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Foam nest in *Scinax rizibilis* (Amphibia: Anura: Hylidae)

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ABSTRACT. During the intervals of February 1993 to January 1994 and November 1994 to February 1995, in the southern São Paulo state, we studied the breeding activity of *Scinax rizibilis* (Bokermann, 1964), the only known hylid species with oviposition in foam nests. The foam nests were constructed by female jumps, during the oviposition. The clutches contained 850-1250 eggs, which were almost black, except for the small clear vegetative pole. The construction of foam nest in *S. rizibilis* is unique among the other species with this characteristic. The complexity of a foam nest is intermediate, and egg development was faster when eggs were surrounded by foam. It is possible to recognize a progression from less developed structures, represented by the bubble nests of some microhylid frogs, to more complex examples, such as the foam nests of Leptodactylidae or Leiuperidae.

KEY WORDS. Oviposition; reproduction; treefrog.

The combination of oviposition and developmental factors, including oviposition site, ovum and clutch characteristics, rate and duration of development, stage and size at hatching and any type of parental care, is defined as a reproductive mode (Duellman & Trueb 1986). The greatest diversity of anuran reproductive mode is found in the Neotropical region (Duellman & Trueb 1986, Haddad & Prado 2005). Foam nests are found in several different reproductive modes (modes 11-14, 28-33 *sensu* Haddad & Prado 2005) and have been reported for anuran species belonging to the families Hylidae, Hyperoliidae, Leiuperidae, Leptodactylidae, Limnodynastidae, Microhylidae, and Rhacophoridae (Littlejohn 1963, Amiet 1974, Tyler & Davies 1979, Haddad *et al*. 1990, Hödl 1990, 1992, Kadadevaru & Kanamadi 2000). The construction of foam nests may differ among these families (Tyler & Davies 1979, Haddad & Hödl 1997, Hödl 1990, 1992, Jennions *et al*. 1992).

Scinax rizibilis (Bokermann, 1964) is the only known hylid that deposits eggs in foam nests (Haddad *et al*. 1990). In the reproductive season, males interact acoustically and physically with each other (Bastos & Haddad 2002), and females apparently select them (Bastos & Haddad 1999). This mating system may have a directional effect on the larval characteristics (Bastos & Haddad 2001). Herein, we: (a) describe the foam nest construction in *Scinax rizibilis*, (b) analyze the clutch characteristics, and (c) verify the influence of the foam nesting on egg development.

MATERIAL AND METHODS

Scinax rizibilis was observed in a temporary pond at the Fazendinha São Luiz (24°21'S, 48°44'W, 800 m altitude) in the municipality of Ribeirão Branco, southern São Paulo state, Brazil, from February 1993 to January 1994, and from November 1994 to February 1995. We visited the pond either fortnightly or monthly, and monitored it for 2-6 nights during each visit, totaling 148 hours in 46 visits. The pond area was approximately 1,950 m^2 and the distribution of vegetation was regular with predominance of Juncaceae. The pond was bordered by typical Atlantic Forest flora.

Nocturnal observations were conducted with a 6 V spotlight covered with sheets of thin red plastic to reduce the stress on the animals (Robertson 1990). Focal-animal, all occurrences, and sequence samples were used for behavioral records (Lehner 1996).

Pairs found in amplexus were collected manually. We measured the snout-vent length (SVL) of individuals to the nearest 0.1 mm with a caliper ruler and weighted them with a Pesola® balance to the nearest 0.05 g. The clutches obtained were preserved in 5% formalin. Ten eggs of each clutch were measured under a stereomicroscopic using a micrometric ocular.

The pairs in amplexus (n = 5) were put into separate aquariums (25 x 8 x 20 cm) with water at a depth of 3 cm. The subjects were filmed with a video camera (two pairs) and photographed (three pairs) for description of the entire oviposition process. Environmental light was kept minimal.

In order to test the foam influence on egg development, we monitored four egg masses over 24 hours, two with foam and two without foam, maintained in a plastic recipient (10 x 10 x 10 cm) with water at a depth of 8 cm. We extracted some eggs from the foam nest with a fine-mesh dipnet. Developmental stages following Gosner (1960) were determined using a stereomicroscopic. For statistical analyses, we used Pearson correlation coefficient (Zar 1996) with the significance level of 0.05.

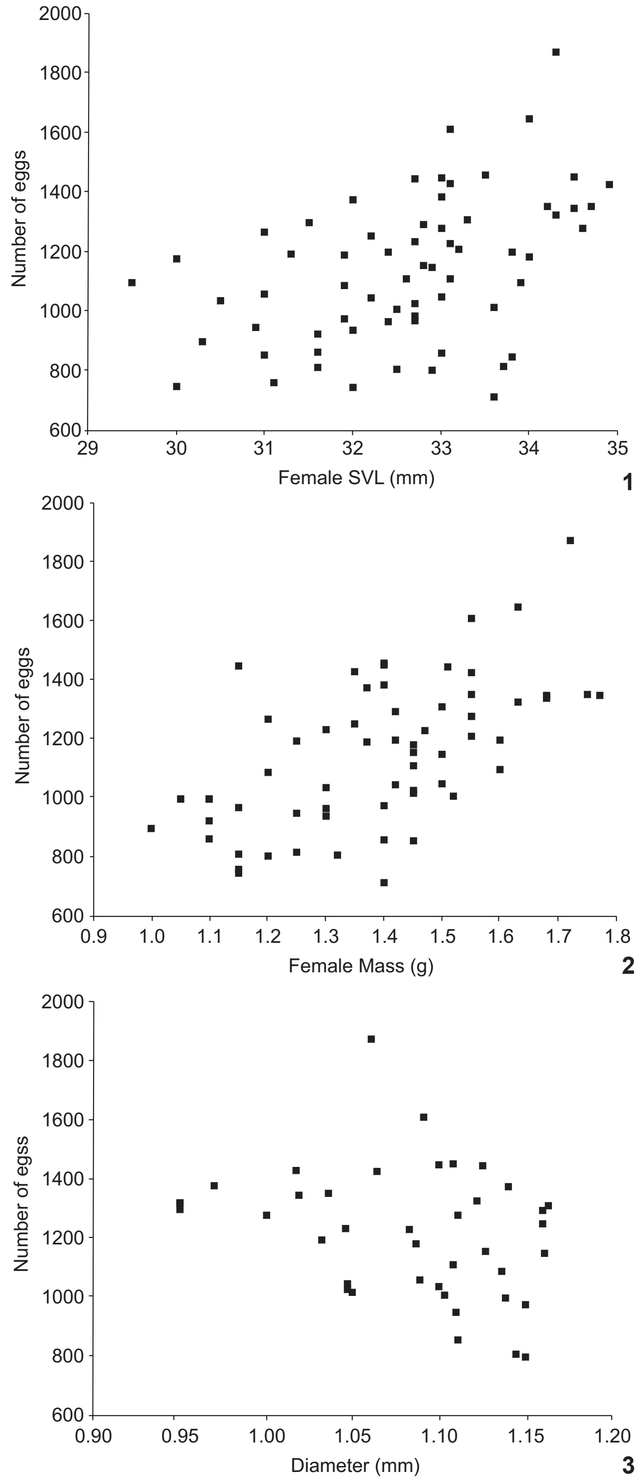
RESULTS

The clutches of pairs collected under natural conditions contained 850-1250 eggs (mean ± SD = 1,125 ± 245; n = 65). We found a significant positive correlation between clutch size and SVL (r = 0.48, $p < 0.01$, n = 65, Fig. 1), and between clutch size and the mass of females after oviposition (r = 0.57, $p < 0.01$, n = 65; Fig. 2).

The eggs were almost totally black except for the small clear vegetative pole, and presented diameters ranging from 1.02 to 1.15 mm (1.09 ± 0.05 mm; n = 380 eggs of 38 clutches). We did not find significant correlation between egg diameter and SVL (r = -0.06, $p > 0.05$, n = 38 clutches), or between egg diameter and female mass after oviposition (r = -0.15, $p > 0.05$, n = 38). However, we found a significant negative correlation between egg diameter and egg number (r = -0.33, $p < 0.05$, n = 38 clutches, Fig. 3).

Foam nest construction (n = 2) lasted 38 or 40 minutes, and was performed by females during the amplexus (Fig. 4). The beginning of the oviposition is marked by a circular swimming by the females. The male pushed its two feet very close, forming a channel between its cloaca and the female cloaca. After that, the pigmented eggs appeared. Since the mucus secreted by the reproductive traits of the females is transparent, we were not able to observe the exact moment of its release.

This sequence of oviposition was repeated several times. During the entire process the female performed alternate movements of the legs to join mucus and eggs. After egg expulsion, the female jumped up in the water and the impact of its body against the water allowed the retention of air bubbles in the mucus, forming the foam nest. The end of oviposition is marked by a characteristic posture signalization of the female; the female arched its back inwardly, the head was elevated (at 45° to it body), and legs and arms were distended. Then, the male slipped laterally off the female body and the process of oviposition ceased (Fig. 4F). The egg development was faster when eggs were surrounded by foam (Fig. 5).



Figures 1-3. Relationship between the: (1) snout-vent length and number of eggs of females of *S. rizibilis*; (2) mass and number of eggs of females of *S. rizibilis*; (3) diameter and number of eggs of *S. rizibilis*.

DISCUSSION

The clutch size is positively correlated with SVL and female mass, as observed in other Neotropical anuran species (Martins 1988, Bastos & Haddad 1996), according to the model

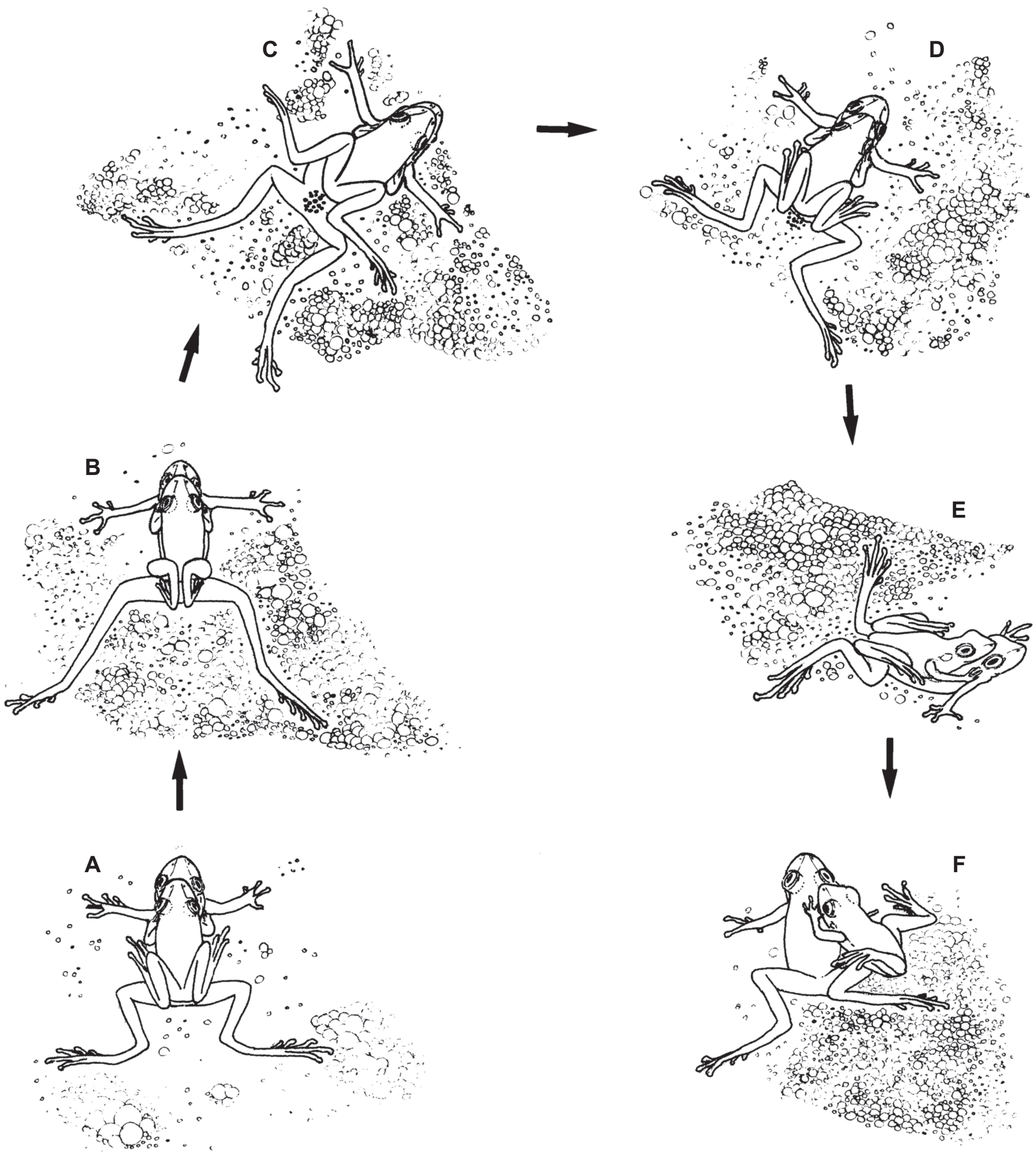


Figure 4. Stages of foam nest construction in *S. rizibilis*. Drawing based in slides.

of SALTHE & DUELLMAN (1974). By contrast, the diameter and number of eggs are negatively correlated. This is an advantage, because smaller eggs develop faster (CRUMP 1982). The eggs of *S. rizibilis* are pigmented, as the eggs of other open-area breeders (BASTOS & HADDAD 1996, HADDAD & HÖDL 1997). This pigment probably offers a protection against ultraviolet radiation (SALTHE & DUELLMAN 1974). In conclusion, larger females of *S. rizibilis* may be twice favored, because they deposit clutches with higher number of eggs that develop faster.

HADDAD *et al.* (1990), in his description of the foam nest of *S. rizibilis* (as *Hyla* cf. *rizibilis*), suggested the females have an active part in nest construction; herein we confirm it. The characteristic posture of the female for signalizing the end of oviposition was observed in the leiuperid *Physalaemus cuvieri* (J.P. Pombal Jr pers. obs.), and may be common in anurans.

The function of foam nests is controversial. Some authors have suggested that it might: 1) reduce exposure to aquatic predators (HEYER 1969); 2) protect the eggs from desiccation (HÖDL

1986, DOWNIE 1988); 3) provide thermal advantage to the eggs (DOBKIN & GETTINGER 1985); and 4) supply oxygen for eggs and embryos (SEYMOUR 1999, SEYMOR & LOVERIDGE 1994), accelerating development (HADDAD & HÖDL 1997). HADDAD *et al.* (1990) suggested that foam nests in *Scinax rizibilis* evolved mainly as a protection against insolation and desiccation of eggs and embryos. However, the data obtained in this study corroborates HADDAD & HÖDL (1997), because eggs without foam (little oxygen) developed slower than eggs with foam (more oxygen) (Fig. 5). Development acceleration and protection are not mutually exclusive functions. Therefore, we cannot say thatthe foam nests of *S. rizibilis* only provide either one of these two benefits. For example, eggs of species that breed in temporary ponds, such as *S. rizibilis*, hatch quickly into larvae, decreasing the risk of desiccation and predation by conspecifics (HÖDL 1992).

It is possible to recognize a sequence from a less elaborate floating device, represented by the bubble nest, to an elaborate structure represented by the foam nest (Tab. I). The foam

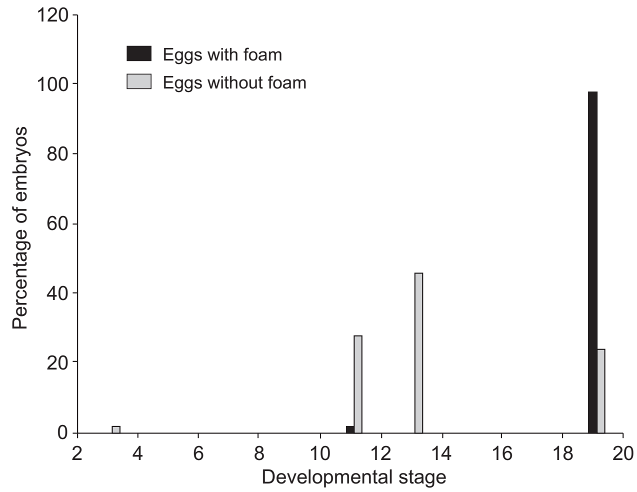


Figure 5. Developmental stages of tadpoles of *S. rizibilis* with and without foam.

Table I. Characteristics of anuran foam nest of selected species.

Species	Clutch site	Mode of foam production	Air bubble quantity/size	Foam complexity	Reference
Limnodynastidae					
Adelotus brevis					
Lechriodus brevis	Water	Water fluxus of females's hands	Few/large	Small	TYLER & DAVIES (1979)
Limnodynastes spp.					
Megistolotis lignarius					
Limnodynastidae					
Philoria frosti	Water	Females beating flanged fingers in water	Few/large	Small	LITTLEJOHN (1963)
Microhylidae					
Chiasmocleis leucosticta	Water	Downing air through the female/male's narines	Few/large	Small	HADDAD & HÖDL (1997)
Hylidae					
Scinax rizibilis	Water	Jump of female up water	High/large	Intermediate	Present study
Hyperoliidae					
Opisthothylax immaculatus	Leaf	Unknown	High/small	High	AIMET (1974)
Leiuperidae					
Pleurodema diplolister	Water	Leg kick by male	High/small	High	HÖDL (1992)
Physalaemus ephippifer	Water	Leg kick by male	High/small	High	HÖDL (1990)
Physalaemus pustulosus	Water	Leg kick by male	High/small	High	HEYER & RAND (1977)
Physalaemus spiniger	Water	Leg kick by male	High/small	High	HADDAD & POMBAL (1998)
Leptodactylidae					
Leptodactylus pentadactylus	Water	Leg kick by male	High/small	High	HEYER & RAND (1977)
Rhacophoridae					
Chiromantis xerampelina	Leaf	Leg kick by female	High/small	High	JENNIONS *et al.* (1992)
Rhacophorus malabaricus	Leaf/Litter	Leg kick by female/male	High/small	High	KADADEVARU & KANAMADİ (2000)

nest produced by *S. rizibilis* is intermediate between the two extremes (Haddad & Hödl 1997). The less complex foam nests are built by Limnodynastidae, Microhylidae, and Myobatrachidae, which deposit eggs on the water surface surrounded by few large air bubbles which are produced by male and female (Haddad & Hödl 1997), or by females that paddle the forelimbs to start a flux of water (Tyler & Davies 1979). The more complex foam nests are those built by Hyperolidae, Leiuperidae, Leptodactylidae, and Rhacophoridae whose eggs are surrounded by many small air bubbles, being deposited on the water surface (Heyer 1969, Hödl 1992) or on leafs/litter (Jennions *et al.* 1992, Kadadevaru & Kanamadi 2000).

As stated by Heyer (1969), the pre-adaptations to foam nest construction are widespread among anurans because many species are able to secrete mucus during oviposition. Haddad *et al.* (1990) manually beat the mucus of *Scinax hiemalis* and obtained a foam nest. The fact that foam nests are known from seven anuran families and that the construction procedures differ among them indicates that the evolution of the foam nest may have originated independently among these families.

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