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REPRODUCTIVE BIOLOGY OF *Lysapsus limellus* COPE, 1862 (ANURA, PSEUDIDAE) IN THE PANTANAL, BRAZIL

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ABSTRACT

The reproductive biology of *Lysapsus limellus* was studied in Southern Pantanal, Mato Grosso do Sul state, southwestern Brazil, from May 1993 to April 1994. Reproduction occurred throughout the year in permanent ponds and flooded areas. The species exhibits sexual dimorphism in size, females being larger than males. Females deposited an average of 63 eggs±20.4 per clutch (N=18) and the average size of eggs was 1.16 mm±0.05 (N=16). During the dry season, females produced less ovarian eggs (36.9±13.2; N=46) than in the rainy season (51.8±28.6; N=50; t=3.2; p<0.001). Number of eggs per clutch was positively correlated to female snout-vent length (r^2=0.21; p<0.05; N=18). Gravid females were more frequent in August-September 1993 and February 1994, indicating the occurrence of two reproductive peaks during the studied period.

Key Words: *Lysapsus limellus*. Pseudidae. Anura. Reproduction. Pantanal. Brazil.

BIOLOGIA REPRODUTIVA DE *Lysapsus limellus* COPE, 1862 (ANURA, PSEUDIDAE) NO PANTANAL, BRASIL

RESUMO

A biologia reprodutiva de *Lysapsus limellus* foi estudada no Pantanal sul-mato-grossense no período de maio/93 a abril/94. A reprodução ocorreu durante o ano todo em lagoas permanentes e áreas inundadas pelos rios. A espécie exibe dimorfismo sexual no tamanho, sendo as fêmeas maiores que os machos. São depositados, em média, 63 ovos±20,4 por desova (N=18) e os ovos medem, em média, 1,16 mm±0,05 (N=16). Durante o período de estiagem as fêmeas produziram um menor número de óvulos (36,9±13,2; N=46) se comparado ao período de chuvas (51,8±28,6; N=50; t=3,2; p<0,001). O número de ovos por desova está positivamente correlacionado com o comprimento rostro-anal das fêmeas (r^2=0,21; p<0,05; N=18). Fêmeas grávidas foram mais freqüentes em agosto-setembro/93 e fevereiro/94, indicando a ocorrência de dois picos reprodutivos durante o período estudado.

Palavras Chave: *Lysapsus limellus*. Pseudidae. Anura. Reprodução. Pantanal. Brasil.

FICHA:

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INTRODUCTION

The family Pseudidae, endemic to South America (Duellman, 1988), has two genera: *Pseudis*, with two species, and *Lysapsus*, with one (Bosch et al., 1996). They are typically aquatic anurans with well-developed webs (Gallardo, 1988). They occur in open habitats such as the Chaco and the Cerrado, but are also found in open environments in rain forests (Bosch et al., 1996).

Lysapsus limellus has a small size, varying from 16 to 21 mm, and is usually associated with aquatic macrophytes. This species can withstand periods of drought in small volumes of water (Gallardo, 1987). It occurs in Guyana, Surinam, French Guiana, Brazil, Paraguay, Uruguay, eastern Bolivia, and northern Argentina (Frost, 1985). Few studies, however, have been conducted on the biology of *L. limellus* (Kehr & Basso, 1990; Bosch et al., 1996). Studies on its reproductive biology, particularly in Brazil, are unknown.

The Pantanal is a floodplain of seasonal climate with one dry and one wet well-defined periods. Although inundation and seasonal floods are common in this region, they are not related to local rainfall alone, but also to drainage constraints, evidenced in the slow outflow of waters (Amaral Filho, 1986). The exact period and intensity of floods may vary from year to year. These features make this a favorable environment for the study of reproductive strategies, especially of those animals that are extremely sensitive to such environmental changes, like anurans, for which humidity constitutes an important limiting factor.

The present work focused on some aspects of the reproduction of *L. limellus* in Southern Pantanal, where the species occurs in high density year-round. The aims of this investigation were: to identify the species reproductive period and breeding sites; to verify the existence of sexual dimorphism in size; to determine clutch and egg sizes; to compare ovarian egg production in the dry and rainy seasons, and to verify the relationship between female snout-vent length and the number of eggs per clutch.

MATERIALS AND METHODS

STUDY SITE

Because of its great extension and the topographic, climatic and floristic features that characterize each of its areas, a number of sub-regions have been proposed for the Pantanal. Adamoli (1982) identified 10 sub-regions, which he named Cáceres, Poconé, Barão de Melgaço, Paiaguás, Nhecolândia, Aquidauana, Paraguai, Miranda, Nabileque, and Abobral (Figure 1). Climate is hot and humid, with a dry season extending from May to September and a rainy period from October to April. Precipitation, however, is low, with an annual average of 972 mm recorded in the municipality of Corumbá, MS (Amaral Filho, 1986). Floods are common from January to April.

Individuals were collected in ponds and flooded areas at Base de Estudos do Pantanal-Universidade Federal de Mato Grosso do Sul (19º34'S, 57º00'W), in the Miranda and Abobral sub-regions of the Pantanal (Figure 1), in the municipal district of Corumbá, State of Maro Grosso do Sul, and also along the MS-184 Road, Brazil.

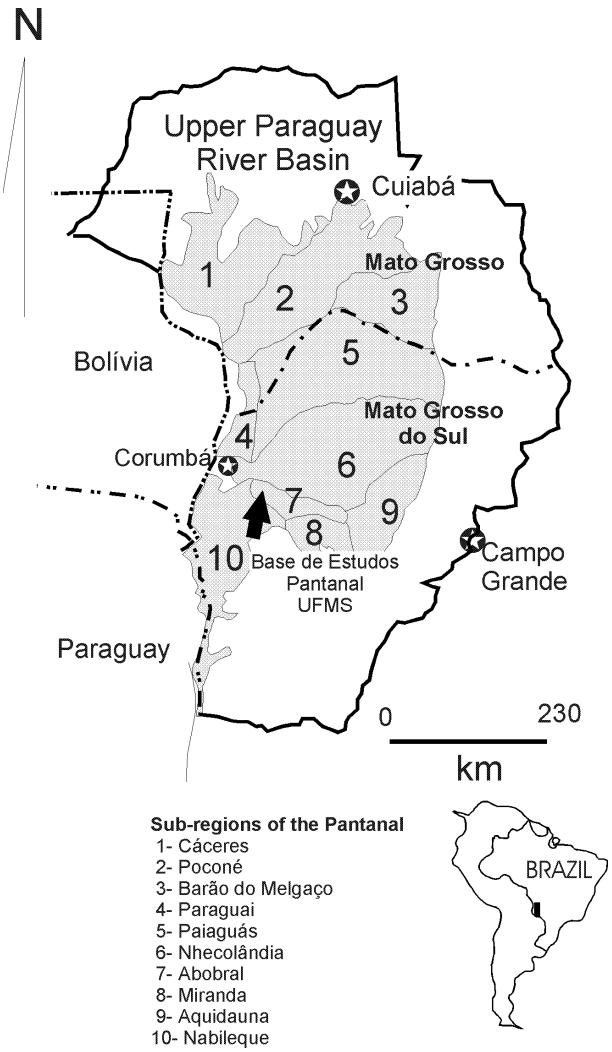

Figure 1- Sub-regions of the Pantanal accorging to Adamoli (1982) and location of the study site (arrow).

DATA COLLECTION

Data were collected monthly from May 1993 to April 1994. Individuals were captured by hand, both diurnally and nocturnally. To determine the reproductive period, direct observations were performed, based either on vocalization or on the observation of gonads. Water depth and plant cover were used to characterize the environments. Water bodies were classified as rain puddles, flooded areas, and permanent ponds.

Following capture, animals were transported to the laboratory, killed in ether vapor, and their snout-vent lengths (SVLs) were determined to the nearest 0.1 mm with a vernier calliper. Ovaries were removed and the number of mature ovarian eggs was determined using a stereo microscope (Zeiss). The largest and most pigmented ones were considered mature (Crump, 1974).

For determining the number of eggs per clutch (clutch size), amplectant pairs were collected so that clutches could be obtained in the laboratory. Egg diameters were measured to the nearest 0.1 mm with a graduated slide. Student's *t*-test was used to compare male and female SVLs, and to compare the number of ovarian eggs produced in the dry and wet seasons. Linear regression analysis was performed to verify the relationship between female SVL and number of eggs per clutch.

RESULTS

Reproduction of *L limellus* ocurred throughout the year, revealed by the observation of gravid females (except in November 1993) and of calling males (except in June 1993) during the 12 months of study. Both permanent ponds and river-flooded areas were used as reproduction sites. Generally, the individuals were observed associated with plants whose leaves are positioned on the water surface, especially *Salvinia minima*, *Salvinia auriculata*, *Ludwigia* spp., and *Nymphaea amazonum*. On such leaves males were found calling in horizontal position, both nocturnally and diurnally.

SVLs ranged from 15.9 to 23.0 mm for females, and from 14.4 to 20.2 mm for males. Data analysis revealed the existence of sexual dimorphism in size, females being larger (18.6 mm±1.4; N=451) than males (16.6 mm±1.0; N=430; t=21.8; p<0.001). These results indicate that females become mature when they reach about 16.0 mm in SVL, while males reach maturity at around 14.5 mm.

Females collected in amplexus deposited from 27 to 117 eggs (63±20.4; N=18), and the average diameter of eggs was 1.16 mm±0.05 (N=16). Following spawning, ovaries were analyzed, usually revealing some mature ovarian eggs, in addition to others in different developmental stages. Although females bearing mature ovarian eggs were found in nearly every month (except November 1993), gravid females were more frequent in August-September 1993 and February 1994 (Figure 2).

Females produced more ovarian eggs in the rainy season (51.8±28.6; range=20-166; N=50) compared to the dry season (36.9±13.2; range=20-68; N=46; t=3.2; p<0.001). Amplectant pairs were found only in the rainy season, so we could not analyze variation in clutch size during the dry and rainy seasons. Clutch size was positively correlated to female SVL (r^2=0.21; p<0.05; N=18; Figure 3).

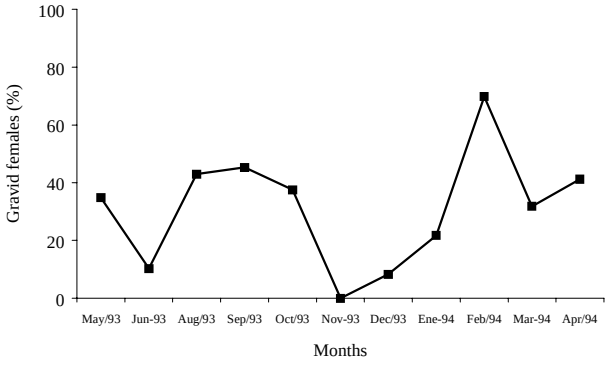

Figure 2- Monthly variation in percentage distribution of gravid females of *Lysapsus limellus* in the Pantanal, Brazil.

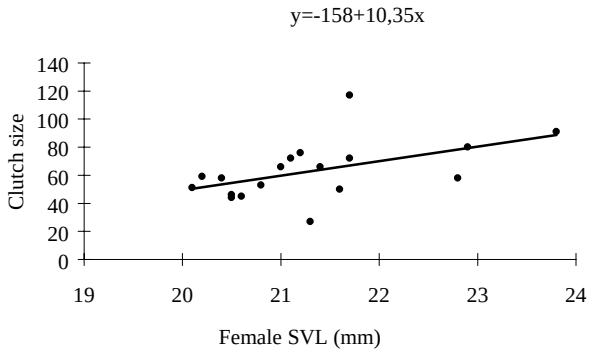

Figure 3- Relationship between snout-vent length (SVL) and clutch size for 18 females of *Lysapsus limellus* in the Pantanal, Brazil (r^2=0.21; p<0.05).

DISCUSSION

According to Wells (1977), *L. limellus* has a prolonged breeding. Females bearing mature ovarian eggs were not found in November 1993 only, when individuals could be found in high concentrations in small volumes of water, due to lack of rain. Males were not heard in June 1993 only, when temperatures were very low (8.5 to 10.0 °C). The reproductive activity of anurans living in seasonal environments is generally associated with the rainy periods, both in tropical climates (Hoogmoed & Gorzula, 1979; Aichinger, 1987) and in temperate ones (Banks & Beebee, 1986; Caldwell, 1987). It is not uncommon, however, especially in tropical areas, to find anurans reproducing year-round even in seasonal environments (Aichinger, 1987; Barreto & Moreira, 1996), provided favorable sites for reproduction are available throughout the year. This is probably what occurs with *L. limellus*, which reproduces both in permanent ponds and in areas that are inundated when rivers overflow.

Females of *L. limellus* have a larger SVL compared to males. Females are larger than males in 90% of anuran species (Shine, 1979), and there are several possible reasons for this feature. Among them, the most emphasized is that larger females are able to produce larger eggs, or lay eggs in larger quantities (Crump, 1974; Crump & Kaplan, 1979). However, other studies involving different species of anurans have proposed other explanations for the larger size of females, such as a higher predation pressure over larger males due to increased risks involved in territorial defense and mating (Howard, 1981), or even restrictions to the growth of males due to energy demands related to reproductive activity (Woolbright, 1989). In fact, sexual dimorphism is the result of complex interactions among many different selective pressures to wich a species is subjected in its environment (Woolbright, 1989).

During the study period, all stages of ovarian development could be observed in females in a same month, from ovaries with early egg formation to ovaries already bearing many mature eggs, as well as all other intermediate stages. Studies on anurans have reported the occurrence of asynchrony in ovarian development among individuals of a population (Pancharatna & Saidapur, 1985; Jorgensen et al., 1986; Silverin & Andrén, 1992), particularly in tropical species that can reproduce year-round (Jorgensen et al., 1986).

The highest frequency of gravid females in the months of August-September 1993 and February 1994 may be related to an increase in the mean temperature (August-September) and to an increase in the local rainfall and the beginning of the period of floods (February). Generally the rainy period begins in September or October, but in 1993 the region studied underwent a prolonged drought that extended to early December, thus causing some permanent ponds to dry completely. Thus, since October 1993 the number of gravid females began to decrease, and in November 1993 no gravid females were found (Figure 2). Individuals collected were in a high density in the pond, and the environmental pressure to which the animals were subjected may have been the cause of the absence of gravid females. As previously observed for other anuran species, climatic factors, especially rainfall and temperature, exert a strong influence on the reproductive activity (Telford and Dyson, 1990; Silverin and Andrén 1992), and conditions of stress can even inhibit ovarian development (Silverin and Andrén 1992). Furthermore, other factors may interfere with reproduction, such as intrinsic (morphophysiologic) components, water temperature, predation pressure, and competition for food.

During the rainy season, females of *L. limellus* produced a higher number of ovarian eggs than in the dry season. Praderio & Robinson (1990), in a study on *Colostethus trinitatus* in a seasonal environment in Venezuela, also observed an increase in ovarian egg production during the rainy season, and attributed this fact to an increase in the availability of food to females during this period. Although such aspect has not been analyzed in the present work, this explanation could apply to *L. limellus*.

Many studies have demonstrated that female size correlates positively with the number of eggs laid or ovarian eggs produced (Martins, 1988; Ryser, 1988; Praderio and Robinson, 1990). This also occurs in *L. limellus*, since 21% of the variation in the number of eggs laid per clutch could be explained by female SVL in the present study (Figure 3). A larger body size confers reproductive advantages to females, such as an increase in the number or size of ovarian eggs (Crump, 1974; Howard, 1978). Furthermore, larger females have higher chances of producing more than one clutch in each reproductive season (Howard, 1978; Telford and Dyson, 1990).

As *L. limellus* reproduces throughout the year, it is possible that females are capable of producing more than one clutch per year. The main evidences are the low number of eggs laid per clutch and the presence of gravid females in all months. The low correlation between female SVL and clutch size could be also due to the ocurrence of multiple egg clutches.

CONCLUSIONS

L. limellus exhibits prolonged breeding, reproduction ocurring throughout the year in Southern Pantanal, Mato Grosso do Sul state, in southwestern Brazil.

The species reproduces in permanent ponds and flooded areas.

Females deposit an average of 63±20.4 eggs per clutch (N=18) and the average diameter of eggs is 1.16 mm±0.05 (N=16).

There is sexual dimorphism in size, females being larger (18.6 mm±1.4; N=451) than males (16.6 mm±1.0; N=430; t=21.8; p<0.001).

Females produced more ovarian eggs was during the rainy season (51.8±28.6; range=20-166; N=50) than in the dry period (36.9±13.2; range=20-68; N=46; t=3.2; p<0.001).

Females bearing mature ovarian eggs were found in nearly every month (except November 1993), but they were more frequent in August-September 1993 and February 1994 (Figure 2), indicating the existence of two reproductive peaks.

There is a positive relationship between female SVL and the number of eggs per clutch (r^2=0.21; p<0.05; N=18; Figure 3).

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