Reproductive biology of *Leptodactylus fuscus* (Anura, Leptodactylidae) in the subtropical climate, Rio Grande do Sul, Brazil

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**ABSTRACT.** The aim of this study was to characterize, for the central region of the State of Rio Grande do Sul, Brazil, the reproductive biology of *Leptodactylus fuscus* (Schneider, 1799), based on the analysis of gonadal development of males and females, reproductive effort, size-fecundity relationships, and occurrence of sexual dimorphism in body size. Mature individuals were found from October 1996 to February 1997 and from October 1997 to December 1997. The highest input of juveniles in the population was recorded in March 1997. There was a positive and significant correlation between the number of mature individuals and the mean monthly temperature. The population did not present sexual dimorphism in size. Males presented significant correlation only between snout-vent length and testes length. All females had oocytes at four different maturation stages and there were no significant correlations regarding size-fecundity variables. The correlation between ovarian size factor and females snout-vent length was not significant either. The main difference between this population and those that inhabit tropical climate was that temperature was responsible for stimulating the reproduction activity, instead of rainfall.

**KEYWORDS.** Amphibians, reproduction, subtropical climate.


**PALAVRAS-CHAVE.** Anfíbios, reprodução, clima subtropical.

Amphibian reproductive cycles are under hormonal control, which responds to environmental variation. Anurans from tropical regions tend to present continuous reproductive cycles; however, they reproduce in the season during which rainfall is sufficient to provide oviposition sites. On the other hand, in temperate and subtropical climates, temperature seems to be the main factor that stimulates the onset of the reproductive period (Duellman & Trueb, 1994).

*Leptodactylus* Fitzinger, 1826 is divided into five large species groups, including the *L. fuscus* group (Heyer, 1969). In this group reproduction generally occurs during rainy periods with high temperature. Males dig subterranean nests close to water bodies and attract females to enter, where oviposition occurs. Eggs are deposited in foam produced by the pair and tadpoles develop up to a certain stage inside the nest, which is destroyed by flooding or inundated when rains start, thus releasing the larvae to complete their development in the water (Heyer, 1969).

*Leptodactylus fuscus* (Schneider, 1799) is widely distributed from Panama to Argentina (Heyer, 1978). Reproductive biology and behavior of this species were investigated in Trinidad (Kenny, 1969) and French Guyana (Lescure, 1972) with the description of burying nest. Seasonality and embryonic development were studied in Venezuela (Solano, 1987). In his revision of the *fuscus* group, Heyer (1978) considered *L. fuscus* a unique species according to morphologic issues. After that, strong genetic differentiation was observed within *L. fuscus* populations along its distribution (Heyer & Reid, 2003; Camargo et al., 2006).

In Brazil, many studies were carried out on *L. fuscus*. In Roraima, reproductive biology and mating behavior were described (Martins, 1988). In the southeast of Brazil, Brasilheiro et al. (2005) and Oliveira-Filho et al. (2005) also studied seasonality reproductive patterns. In Pantanal of Mato Grosso do Sul, the reproduction occurs during rainy season (Avila & Ferreira, 2004) and the reproductive biology was studied by Prado & Haddad (2003, 2005).

Recently, a revision on *L. fuscus* reproductive ecology indicated that the species has a high reproductive plasticity (Lucas et al., 2008). For the subtropical region, males of *L. fuscus* vocalize mainly in the warmest months of the year (Bott et al., 2008; Santos et al., 2008). Even though the reproductive biology of *L. fuscus* was well studied in tropical areas, there are no studies in subtropical climate.

Thus, the aim of this study is to characterize the reproductive biology of *Leptodactylus fuscus* based on the analysis of gonadal development of males and females, reproductive effort, size-fecundity correlation and existence of sexual dimorphism in body size, at a locality in the central region of Rio Grande do Sul State, Brazil.
MATERIAL AND METHODS

The study area is located in the municipality of Santa Maria, central region of the State of Rio Grande do Sul, Brazil (Fig. 1). Sampling was carried out from October 1996 to March 1998 in the Campo de Instrução do Exército de Santa Maria (CISM), which is characterized as a deciduous seasonal forest (Veloso & Goes-Filho, 1982) but currently presents anthropic alterations. There are natural fields near the study area.

According to Köppen’s system classification the climate in the region is subtropical humid. The mean annual temperature oscillates from 17.9 to 19.2ºC. However the four seasons are well defined, with summer temperatures reaching about 40ºC and winter temperatures, 0ºC. Rainfall is regular throughout the year with annual rainfall index from 1500 mm to 1750 mm. March, November and December are the months with lower rainfall (Pereira et al., 1989).

Pitfall traps with drift fences (barrels of 100 l) were used to sample the population of the CISM (Cechin & Martins, 2000). Three lines of ten containers were installed: one line in the natural grassfield, one along the forest border and one inside the forest. The traps remained open during all study, from October 1996 to April 1998, and were inspected three times a week. The individuals trapped were deposited in the scientific collection of the Zoology Section at Universidade Federal de Santa Maria, Rio Grande do Sul.

Following fixation in 10 % formalin and transference to 70 % ethanol, snout-vent length (SVL) and mass were measured for each individual using a caliper (resolution 0.1 mm) and a precision scale (resolution 0.1 g), respectively. The testes length was measured using a stereomicroscope with ocular scale and mass using a precision scale with resolution 0.0001 g. In order to assess maturity, monthly samples of testes were prepared for histological sections survey. Males with spermatozoa in the seminiferous tubules were considered mature. Males with SVL larger than the smallest mature male were considered mature. After histology observations, the pigmentation of vocal sacs was also checked to evaluate male maturity. Analyses were based on the left testis. The following size-fecundity correlations were investigated for mature individuals: 1. SVL x testes length; 2. SVL x testes mass; 3. body mass x testes length; 4. body mass x testes mass.

Ovaries were classified by maturity following to Hermosilla et al. (1986), with modifications for this species. It is known that in this species, oocytes are not pigmented (Heyer, 1969). Those with darker yellow coloration and pronounced larger size were considered post-vitellogenic (mature). Mature oocytes of both ovaries were counted and the diameter of the ten largest ones was measured for each female. To facilitate counting, the oocytes were separated by immersion into a solution of 3% sodium hypochlorite. Fecundity was estimated based on the ovarian complement (number of mature oocytes in the ovary) and on the “ovary size factor” (OSF) (Duellman & Crump, 1974), which correlates number and size of post-vitellogenic oocytes to body length: OSF = OC x DO / SVL, where OC: ovarian complement; DO: mean diameter of the ten largest post-vitellogenic oocytes; SVL = females snout-vent length. Size-fecundity correlations were evaluated among the following variables: 1. Females SVL x ovarian complement; 2. SVL x mass of mature ovaries; 3. Body mass x ovarian complement; 4. Body mass x mass of mature ovaries.

Reproductive effort was measured as percentage of gonad mass in relation to body mass (Prado et al., 2000). For males, the value recorded for the left testis was multiplied by two.

The Spearman (r) Correlation Coefficient was used to calculate correlation between size and fecundity, OSF and female SVL, and temperature and rainfall and abundance of mature individuals. Sexual dimorphism in SVL was tested through Student’s t Test. Normality was evaluated with the Shapiro-Wilk test.

RESULTS

Mature individuals were found from October 1996 to February 1997 and from October 1997 to December 1997. Monthly mean temperature was higher in January and lower in June and July. The monthly rainfall varied along the year, with the most rainy months at the end of 1997 (Fig. 2).
There was a significant positive correlation between the number of mature individuals and the mean monthly temperature ($r_t = 0.539; p = 0.02; n = 18$), but there was no correlation to the mean monthly rainfall ($r_t = 0.117; p = 0.643; n = 18$).

The population had no sexual dimorphism in SVL ($t = 1.55; p = 0.132; FD = 28$), but females were larger in body mass than males ($t = 3.00; p = 0.005; FD = 28$). Among the testes analyzed by histological sections, 18 (43.9 %) had spermatozoa in the seminiferous tubules. Of these, 13 had vocal sacs pigmented and five had not. In addition, 11 individuals had larger SVL than the smallest mature male. Eight of them had vocal sacs pigmented and three had not. There was significant correlation only between SVL and testes lengthsize ($r_t = 0.45; p = 0.016; n = 29$).

Twelve mature females of *L. fuscus* were collected and all had oocytes in four different stages: 1: previtellogenic – with no accumulation of yolk in the cytoplasm, small and transparent; 2: primary vitellogenesis – accumulation of vitellus started, small and whitish; 3: late vitellogenesis – larger accumulation of vitellus, slightly larger and yellow; 4: post-vitellogenic – mature oocytes, large and dark yellow. Some females presented a small number of post-vitellogenic oocytes (minimum = 42). No significant correlation ($p > 0.05; n = 12$) was found to the size-fecundity variables. The correlation between OSF and SVL was not significant ($r_s = -0.09; p = 0.78; n = 12$).

The number of juveniles in the population greatly increased in March 1997. The smallest juvenile caught in that month had 17.07 mm; the smallest juvenile was caught in January and had 15.90 mm. During the first three months of the year (Jan – Mar) juveniles of different sizes were found.

**DISCUSSION**

The studied population of *L. fuscus* may be classified in the prolonged reproductive activity pattern, as found for a tropical population at Corumbá, western Brazil (Prado & Haddad, 2005). Analysis of gonads of the present study population indicated that reproduction occurred from October to March. This result agrees with that of Santos et al. (2008), who observed *L. fuscus* engaged in vocalization activity from November to March in our study region.

All previous studies with *L. fuscus* in tropical areas identified rainfall as the most important factor for the onset of reproduction (Lucas et al., 2008). In our study, the capture of mature individuals showed positive correlation only with monthly temperature. The number of vocalizing males for the whole anuran community also showed correlation with monthly temperature in a previous study conducted in this same area (Santos et al., 2008). In south Brazil, characterized by a subtropical climate, temperature presents high variation along the year, while rainfall is observed well distributed round-year (with no dry season). Thus, the reproductive behavior variation of *L. fuscus* is a consequence of this climatic variation. Besides, the population studied here reacted to a different climate factor from those of the same genetic lineage, but that inhabiting tropical environment (Camargo et al., 2006; Lucas et al., 2008).

The population of *L. fuscus* here studied did not present sexual dimorphism in SVL. The length of males and females was similar to that found by Solano (1987), in Venezuela. On the other hand, length of males and females was larger than that found by Martins (1988), in Roraima and smaller than found by Lucas et al. (2008), in São Paulo, Brazil. Among the studies about the species

<table>
<thead>
<tr>
<th>Study</th>
<th>This study</th>
<th>Lucas et al., 2008</th>
<th>Prado &amp; Haddad, 2003</th>
<th>Prado &amp; Haddad, 2005</th>
<th>Martins, 1988</th>
<th>Solano, 1987</th>
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<tbody>
<tr>
<td>MFSVL (mm)</td>
<td>43.7(41.9-46.3)</td>
<td>45.6</td>
<td>43.6(40-46.2)</td>
<td>39.5</td>
<td>42</td>
<td></td>
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<tr>
<td>MMSVL (mm)</td>
<td>43(39.9-46.8)</td>
<td>43.6</td>
<td>36.2</td>
<td>43</td>
<td></td>
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</tr>
<tr>
<td>MFBM (g)</td>
<td>10.4(9.0-13.2)</td>
<td>8.6(6.9-11)</td>
<td>7.7</td>
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<tr>
<td>MMBM (g)</td>
<td>8.92(7-11.2)</td>
<td>1.0-2.0</td>
<td>2.2(1.2-2.3)</td>
<td>1.8</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>DPvO (mm)</td>
<td>1.5(1.2-1.6)</td>
<td>128-385</td>
<td>214(185-248)</td>
<td>182-248</td>
<td>245-296</td>
<td></td>
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<tr>
<td>NPvO</td>
<td>191.8(42-441)</td>
<td>0.72</td>
<td>0.58(0.18-0.87)</td>
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<tr>
<td>MOM (g)</td>
<td>6.47(0.22-1.0)</td>
<td>0.003(0.0012-0.0052)</td>
<td>0.005</td>
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<td>M OSI</td>
<td>6.47(0.22-1.0)</td>
<td>2.3(1.6-3.0)</td>
<td>1.6-2.1</td>
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<tr>
<td>MMRE (%)</td>
<td>0.07(0.015-0.05)</td>
<td>7.47(2.6-10.8)</td>
<td>0.06</td>
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<tr>
<td>MFRE (%)</td>
<td>7.47(2.6-10.8)</td>
<td>6.8(2.6-12.6)</td>
<td>11</td>
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only Martins (1988) and Lucas et al. (2008) recorded sexual dimorphism, with females larger than males. The highest recruitment of juveniles of L. fuscus occurred in February and March, which corresponds to the end of the reproductive period. In Venezuela, Solano (1987) observed that, in the laboratory, the metamorphosis of this species takes 50 days and the size of the newly metamorphosed individuals is around 11 to 12 mm, reaching 20 mm after only two months. If we do not consider population or climate differences, the observation of juveniles of 20 mm or larger in January 1997 in the present study suggests that clutches were laid in October 1996, which corroborates the findings from gonad analysis that the reproductive activity begins in October. The large number of juveniles captured at the end of the reproductive period is probably related to their displacements searching for shelter to undergo colder months, since the species was not found in the winter (June to August).

The size and mass of anuran testes increase during spermatogenesis due to maturation of the spermatogenic cells (Duellman \& Trub, 1994). This increase was recorded for L. fuscus in this study, since length of testes was positively correlated to the SVL. In the present study, some males with mature testes had not pigmented vocal sacs. The development of this secondary characteristic may have been retarded in these individuals. On the other hand, some males with pigmented vocal sacs had no mature testes. Only the left testis was evaluated here and it is possible that these individuals had spermatozoids on the right test. Therefore, these results highlight the importance of carrying out histological analysis of at least one testis to assess male maturity.

Males of a population of L. fuscus studied in Mato Grosso do Sul (Prado \& Haddad, 2003) presented smaller mean body mass and reproductive effort than those found in this study; however, the mean testes mass was larger in that study. Otherwise, these populations may be considered very similar. Camargo et al. (2006) found that the mtDNA data support the hypothesis of three groups within L. fuscus, occurring in northern, southern and south-eastern, and western South America. They did not evaluate individuals from Pantanal and Rio Grade do Sul, but it is possible that these two populations belong to the same group.

High gonad investment in males may indicate sperm competition, as recorded by Prado \& Haddad (2003) in Leptodactylus chaquensis Cej, 1950 and L. podicipinus (Cope, 1862). Their record for L. fuscus was similar to that found in this study. The low reproductive effort found in both studies is probably related to the reproductive strategy used by the species, in which the male builds a subterranean nest, attracts a female and the clutch is laid there, which may prevent eliminates sperm competition.

In comparison to this study, in Mato Grosso do Sul, females of L. fuscus had smaller body mass and lighter ovaries, but contained larger and fewer post-vitellogenic oocytes (Prado \& Haddad, 2005). The population studied here presented most of the body filled by the gonads, which were heavier probably because the post-vitellogenic oocytes were smaller but occurred in greater number. In southeast Brazil, the oocytes were larger than observed here and found in lower numbers (Lucas et al., 2008). In Roraima, post-vitellogenic oocytes had similar size of that recorded herein, but found in a smaller number. This could be related with the small body size of mature females from Roraima (Martins, 1988). In Venezuela (Solano, 1987), female reproductive effort was higher than in this study, probably due to larger oocytes found in a great number, once the body size was similar. The trend to decrease egg number with the increase in egg diameter was suggested to be related to more terrestrial reproductive modes (Heyer, 1969). Comparing to other studies, the population herein is the one that least represents this trend, but the values found are in accordance to what Heyer (1969) proposed for the L. fuscus group.

The absence of correlation between the size-fecundity variables in females of this study was also verified by Solano (1987) and Prado \& Haddad (2005) in other populations of L. fuscus. Only Martins (1988) found significant correlation between SVL and number of eggs. According to Crump (1974), the ovary size factor increases together with the increase of the female SVL in each reproductive mode, but this correlation was not verified in this study. Neither was it recorded by Solano (1987), which suggests a hypothesis of influence of nutritional and environmental condition on the oocytes production.

The reproductive biology of L. fuscus in southern Brazil presented some differences comparing to other populations already studied. The main difference concerns the factor responsible for stimulating the reproduction, which is rainfall in seasonally dry tropical regions whereas in the area of this study, where rainfall is well distributed along the year, temperature played this role. Such data demonstrate the environmental plasticity of the species and evidence that populations of the same species may present different patterns according to the climate under which they live.

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