

Foam making behaviour of tadpoles of the pepper frog *Leptodactylus vastus* in north-eastern Brazil

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The pepper frog *Leptodactylus vastus* Lutz, 1930 belongs to the *Leptodactylus pentadactylus* group and is the largest species of leptodactylid to occur in north-eastern Brazil (Heyer, 2005). This species is endemic to Brazil and occurs exclusively in open-formation habitats (Heyer, 2005).

At the time of the first rains, adult leptodactylids produce foam nests near the margins of temporary or permanent pools, or in cavities excavated in the soil. The eggs are enclosed in a dense layer of foam (Silva et al., 2005). In some leptodactylids, foam production is not exclusive to adults, with tadpoles of the species also showing this behaviour. This has been recorded in *Leptodactylus labyrinthicus*, which also belongs to the *L. pentadactylus* group (Kokubum & Giaretta, 2005), in five species of the *Leptodactylus fuscus* group, namely *L. fuscus* (Downie, 1984; Downie, 1989), *L. mystaceus* (Caldwell & Lopez, 1989), *L. furnarius* (Giaretta & Kokubum, 2004), *L. latinasus* (Ponssa & Barrionuevo, 2008) and *L. troglodytes* (Kokubum et al., 2009). It is also known in two species of the genus *Adenomera* (Almeida & Angulo, 2002; Kokubum & Giaretta, 2005).

Foam production by tadpoles assists in obtaining soil moisture, preventing tadpoles contacting the bottom of the nest, preventing compaction and overcrowding, and facilitating respiratory processes and excretion (Downie & Smith, 2003; Kokubum & Giaretta, 2005). Here, we report the results of a laboratory test designed to show whether or not tadpoles of *L. vastus* can produce foam and to what extent the addition of small amounts of water is required for foam production.

For the tests, two egg clutches were collected in a flooded, grassy area on the campus of Universidade Federal de Campina Grande, municipality of Patos, Paraíba State, Brazil (7° 1'32" S, 37° 16'40" W). Species identity was inferred from traits of the foam nests, in particular the large size (>30 cm diameter) and thickness of the foam, also taking into account the species present in the area (Heyer, 2005). Identity was subsequently confirmed by following the tadpoles through to complete metamorphosis. The first clutch (471 tadpoles) was collected on January 18th 2021, and the second (295 tadpoles) on February 5th 2021. In the first clutch, we found that the tadpoles had already hatched and were in stage 27-31 of Gosner (1960), there were also 143 possibly trophic

eggs. The second clutch also had some possible trophic eggs but they could not be counted due to the dryness of the clutch.

The test followed the design of Kokubum & Giaretta (2005). Sixty tadpoles in stages 27-31 (Gosner, 1960) were removed from each clutch, washed in dechlorinated tap water and then placed in 30 cm³ Falcon® tubes (Fig. 1A). For each clutch two replicates were prepared with 5, 10 or 15 tadpoles in the tube. Each group was kept for a period of up to six days in the Falcon® tube (Fig. 1A). The test with clutch 1 was initiated on 18th January 2021 and with clutch 2 on 5th February 2021. At the start, a shallow film of water (two drops with a doser) was added to all tubes just covering the tadpoles, this was to prevent desiccation; the tubes were kept open. In test 1, the water film was replenished as required, when the foam production was stopped, while in Test 2 it was not replenished immediately, to observe the influence of moisture on foam production. We measured the air temperature and air humidity with a digital thermohygrometer. The mean air temperature in Test 1 was 31.5° C (29.0°-33.4° C) and the mean relative humidity 39 % (30 %-48 %). In the second test, the mean temperature was 32.6° C (30.4°-34.4° C) and the mean relative humidity 43 % (29 %-50 %). Once the tests had started the tubes were observed every three hours.

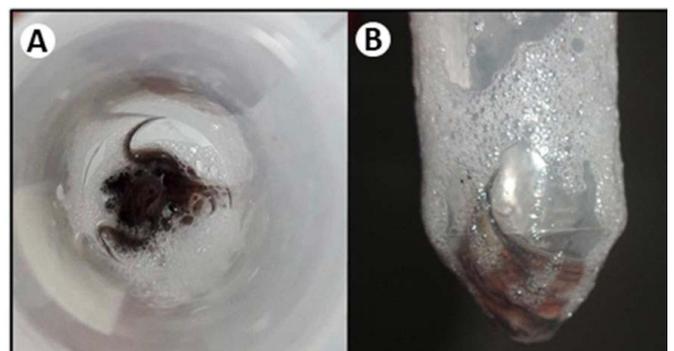


Figure 1. A group of 15 *Leptodactylus vastus* tadpoles in a plastic tube three hours after the beginning of a test to observe foam production-**A.** Tadpoles viewed from above, **B.** Tadpoles viewed from the side

The tests were considered successful, since the tadpoles began to produce enough foam to move around in the tube after the first three hours in both tests (Fig. 1B). In those tubes with 15 tadpoles, foam had appeared by 90 minutes, indicating that with greater numbers of tadpoles the foam is produced more quickly. In Test 1, tubes containing 5 or 10 tadpoles required the film to be replenished after 37 h while in the tubes containing 15 tadpoles the foam lasted longer and did not need replenishment until 55 h after the start. This corroborates the observations of Downie & Smith (2003) for the need of a shallow film of water for foam production and that with greater numbers of tadpoles the water film does not need replenishment so quickly.

In Test 2, the tadpoles in the two tubes with 5 individuals had all died after about two days as the water film was not replenished; death occurred about 7.5 hours after the foam production had ceased. The tadpoles in the two tubes with 10 individuals maintained a considerable quantity of foam until three days after the start of the experiment, on the fourth day the foam production had declined and after a further 8 hours the tadpoles had died. The tadpoles in the two tubes with 15 individuals managed to maintain a considerable amount of foam until the fourth day when the foam production declined and within 7 hours the water film was replaced which prevented the death of the tadpoles.

We observed that the production of foam by the tadpoles was caused by the release of small bubbles from the mouth parts of the tadpoles and was facilitated by the movement of their tails, in a similar manner to the tadpoles of *L. fuscus* (Downie, 1989), *Adenomera* sp. and *L. labyrinthicus* (*pentadactylus* group) (Kokubum & Giaretta, 2005). Three leptodactylids foam-producing tadpoles have been shown to have oral epithelial mucous glands - *L. furnarius*, *L. labyrinthicus* and a member of the *L. marmoratus* group (= *Adenomera*) (Giaretta et al., 2011).

The ability of *L. vastus* tadpoles to produce foam, independent of their parents, allows tadpoles to remain in the foam nest for longer, preventing nest desiccation. Additionally, this behaviour occurs before or after periods of irregular rainfall, which allows tadpoles to survive and develop until frequent rains provide temporary water bodies where they complete their development. This is before other species that begin their reproduction only in the period of regular rains and provides an advantage to the foam-producing species. In the species of the *fuscus* group, tadpoles in foam nests reach a specific stage, but do not progress any further until the rain washes them into a pool (Downie, 1984). But those of the *pentadactylus* group that have been studied, continue to develop and grow because their tadpoles are able to feed on trophic eggs (Kokubum & Giaretta, 2005).

The individuals involved in this test were sacrificed using the liquid anaesthetic lidocaine, fixed in 5% diluted formaldehyde and deposited in the Collection of Amphibians and Reptiles of the Laboratório de Herpetologia da Universidade Federal de Campina Grande (LHUFGL0115, LHUFGL0116, LHUFGL0117, LHUFGL0118, LHUFGL0119, LHUFGL0120, LHUFGL0121), municipality of Patos, state of Paraíba, north-eastern Brazil, under the license (#25267-1)

of the Biodiversity Authorization and Information System (SISBIO-ICMBio).

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