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Observations on nest dynamics and embryonic and larval development in the nest building gladiator frog, *Hyla faber*

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Abstract. Nests and larval development of the nest building gladiator frog, *Hyla faber* were studied in southeastern Brazil, during the rainy season of 1988-1989. Nests were built at the pond margins, exclusively by males, and varied in shape, size, and composition in relation to the substrate. Nests were used by 1-4 individual males and housed larvae for a mean of 26 days; 0-6 egg clutches were deposited in a nest. Larvae from individual clutches stayed inside the nests for 8-38 days. Embryonic development occurred within the first 210 h after fertilization and larval development, inside an enclosure installed in a pond, lasted over 8 months. Mortality inside the nests was due to nest water evaporation and/or drainage, to eggs sinking in the first hours after fertilization, or to predation by aquatic insects. Slow larval development in *Hyla faber* seems to be related to breeding in permanent ponds. Nest building in *Hyla faber* and related species may have evolved from the habit of using natural depressions for egg laying observed in other morphologically similar species.

Introduction

Nest building for egg deposition is known in relatively few frog species and probably arose independently in distinct lineages within the Anura (see Duellman and Trueb, 1986). Members of the *Hyla boans* group ("nest building gladiator frogs", Kluge, 1979) build sand or clay nests at the margins of ponds and streams where embryonic and early larval development take place. This habit has been interpreted as a method of protecting the early developmental stages against aquatic predators in the adjacent waterbody (see Kluge, 1981).

Hyla faber, a member of the *boans* group, occurs from eastern Brazil to northern Argentina (see taxonomic comments on the *boans* group in Martins and Haddad, 1988). Fragmented data on *H. faber* natural history are found in Goeldi (1895), Lutz (1960a, 1973), and Cei (1980). Recently, Martins and Haddad (1988) and Martins (1993) described the reproductive behaviour of this species in forested and open areas, respectively.

Six species related to *H. boans* are known to build nests for egg deposition: *H. biobeba, H. boans, H. faber, H. pardalis, H. rosenbergi,* and *H. wavrini* (Goeldi, 1895; Lutz, 1960b; Duellman, 1970; Jim, 1980; Kluge, 1981; Martins and Moreira, 1991). However, detailed observations on nests and larval development have been published only for *H. rosenbergi* (Kluge, 1981). Here I describe nest dynamics and the embryonic and larval development of *H. faber* observed in an open area in southeastern Brazil. Additional data on this population are in Martins (1993) and Martins et al. (1993).

Materials and methods

Observations were made in an artificial permanent pond at the Fazenda Santa Mônica (22°54' S; 46°53' W; elevation about 800 m), located 8.5 km west of Joaquim Egidio, Municipality of Campinas, the State of São Paulo, Brazil. The pond is located within pastures and had a nearly rectangular shape (fig. 1); its deepest point is up to 1 m deep. The lower end of the pond has muddy banks with small sedges and grasses and the upper part is nearly completely covered by cattails (fig. 1). A detailed description on the study area is found in Martins (1993).

Observations on nest dynamics and early larval development were made from early November 1988 to mid-March 1989. I made visits to the area every two or three days, totalling 57 observation-nights; observations began at 1630-1830 h and ended at 2100-0130 h. Twenty five males and 20 females were marked during the study; methods involving adults are described in Martins (1993).

The location of each nest was recorded on a map of the study area. Each nest was photographed and marked with aluminium stakes bearing a plastic tape with its reference number. For each nest I recorded: (1) date of appearance, (2) presence and stage of larvae, (3) maximum and minimum axis of water surface, (4) maximum water depth, (5) mean wall height, (6) distance from the nearest nest, (7) distance from pond water, and (8) male that built it (when known). Water volume of each nest was estimated later by calculating the volume of an elypsoid section. Further analysis of material on the walls (clay and/or vegetation) and number of eggs in clutches were made on the photographs.

I made observations of nests each evening by walking slowly through the pond margins recording any new nest and collecting 2-5 larvae from each nest (immediately fixed in 5% formalin); a total of 42 clutches were sampled. To calculate time after fertilization I considered that all clutches were deposited at 0300 h (although I never observed *H. faber* laying eggs, this is the mean time the related *H. rosenbergi* laid their eggs, Kluge, 1981). Larvae were staged (Gosner, 1960) and measured under a dissecting microscope with an ocular micrometer. Each metamorph leaving the water was captured, staged, measured with a calliper rule, and released.

To estimate larval development, I installed a 80x80x50 cm enclosure, made of aluminium wire and plastic mesh, in the pond shallows on 19 February 1989. The enclosure was installed so that half of its height was inside the water. Fifteen days after installation, 200 *H. faber* embryos (stage 22) from a single clutch were placed inside the

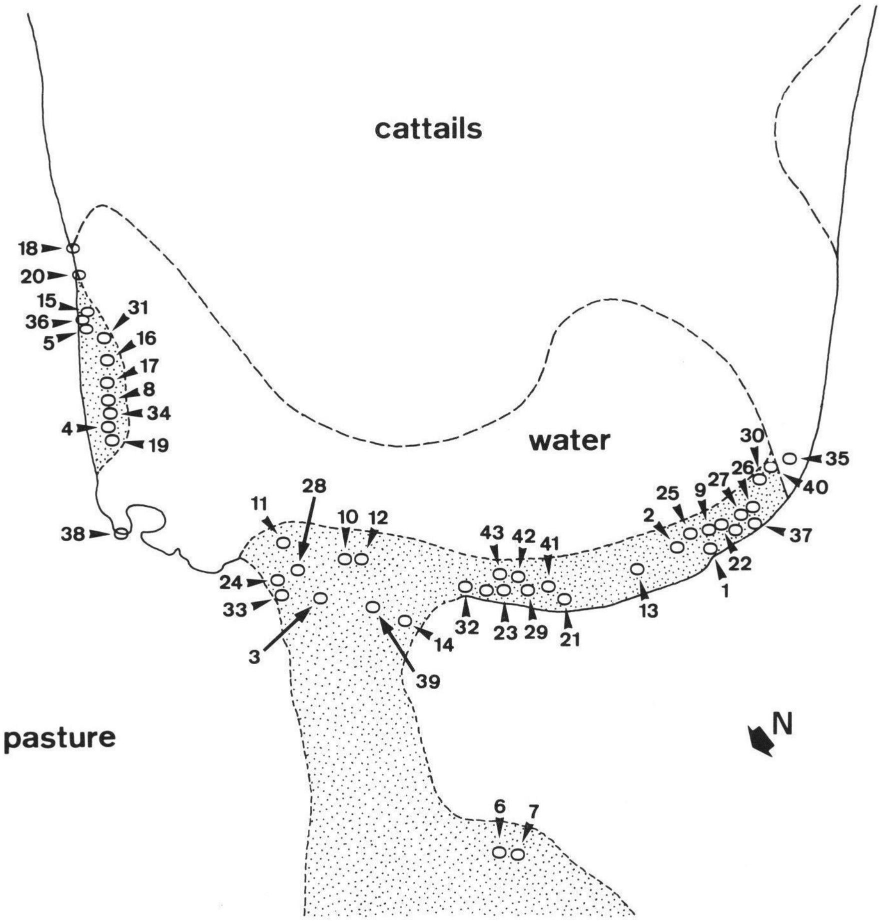

Figure 1. Map of the study site showing the location of nests built throughout the study by male *Hyla faber*. Stippled areas indicate muddy substrate.

enclosure. From March to November 1989, approximately every two weeks, a sample of 9-21 tadpoles were collected inside the enclosure, measured, staged, and immediately released.

Values are presented as means ± one standard deviation and sample size (when necessary).

Results

Nests were built on the muddy margins of the pond, exclusively by males. A typical nest was a nearly round water-filled depression surrounded by walls of clay or plants. I observed males building nests on three occasions. To build nests, males entered a water-filled depression and began to push away the surrounding clay or plants with its hands. Males then began to describe a circle, making a nearly round depression surrounded by the clay or plants it just pushed away. One male spent 25 and 35 minutes to build each of two nests in a single night. Further nest renewal by males or by females (in amplexus) was similar to nest building: clay was pushed toward the perimeter and spread along the walls with the hands and snout (see fig. 6 in Martins and Haddad, 1988). While building or renovating a nest, males stopped calling. Abandoned nests deteriorated in a few days; rains and floodings destroyed the walls and filled the basin with silt.

Forty five nests were built at the pond margins during this study; two of them were not marked because they were found several days after having being built. The shape and size of 43 nests, at the time they were marked, varied considerably: maximum and minimum axis of water surface were 11-30 cm (21.2 ± 3.6 cm) and 11-26 cm (17.0 ± 2.7 cm), respectively; water surface 95.0-612.6 cm^2 (286.9 ± 87.9 cm^2); water depth 4-13 cm (8.2 ± 1.8 cm); water volume 261-3587 cm^3 (1528.0 ± 674.4 cm^3); and wall height 0-6 cm (1.8 ± 1.6 cm). Neither water surface nor water volume were significantly correlated to the size of the males that built each nest ($r = -0.239$, and $r = -0.116$, respectively, $n = 26$). Considering 41 nests built along the pond margins (fig. 1), distance between nests and pond water was 0-96 cm (22.5 ± 24.7 cm). Distance between adjacent nests, at the time they were marked, was 0-350 cm (77.4 ± 82.9 cm, 41).

The composition of the walls was clearly related to the substrate where nests were built. Of 43 marked nests, 14 (32.6%) had only plants on the walls whereas the remaining 29 (67.4%) had clay on at least one quarter of the walls; only four nests had walls made entirely of clay. Considering 41 nests built on the pond margins, the ratio of clay/plants on the walls was negatively correlated with water depth ($r = -0.397, p = 0.01$) and positively correlated with the distance from nests to pond water ($r = 0.481$, $p = 0.001$), i.e., nests with a higher clay/plants ratio tended to be shallower and farther to pond water.

The number of different males observed occupying each nest was 1-4 (1.3 ± 0.7 males, 43). Zero to six clutches were laid in each nest (1.7 ± 1.3 clutches, 41). The number of clutches laid in each nest was not significantly correlated with water surface ($r = 0.197$), water volume ($r = 0.223$). water depth ($r = 0.227$), distance between nest and pond water ($r = -0.217$), or ratio of clay/plants on nest walls ($r = -0.140$). The interval between successive egg clutches in individual nests was 2-70 days (20.3 ± 15.6 days, 36). Nests housed larvae for a total of 0-97 days (25.8 ± 22.0 days, 41); nearly two thirds (65.1%) of the nests housed larvae for less than 20 days.

All nests were built on the muddy banks of the pond, except five built elsewhere (three within the cattails and two in a swamp some meters below the pond; fig. 1). Nests built by the same male on consecutive days tended to be grouped (nests nos. 10, 11, and 12 built by male no. 1 in 7, 9, and 9 November, respectively; nests nos. 16, 17, and 19, male no. 16 in 7, 14, and 16 December; and nests nos. 22, 25, 26, and 27, male 13 in 26, and 30 December and 1 and 2 January; fig. 1). On most occasions (83%) males observed calling inside nests attracted females and mated with them. The rate of new nest appearance on the pond margins throughout the study was 0.36 nests. day^{-1}; this rate was nearly constant, except that no nest was built during a drought from mid-November to early December (see Martins, 1993).

Clutches consisted of a floating monolayer of eggs (diameter 1.9-2.1 mm), embedded in transparent gelatinous capsules (thickness 1.6-2.1 mm), which occupied most of the water surface of nests. No clutch was found outside a nest. Nine clutches had 1614-3576 eggs (2276 ± 635 eggs).

Figure 2 shows the embryonic and early larval growth inside the nests in the first 600 h after fertilization. Growth was fast in the first 250 h (about 1 mm.day^{-1}) and gradually diminished in the following 350 h (about 0.2 mm.day^{-1}). Figure 3 shows the developmental stages of embryos and tadpoles in the first 600 h after fertilization. Forty hours after fertilization embryos began to move within their capsules (stages 18 and 19), and after 65 h they had already large external gills and aggregated near the water surface (stages 20-24). After about 210 h, all larvae were in stage 25; they swam freely inside the nest and respired through internal gills.

Tadpoles stayed inside the nests for 8-38 days (15.9 ± 6.7 days, 29). Although I never saw tadpoles leaving their nests, on three occasions I found tadpole aggregations (stage 25, total length 10-15 mm) at the pond margins, always close (10-30 cm) to recently abandoned nests. When the pond margins flooded during heavy rains larvae younger than about 10 days after fertilization (stage 20-25) remained in the nest bottom until the water level fell.

Larval development inside the enclosure, from stage 25 until metamorphosis, lasted over 8 months (fig. 4). This length of larval development is similar to that which occurred in the pond during 1988 because most metamorphosing froglets found during 1988 appeared in November (see below). Growth was fast, about 0.45 mm. day^{-1}, and nearly linear in the first three months (March to May), fell to about 0.03 mm. day^{-1} in the colder days (June to August) and accelerated to about 0.15 mm. day^{-1} in September and October (fig. 4). The first tadpole that attained stage 26 was found on 6 October and the first metamorph (stage 42) was found on 17 November.

Figure 5 shows total length of embryos and tadpoles in relation to stage of development both from the nests and the enclosure. Between stages 16 and 24 growth was nearly constant (about 1 mm each stage). About 90% of growth occurred during stage 25; in this stage, tadpoles grew from 10 mm up to 81 mm. Metamorphosis (stages 26-42) began when tadpoles were above 72 mm and growth virtually stopped during these late stages.

After abandoning their nests, tadpoles aggregated in shallows until attaining a total length of about 20 mm; from this size to leaving the water (stages 43 and 44), tadpoles were found isolated, resting in shallows at night; by day they were found in greater depths (up to 1 m). The SVL of metamorphs (stages 43 and 44) was 30.8-37.2 mm (33.7 ± 1.6 mm, 30). Eighty percent of these froglets were found in November; the last froglet was found on 10 March.

Three factors were responsible for most premetamophic mortality inside the nests: (1)

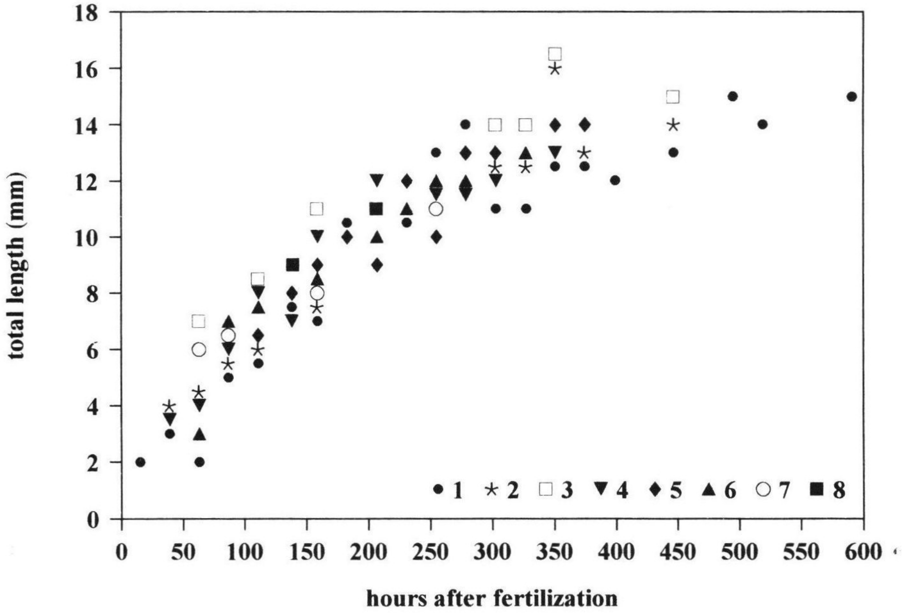

Figure 2. Growth of *Hyla faber* embryos and tadpoles inside the nests.

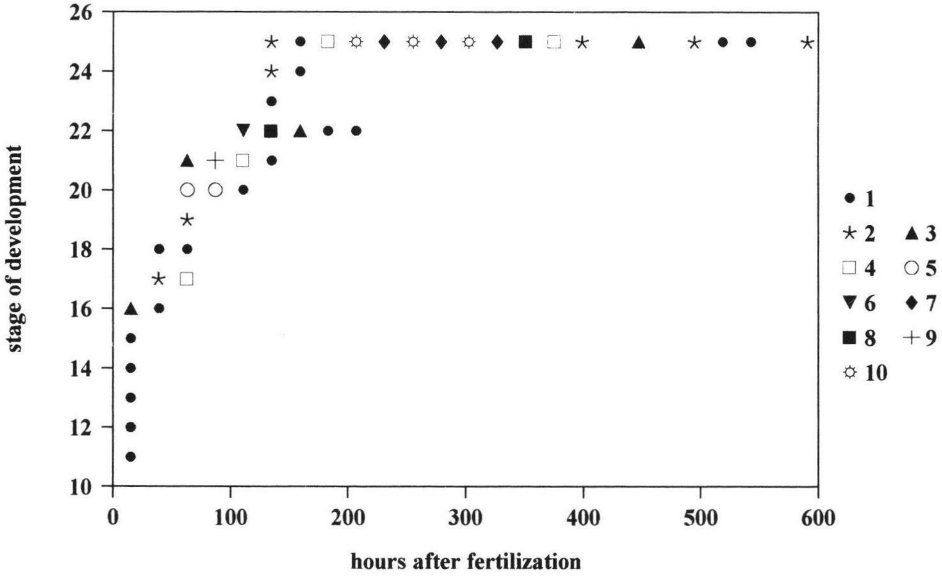

Figure 3. Developmental stages (Gosner, 1960) of *Hyla faber* embryos and tadpoles in relation to time after fertilization.

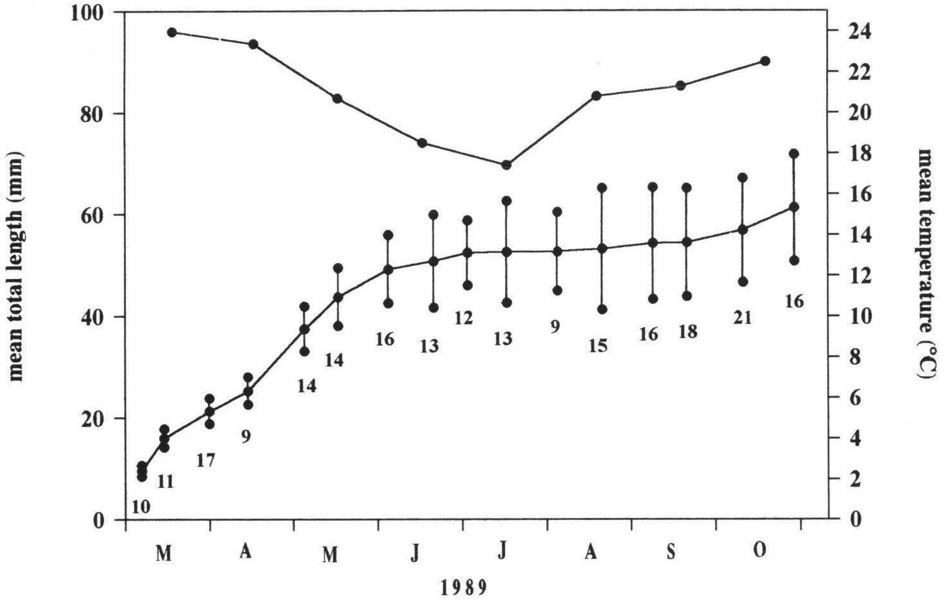

Figure 4. Growth of *Hyla faber* tadpoles inside the enclosure installed in the pond shallows and mean monthly temperatures. Vertical lines indicate standard deviation and the values below them, sample sizes.

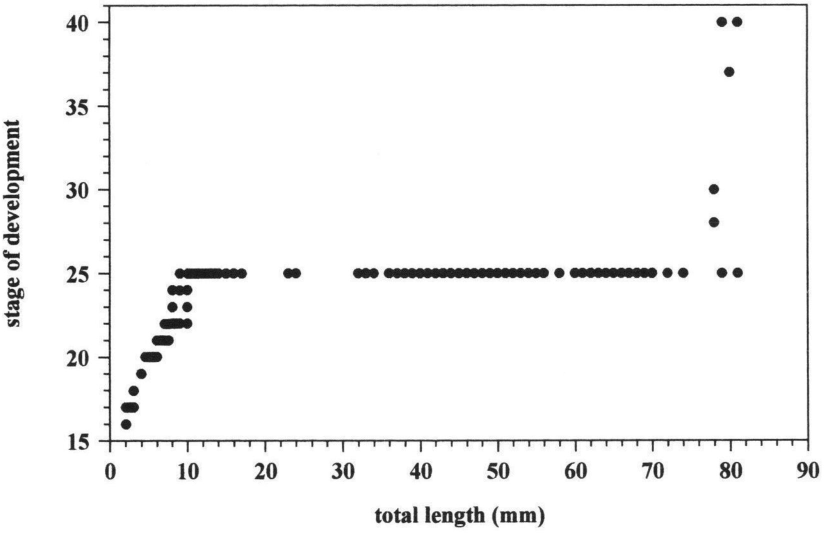

Figure 5. Total length of *Hyla faber* tadpoles in relation to developmental stages of Gosner (1960); each point indicates one to several observations. Note that most growth occurred during stage 25.

nest water evaporation and/or draining ($n = 2$ clutches), (2) egg sinking (e.g., by heavy rains and cattle walking on the nests) in the first hours after fertilization ($n = 3$), and (3) predation by aquatic insects (see Martins et al., 1993). In two nests water volume diminished considerably during long droughts; however, further rains reversed this situation. These partial droughts caused extremely high densities inside the nests and, in this situation, most tadpoles had their tail tips lacking, probably eaten by their siblings.

Discussion

The nests of *H. rosenbergi* (Kluge, 1981) and *H. boans* (Duellman, 1970, 1973; Caramaschi and Cardoso, 1978; pers. obs.) are very similar to those of *H. faber*; the nests of *H. biobeba* (Jim, 1980), *H. pardalis* (Bokermann, 1968; Lutz, 1960b), and *H. wavrini* (Martins and Moreira, 1991) are less elaborate. As observed in *H. rosenbergi* (Kluge, 1981) and *H. boans* (Duellman, 1970), only males of *H. faber* build nests (Lutz, 1960a, 1973; Martins and Haddad, 1988; this study); females only renovate them (while in amplexus). Nest building behaviour in *H. faber* (Lutz, 1960a; this study) is similar to that described for *H. rosenbergi* (Kluge, 1981) and may be the rule for the *boans* group.

Kluge (1981) also observed a high variability in shape and dimensions in the nests of *H. rosenbergi*. Mean water surface in *H. rosenbergi* (282.9 cm^2, Kluge, 1981) was nearly the same as that of *H. faber* (286.9 cm^2), probably due to the similar adult size and egg number in both species (see Kluge, 1981; Martins, 1993). However, mean water depth appears to be greater in *H. faber* (8.2 cm) than in *H. rosenbergi* (4.5 cm, Kluge, 1981).

Hyla faber females inspect nests before mating with their owners (Martins and Haddad, 1988; Martins, 1993). Nest volume might be important during nest evaluation by females since the risk of death by overpopulation and water evaporation and/or draining are lower in nests with higher water volume. However, the intensity of nest use (number of clutches laid in each nest), that would reflect female choice, was not significantly correlated to water volume.

The number of clutches per nest appeared to be higher in *H. faber* (1.70) than in *H. rosenbergi* (0.97, Kluge, 1981). This difference may be related to the space available for nest construction in the study areas considered: Kluge (1981) studied *H. rosenbergi* in 180 m of creek margins versus 9 m of pond margins in this study. However, re-occupying pre-existing nests results in time and energy saving and may be selected for in nest building frogs.

Wassersug (1974, 1975) and Wilbur (1980) showed that larval development tends to be fast in frogs that breed in temporary habitats and slow in those that breed in permanent habitats. Interestingly, these tendencies occur even in closely related species: *H. faber* breeds in permanent ponds and has a long larval development (over 8 months) while *H. rosenbergi* breeds in temporary streams and has a short larval development (40 days, Kluge, 1981). However, other factors can also lead to the appearance of short developmental periods in frogs (see, e.g., Wilbur, 1984); the Amazonian *H. wavrini*, another nest building gladiator frog, breeds at the margins of lakes and rivers in areas of flooded forests where water level is highly unpredictable and predators (mainly fishes) are very abundant; this species seems to have a very short developmental period (about 20 days, pers. obs.).

Embryos of *H. boans, H. faber,* and *H. rosenbergi* have large filamentous gills in stages 20-24. Noble (1927) suggested that these large gills may be a consequence of the low levels of dissolved oxygen in the nests. In support of Noble's (1927) hypothesis, Kluge (1981) observed that mean dissolved oxygen of several *H. rosenbergi* nests was significantly lower than that of the adjacent stream. Tadpoles of *H. faber* (Martins and Haddad, 1988; this study) and *H. rosenbergi* (Kluge, 1981) in stages 20-24 congregate adjacent to the water surface in a manner that their gills contact the oxygen rich surface tension layer of the water column (see fig. 8 in Martins and Haddad, 1988). This habit, associated with large gills, may improve oxygen absorption in an oxygen-poor environment.

Embryonic stages are less able to avoid predation by swimming and small tadpoles are more vulnerable to predation than large ones (e.g., Brodie and Formanowicz, 1987). As observed by Kluge (1981) in *H. rosenbergi,* the larvae of *H. faber* leave their nests only after 8 days, always in stage 25. In *H. faber,* the habit of young larvae (stage 20-25) is to remain in the nest bottom during floods; additionally, the gregariousness of tadpoles below 20 mm, may increase the possibility that the vulnerable early larval stages occur inside the nests.

The habit of building nests in the *boans* group has been interpreted as a way to avoid the contact between early developmental stages (more vulnerable to predation, see above) and aquatic predators in the adjacent waterbody (e.g., fishes, aquatic insects, tadpoles). However, this habit may expose early stages to alternative risks and constraints: (1) predation by terrestrial, semi-aquatic and aquatic predators, (2) death due to evaporation and/or draining of nest water, (3) low levels of dissolved oxygen in the nests, and (4) tail mutilation by siblings, the three latter during droughts. A high predation risk for larvae in nests was observed by Martins (1988) for *Leptodactylus fuscus,* a leptodactylid that builds subterranean nests for egg deposition. Besides these risks and constraints, the habit of building nests in gladiator frogs involves costs (energy and time) to build or renovate them. As suggested by Kluge (1981), the selective pressures imposed by aquatic predators may have been sufficiently strong to lead to the appearance of this habit in these species.

Nest building is known in all species of the *boans* group as behaviourally defined by Martins and Haddad (1988). Kluge (1981) named gladiator frogs all those species that have well developed prepollices, encompassing those species considered in the *boans* group and others considered by other authors as belonging to the *Hyla circumdata* group (see Frost, 1985). However, there is no clear definition of any of these groups in the literature (see Cardoso, 1983). The *circumdata* group as currently considered encompasses many small to medium-sized treefrogs very similar in morphology to the *boans* group; although their biology is very poorly known, none of its members is known to build nests. However, *Hyla ibitipoca, H. crepitans,* and at least two undescribed species (of the *circumdata* group) from southeastern Brazil may use natural depressions and cavities in the soil to lay their eggs (Caramaschi and Feio, 1990; Haddad, 1992; pers. obs.). It is possible that the nest building behaviour of frogs in the *boans* group could have arisen as an elaboration of similar behaviour in the *circumdata* group if a strong predation pressure developed in the larval habitat. The nests built by the frogs in the *boans* group are variable intra- (Kluge, 1981; this study) and interspecifically: from simply pushing away debris from a natural water filled depression (e.g., *H. wavrini*) to building elaborate nests (e.g., *H. boans, H. faber* and *H. rosenbergi*). Further observations on the reproductive biology in the *boans-circumdata* complex may show distinct stages in the evolution of nest building within this assemblage.

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