capsule is 1.44mm (SD=0.07, range=1.29-1.61, n=83). The snout-vent length for females ranged from 39.1 to 44.2mm (41.7mm±1.7; n=17) and the average number of eggs per foam nest was 1 137 (SD=275, range=900-1 500, n=4).

The hatching under laboratory conditions of the *P. guayapae* took between 26 to 30 hours (Stage 19).

**DISCUSSION**

The reproductive sites where *P. guayapae* was found the night of January 18 were formed after the rain (ca. 30mm). *P. guayapae* males have called only at that night and they were not detected in the subsequent nights. The species-specific breeding phenologies are adaptive and often correlate with breeding habitat, seasonal changes in climate, or the presence of potential competitors or predators (Wilbur & Alford, 1985; Duellman & Trueb, 1986). In this sense, Wells (1977), considering the temporal patterns of anuran reproduction, classified species into
two broad types: explosive breeders and prolonged breeders. Most tropical anurans breed in every month of the year and therefore they are classified as prolonged breeders. By contrast, temperate species are more likely to breed explosively and this is common in many species that use seasonally ephemeral habitats such as rain ponds, and they may involve a single annual bout (Heyer, McDiarmid & Weigmann, 1975; Crump, 1983). According to our results, *P. guayapae* fits the characteristics of the explosive breeder species.

Although individuals of *P. guayapae* breed in ephemeral ponds, individuals of both sexes were observed outside of permanent and semi-permanent ponds, but never inside the ponds. Such situations were detected in many occasions during the field season 2007-08 (January-February, J. Valetti, personal observation). In addition, it was not possible to verify the formation of breeding aggregations in such opportunities. A possible explanation may be the abundance of predators inside of these ponds. These ponds were densely inhabited by *Lepidobatrachus llanensis* (Anura: Ceratophryidae) individuals (Approximately 1 individual/m²), a natural predator of *P. guayapae* (Hulse, 1978; J. Valetti, personal observation). At these sites, adults of *P. guayapae* were distant from the water bodies (between 5 and 50m) and *L. llanensis* individuals were not observed outside of the ponds. In addition, as Hulse (1978) established, *L. llanensis* individuals were not observed inside of ephemeral ponds where *P. guayapae* breeding. Nevertheless, an appropriate experimental design should be done to evaluate this alleged relationship.

The amplexus observed in *P. guayapae* is axillary and the eggs are deposited in foam nests floating on water, as the syntopic congeneric species *P. nebulosum* (Barrio, 1964). The calling sites of *P. guayapae* males differ from that of *P. diplolister*. Males of *P. diplolister* call floating on the surface of puddles (Hödl, 1992) whereas those of *P. guayapae* always call attached to vegetation into the ponds, on stones or even outside the ponds. Observations in nearby ponds allowed us to determine that the sympatric congeneric species *P. tucumanum* and *P. nebulosum* also showed diverse acoustic behaviour. The males of these species emitted the calls floating in ponds or from outside them. In *P. guayapae*, several amplexant pairs were observed floating on the surface of ponds and others were out of water bodies. Regarding the latter, Hödl (1990) indicated that *Physalaemus ephippifer* performs the amplexus into the water, but the pair usually leaves the amplexus site and returns to the water several minutes before oviposition. Respect to the breeding site of *P. guayapae*, there is an important difference with regard to the report by Barrio (1964). This author indicated that the breeding sites are represented by temporary ponds of brackish water, while we find that *P. guayapae* breed in ephemeral ponds of fresh water, product of a recent rain. In fact, the first foam nests were constructed four hours after the pond formation.

Nine species of *Pleurodema* lay eggs in aquatic foam nest (Barrio, 1964; Duellman & Veloso, 1977; Hulse, 1979; Cei, 1980; Peixoto, 1982; Hödl, 1992). Halloy & Fiaño (2000) indicate that *P. borellii* males use their hind legs to whip up a foam nest that prevents the eggs from desiccation. This behaviour is similar to that described for *P. diplolister* (Hödl, 1992). Our results indicate that *P. guayapae* lays eggs in aquatic foam nest and males also use their hind legs to construct the nest, as was described for *P. borellii* and *P. diplolister*.

According to the terminology used by Hödl (1990, 1992), *P. guayapae* constructs the foam nest by alternating rest and activity phases. The activity phases composed by three stages or phases were also described for *Physalaemus ephippifer* (Hödl, 1990) and *Pleurodema diplolister* (Hödl, 1992). The activity of these phases is similar to that described here for *P. guayapae*. Moreover, the second stage of the activity phase of *P. guayapae* (foam beating phase of Hödl, 1992) is the longest, a feature shared with *P. diplolister*. Furthermore, both species show series of cyclic movements of the legs at this stage (12 in *P. guayapae*, and 10 in *P. diplolister*: Hödl, 1992). However, the foam beating phase of *P. diplolister* was
characterized by Hödl (1992) as rotational movements of the tibia and tarsus perpendicular to the body axis, whereas the movements of legs in *P. guayapae* differ slightly. In *P. guayapae*, the femur and the tibia rotate perpendicular to the body axis, while the foot is almost parallel to it. Despite that signs of ejaculation were not observed by Hödl (1992) during the process of foam nest construction, the “female lordosis and male basket formation phase” is suggested for the reason of the juxtaposition of their cloacae. We suggest that in *P. guayapae* the sperm release probably occurs in the foam beating phase, after the eggs are released by the female. This is based on the observation of the male cloaca that opens at this phase. However, a more detailed analysis over this process will be needed to determine the exact moment of ejaculation.

*Pleurodema guayapae* lays eggs in foam nests and demonstrated a reproductive strategy adapted to the unpredictable rainfall patterns of the region. Thus, the reproductive mode that corresponds to *P. guayapae* is 8 (sensu Duellman & Trueb, 1986) or 11 (sensu Haddad & Prado, 2005): “Foam nests floating on the water in ponds; exotrophic tadpoles in ponds”. It has already been demonstrated that species inhabiting environments where water resources are restricted to only short periods during the year, generally have generalized reproductive modes more resistant to the desiccation (Haddad & Prado, 2005; Vieira, Santana & Arzabe, 2009). Several authors suggested that foam nest construction is an adaptation to avoid desiccation of eggs and early larval stages (Dobkin & Gettinger, 1985; Downie, 1988; Cardoso & Arzabe, 1993; Prado, Uetanabaro & Haddad, 2002). Eggs and larvae that develop within foam nests are more protected from predators and against desiccation (Crump, 1974; Haddad, Pombal & Gordo, 1990; Silva, Giaretta & Facure, 2005; Dalgetty & Kennedy, 2010) and they are exposed to adequate supplies of oxygen and to temperatures that favour their development (Petranka, Hopey, Jennings, Baird & Boone, 1994; Haddad & Hödl, 1997). Moreover, Hödl (1992) suggested that the foam nest of *Pleurodema diplolister* reduced egg predation by conspecific larvae. Also, several inter-connected communal nests of *P. guayapae* were observed. Zina (2006), in a work about communal nests in *Engystomops pustulosus*, reported that the foam protects eggs and tadpoles from desiccation for at least four days after oviposition, and nesting communally can extend this protection even further. Therefore, communal nesting in species that deposits eggs in foam may be an important adaptation enhancing survival of these species inhabiting environments of unpredictable rainfall patterns (Downie, 1988; Hödl, 1990; Zina, 2006). In this regard, it has already been confirmed the presence of communal nests in some species of Leiuiperinae (Barreto & Andrade, 1995; Tára-no, 1998; Giaretta & Menin, 2003; Dalgetty & Kennedy, 2010) and also within the genus *Pleurodema* in *P. diplolister* (Höld, 1992; Cardoso & Arzabe, 1993) and *P. cinereum* (Agostini, Cajade & Roesler, 1997).

Faivovich et al. (2012) suggested that eggs laid in a foam nest built by the mating pair during the amplexus is a synapomorphy for the clade composed by *Edalorhina, Engystomops, Physalaemus* and *Pleurodema*, but their results also showed that *Pleurodema* has experienced subsequent evolutive transformations from foam nest clutches to eggs laid in gelatinous structures, being subspherical masses, ovoid and plate-like, or egg strings.

The size and form of *P. guayapae* foam nests were similar to those declared for *P. diplolister* (Höld, 1992; Cardoso & Arzabe, 1993). However, the number of eggs per foam nest was greater in *P. guayapae* (900-1500 against 528-748 in *P. diplolister*; Höld 1992). The number of eggs per clutches remains unknown for most species of *Pleurodema*. In this regard, the foam nests of *P. borellii* produce, on average, about 1300 hatchlings (Halloy & Fiaño, 2000) and the semi-submerged gelatinous eggs-masses of *P. cordobae* (Valetti et al., 2009) have between 74 and 215 eggs (Valetti et al., 2011). The egg diameter of *P. guayapae* without the gelatinous capsule was somewhat smaller (between 1.29 and 1.61 mm) that the reported for *P. bufoninum* (1.26 mm to 2.94 mm), a species that lays
eggs in solid and cylindrical strings (Weigandt et al., 2004).

The hatching time of the *P. guayapae* tadpoles was short (26 to 30 hours at the developmental Stage 19), inferring a rapid larval development. This pattern of development is typical of species that breed explosively in temporary or ephemeral environments, so the larvae metamorphose in a short period, before desiccation of the water bodies. Rapid embryonic and larval development in *P. diplolister* was interpreted by Peixoto (1982) as an adaptation to highly xeric environments. We corroborated that 48 hours after the start of foam nest construction, the water level of ponds decreased considerably. In this sense, some foam nests stayed out of the water and all eggs and small tadpoles recently hatching were found dead.

In summary, our results indicated that the reproduction period of *P. guayapae* is associated to the summer rainfalls and ephemeral environments generated after these rains. Moreover, the short time at which the populations of *P. guayapae* are reproductively active, the type of clutches in foam nest, the presence of communal nests, and the rapid embryonic development, allow us to classify this species as an extreme explosive breeder.

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**RESUMEN**

**Biología reproductiva de Pleurodema guayapae** (Anura: Leptodactylidae: Leiuperinae). Pleurodema *guayapae* es una especie que habita en zonas semidesérticas y salinas del área central de Argentina. Los conocimientos actuales sobre la biología reproductiva de esta especie son muy pobre, por lo que nuestro objetivo fue contribuir a su conocimiento con la descripción de algunos aspectos reproductivos importantes. Para ello se realizaron tres campañas de muestreo durante los veranos (2006-2007, 2007-2008 y 2008-2009) en el área de Chamical-Patquía, provincia de La Rioja, donde se encontraron poblaciones reproductivamente activas. Se observaron y describieron los sitios reproductivos, tipo de puesta, proceso de construcción del nido de espuma, tamaño de la puesta y de los huevos, número de huevos por puesta y tiempo y estado de eclosión de la larva. Las observaciones de comportamiento se realizaron desde el momento en que los machos comenzaron a emitir los cantos hasta que la pareja en amplexo terminó de construir el nido de espuma con los huevos en su interior. Además, se observó y filmó una pareja en amplexo, en el proceso de construcción del nido de espuma y se recolectaron cuatro parejas en amplexo y se colocaron separadamente en recipientes plásticos para que sus puestas pudieran ser observadas en el laboratorio. El tiempo de eclosión se determinó en tres nidos de espuma de edad conocida. Se encontró que las poblaciones de *P. guayapae* solo estuvieron acústicamente activas luego de una lluvia torrencial. Los sitios de reproducción fueron charcas efímeras de agua de lluvia. Los machos emitieron sus cantos de advertencia desde adentro o fuera de esas charcas. Se realizó una descripción detallada del proceso de construcción del nido de espuma por parte de la hembra y el macho de *P. guayapae*. El tipo de la puesta en esta especie es un nido de espuma flotante con forma de domo de 6 a 9 cm de diámetro y de 1 a 3 cm de alto, algunos de los cuales estaban unidos entre sí formando puestas comunales. Los nidos de espuma poseen promedio 137 huevos pigmentados. Esta especie mostró un tiempo de eclosión corto. Nuestros resultados nos permiten concluir que *P. guayapae* debe ser considerada como reproductor explosivo extremo. Nuestros resultados son discutidos con los obtenidos en otras especies afines.

**Palabras claves:** nidos comunales, ambiente efímero, reproductor explosivo, construcción de nido de espuma.

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