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The reproductive ecology of *Leptodactylus fuscus* (Anura, Leptodactylidae): new data from natural temporary ponds in the Brazilian Cerrado and a review throughout its distribution

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Recent studies indicate that populations historically called *Leptodactylus fuscus* (Schneider, 1799) comprise at least three well-differentiated lineages. Herein, we describe the reproductive ecology of a southeastern clade population of *L. fuscus*, and review the characters of the reproductive ecology for several populations of the northern and southeastern clades. Most reproductive activity occurred in December and January, which coincided with the highest rainfall period. Males had an aggregated spatial distribution in the central area of the ponds. Three courtship interactions were observed. A female was observed closing the entrance to a burrow with moist sand after oviposition. This behaviour is described for the first time in *L. fuscus*. The review of the reproductive biology of the northern and southeastern clades indicates a relatively high plasticity in *L. fuscus*. Furthermore, the results corroborate the suggestion, based on molecular data, that the northern and southeastern clades of *L. fuscus* represent distinct evolutionary units.

Keywords: reproductive ecology; *Leptodactylus fuscus*; Cerrado; Brazil

Introduction

The genus *Leptodactylus* currently consists of five species groups: *fuscus*, *marmoratus*, *melanonotus*, *pentadactylus* and *ocellatus*, based on morphological and behavioural comparisons (Heyer 1969; Frost et al. 2006). Recent molecular studies give support to this arrangement (e.g. de Sá et al. 2005; Hedges and Heinicke 2007). All species of the *L. fuscus* group use underground burrows, where they lay their eggs within foam nests (Heyer 1978). Following floods or strong rains, tadpoles are generally washed to a nearby water body, where they complete their development.

Leptodactylus fuscus (Schneider, 1799) is widely distributed occurring from Panama to Argentina, in predominantly open habitats (Heyer 1978; Wynn and Heyer 2001; Camargo et al. 2006). Recent studies on the genetics of populations historically considered as *L. fuscus* indicate that at least three well-differentiated lineages can be distinguished (Wynn and Heyer 2001; Camargo et al. 2006): one that includes Central American, Guianan and Amazonian populations (hereafter called the northern clade), a second including populations from Bolivia and Argentina, and a third comprising those populations distributed throughout southeastern South America (hereafter called the southeastern clade; following Camargo et al. 2006).

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Morphology (Heyer 1978) and call characteristics (Heyer 1978; Heyer and Reid 2003) are highly variable and the range of variation overlaps among these clades (Camargo et al. 2006).

Aspects of the reproductive biology of populations of the southeastern clade have been studied in many parts of its range (e.g. Sazima 1975; Rossa-Feres et al. 1999; Freitas et al. 2001; Ávila and Ferreira 2004; Brasileiro et al. 2005; Oliveira-Filho et al. 2005). The existing literature provides information about reproductive and/or calling season (Sazima 1975; Rossa-Feres et al. 1999; Freitas et al. 2001; Ávila and Ferreira 2004), courtship and territorial behaviour (Freitas et al. 2001), nest building (Sazima 1975), and embryo and larval development (Sazima 1975). In summary, regarding populations of the southeastern clade, males of *L. fuscus* build ovoid subterranean chambers in the dry margins of ponds, where mating takes place. Males call near these burrows and defend the area around them with territorial calls and fights. Calling activity occurs throughout most of the rainy season. Attracted females are guided by males to the chamber where oviposition takes place.

Several aspects of the reproductive biology of the northern clade of *L. fuscus* (cf. Camargo et al. 2006) have also been reported (e.g. Lescure 1972; Solano 1987; Martins 1988; Walsh and Downie 2005). Overall, the biology of the northern clade is very similar to that of the southeastern clade. Martins (1988) compared the reproductive biology of *L. fuscus* in extreme northern Brazil (northern clade) with those from a few other areas throughout the wide distribution of the species (northern and southeastern clades) and suggested that this species shows a relatively high behavioural plasticity.

With the exception of a few studies conducted in natural habitats (e.g. Solano 1987; Martins 1988; Brasileiro et al. 2005), most available studies on the reproductive biology of both northern and southeastern clades of *L. fuscus* were carried out in disturbed habitats. These studies show that *L. fuscus* is able to survive in several kinds of disturbed habitats and has been described as a "weedy" (Wynn and Heyer 2001).

Herein, we describe the reproductive ecology of a southeastern clade population of *L. fuscus* in natural, temporary ponds of the Cerrado of southeastern Brazil. We report data on calling activity, spatial distribution of breeding individuals, adult size, reproductive behaviour, burrow characteristics, foam nests and tadpoles. Furthermore, to find out how plastic *L. fuscus* is throughout its distribution and to compare the reproductive biology of the northern and southeastern clades, we review these characters in the available literature for several populations from Trinidad to Argentina.

Material and methods

Our focal ponds were located at the Estação Ecológica de Itirapina (EEI), in the municipalities of Itirapina and Brotas (22°00′ and 22°15′S 47°45′ and 48°00′W), State of São Paulo, Brazil. The total area of the EEI is 2430 ha, with natural Cerrado vegetation (see Ratter et al. 1997), including grasslands, grasslands with shrubs, grasslands with shrubs and trees, swamps, flooded areas and gallery forests. The climate is mesothermic with a marked dry season. The mean annual precipitation from 2000 to 2002 was 1351 mm, with a dry season between April and August (17–53 mm/month) and a rainy season between September and March (78–311 mm/month). We visited our sites every 15 days from August 2002 to April 2003. All observations were carried out in three natural temporary ponds (hereafter called Nova, Tuiuiu and Ponte). The three ponds have similar plant communities, which are typical of floodable areas (Fantinati 2004). Ponds are located 300–1670 m from each other. The biodiversity of anurans at EEI has been previously described (Brasileiro et al. 2005).

To study the spatial distribution of calling males and burrows, the three ponds were marked with a 10 × 10-m grid with PVC pipes identified by a combination of letters and numbers. Tuiuiu had the largest mapped area, followed by Ponte and Nova (Table 1). The presence of water in the ponds depends on precipitation during the rainy season. During this study, water accumulation occurred differently in the three ponds (Table 1). The first puddles were observed in early January in the central area of Ponte. In mid-January, Ponte filled out, remaining full throughout the study period. Three filling events occurred in Nova, the first at the end of January and the last at the beginning of March. Tuiuiu did not accumulate water.

Behavioural observations were initiated at dusk and stopped when the calling activity of *L. fuscus* decreased or ceased. We recorded air temperature, number of calling and non-calling males, and number of females each night of observation. For each individual observed, we recorded the degree of exposure of the body (body exposed, partially exposed or hidden), and distance to and height of the nearest clump of vegetation (in cm). The latter was divided into three categories: low (<5 cm), medium (>5 and <20 cm), and high (>20 cm).

The locations of individuals and of burrows were mapped. Approximately 70% of the individuals found were tagged with a soft plastic waistband (Martins 1993). A map of each pond was used to record the locations of the individuals for each sample, using a mapping program (Santos 1994). To test whether smaller males differed from larger males in their spatial dynamics, we separated small males (<41 mm) from large males (>42 mm) following Rossa-Feres et al. (1999).

For each tagged individual, we recorded sex, mass (measured in grams with a Pesola® scale), and snout–vent length (SVL; in mm, measured with a metal calliper to 0.01 mm). We characterized the burrows recording their length (cm), depth (cm), diameter of tunnel entrance and of chamber (at its widest and highest points; cm), presence of foam nests, presence of tadpoles, and presence of predators inside or near the burrows.

Adult behaviour was studied with the method of continuous observations of all occurrences (Lehner 1979). Foam nests and tadpoles were collected from inside burrows and additional tadpoles were collected in the ponds with a net, in a non-systematic fashion. Tadpoles were preserved in 5% formalin, and larval stages were

Table 1. Characteristics of the three temporary ponds studied at the Estação Ecológica de Itirapina, São Paulo, including the total accumulated rainfall (mm), and greatest depth of the water column (cm) from November 2002 to March 2003.

Pond	Elevation (m)	Mapped area (m^2)	Total accumulated rainfall (mm)	Greatest depth of the water column (cm)
Ponte	716	10.100	299.3	30.6
Nova	728	7.600	272.9	14.1
Tuiuiu	729	12.900	270.5	–

identified according to Gosner (1960). Females collected previously in the EEI were dissected, and mature eggs were counted and measured. The diameters of 10 eggs from each female were measured under a stereoscopic microscope. These females were used in analyses to test sexual dimorphism in body size (SVL).

The spatial distribution patterns (random, aggregated or uniform) of individuals and burrows around the ponds were analysed using the Dispersion Index ($DI=\bar{x}/S2$; where S2 is the variance and $\bar{x}$ is the mean of the observed distances; Ludwig and Reynolds 1988) and a χ^2 test. Correlations between variables were tested with the Spearman correlation coefficient (r). For the remaining analyses, Student's t-test was used for parametric tests, and Kruskal–Wallis's H analysis of variance, χ^2 test and Mann–Whitney U-test, for non-parametric tests. All analyses were implemented in the program STATISTICA (StatSoft 2003), with a significance level of $P<0.05$; recaptured individuals were not considered in the analyses.

Data on temperature and rainfall were obtained from a weather station at Fazenda Siriema, Ripasa S.A. Celulose & Papel, located approximately 10 km from the study site, as well as at each pond on the days of fieldwork.

Results

Leptodactylus fuscus was the first anuran to occupy the studied ponds following the beginning of the rainy season in November. The months with greatest reproductive activity were December and January, coinciding with the highest recorded rainfall (Figure 1). There was no significant difference in abundance of *L. fuscus* among ponds ($H=1.03$; df=2; $N=28$; $P=0.59$; Figure 1). Calling activity began at about 18:30 h and diminished after 23:00 h to 24:00 h (Figure 2). The number of calling males throughout the study period did not differ significantly ($\chi^2=5.90$; df=7; $P=0.55$) among the three ponds studied. Air temperature during the period of study varied from 18 to 25°C. We found no significant correlation between the number of calling males and air temperature at each hour of observation ($r=0.048$; $P=0.56$). The number of males observed was much higher (232) than the number of females (16) throughout the entire study period, resulting in an operational sex ratio of 15 : 1 in Nova Pond, 12.5 : 1 in Tuiuiu Pond and 15 : 1 in Ponte Pond.

Adults were found mainly exposed in sites of predominantly low vegetation (Table 2).

In total, 124 individuals were tagged. The recapture rate was 23.2% in Ponte Pond ($N=13$ individuals), 19.0% in Tuiuiu Pond ($N=6$ individuals), and 27.0% in Nova Pond ($N=10$ individuals). Individuals were recaptured a maximum of three times ($N=3$ individuals) after tagging. The distance moved by tagged individuals from one location to the next was greater in Nova Pond (9.5 ± 5.1 m; $N=16$; range: 1.8–18.2 m) followed by Ponte Pond (5.8 ± 4.9 m; $N=17$; range: 1.2–16.3 m), and Tuiuiu Pond (4.3 ± 0.8 m; $N=7$; range: 3.1–5.3 m). The time between capture and recapture varied from 1 to 57 days (10.5 ± 11.7 days; $N=40$) and was positively correlated with the distance moved between two consecutive locations ($r=0.489$; $P<0.01$; $N=40$). Migration from one pond to another was not recorded.

In Ponte Pond, the distances between males (both calling and non-calling) were significantly greater in December and January than in the remaining months ($U=624.0$; $P<0.01$); calling males were found at a greater distance from each other than non-calling males (Figure 3). In Nova and Tuiuiu Ponds, distances between

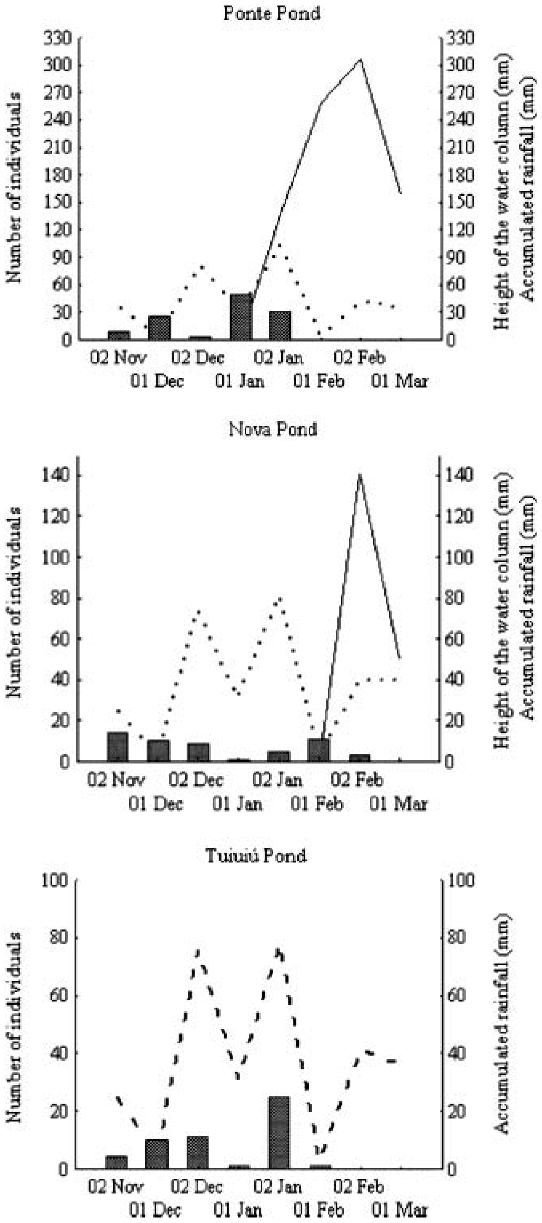

Figure 1. Maximum number of individuals of *Leptodactylus fuscus* (bars), accumulated rainfall (dotted lines; mm) and height of the water column (continuous line; mm) reported in each sampling period, in the three temporary ponds at the Estação Ecológica de Itirapina, São Paulo, from November 2002 to March 2003.

males (both calling and non-calling) did not differ significantly among months ($H_{\mathrm{Nova}}=0.68$; df=3; $N=56$; $P=0.87$; $H_{\mathrm{Tuiuiu}}=4.31$; df=2; $N=42$; $P=0.11$); the distance between small and large males was not compared for these ponds because of small sample sizes. In Ponte Pond, there was no difference in intra-specific distance between small (<41 mm; 2.5 ± 2.2 m; $N=6$) and large males (>42 mm; 4.3 ± 5.2 m;

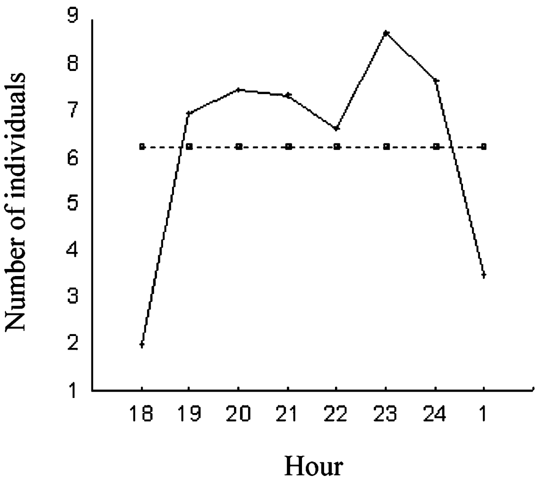

Figure 2. Corrected number *Leptodactylus fuscus* males observed in calling activity every hour in the three temporary ponds at the Estação Ecológica de Itirapina, São Paulo, from November 2002 to March 2003. Dotted line represents number expected; continuous line represents number observed.

Table 2. Number (and % occurrence between parentheses) of adult individuals of *Leptodactylus fuscus* in the ponds studied at the Estação Ecológica de Itirapina, from November 2002 to March 2003.

	Degree of exposure of the body			Height of the nearest clump of vegetation		
	Exposed	Partially exposed	Hidden	Low	Medium	High
Tuiuiu	89 (72)	27 (22)	8 (6)	57 (45)	46 (37)	22 (18)
Ponte	37 (73)	12 (23)	2 (4)	38 (60)	23 (37)	2 (3)
Nova	63 (85)	11 (15)	0	44 (57)	28 (36)	5 (7)

Note: Categories for the height of the nearest clump of vegetation are: low, up to 5 cm; medium, >5 cm and up to 20 cm; and high, >20 cm.

N=33; U=81.5, P=0.49). The distance between small and large males was not compared for the other ponds because of small sample sizes.

Adults showed an aggregated spatial distribution in the central area of each of the three ponds; except in December at the Nova Pond, in which the spatial distribution was uniform (Table 3). The burrows also had an aggregated spatial distribution in the central area of Ponte Pond (December: DI=3.51; χ_2=361.51; df=103; January: DI=6.76; χ_2=697.34; df=103).

Adult males (SVL 43.6 ± 2.4 mm, N=135; range: 38–50.5 mm) were significantly smaller than adult females (45.6 ± 2.2 mm; N=13; range: 42.9–48.6 mm; t=2.83; df=146; P<0.01). Males found at the beginning of the reproductive season were larger (November: 44.3+1.9 mm; N=35) than those found at the end of the season, although the difference was marginally non-significant (February: 42.5 ± 1.5 mm; N=7; H=7.68; df=3; N=133; P=0.06).

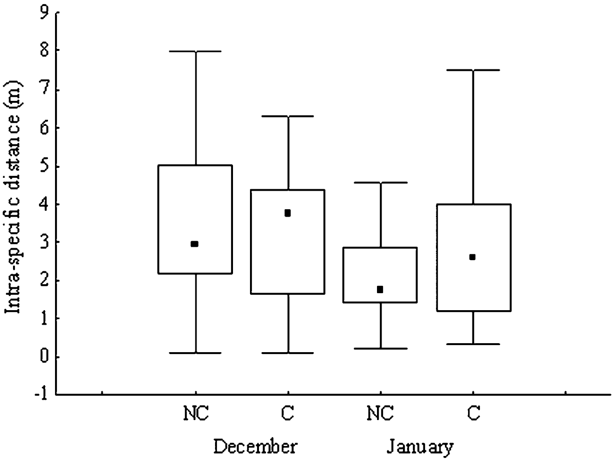

Figure 3. Intra-specific distance of *Leptodactylus fuscus* males in the Ponte Pond in the Estação Ecológica de Itirapina, São Paulo, from December 2002 and January 2003. In December: NC, non-calling males (N=28) and C, calling males (N=9); in January: NC, non-calling males (N=36) and C, calling males (N=19). Points indicate medians; boxes represent first and third quartiles (between 25% and 75% of the variation); bars indicate minimum and maximum values.

Table 3. Spatial distribution of *Leptodactylus fuscus* in three temporary ponds in the Estação Ecológica de Itirapina, São Paulo, from November 2002 to January 2003.

Pond	Month	Distribution	Mean	s^2	DI	χ^2	*Ns*	*N*
Nova	November	aggregated	0.22	0.34	1.54	75.14	49	11
Nova	December	uniform	0.79	2.91	3.68	17.16	49	39
Nova	January	aggregated	0.16	0.22	1.37	66.84	49	8
Tuiuiu	December	aggregated	0.28	0.61	2.18	236.65	110	31
Tuiuiu	January	aggregated	0.07	0.12	1.71	191.70	110	8
Ponte	November	aggregated	0.09	0.12	1.33	135.80	104	9
Ponte	December	aggregated	0.40	1.85	4.62	477.60	104	42
Ponte	January	aggregated	0.59	3.52	5.96	615.32	104	62

s^2=variance; DI=dispersion index; χ^2=chi-squared test value; *Ns*=number of squares sampled; N=number of individuals.

The burrow consisted of an oval chamber with an access tunnel (Table 4). The first burrows were observed at the beginning of December in Ponte Pond and the largest number of burrows was recorded in January at this pond (N=100). In Tuiuiu and Nova Ponds, only one and three burrows were observed, respectively. In mid-December, Ponte Pond was still dry and the majority of burrows observed (69.7%; N=30) were partially destroyed and/or unfinished. The unfinished burrows were found with the access tunnel in various stages of construction (just beginning to completed) and with the chamber open. Males were observed inside these unfinished burrows at night and, in some occasions, they emitted territorial calls (N=4 males). Burrows with foam nests were found exposed, suggesting predation or collapse of the chamber. No individuals were observed using the burrows during the day.

Table 4. Characteristics of the burrows of *Leptodactylus fuscus* at the Estação Ecológica de Itirapina.

	N	Mean (cm)	SD (cm)	Range (cm)
Total length	41	9.0	2.3	4.3–13.5
Greatest height of tunnel entrance	40	1.8	0.5	1.0–3.2
Greatest width of tunnel entrance	40	2.0	0.4	1.2–3.2
Height of chamber	72	5.4	1.4	2.4–10.6
Greatest length of chamber	77	4.0	1.1	1.6–7.4
Greatest width of chamber	78	4.8	1.1	1.7–9.0

N=number of burrows characterized; SD=standard deviation.

Territorial behaviour among males was observed on one occasion and lasted 25 minutes. A resident male was calling when a second male (intruder) approached and stopped about 10 cm from the resident male. As the intruder male drew nearer, the resident male emitted advertisement calls, with apparently shorter intervals between the calls. The intruder male also began to emit advertisement calls at short intervals, initiating a vocal duel. At that moment, intercalated with the advertisement calls, both males emitted another type of call, similar to the courtship call. During this vocal interaction, the resident male circled around the intruder male approaching him and, on one occasion, leapt over the intruder. At times, the resident male moved away, but continued to circle around the intruder. During one of its approaches, the resident remained face-to-face with the intruder at a close distance (about 1 cm). The resident male moved away about 1 m from the intruder. Both began to emit advertisement calls. The resident emitted territorial calls on two occasions. At the site where this territorial interaction took place no burrows were found within a radius of approximately 40 cm. The two males were collected and similar in size (resident male SVL=42.1 mm; intruder male SVL=42.7 mm).

Three courtship interactions were observed, two partial sequences and one complete. The courtship interactions were initiated when a female approached a calling male. With the approach of the female, the male emitted courtship calls and led the female to the burrow (two observations). On their way to the burrow, when the female fell behind, the male stopped and emitted advertisement calls until the female again drew closer (one observation). The trajectories from the calling sites to the burrows were observed on two occasions and covered distances of 20 cm and 3.5 m. Before entering the burrow, the male stopped and was touched by the female in the posterior region (one observation) or on the side (one observation) of the body. The male entered the burrow, immediately followed by the female (two observations). The male remained with its nose obstructing the entrance to the burrow (two observations).

During one of the observations, at the moment when the female was sighted near the male, a second male approached, emitting advertisement calls. Both males began to emit territorial calls intercalated with advertisement calls at a distance of about 10 cm from one another. The female followed the second male into the burrow (20 cm). The male chosen by the female was smaller (SVL=44.0 mm) than the rejected male (SVL=45.9 mm). Right after the couple entered the burrow, the male positioned itself at the entrance; after a few minutes, it entered the burrow and began emitting advertisement and courtship calls, intercalated and at varying intervals. Approximately 20 minutes after the pair had entered the burrow, the female (still bearing eggs) abandoned the burrow. The male remained inside the burrow for some time emitting advertisement calls, and then left silently. The burrow was examined, and no eggs were found.

On another occasion, following spawning, the male was the first to leave the burrow and immediately left the site. The female emerged, remaining next to the burrow for almost 10 minutes, and then began to close the entrance to the burrow with sand. Initially she pressed her nose against the substrate, pushing small grains of moist sand toward the burrow entrance. With movements of her legs, she loosened the sand, which she then pushed with her nose; while with her front limbs and with her chin, she compacted the loose sand as she stretched and contracted her body. This activity lasted for about 50 min. The female remained motionless for some time alongside the burrow. A foam nest was found in this burrow. Other burrows ($N=6$) were found closed off in a similar fashion.

The first foam nests were observed at the beginning of December in Ponte Pond ($N=17$) and, of these, five (29.4%) were found exposed but intact, inside partially destroyed burrows, and four (23.5%) were being preyed upon by ants of the genus *Camponotus*. In January, 14 foam nests were observed in this pond, 11 (78.5%) were found exposed and intact. Tadpoles were observed inside one of the burrows in mid-December ($N=164$ tadpoles; stage 25) at Ponte Pond. Tadpoles in stages 25, 26 and 27 were observed in the early puddles of water in the central area of Ponte Pond in mid-January. However, the puddles dried up and many dead tadpoles were observed being carried off by ants. In Tuiuiu Pond, only one foam-nest was observed inside a burrow in early January; after 25 days, the foam nest dried up (the burrow continued intact). In Nova Pond, neither foam nests nor tadpoles were observed. At the end of January, when Ponte Pond had filled up, stage 25 tadpoles were collected ($N=24$), and in early February, tadpoles in stages 41 and 42 were collected ($N=7$). Beginning in mid-February, recently metamorphosed (state 46) juveniles were observed in Ponte ($N=6$) and in Nova ponds ($N=3$).

The females collected at the study site ($N=8$) had an average SVL of 46.2 ± 1.9 mm (range: 43.8–48.6 mm) and contained 242 ± 105 mature eggs (range: 128–385 eggs), with diameters of 1.15 ± 0.18 mm (range: 1.0–2.0 mm; $N=60$).

Discussion

Various studies have shown that climatic factors influence the reproductive activity of many species of anurans in tropical and temperate regions (e.g. Aichinger 1987; Oseen and Wassersug 2002). The initiation of reproductive activity of *L. fuscus* with the first rains of the rainy season was reported previously for populations of the southeastern clade (Sazima 1975; Rossa-Feres et al. 1999) and for populations of the northern clade (Lescure 1972; Solano 1987; Martins 1988). In populations of both clades, the greatest reproductive activity normally occurs during periods of higher precipitation (Sazima 1975; Rossa-Feres et al. 1999; present study), whereas the decrease in activity may be related to flooding of the breeding site (Sazima 1975; Martins 1988; present study). The number of males observed was greater than the number of females at the breeding sites, as in many other species with prolonged breeding seasons (Bastos and Haddad 1999; Goldberg and Schwalbe 2004).

Calling males were observed exposed in predominantly open sites (low vegetation), in the central area of the three ponds studied. Grouped distribution patterns normally suggest a choice of more favourable parts of the environment (Ludwig and Reynolds 1988). The central areas of the three ponds are more open (lower density of tree stumps and grasses), more humid and slightly lower than the edges (personal observations). Perhaps the more open areas result in sites that are more appropriate for the establishment of sites for building burrows and calling. A wet soil may facilitate the construction of the burrows (Solano 1987). Additionally, the fact that the central areas of the ponds are lower results in a more rapid filling as the water column rises. Among hylids, the establishment of calling sites in more visible sites may facilitate the defence of the territory or encounters with females (Abrunhosa and Wogel 2004). The males that used Ponte Pond were more closely grouped at the peak of the reproductive season (January), indicating that the abundance of individuals influenced the spacing between them at the breeding site.

The recorded adult movements (from 1.2 to 18.2 m) for *L. fuscus* support the theory that the location of calling males can vary on consecutive nights as previously reported (Rossa-Feres et al. 1999; Freitas et al. 2001). Perhaps males search for more appropriate sites for building burrows or better calling sites.

Although territoriality in *L. fuscus* has been described for both the northern and the southeastern clade (Martins 1988; Rossa-Feres et al. 1999; Freitas et al. 2001; present study), the possible resources defended by males remain unknown. The fact that the location of males can vary on different nights and that the same has been observed with respect to the distance that the males call from their burrows (Rossa-Feres et al. 1999; Freitas et al. 2001; present study), suggests that *L. fuscus* may not defend a constructed burrow, but rather an appropriate area for building a burrow and/or establishing a calling site. The defence of a calling site may be important to avoid interruption of courtship and/or amplexus (Wells 1977).

The SVL of males was significantly smaller than that of females in this study. Sazima (1975) and Heyer (1978) observed that females were slightly larger than males and Martins (1988) later observed that females were significantly larger than males in a population in Roraima, Brazil. On the other hand, Solano (1987) found no sexual dimorphism in size in a population in Venezuela. Further studies are necessary to understand how sexual dimorphism in size varies among populations of *L. fuscus* throughout its distribution.

Although data are still scarce, fecundity seems not to vary significantly between the southern and the northern clades: the number of mature eggs (241.8 ± 105.4 eggs) found in Itirapina was similar to that found by Martins (1988; 232.3 ± 31.5) and by Sazima (1975; 298 and 304 eggs).

The larger size of the males at the beginning of the reproductive season compared to those at the end of the season has also been previously reported as the result of competition for calling sites (Rossa-Feres et al. 1999). However, the males found at the ponds early in the rainy season may be older than those found later in the season, as would result from adult male recruitment during the rainy season.

We found a number of collapsed burrows before the flooding. Martins (1988) also observed burrows that had collapsed or been preyed upon before the flooding. The collapse of the burrows may be related to the characteristics of the soil or the fragility of the burrow (Sazima 1975). Martins (1988), studying *L. fuscus* in Roraima, suggested that burrows had probably been opened by a wading bird (see below);

however we found no direct evidence for vertebrate destruction of burrows. The different stages of construction of the access tunnels to the burrows on different nights suggest that the construction of burrow by male *L. fuscus* may occur over more than one night. The ability to learn the location of the oviposition site was suggested by Lüddecke (2003) for *Colostethus palmatus*. Rossa-Feres et al. (1999) reported that *L. fuscus* use the burrows as daytime shelters; however, we did not observe adults in burrows during the day as indicated by Freitas et al. (2001). These differences may be the result of environmental characteristics of the study sites (e.g. humidity, shade, temperature, etc.).

We observed a male leaping over another in the dispute for territory. Freitas et al. (2001) reported resident males pursuing intruder males. The "face to face" encounters by male *L. fuscus* defending their territory may be limited to expel the intruder male without engaging in physical fighting and its consequences (e.g. Martins et al. 1998).

The exchange of tactile signals during courtship observed in *L. fuscus* appears to be common in anuran species whose males guide the females to a previously constructed burrow (Haddad and Sawaya 2000). As in this study, Freitas et al. (2001) also reported the behaviour of abandonment of the burrow by the female in *L. fuscus*. The behaviours of evaluating and abandoning the burrow before oviposition have been reported for other species (e.g., Martins 1993 for *Hypsiboas faber*; Haddad and Giaretta 1999 for *Hylodes asper*; Haddad and Sawaya 2000 for *Aplastodiscus leucopygius*). Like the physical characteristics of the calls (Gerhardt 1982; Ryan 1985), the characteristics of the burrow may represent a criterion for mate selection by females, because the conditions of the oviposition site can compromise the survival of the eggs and development of premetamorphic larval stages (Lüddecke 2003). In the present study, a female that abandoned the burrow had previously chosen between two calling males. Despite being a single observation, the behaviour suggests that the choice of mate by the female *L. fuscus* may be made in three non-exclusive ways: (1) calls emitted by the males; (2) characteristics of the burrow; and (3) via tactile signals during courtship. However, additional data are needed to determine how females evaluate each of these parameters.

Herein we described the female behaviour of closing the entrance to the burrow with sand after oviposition. A similar behaviour has been described in *Leptodactylus bufonius* (Crump 1995). Reading and Jofré (2003) reported that the entrances of some burrows of *L. bufonius* in Argentina had been closed whereas others had not, and that closed burrows were farther from water than open burrows. In our study, the spatial distribution of closed and non-closed burrows was not compared. Perhaps this behaviour, which apparently serves to protect the foam nest from extreme temperatures (Reading and Jofré 2003) or predation, is optional in female *L. fuscus*, as a result of intrapopulation variability or climatic conditions. The description of burrow closing for two species of the group *L. fuscus* (*L. fuscus*, present study, and *L. bufonius*, Reading and Jofré 2003) suggests that this behaviour may be widespread in this group.

We found foam nests concurrently with the first burrows, and the appearance of both preceded the accumulation of water in the ponds by several days. Apparently, much of the reproductive investment was lost during our study year because of climatic instability and/or predation. Martins (1988) reported burrows with the remains of foam nests that had been preyed upon by invertebrates and, possibly, birds. Predation of

Table 5. A summary of information on the biology of populations of the northern (N) and southeastern (S) clades of *Leptodactylus fuscus*.

Clade	Waterbody	Calling season	Male SVL (mm)	Female SVL (mm)	Number of eggs in female	Egg diameter (mm)	Duration of premetamorphic development (weeks)	Burrow length	Burrow height	Frequency range of advertisement call (MHz)	Burrow building behaviour observed	Territorial behaviour observed	Territorial call observed	Courtship behaviour described	Study
N	A?	–	52.1	–	–	–	–	–	–	0.6–2.4	No	No	No	No	1
N	–	E+M	40	50	–	–	–	–	–	–	No	No	No	No	2
N	?	?	37.8–43.0	41.9–43.4	–	–	–	10	5	0.3–3.0	No	No	No	No	3
N	NT	E	36.2	38.6–41.9	182–284	2.2–2.6	3	10–12	4–6	0.7–3.1	Yes	No	Yes	Yes	4
N	A	T	43.0	42.0	245–369	2	–	8–10	–	–	No	No	No	No	5
N	NT	E	36–40	37–43	–	–	3	–	–	–	No	No	No	No	6
S	?	–			–	–	–	5–30	4.5	–	No	No	No	No	7
S	A	E+M	–	–	–	–	–	–	–	–	No	No	No	No	8
S	NT	E+M			–	–	–	–	–	–	No	No	No	No	9
S	A	–	–	–	–	–	–	–	–	0.8–2.3	No	Yes	Yes	Yes	10
S	A	E+M	43.7	–	–	–	–	–	–	–	No	Yes	Yes	No	11
S	A	E+M	44.0	47.0	146–225	3.0–3,2	2	7–7.5	4.5–5.5	0.8–2.6	No	No	No	No	12
S	–	–	–	–	–	–	–	–	–	0.8–2.8	No	No	No	No	13
S	A	T	–	–	–	–	–	–	–	–	No	No	No	No	14
S	A/NT	E+M	–	–	–	–	–	–	–	–	No	No	No	No	15
S	A	T	–	–	–	–	–	–	–	–	No	No	No	No	16
S	NT	E+M	43.6	45.6	128–385	1.0–2.0	–	4.3–13.5	2.4–10.6	–	No	Yes	Yes	Yes	17

A=artificial; E=early rainy season; M=middle of the rainy season; NT=natural; T=throughout the rainy season. References: 1, Bernal et al. (2004), Colombia; 2, Kenny (1969), Trinidad; 3, Lescure (1972), French Guiana; 4, Martins (1988), Roraima, northern Brazil; 5, Martins (1998), Roraima, northern Brazil; 6, Solano (1987), Venezuela; 7, Arzabe and Prado (2006), Mato Grosso do Sul, southwestern Brazil; 8, Ávila and Ferreira (2004), Mato Grosso do Sul, southwestern Brazil; 9, Brasileiro et al. (2005), São Paulo, southeastern Brazil, Brazil; 10, Freitas et al. (2001), São Paulo, southeastern Brazil; 11, Rossa-Feres et al. (1999), São Paulo, southeastern Brazil; 12, Sazima (1975), São Paulo, southeastern Brazil; 13, Straughan and Heyer (1976), Argentina; 14, Toledo et al. (2003), São Paulo, southeastern Brazil; 15, Vaira (2002), Argentina; 16, Vasconcelos and Rossa-Feres (2005), São Paulo, southeastern Brazil; 17, present study, São Paulo, southeastern Brazil.

nests of *L. fuscus* by invertebrates has been reported also by Langone (1994) and Downie et al. (1995). In this study, ants were observed preying on the foam nests in Ponte Pond, although they did not appear to be responsible for the destruction of the burrows. We believe that, in addition to the possible destruction of the burrows by terrestrial predators, many of the exposed foam nests were found in this condition due to the collapse of the burrow, perhaps during rainstorms.

Tadpoles of *L. fuscus* can take refuge beneath rocks or fallen leaves for several days after temporary ponds dry up (Downie 1984; Downie and Smith 2003). In our study, no tadpoles were observed taking refuge, but the presence of tadpoles and recently metamorphosed juveniles in the pond later in the season indicate that many foam nests and/or tadpoles survived the apparently unstable climatic conditions. We found tadpoles at stage 27 inside the burrows and Downie (1984) reported *L. fuscus* tadpoles at stage 28 inside burrows. During periods of climatic instability, the survival of *L. fuscus* tadpoles inside the burrows appeared to be related to their capacity to generate foam (see Downie 1984; Caldwell and Lopez 1989) and temporarily decreased or interrupted growth (Downie 1994). The permanence of the tadpoles in the first stages of development inside the burrows may represent an advantage for *L. fuscus* that reproduces in temporary environments, when compared to species that begin their development after water has accumulated in the ponds (Downie and Weir 1997).

Variations in characters for which enough data are available to explore the degree of reproductive plasticity in *L. fuscus* (timing and duration of the calling season, male and female body size, burrow morphology, frequency range of the advertisement call) indicate a relatively high plasticity when both clades are considered together as well as when each is considered separately, especially in the northern clade (Table 5). A high plasticity in the behaviour of *L. fuscus* was reported by Martins (1988).

When the reproductive biology of the northern and southeastern clades are compared (Table 5), it seems that northern populations tend to breed earlier in the rainy season and for a longer period than southeastern populations. Based on the studies reviewed in Table 5, the variation in body size is higher in the northern clade, both for males (36–52 mm) and females (37–50 mm). Although few studies reporting on male body size are available for the southeastern populations, males in the northern populations (mean SVL approximately 42 mm) seem to be slightly smaller than those from southeastern populations (mean SVL approximately 44 mm). A similar pattern seems to occur in females (Table 5). Sample size precludes comparisons of number of eggs, egg diameter, and duration of premetamorphic development between northern and southeastern populations. Although data are limited, burrows seem to be longer in the northern clade compared to the southeastern clade, whereas burrow height seems not to be different between clades. The frequency range of the advertisement call in the northern clade (1.8–2.7 kHz) seems to be a little larger than that in the southeastern clade (1.5–2.0 kHz). Although not always recorded, territoriality was observed in both northern and southeastern populations (Table 5). Perhaps the apparent differences described herein are the result of the northern and southeastern clades of *L. fuscus* representing distinct evolutionary units (perhaps species; Camargo et al. 2006).

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