

## TROPHIC EGGS IN THE FOAM NESTS OF *LEPTODACTYLUS LABYRINTHICUS* (ANURA, LEPTODACTYLIDAE): AN EXPERIMENTAL APPROACH

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The South American pepper frog, *Leptodactylus labyrinthicus*, is a large species that lays eggs in foam nests in holes dug out of the banks of different bodies of water. Recently, it was reported that only 6-10% of eggs are fertilized in foam nests of *L. labyrinthicus* and the remaining unfertilized eggs are consumed by the tadpoles inside the nest. Here we tested experimentally the influence of the ingestion of trophic eggs on the survivorship and growth of *L. labyrinthicus* tadpoles. Tadpoles fed on trophic eggs and subsequently fed on dry fish food grew larger than those fed only on dry fish food, and this suggests that the ingestion of trophic eggs is an adaptation to improve tadpole growth. The ingestion of trophic eggs also seems to be important for the maintenance of tadpoles in environments with unpredictable rainfall, as they were able to survive for about 70 days feeding only on these trophic eggs and one tadpole managed to complete metamorphosis feeding on trophic eggs only. Details of the spawning behaviour observed in the field, occurrence of multiple mating, and predation on eggs by terrestrial invertebrates and vertebrates are also reported.

**Key words:** egg predation, multiple mating, frog, reproduction, spawning behaviour

### INTRODUCTION

The genus *Leptodactylus* is characterized by the deposition of eggs embedded in foam nests (Heyer, 1969), showing an evident tendency toward a terrestrial existence, i.e. reproduction becoming gradually independent of water (Heyer, 1969). Studies on *Leptodactylus* published since the revision made by Heyer (1969), report several novelties in the reproductive modes of the frogs in this genus (e.g. Hero & Galatti, 1990; Davis *et al.*, 2000; Prado *et al.*, 2002). For the *L. pentadactylus* group, with approximately 13 species (Frost, 2004), two different reproductive modes have been described. One of them is the mode described by Heyer (1969), with foam nests deposited in depressions or holes close to water and exotrophic tadpoles that develop in water (e.g. *L. knudseni*: Hero & Galatti, 1990; Rodríguez & Duellman, 1994; *L. labyrinthicus*: Agostinho, 1994; Rodrigues Silva *et al.*, 2005). A totally terrestrial mode in the group was reported for *L. fallax* (Davis *et al.*, 2000) and *L. pentadactylus* (Hero & Galatti, 1990; Rodríguez & Duellman, 1994), with foam nests inside burrows in the ground and development of larvae inside the nest. At least for *L. fallax*, it was recently reported that tadpoles display obligatory oophagy, feeding only on trophic eggs deposited by the females (Gibson & Buley, 2004), as previously suggested by Prado *et al.* (2002).

Although the presence of a foam nest has been widely reported for leptodactylines, foam functions are still scarcely understood (Downie, 1993). Many functions are attributed to foam nests made by several anuran species, namely, defence against predators (Kluge, 1981; Ryan,

1985; Downie, 1988; 1990; 1993), temperature control (Downie, 1988), protection against desiccation (Ryan, 1985; Downie, 1988), inhibition of tadpole growth (Pisano & Del Rio, 1968), improvement of oxygen supply (Seymour & Loveridge, 1994), and food source (Vinton, 1951; Tanaka & Nishihira, 1987). Tadpoles of different *Leptodactylus* species in the *Pentadactylus* group have been recorded feeding on foam in the nest (*L. pentadactylus*: Vinton, 1951), as well as on conspecific eggs inside the same nest (*L. pentadactylus*: Muedeking & Heyer, 1976; *L. labyrinthicus*: Agostinho, 1994), allowing tadpoles to survive for long periods before being washed to the water by rain.

The South American pepper frog, *Leptodactylus labyrinthicus*, is a large species that lays eggs in foam nests in holes dug out on the banks of different bodies of water (Rodrigues Silva *et al.*, 2005), occurring throughout South America (Heyer, 1979). However, its reproductive biology is still poorly known (e.g. Cardoso & Sazima, 1977; Agostinho, 1994; Rodrigues Silva *et al.*, 2005). Recently, it was reported that only 6-10% of eggs are fertilized in the foam nests of *L. labyrinthicus* (Agostinho, 1994; Rodrigues Silva *et al.*, 2005), and the remaining unfertilized eggs are consumed by the tadpoles inside the nest. Based on this information, we tested in the laboratory the influence of the ingestion of trophic eggs on the survivorship and growth of *L. labyrinthicus* tadpoles. As the species lives in environments with unpredictable rainfall and may remain in the nest for more than 30 days (Agostinho, 1994; Eterovick & Sazima, 2000b), we tried to determine the importance of the trophic eggs in the diet of *L. labyrinthicus* tadpoles by simulating field conditions to answer the following questions: (1) do tadpoles remaining in the foam nest and feeding on trophic eggs grow larger than those that leave the foam

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prematurely? And (2) how long can the tadpoles survive by remaining in the foam nest and feeding only on trophic eggs? Furthermore, details of the spawning behaviour observed in the field, multiple mating, and predation on eggs by terrestrial invertebrates and vertebrates are reported.

## MATERIALS AND METHODS

### SPAWNING BEHAVIOUR AND PREDATION EVENTS

Field observations were made at the Estação Ecológica de Itirapina (EEI), between the municipalities of Brotas and Itirapina (22°13'S; 47°54'W), State of São Paulo, south-eastern Brazil. This area represents one of the last remnants of Cerrado in the State of São Paulo, which is an open formation covered by savannah-like vegetation. Monthly field observations were made during the day and at night in two distinct periods: October 2002 through February 2003 and October 2003 through January 2004, which correspond to the breeding period of this species at the site. Individuals of *L. labyrinthicus* were observed breeding at the edge of temporary ponds and small streams. We used all occurrences sampling (Altmann, 1974) to describe spawning and nest excavation behaviours. All foam nests observed were checked for predators.

### TADPOLE GROWTH AND SURVIVORSHIP

Foam nests of *L. labyrinthicus* were collected at the Estação Ecológica de Itirapina (EEI: 22°13'S; 47°54'W) and in the municipality of Rio Claro (22°24' S; 47°33' W), State of São Paulo, south-eastern Brazil, between October - December 2001 and October-December 2002. When collected, clutches contained tadpoles at stages 18 and 19 (Gosner, 1960); total number of eggs and percentage of fertilized eggs were recorded. For the experiments, tadpoles were kept in the foam nests until they reached 6.0 mm of body length (stage 25; Gosner, 1960). All foam nests and tadpoles were maintained in the laboratory in plastic boxes (30 x 40 cm) with 3.0 cm of well water, with water temperature between 25 and 27°C (mean temperatures registered in the field), and natural photoperiod (sunlight coming through the windows). As tadpoles of *L. labyrinthicus* are carnivorous (Eterovick & Sazima, 2000a), pellets of dry fish food (Alcon®) were used in the experiment as the alternative food source.

To test for differences in growth rate and survivorship, tadpoles were divided into three groups: Treatment I, 25 individuals ( $N=3$  clutches) reared in foam nests with trophic eggs, and subsequently fed dry fish food pellets *ad libitum* once a day; Treatment II, 33 individuals ( $N=3$  clutches) reared in water and fed only dry fish food *ad libitum* once a day; Treatment III, groups of 14 and 20 tadpoles ( $N=2$  clutches) were reared in foam nests with trophic eggs, without additional food. The body length of 10-20 larvae was measured for each treatment at intervals of about five days with a calliper ruler to the nearest 0.1 mm. Snout-vent length (SVL) of metamorphosed specimens was recorded with a calliper

ruler to the nearest 0.1 mm and body mass was measured on an electronic balance to the nearest 0.01 g. Some tadpoles and metamorphs were deposited at the Célio F. B. Haddad collection (CFBH 5879-5902), in the Departamento de Zoologia, Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil, but most metamorphs were released at the site where they had been collected after being health-screened.

## RESULTS

### SPAWNING BEHAVIOUR

On 9 January 2004 at the Estação Ecológica de Itirapina, we recorded three amplexant pairs building foam nests. Spawning behaviour was similar in the three nests, the main steps being summarized as follows. Males called from shallow natural cavities next to lentic water bodies, or from old hollows that had already been used for foam nest deposition. The female approached a calling male, which began to emit courtship call. The male clasped the female's flanks; the female arched its body down and discharged a jelly-like secretion throughout its vent. At this moment, the male started the foam production by stirring the secretion with its hind limbs. Next, the female pushed the mud with its arms from the centre to the edges of the nest, digging it deeper. When the female's movements ended, the couple rotated about 20° to their right. It took approximately 19 short turns to complete 360° in the nest, which lasted about 14 min. This behaviour was repeated until the end of the foam nest construction. Nest excavation by the female and spawning occurred simultaneously and lasted about three hours, starting between three and four hours after sunset. We observed that the eggs were not laid at the beginning of the foam construction, but only when the foam reached a larger size. In all three cases, males left the nest before the females, which stayed inside the nest for up to another 10 min.

On the same night we observed two nests in which three individuals tried to build the foam nest in a hollow, all at the same time. In one case it was a multimale spawning (two males and one female) and in the other, a multifemale spawning (two females and one male). We do not know the moment when the multimale group formed. However, we observed that the multifemale group formed when a second female entered a nest between the amplexant pair. The presence of a third individual in both nests made the foam production movements awkward. Males could not move their legs properly and females could hardly move in the nest or even push the mud forward. The multiple mating groups remained together for up to three hours, trying to raise a foam nest; suddenly they gave up and left the nests without spawning.

### PREDATION

Predation within the nests was recorded several times at the Estação Ecológica de Itirapina. On 2 December 2003 at one temporary pond edge, ants (*Camponotus rufipes*, Formicidae) were observed preying upon *L.*

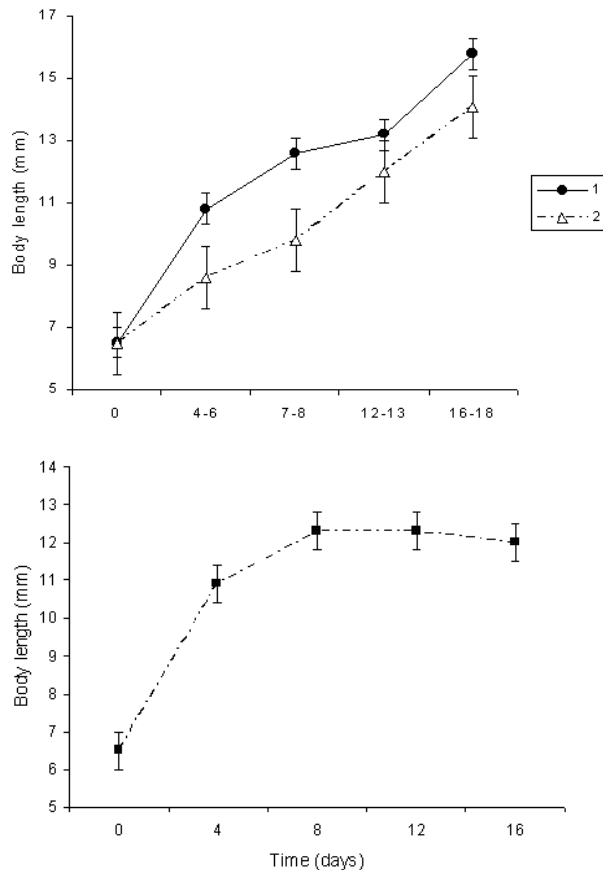


FIG. 1. (A) Mean body length of *Leptodactylus labyrinthicus* tadpoles reared (1) in foam nest with trophic eggs, and subsequently fed on dry fish food (solid circles;  $N=15$ ); and (2) in water fed only on dry fish food (open triangles;  $N=20$ ). The arrow indicates the time when tadpoles changed their diet to dry fish food. (B) Mean body length of tadpoles ( $N=15$ ) reared in foam nest with trophic eggs but no additional food. The arrow indicates the time when the trophic eggs finished; Bars = 1 SD.

*labyrinthicus* eggs that were on the outermost layer of the foam nest. The ants pulled the eggs from the foam with the mandibles and carried them away. On 9 January 2004 at another pond, two other nests were also being preyed upon by the same ant species. On 2 December 2003, a colubrid snake was observed in a *L. labyrinthicus* foam nest that contained tadpoles. The snake fled before we could catch it and thus its identity remains unknown.

On several occasions throughout the study period, some foam nests of *L. labyrinthicus* were destroyed during the night, but the predator was never seen. On 9 January 2004, one foam nest was found at 19.20 hr. At 20.00 hr, an adult maned wolf (*Chrysocyon brachyurus*) was seen at a temporary pond next to the foam nest (N.L. Hulle, pers. comm.). At 21.30 hr, we returned to the pond and found only 10 % of the foam nest in a ruined cavity where footprints of *C. brachyurus* were found. At the same pond, 2 m away from recent wolf faeces we found a forelimb and parts of the abdomen (with ovaries and mature oocytes) of an adult female *L. labyrinthicus*. We presume the wolf ate the frog since these body parts had not been seen in the pond beforehand.

#### TADPOLE GROWTH AND SURVIVORSHIP

Foam nests contained an average of  $2756 \pm 821$  eggs ( $N=4$ ; range=1817 - 3786), and the mean ( $\pm$ SD) percentage of fertilized eggs was  $6.5 \pm 3.1\%$  eggs ( $N=5$ ; range=3.4-11.5%). Individuals reared in foam nests with trophic eggs and subsequently fed on dry fish food (Treatment I) also consumed the foam and grew larger than those reared in water and fed only on dry fish food (Treatment II; Fig. 1A). All larvae from Treatments I and II reached metamorphosis within about 40 days from the date they were collected (clutch about 3-5 days old). Mean ( $\pm$ SD) SVL of metamorphs from Treatment I was  $23.0 \pm 1.4$  mm ( $N=25$ ; range=20.1-25.8 mm) and mean ( $\pm$ SD) mass was  $1.38 \pm 0.2$  g ( $N=23$ ; range=0.83-1.77 g). From Treatment II, metamorphs reached a mean ( $\pm$ SD) SVL of  $21.5 \pm 1.5$  mm ( $N=33$ ; range=18.4-24.8 mm) and a mean ( $\pm$ SD) mass of  $1.17 \pm 0.2$  g ( $N=31$ ; range=0.78-1.5 g). Metamorphs from Treatment I were significantly larger ( $t=3.6$ ,  $df=56$ ,  $P=0.0006$ ) and heavier ( $t=3.5$ ,  $df=52$ ,  $P=0.001$ ) than those from Treatment II. Tadpoles reared in foam nests with trophic eggs but no additional food also consumed foam (Treatment III), but these shrank and their development slowed down after 12-13 days (Fig. 1B), when the trophic eggs and foam finished. However, some of them survived for about 40 days ( $N=6$  from 14 tadpoles) in the first clutch tested, and even for about 70 days ( $N=18$  from 20) in the second. Among these tadpoles, only one reached metamorphosis after 34 days, but with a small size (SVL=15.8 mm; mass=0.4 g).

#### DISCUSSION

Despite its wide geographical distribution, the spawning behaviour of *L. labyrinthicus* and the gender responsible for nest excavation remained unknown until the present study and the study carried out by Rodrigues Silva *et al.* (2005). Our observations on nest excavation by the amplexed female and foam construction are consistent with those described by Rodrigues Silva *et al.* (2005) for another *L. labyrinthicus* population. Furthermore, this is the first report of multiple mating for the species. Multimale spawning was reported for *Leptodactylus chaquensis* and *L. podicipinus* (Prado & Haddad, 2003), as well as for other species in some other families (e.g. Kusano *et al.*, 1991; Roberts *et al.*, 1999). In multimale breeder species, males have much larger relative testes size compared with other species, probably a trait related to sperm competition (e.g. Kusano *et al.*, 1991; Jennions & Passmore, 1993; Prado & Haddad, 2003). However, males of *L. labyrinthicus* have much smaller testes mass relative to body mass compared with other leptodactylids (Prado & Haddad, 2003) and we suggest that this could be related to the fact that males do not need to produce a great amount of sperm, since only about 10% of eggs are fertilized. Furthermore, polyandry seems to be accidental in this species since it prevents egg laying. Occurrence of simultaneous polygyny, as observed for *L. labyrinthicus*

in the present study, is unrecorded for frogs as far as we know. The trios observed in the hollows (multifemale and multimale) abandoned the foam construction before spawning, probably due to spatial limitation. We suggest that the explosive reproductive pattern allied to an almost 1:1 sex ratio (L.F. Toledo, unpublished data) of *L. labyrinthicus* could be related to the multiple mating behaviour observed at the study site.

The deposition of eggs outside the water is suggested to be an adaptation against aquatic predators (e.g., Magnusson & Hero, 1991; Haddad & Sawaya, 2000). For *L. labyrinthicus*, whose tadpoles are known to be carnivorous (Cardoso & Sazima, 1977; Eterovick & Sazima, 2000a), it has been suggested that the deposition of foam nests in depressions isolated from the water body might protect the tadpoles from predation by conspecific tadpoles (Rodrigues Silva *et al.*, 2005). Although nests of *L. labyrinthicus* may protect eggs, embryos, and tadpoles from aquatic predators, it seems to imply in an additional cost, as they are vulnerable to some terrestrial predators, both invertebrates and vertebrates (Menin & Giaretta, 2003; present study). Terrestrial invertebrates preying on eggs inside foam nests had already been reported for other *Leptodactylus* species, including those that place eggs inside subterranean chambers outside the water, such as *L. fuscus* and *L. latinasus* (e.g. Villa *et al.*, 1982; Downie *et al.*, 1995).

Our results confirm the occurrence of trophic eggs in the foam nests of *L. labyrinthicus*, as previously reported (Agostinho, 1994; Rodrigues Silva *et al.*, 2005). Histological study of the ovary, unfertilized eggs, and testis of *L. labyrinthicus* (Prado *et al.*, 2004) did not reveal any abnormality that could explain the low fertilization rate. However, it was observed that females remain in the nest after spawning (Rodrigues Silva *et al.*, 2005; present study), and thus the most plausible explanation for the low fertilization rate would be that females place additional eggs following spawning, as previously suggested by Rodrigues Silva *et al.* (2005). The observation that *L. fallax* females return to the nest in several occasions to place trophic eggs (Gibson & Buley, 2004) seems to support the hypothesis that females of *L. labyrinthicus* also deposit trophic eggs, since both species are phylogenetically related and are usually placed within the same species group (Heyer, 1979; Eterovick & Sazima, 2000a). The origin of carnivory in *L. labyrinthicus* tadpoles may be explained by a few simple behavioural steps, from the ingestion of trophic eggs in the nest (e.g. Agostinho, 1994; present study) to the predation on eggs and larvae of other frog species (e.g., Cardoso & Sazima, 1977; Rodrigues Silva *et al.*, 2005) going through cannibalistic behaviour by preying upon conspecific tadpoles and eggs (e.g. Rodrigues Silva *et al.*, 2005).

Among leptodactylids that build foam nests outside the water, several adaptations have evolved in order to maintain tadpole development, from production of additional foam by the tadpoles to remain in the nest waiting for rain (e.g. *Leptodactylus fuscus*: Downie, 1989) to the

deposition of eggs rich in yolk to nourish tadpoles that do not leave the nest (e.g. some *Adenomera* species: De la Riva, 1995). The deposition of a large amount of eggs that will not be fertilized, but instead will be consumed by the tadpoles, as recorded for *L. labyrinthicus* (Rodrigues Silva *et al.*, 2005; present study) can be considered a new reproductive strategy within the genus. Among the five reproductive modes recognized for the genus *Leptodactylus* by Prado *et al.* (2002), the reproductive mode of *L. labyrinthicus* herein described, with foam nests in excavated basins close to water, initial larval nourishment on trophic eggs, and late larval phase in the water, may be considered as an intermediate step between the mode described for *L. podicipinus*, with foam nests placed in open excavated basins close to water, and that of *L. fallax*, with foam nests placed in burrows far from the water and female provisioning of trophic eggs, allowing whole larval development inside the nest. As suggested previously (Prado *et al.*, 2002; Rodrigues Silva *et al.*, 2005), this strategy may be widespread among species in the *L. pentadactylus* group. The evolution of terrestrial reproductive modes in the genus *Leptodactylus*, with the deposition of eggs in places far from the water (Prado *et al.*, 2002), was apparently accompanied by the evolution of terrestrial modes of larval nourishment based on trophic eggs.

*Leptodactylus labyrinthicus* tadpoles and metamorphs that were fed on trophic eggs and subsequently on dry fish food reached larger sizes than those fed only on dry fish food. This suggests that the ingestion of trophic eggs is an adaptation to improve tadpole growth and not a fortuitous feature. Our results seem to support the hypothesis that the deposition of trophic eggs is important for the maintenance of the tadpoles, which can eventually reach metamorphosis by feeding only on these eggs (Rodrigues Silva *et al.*, 2005; present study). In natural conditions, it may be advantageous for the tadpoles to remain in the foam nest feeding on nutritive trophic eggs instead of going to the water to feed, since this implies predatory risks and energy expenditure related to prey search and capture. Furthermore, since *L. labyrinthicus* occurs in environments with unpredictable rainfall (Agostinho, 1994; Eterovick & Sazima, 2000b), tadpoles may perhaps be able to remain in the foam nest for a longer period of time until the next rainfall. However, alternative explanations could be responsible for the differences in the size of the metamorphs. It is possible that dry fish food is less nutritive than the trophic eggs or, as tadpoles fed on dry fish food were reared in the water without foam, we can not exclude the possibility that the higher growth rate of tadpoles may have been influenced by some chemical compound in the foam that promotes faster growth (e.g., growth hormone). Moreover, foam nests were also consumed by tadpoles reared in Treatments I and III. Contrary to this idea, it has been suggested that some biological properties of the natural foam or the foam made by the tadpoles, inhibit growth of tadpoles (Pisano & Del Rio, 1968; Downie, 1994). Although these stud-

ies were made with *Leptodactylus* species belonging to the *L. fuscus* group (Heyer, 1969), *L. labyrinthicus* tadpoles are also capable of making foam (W. R. Rodrigues Silva & A. A. Giaretta, pers. comm.). Additional experiments are necessary to study the effects of foam on *Leptodactylus* tadpole growth and survivorship.

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