

## SIZE-FECUNDITY RELATIONSHIPS AND REPRODUCTIVE INVESTMENT IN FEMALE FROGS IN THE PANTANAL, SOUTH-WESTERN BRAZIL

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The ovarian complement of anurans exhibiting different reproductive modes is highly diverse, and intraspecific variation in reproductive output of anurans is generally related to variation in female properties and/or environmental conditions. The size-fecundity relationships, reproductive investment, and correlation between ovary mass and fat body mass were investigated for females of an anuran assemblage in the Pantanal, Brazil. Female body size was positively correlated with clutch size among seven of the eight species analyzed. However, these results seem to be influenced by seasonal variation in fecundity. Interspecific size-fecundity relationships revealed that female SVL was positively correlated with clutch size and egg size regardless of reproductive mode. Among 11 species analyzed, the reproductive investment (RI: ovary mass relative to body mass) varied from 5.5 to 18%, and there were no differences among reproductive modes and activity patterns (explosive / prolonged). RI correlated negatively with female size. Among three *Leptodactylus* species examined, negative correlation between ovary mass and fat body mass was verified for two species. Variations in the reproductive strategies are closely related to the reproductive activity patterns and reproductive modes exhibited by individual species, but are also influenced by environmental conditions. However, regardless of reproductive mode or activity pattern, each species seems to exhibit a reproductive strategy that allows them to respond differently to the same environmental restrictions.

*Key words:* anurans, clutch size, fat body mass, reproductive investment

### INTRODUCTION

As life history traits affect organism reproduction and survivorship, most of the interest in studying life history evolution comes from the different possible combinations of these traits which affect fitness (Stearns, 1992). Tropical anuran species exhibit a great diversity of reproductive patterns (Duellman & Trueb, 1986). The ovarian complement of anurans with different modes of reproduction is highly diverse (Salthe & Duellman, 1973). Generally, large species produce more eggs than smaller ones, and species that exhibit the generalized reproductive mode produce larger clutches than those with specialized modes. Furthermore, within a given reproductive mode there is a positive correlation between female body size and clutch size, and a negative correlation between clutch size and ovum size (Duellman, 1989). Information on size-fecundity relationships for anurans has been published by Salthe & Duellman (1973), Crump (1974), and more recently, by Lang (1995) and Perotti (1997).

Reproductive investment – also called reproductive effort – has been defined as the proportion of resources available to an organism of certain age, which is invested in reproduction during a defined period of time (Gadgil & Bossert, 1970). However, it is much easier to define than to measure such investment, and the most common

method used consists of establishing proportions as gonad mass relative to body mass or clutch volume relative to body volume (e.g. Crump, 1974; Lemckert & Shine, 1993; Perotti, 1997). In female frogs, which devote a great amount of energy into egg production, interspecific variation in reproductive investment is related to reproductive mode (Ryan, 1992). While intraspecific variation in reproductive output of anurans may be related to variation in female properties and environmental conditions (e.g. Berven, 1988; Lüddecke, 2002). However, comparative studies on reproductive investment and fecundity are scarce for Neotropical frogs inhabiting the seasonal Chaco-Pantanal domain (e.g. Perotti, 1997; Prado *et al.*, 2000).

The size-fecundity relationships and reproductive investment (RI) of female frogs from a Neotropical assemblage in the Pantanal, south-western Brazil, are herein described. As this region is markedly seasonal with a long dry period, main predictions tested in the present study were: (1) that RI will be greater in explosive breeders compared to prolonged breeders regardless of reproductive mode; and (2) that females of prolonged breeders will produce larger clutches in the wet season compared to the dry season. Regardless of reproductive mode, clutch size correlates negatively with egg size in anurans (e.g. Crump, 1974). Consequently, we expect little variation in RI among species exhibiting different reproductive modes. Furthermore, individual variation in ovary mass and fat body mass was also investigated for three *Leptodactylus* species.

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## MATERIALS AND METHODS

## STUDY SITE

The Pantanal is a sedimentary floodplain located in south-western Brazil, with an area of approximately 140,000 km<sup>2</sup> between the parallels 16°-22° S and 55°-58° W. The study was conducted in the municipality of Corumbá, MS, at the Pantanal Research Station, Federal University of Mato Grosso do Sul (19° 34'S - 57° 00'W). Climate is markedly seasonal ("Aw" type in Köppen's classification), with wet summers from October to April and dry winters from May to September. Rainfall concentrates from November to January, with an annual mean of 1215 mm at the study site, between 1995 and 1998. For the same period, mean annual temperature was 25.1 °C, varying from 15.8 °C (June 1996) to 29.5 °C (January 1998). The topography is plain, formed mostly by hydromorphic soils, causing drainage problems, that partially explains its tendency for periodic and prolonged floods (Por, 1995), which are common in the region from January to April. The region is characterized by open formations included in the Cerrado domain (savanna-like vegetation).

## STUDIED FROG ASSEMBLAGE

The anuran assemblage at the study site is composed by 24 frog species belonging to four families: Bufonidae, Hylidae, Leptodactylidae, and Microhylidae, and the reproductive activity concentrates in the rainy season (November-January; Prado *et al.*, 2005). Herein we present data for 16 anuran species belonging to three families (Table 1), with three continuous breeders (*Hyla nana*, *Lysapsus limellus*, and *Leptodactylus*

*podicipinus*), seven prolonged breeders, reproducing for more than three consecutive months, and six explosive breeders. Five reproductive modes occur among the frog species, with the majority exhibiting the generalized aquatic mode, with eggs deposited in lentic water bodies and exotrophic aquatic tadpoles (Prado *et al.*, 2005; Table 1). Terrestrial modes are exhibited by *Phyllomedusa hypochondrialis*, which deposits eggs in leaves above water and tadpoles develop in lentic water, *Leptodactylus fuscus* and *Adenomera cf. diptyx*, which deposit eggs in foam nests in terrestrial subterranean chambers and tadpoles are carried to the pond subsequent to flooding.

## FIELD WORK

Data were collected monthly between January 1996 and December 1998. Additional data were collected in the rainy season (October-March) in the years 1995, 2001 and 2002. Clutches were collected in the field whenever possible, but most clutches were obtained from amplexed pairs or estimated based on number and size of mature ovarian eggs from gravid females caught in the field.

Eggs of each clutch were counted (= clutch size) and individual egg diameter from different clutches was measured to the nearest 0.1 mm with an ocular micrometer in a Zeiss stereomicroscope. Snout-vent length (SVL) of individuals was measured with a caliper ruler to the nearest 0.1 mm. Body, mature ovaries, and ovarian fat bodies were measured on electronic balances to the nearest 0.001 g for the larger species, and 0.0001 g for the smaller ones, after being blotted to remove ex-

TABLE 1. Reproductive modes, reproductive activity patterns, and reproductive period for 16 anuran species in the south Pantanal (source Prado *et al.*, 2005). Reproductive modes: (1) eggs and exotrophic tadpoles in lentic water; (8) foam nest and exotrophic tadpoles in lentic water; (18) eggs on leaves above water; exotrophic tadpoles in lentic water; (21) foam nest in subterranean chamber; exotrophic tadpoles in lentic water (Duellman and Trueb, 1986); (3) eggs and early larval stages in foam nests in water-filled basins constructed by males; exotrophic tadpoles in lentic water (Prado *et al.*, 2002).

Family/species	Mode	Reproductive pattern	Reproductive period
HYLIDAE			
<i>Hyla nana</i>	1	continuous	Jan-Dec
<i>H. raniceps</i>	1	prolonged	Sep-Apr
<i>Lysapsus limellus</i>	1	continuous	Jan-Dec
<i>Phrynohyas venulosa</i>	1	explosive	Oct-Jan
<i>Phyllomedusa hypochondrialis</i>	18	prolonged	Sep-Mar
<i>Pseudis paradoxa</i>	1	prolonged	Oct-May
<i>Scinax acuminatus</i>	1	explosive	Oct-Mar
<i>S. fuscomarginatus</i>	1	prolonged	Jan-May
LEPTODACTYLIDAE			
<i>Adenomera cf. diptyx</i>	21	prolonged	Oct-Mar
<i>Leptodactylus chaquensis</i>	8	explosive	Oct-Mar
<i>L. fuscus</i>	21	prolonged	Sep-Mar
<i>L. podicipinus</i>	3	continuous	Jan-Dec
<i>Physalaemus albonotatus</i>	8	prolonged	Oct-Apr
<i>Pseudopaludicola cf. falcipes</i>	1	explosive	Nov-Mar
MICROHYLIDAE			
<i>Chiasmocleis mehelyi</i>	1	explosive	Nov
<i>Elachistocleis cf. bicolor</i>	1	explosive	Sep-Mar

cess liquid. Percentage of mature ovarian mass relative to body mass was used as a measure of the reproductive investment (RI).

DATA ANALYSIS

Variables were tested for normality (Shapiro - Wilk *W* test) before each analysis. Size-fecundity relationships were examined through linear regression analysis (Zar, 1999). Intraspecific relationships between clutch size and female SVL and clutch size and female body mass (body mass - ovary mass) were determined. Relationships between clutch size and female SVL, egg size and female SVL, ovary mass and female mass, and reproductive investment and female mass were compared

among different species. Considering allometric relationships between organ size and body size, to meet statistical assumptions, and to allow biological interpretation and comparison (King, 2000), we calculated linear regressions and correlations with log-transformed data. The reproductive investment (RI) was compared among different reproductive modes and between explosive and prolonged (including continuous) breeders. Comparisons of ovarian egg production in the dry and wet seasons were made for two continuous breeders, *Hyla nana* and *Leptodactylus podicipinus*. As most frog species disappear out of the reproductive season, correlation between ovary mass and fat body mass was examined only for three species, *Leptodactylus*

TABLE 2. Mean±SD female SVL and mass, clutch size (number of eggs per clutch), egg diameter, ovary mass, and reproductive investment (RI) for the anurans in the Pantanal, Brazil. Range and sample size in parenthesis. \*Parameters based on ovarian eggs.

Family/Species	Female SVL (mm)	Female mass (g)	Clutch size	Egg diameter (mm)	Ovary mass (g)	RI (%ovary/body)
<b>HYLIDAE</b>						
<i>H. nana</i>	21.3±1.5 (18.8-23.8;13)	0.6±0.1 (0.3-0.7;13)	242±125 (120-551;15)	0.9±0.05 (0.8-1.0;20)	0.09±0.01 (0.07-0.10;5)	15.6±4.6 (10.7-21.4;5)
<i>H. raniceps</i>	60.2±4.4 (51.0-70.8;34)	12.3±3.4 (7.2-22.5;32)	1991±533 (1220-3096;23)*	1.3±0.10 (1.1-1.5;30)*	1.64±0.73 (0.94-3.04;6)	11.9±6.0 (7.4-23.8;6)
<i>L. limellus</i>	18.6±1.4 (16.0-23.0;45)	–	63±20 (27-117;18)	1.2±0.05 (1.1-1.2;16)	–	–
<i>P. venulosa</i>	77.4±6.0 (67.7-86.0;10)	34.5±10.8 (20.3-56.9;10)	3981±271 (3625-4310;6)*	1.7±0.11 (1.4-1.8;30)	3.9±1.22 (2.52-4.86;3)	8.2±1.3 (6.7-9.3;3)
<i>P. hypochondrialis</i>	43.0±1.5 (41.3-46.0;10)	4.0±0.5 (3.1-4.8;10)	89±30 (25-136;15)	2.1±0.12 (2.0-2.4;26)	0.66±0.28 (0.47-1.17;6)	16.2±5.6 (10.8-25.2;6)
<i>P. paradoxa</i>	57.5±8.0 (45.5-73.2;17)	27.2±13.5 (8.6-62.2;14)	1834±1,194 (1000-4624;8)*	1.4±0.08 (1.3-1.5;15)	1.82±1.40 (0.80-4.90;7)	5.5±3.1 (2.6-11.1;7)
<i>S. acuminatus</i>	43.1±2.0 (40.4-48.0;34)	6.5±1.2 (4.9-9.3;33)	879±274 (594-1352;9)	1.1±0.07 (1.0-1.2;65)	0.77±0.36 (0.27-1.70;21)	11.1±5.3 (3.9-26.4;21)
<i>S. fuscomarginatus</i>	22.0±0.9 (21.0-22.7;3)	0.5±0.0 (0.5-0.6;3)	337±132 (185-421;3)	0.9±0.05 (0.9-1.0;10)	–	–
<b>LEPTODACTYLIDAE</b>						
<i>A. cf. diptyx</i>	25.7 (1)	–	36 (1)*	1.4±0.14 (1.2-1.5;5)*	–	–
<i>L. chaquensis</i>	71.3±4.5 (60.7-80.1;50)	34.8±6.5 (21.9-49.0;50)	4936±1720 (3007-8375;32)*	1.5±0.09 (1.4-1.7; 5)	5.87±1.72 (2.97-8.92;26)	16.0±2.9 (9.9-21.2; 26)
<i>L. fuscus</i>	43.6±1.9 (40.0-46.2;21)	8.6±1.3 (6.9-11.3;21)	214±27 (185-248;4)	2.2±0.07 (2.1-2.3;12)	0.58±0.24 (0.18-0.87; 8)	6.8±3.3 (2.6-12.6; 8)
<i>L. podicipinus</i>	39.5 ± 2.1 (35.6-44.0;36)	6.1±1.0 (4.4-8.2;27)	2102±442 (1750-2953;6)	1.1±0.05 (1.0-1.2; 20)	1.08±0.28 (0.66-1.34; 10)	15.0±2.6 (11.3-18.5; 10)
<i>P. albonotatus</i>	23.5±2.3 (20.1-27.0;41)	1.2±0.4 (0.5-2.3;37)	719 ± 433 (246-1562;19)	1.0±0.04 (0.9-1.0;43)	0.21±0.10 (0.12-0.52; 12)	16.6±4.3 (11.0-22.4;12)
<i>P. cf. falcipes</i>	14.3±0.8 (13.5-15.0;3)	0.30±0.07 (0.25-0.35;2)	32(1)	0.8 ± 0.02 (0.8-0.9;9)	–	–
<b>MICROHYLIDAE</b>						
<i>C. mehelyi</i>	23.8±0.6 (23.3-24.5;3)	–	217±33 (183-250;3)*	1.3±0.09 (1.2-1.4;30)*	–	–
<i>E. cf. bicolor</i>	26.1±1.5 (23.3-28.7;13)	1.4±0.4 (0.8-2.3;9)	478±279 (178-888;5)*	1.0±0.04 (0.9-1.1;15)*	0.32±0.18 (0.21-0.53;3)	18.0±4.5 (15.0-23.3;3)

*chaquensis*, *L. fuscus*, and *L. podicipinus*. These species occurred throughout the year and sufficient data were collected for them. When variables were not normally distributed, nonparametric tests were performed. Results were considered statistically significant at the level of  $P < 0.05$ .

## RESULTS

### SIZE-FECUNDITY RELATIONSHIPS

Data on clutch and egg size were obtained for 16 species (Table 2). However, due to the small sample size for some species, intraspecific relationships between clutch size (= number of eggs per clutch) and female SVL were only possible for eight species, and between clutch size and female body mass for six species (Table 3). Body size was positively correlated with clutch size in the majority of the species, with body mass explaining more variation in clutch size compared to SVL (Table 3). For *L. fuscus*, neither SVL nor body mass were significantly associated with clutch size. For *P. albonotatus* and *S. acuminatus* SVL was not associated with clutch size. For *L. podicipinus*, neither SVL nor body mass correlated with clutch size ( $R^2=0.09$ ;  $P=0.23$ ;  $n=17$ ; and  $R^2=0.12$ ;  $P=0.21$ ;  $n=15$ ; respectively). But when females were analyzed separately by seasons (dry/rainy), both SVL and body mass were highly correlated with clutch size in the rainy season (Table 3), period when peak of activity occurred for the species.

Interspecific size-fecundity relationships were also examined (Table 2). Clutch size was positively correlated with female SVL among the species compared ( $R^2 = 0.60$ ;  $P < 0.01$ ;  $n=16$ ). However, the species with terrestrial reproductive modes, *P. hypochondrialis*, *A. cf.*

*diptyx*, and *L. fuscus*, exhibited much smaller clutches than expected (Fig. 1), and two species that deposit eggs in foam nests on the water, *L. podicipinus* and *P. albonotatus*, exhibited larger clutches compared to the remaining species. Egg diameter also correlated positively with female SVL ( $R^2 = 0.43$ ;  $P < 0.01$ ;  $n = 16$ ). Species with terrestrial modes, mainly *P. hypochondrialis* and *L. fuscus*, exhibited larger egg sizes compared to the remaining species (Fig. 2). Excluding the three species with terrestrial modes, the relationship between body size and egg size became even stronger ( $R^2=0.68$ ;  $P < 0.01$ ;  $n=13$ ).

### REPRODUCTIVE INVESTMENT

Mean reproductive investment (RI), including all species and reproductive modes, was  $12.8 \pm 4.4\%$  ( $n=11$ ), ranging from 5.5 to 18% (Table 2). For the species exhibiting aquatic eggs, mean RI was  $11.7 \pm 4.6\%$  (range=5.5-18%;  $n=6$ ), for the frogs with aquatic foam nests, it was  $15.9 \pm 0.8\%$  (range=15-16.6%;  $n=3$ ), and for two species with terrestrial eggs, values of RI were 6.8% for *L. fuscus* and 16.2% for *P. hypochondrialis*. The reproductive investment was not significantly different among these three categories of reproductive modes (Kruskal-Wallis,  $H=0.92$ ,  $df=2$ ,  $P=0.63$ ). For explosive breeders, mean RI was  $13.3 \pm 4.5\%$  (range=8.2-18%;  $n=4$ ) and for prolonged species it was  $12.5 \pm 4.6\%$  (range=5.5-16.6%;  $n=7$ ), showing no significant difference (Mann Whitney  $U$ -test:  $W=12.0$ ;  $P=0.70$ ). Ovary mass was strongly associated with female mass among the species analyzed ( $R^2 = 0.91$ ;  $P < 0.001$ ;  $n=11$ ; Fig. 3). Results of linear regression analysis revealed a negative relationship between RI and female mass ( $R^2=0.38$ ;

TABLE 3. Results of linear regression analysis between female SVL and clutch size and female body mass and clutch size for females of anurans in the Pantanal, Brazil. NS = non significant results.

Family/Species	log SVL (mm) vs. log clutch size	log body mass (g) vs. log clutch size
<b>HYLIDAE</b>		
<i>H. nana</i>	$R^2=0.59$ ; $P < 0.01$ ; $N=12$ $y = -4.0 + 4.8x$	—
<i>H. raniceps</i>	$R^2=0.25$ ; $P=0.03$ ; $N=23$ $y = -0.15 + 1.9x$	$R^2=0.34$ ; $P < 0.01$ ; $N=23$ $y = 2.6 + 0.57x$
<i>L. limellus</i>	$R^2=0.25$ ; $P=0.03$ ; $N=19$ $y = -2.8 + 3.5x$	—
<i>S. acuminatus</i>	$R^2=0.41$ ; $P=0.06$ ; $N=9$ NS	$R^2=0.51$ ; $P=0.03$ ; $N=9$ $y = 1.61 + 1.8x$
<b>LEPTODACTYLIDAE</b>		
<i>L. chaquensis</i>	$R^2=0.30$ ; $P < 0.01$ ; $N=26$ $y = -0.89 + 2.5x$	$R^2=0.57$ ; $P < 0.01$ ; $N=26$ $y = 2.0 + 1.1x$
<i>L. fuscus</i>	$R^2=0.19$ ; $P=0.21$ ; $N=10$ NS	$R^2=0.05$ ; $P=0.5$ ; $N=10$ NS
<i>L. podicipinus</i>	$R^2=0.65$ ; $P < 0.01$ ; $N=9$ $y = -4.0 + 4.6x$	$R^2 = 0.62$ ; $P=0.01$ ; $N=9$ $y = 2.2 + 1.5x$
<i>P. albonotatus</i>	$R^2=0.18$ ; $P=0.16$ ; $N=12$ NS	$R^2=0.42$ ; $P=0.02$ ; $N=12$ $y = 2.6 + 0.82x$

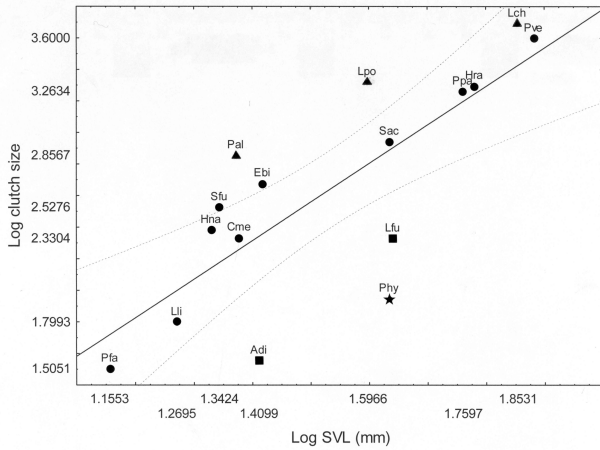


FIG. 1. Relationship between mean log female SVL and log clutch size for 16 anuran species in the Pantanal ( $\log y = -1.1 + 2.4 \log x$ ). Species: (Adi) *Adenomera cf. diptyx*; (Cme) *Chiasmocleis mehelyi*; (Ebi) *Elachistocleis cf. bicolor*; (Hna) *Hyla nana*; (Hra) *H. raniceps*; (Lch) *Leptodactylus chaquensis*; (Lfu) *L. fuscus*; (Lpo) *L. podicipinus*; (Lli) *Lysapsus limellus*; (Pve) *Phrynohyas venulosa*; (Phy) *Phyllomedusa hypochondrialis*; (Pal) *Physalaemus albonotatus*; (Pfa) *Pseudopaludicola cf. falcipes*; (Ppa) *Pseudis paradoxa*; (Sac) *Scinax acuminatus*; (Sfu) *S. fuscomarginatus*. Curved lines represent 95% confidence interval. Circles, eggs aquatic; triangles, aquatic foam nests; squares, terrestrial foam nests; stars, terrestrial eggs on leaves above water.

$P=0.04$ ;  $n=11$ ; Fig. 4), i. e., females of larger species tend to invest less in ovaries. However, RI in females of *L. chaquensis*, an explosive breeder that deposits eggs in foam nests on the water, was much higher than expected (Fig. 4). In contrast, females of two species, *Pseudis paradoxa*, a prolonged breeder that exhibits the generalized aquatic mode, and *L. fuscus*, another prolonged breeder, but with a terrestrial reproductive mode, invested much less in ovaries (Fig. 4).

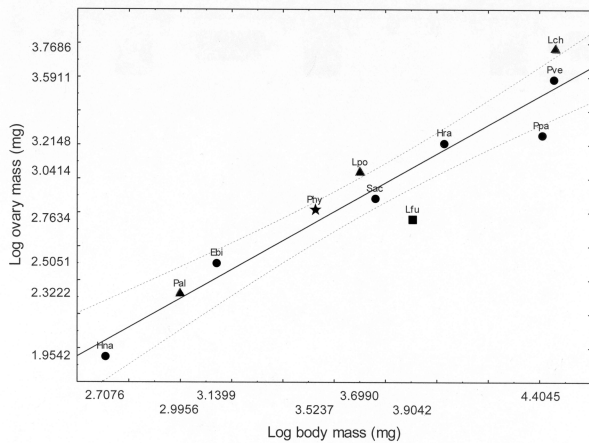


FIG. 3. Relationship between mean log female body mass and log ovary mass for 11 anuran species in the Pantanal ( $\log y = -0.27 + 0.85 \log x$ ). Species: (Ebi) *Elachistocleis cf. bicolor*; (Hna) *Hyla nana*; (Hra) *H. raniceps*; (Lch) *Leptodactylus chaquensis*; (Lfu) *L. fuscus*; (Lpo) *L. podicipinus*; (Pve) *Phrynohyas venulosa*; (Phy) *Phyllomedusa hypochondrialis*; (Pal) *Physalaemus albonotatus*; (Ppa) *Pseudis paradoxa*; (Sac) *Scinax acuminatus*. Curved lines represent 95% confidence interval. Circles, eggs aquatic; triangles, aquatic foam nests; squares, terrestrial foam nests; stars, terrestrial eggs on leaves above water.

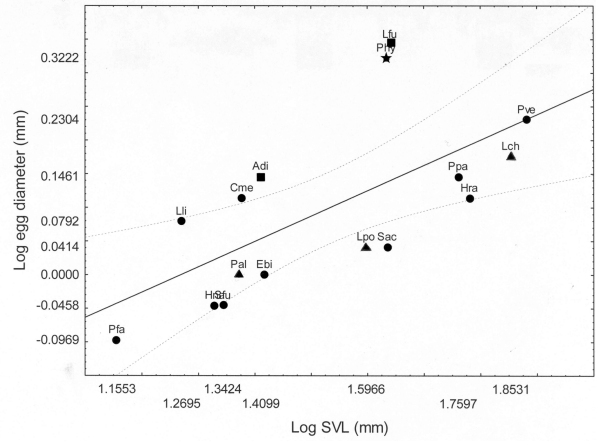


FIG. 2. Relationship between mean log female SVL and log egg size for 16 anuran species in the Pantanal ( $\log y = -0.48 + 0.38 \log x$ ). Species: (Adi) *Adenomera cf. diptyx*; (Cme) *Chiasmocleis mehelyi*; (Ebi) *Elachistocleis cf. bicolor*; (Hna) *Hyla nana*; (Hra) *H. raniceps*; (Lch) *Leptodactylus chaquensis*; (Lfu) *L. fuscus*; (Lpo) *L. podicipinus*; (Lli) *Lysapsus limellus*; (Pve) *Phrynohyas venulosa*; (Phy) *Phyllomedusa hypochondrialis*; (Pal) *Physalaemus albonotatus*; (Pfa) *Pseudopaludicola cf. falcipes*; (Ppa) *Pseudis paradoxa*; (Sac) *Scinax acuminatus*; (Sfu) *S. fuscomarginatus*. Curved lines represent 95% confidence interval. Circles, eggs aquatic; triangles, aquatic foam nests; squares, terrestrial foam nests; stars, terrestrial eggs on leaves above water.

SEASONAL CHANGES IN FECUNDITY AND RELATIONSHIP BETWEEN OVARIAN MASS AND FAT BODY MASS

For two continuous breeders, *H. nana* and *L. podicipinus*, ovarian egg production in the wet and dry season was highly variable. Females of *H. nana* produced, on average,  $250 \pm 130$  (range = 139-551;  $n=10$ ) mature ovarian eggs in the wet season and  $218 \pm 106$  (range = 120-364;  $n=7$ ) in the dry season, but this difference was not significant (Mann Whitney  $U$ -test:

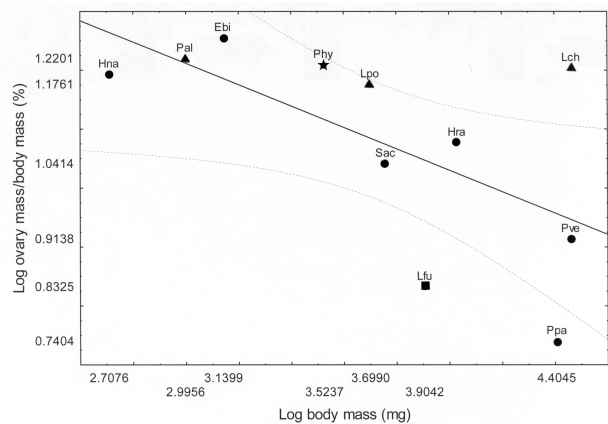


FIG. 4. Relationship between mean log female body mass and log ovary mass/body mass (reproductive investment) for 11 anuran species in the Pantanal ( $\log y = 1.7 - 0.18 \log x$ ). Species: (Ebi) *Elachistocleis cf. bicolor*; (Hna) *Hyla nana*; (Hra) *H. raniceps*; (Lch) *Leptodactylus chaquensis*; (Lfu) *L. fuscus*; (Lpo) *L. podicipinus*; (Pve) *Phrynohyas venulosa*; (Phy) *Phyllomedusa hypochondrialis*; (Pal) *Physalaemus albonotatus*; (Ppa) *Pseudis paradoxa*; (Sac) *Scinax acuminatus*. Curved lines represent 95% confidence interval. Circles, eggs aquatic; triangles, aquatic foam nests; squares, terrestrial foam nests; stars, terrestrial eggs on leaves above water.

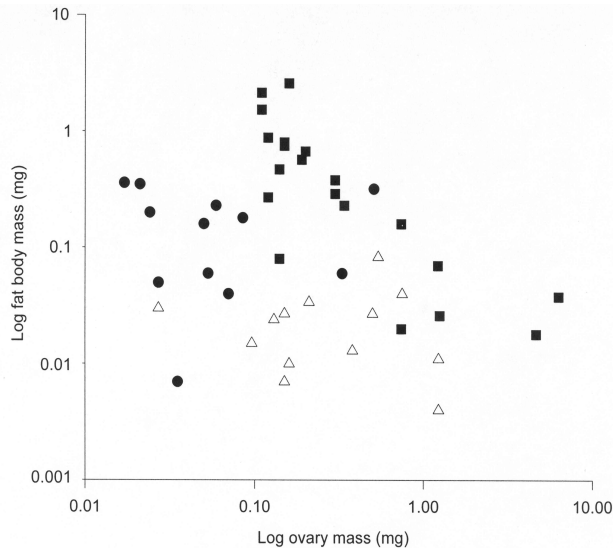


FIG. 5. Correlation between log ovary mass and fat body mass in females of *Leptodactylus chaquensis* (squares), *L. fuscus* (circles), and *L. podicipinus* (triangles) in the Pantanal, Brazil.

$W=28.0$ ;  $P=0.49$ ). Females of *L. podicipinus* produced a mean of  $2272\pm 464$  (range = 1700-2960;  $n=9$ ) ovarian eggs in the wet season and a mean of  $1766\pm 393$  (range=1304-2526;  $n=8$ ) in the dry season. For *L. podicipinus*, ovarian egg production in the wet and dry season differed significantly ( $t=2.4$ ;  $P=0.01$ ).

A significant negative relationship between ovary mass and fat body mass was verified for the explosive breeder *L. chaquensis* (Spearman rank correlation:  $r_s = -0.74$ ;  $P < 0.05$ ;  $n=50$ ; Fig. 5) and for the prolonged breeder *L. fuscus* ( $r_s = -0.69$ ;  $P < 0.05$ ;  $n=21$ ; Fig. 5). For the continuous breeder *L. podicipinus*, there was no significant correlation between ovary mass and fat body mass ( $r_s = -0.16$ ;  $P > 0.05$ ;  $n=27$ ; Fig. 5).

## DISCUSSION

### SIZE-FECUNDITY RELATIONSHIPS

Although female size correlated positively with clutch size in most species (7/8; Table 3), body size explained between 25% (*H. raniceps* and *L. limellus*) and 65% (*L. podicipinus*) of the variation in clutch size. Female size is positively correlated with fecundity in many species of frogs (e.g. Berven 1988; Lemckert & Shine, 1993; Lüddecke, 2002), but egg production may exhibit considerable variation among females in a single population (e.g. Berven 1988; Lemckert & Shine, 1993). Besides size, other factors may influence female fecundity, as environmental conditions and female nutritional state (e.g. Ryser, 1988, 1989; Lemckert & Shine, 1993). In prolonged or continuous breeding species, egg production may also differ if measured at different times (e.g. early, mid, or late season) during the breeding season (e.g. Praderio & Robinson, 1990; Giaretta & Kokubum, 2004). Variation in the regression coefficients in the present study could be related to the fact that, for most species, samples included females and clutches collected in different years, or different seasons

(dry/rainy) in the cases of the prolonged and continuous breeders. In the present study, neither SVL nor body mass correlated with clutch size in *L. fuscus*, but in another population studied in northern Brazil (Martins, 1988) SVL and number of ovarian eggs were positively correlated. An explanation could be that for the population in the Pantanal, females collected in different reproductive seasons were analyzed together and in the study conducted in northern Brazil, only females from a single reproductive episode were analyzed. But such differences could also be due to multi clutching, or related to population differences, or even it is possible that more than one species are being currently identified under the name *L. fuscus* (Wynn & Heyer, 2001).

Our results on variation in fecundity of *L. podicipinus* and *H. nana* support seasonal predictions (described above). Females of *L. podicipinus*, a continuous breeder at the study site, produced less ovarian eggs in the dry season compared to the rainy season. Analyzing all available females and clutches of *L. podicipinus*, neither SVL nor body mass correlated significantly with clutch size. But when females were analyzed separately by seasons (dry/rainy), both SVL and body mass were highly correlated with clutch size. In contrast, the other continuous breeder, *H. nana*, did not exhibit differences in egg production between the dry and rainy season at the study site. When all females from all seasons were analyzed, body size was positively correlated with clutch size. Although both species are continuous breeders, our results suggest that they exhibit different responses to environmental changes. As for *L. podicipinus* in the present study, variation in egg production between dry and rainy seasons was also reported for another continuous breeder in the Pantanal, *L. limellus* (Prado & Uetanabaro, 2000). Such variation was also observed for *Colostethus trinitatus* in a seasonal region in Venezuela (Praderio & Robinson, 1990), and the authors suggested that prey availability may diminish during the dry season affecting female nutritional state. This explanation could be applied for *L. podicipinus*, or, as noted by Crump (1974), it is possible that females regulate egg production according to climatic conditions.

Considering the reproductive modes in amphibians, from the most generalized aquatic to the most terrestrial modes, number of eggs deposited tends to decrease while egg size increases (Salthe & Duellman, 1973; Crump, 1974; Duellman & Trueb, 1986). Such a negative correlation was verified in many studies on reproductive modes in anurans (e.g. Crump & Kaplan, 1979; Hödl, 1990; Perotti, 1997). Although only three terrestrial modes were analyzed herein, such tendency was also observed. The species *P. hypochondrialis*, which deposits eggs on leaves above water, and *Adenomera* cf. *diptyx* and *L. fuscus*, which deposit eggs in foam nests in subterranean chambers, displayed smaller clutches and larger eggs compared to species exhibiting aquatic modes (aquatic eggs and aquatic foam nests). Two species that exhibit aquatic foam

nests, *L. podicipinus* and *P. albonotatus*, had relatively larger clutches compared to other species. Perotti (1997) compared the ovary size factor (Duellman & Crump, 1974), a method to measure fecundity which takes into account egg size, among species in different families and different reproductive modes in the Chaco, Argentina. Two leptodactylid species present in the Chaco – *L. chaquensis* and *Physalaemus biligonigerus* – that also deposit eggs in foam nests on the water, also had higher values of fecundity compared to other species (Perotti, 1997). It would be necessary to have data on other species with aquatic foam nests to test whether such larger clutches are phylogenetic determined or a common trait associated to this reproductive mode.

Interspecific analysis showed that female SVL was positively correlated with clutch size in the present study, as registered before for other frog assemblages (e.g. Crump, 1974; Lang, 1995). However, considering intra- and interspecific analysis, body mass and mature ovary mass showed a much stronger correlation. Regarding size-fecundity relationships, in comparison to SVL, body volume or mass give a more accurate measure of the size of the frog's internal cavity and more clearly defines the ovarian capacity of anurans (e.g. Crump, 1974; Lang, 1995; Prado *et al.*, 2000). Our results support previous studies cited above, with body mass explaining more variation in clutch size compared to SVL. Egg size and body size were not significantly correlated in a study conducted by Lang (1995). However, Lang (1995) tested the correlation with stream-breeding hylid frogs, which exhibited low egg size variation. All the stream-breeding species had relative large eggs, probably related to the water current of these habitats, which could explain the lack of correlation. When analysing species with different reproductive modes, depositing eggs at different habitat types, egg size correlates positively with body size (Perotti, 1997; present study). This fact may probably be related with many different selective pressures leading to differences in egg sizes.

#### REPRODUCTIVE INVESTMENT

Reproductive investment (RI), measured as percentage of ovary mass relative to body mass, varied from 5.5 to 18% among frog species in the present study. However, the RI did not differ significantly among the three categories of reproductive modes tested, neither between prolonged and explosive breeders. Percentage of clutch volume relative to body volume was examined for 23 species in different families and with different reproductive modes at Santa Cecilia, Ecuador (Crump, 1974). Similar to values observed for the frogs in the present study, relative clutch size at Santa Cecilia varied from 3.1 to 18.2%, the smallest value being that of the largest species, *Bufo marinus*, and largest percentage being that of the small treefrog *Hyla cruentomma*. Crump (1974) found an inverse relationship between the reproductive investment and body size, and so did

Perotti (1997), in Argentina. Such inverse relationship was also observed for the frog species in the present study. This negative trend indicates that as body size increases, proportionately less of the body size is accounted for by the ovary size, or less is invested in gonads. Crump (1974) suggested that probably this results from the fact that large species have a proportionately larger amount of supportive tissue.

The frog *L. chaquensis* invested more in gonads than expected, and *L. fuscus* and *P. paradoxa* much less. Ecological differences among these species mainly regarding reproductive activity patterns and number of clutches a female can deposit during a single reproductive period may explain such differences. Many studies showed that individual females can produce more than one clutch per reproductive season (e.g. Perrill, 1983; Lemckert & Shine, 1993). Based on presence of immature ovarian eggs in nearly every gravid female, Crump (1974) suggested that females breed repeatedly throughout the year in the Neotropics. Such situation was also observed for all the species in the present study (C. P. A. Prado, pers. obs.), and it is also suggested that females of all studied species may produce more than a clutch per season. But the number of clutches a female can produce may be under the control of environmental conditions as well as the species reproductive activity pattern. In contrast to other explosive breeders in the Pantanal that breed many times during the rainy season (Prado *et al.*, 2005), *L. chaquensis* is an explosive breeder that only breeds during the first heavy rains in the rainy season (Prado *et al.*, 2000), a condition that is rare at the study site. As a consequence, females of this species may not have the opportunity to lay many clutches during a single breeding season, which could lead to a high reproductive investment. In contrast, *L. fuscus* and *P. paradoxa* are prolonged breeders that reproduce continuously for more than six consecutive months at the study site. Females of these species may lay many small clutches during a single season, which would explain the extremely low investment in gonads.

#### SEASONAL CHANGES IN FAT BODY MASS IN LEPTODACTYLUS

In amphibians and reptiles fat is stored in special organs, the fat bodies, located anterior to the gonads, and their sizes are good indicators of the nutritional state of the organism (Jorgensen, 1992). Three *Leptodactylus* species exhibited differences regarding patterns of fat deposition in the present study, indirectly indicated by the correlation between fat body mass and ovary mass. For *L. chaquensis* and *L. fuscus*, fat body mass correlated negatively with ovary mass, i.e. females with ovaries containing eggs exhibited smaller fat bodies. However, for *L. podicipinus*, fat body mass was not correlated with ovary mass. In temperate regions, fat bodies in anurans are primarily known to serve as nutritional reserves, mainly during hibernation (Saidapur & Hoque, 1996). However, involvement of fat bodies in game-

togenesis has been reported for many species both in temperate (e.g. Long, 1987) and in tropical regions (e.g. Pancharatna & Saidapur, 1985; Saidapur & Hoque, 1996). An inverse relationship between fat body size and ovary size was reported for the temperate frog *Acris crepitans* (Long, 1987) and for the tropical frog *Rana cyanophlyctis* (Pancharatna & Saidapur, 1985), suggesting that lipids are used for vitellogenesis, as for *L. chaquensis* and *L. fuscus* in the present study. For *L. podicipinus*, fat body mass did not correlate negatively with ovary mass. In a study with *Bufo woodhousei* (Long, 1987), it was observed that although fat body size did not correlate negatively to ovary mass, lipid reserves were mobilized for egg production, and such a situation could be the case for *L. podicipinus* in the present study. Differences in fat deposition patterns among the *Leptodactylus* species may be due to ecological differences in reproduction. While *L. chaquensis* – an explosive breeder – and *L. fuscus* – a prolonged breeder – only reproduce in the rainy season, *L. podicipinus* reproduces throughout the year, which could explain the lower variation in lipid reserves throughout the year.

The Pantanal is included in the Cerrado-Caatinga-Chaco domain (Duellman, 1999), which is characterized by a subhumid to semiarid climate, high temperatures, and low rainfall, that falls mainly in a short rainy season. The climate is markedly seasonal in the Pantanal, with a defined wet period with unpredictable rains and a long dry season (Por, 1995). Floods are common in the region, but the exact period and intensity of floods may vary from year to year. Results herein described for the frog assemblage in the Pantanal suggest that variations in reproductive traits – as variation in egg production, pattern of fat deposition, and reproductive investment – although influenced by reproductive activity patterns and modes of reproduction, seem to be strongly influenced by environmental conditions. However, the most important conclusion is that, regardless of reproductive mode or activity pattern, each species seems to exhibit a unique reproductive strategy which allows them to respond differently to the same environmental restrictions.

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