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UNIVERSIDADE ESTADUAL PAULISTA
Instituto de Biociências de Rio Claro
Departamento de Zoologia

ESTRATÉGIAS REPRODUTIVAS EM UMA COMUNIDADE DE ANUROS NO PANTANAL, ESTADO DE MATO GROSSO DO SUL, BRASIL

CYNTHIA PERALTA DE ALMEIDA PRADO

Tese apresentada ao Instituto de Biociências da Universidade Estadual Paulista, Campus de Rio Claro, como parte dos requisitos para a obtenção do título de Doutor em Ciências Biológicas (Área de Zoologia).

RIO CLARO
Estado de São Paulo – Brasil
Novembro de 2003

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RIO CLARO
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Nasci para administrar o à toa

o em vão

o inútil.

Pertenço de fazer imagens.

Opero por semelhanças.

Retiro semelhanças de pessoas com árvores

de pessoas com rãs

de pessoas com pedras

etc etc.

Retiro semelhanças de árvores comigo.

Não tenho habilidade pra clarezas.

Preciso de obter sabedoria vegetal.

(Sabedoria vegetal é receber com naturalidade uma rã no talo.)

E quando esteja apropriado para pedra, terei também sabedoria mineral.

Manoel de Barros

Aos meus pais,

Wilma e Francisco

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RESUMO

O Pantanal mato-grossense, com aproximadamente 140.000 km², constitui a maior planície inundável do mundo. É uma região de clima marcadamente sazonal, com cheias durante o verão, as quais não estão relacionadas à precipitação local. Este estudo descreve as estratégias reprodutivas presentes em uma comunidade de anfíbios anuros do Pantanal, Estado de Mato Grosso do Sul, região Central do Brasil. Os dados foram coletados no município de Corumbá (57°00'W, 19°34'S), entre os anos de 1995 e 2001. Foram investigados: o período reprodutivo e o padrão reprodutivo exibido pelas espécies da comunidade, ocupação ambiental, modos reprodutivos, número de ovos por desova, tamanho dos ovos, relações de tamanho-fecundidade, investimento reprodutivo (IR) em machos e fêmeas, dimorfismo sexual em tamanho, além de comportamentos envolvendo corte, acasalamento, territorialidade e cuidado parental. Vinte e quatro espécies de anuros foram registradas, distribuídas entre as famílias Bufonidae, Hylidae, Leptodactylidae e Microhylidae. A atividade reprodutiva concentrou-se no período chuvoso, apresentando um padrão sazonal, com 50% das espécies exibindo atividade reprodutiva explosiva. Dimorfismo sexual em tamanho foi observado para a maioria das espécies, as fêmeas sendo maiores que os machos. O tamanho da fêmea correlacionou-se positivamente com o tamanho da desova e dos ovos. O investimento reprodutivo (IR) em fêmeas, medido como a porcentagem da massa do ovário em relação à massa do corpo, variou de 5,5 a 18%. Comportamentos de desova múltipla, com mais de um macho participando da oviposição, foram observados para *Leptodactylus chaquensis* e *L. podicipinus*. O tamanho relativo de testículos de espécies poliândricas foi maior comparado ao de outras espécies, e a competição de esperma é discutida. Um novo modo reprodutivo para o gênero *Leptodactylus* é apresentado, tendo sido observado para *L. podicipinus.* Os padrões de atividade reprodutiva e modos reprodutivos registrados para a comunidade do presente estudo são comuns em ambientes abertos e sazonais, como o Pantanal. Além disso, variações em características reprodutivas, principalmente relacionadas à fecundidade e IR, parecem ser um reflexo do padrão de atividade e modos reprodutivos das espécies, mas também sofrem influências do clima sazonal da região.

ABSTRACT

The Pantanal, with an area of approximately 140,000 km², constitutes the world's largest floodplain. The climate is markedly seasonal, with floodings occurring in the summer, which are not related to local rainfall. The present study describes the reproductive strategies of an amphibian anuran community in the Pantanal, Mato Grosso do Sul State, central Brazil. Data were collected in the municipality of Corumbá (57°00'W, 19°34'S), between the years 1995 and 2001. Were investigated: the reproductive period and reproductive activity patterns exhibited by the species, habitat use, reproductive modes, number of eggs per clutch and size of eggs, size-fecundity relationships, reproductive investment (RI) in males and females, and sexual size dimorphism (SSD). Additionally, behaviors involved in courtship, mating, territoriality, and parental behavior were also registered. Twenty four species were registered, distributed in the families Bufonidae, Hylidae, Leptodactylidae, and Microhylidae. The reproductive activity concentrated in the rainy period, exhibiting a seasonal pattern. 50% of the species presented the explosive breeding pattern. Sexual size dimorphism was observed for the majority of the species, with females being larger than males. Size of females was positively correlated to clutch and egg size. The RI in females, measured as the percentage of ovary mass relative to body mass, varied from 5.5 to 18%. Multimale spawning behavior was observed for two species, *Leptodactylus chaquensis* and *L. podicipinus*. Relative testes size of poliandric species was larger compared to other species, and sperm competition is discussed. A new reproductive mode for the genus *Leptodactylus* is described, which was observed for *L. podicipinus*. Reproductive activity patterns and reproductive modes registered for the studied community are common in open and seasonal habitats like the Pantanal. Furthermore, variations in the reproductive traits, especially those concerning fecundity and RI, seem to be related to the species reproductive activity pattern and reproductive mode, but also are under the control of the seasonal environmental conditions of the region.

INTRODUÇÃO GERAL

As características das histórias de vida afetam diretamente a reprodução e a sobrevivência dos organismos. A complexidade e o interesse na evolução das histórias de vida surgem devido ao fato de que os organismos possuem diferentes combinações entre essas características, as quais afetam a aptidão (Stearns, 1992). Além disso, as características das histórias de vida têm efeito sobre o crescimento e a manutenção das populações nos diversos tipos de ambientes (Cole, 1954).

A história de vida de um organismo pode ser vista como sendo o resultado de três processos biológicos: a manutenção, o crescimento e a reprodução, onde, para um organismo com tempo e energia limitados, tais processos irão competir por esses recursos (Gadgil & Bossert, 1970). Desta forma, se um organismo destina todos os seus recursos à reprodução, não pode ao mesmo tempo destiná-los todos à sobrevivência; e se dedica muito do seu tempo e energia em cuidado da prole, não pode ao mesmo tempo continuar produzindo um grande número de descendentes. Os benefícios procedentes de um processo que são conseguidos às expensas de outro é denominada permuta ou balanço (“trade-off”) (Stearns, 1992). As permutas têm desempenhado um papel central no desenvolvimento da teoria referente às histórias de vida (Stearns, 1989), sendo que a mais evidente é a que envolve o custo da reprodução. Uma das melhores revisões sobre o tema é apresentada no trabalho de Bell & Koufopanou (1986), que citam duas formas principais de custos associados à reprodução: custo da fecundidade (correlação negativa entre fecundidade atual e futura, devido a restrições na obtenção de energia) e custo da sobrevivência (riscos associados com as atividades reprodutivas que levam a uma correlação negativa entre reprodução e sobrevivência).

A idéia de que a reprodução compete por energia com outras funções no indivíduo é tão antiga quanto a própria biologia (Jönsson & Tuomi, 1994). Desde o surgimento das idéias de Fisher (1930) sobre valor reprodutivo, muitos trabalhos foram feitos com o objetivo de desenvolver modelos que melhor descrevam os custos associados à reprodução nos mais variados grupos taxonômicos (e.g., Williams, 1966; Gadgil & Bossert, 1970; Ricklefs, 1977; Clutton-Brock, 1984; Shine & Schwarzkopf, 1992; Niewiarowski & Dunham, 1998; Sinervo, 1999).

A visão de custo da reprodução discutida até aqui tem relação com a biologia evolutiva, pois está intimamente ligada a uma medida usada para quantificar os efeitos evolutivos da seleção natural: a aptidão, como já mencionado. Porém, todos os comportamentos têm algum custo fisiológico que potencialmente pode ser medido como gasto de energia (Ryan, 1992). Os animais não podem se reproduzir sem gastar energia e diversos pesquisadores têm se empenhado em medir tal gasto energético (e.g., Fitzpatrick, 1973; Bevier, 1997; Testa & Adams, 1998; Buffrenil & Rimblot-Baly, 1999). Assim, o custo da reprodução pode ser estimado no contexto fisiológico, através do gasto energético, ou evolutivo, através da diminuição da aptidão (Ryan, 1992).

O esforço reprodutivo é muito importante na evolução da história de vida, porque através dele é possível medir o custo da reprodução (Stearns, 1992). Segundo o modelo de história de vida proposto por Gadgil & Bossert (1970), esforço reprodutivo é a proporção de recursos disponíveis para um organismo, de determinada idade, que é destinada à reprodução ao longo de um período definido de tempo. Porém, é muito mais fácil definir do que medir tal esforço e, sem dúvida, o modo mais habitual de medi-lo consiste em estabelecer proporções como: massa de gônadas em relação à massa do corpo ou o volume da desova em relação ao volume corporal (e.g., Clark *et al.*,1997; Poizat *et al.*, 1999). Mesmo estando longe do ideal, tais medidas podem ser aproximações úteis quando se pretende unicamente comparar os esforços reprodutivos (Begon *et al.*, 1996). De maneira geral, os métodos utilizados para medidas de custo reprodutivo têm sido alvo de críticas e discussões nos últimos 20 anos (e.g., Stearns, 1989; Reznick, 1992; Sinervo & DeNardo, 1996).

Os anfíbios, pela alternância, na maioria dos casos, de uma fase larval aquática e uma adulta terrestre, e pela estreita dependência de fatores físicos do meio, constituem um grupo interessante para se estudar histórias de vida (Barbault, 1984). Além disso, exibem uma grande diversidade de modos reprodutivos, muito maior que a observada em outros grupos de vertebrados, principalmente os amniotas (Duellman & Trueb, 1986). Mais especificamente, os anuros são organismos que exibem diversas vantagens em estudos de campo, incluindo facilidade de captura, visibilidade e adequação em estudos de experimentação (Howard, 1988), o que se nota pelo aumento do interesse nesse grupo nos últimos anos, principalmente com relação ao comportamento reprodutivo (e.g., Haddad & Giaretta, 1999; Donnelly, 1999; Bastos & Haddad, 2002; Giaretta & Kokubum, 2003).

O conceito de modo de reprodução em anfíbios tal como definido por Salthe (1969) e Salthe & Duellman (1973), é uma combinação de fatores que incluem o local de desova, características da desova e do ovo, taxa e duração do desenvolvimento larval, estágio e tamanho da larva recém eclodida e tipo de cuidado parental, se existir. Para os anuros, Duellman & Trueb (1986) citam 29 modos de reprodução e com o aumento de estudos ecológicos e de história natural, principalmente das espécies Neotropicais, diferentes modos reprodutivos têm sido descritos (e. g., Haddad & Hödl, 1997; Haddad & Pombal Jr., 1998; Haddad & Sawaya, 2000; Prado *et al*., 2002). Cabe então enfatizar que, devido a essas características comportamentais e reprodutivas peculiares, os anuros apresentam-se como organismos-modelo para estudos de diversos aspectos da teoria de histórias de vida, principalmente aquelas relacionadas ao custo da reprodução. Segundo Ryan (1992), os anuros talvez forneçam os melhores exemplos de dois dos custos mais óbvios associados ao comportamento de corte dos machos: risco de predação e gasto energético com vocalização.

Do ponto de vista evolutivo, estudos têm documentado relações entre variações nas características dos machos, como tamanho e persistência na vocalização, e variações no sucesso reprodutivo e susceptibilidade à predação (e.g., Howard, 1981; Woodward, 1982; Wells & Taigen, 1986; Hinshaw & Sullivan, 1990; Haddad & Bastos, 1997). Com relação às fêmeas de anuros, que devotam uma grande quantidade de energia para a reprodução, principalmente na forma de produção de óvulos, o maior interesse na variação do investimento reprodutivo refere-se à relação entre investimento e diferenças interespecíficas em cada modo reprodutivo (Ryan, 1992). Medidas do número, tamanho e conteúdo calórico dos óvulos têm sido usadas para se estimar o custo energético da reprodução em fêmeas (e.g., Salthe & Duellman, 1973; Crump & Kaplan, 1979; Basso, 1990; Lemckert & Shine, 1993).

Características reprodutivas, tais como freqüência de oviposição, número de ovos depositados em um período reprodutivo, o tamanho dos ovos e a quantidade de energia contida neles refletem o modo de vida da espécie e as condições do ambiente em que elas vivem (Stebbins & Cohen, 1995). Um aumento no conhecimento da biologia de anfíbios implica em um aumento do nosso entendimento dos mecanismos da seleção natural e evolução (Pough, 1992). Informações sobre anfíbios podem ser usadas para se testar a generalidade de hipóteses derivadas de estudos com outros taxa e podem, também, gerar hipóteses que poderão ser testadas, por sua vez, em estudos comparativos.

A maior diversidade de espécies de anuros, conseqüentemente de modos reprodutivos, ocorre na região Neotropical, que possui 43,7% das espécies já descritas (Duellman, 1988). Apesar dessa grande diversidade, pode-se dizer que a anurofauna Neotropical é pouco conhecida, principalmente sob o ponto de vista reprodutivo, apesar dos estudos já realizados (e.g., Duellman, 1967; Crump, 1974; Hödl, 1977; Duellman, 1978; McDiarmid & Gorzula, 1989; Praderio & Robinson, 1990; Gascon, 1993; Marquez *et al.*, 1993; Krügel & Richter, 1995). Estudos sobre investimento reprodutivo são ainda mais raros (e.g., Lampo & Medialdea, 1996; Perotti, 1997). No Brasil, a maior parte dos trabalhos com anuros foi realizada na região Sudeste (e.g., Martins *et al.*, 1998; Bastos & Haddad, 1999; Eterovick & Sazima, 2000; Bertoluci & Rodrigues, 2002) e na Floresta Amazônica (e.g., Martins & Moreira, 1991; Magnusson *et al.*, 1999; Neckel-Oliveira *et al.*, 2000).

O Pantanal mato-grossense, com aproximadamente 140.000 km², constitui a maior planície inundável do mundo (Alho *et al.*, 1988). É uma região de clima marcadamente sazonal, com um período de chuvas de outubro a abril e um período de seca de maio a setembro. Apesar de cheias sazonais serem comuns na região, elas não estão relacionadas com a precipitação local, ocorrendo devido à dificuldade de escoamento das águas (Amaral Filho, 1986). O exato período e intensidade das cheias podem variar de um ano para o outro. Essas características fazem do Pantanal um ambiente favorável para o estudo de estratégias reprodutivas, especialmente de animais como anuros, extremamente sensíveis a tais variações do meio físico. Apesar disso, o Pantanal talvez seja uma das áreas menos conhecidas do mundo sob o ponto de vista científico (Por, 1995). Em relação à anurofauna do Pantanal, os estudos são também escassos, tanto sob o ponto de vista de sua composição taxonômica, quanto sob o aspecto da biologia das espécies (Lutz, 1946, 1972; Bosch *et al.*, 1996; Prado & Uetanabaro, 2000; Prado *et al.*, 2000; Strüssmann *et al*., 2000).

Este estudo trata das estratégias reprodutivas presentes em uma comunidade de anfíbios anuros do Pantanal sul, Estado de Mato Grosso do Sul, região Central do Brasil. Os principais componentes das estratégias reprodutivas investigadas foram: o período reprodutivo e o padrão reprodutivo exibido pelas diferentes espécies da comunidade, ocupação ambiental, modos reprodutivos, número de ovos por desova, tamanho dos ovos e as relações de tamanho-fecundidade, investimento reprodutivo em machos e fêmeas, dimorfismo sexual em tamanho, além de comportamentos envolvendo corte, acasalamento, territorialidade e cuidado parental.

A presente tese é apresentada na forma de quatro capítulos. O primeiro capítulo trata dos períodos reprodutivos das espécies de anuros que ocorrem na área de estudo, seus modos reprodutivos e uso do ambiente. O padrão de atividade reprodutiva foi também correlacionado a fatores ambientais, como precipitação, temperatura, umidade relativa do ar e nível do rio. O segundo capítulo descreve as relações de tamanho-fecundidade em fêmeas, ou seja, a relação entre o tamanho do corpo das fêmeas e o número e tamanho de ovos, massa do corpo e massa dos ovários e o investimento reprodutivo (massa dos ovários em relação à massa do corpo), variações sazonais em fecundidade, padrão de deposição do corpo gorduroso, dimorfismo sexual em tamanho e relação entre tamanho das fêmeas e machos amplectados. O terceiro capítulo, publicado recentemente no periódico Journal of Herpetology, descreve a ocorrência de poliandria simultânea, i. e., comportamento de desova múltipla envolvendo vários machos com uma fêmea, em duas espécies do gênero *Leptodactylus*, *L. chaquensis* e *L. podicipinus*, com a análise do tamanho relativo dos testículos em machos. A massa relativa dos testículos foi comparada entre 11 espécies da família Leptodactylidae e mais 22 espécies em diferentes famílias, para as quais existiam dados disponíveis na literatura. No quarto capítulo, publicado no periódico Copeia, é apresentada a descrição de um novo modo reprodutivo para a espécie de rã *Leptodactylus podicipinus* (Anura, Leptodactylidae), com uma revisão sobre os modos reprodutivos apresentados pelo gênero.

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CHAPTER 1

BREEDING ACTIVITY PATTERNS, REPRODUCTIVE MODES, AND HABITAT USE BY ANURANS (AMPHIBIA) IN A SEASONAL ENVIRONMENT IN THE PANTANAL, BRAZIL

PRADO, C. P. A., M. UETANABARO, and C. F. B. HADDAD

BREEDING ACTIVITY PATTERNS, REPRODUCTIVE MODES, AND HABITAT USE BY ANURANS (AMPHIBIA) IN A SEASONAL ENVIRONMENT IN THE PANTANAL, BRAZIL

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Key words: Breeding activity, habitat use, reproductive modes, anurans, Pantanal, seasonality

ABSTRACT. Annual patterns of breeding activity, reproductive modes, and habitat use are described for a frog community in a seasonal environment, in the southern Pantanal, Mato Grosso do Sul state, Brazil. Data were collected monthly between January 1995 and December 1998. A total of 24 species were registered, distributed in the families Bufonidae (3 species), Hylidae (10 species), Leptodactylidae (9 species), and Microhylidae (2 species). Three reproductive activity patterns may be recognized among the studied species: continuous, explosive, and prolonged; 50% of the species were explosive breeders. Seasonal pattern of reproduction was verified for three analyzed years (1995-1997), with most species breeding during the rainy season (Nov-Jan). The reproduction was aseasonal in 1998 due to unexpected rains in the dry season, leading to an unusual breeding activity. Five reproductive modes were registered, with 62.5% of the species exhibiting the generalized aquatic mode, and 33.3% depositing eggs embedded in foam nests. There was a great overlap relative to habitat types used for reproduction, although temporal partitioning and some degree of calling site segregation could be observed. The great occurrence of species that breed early in the rainy season and species with explosive breeding pattern is common in seasonal and open environments as the Pantanal, with a variable and unpredictable rain distribution.

INTRODUCTION

Environmental conditions affects organisms life history traits, playing an important role in structuring and regulating ecological communities (e. g., Toft, 1985; Menge and Olson, 1990; Walther et al., 2002). Amphibian anurans are especially dependent on water and/or atmospheric humidity for reproduction, mainly because they are vulnerable to desiccation, at least in one phase of their lives (e. g., egg, tadpole, or post metamorphic). Generally, anurans are capable of breeding throughout the year, but slight variation in environmental conditions tends to establish breeding seasonality, and annual variation in rainfall seems to be the main factor inducing such periodicity (Jørgensen, 1992). Regarding breeding seasonality, anurans are known to exhibit two basic patterns: 1) tropical species, which are capable of breeding throughout the year; rainfall being the major extrinsic factor controlling reproduction; 2) temperate species, where breeding activity is seasonal and dependent on a combination of temperature and rainfall (Duellman and Trueb, 1986).

Temporal and/or spatial partitioning of reproductive resources constitute mechanisms by which syntopic taxa may avoid competition (Crump, 1971). Anurans may differ in habitat use for breeding, calling site, annual reproductive period, daily period of calling activity, and acoustic features of advertisement call, which are also interpreted as important isolating mechanisms (e. g., Wells, 1977; Haddad et al., 1990). Furthermore, frogs exhibit a great diversity of reproductive modes, mainly in the Neotropics (Duellman and Trueb, 1986), and the comprehension of such diversity is crucial in understanding anuran community organization (Duellman, 1989; Hödl, 1990; Magnusson and Hero, 1991), along with information on temporal and spatial distribution. In spite of this, descriptive data on reproductive patterns and habitat use in anurans, which are important in determining patterns, are available for only a few tropical assemblages (Donnelly and Guyer, 1994). Most studies on breeding activity patterns and habitat use of Neotropical anuran communities have been carried out in the Amazon Basin (e. g., Crump, 1974; Aichinger, 1987; Neckel-Oliveira et al., 2000) and in the Atlantic Rainforest, southeastern Brazil (e. g., Haddad and Sazima, 1992; Bertoluci, 1998; Bertoluci and Rodrigues, 2002). Studies on the phenology and habitat use of anuran communities in open seasonal environments in Brazil are scarce (e. g., Arzabe, 1999; Eterovick and Sazima, 2000).

The Pantanal constitutes the world's largest flood plain (Alho et al., 1988) in Central South America, Brazil. The climate is markedly seasonal, with a defined wet period and a long dry season. Floods are common in the region, but the exact period and intensity of floods may vary from year to year. These features make the Pantanal a favorable environment to study reproductive strategies, especially of anurans, which are extremely sensitive to environmental variations. In spite of this, the anuran fauna of the Pantanal is extremely unknown both regarding its taxonomic composition and biology (e. g., Lutz, 1946, 1972; Bosch et al., 1996; Prado and Uetanabaro, 2000; Prado et al., 2000; Strüssmann et al., 2000; Prado et al., 2002), and studies on anuran community phenology are lacking for this region.

Herein, we present data on an anuran community of a seasonal environment in the southern Pantanal, western Brazil. Data collected during four consecutive years regarding reproductive activity patterns, habitat use, and reproductive modes are described. The influence of climatic conditions on the breeding activity of the species are also verified.

MATERIALS AND METHODS

Study site

The Pantanal is a sedimentary floodplain located in the southwestern Brazil, with an area of approximately 140,000 km² (16° and 22° S; 55° and 58° W), and elevation between 75 and 200 m above sea level. The topography is plain, formed mostly by hidromorphic soils, causing drainage problems, what partially explains its tendency for periodic and prolonged floods (Por, 1995). The low water flow, frequently obstructed by transported aluvional sediments, causes the formation of peculiar water bodies, locally named "baías" (lakes or ponds), "vazantes" (natural water drainage channels during floods), and "corixos" (small permanent water courses with defined channels) (Willink et al., 2000). Climate is 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 (fig. 1), with an annual mean of 1,215 mm at the study site, along the Miranda river, between 1995 and 1998. For the same period, mean annual temperature was 25.1 ºC, varying from 15.8 ºC (June 1996) to 29.5 ºC (January 1998). Floods are common in the region from January to April.

Due to its great extension, and to the diversified topographic, climatic, and floristic traits in each region, the Pantanal is commonly divided into many sub-regions. We use the division proposed by Adámoli (1982), with 10 sub-regions. The study was conducted in the sub-regions Miranda and Abobral, municipality of Corumbá, MS (57°00'W, 19°34'S), which are characterized by open formations included in the Cerrado domain (savanna-like vegetation), with grassland fields and patches of semideciduous forest. Furthermore, vegetation types include riparian forests and patches of monotypical formations as the “paratudal” (composed by trees ‘paratudo’, *Tabebuia aurea*), and the “carandazal” (composed by palm trees ‘carandá’, *Copernicia australis*).

Field activities and statistical analyses

Data were collected monthly from January 1995 to December 1998, with a minimum permanence in the field of four days per month. The reproductive period of each species was determined based on calling males observed or heard during diurnal and nocturnal surveys, and/or presence of gravid females, amplectant pairs, and/or tadpoles. Whenever necessary, tadpoles were collected and reared in the laboratory up to metamorphosis to be identified. Reproductive activity patterns exhibited by the species were classified as: (1) continuous (*sensu* Crump, 1974); (2) prolonged; and (3) explosive (*sensu* Wells, 1977). Sites used for breeding were classified as: rain puddles, flooded areas, permanent and temporary ponds. Reproductive modes were classified according to Duellman and Trueb (1986) and Prado et al. (2002).

Occurrence of seasonal reproductive pattern in a given year was verified through a circular statistical analysis performed for the years 1995 to 1998 (Zar, 1999). Months were converted to angles (e.g., 0° = January to 330° = December, intervals of 30°), and the frequency of occurrence of breeding species within each angle was calculated. The following parameters were estimated for each study year: (1) the mean angle (a), which corresponds to the mean date of the year around which most species were breeding; (2) the circular standard deviation ($\underline{s}$); and (3) the vector $\underline{r}$, a measure of concentration of data around the circle or year. Circular data analyses were performed using the ORIANA software (Kovach, 1994).

The significance of the mean angle ($\underline{a}$), or the occurrence of seasonality, was tested using the nonparametric Rayleigh test ($\underline{z}$). We tested the hypotheses: H_0: breeding species are distributed uniformly (or randomly) around the circle or year; there is no mean direction and consequently, no seasonality; H_A: breeding species are not uniformly distributed around the year; there is a significant mean angle or mean direction and consequently, there is some seasonality. The vector $\underline{r}$, which may vary from 0 (when data is uniformly distributed) to 1 (when all data are concentrated at the same direction), was used as a measure of the "degree" of seasonality (see Morellato et al., 2000). Breeding patterns were also compared among years. When the mean angle ($\underline{a}$) was significant, i. e., when annual breeding pattern was seasonal, we performed two-sample Watson-Williams test ($\underline{F}$) to verify if each year exhibited the same seasonal pattern, or mean angle ($\underline{a}$) (Zar, 1999).

Climatic data were obtained from a meteorological station of the Universidade Federal de Mato Grosso do Sul, located at the study site, at the research station Base de Estudos do Pantanal, and from INPE – Instituto Nacional de Pesquisas Espaciais, in Brazil. River level variation at the study site was obtained from the Departamento de Hidráulica e Transportes/CCET - Universidade Federal de Mato Grosso do Sul. Number of species breeding per month was correlated to monthly maximum river level, mean monthly temperature, monthly total rainfall, and monthly relative atmospheric humidity.

As most variables were not normally distributed (Shapiro-Wilk W test), Spearman's rank correlation coefficients (r_s) were computed (Zar, 1999).

RESULTS

Annual breeding activity patterns

A total of 24 anuran species were registered at the study site, in 13 genera distributed among four families (table 1): Bufonidae (1 genus; 3 species), Hylidae (6 genera; 10 species), Leptodactylidae (4 genera; 9 species), and Microhylidae (2 genera; 2 species). The reproductive period registered for the species are shown in table 1. Three distinct annual breeding patterns were registered for the local anuran community (table 2): (1) continuous breeding, with three species (Hyla nana, Leptodactylus podicipinus, and Lysapsus limellus) reproducing throughout the year; (2) prolonged breeding, with nine species reproducing for at least three consecutive months; and (3) explosive breeding, presented by 50% of the species at the study site, with short periods of reproduction, occurring mainly during the rainy season. Among the prolonged breeders, the majority also reproduced in the rainy and warm season (October to March; fig. 1), but two hylids, Hyla punctata and Scinax fuscomarginatus, began reproduction late in the rainy season, after the flood has occurred, entering the dry months (January to May; table 1). Two explosive breeders, Bufo schneideri and Leptodactylus cf. macrosternum, reproduced sporadically during the dry months (table 1); the latter species is naturally rare at the study site and its reproductive period could be underestimated. Males of L. cf. macrosternum were heard calling following unexpected rains or floods.

Reproduction occurred mainly during the rainy season at the study site, in the years 1995 - 1997, with most of the species breeding between November and January (fig. 1). In 1998, after three months almost without rain, unexpected rains fell in August and September, what caused the occurrence of an explosive breeding activity out of the expected pattern for the region (fig. 1). The circular statistical analysis (Rayleigh test of uniformity) revealed that the breeding activity of frogs was significantly seasonal (table 3) for three of the four years tested, the exception being the year 1998.

Among the years with breeding activity significantly seasonal, the degree of seasonality and mean date of peak of breeding activity were much variable. The degree of seasonality ($\underline{r}$) varied from 0.92 to 0.57 (table 3), which can be considered high values. Comparison of the mean angle among the years showed that the year 1996 (05 January) differed significantly from the years 1995 (14 November; $F = 14.22$; $p < 0.001$) and 1997 (30 November; $F = 6.31$; $p = 0.01$) regarding the peak of breeding activity. The years 1995 and 1997 were not significantly different concerning to the mean date where most species were breeding ($F = 1.46$; $p = 0.23$). Number of species reproducing per month was positively correlated to local rainfall ($r_s = 0.60$; $p < 0.001$; $N = 44$) and temperature ($r_s = 0.51$; $p < 0.001$; $N = 44$), but was not correlated to relative atmospheric humidity ($r_s = 0.18$; $p = 0.33$; $N = 32$) and river level at the study site ($r_s = -0.15$; $p = 0.40$; $N = 32$); river level was not correlated to local rainfall ($p = 0.30$; $N = 32$).

Reproductive modes and habitat use

Among the 24 anuran species registered at the study site, five reproductive modes were observed (table 1). The generalized mode 1 (sensu Duellman and Trueb, 1986), where eggs are deposited in lentic water and exotrophic tadpoles (sensu Thibaudeau and Altig, 1999) develop in water, was the most common mode, occurring in 15 species (62.5%) in the families Bufonidae, Hylidae, Leptodactylidae, and Microhylid (table 1). Eggs embedded in foam nests occurred in eight species (33.3%) in the family Leptodactylidae, but doubts remained concerning the occurrence of a sixth mode in the leptodactylid Adenomera cf. diptyx, because we failed finding nests and tadpoles. The Mode 18, with eggs deposited on leaves above water and exotrophic tadpoles in lentic water, occurred in only one hylid species (4.2%), Phyllomedusa hypochondrialis.

Habitat use was temporally and spatially partitioned by the anuran community at the study site. Three distinct periods could be distinguished based on the breeding activity of the anurans (fig. 2): dry season, rainy season, and flooding season. During the dry season, the three continuous breeders, Hyla nana, Lysapsus limellus, and Leptodactylus podicipinus, were observed reproducing syntopically in permanent ponds ("baías" and/or "corixos"), although, calling site segregation has occurred (fig. 2A). Males of H. nana generally called from grass clumps or emergent aquatic vegetation at the edge or in the middle of the water bodies, L. limellus called from the emergent aquatic vegetation whose leaves lay on the water surface, and L. podicipinus called from the ground at the edge of ponds. Another species that reproduced during the dry season was the explosive breeder B. schneideri, whose males called from the margin of large ponds, generally in places lacking aquatic vegetation. Males of L. cf. macrosternum were observed calling during the dry season in flooded areas formed by unexpected rains or floods.

In the early rainy season, a maximum of 18 species could be heard simultaneously emitting advertisement calls. Many species used the same sites for reproduction (fig. 2B); at least nine species, some of them explosive breeders, could be observed reproducing syntopically in recently formed rain puddles. Among these nine species, males differed regarding calling sites used (fig. 2B). Other species called from large ponds ("baías" and/or "corixos"), as H. nana, Hyla raniceps, L. limellus, and Pseudis paradoxa, but they eventually also occurred in large rain puddles. Adenomera cf. dyptix, Leptodactylus elenae, and L. fuscus are leptodactylids that deposit eggs in foam nests in subterranean chambers out of the water bodies. Males of A. cf. dyptix called from the ground, among grass clumps, in the grassland field subjected to periodic floods. Males of L. elenae called from the ground, among the forest litter in the gallery forest subjected to floods, and males of L. fuscus called from the ground, in open areas at the edge of temporary ponds or depressions also subjected to floods. Phyllomedusa hypochondrialis deposited eggs on leaves of grass above water. Males of this species were observed emitting advertisement calls from the grass leaves in temporary ponds or recently flooded areas with clean and transparent water.

Periodic floods were common from January to April at the study site, and after the water had invaded the grassland fields, the number of species reproducing decreased from about 18 to eight (fig. 2C). During this period, along with the three continuous breeders, some prolonged breeders, as H. raniceps, P. paradoxa, and Physalaemus albonotatus, continued breeding in permanent ponds and flooded areas. The hylids H.

punctata and S. fuscomarginatus, that began reproduction after the floods, were observed reproducing at peculiar sites. Males of H. punctata called from the emergent aquatic vegetation in the middle of large water bodies, generally associated to the river. Males of S. fuscomarginatus called from the emergent grass clumps in flooded grassland fields. Among the continuous breeders, H. nana and L. limellus reproduced in all types of water bodies (permanent ponds, rain puddles, and flooded areas), and Leptodactylus podicipinus occurred in permanent ponds and flooded areas.

DISCUSSION

Annual breeding activity patterns

The Pantanal is included in the Cerrado-Caatinga-Chaco domain (Duellman, 1999), which extends from northeastern Brazil to northern Argentina. This domain is characterized by a subhumid to semiarid climate, high temperatures, and low rainfall, that concentrates in a short rainy season; concerning the topography, it is included in the cis-Andean lowland region (Duellman, 1999). Approximately 100 species of frogs occur in the Cerrado-Caatinga-Chaco domain, and this number is considered low regarding its extension, ca. 4,655,000 km². This low diversity is attributed mainly to the low annual rainfall, long dry season, and plain topography of the region (Duellman, 1999). Rainfall and topography are considered important factors determining diversity and degree of endemism among amphibians in South America (Duellman, 1999). To date, 41 anuran species are known to occur in the Pantanal and surrounding plateau (PCBAP, 1997; Strüssmann et al., 2000), although many regions have not been adequately sampled yet (Strüssmann et al., 2000). Thus, the low diversity of anurans registered for the study site was expected, mainly when compared to forested and humid areas known to exhibit great anuran diversity through South America (e. g., Crump, 1974; Heyer et al., 1990; Duellman, 1999).

Breeding activity in anurans can be divided into two basic patterns: explosive and prolonged (Wells, 1977), where explosive breeding refers to breeding periods of a few days to a few weeks, whereas prolonged breeding refers to breeding periods of more than a month. However, Wells (1977) noted that these categories represent two ends of a continuum, and in fact it is difficult to assign a pattern to tropical species which exhibit variable breeding patterns. As a consequence, many studies present alternative classifications regarding anuran breeding activity patterns (e.g., Crump, 1974; Bertoluci, 1998; Bertoluci and Rodrigues, 2002). At the study site, most species reproduced during the rainy season, where 50% were explosive breeders, 37.5% were prolonged breeders, and 12.5% exhibited continuous reproduction. This seems to be the common pattern in seasonal environments throughout the Neotropics. In southeastern Brazil, at two seasonal sites in the Atlantic Rainforest, only 16 to 11% of the species exhibited continuous breeding, and 60 to 70% of the species reproduced exclusively or predominantly during the rainy season (Bertoluci, 1998; Bertoluci and Rodrigues, 2002, respectively). At two sites in northeastern Brazil, in the Caatinga domain, with a severe dry season, among 20 species registered, most reproduced in the rainy season, but none was observed reproducing throughout the year (Arzabe, 1999).

Although rainfall is one of the most important extrinsic factors determining reproduction in anurans (Duellman and Trueb, 1986; Donnelly and Guyer, 1994), species capable of reproducing throughout the year are not rare in seasonal environments

(Barreto and Moreira, 1996; Prado et al., 2000; Bertoluci and Rodrigues, 2002; present study). In general, continuous breeding species are not habitat specialists, and reproduction can occur due to availability of water bodies along with other favorable abiotic and biotic conditions. This seems to be the case of H. nana, L. podicipinus, and L. limellus in the Pantanal. However, calling activity by male frogs occurs more frequently than actual reproduction (Donnelly and Guyer, 1994), and as a consequence, the link between calling and breeding in the Neotropics should be made with caution. For the continuous breeders in the southern Pantanal, actual reproduction was verified for L. limellus and L. podicipinus in almost all months sampled, including the dry months (see table 1).

Breeding activity of anurans was seasonal at the study site, with most species reproducing in the rainy season in three of the four studied years. Number of species breeding correlated positively to rainfall and temperature. The breeding activity of anurans that inhabit seasonal environments is generally associated to periods of rain, both in the tropics (e. g., Aichinger, 1987; Donnelly and Guyer, 1994, Bertoluci and Rodrigues, 2002) and in temperate regions (e. g., Banks and Beebee, 1986; Caldwell, 1987). However, the occurrence and quantity of rains varied among years at the study site, and this had a direct effect in the breeding period of the anurans; years differed regarding number of active species and peak of breeding activity. The effect of rainfall in regulating anuran breeding activity was most evident when many species that generally reproduce in the rainy season, could also breed in the dry season in 1998, during unexpected heavy rains. A similar situation was registered for an anuran community in another seasonal region in Venezuela (Hoogmoed and Gorzula, 1979), where anuran reproduction is generally seasonal; unexpected reproductive activity of some species occurred throughout the year because there was no dry season. Nevertheless, Donnelly and Guyer (1994) raised the question that assigning pond-breeding anuran assemblages as seasonal, aseasonal, or continuous depends on the sampling frequency, and should be done with care because many species can be explosive and active for only a few days, being not detected in a few-days sample method.

Reproductive modes and habitat use

Reproductive mode in amphibians was defined by Salthe (1969) and Salthe and Duellman (1973) as being a combination of traits that includes oviposition site, ovum and clutch characteristics, rate and duration of development, stage and size of hatchling, and type of parental care, if any. More than 30 modes have been recognized for the anurans (Duellman and Trueb, 1986; Haddad and Sawaya, 2000; Prado et al., 2002), and the greatest anuran diversity regarding reproductive modes occurs in the Neotropics (Duellman, 1988), with 30 known reproductive modes (Haddad and Sawaya, 2000). Reproductive modes tend to be more diversified in forest habitats, with the occurrence of many terrestrial modes, mainly due to high temperatures, high rainfall, and high atmospheric moisture, which in combination prevent eggs from desiccation (Hödl, 1990). In seasonal habitats with a pronounced dry season, the generalized mode, with eggs deposited in lentic water bodies and aquatic tadpoles, is most widespread (Duellman and Trueb, 1986). The generalized reproductive mode occurred in 62.5% of the species in the present study. Such proportion is higher compared to other seasonal environments in

South America, like the Chaco, in Argentina, with 50% of the species exhibiting the generalized reproductive mode (Perotti, 1997), and in a savanna area in Venezuela, where 57.7% of the frog species exhibit the generalized mode (Hoogmoed and Gorzula, 1979). Apart from the markedly seasonal climate, other factors may contribute to the great occurrence of species that exhibit the generalized mode in the Pantanal, as the habitat homogeneity, with absence of small streams and rocky habitats, mainly due to the plain relief which characterizes the region.

The modes that include eggs embedded in foam nests were the second more frequent among the frogs at the study site, being present in 33.3% of the species. High proportions of modes with eggs in foam nests were registered in other seasonal environments: in a study in the Caatinga domain, northeastern Brazil (Arzabe, 1999), 35% of the species exhibited such modes, in the Cerrado domain (Eterovick and Sazima, 2000) approximately 38% of the species were registered laying eggs in foam nests, and in a savanna-like area in Venezuela (Hoogmoed and Gorzula, 1979) 30.8% of the species deposited eggs in foam nests. In contrast, in more humid and forested habitats, these proportions decrease substantially. Studies conducted at the Atlantic Rainforest, southeastern Brazil, registered the occurrence of species with foam nests at proportions varying from 8.7 to 19.2% (Haddad and Sazima, 1992; Bertoluci, 1998; Bertoluci and Rodrigues, 2002), and in the Amazon Basin the proportions varied from 6.2 to approximately 20% (Crump, 1974; Aichinger, 1987; Hödl, 1990). The high proportion of modes with foam nests in the Pantanal, and other seasonal regions, could result from the fact that the foam nest is considered to be an adaptation to seasonal environments, unpredictable regarding rainfall, as the foam prevents egg desiccation when water bodies dry up (Heyer, 1969). Furthermore, other functions of foam nests have been proposed, as the maintenance of a thermally advantageous environment for larval development, as the white foam reflects heat (Gorzula, 1977; Dobkin and Gettinger, 1985; Downie, 1988), which is also important in open and warm habitats as the study site in the Pantanal.

According to De la Riva (1996), species in the genus *Adenomera* can exhibit two reproductive modes: mode 22 (Duellman and Trueb, 1986), with eggs in foam nests deposited in subterranean chambers and endotrophic tadpoles that complete metamorphosis inside the chambers, and mode 21 (Duellman and Trueb, 1986), typical of species in the *Leptodactylus fuscus* group, where foam nests are deposited in subterranean chambers and exotrophic tadpoles, subsequent to floods or rains, are carried to natural water bodies to develop. *Adenomera diptyx* or *Adenomera hylaedactyla* can occur at the study site. These species are parapatric, with a broad zone of contact in the state of Mato Grosso and eastern Bolivia (De la Riva, 1996), but exhibit different reproductive modes. *Adenomera hylaedactyla* presents the reproductive mode 22 and *A. diptyx* exhibits the mode 21, which is considered to be an adaptation to xeric environments where the latter species occurs - the Paraguay River Basin (De la Riva, 1996). Based on morphological description, environmental traits, and geographic distribution provided by De la Riva (1996), we considered the species of *Adenomera* in the present study as being *A. diptyx*. The observation of the reproductive mode and tadpoles are necessary to confirm the identity of this taxon, as well as the occurrence of a sixth reproductive mode in the region.

Many species used the same sites for reproduction at the study area in the southern Pantanal, although, year-round temporal partitioning could be observed (fig. 2). Interspecific competition for reproductive resources (e.g., calling sites, oviposition sites) was suggested to be important in organizing tropical frog assemblages (Crump, 1971). However, many studies have been suggesting that resource partitioning in amphibians may result from three main causes, of which competition is only one; the other two are predation and factors that operate independently of interspecific interactions, as physiological constraints (e.g., Toft, 1985; Duellman, 1989; Donnelly and Guyer, 1994). Duellman (1989) pointed out that in seasonal habitats, abiotic factors, especially seasonal droughts, have detrimental effects on some species populations, mainly anurans. During the dry months, only four species reproduced in permanent ponds at the study site, and this low activity could be attributed to physiological constraints related to lower temperatures and decrease in water availability.

More than two third of the species, most of them explosive breeders, reproduced in the early rainy season at the study site. In this period, after the first heavy rains and soil saturation, temporary ponds and rain puddles formed and were rapidly colonized. At least nine species could be observed breeding at the same habitat type, forming huge and noisy choruses. However, males differed in relation to calling site utilization. For frog communities breeding synchronically, differences in the advertisement call characteristics, and partitioning of calling and oviposition sites are assumed to be important reproductive isolation mechanisms, facilitating species recognition and preventing mismatings (Hödl, 1977; Duellman and Pyles, 1983; Haddad et al., 1990, 1994). Many studies on frog assemblages have demonstrated differential use of calling and oviposition sites (Crump, 1974; Hödl, 1977; Eterovick and Sazima, 2000), as observed for the anuran assemblage in the Pantanal.

However, hybridization between two close related species, included in the Bufo granulosus group, was registered at the study site. Although, hybrid pairs have not been observed during this study, analysis of preserved specimens from that locality revealed the presence of hybrids (P. Narvaes and C. P. A. Prado, pers. obs.). Haddad et al. (1990) also registered hybridization between two species of Bufo, Bufo ictericus and Bufo crucifer, in southeastern Brazil. Although the species differed regarding advertisement calls, hybridization was facilitated by two reasons: (1) the explosive breeding behavior exhibited by the species and (2) the overlap of period and site of reproduction. As explosive breeding is characterized by active search and pair displacement performed by males, there could be reduced opportunities for mate selection by females (see Wells, 1977, 1979), causing the formation of interspecific pairs. Hybridization between species of Bufo in the "granulosus" group at the study site may result from the same reasons, as they are also explosive breeders and breeding period, calling, and oviposition sites were totally overlapped.

In the flooding season, almost all water bodies become connected at the study site, functioning as a big and shallow lake. The number of species of frogs breeding in the flooding season decreased from 18 to about eight. By this period, fish colonization and insect larvae proliferation occur in the flooded field. Although the great water availability, predation pressure on eggs and tadpoles, and maybe, alterations in water chemistry (e. g., pH, oxygen dissolved), may act as inhibitor factors, restricting reproductive activity of many species. Predation on anuran eggs and larvae, the latter mainly by water bugs (Belostomatidae, Hemiptera) and dragonfly nymphs (Odonata), was frequently observed at the aquatic sites during the study. The utilization of recently formed and ephemeral water bodies in the early rainy season, mainly by explosive breeders in seasonal environments, is generally correlated to aquatic predator avoidance (Crump, 1974; Heyer, 1976; Eterovick and Sazima, 2000). Frog species that breed early in the rainy period in seasonal environments, generally, reproduce in rain puddles, deposit many small eggs, exhibit multiple clutches, and rapid larval development, which allow the larvae to explore food sources before insect and fish colonization, avoiding predators at the same time (Barreto and Moreira, 1996; Eterovick and Sazima, 2000). These features make the early breeder species extremely successful in seasonal and unpredictable habitats, which seems to be confirmed by the great occurrence of early breeders and explosive species at the study site in the southern Pantanal.

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Table 1. Frog species registered for the southern Pantanal, Brazil, their reproductive modes, and breeding period based on calling males, recorded from January 1995 to December 1998. ^{R} presence of gravid females, clutches, and/or tadpoles.

Species	Mode	Reproductive Period											
Bufonidae													
Bufo sp. 1 (gr. granulosus)	1	●	●^{R}	●							●^{R}	●^{R}	●
Bufo sp. 2 (gr. granulosus)	1	●	●^{R}	●							●^{R}	●^{R}	●
B. schneideri	1							●^{R}	●^{R}	●^{R}	●^{R}		
Hylidae													
Hyla nana	1	●^{R}	●^{R}	●	●	●	●	●	●^{R}	●^{R}	●^{R}	●^{R}	●
H. punctata	1	●		●	●	●							
H. raniceps	1	●^{R}	●^{R}	●^{R}	●					●^{R}	●	●^{R}	●
Lysapsus limellus	1	●^{R}	●^{R}	●^{R}	●^{R}	●^{R}	●^{R}	●	●^{R}	●^{R}	●^{R}	●	●
Phrynohyas venulosa	1	●^{R}									●^{R}	●	●
Phyllomedusa hypochondrialis	18	●^{R}	●	●						●	●^{R}	●^{R}	●
Pseudis paradoxa	1	●^{R}	●^{R}	●	●^{R}	●					●	●^{R}	●
Scinax acuminatus	1	●^{R}	●^{R}	●							●^{R}	●^{R}	●
S. fuscomarginatus	1	●	●^{R}	●	●	●							
S. nasicus	1	●^{R}	●^{R}	●						●^{R}	●^{R}	●^{R}	●
	Months	J	F	M	A	M	J	J	A	S	O	N	D

Continuation Table 1.

Species	Mode	Reproductive Period											
Leptodactylidae													
Adenomera cf. diptyx	21 or 22	●	●	●							●	●	●
Leptodactylus chaquensis	8	●R	●R	●							●R	●R	●
L. elenae	21	●	●	●							●	●	●
L. fuscus	21	●R	●	●						●	●	●R	●
L. cf. macrosternum	8						●	●	●				
L. podicipinus	8a	●R	●	●R	●R	●R	●	●	●R	●R	●	●R	●
Physalaemus albonotatus	8	●R	●R	●	●						●R	●R	●
P. cf. biligonigerus	8	●		R									R
Pseudopaludicola cf. falcipes	1	●R	●	●								●	●
Microhylidae													
Chiasmocleis mehelyi	1											R	
Elachistocleis cf. bicolor	1	●	●R	●						●	●R	●R	●
	Months	J	F	M	A	M	J	J	A	S	O	N	D

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; (22) foam nest in subterranean chamber; endotrophic tadpoles inside the chamber (Duellman and Trueb, 1986); (8a) eggs and early larval stages in foam nests in basins constructed by males; exotrophic tadpoles in lentic water (Prado et al., 2002).

Table 2. Reproductive activity patterns registered for the frog species in the southern Pantanal, Brazil.

Explosive	Prolonged	Continuous
Bufo sp. 1 (gr. *granulosus*)	*Hyla punctata*	*Hyla nana*
Bufo sp. 2 (gr. *granulosus*)	*H. raniceps*	*Lysapsus limellus*
B. schneideri	*Phyllomedusa hypochondrialis*	*Leptodactylus podicipinus*
Phrynohyas venulosa	*Pseudis paradoxa*	
Scinax acuminatus	*Scinax fuscomarginatus*	
S. nasicus	*Adenomera* cf. *diptyx*	
Leptodactylus chaquensis	*Leptodactylus elenae*	
L. cf. *macrosternum*	*L. fuscus*	
Physalaemus cf. *biligonigerus*	*Physalaemus albonotatus*	
Pseudopaludicola cf. *falcipes*		
Chiasmocleis mehelyi		
Elachistocleis cf. *bicolor*		

Table 3. Results of circular statistical analysis testing for the occurrence of seasonality on breeding activity of frogs in the southern Pantanal, Brazil, in four consecutive years. Mean date (=mean angle) when most species were observed reproducing is shown in parentheses when significant. N = sum of frequencies of breeding species registered at each angle.

	Years			
	1995	1996	1997	1998
Observations (N)	72	101	111	75
Mean angle (a)	314.14º	4.71º	331.04º	215.59º
	(14 Nov)	(5 Jan)	(30 Nov)	
Circular standard deviation (s)	75.82º	83.48º	91.92º	115.33º
Length of mean vector (r)	0.92	0.74	0.57	0.27
Rayleigh test of uniformity (p)	<0.001	<0.001	<0.001	0.27

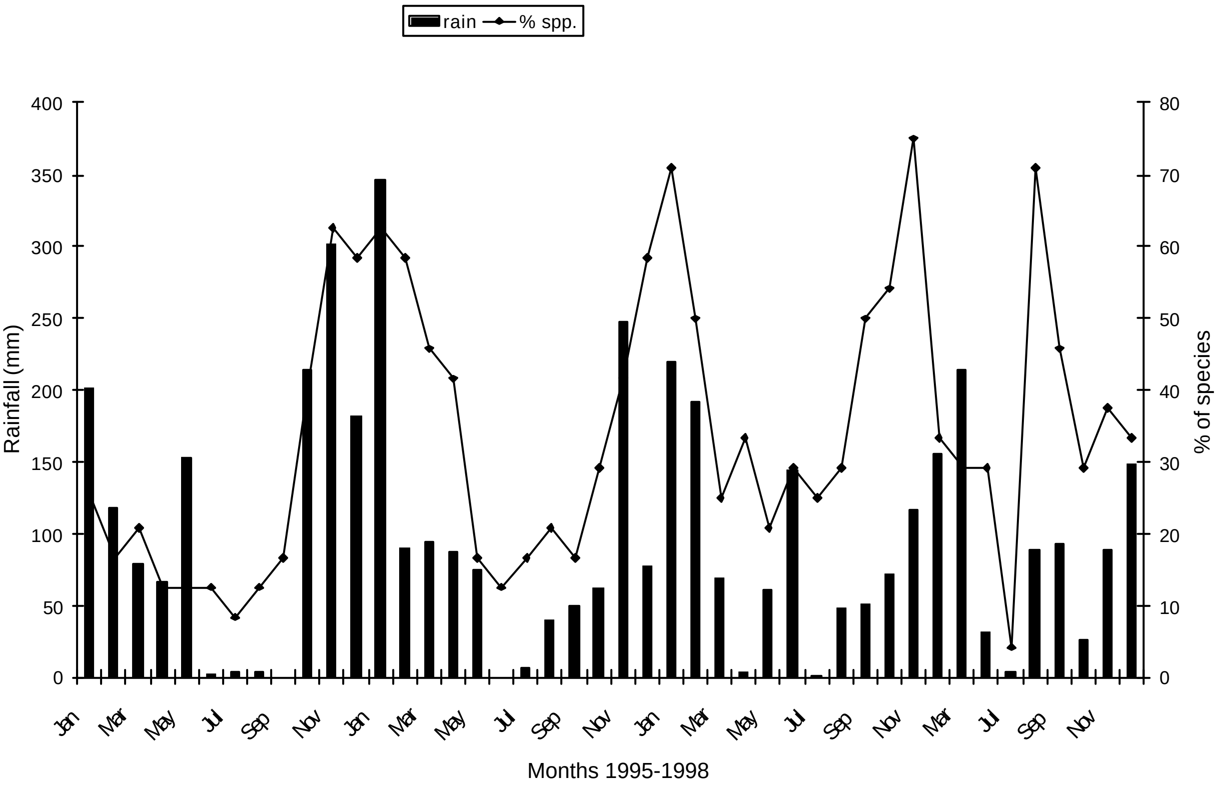

Figure 1. Percentage of species breeding per month and monthly rainfall registered at the study site in the southern Pantanal, Brazil, in the years 1995, 1996, 1997, and 1998. Months not sampled are Dec 1997, Feb, Apr, and Jun 1998.

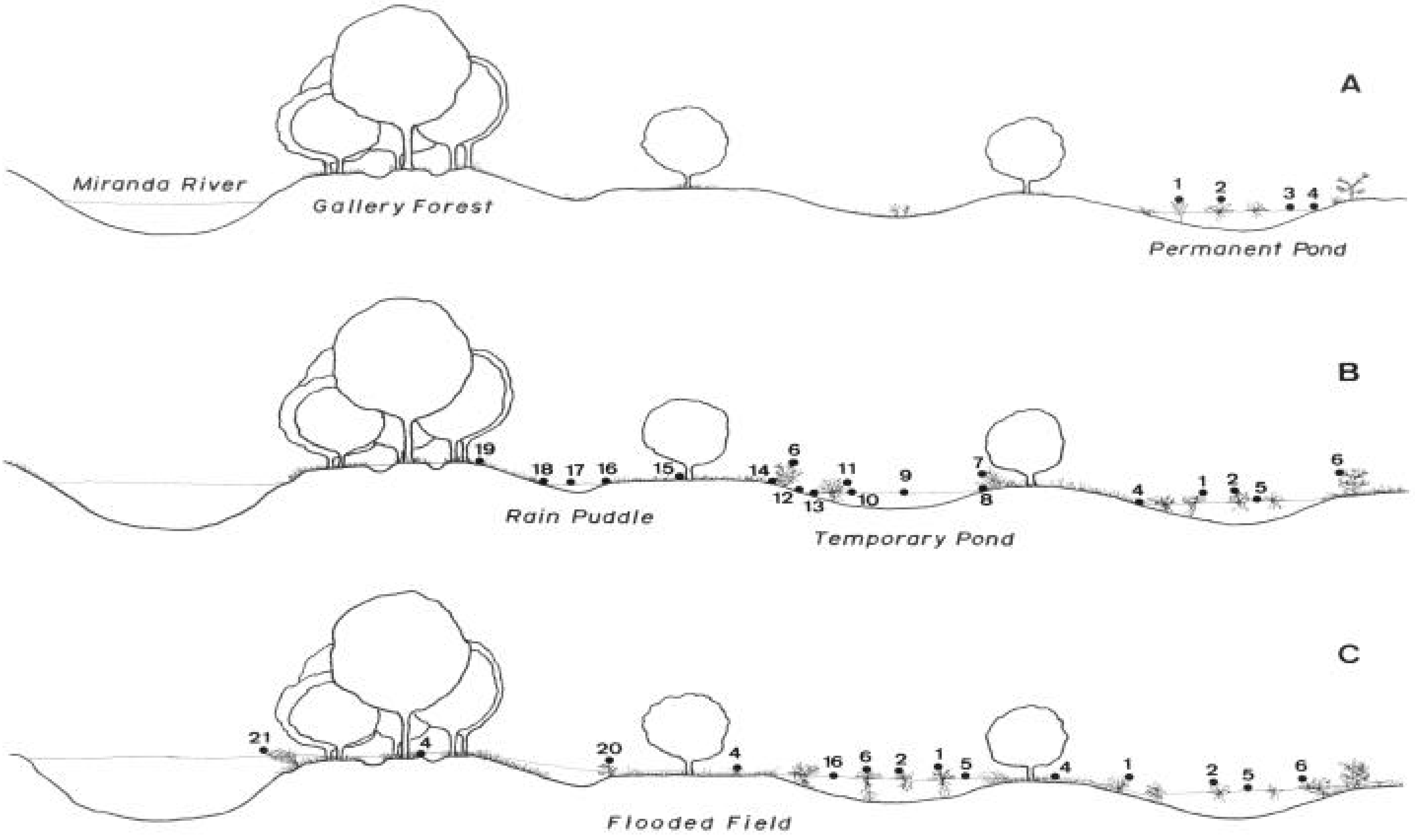

Figure 2. Schematic representation of the spatial and temporal distribution of the frog species registered for the south Pantanal. (A) Dry season; (B) rainy season; (C) flooding season. Species: 1- Hyla nana, 2- Lysapsus limellus, 3- Bufo schneideri, 4- Leptodactylus podicipinus, 5- Pseudis paradoxa, 6- H. raniceps, 7- Phyllomedusa hypochondrialis, 8- L. chaquensis, 9- Phrynohyas venulosa, 10- Scinax nasicus, 11- S. acuminatus, 12- Bufo sp. 1 (gr. granulosus), 13- Bufo sp. 2 (gr. granulosus), 14- L. fuscus, 15- Adenomera cf. diptyx, 16- Pseudopaludicola cf. falcipes, 17- Physalaemus albonotatus, 18- Elachistocleis cf. bicolor, 19- L. elenae, 20- S. fuscomarginatus, and 21- H. punctata.

CHAPTER 2

REPRODUCTIVE STRATEGIES IN AN ANURAN ASSEMBLAGE IN A SEASONAL ENVIRONMENT IN THE PANTANAL, SOUTHWESTERN BRAZIL

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REPRODUCTIVE STRATEGIES IN AN ANURAN ASSEMBLAGE IN A SEASONAL ENVIRONMENT IN THE PANTANAL, SOUTHWESTERN BRAZIL

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ABSTRACT. The reproductive strategies present in an anuran assemblage in the Pantanal, Brazil, were studied. The frog assemblage at the study site was composed by 24 frog species in four families. Three reproductive patterns, explosive, prolonged, and continuous, and five reproductive modes were verified among the species. Sexual size dimorphism in snout-vent length (SVL) and mass was verified for the majority of the species, females being larger than males. Correlation between male and female size in amplectant pairs was investigated for three species. Male size was positively correlated to female size only in Lysapsus limellus. Non-random and assortative mating in these species are herein discussed. Female body size was positively correlated to clutch size among seven of the eight species analyzed. However, results of linear regression analysis between body size and clutch size may be influenced by seasonal variation in fecundity. Interspecific size-fecundity relationships revealed that female SVL was positively correlated to clutch size and egg size, independent on the reproductive mode. Species exhibiting terrestrial reproductive modes presented small clutches and large eggs compared to species with aquatic modes. Among 11 species analyzed, the reproductive investment (ovary mass relative to body mass - RI) varied from 5.5 to 18%, and there were no differences in RI among the reproductive modes. RI correlated negatively to female size; proportionately, larger females tended to invest less in gonads. Among three Leptodactylus species examined, negative correlation between ovary mass and fat body mass was verified for two species. Variations in the reproductive strategies seem to be close related to the reproductive activity patterns and modes exhibited by individual species, but may also be under the control of environmental conditions.

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 the fitness (Stearns, 1992). An important component of the life history is the reproductive strategy, which can be defined as the combination of physiological, morphological, and behavioral traits that act together to produce an optimal number of offspring under certain conditions (Duellman and Trueb, 1986). Some components of the reproductive strategies include gametogenic cycle, reproductive period, reproductive life span, fecundity, duration of development, and reproductive investment, including parental care (Duellman and Trueb, 1986).

According to the life history model proposed by Gadgil and Bossert (1970), the reproductive investment, also called reproductive effort, is the proportion of resources available to an organism of certain age, which is invested in reproduction during a defined period of time. 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 and Shine, 1993; Perotti, 1997). In female frogs, which devote a great amount of energy in reproduction, mainly in egg production, the major interest in reproductive investment is related to interspecific variations occurring in each reproductive mode (Ryan, 1992). Similar interest is related to variation in fecundity and egg size among species with different reproductive modes (e. g., Salthe and Duellman, 1973; Crump, 1974). 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).

Concerning the evolution of life histories, sexual size dimorphism (SSD) is a very studied and controversial point in amphibians (e. g., Shine, 1979, 1989; Halliday and Verrell, 1988; Monnet and Cherry, 2002). Female frogs are usually larger than males, and two main hypotheses have been proposed to explain both the extent and direction of SSD: (1) sexual selection, occurring through male-male competition or female choice of mates (e. g., Shine, 1979; Katsikaros and Shine, 1997; Emerson, 2001) and (2) ecological divergence between the sexes, as different growth rates, age at first maturity, survivorship, and differential energetic constraints (e. g., Howard, 1981; Woolbright, 1989; Monnet and Cherry, 2002). Increase in fecundity has been suggested to be an important selective pressure leading to an increase in female body size (Shine, 1989), at the other side, male-male agonistic interactions could favor larger males (e. g., Shine, 1989; Katsikaros and Shine, 1997). According to Shine (1989), potentially, SSD may evolve in response to any of these factors alone, or through a combination of these evolutionary forces acting simultaneously or in a sequence.

Sexual selection through male-male competition or female choice of mates is also invoked to explain patterns of mating in anurans (e. g., Howard, 1978; Wells, 1979; Tejedo, 1988). Wells (1977) classified anuran reproductive activity into two basic types based on the duration of the breeding season, which he namely prolonged and explosive breeding. Intensity of male-male competition and opportunities for mate choice by females are highly influenced by these two patterns of reproduction, which in consequence influence pair formation (e. g., Tejedo, 1988; Bourne, 1993; Bastos and

Haddad, 1996). In explosive species, breeding period is very short, females arrive at the site simultaneously, males active search for mates and attempt to displace males already in amplexus (Wells, 1979). As a consequence, opportunities for mate selection by females are reduced (Wells, 1979). Females of species with prolonged breeding season arrive at the breeding site asynchronously, males defend territories used as calling sites or for oviposition, and females choose mates among the available males (Wells, 1979).

The reproductive strategies of frog species in a Neotropical seasonal assemblage are herein described. Components of the reproductive strategies investigated were: fecundity and egg size, sexual size dimorphism, pair formation, size-fecundity relationships, and reproductive investment. Seasonal variation in fecundity was investigated, comparing egg production in the wet and dry season. Individual variation in gonad mass and fat body mass was also investigated. Variations in reproductive strategies were discussed in relation to variation in reproductive period, reproductive activity pattern, and reproductive mode exhibited by individual species.

MATERIALS AND METHODS

Study site. The Pantanal is a sedimentary floodplain located in the southwestern Brazil, with an area of approximately 140,000 km² between the paralels 16° and 22° S and 55° and 58° W. It extends through the states of Mato Grosso and Mato Grosso do Sul. Climate in the Pantanal 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 1,215 mm at the study site, between 1995 and 1998. For the same period, mean annual temperature was 25.1 ºC, varying from 15.8 ºC (June 1996) to 29.5 ºC (January 1998). The topography is plain, formed mostly by hidromorphic soils, causing drainage problems, what partially explains its tendency for periodic and prolonged floods (Por, 1995), which are common in the region from January to April.

The study was conducted in the municipality of Corumbá, MS, at the Pantanal Research Station, Federal University of Mato Grosso do Sul (57°00'W, 19°34'S). The region is characterized by open formations included in the Cerrado domain (savanna-like vegetation), with grassland fields and patches of semideciduous forest. Furthermore, vegetation types include riparian forests and patches of monotypical formations as the "paratudal" (composed by trees 'paratudo', Tabebuia aurea), and the "carandazal" (composed by palm trees 'carandá', Copernicia australis).

Data collection. Data were collected from January 1996 to December 1998, with a minimum permanence in the field of four days per month. Additional data were collected in the rainy season (October-March) in the years 1995, 1996, 2001 and 2002. Clutches were collected in the field, or obtained from amplected pairs, and fixed in 5% formalin. Eggs of each clutch were counted (= clutch size) and individual egg diameters were measured to the nearest 0.1 mm with an ocular micrometer in a Zeiss stereomicroscope. When few clutches were found, number and size of mature ovarian eggs were used to estimate clutch and egg size.

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 excess liquid. Amplectant pairs were collected and the ratios male SVL to female SVL, and male mass to female mass were also determined. Percentage of mature ovarian mass relative to body mass was used as a measure of reproductive investment (RI).

The reproductive period of each species was determined based on calling males observed or heard during diurnal and nocturnal surveys, and/or presence of gravid females, amplectant pairs, and/or tadpoles. Reproductive activity patterns exhibited by the species were classified as: (1) continuous (sensu Crump, 1974); (2) prolonged; and (3) explosive (sensu Wells, 1977). Reproductive modes were classified according to Duellman and Trueb (1986) and Prado et al. (2002).

Statistical analysis. Variables were tested for normality (Shapiro Wilk W test) before each analysis. Size-fecundity relationships were verified through linear regression analysis (Zar, 1999). Relationships between clutch size and female SVL and clutch size and female body mass (body mass – ovary mass) were verified for individual species. 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 with log-transformed data. Differences in mean SVL and mass of males and females (including gravid and non gravid females), and between mated and unmated males, were verified through one-tailed Student's t-test. Pearson correlation coefficient was computed to verify correlation between the size of males and females in amplexus. Reproductive investment (RI) was compared among different reproductive modes through the Kruskal-Wallis test. When variables were not normally distributed, nonparametric tests were performed (Mann Whitney U-test and Spearman rank correlation). Results were considered statistically significant at the level of P = 0.05. Statistical analyses were computed in the Excel XP and Statistica 6.0.

RESULTS

Studied frog assemblage. The anuran assemblage at the study site was composed by 24 frog species, distributed among four families: Bufonidae (1 genus; 3 species), Hylidae (6 genera; 10 species), Leptodactylidae (4 genera; 9 species), and Microhylidae (2 genera; 2 species; Table 1). Reproductive activity concentrated in the rainy season (November to January), with 50 % of the species exhibiting the explosive breeding pattern. Three species were continuous breeders, reproducing throughout the year (Hyla nana, Lysapsus limellus, and Leptodactylus podicipinus). Nine species were considered prolonged breeders, reproducing for more than three consecutive months. Five reproductive modes were observed among the frogs at the study site, with 62.5 % exhibiting the generalized aquatic mode, and 33.3 % depositing eggs embedded in foam nests in the water (Table 1; Chapter 1 for details). Terrestrial modes were exhibited by the phyllomedusinae Phyllomedusa hypochondrialis, which deposits eggs in leaves above water and tadpoles develop in lentic water, and the leptodactylinae Leptodactylus fuscus, which deposits eggs in foam nests in terrestrial subterranean chambers and tadpoles are carried to the pond subsequent to flooding. Doubts on the occurrence of a sixth reproductive mode remains relative to Adenomera cf. diptyx. However, based on data presented by De la Riva (1996), herein we assume that this species presents a terrestrial reproductive mode similar to that described above for L. fuscus.

Sexual size dimorphism (SSD) and amplectant pairs. Among 17 species examined, SSD in snout-vent length (SVL) occurred in 13 (76.5%) species, with females being larger than males (Table 2). Females of Scinax fuscomarginatus, Leptodactylus chaquensis, Leptodactylus cf. macrosternum, and Physalaemus albonotatus were not significantly larger than males. Sexual dimorphism in mass was examined for 13 species, and females were heavier in nine species (69%; Table 2). Body mass of males and females were not significantly different in Phrynohyas venulosa, S. fuscomarginatus, L. chaquensis, and P. albonotatus.

Correlation between size of males and females in amplexus was verified for three species: Scinax acuminatus, an explosive breeder, L. limellus, a continuous breeder, and P. albonotatus, a prolonged breeder. Neither SVL nor mass of females were correlated to male size in S. acuminatus and P. albonotatus (Table 3; Fig. 1 A, C). For L. limellus only SVL was investigated, and female SVL was positively correlated to male SVL (Table 3; Fig. 1 B). The ratio male to female size showed that females in amplexus tended to be larger than males in S. acuminatus and L. limellus (Table 3; Fig. 1 A, B). In contrast, for P. albonotatus, males in amplexus tended to be larger and heavier than females (Table 3; Fig. 1 C). Mated and unmated males did not differ significantly in size in S. acuminatus and L. limellus, but mated males were significantly larger and heavier than unmated males in P. albonotatus (Table 4).

Larger females tended to mate with relatively small males compared to small females in S. acuminatus and P. albonotatus. The ratio male to female SVL correlated negatively to female SVL in S. acuminatus ($r = -0.66$; $P < 0.01$; $N = 23$; Fig. 2 A) and P. albonotatus ($r = -0.90$; $P < 0.001$; $N = 14$; Fig. 2 C), but such correlation was not significant for L. limellus (r = -0.28; P = 0.28; N = 17; Fig. 2 B). The ratio male to female mass also correlated negatively to female mass both in S. acuminatus (r = -0.79; P < 0.01; N = 14) and P. albonotatus (r = -0.72; P = 0.04; N =8). Variances in size of males and females in amplexus were then verified. For S. acuminatus, variances in SVL were not significant different (F = 1.51; p = 0.17; N = 23), but females exhibited more variance in mass compared to males (F = 3.93; p < 0.01; N = 14). For P. albonotatus, there were not significant differences in mass (F = 1.01; p = 0.49; N = 8), but variance in female SVL was significantly higher compared to males (F = 4.67; p < 0.01; N = 14). For males and females of L. limellus, variances in SVL were not significantly different (F = 0.80; p = 0.33; N = 17).

Size-fecundity relationships. Of the 24 species occurring at the study site, data on clutch and egg size were obtained for 17 species (Table 5). However, due to the small sample size for some species, relationship between clutch size (= number of eggs per clutch) and female SVL was verified for eight species, and between clutch size and female body mass for six species (Table 6). Body size was positively correlated to clutch size in the majority of the species, with body mass explaining more variation in clutch size compared to SVL (see values of regression coefficients - r^2 in Table 6). For L. fuscus, neither SVL nor body mass were significantly correlated to clutch size.

Using data shown in Tables 2 and 5, interspecific size-fecundity relationships were also verified. Clutch size was positively correlated to 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. 3), 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 to 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. 4). 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% ± 4.4 (N = 11), ranging from 5.5 to 18% (Table 5). For the species exhibiting aquatic eggs, mean RI was 11.7% ± 4.6 (range = 5.5-18%; N = 6), for the frogs with aquatic foam nests, it was 15.9% ± 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. RI was not significantly different among these three categories of reproductive modes (Kruskal-Wallis, $H = 0.92$, df = 2, $P = 0.63$). Ovary mass showed to be strongly correlated to female mass among the species analyzed ($r^2 = 0.91$; $P < 0.001$; N = 11; Fig. 5). Although, results of linear regression analysis revealed a negative correlation between RI and female mass ($r^2 = 0.38$; $P = 0.04$; N = 11; Fig. 6), i. e., females of larger species tend to invest less in ovaries. RI in females of L. chaquensis, an explosive breeder that deposits eggs in foam nests on the water, was much higher than expected (Fig. 6). 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. 6).

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 compared. Females of *H*. *nana* produced, on average, 250 ± 130 (range = 139 - 551; N = 10) mature ovarian eggs in the wet season and 218 ± 106 (range = 120 - 364; N = 7) in the dry season, but such difference was not significant (Mann Whitney *U*-test: W = 28.0; *P* = 0.49). Females of *L*. *podicipinus* produced a mean of 2,272 ± 464 (range = 1,700 - 2,960; N = 9) ovarian eggs in the wet season and a mean of 1,766 ± 393 (range = 1,304 - 2,526; 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).

Correlation between ovary mass and fat body mass was verified for females of three *Leptodactylus* species, *L*. *chaquensis*, *L*. *fuscus*, and *L*. *podicipinus*. A significant negative correlation 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. 7 A) and for the prolonged breeder *L*. *fuscus* (r_s = -0.69; *P* < 0.05; N = 21; Fig. 7 B). For the continuous breeder *L*. *podicipinus*, there was no correlation between ovary mass and fat body mass (r_s = -0.16; *P* > 0.05; N = 27; Fig. 7 C).

DISCUSSION

In anurans, females are larger than males in 90% of the species (Shine, 1979). In the present study, females were larger and heavier than males in the majority of the species. Sexual selection and/or ecological factors have been suggested as responsible for differences in size between males and females (e. g., Shine, 1989; Emerson, 2001;

Monnet and Cherry, 2002). Increase in fecundity is pointed out as being one of the main forces leading to an increase in female body size (e. g., Crump, 1974; Shine, 1989). Herein, clutch size correlated positively to female body size in seven of the eigth species examined, even in those species where SSD was absent (L. chaquensis and P. albonotatus). For several frog species where females are larger than males, it has been shown that larger females produce larger clutches or eggs (e. g., Howard, 1978; Woolbright, 1989). However, in the studies mentioned above, other ecological factors were suggested to explain the larger size of females, as higher predation pressure on larger males, due to their exposure during breeding activities, as territorial defense or calling activity (e. g., Rana catesbeiana: Howard, 1981), or energetic constraints, resulting from male reproductive behavior, that reduced growth (e. g., Eleutherodactylus coqui: Woolbright, 1989).

Of the 17 species examined in the present study, four did not exhibit SSD. Presence of male-male combats was pointed out as leading to an increase in male body size, being responsible for the absence of SSD in some species (Shine, 1979, 1989). For L. chaquensis, male-male combats were already reported (Prado and Haddad, 2003), and it is possible that combats between males are also present in L. cf. macrosternum, a species included in the same group of L. chaquensis, the Leptodactylus ocellatus group (Heyer, 1969). Sexual size dimorphism was also absent in P. albonotatus at the study site, and in a population in Argentina, males are slightly larger and heavier than females (Yanosky et al., 1997). Male-male combats in this species, which is included in the Physalaemus cuvieri group, were not recorded during the present study. However, combats between males were observed for other species in the same group, as P. centralis

(Brasileiro, 1998b), where SSD is also absent (Brasileiro, 1998a), and P. cuvieri (Barreto and Andrade, 1995). These data suggest that it is possible that male-male combats are also present in P. albonotatus. In the case of the hylid S. fuscomarginatus, few females (N = 3) were included in the analysis, what could explain the absence of SSD.

The idea of male-male combats leading to absence of SSD, or males larger than females, was hardly criticized (e. g., Halliday and Verrell, 1986; Halliday, 1987; Halliday and Verrell, 1988) because combats between males were extensively recorded also for species where females are larger than males (e. g., Hyla raniceps: Guimarães, 2000; Guimarães et al. 2001; Phyllomedusa hypochondrialis: C. P. A. Prado, pers. obs.; Scinax rizibilis: Bastos and Haddad, 1999, 2002). More recently, four models have been proposed to explain SSD in anurans (see Halliday and Tejedo, 1995) based on differences in growth trajectories and age at maturation between the sexes. According to this model, divergence in size occurs when growth rates are similar but one sex mature first, or both sexes mature at the same age but continue to grow at different rates, or when age at maturity and growth rates are different in both sexes. There is no SSD when sexes mature at the same age and grow at similar rates. Indeed, most studies show that males mature at earlier ages (e. g., Howard, 1981; Márquez et al., 1997), and in the minority of species in which females are smaller than males, males are the older sex (Monnet and Cherry, 2002). In a recent study including 30 species in eight genera, Monnet and Cherry (2002) also suggest that SSD can be explained in terms of differences in age between the sexes, and that if sexual selection has an effect, it may be secondary.

Among the species where size of males and females in amplexus were analyzed, male size was not significantly correlated to female size in S. acuminatus and P.

albonotatus. For L. limellus, male SVL was positively correlated to female SVL. Mating systems of most anurans are non-random, which is generally attributed to male-male competition or female choice (e. g., Wells, 1977; Robertson, 1990). Positive correlation between size of males and females in amplexus has been reported for many anuran species, indicating assortative mating, with females choosing their mates by size (e. g., Robertson, 1990; Bourne, 1993; Bastos and Haddad, 1996). Lysapsus limellus is a continuous breeder (Prado and Uetanabaro, 2000; present study), which implies that females may have the opportunity to choose calling males that are resident and defend territories (C. P. A. Prado, pers. obs.). For S. acuminatus and P. albonotatus, where size of males and females in amplexus were not correlated, intensity of male-male competition could explain the lack of such correlation. Scinax acuminatus exhibits an explosive breeding pattern, which is characterized by alternative tactics employed by males to obtain mates, as active search of females and attempts to displace amplexed males (Wells, 1977). Even if females of S. acuminatus exhibit preferences, males' employment of alternative tactics may mask female choice, what was observed for other species (e. g., Wells, 1979; Bourne, 1993). Physalaemus albonotatus is a prolonged breeder and is included among the minority of the species where sexes are not dimorphic in size (present study). In P. centralis, another species in the P. cuvieri group where SSD is also absent, size of males and females in amplexus were not correlated (Brasileiro, 1998a) and male figths are relatively common (Brasileiro, 1998b). Male-male competition in these species may also affect mating strategy, with larger males excluding or diminishing mating sucssess of smaller males by controlling better territories. Similar situation was reported for Adelotus brevis, a species where males are larger than females

(Katsikaros and Shine, 1997). An evidence which may support above prediction was the fact that among the three species analyzed, only in P. albonotatus unmated males were significantly smaller than mated ones.

For the species dimorphic in size, amplexed males were smaller than females. On average, amplexed males of L. limellus attained 85% of the females' SVL, and males of S. acuminatus reached 84% of females' mass and 98% of females' SVL. For some anuran species with axillary amplexus, it has been shown that females choose males of a certain size, proportionately smaller, to better juxtapose their cloacae and optimize egg fertilization (e. g., Licht, 1976; Robertson, 1990; Bourne, 1993; Bastos and Haddad, 1996). However, amplexed males of P. albonotatus were slightly larger and heavier than females, and, apparently, this fact did not affect egg fertilization or ovipisition behavior (C. P. A. Prado, pers. obs.). Variances in size of males and females in amplexus were analyzed. For S. acuminatus and P. albonotatus, females were more variable in size than males, what could explain the negative correlation between the ratio male to female size and female size. Larger females may not have males proportionately large to mate with compared to small females. For L. limellus, variances in size of males and females did not differ, and no negative correlation was found.

Although female size correlated positively to clutch size in most species, values of the regression coefficients (r^2) were much variable among species herein analyzed. Body size explained from 25% of variation in clutch size in H. raniceps and L. limellus, up to 65% in L. podicipinus. Female size is positively correlated to fecundity in many species of frogs (e. g., Berven 1988; Lemckert and Shine, 1993; Lüddecke, 2002), but egg production may exhibit considerable variation among females in a single population (e.

g., Berven 1988; Lemckert and Shine, 1993). Besides size, other factors may influence female fecundity, as environmental conditions and female nutritional state (e. g., Ryser, 1988, 1989; Lemckert and Shine, 1993). In prolonged or continuous breeder species, egg production may also differ if measured at different moments (e. g., early, mid, or late season) during the breeding season (e. g., Praderio and Robinson, 1990; Giaretta and Kokubum, 2003). Variation in the regression coefficients (r^2) 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 to 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, as mentioned above. In the study conducted in northern Brazil, only females from a single reproductive episode were included in the analysis. But such differences could also be related to population differences, or even, they could be explained by the existence of more than one species being currently identified under the name *L. fuscus* (Wynn and Heyer, 2001).

Our results on variation in fecundity of *L. podicipinus* and *H. nana* seem to support predictions mentioned 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, and similar results were registered for another continuous breeder in the Pantanal, *L. limellus* (Prado and Uetanabaro, 2000), and for *Colostethus trinitatus* in a seasonal region in Venezuela (Praderio and Robinson, 1990). Analysing all available females and clutches of L. podicipinus, nor SVL neither body mass correlated significantly to 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 separetely by seasons, both SVL and body mass were highly correlated to clutch size (Table 6). In contrast, the other continuous breeder, H. nana, did not exhibit differences in egg production between the dry and rainy seasons at the study site. Even including females from different seasons in the analysis, body size was positively correlated to clutch size. Although both species are continuous breeders, our results suggest that they exhibit different responses to environmental changes. In the case of L. podicipinus, prey availability may diminish during the dry season affecting female nutritional state (Praderio and Robinson, 1990) 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 and Duellman, 1973; Crump, 1974; Duellman and Trueb, 1986). Such negative correlation has been extensively confirmed by many studies on reproductive modes in anurans (e. g., Crump and Kaplan, 1979; Hödl, 1990; Perotti, 1997). Although only three terrestrial modes occur at the study site, such tendency could also be 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, presented smaller clutches and larger eggs (Figs. 3 and 4) compared to species exhibiting aquatic modes (aquactic eggs and aquatic foam nests). Two species that exhibit aquatic foam nests, L. podicipinus and P. albonotatus, presented larger clutches compared to other species. Perotti (1997) compared the ovary size factor

(Duellman and 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 community, L. chaquensis and Physalaemus biligonigerus, that also deposit eggs in foam nests on the water, presented higher values of fecundity compared to other species (Perotti, 1997). It would be necessary data on other species with aquatic foam nests to test whether such large clutches are phylogenetic determined or a common trait associated to their reproductive mode.

Female SVL was positively correlated to clutch size in 16 species in different anuran families in this study, as registered before for other frog assemblages (e. g., Crump, 1974; Lang, 1995). However, body mass and mature ovary mass showed a much higher 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, 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 variation relative to egg size. All the stream-breeding species exhibited relative large eggs, probably a restriction associated to the oviposition environment. Perotti (1997) compared egg size among species with different reproductive modes and found that egg size was positively correlated to body size, as observed in the present study.

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: aquatic eggs (RI of 11.7%), aquatic foam nests (RI of 15.9%), and terrestrial eggs (RI of 11.5%). Percentage of clutch volume relative to body volume was examined for 23 species in different families and with different reproductive modes in Santa Cecilia, Ecuador (Crump, 1974). Similarly to values observed for the frogs in the present study, at Santa Cecilia relative clutch size 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. An inverse relationship was also observed for the frog community in the present study. This negative trend indicates that as body size increases, proportionately less of the body size is accounted 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 (Figs. 5 and 6). Ecological differences among these species regarding reproductive activity patterns, reproductive modes, 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 and Shine, 1993). Based on presence of immature ovarian eggs in nearly every gravid female, Crump (1974) suggested that females breed repeatedly throughout the year. 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 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 pattern. In the Pantanal, L. chaquensis is an explosive breeder that breeds during few days in the rainy season, and only on rainy days (Prado et al., 2000). As a consequence, females of this species may not have the opportunity to lay many clutches during a single breeding season, what could lead to a great investment in gonads. 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, what could explain the extremely low investment in gonads.

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 to ovary mass, i. e., females with ovaries containing eggs exhibited smaller fat bodies. However, for L. podicipinus, fat body mass was not correlated to ovary mass. In temperate regions, fat bodies in anurans are primarily known to serve as nutritional reserves, mainly during hibernation (Saidapur and Hoque, 1996). However, involvement of fat bodies in gametogenesis was reported for many species both in temperate (e.g., Long, 1987) and in tropical regions (e. g., Pancharatna and Saidapur, 1985; Saidapur and Hoque, 1996). 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 and Saidapur, 1985), suggesting that lipids are used for vitellogenesis, what seems to be the cases of L. chaquensis and L. fuscus in the present study. For L. podicipinus, fat body mass did not correlate negatively to 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 situation could the case of 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, reproduce only in the rainy season, L. podicipinus reproduces throughout the year, which could imply in less 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 concentrates in a short rainy season. Then, 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. These features make the Pantanal an interesting environment to study the reproductive strategies of organisms like the majority of the anurans, which are extremely sensitive to environmental variations. Results herein described for the frog assemblage at the study site in the Pantanal suggest that variations among reproductive strategies, as variation in mating systems, egg production, pattern of fat deposition, and reproductive investment, may be related to the reproductive activity patterns and modes of reproduction exhibited by each species, but also may be under the control of the environmental conditions, i. e., adapted to the seasonal climate of the region.

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Table 1. Reproductive modes, reproductive activity patterns, and reproductive period registered for the anuran species in the south Pantanal. 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); (8a) eggs and early larval stages in foam nests in water-filled basins constructed by males; exotrophic tadpoles in lentic water (Prado et al., 2002). * Reproductive period based on presence of gravid females.

Species	Mode	Reproductive Pattern	Reproductive period
Bufonidae			
Bufo sp. 1 (gr. *granulosus*)	1	explosive	Oct-Mar
Bufo sp. 2 (gr. *granulosus*)	1	explosive	Oct-Mar
B. schneideri	1	explosive	Jul-Oct
Hylidae			
Hyla nana	1	continuous	Jan-Dec
H. punctata	1	prolonged	Jan-May
H. raniceps	1	prolonged	Sep-Apr
Lysapsus limellus	1	continuous	Jan-Dec
Phrynohyas venulosa	1	explosive	Oct-Jan
Phyllomedusa hypochondrialis	18	prolonged	Sep-Mar
Pseudis paradoxa	1	prolonged	Oct-May
Scinax acuminatus	1	explosive	Oct-Mar

Continuation Table 1.

Species	Mode	Reproductive Pattern	Reproductive period
Hylidae			
S. *fuscomarginatus*	1	prolonged	Jan-May
S. *nasicus*	1	explosive	Sep-Mar
Leptodactylidae			
Adenomera cf. *diptyx*	21 (?)	prolonged	Oct-Mar
Leptodactylus *chaquensis*	8	explosive	Oct-Mar
L. *elenae*	21	prolonged	Oct-Mar
L. *fuscus*	21	prolonged	Sep-Mar
L. cf. *macrosternum*	8	explosive	Jun-Aug
L. *podicipinus*	8a	continuous	Jan-Dec
Physalaemus *albonotatus*	8	prolonged	Oct-Apr
P. cf. *biligonigerus*	8	explosive	Dec-Mar*
Pseudopaludicola cf. *falcipes*	1	explosive	Nov-Mar
Microhylidae			
Chiasmocleis *mehelyi*	1	explosive	Nov*
Elachistocleis cf. *bicolor*	1	explosive	Sep-Mar

Table 2. Comparisons (t-values) of mean±SD snout-vent length and mean±SD mass of males and females of anuran species in the Pantanal, Brazil. Range and sample size in parenthesis. In bold results statistically not significant.

Species	SVL (mm)		Mass (g)		*SVL*		*Mass*	
	female	male	female	male	t	*P*	t	*P*
Bufonidae								
Bufo schneideri	150 ± 24	128 ± 14	___	___	2.7	<0.01	___	___
	(117-186; 7)	(103-155; 17)	___	___				
Hylidae								
Hyla nana	21.3 ± 1.5	19.5 ± 0.6	0.6 ± 0.1	0.4 ± 0.1	3.6	<0.01	2.7	<0.01
	(18,8-23,8; 13)	(18,6-20,4; 7)	(0,3-0,7; 13)	(0,3-0,6; 7)				
H. raniceps	60.2 ± 4.4	55.7 ± 3.4	12.3 ± 3.4	9.3 ± 2.1	5.4	<0.01	4.5	<0.01
	(51.0-70.8; 34)	(44.1-66.2; 73)	(7.2-22.5; 32)	(4.5-13.6; 73)				
Lysapsus limellus	18.6 ± 1.4	16.6 ± 1.0	___	___	21.8	<0.01	___	___
	(16.0-23.0; 45)	(14.5-20.2; 43)	___	___				
Phrynohyas venulosa	77.4 ± 6.0	72.7 ± 5.7	34.5 ± 10.8	27.3 ± 9.0	1.7	0.05	**1.5**	**0.07**
	(67.7-86.0; 10)	(64.0-80.0; 8)	(20.3-56.9; 10)	(16.1-39.4; 8)				
Phyllomedusa hypochondrialis	43.0 ± 1.5	39.0 ± 1.8	4.0 ± 0.5	2.7 ± 0.5	6.0	<0.01	6.5	<0.01
	(41.3-46.0; 10)	(36.5-43.0; 19)	(3.1-4.8; 10)	(1.8-3.5; 18)				
Pseudis paradoxa	57.5 ± 8.0	50.4 ± 4.5	27.2 ± 13.5	16.6 ± 5.1	3.3	<0.01	2.8	<0.01
	(45.5-73.2; 17)	(40.6-60.0; 25)	(8.6-62.2; 14)	(8.5-24.0; 23)				
Scinax acuminatus	43.1 ± 2.0	42.0 ± 1.5	6.5 ± 1.2	5.8 ± 0.8	2.6	<0.01	2.6	<0.01
	(40.4-48.0; 34)	(39.4-46.3; 37)	(4.9-9.3; 33)	(3.9-7.9; 37)				
S. fuscomarginatus	22.0 ± 0.9	21.4 ± 0.7	0.5 ± 0.0	0.6 ± 0.1	**1.4**	**0.08**	**0.2**	**0.41**
	(21.0-22.7; 3)	(19.7-23.4; 28)	(0.5-0.6; 3)	(0.4-0.7; 26)				

Continuation Table 2.

Species	SVL (mm)		Mass (g)		SVL		Mass	
	female	male	female	male	t	P	t	P
Leptodactylidae								
Leptodactylus chaquensis	71.3 ± 4.5	71.3 ± 5.1	34.8 ± 6.5	35.9±8.4	**0.0**	**0.98**	**0.7**	**0.50**
	(60.7-80.1; 50)	(58.2-82.8; 34)	(21.9-49.0; 50)	(18.4-63.0; 34)				
L. fuscus	43.6 ± 1.9	42.6 ± 1.7	8.6 ± 1.3	7.6 ± 1.0	2.0	0.02	3.4	<0.01
	(40.0-46.2; 21)	(37.6-45.8; 47)	(6.9-11.3; 21)	(4.8-9.2; 47)				
L. cf.. macrosternum	103 ± 17	111 ± 6.8	___	___	**0.9**	**0.20**	___	___
	(91-115; 2)	(102-119; 4)	___	___				
L. podicipinus	39.5 ± 2.1	35.2 ± 1.3	6.1 ± 1.0	4.3 ± 0.5	9.3	<0.01	6.8	<0.01
	(35.6-44.0; 36)	(31.6-37.0; 21)	(4.4-8.2; 27)	(3.2-5.3; 20)				
Physalaemus albonotatus	23.5 ± 2.3	24.1 ± 2.6	1.2 ± 0.4	1.2 ± 0.4	**0.8**	**0.21**	**0.2**	**0.41**
	(20.1-27.0; 41)	(16.6-27.2; 33)	(0.5-2.3; 37)	(0.3-1.8; 29)				
Pseudopaludicola cf. falcipes	14.3 ± 0.8	12.5 ± 0.6	0.30 ± 0.07	0.20 ± 0.02	4.6	<0.01	2.9	0.02
	(13.5-15.0; 3)	(11.6-13.4; 10)	(0.25-0.35; 2)	(0.19-0.25; 5)				
Microhylidae								
Chiasmocleis mehelyi	23.8 ± 0.6	20.8 ± 0.3	___	___	8.8	<0.01	___	___
	(23.3-24.5; 3)	(20.6-21.1; 4)	___	___				
Elachistocleis cf. bicolor	26.1 ± 1.5	22.8 ± 2.0	1.4 ± 0.4	1.1 ± 0.4	5.0	<0.01	1.7	0.04
	(23.3-28.7; 13)	(20.5-27.5; 22)	(0.8-2.3; 9)	(0.6-1.8; 21)				

Table 3. Mean±SD ratio of male to female body size in amplectant pairs of three anuran species in the Pantanal, Brazil. Pearson correlation coefficients computed between male and female body size and occurrence of sexual size dimorphism (SSD) are shown. Range of the ratio male size to female size in parenthesis.

	SVL (mm)			Mass (g)			SSD
Species	N	M/F ratio	r	N	M/F ratio	r	
S. *acuminatus*	23	0.98 ± 0.0	0.37	14	0.84 ± 0.1	0.12	yes
		(0.86-1.04)	*P* = 0.08		(0.57-1.07)	*P* = 0.68	
L. *limellus*	17	0.85 ± 0.0	0.52	—	—	—	yes
		(0.78-0.91)	*P* = 0.03				
P. *albonotatus*	14	1.08 ± 0.1	0.43	08	1.40 ± 0.4	0.16	no
		(0.95-1.28)	*P* = 0.13		(1.04-2.17)	*P* = 0.70	

Table 4. Comparisons (*t*-values) of mean±SD snout-vent length and mean±SD mass of mated and unmated males of three anuran species in the Pantanal, Brazil. Range and sample size in parenthesis.

	SVL (mm)				Mass (g)			
Species	Mated	Unmated	*t*	P	Mated	Unmated	*t*	P
S. acuminatus	42.1±1.6	41.5±1.6	0.96	0.17	5.7±0.5	6.0±0.7	0.96	0.17
	(39.6-46.3; 23)	(39.4-44.4; 10)			(4.7-6.5; 14)	(4.9-6.9; 10)		
L. limellus	18.1±1.0	17.9±1.7	0.25	0.40	—	—	—	—
	(16.6-20.8; 17)	(16.0-20.3; 8)						
P. albonotatus	25.6±1.1	22.9±2.8	3.7	<0.01	1.5±0.2	1.0±0.4	2.9	<0.01
	(23.4-27.2; 14)	(16.6-26.6; 18)			(1.1-1.8; 8)	(0.3-1.6; 18)		

Table 5 – Mean±SD of clutch size (number of eggs per clutch), egg diameter, ovary mass, and reproductive investment (RI) for females of anurans in the Pantanal, Brazil. Range and sample size in parenthesis. * Parameters based on ovarian eggs.

Species	Clutch size	Egg diameter (mm)	Ovary mass (g)	RI (%) (ovarymass/bodymass)
Hylidae				
Hyla nana	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)
H. raniceps	1,991 ± 533 (1,220-3,096; 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)
Lysapsus limellus	63 ± 20 (27-117; 18)	1.2 ± 0.05 (1.1-1.2; 16)	___	___
Phrynohyas venulosa	3,981 ± 271 (3,625-4,310; 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)
Phyllomedusa hypochondrialis	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)
Pseudis paradoxa	1,834 ± 1,194 (1,000-4,624; 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)
Scinax acuminatus	879 ± 274 (594-1,352; 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)
S. fuscomarginatus	337 ± 132 (185-421; 3)	0.9 ± 0.05 (0.9-1.0; 10)	0.034 (1)	6.6 (1)

Continuation Table 5.

Species	Clutch size	Egg diameter (mm)	Ovary mass (g)	RI (%) (ovarymass/bodymass)
Leptodactylidae				
Adenomera cf. diptyx	36 (1)*	1.4 ± 0.14 (1.2-1.5; 5)*	___	___
Leptodactylus chaquensis	4,936 ± 1,720 (3,007-8,375; 32)*	1.5 ± 0.09 (1.4-1.7; 35)	5.87 ± 1.72 (2.97-8.92; 26)	16.0 ± 2.9 (9.9-21.2; 26)
L. fuscus	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)
L. podicipinus	2,102 ± 442 (1,750-2,953; 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)
Physalaemus albonotatus	719 ± 433 (246-1,562; 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)
Physalaemus cf. biligonigerus	281 (1)*	0.8 ± 0.05 (0.7-0.9; 10)*	___	___
Pseudopaludicola cf. falcipes	32 (1)	0.8 ± 0.02 (0.8-0.9; 9)	___	___
Microhylidae				
Chiasmocleis mehelyi	217 ± 33 (183-250; 3)*	1.3 ± 0,09 (1.2-1.4; 30)*	___	___
Elachistocleis cf. bicolor	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)

Table 6. 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. Results statistically not significant marked in bold.

Species	Log SVL (mm) *Vs*. Log clutch size	Log body mass (g) *Vs*. Log clutch size
Hylidae		
Hyla nana	$r^2 = 0.59$; $P < 0.01$; $N = 12$ $y = -4.0 + 4.8\ x$	—
H. raniceps	$r^2 = 0.25$; $P = 0.03$; $N = 23$ $y = -0.15 + 1.9\ x$	$r^2 = 0.34$; $P < 0.01$; $N = 23$ $y = 2.6 + 0.57\ x$
Lysapsus limellus	$r^2 = 0.25$; $P = 0.03$; $N = 19$ $y = -2.8 + 3.5\ x$	—
Scinax acuminatus	**$r^2 = 0.41$; $P = 0.06$; $N = 9$**	$r^2 = 0.51$; $P = 0.03$; $N = 9$ $y = 1.61 + 1.8\ x$
Leptodactylidae		
Leptodactylus chaquensis	$r^2 = 0.30$; $P < 0.01$; $N = 26$ $y = -0.89 + 2.5\ x$	$r^2 = 0.57$; $P < 0.01$; $N = 26$ $y = 2.0 + 1.1\ x$
L. fuscus	**$r^2 = 0.19$; $P = 0.21$; $N = 10$**	**$r^2 = 0.05$; $P = 0.5$; $N = 10$**
L. podicipinus	$r^2 = 0.65$; $P < 0.01$; $N = 9$ $y = -4.0 + 4.6\ x$	$r^2 = 0.62$; $P = 0.01$; $N = 9$ $y = 2.2 + 1.5\ x$
Physalaemus albonotatus	**$r^2 = 0.18$; $P = 0.16$; $N = 12$**	$r^2 = 0.42$; $P = 0.02$; $N = 12$ $y = 2.6 + 0.82\ x$

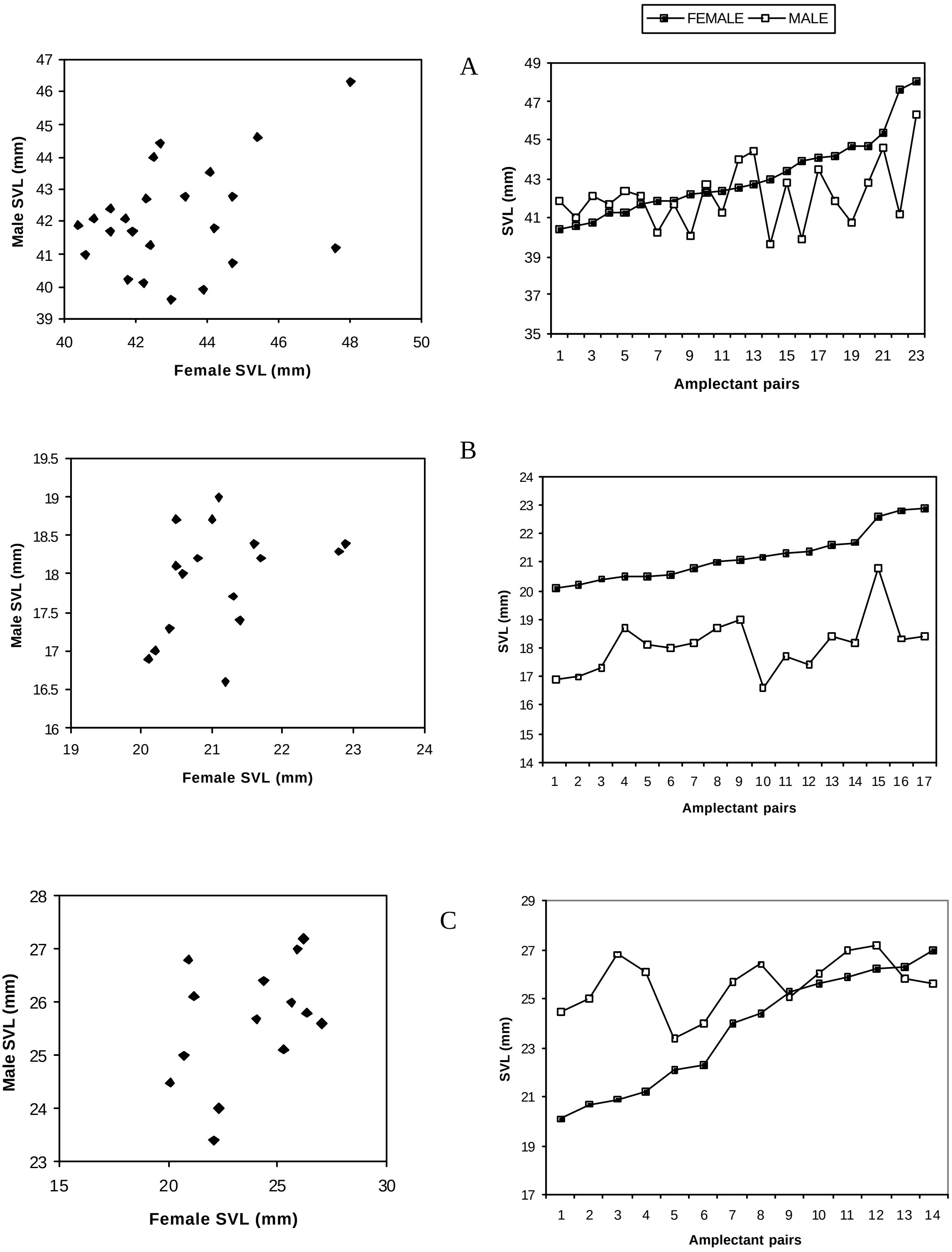

Fig. 1. Relationship between the snout-vent length (SVL) of males and females in amplexus (left) and body size variation between amplectant males and females (right). (A) Scinax acuminatus, (B) Lysapsus limellus, and (C) Physalaemus albonotatus.

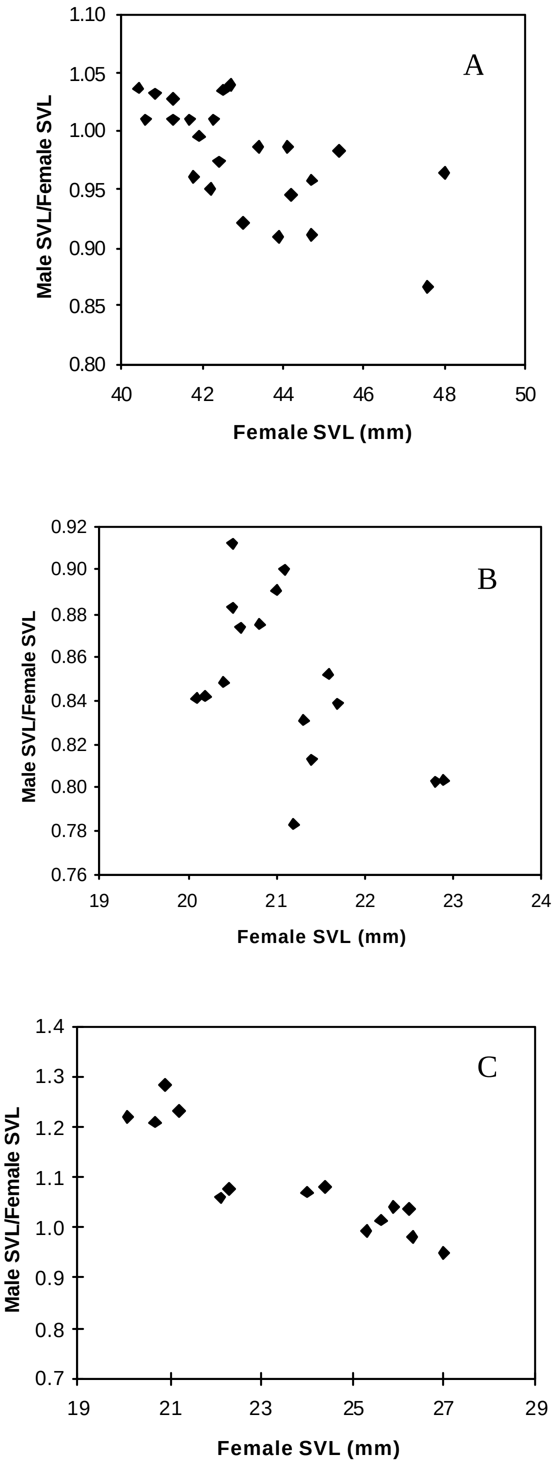

Fig. 2. Relationship between the snout-vent length (SVL) of females and the ratio male SVL to female SVL in amplexed pairs of (A) Scinax acuminatus, (B) Lysapsus limellus, and (C) Physalaemus albonotatus.

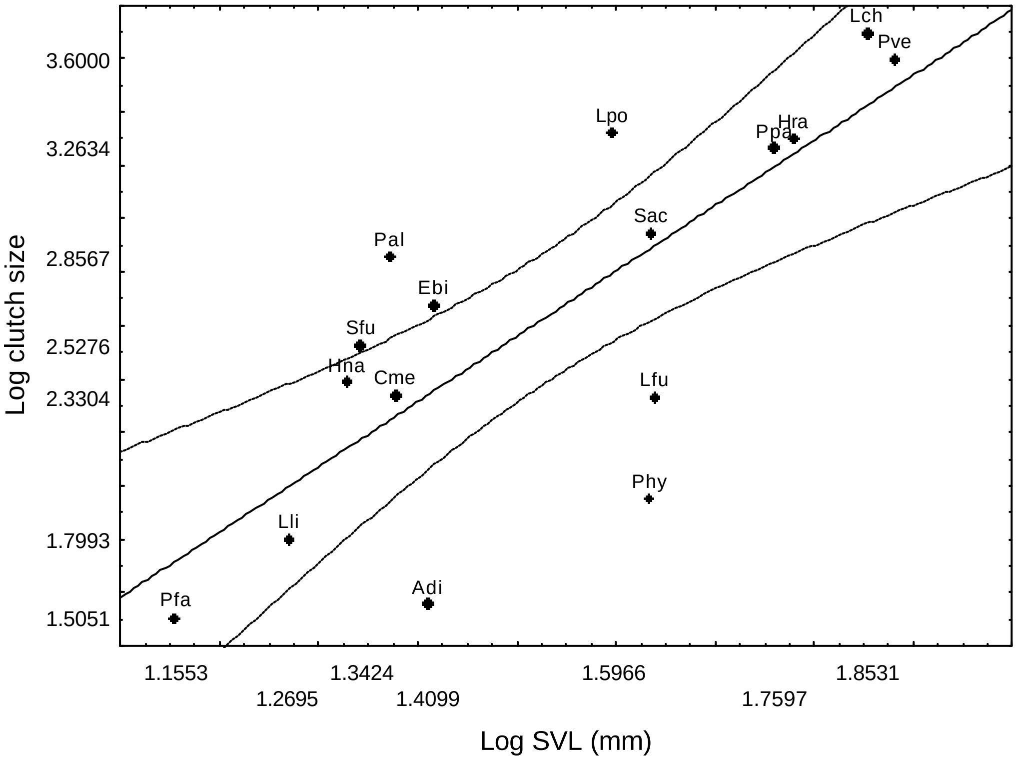

Fig. 3. Relationship between mean log female SVL and log clutch size for 16 anuran species in the Pantanal (log y = -1.1 + 2.4 log x). Species: (Adi) Adenomera cf. diptyx; (Cme) Chiasmocleis mehelyi; (Ebi) Elachistocleis cf. bicolor; (Hna) Hyla nana; (Hra) H. raniceps; (Lch) Leptodactylus chaquensis; (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. Dashed lines represent 95% confidence interval.

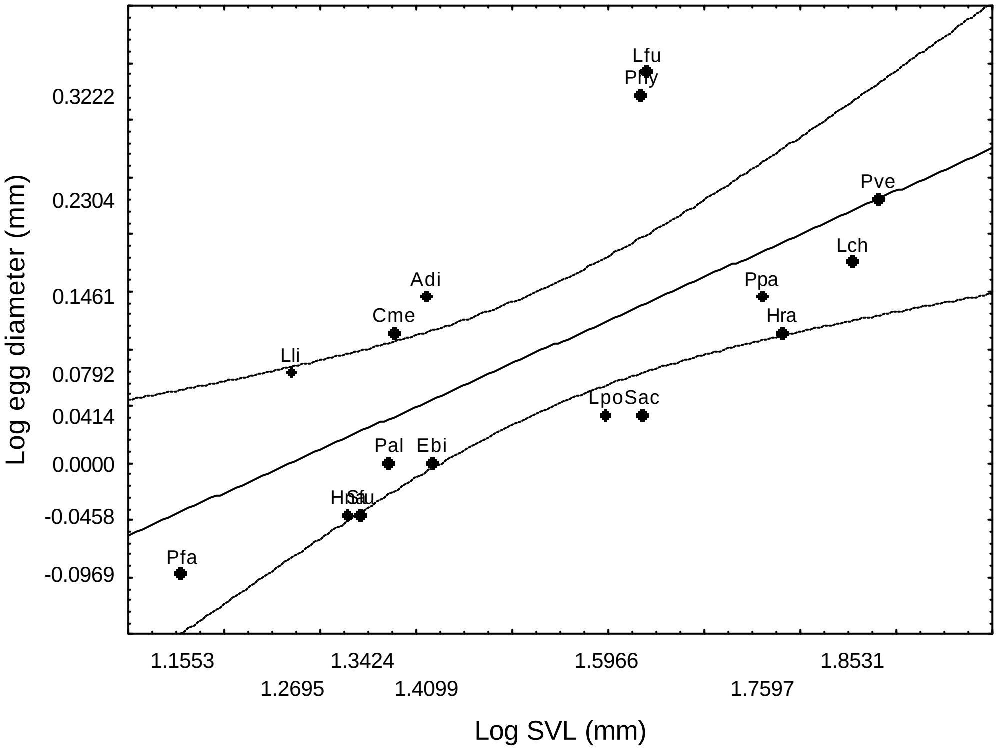

Fig. 4. Relationship between mean log female SVL and log egg size for 16 anuran species in the Pantanal (log y = -0.48 + 0.38 log x). Species: (Adi) Adenomera cf. diptyx; (Cme) Chiasmocleis mehelyi; (Ebi) Elachistocleis cf. bicolor; (Hna) Hyla nana; (Hra) H. raniceps; (Lch) Leptodactylus chaquensis; (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. Dashed lines represent 95% confidence interval.

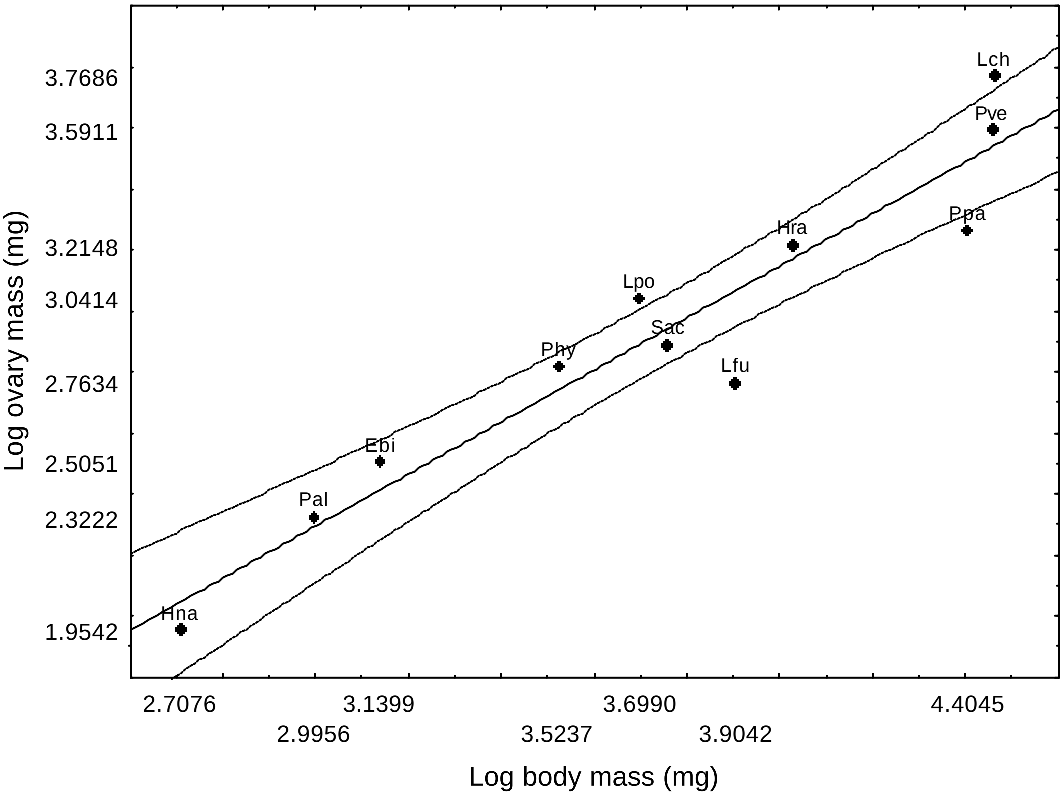

Fig. 5. Relationship between mean log female body mass and log ovary mass for 11 anuran species in the Pantanal (log y = -0.27 + 0.85 log x). Species: (Ebi) Elachistocleis cf. bicolor; (Hna) Hyla nana; (Hra) H. raniceps; (Lch) Leptodactylus chaquensis; (Lfu) L. fuscus; (Lpo) L. podicipinus; (Pve) Phrynohyas venulosa; (Phy) Phyllomedusa hypochondrialis; (Pal) Physalaemus albonotatus; (Ppa) Pseudis paradoxa; (Sac) Scinax acuminatus. Dashed lines represent 95% confidence interval.

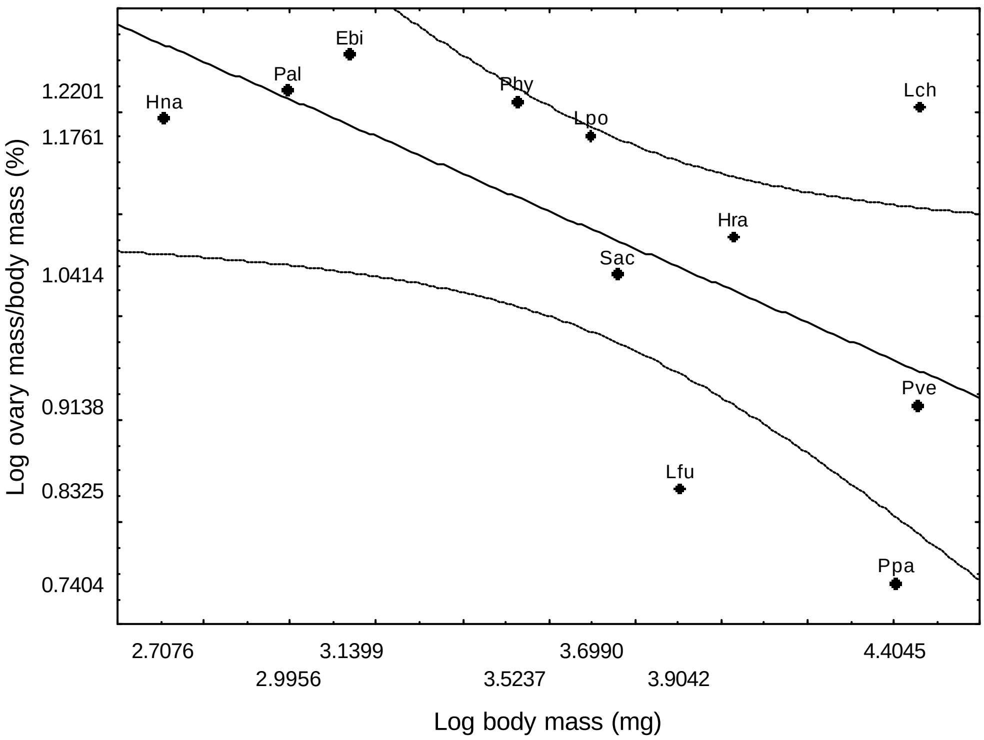

Fig. 6. Relationship between mean log female body mass and log ovary mass/body mass (reproductive investment) for 11 anuran species in the Pantanal (log y = 1.7 - 0.18 log x). Species: (Ebi) Elachistocleis cf. bicolor; (Hna) Hyla nana; (Hra) H. raniceps; (Lch) Leptodactylus chaquensis; (Lfu) L. fuscus; (Lpo) L. podicipinus; (Pve) Phrynohyas venulosa; (Phy) Phyllomedusa hypochondrialis; (Pal) Physalaemus albonotatus; (Ppa) Pseudis paradoxa; (Sac) Scinax acuminatus. Dashed lines represent 95% confidence interval.

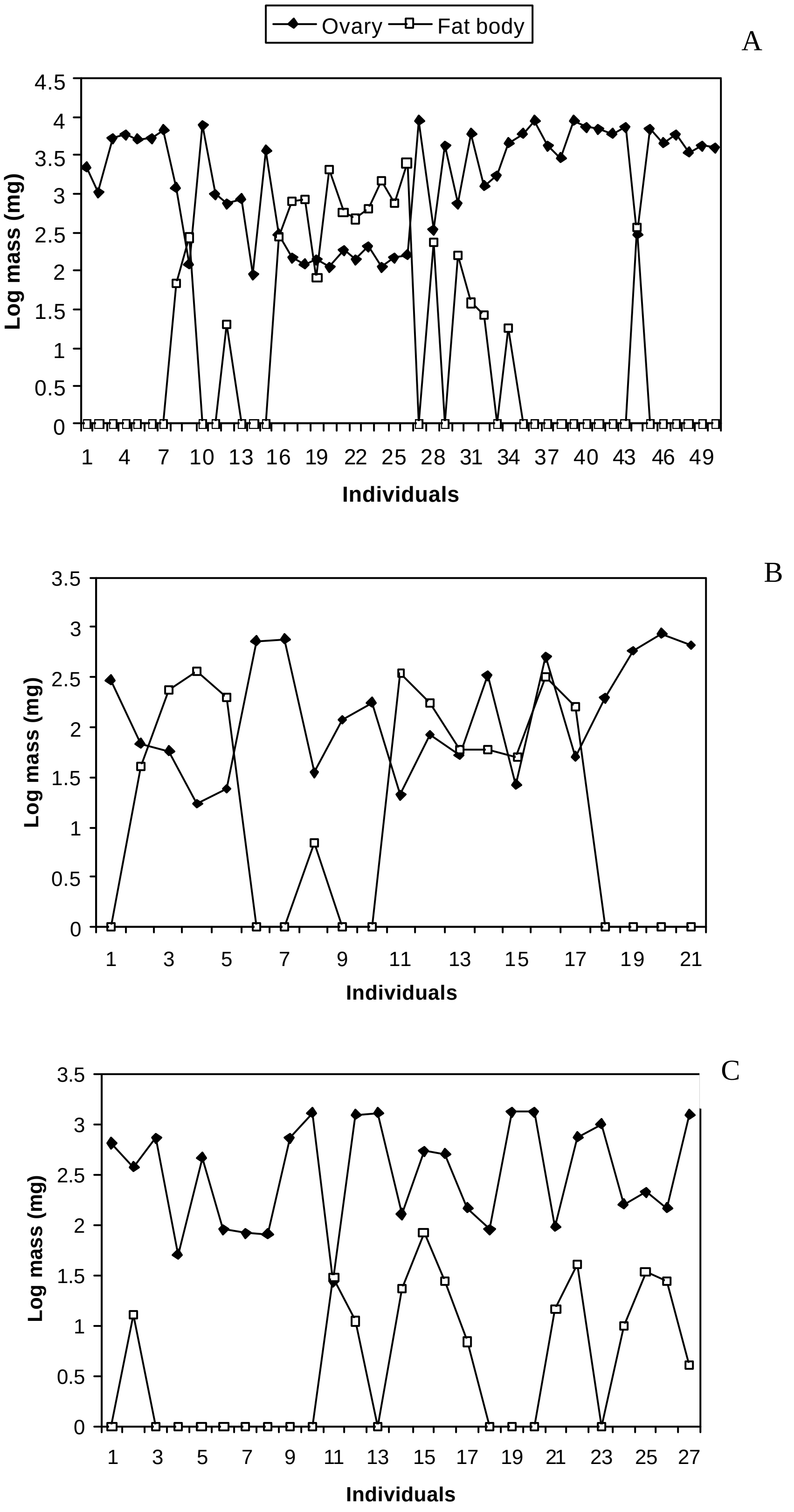

Fig. 7. Variation in ovary mass and fat body mass in females of (A) Leptodactylus chaquensis, (B) L. fuscus, and (C) L. podicipinus in the Pantanal, Brazil.

CHAPTER 3

TESTES SIZE IN LEPTODACTYLID FROGS AND OCCURRENCE OF MULTI-MALE SPAWNING IN THE GENUS *LEPTODACTYLUS* IN BRAZIL

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TESTES SIZE IN LEPTODACTYLID FROGS AND OCCURRENCE OF MULTI-MALE SPAWNING IN THE GENUS LEPTODACTYLUS IN BRAZIL

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Key words: Multi–male Spawning, Testes Size, Sperm Competition, Anura, Leptodactylidae, Leptodactylus

ABSTRACT. - Although rare, some anuran species are known to show multi-male spawning, and multiple paternity and testes size positively related to sperm competition intensity have been demonstrated. Herein we present data on testes mass relative to body mass for 11 leptodactylid species, and report the occurrence of additional males taking part in spawning of Leptodactylus chaquensis and L. podicipinus. Testes mass averaged 4.13 % of body mass for L. chaquensis and 0.75 % for L. podicipinus. Testes size of both leptodactylid species with multi–male spawning show that they are large and close to values recorded for rhacophorid frogs with multi-male breeding (0.71–7.79 %). Large testes size in frogs with multi–male spawning supports the sperm competition hypothesis, and indicates that sperm competition and multiple paternity may be commoner in frogs than currently thought.

INTRODUCTION

Parker (1970) defined sperm competition as competition between sperm of two or more males to fertilize ova of a single female. Sperm competition has been invoked based on two criteria: (1) direct observation of multiple mating by individual females during a single reproductive episode, and (2) indirect inference of multiple mating through paternity analyses (Birkhead, 1995). Although this phenomenon is taxonomically widespread, including animals with external and internal fertilization, much more attention has been devoted to animals with internal fertilization (Byrne, 2002).

Fertilization is external in most frogs, and generally a single male grasps a female dorsally in amplexus until the female spawns (Kusano et al., 1991). The male sheds sperm onto the eggs almost simultaneously with spawning. Although sperm competition is poorly studied in anurans, some species are known to show multi-male breeding, in which two or more males grasp, or associate with, a single female (e.g., Pyburn, 1970; Fukuyama, 1991; Jennions and Passmore, 1993; Kasuya et al., 1996; Kaminsky, 1997). Multiple paternity has recently been demonstrated through genetic analyses for the phyllomedusine Agalychnis callidryas (D'Orgeix and Turner, 1995) and the myobatrachid Crinia georgiana (Roberts et al., 1999). Indirect evidences showed that it also occurs in the rhacophorid Chiromantis xerampelina (Jennions and Passmore, 1993).

Since sperm number affects likelihood of paternity when females breed with more than one male, selection will favor males with large testes and large, high quality ejaculates (see Birkhead, 1995). Several studies have demonstrated that there is a positive relationship between testes size and intensity of sperm competition, estimated by the number of males a female is likely to breed with, in several vertebrate groups (e.g., Warner and Robertson, 1978; Kenagy and Trombulak, 1986, Birkhead and Moller, 1992). In frogs, testes size is also related to the intensity of sperm competition. Many species that show multi-male breeding have relatively large testes compared with other species where this behavior is unknown (Kusano et al., 1991; Jennions and Passmore, 1993; Emerson, 1997).

To date, multi-male spawning in anurans has been directly observed in six rhacophorids (Coe, 1967, 1974; Fukuyama, 1991; Feng and Narins, 1991; Jennions et al., 1992; Kasuya et al., 1996), three hylids (Pyburn, 1970; Roberts, 1994), one bufonid (Kaminsky, 1997), and one myobatrachid (Roberts et al., 1999). In this paper, we describe multi-male spawning behavior in two foam nest-building species, Leptodactylus chaquensis and L. podicipinus (Leptodactylidae), and compare testes size relative to body size among foam nest-building leptodactylids exhibiting different reproductive modes. Testes size was also compared among 33 frog species in five families: Bufonidae, Hylidae, Leptodactylidae, Ranidae, and Rhacophoridae.

MATERIALS AND METHODS

Multi-male Spawning Behavior. - Observations were carried out in the Pantanal, a great floodplain with an area of about 140,000 km², in the municipality of Corumbá (19º34'S, 57º00'W), Mato Grosso do Sul state, southwestern Brazil. Climate is of the "Aw" type in Köppen's classification, with a dry winter from May to September and a rainy summer from October to April. Floods are common, generally from January to April. The region is dominated by savanna-like formations ("cerrado") and gallery forests.

Field observations of mating behavior of L. chaquensis and L. podicipinus were made from October 2000 to April 2001. Leptodactylus chaquensis breeds during the rainy period at the study site, from October to March, in puddles and flooded areas, and males typically call during rain (Prado et al., 2000). Eggs embedded in foam nests are deposited on the water surface. *Leptodactylus podicipinus* breeds at the edges of permanent ponds and flooded areas throughout the year; eggs in foam nests are deposited in basins constructed by males (Prado et al., 2002). In both species females attend nests and tadpoles (Prado et al., 2000; Martins, 2001). Calling males, amplectant pairs, and foam nests were located by active searching. The sites were marked and behavioral sequences at breeding sites were recorded. Only individuals involved in multi-male spawning were collected, measured to the nearest 0.1 mm, and preserved.

Testes Size. - Testes and body sizes were analyzed for 11 leptodactylids in two genera: *Leptodactylus* and *Physalaemus*. Both genera are characterized by depositing eggs embedded in foam nests. *Leptodactylus* species analyzed were: *L. chaquensis*, *L. fuscus*, *L. labyrinthicus*, *L. macrosternum*, *L. mystacinus*, *L. notoaktites*, *L. ocellatus*, and *L. podicipinus*. Reproductive modes among the *Leptodactylus* species exhibit a continuum, from the deposition of the foam nest on the surface of water, to deposition of the foam inside burrows far from the water (Heyer, 1969; Prado et al., 2002). *Physalaemus albonotatus*, *P. crombiei*, and *P. nattereri*, three species that deposit foam nests on the surface of water, were also investigated.

Data from *L. chaquensis*, *L. podicipinus*, *L. fuscus*, and *P. albonotatus* were obtained from males collected in the Pantanal (19º34'S, 57º00'W), Brazil, from December 1996 to November 1997, and in January 1999. Data on the seven other Brazilian leptodactylids were obtained from preserved specimens housed in the Célio F. B. Haddad collection, deposited in Departamento de Zoologia, Universidade Estadual Paulista, Rio Claro, São Paulo state, Brazil.

Body and testes mass was measured in the laboratory after the frogs were blotted to remove excess liquid. Body and testes mass of each specimen was determined to the nearest 0.001 g for larger species, and 0.0001 g for the smaller ones on electronic balances. Our data on relative testes size in leptodactylids (2 genera, 11 species) were compared to data published for species in the families Bufonidae (1 genus, 4 species), Hylidae (1 genus, 1 species), Ranidae (3 genera, 5 species), and Rhacophoridae (4 genera, 8 species) (Kusano et al., 1991; Jennions and Passmore, 1993). Leptodactylids were also compared to species in the family Hylidae (2 genera, 4 species), housed in the Célio F. B. Haddad collection. Considering allometric relationships between organ size and body size, and to allow biological interpretation and comparison between studies and taxa (King, 2000), we calculated linear regressions of variables using log-transformed data (Zar, 1999). Only males captured during the breeding season and with well-developed secondary sexual characteristics (e.g., vocal sacs, nuptial excrescences) were included in this study, as testes size can vary seasonally (Ko et al., 1998).

Comparative studies are generally confounded by phylogenetic effects, and comparative methods based on reconstructed phylogenies are recommended (Harvey and Pagel, 1995). However, phylogenies of Brazilian anuran species are not available. Alternatively, we used taxonomic classification as an approximate evolutionary tree (Harvey and Purvis, 1991), and, to minimize the problem of non-idependence of species data points (Felsenstein, 1985), we included, whenever possible, more than one genus in each analyzed family (Emerson, 1997). Testes mass relative to body mass was also compared between species that show multi-male spawning and those where this behavior is unknown.

RESULTS

Multi-male Spawning Behavior in Leptodactylus chaquensis. - On 13 November 2000, males of L. chaquensis were observed calling during the day in a recently formed puddle. We returned to this site at dusk, and observed two males calling and fighting in the middle of a nest. An hour later, four males gathered around the nest and began to emit low calls. The largest male, which was always in the center of the nest, emitted louder calls, jumped toward the other frogs, and hit them with its head. Sometimes these encounters ended in wrestling bouts, with the largest male clasping the other around the pectoral region with the forelimbs, with throats and venters pressed together. Additional males arrived, and finally, eight males were within the nest (the largest in the middle and others on the periphery), when a female jumped within the nest and one of the males grasped her. The other males attempted to displace the amplectant male by pushing their heads between the pair; minutes later the pair broke apart. Then, the largest male grasped the female and immediately they started spawning in bouts of churning. At the same time the seven peripheral males began to churn into the foam nest in synchrony with the pair, with their legs kicking the foam in the same way of the amplexed male, but none of them touched the pair in amplexus (Fig. 1). All individuals within the nest were collected. The female SVL was 75.1 mm, the amplectant male measured 86.0 mm, and mean (±SD) SVL of the peripheral males was 72.5 ± 3.74 mm (range = 68.0-76.9 mm, N = 7).

In two other foam nests we observed large males calling in the center of each nest surrounded by four males. Additionally, in December 2000, two nests were observed: one with four males and two females, the other with three males and one female. Male-male combats were recorded in three nests; however, pair formations or multi-male spawning behavior were not observed.

Multi-male Spawning Behavior in Leptodactylus podicipinus. - On 4 April 2001 at 2310 h, at the edge of a permanent pond, we found a male L. podicipinus calling inside a basin under a leaf. We found a female inside the basin close to the calling male; around 0135 h the pair entered in amplexus, and immediately began to spawn. We noticed that, in fact, there were two males grasping the female, a small one in the middle and the larger, resident male on the top (Fig. 2). We failed to notice the exact moment the second male approached, but no calling or movements were detected. The three frogs remained together, both males churning into the foam nest. The eggs were deposited during circular movements performed by the frogs. The larger male released its hold of the still amplectant pair, at which time, the frogs were collected. The female SVL was 36.8 mm, the smaller male measured 32.3 mm, and the larger 36.8 mm.

Testes Size. - Percentage of testes mass relative to body mass in species of the family Leptodactylidae ranged 0.04-4.13 % (Table 1). Two species presented much larger relative testes size compared to the other leptodactylids; testes mass relative to body mass averaged 4.13 % for L. chaquensis, and 0.75 % for L. podicipinus. In the remaining leptodactylids, testes size ranged from 0.04 to 0.19 %. Testes mass relative to body mass was much smaller in L. fuscus, L. labyrinthicus, L. mystacinus, and L. notoaktites (range = 0.04-0.06 %). Relative testes size varied less among species in the genus Physalaemus (range = 0.12-0.19 %). Linear regression calculated for the leptodactylids revealed a significant positive relationship between body mass and testes mass ($r^2 = 0.63$, $P = 0.003$, $N = 11$) (Fig. 3). Figure 3 shows that both Leptodactylus species with multi-male spawning herein described lie above the 95 % confidence interval of the calculated regression.

Considering the species analyzed in Table 1, testes size relative to body size varied less among bufonids (range = 0.13-0.39 %), hylids (range = 0.14-0.53 %), and ranids (range = 0.17-0.45 %). Similarly to leptodactylids, rhacophorid species exhibited a great variation in relative testes size (range = 0.25-7.79 %). Rhacophorids that show multi-male breeding presented larger relative testes size (Table 1). Among the rhacophorid multi-breeders, percentage of testes mass relative to body mass range from 0.71 % in Polypedates leucomystax to 7.79 % in Chiromantis xerampelina. Testes mass correlated positively to body mass for the 33 frog species in five families analyzed ($r^2 = 0.53$, $P < 0.0001$, $N = 33$) (Fig. 4): larger species tend to have larger testes. The testes of the leptodactylids L. chaquensis and L. podicipinus, and the rhacophorids C. xerampelina, P. leucomystax, Rhacophorus arboreus, R. owstoni, R. schlegelii, and R. viridis lie outside the upper 95 % confidence interval of the calculated regression (Fig. 4). Testes size of L. fuscus, L. mystacinus, and L. notoaktites were much smaller than predicted (Fig. 4). Testes mass relative to body mass averaged 0.033 ± 0.03 (range = 0.007-0.078, N = 6) in species known to show multi-male breeding, and 0.003 ± 0.002 (range = 0.0004-0.011) in the remaining 27 species where this behavior was not observed. Ignoring phylogenetic relationships, testes mass relative to body mass was significantly larger in multi-male breeders than in species where multi-male spawning was not recorded yet (Mann Whitney U-Test: $W = 5.0$, $P < 0.001$).

DISCUSSION

Testes size relative to body size showed much variation among leptodactylid species. Larger testes were observed for L. chaquensis, where testes mass averaged 4.13 % of body mass, and for L. podicipinus it averaged 0.75 % of body mass. Compared to other 31 anuran species distributed in the families Bufonidae, Hylidae, Leptodactylidae, Ranidae, and Rhacophoridae, our results on testes size of both leptodactylid species show that they are large and close to values recorded for rhacophorid frogs. Extremely low values of testes mass relative to body mass were verified for L. labyrinthicus, L. fuscus, L. mystacinus, and L. notoaktites (0.04-0.06 %). In 19 species from four families of Asian anurans, testes mass averaged 0.2-0.5 % of body mass, except for the foam nest-building rhacophorids of the genera Rhacophorus and Polypedates (Kusano et al., 1991), where relative testes mass ranged 0.7-5 %. For 18 species of African anurans, included in 10 genera and four families, relative testes mass was small (0.1-0.6 %) in 17 species, the exception being the rhacophorid Chiromantis xerampelina with large testes (average 7.8 %) (Jennions and Passmore, 1993).

Observations of more than one male with a female during oviposition in L. chaquensis and L. podicipinus is an indirect evidence on the occurrence of multi-male breeding. Although peripheral males of L. chaquensis were not grasping the amplexed pair, as recorded for other species (e.g., Chiromantis rufescens: Coe, 1967; Agalychnis saltator: Roberts, 1994; Crinia georgiana: Roberts et al., 1999; L. podicipinus, this study), their movements were similar and synchronized with the reproductive movements of the amplectant pair, and they showed the same churning behavior described for other leptodactylid males during oviposition (e.g., Hödl, 1986, 1990; this study). The behavior observed in both species here studied indicates that males probably were trying to fertilize the eggs. Multi-male spawning is reported for the following foam nest-building rhacophorids herein compared to leptodactylids: C. xerampelina (Jennions et al., 1992), P. leucomystax (Feng and Narins, 1991), R. arboreus (Kasuya et al., 1996), and R. schlegelii (Fukuyama, 1991). At least for one species, C. xerampelina, peripheral males in the same foam nest are capable of fertilizing the eggs (Jennions and Passmore, 1993). Jennions and

Passmore (1993) predicted that multi-male breeding may also occur in the rhacophorids *R. viridis* and *R. owstoni* as they present relative large testes.

In species with external fertilization, two strategies are available for males to increase their fertilization success: (1) to increase the number of sperm released and (2) to maintain proximity to the female (Gross, 1985; Jennions and Passmore, 1993). Therefore, selection may favor males with high sperm production, consequently with large testes (Jennions and Passmore, 1993). The relative large testes in the rhacophorid multi-male breeders, as well as in the two leptodactylids of the present study, seem to support the above prediction.

Several factors have been suggested as important for the occurrence of multi-male spawning in the rhacophorids *R. arboreus* and *C. xerampelina*, as well as for its frequency: (1) conspicuousness of the foam nest, (2) long time required for foam nest construction, (3) operational sex ratio (OSR) strongly male-biased, and (4) possibility of fertilization of the eggs by the sperm retained in the foam nest (Kusano et al., 1991; Jennions and Passmore, 1993). *Leptodactylus chaquensis* exhibits all the above mentioned breeding features (C. P. A. Prado, pers. obs.). Furthermore, male-male combat and encounter calls in the nest are known both for *R. arboreus* (Kasuya et al., 1996) and for *L. chaquensis* (this study); these behaviors possibly allow males to locate the nest and join to other males and amplectant pair (Kasuya et al., 1996). However, presence of foam nest is not essential for the occurrence of multi-male breeding, since such behavior has been reported for anurans exhibiting different reproductive modes (e.g., Pyburn, 1970; Kaminsky, 1997; Roberts et al., 1999). For multi-male breeders exhibiting different reproductive modes, other factors may influence the occurrence of simultaneous polyandry, as high male density, explosive breeding activity, competition for oviposition sites, rate of female arrival, and rainfall (e.g., Kaminsky, 1997; Byrne, 2002).

Leptodactylus podicipinus deposits foam nests in leaf-covered basins constructed by males prior to female arrival (Prado et al., 2002). We suggest that for *L*. *podicipinus* the number of additional males may be restricted by difficulties in finding the leaf-covered basins. This situation is somewhat similar to that described for the rhacophorid multi-male breeder *R*. *schlegelii*. In this species the amplexed female digs the soil, and constructs a hole where the foam nest is made (Fukuyama, 1991). One or two sneakers were more commonly observed in *R*. *schlegelii*; they follow amplectant pairs, entering into the nest digging. Due to difficulties in finding the holes, and as they are narrow, participation of additional males in the spawning process is also limited for this rhacophorid (Kusano et al., 1991).

Leptodactylus chaquensis, *C*. *xerampelina*, and *R*. *arboreus* have much larger testes mass than all other species, including rhacophorids and leptodactylids. Jennions and Passmore (1993) suggested the existence of a positive relationship among the frequency of multi-male breeding, average number of males participating in spawning, and relative testes mass in rhacophorids. *Chiromantis xerampelina* shows the highest frequency of multi-male breeding (92.3 %) and mean number of males present in the nest (5.5±2.8), followed by *R*. *arboreus* (81.4 %, 3.4±1.9) and *R*. *schlegelii* (44.4 %, 1.9±1.3). *Leptodactylus chaquensis* and R. arboreus present exposed foam nests, male-male combat in the nest, and exhibit similar relative testes size. *Leptodactylus podicipinus* and *R*. *schlegelii* deposit foam nests in hidden places, and their testes size are also similar. Based on relative testes size and breeding behavior, we predict that frequency of multi-male breeding and number of males participating in the mating may be higher for *L*. *chaquensis* than for *L*. *podicipinus*.

Among the species analyzed here, L. fuscus, L. mystacinus, and L. notoaktites are members of the 'fuscus' group (Heyer, 1969), where foam nests are deposited in subterranean chambers constructed by males prior to female arrival. Reproductive behavior has been studied for L. mystacinus (Sazima, 1975), and mainly for L. fuscus (e.g., Solano, 1987; Freitas et al., 2001), but multi-male spawning remains unrecorded. The small testes relative to body size may be a common feature in the 'fuscus' group, in which access to female by additional males is more difficult due to chamber structure, that presents a very small and hidden aperture, and male behavior, that uses its body to obstruct the chamber tunnel subsequent to female entrance (Martins, 1988).

Percentage of testes mass relative to body mass in L. labyrinthicus (0.04 %), a member of the 'pentadactylus' group (Heyer, 1969), was comparable to those in members of the 'fuscus' group (0.04-0.06 %). The 'pentadactylus' group exhibits at least two reproductive modes (Prado et al., 2002): (1) foam nests in water-filled depressions at the edges of water bodies, and exotrophic tadpoles in water (e.g., L. knudseni: Hero and Galatti, 1990; L. labyrinthicus: Agostinho, 1994); (2) foam nests placed in burrows in the ground, and tadpoles develop inside the nests (e.g., L. fallax: Davis et al., 2000; L. pentadactylus: Hero and Galatti, 1990). However, reproductive behavior is poorly known in this group. Leptodactylus chaquensis, L. macrosternum, and L. ocellatus belong to the 'ocellatus' group (Heyer, 1969), depositing eggs in foam nests on the surface of water. Testes size seems not to be related to the species groups, at least in the 'ocellatus' group, with L. chaquensis exhibiting a testes mass much larger than the other two species of the same group, contrasting to the 'fuscus' group.

The great variation in testes size in Leptodactylus, when compared to species in other families (Fig. 4), could be related to the diverse reproductive modes exhibited by the genus, which exert influence on the mating systems, as well as on the alternative reproductive tactics that can be employed by males. Several tactics are precluded when the spawning site is in a hidden and inaccessible place. For instance, males attempting to displace other males in amplexus (Davies and Halliday, 1978; Wells 1979), multi-male spawning (Coe, 1974; this study), sneaking behavior (Kaminsky, 1997), satellite behavior (Haddad, 1991), and males trying to steal exposed structures for egg deposition (Martins et al., 1998) are impracticable tactics for species such as those in the 'fuscus' group, and for some species in the 'pentadactylus' group, that deposit eggs inside subterranean nests or burrows (see Haddad and Sawaya, 2000, and Prado et al., 2002).

Although sperm competition may be a major factor influencing relative testes size in vertebrates, Emerson (1997) tested two other hypotheses for differences in relative testes size among 90 species of frogs belonging to five families: (1) relative clutch size and (2) androgen level. Phyllomedusine hylid frogs known to have multiple-male mating (Pyburn, 1970; Roberts, 1994) have larger testes than hylids without multiple mating, and variation in relative testes size among frogs support both the sperm competition and the clutch size hypotheses (Emerson, 1997). However, clutch size hypothesis was tested by Emerson (1997) only for ranids with no multi-male breeding. Positive relationship between testes size and clutch size, as observed by Emerson (1997), may result from an indirect effect of body size, as testes and clutch sizes increase with body size (e.g., Kusano et al., 1991; this study). For species of Leptodactylus, testes size does not seem to be related to clutch size; L. labyrinthicus (Agostinho, 1994) and L. ocellatus (Vaz-Ferreira and Gehrau, 1975) exhibit clutches as large as clutches of L. chaquensis and L. podicipinus (Prado et al., 2000), but much smaller relative testes size (Table 1). For the myobatrachid multi-male breeder Crinia georgiana, Byrne (2002) commented that testes size relative to body size is at least four times greater than any other Crinia species. As for rhacophorids, phyllomedusines, and myobatrachids, our data on relative testes size for the multi-male breeders L. chaquensis and L. podicipinus indicate that sperm competition may be an important factor affecting relative testes size also in leptodactylids.

Simultaneous polyandry is phylogenetically widespread among frog families, which exhibit different reproductive modes, and reproductive activity patterns (e.g., explosive or prolonged breeders), suggesting convergent evolution. Different selective factors may have favored the evolution of polyandry in different species (Roberts et al., 1999). The evolution of simultaneous polyandry involves many adaptive changes in behavior (see Jennions et al., 1992), and may be restricted to taxa with reproductive modes where sperm are released in a limited space, such as: (1) shallow ponds (e.g., Bufo americanus: Kaminsky, 1997; Crinia georgiana: Byrne, 2002); (2) foam nests (e.g., rhacophorids: Kusano et al., 1991; leptodactylids, present study); and (3) leaf nests (e.g., phyllomedusines: Pyburn, 1970). Current observations of multi-male breeding in many anurans, the wide variation in testes size and occurrence of several types of mating systems strongly indicate that sperm competition and multiple paternity may be commoner than currently thought for frogs, as noted before by Roberts et al. (1999).

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Table 1. Body mass, testes mass (combined mass of both testes), and percentage of testes mass relative to body mass for 33 anuran species in five families. Means and SD are shown. (1) Present study; (2) Jennions and Passmore (1993); (3) Kusano et al. (1991); (4) Unpublished data from C. P. A. Prado. In bold species that exhibit multi-male spawning.

Families and species	N	Body mass (g)	Testes mass (mg)	Testes/body (%)
Leptodactylidae				
Leptodactylus chaquensis[1]	**21**	**32.8±6.3**	**1358.0±423.0**	**4.13±0.99**
L. fuscus[1]	21	7.7±1.1	5.0±1.8	0.06±0.02
L. labyrinthicus[1]	03	561.0±183.5	220.0±15.0	0.04±0.01
L. macrosternum[1]	02	161.0±13.3	180.0±14.0	0.11±0.02
L. mystacinus[1]	06	15.3±2.0	9.4±1.2	0.06±0.01
L. notoaktites[1]	05	10.7±1.3	5.0±2.0	0.04±0.02
L. ocellatus[1]	04	113.4±17.4	130.0±19.0	0.12±0.02
L. podicipinus[1]	**20**	**4.3±0.5**	**33.0±13.6**	**0.75±0.26**
Physalaemus albonotatus[1]	07	1.2±0.4	1.4±0.5	0.12±0.04
P. crombiei[1]	06	1.0±0.1	1.6±0.6	0.15±0.05
P. nattereri[1]	04	8.8±0.1	17.0±3.0	0.19±0.04
Rhacophoridae				
Buergeria buergeri[3]	10	4.1±0.5	21.2±5.2	0.52±0.14
B. japonica[3]	19	1.8±0.3	4.3±1.6	0.25±0.08
Chiromantis xerampelina[2]	**12**	**13.4±2.7**	**1030.0±340.7**	**7.79±2.38**
Polypedates leucomystax[3]	**03**	**7.2±1.1**	**49.7±36.3**	**0.71±0.54**
Rhacophorus arboreus[3]	**22**	**13.9±4.6**	**753.9±427.9**	**5.15±2.00**
R. owstoni[3]	03	7.6±1.0	73.1±29.1	0.95±0.27

R. schlegelii[3]	**27**	**3.2±0.5**	**33.5±10.9**	**1.06±0.35**
R. viridis[3]	21	9.1±3.0	96.3±38.5	1.11±0.44
Bufonidae				
Bufo garmani[2]	05	42.7±5.7	55.5±19.6	0.13±0.06
B. gutturalis[2]	09	38.7±6.5	118.7±38.1	0.31±0.08
B. japonicus[3]	04	94.1±19.1	331.7±173.5	0.35±0.15
B. maculatus[2]	11	9.8±1.3	37.3±11.9	0.39±0.14
Hylidae				
Hyla japonica[3]	10	2.5±0.3	6.8±2.2	0.28±0.10
Phyllomedusa hypochondrialis[4]	13	2.7±0.5	12.0±4.0	0.53±0.13
Scinax acuminatus[4]	32	5.8±0.8	8.4±3.0	0.14±0.05
S. fuscomarginatus[4]	11	0.6±0.1	1.8±0.5	0.30±0.08
S. nasicus[4]	17	1.8±0.8	6.0±3.0	0.29±0.16
Ranidae				
Leptopelis natalensis[2]	03	4.7±0.8	9.1±2.0	0.19±0.04
Ptychadena anchietae[2]	10	5.6±0.8	15.2±2.0	0.28±0.05
Rana ornativentris[3]	09	13.8±3.8	36.0±13.4	0.26±0.05
R. porosa[3]	09	14.9±3.9	24.8±9.0	0.17±0.05
R. tagoi[3]	14	5.9±2.7	23.2±11.9	0.45±0.26

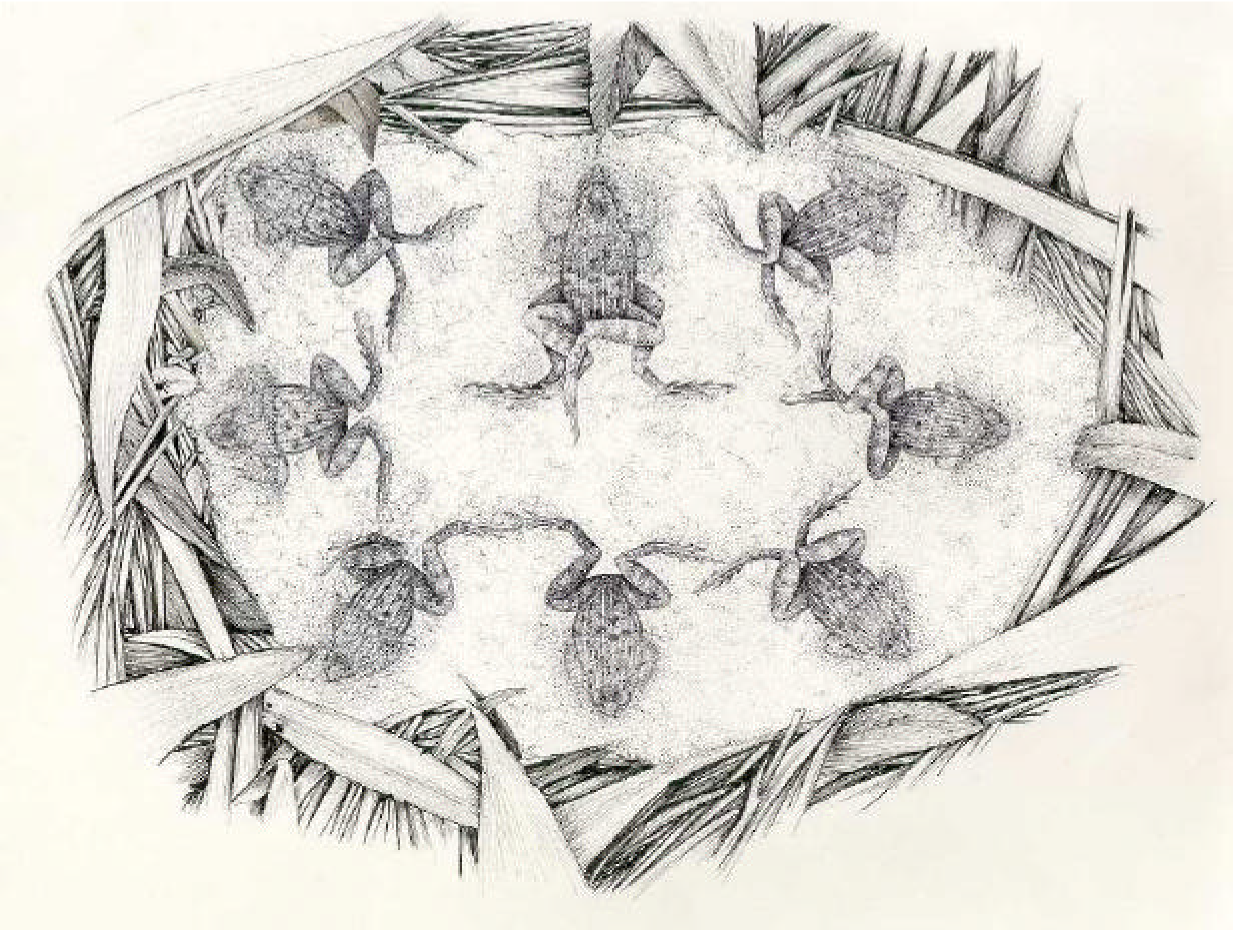

Fig. 1. Illustration of multiple spawning in Leptodactylus chaquensis, observed in the field on 13 November 2000, South Pantanal, Brazil. A couple spawning in the top center, surrounded by seven peripheral males churning into the foam nest in synchrony with the couple. Note that individuals have their bodies partially submerged in the foam. Drawn after slides and observations.

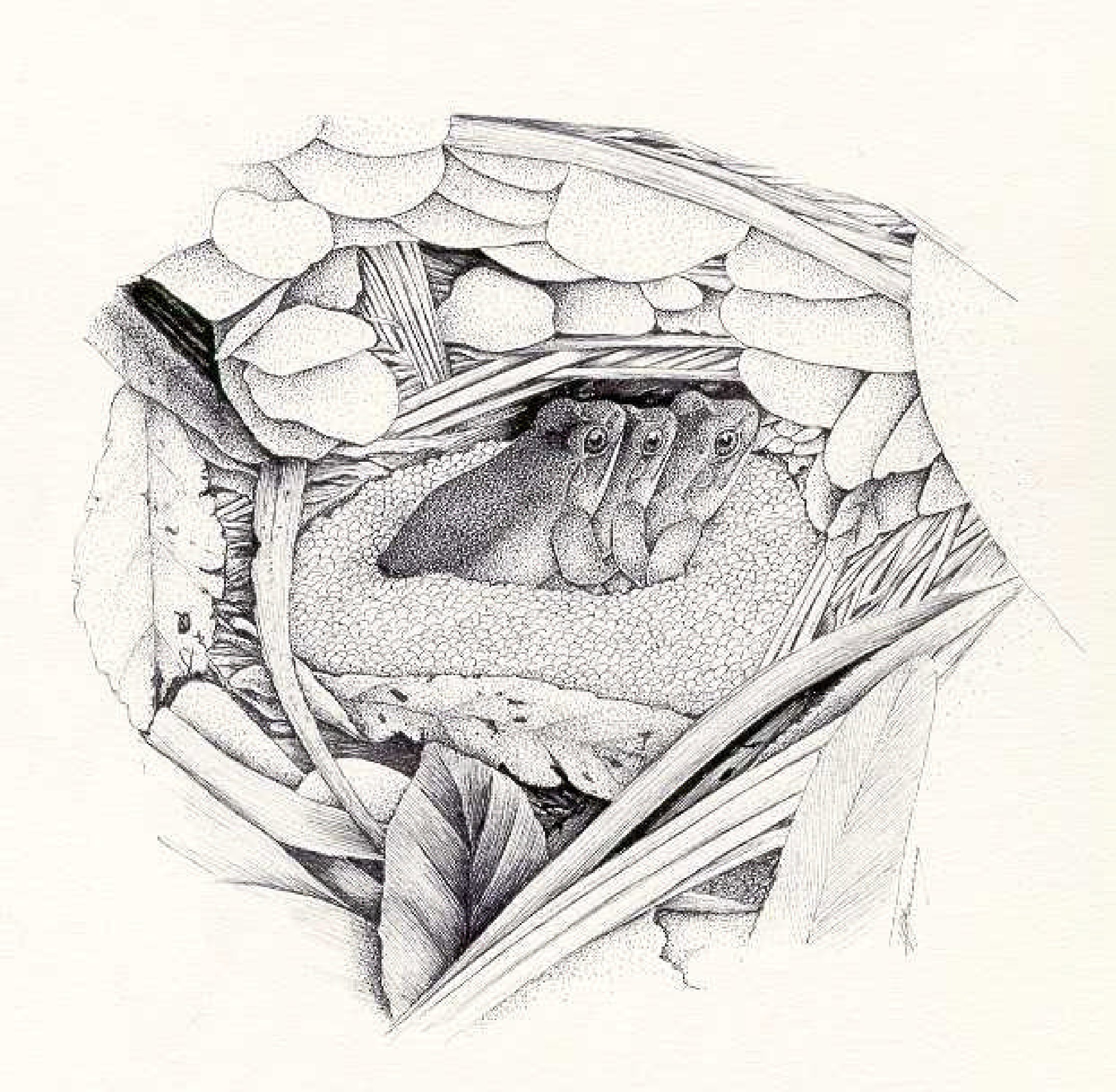

Fig. 2. Illustration of multiple spawning in Leptodactylus podicipinus, observed in the field on 4 April 2001, South Pantanal, Brazil. Two males grasping a female, a small one in the middle and the larger, resident male on the top. Drawn after slides.

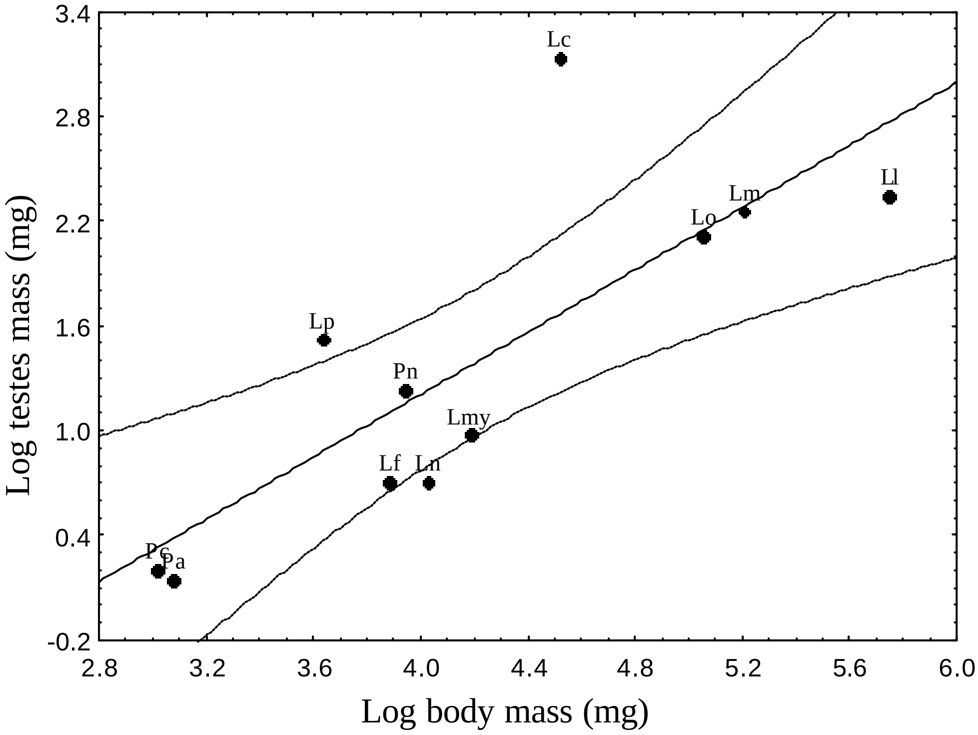

Fig. 3: Relationship between log body mass and log testes mass for 11 leptodactylid species (log y = - 2.37 + 0.89 log x). Curved lines represent 95 % confidence intervals. Species - Lc: L. chaquensis, Lf: L. fuscus, Ll: L. labyrinthicus, Lm: L. macrosternum, Lmy: L. mystacinus, Ln: L. notoaktites, Lo: L. ocellatus, Lp: L. podicipinus, Pa: Physalaemus albonotatus, Pc: P. crombiei, Pn: P. nattereri. Note that multi-male breeder species lie above the 95 % confidence interval.

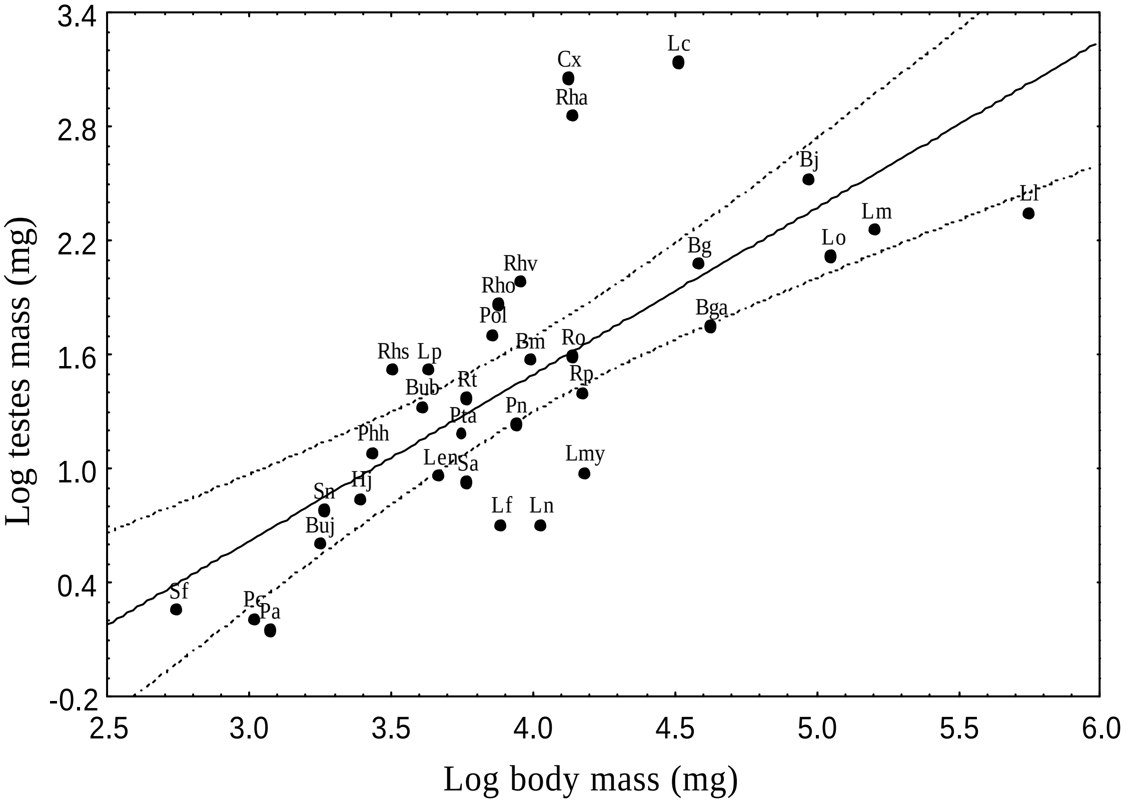

Fig. 4. Relationship between log body mass and log testes mass for 33 frog species in five families ($\log y = - 2.0 + 0.88 \log x$). Curved lines represent 95 % confidence intervals. Families and species - Bufonidae: Bga: Bufo garmani, Bg: B. gutturalis, Bj: B. japonicus, Bm: B. maculatus; Hylidae: Hj: Hyla japonica, Phh: Phyllomedusa hypochondrialis, Sa: Scinax acuminatus, Sf: S. fuscomarginatus, Sn: S. nasicus; Leptodactylidae: Lc: Leptodactylus chaquensis, Lf: L. fuscus, Ll: L. labyrinthicus, Lm: L. macrosternum, Lmy: L. mystacinus, Ln: L. notoaktites, Lo: L. ocellatus, Lp: L. podicipinus, Pa: Physalaemus albonotatus, Pc: P. crombiei, Pn: P. nattereri; Ranidae: Len: Leptopelis natalensis, Pta: Ptychadena anchietae, Ro: Rana ornativentris, Rp: R. porosa, Rt: R. tagoi; Rhacophoridae: Bub: Buergeria buergeri, Buj: B. japonica, Cx: Chiromantis xerampelina, Pol: Polypedates leucomystax, Rha: Rhacophorus arboreus, Rho: R. owstoni, Rhs: R. schlegelii, Rhv: R. viridis. Note that multi-male breeder species lie above the 95 % confidence interval.

CHAPTER 4

DESCRIPTION OF A NEW REPRODUCTIVE MODE IN *LEPTODACTYLUS* (ANURA, LEPTODACTYLIDAE), WITH A REVIEW OF THE REPRODUCTIVE SPECIALIZATION TOWARD TERRESTRIALITY IN THE GENUS

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DESCRIPTION OF A NEW REPRODUCTIVE MODE IN LEPTODACTYLUS (ANURA, LEPTODACTYLIDAE), WITH A REVIEW OF THE REPRODUCTIVE SPECIALIZATION TOWARD TERRESTRIALITY IN THE GENUS

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Key words – Leptodactylidae; Leptodactylus; reproductive modes; new mode; Pantanal

ABSTRACT. The genus *Leptodactylus* provides an example among anurans where there is an evident tendency towards terrestrial reproduction. Herein we describe a new reproductive mode for the frog *Leptodactylus podicipinus*, a member of the '*melanonotus*' group. This new reproductive mode represents one of the intermediate steps from the most aquatic to the most terrestrial modes reported in the genus. Three reproductive modes were previously recognized for the genus *Leptodactylus*. However, based on our data, and on several studies on *Leptodactylus* species that have been published since the last reviews, we propose a new classification, with the addition of two modes for the genus.

INTRODUCTION

The concept of reproductive mode in amphibians was defined by Salthe (1969) and Salthe and Duellman (1973) as being a combination of traits that includes oviposition site, ovum and clutch characteristics, rate and duration of development, stage and size of hatchling, and type of parental care, if any. For the anurans, Duellman (1985) and Duellman and Trueb (1986) recognized 29 modes of reproduction, although in recent studies on ecology and natural history new reproductive modes have been described (e. g., Haddad and Hödl, 1997; Haddad and Pombal Jr., 1998; Haddad and Sawaya, 2000). Amphibians exhibit a great diversity of reproductive modes, especially in the New World tropics, including clear trend towards terrestriality (Duellman, 1985), i. e., reproduction becoming gradually independent of water bodies.

Members of the family Leptodactylidae exhibit several reproductive modes ranging from aquatic to terrestrial breeding, with many intermediate forms depositing eggs embedded in foam nests (Heyer, 1969). Species of *Leptodactylus* and of the related genus *Adenomera* are an example where a tendency towards terrestriality is evident (Heyer, 1969; Duellman, 1985). Heyer (1969) proposed that *Leptodactylus* species belonging to the '*ocellatus*' and '*melanonotus*' groups have the most primitive reproductive modes. Species of both groups deposit eggs in foam nests on top of the water and tadpoles are exotrophic, feeding and developing in water. According to Heyer's (1969) review, species in the 'pentadactylus' group show the first step to a more terrestrial life (Heyer, 1969), with foam nests deposited in depressions or burrows at the edges or close to water; exotrophic tadpoles develop in water. More specialized modes are demonstrated by species of the '*fuscus*' and '*marmoratus*' groups. Heyer (1974) placed '*marmoratus*' group species in the genus *Adenomera*. Species in the '*fuscus*' group have foam nests that are placed on land in subterranean chambers constructed by males; exotrophic larvae in advanced stages are released through floods or rain to lentic or lotic water bodies. Frogs in the genus Adenomera also deposit foam nests in subterranean chambers, and in most species, tadpoles develop inside these chambers and are endotrophic, i. e., entire developmental energy is obtained from vitellogenic yolk (Heyer, 1969). More recently, De la Riva (1995) described another reproductive mode for the genus Adenomera, which corresponds to the same reproductive mode of the L. fuscus group.

In the present paper we report a new reproductive mode in a leptodactylid frog, Leptodactylus podicipinus, a member of the 'melanonotus' group, in the Pantanal, southwestern Brazil. Based in this new reproductive mode, and on data published after Heyer's (1969) review, we review the reproductive modes exhibited by the species of Leptodactylus, and propose a new classification of modes for the genus.

MATERIALS AND METHODS

The study was conducted in the southern Pantanal, municipality of Corumbá, State of Mato Grosso do Sul, southwestern Brazil (19º34'S, 57º00'W). The Pantanal is a floodplain, with an area of ca. 140,000 km² and elevation between 75 and 200 m above sea level, delimited mostly by the Paraguay river in the west and Brazilian uplands in the east (see Por, 1995). The region is characterized by a seasonal climate ("Aw" type in Köppen's classification), with a rainy summer from October to April and a dry winter from May to September. Annual floods occur in the Pantanal, and at the study site, along the Miranda river, floodings are common from January to April. The "cerrado" (savanna-like vegetation) predominates in the area, with patches of semideciduous forests, gallery forests, and grassland fields.

Observations on L. podicipinus were made at ponds and flooded areas near the Base de Estudos do Pantanal / Universidade Federal de Mato Grosso do Sul (57°00'W; 19°34'S). Data reported here were collected between January 1998 and March 1999, and from January to April 2001. Diameter and depth of the depressions where males were calling were measured with a measuring tape to the nearest 0.1 cm. Calling males were captured and toe clipped according to Waichman (1992) between 23 March and 08 April 2001. Snout-vent length (SVL) was measured in the field to the nearest 0.1 mm with a caliper. Then, individuals were released at their capture point. Calling sites were also marked. Clutches were collected and immediately preserved in 5% formalin; number of eggs per clutch was determined and egg diameter measured with an ocular micrometer in a Zeiss stereomicroscope (± 0.1 mm). The terminology used to describe the larvae nourishment follows Thibaudeau and Altig (1999).

RESULTS

Basin description. – Basins of L. podicipinus were observed at the edges of permanent ponds and flooded areas, among grass clumps or aquatic plants. The format of the depressions was approximately circular, with a mean diameter of 61.0 mm ± 4.5 SD (range = 50–70, n = 16). Water inside the depressions, that permeated from the adjacent water body, averaged 31.0 mm ± 4.5 SD of depth (range = 24–40, n = 16). In general, males call from the interior of the basins with their bodies partially submerged (Fig. 1). Calling males are difficult to visualize because basins are well covered by the marginal vegetation, fallen leaves or dead vegetation, which function as roofs. In 24 basins we observed, all were covered by vegetation and males were calling beside the basin twice; the remaining 22 males were inside the depressions. Mean distance between the nearest occupied basin was 2.5 m ± 2.1 SD (range = 0.6–8.4, n = 12). When water level was decreasing, new nests were being constructed following the water line at the edges of the water bodies, and the older ones were abandoned.

Behavior of males. – Nine vocalizing males that were inside their basins were marked and measured. Five marked males were recaptured from one to three times on successive nights during a 15 day study period. Mean SVL of recaptured males (33.8 mm ± 0.8 SD, range = 34.5-32.7, n = 5) was larger (t = 4.41, P = 0.001) than that of not-recaptured males (31.0 mm ± 1.0 SD, range = 32.4-30.0, n = 4). Two males were recaptured three times at the same sites, although they had moved to new basins near original ones as the water level was decreasing. Two other males were recaptured once; one of them, marked on 29 March 2001, was recaptured six days later calling in a new basin 4.5 m from the original, and the second male was recaptured six days later ca. 10 m from the original site. One male was recaptured twice inside the same basin. Three other males captured calling inside their depressions were marked at night in March 1999. These males were recaptured resting inside the depressions the next morning.

Clutches and female parental care. – Foam nests of L. podicipinus were observed inside basins in January 1998, September 1998, and January 1999. The eggs were pigmented, with a mean diameter of 1.1 mm ± 0.05 SD (range = 1.0-1.2, n = 21 eggs from 2 clutches), and mean number of eggs per clutch was 2102.5 ± 442.1 SD (range = 1750–2953, n = 6 clutches). The foam nests were covered by dead vegetation and mean water depth inside the basins was 26.3 mm ± 3.2 SD (range = 24–30, n = 3). Oviposition was observed once inside a basin covered by aquatic vegetation leaves in April 2001.

Females of L. podicipinus attending tadpoles were observed (n = 14), generally, in the margin of ponds or flooded areas, with water depth with about 5 cm. Females remained close to the tadpole schools. When tadpoles were disturbed, almost all females were aggressive, mainly those with hatchlings, jumping and knocking with their heads against observers' hands. They also performed pumping movements (sensu Wells and Bard, 1988), after what they usually dived and emerged at short distances, being followed by the tadpoles. Females were observed attending tadpoles in different developmental stages. In six foam nests analyzed we did not observe females attending the eggs. Although, we suggest that females also attend eggs because they were observed attending tadpoles since the early larval stages.

DISCUSSION

Reproductive mode in L. podicipinus. – Leptodactylus podicipinus occurs in open formations of Paraguay, Argentina, Bolivia, and in Brazil in the Amazon basin, throughout central region, extending to southeast and south of the country (Heyer, 1994). The reproductive biology of this species, like that of many tropical species, is poorly known (Vizotto, 1967; Rossa-Feres and Jim, 1994). Recent studies reported the existence of female parental care of eggs and tadpoles in this species (Prado et al., 2000; Martins, 2001), but the exact description of calling and oviposition sites is lacking in these previous studies. The exception was the study conducted by Martins (1996), where he reported the occurrence of males of L. podicipinus calling from basins at the edges of water bodies in the State of São Paulo, Brazil. We observed the same situation for this species in the Pantanal.

We did not observe the construction of the basins in the present study, but Martins (1996) observed males constructing basins with their snouts, and suggested that males could use either natural or constructed depressions. Female parental care of tadpoles was observed at our study site but we failed to observe females attending eggs. Nevertheless, Martins (1996, 2001) observed females attending eggs and tadpoles. Based on these information, on our observations on the behavior of males, and given the uniform format and dimensions of the depressions we have measured, we consider that L. podicipinus exhibits a new reproductive mode which can be summarized as follows: pigmented eggs and early larval stages in foam nests, which are deposited in water–filled basins constructed by the males adjacent to water; exotrophic tadpoles in ponds; females perform parental care, attending eggs and tadpoles.

Among the reproductive modes exhibited by the anurans, the generalized mode of aquatic eggs and tadpoles is considered to be primitive (Duellman, 1985). The evolution of specialized modes, with eggs deposited in protected sites or out of water, have been considered to be adaptations against aquatic predators (Magnusson and Hero, 1991; Haddad and Sawaya, 2000). The reproductive mode of L. podicipinus could have evolved in response to predation pressure on eggs, embryos, and even on adults (e.g., calling males, amplexed pairs during oviposition, and females attending eggs). Although females of L. podicipinus attend nests and tadpoles until metamorphosis (Prado et al., 2000; Martins, 2001; present study), the deposition of foam nests inside vegetation covered basins may provide additional protection for the eggs and embryos. Adult males were observed inside leaf–covered basins during the day. Visual predators, like wading birds, were observed preying upon other anuran species (Leptodactylus chaquensis and Pseudis paradoxa) during the day at the study site (C. P. A. Prado, pers. obs.). It seems that the basins could also function as shelter sites for L. podicipinus males, avoiding or diminishing predation risk.

Studies on other species of the 'melanonotus' group show no evidence of basin construction by males (e. g., L. leptodactyloides: Heyer and Bellin, 1973, reported as L. wagneri; either L. leptodactyloides or L. wagneri: Duellman, 1978; L. validus: Downie, 1996). Nevertheless, Downie (1996) pointed out that clutches of L. validus in Trinidad were always found at the edges of small ponds, usually well covered by dead vegetation. It is possible that the reproductive mode described here for L. podicipinus occurs in other species of the 'melanonotus' group, but more detailed studies on their reproductive behavior are necessary.

Female parental care of eggs and tadpoles in species of the 'ocellatus' and 'melanonotus' groups (e. g., Wells and Bard, 1988; Prado et al., 2000; Martins, 2001) reinforces Heyer's (1969) proposition that members of both groups are the most closely related of the four Leptodactylus groups he identified. However, the reproductive mode of L. podicipinus, a member of the 'melanonotus' group, represents a specialization possibly derived from the primitive mode present in species of the 'ocellatus' group and some species of the 'melanonotus' group, representing one more step toward terrestrial reproduction in the genus.

Reproductive modes in the genus Leptodactylus. – Heyer (1969) proposed four reproductive stages for the species groups of the genus Leptodactylus, including the species of Adenomera. Several studies on Leptodactylus species have been published since, resulting in new information regarding their reproductive biology (e. g., Rodríguez and Duellman, 1994; Davis et al., 2000; Eterovick and Sazima, 2000). An interesting group concerning reproduction within the genus Leptodactylus is the ‘pentadactylus’ group, which presents at least two reproductive modes. The first specialization in this group is the deposition of foam nests in water-filled depressions at the edges of water bodies; subsequent to flooding, exotrophic tadpoles develop in water (e. g., L. knudseni: Hero and Galatti, 1990; Rodríguez and Duellman, 1994; L. labyrinthicus: Agostinho, 1994; C. F. B. Haddad, pers. obs.). Whether these depressions are natural or constructed remains unclear. The ultimate specialization in the 'pentadactylus' group is the total terrestrial reproductive mode described for L. fallax (Davis et al., 2000). In this species foam nests are placed in burrows in the ground, and tadpoles develop inside the nests, but some details on reproduction and larval development need to be clarified. Also in this species it is not clear if the cavities used are natural or constructed (Davis et al., 2000). Although Lescure and Letellier (1983) reported that tadpoles of L. fallax develop inside the nest only on their vitelline reserves until metamorphosis, Davis et al. (2000) described tadpoles without yolk sacs. If tadpoles develop only on their yolk, this reproductive mode could be considered comparable to the mode described for some species of Adenomera (Heyer, 1969; De la Riva, 1995).

Leptodactylus pentadactylus apparently has two alternative reproductive modes: (1) deposition of foam nests in open depressions ('potholes'); subsequent to flooding, exotrophic tadpoles develop in water (Breder, 1946), as in other species of the group mentioned above; (2) reproductive mode similar to that described for L. fallax (Hero and Galatti, 1990; Rodríguez and Duellman, 1994), i. e., a foam nest inside burrows in the ground and development of larvae inside the nest. Muedeking and Heyer (1976) observed nests of L. pentadactylus in Panama deposited in apparently excavated potholes, and at a some distance from water bodies. These authors also registered the co–occurrence of larvae and eggs in the same nests, and some of the larvae had yolk colored guts, which was interpreted as ingestion of eggs by the larvae. As suggested by Muedeking and Heyer (1976), possibly the normal pattern for L. pentadactylus is the release of tadpoles in water through floods of potholes, as observed by Breder (1946). Nevertheless, in unfavorable conditions of rain, larvae could remain in the nest feeding on eggs up to metamorphosis (Muedeking and Heyer, 1976). This latter situation was observed by Hero and Galatti (1990) in Central Amazonia, although they did not mention how larvae were nourished. It is possible that the alternative modes present in L. pentadactylus are partially associated with feeding diversity exhibited by the larvae, which can feed on algae, foam (Vinton, 1951), other tadpoles (Heyer et al., 1975), and even on eggs (Muedeking and Heyer, 1976), and also due to the presence of a foam nest that provides protection from desiccation to tadpoles, as previously mentioned by Heyer (1969). Alternatively, the occurrence of these different reproductive modes could also be explained by the existence of more than one species being identified under the name L. pentadactylus (W. R. Heyer, pers. comm.).

Oophagous tadpoles are also known for other species of the 'pentadactylus' group. Tadpoles of L. knudseni were already observed feeding on conspecific eggs placed later in the same pond (C. F. B. Haddad, pers. obs.). Agostinho (1994) verified that a mean of 90% of eggs present in foam nests of L. labyrinthicus were not fertilized, and were consumed by the tadpoles, which could survive in the nest for almost 30 days in good condition before being carried to water by the rain. Female deposition of a great amount of eggs that will not be fertilized, but instead, will supply nourishment for the tadpoles can occur in other species of the 'pentadactylus' group, and could also be another hypothesis for larval nutrition in L. fallax, beyond the three possibilities proposed by Davis et al. (2000).

The reproductive mode of L. podicipinus is one of the transitional steps from an aquatic to a terrestrial breeding within the genus (Fig. 2). In this case, males construct water-filled basins at the edges of ponds, which are well covered by leaves or dead vegetation, and exotrophic tadpoles develop in water. In Table 1 we summarized the reproductive modes known for the genus Leptodactylus. Analysis of all available information reveals that the way leading to a terrestrial reproduction in the genus Leptodactylus is much more complex, with many, and maybe unknown, intermediate steps, which was evidenced by the results presented in our study.

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Table 1. Diversity of reproductive modes in leptodactylid frogs of the genus Leptodactylus, from the most aquatic to the most terrestrial. In bold the new steps proposed since Heyer (1969).

Reproductive mode	Examples
1. Eggs and early larval stages in foam nest on top of the water; exotrophic tadpoles in lentic water.	Species of the L. ocellatus (e. g., L. bolivianus, L. chaquensis, L. ocellatus) and L. melanonotus groups (e. g., L. leptodactyloides, L. validus).
2. Eggs and early larval stages in foam nest in water–filled depressions close to water; subsequent to flooding, exotrophic tadpoles in lentic water.	Some species of the L. pentadactylus group (e. g., L. knudseni, L. labyrinthicus, L. pentadactylus).
3. Eggs and early larval stages in foam nest in water-filled basins constructed by males; exotrophic tadpoles in lentic water.	**One species of the L. melanonotus group (L. podicipinus).**
4. Eggs and early larval stages in foam nest in subterranean chambers constructed by males; subsequent to flooding, exotrophic tadpoles in lentic or lotic water.	L. fuscus group (e. g., L. cunicularius, L. elenae, L. furnarius, L. fuscus, L. jolyi, L. mystaceus, L. notoaktites).
5. Eggs in foam nest in burrows, either close or far from water; larval development in the foam nest inside the burrows[a].	**Some species of the L. pentadactylus group (e. g., L. fallax, L. pentadactylus).**

[a] It remains unclear if tadpoles of L. fallax develop only on their vitelline reserves, or if they have another nutrition source (see text for references).

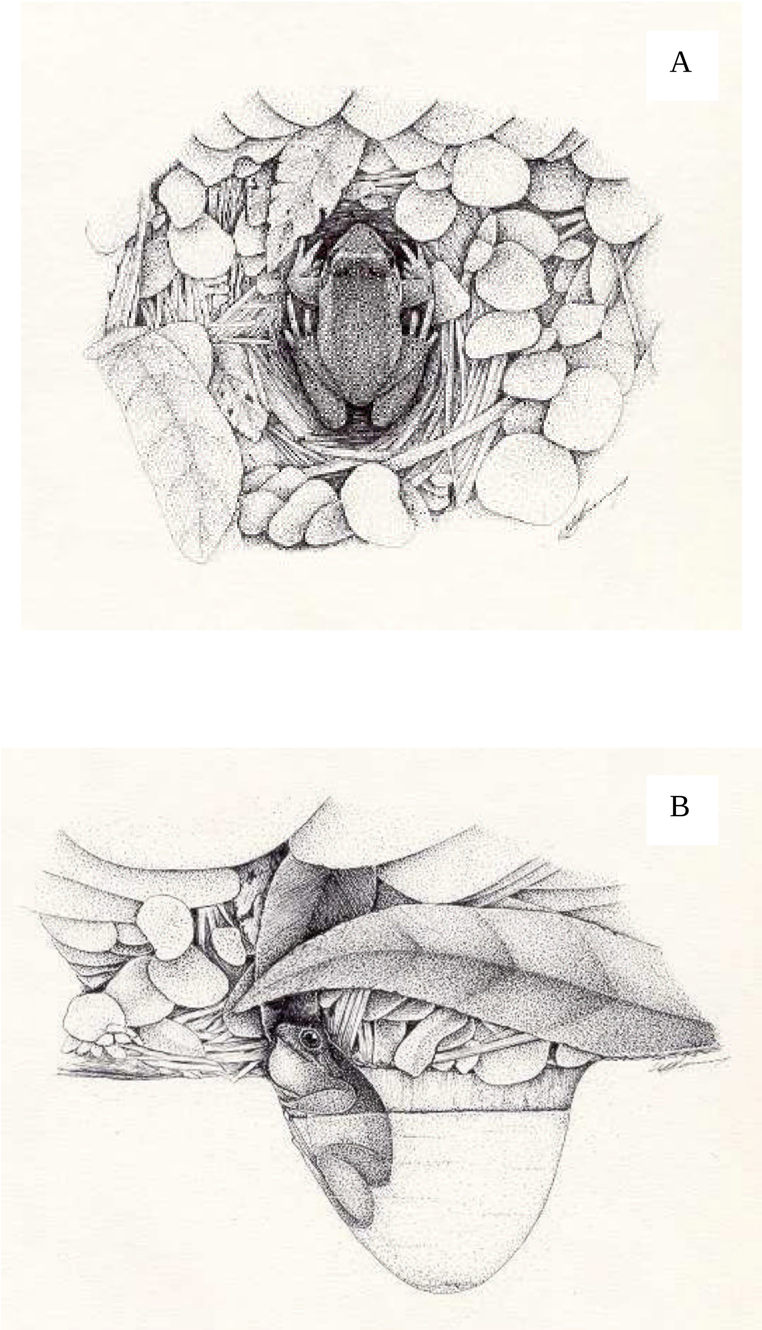

Fig. 1. (A) Dorsal and (B) lateral views of a male of Leptodactylus podicipinus calling inside an excavated basin (Corumbá, Mato Grosso do Sul, South Pantanal, Brazil). (B) Note that leaves function as a roof for the basin. Drawn from photographies.

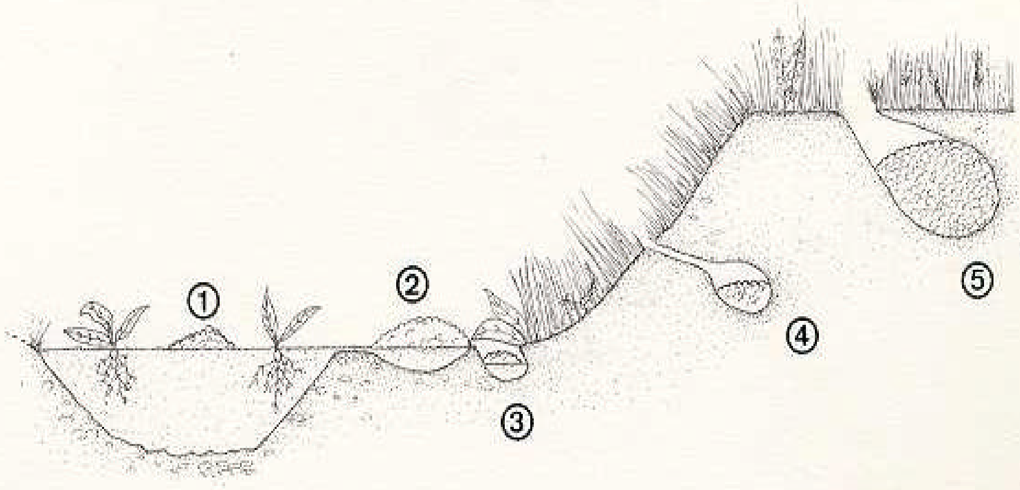

Fig. 2. Schematic representation of sequential steps of the reproductive modes in the genus Leptodactylus, from the most aquatic to the most terrestrial modes. (1) Eggs in foam nest on top of water; tadpoles in lentic water, (2) eggs in foam nest in depressions close to water; tadpoles in lentic water, (3) eggs in foam nest in water–filled basins excavated by males; tadpoles in lentic water, (4) eggs in foam nest in subterranean chambers constructed by males; tadpoles in lentic or lotic water, (5) eggs in foam nest in burrows; larval development in the foam nest inside the burrows. New steps proposed for the genus Leptodactylus since Heyer (1969) are (3) and (5).

CONCLUSÕES

- A comunidade de anfíbios anuros na área do presente estudo, no Pantanal sul, é composta por 24 espécies distribuídas em quatro famílias: *Bufo* sp. 1 (gr. *granulosus*), *Bufo* sp. 2 (gr. *granulosus*), *B. schneideri* (Bufonidae); *Hyla nana*, *H. punctata*, *H. raniceps*, *Lysapsus limellus, Phrynohyas venulosa, Phyllomedusa hypochondrialis, Pseudis paradoxa, Scinax acuminatus, S. fuscomarginatus, S. nasicus* (Hylidae); *Adenomera* cf. *diptyx*, *Leptodactylus chaquensis, L. elenae, L. fuscus*, *L.* cf. *macrosternum*, *L. podicipinus,* *Physalaemus albonotatus*, *P.* cf. *biligonigerus*, *Pseudopaludicola* cf. *falcipes* (Leptodactylidae); *Chiasmocleis mehelyi* e *Elachistocleis* cf. *bicolor* (Microhylidae).

- A maior parte das espécies (50%) exibiu o padrão explosivo de reprodução e nove espécies apresentaram reprodução prolongada, reproduzindo-se por mais de três meses consecutivos. Apenas três espécies exibiram o padrão contínuo, *Hyla nana, Lysapsus limellus* e *Leptodactylus podicipinus*, reproduzindo-se ao longo de todo o ano.

- A atividade reprodutiva das espécies de anuros na área de estudo ocorreu principalmente na estação quente e chuvosa (outubro a março). A atividade reprodutiva é sazonal, sendo influenciada por fatores como a precipitação local e a temperatura.

- Cinco modos reprodutivos foram registrados para a comunidade estudada, sendo um deles novo: (1) ovos e girinos em ambiente lêntico, (2) ovos depositados em ninho de espuma sobre a água e girinos em ambiente lêntico, (3) ovos depositados em folhas acima da água e girinos em ambiente lêntico, (4) ovos em ninho de espuma, depositados dentro de câmaras subterrâneas construídas pelos machos; posteriormente os girinos são carregados pela água e desenvolvem-se em ambiente lêntico, e o novo modo (5) com ovos em ninho de espuma depositados em depressões construídas pelos machos, posteriormente os girinos desenvolvem-se em ambiente lêntico. Há dúvidas quanto à ocorrência de um sexto modo para a espécie *Adenomera* cf. *diptyx*. Porém, com base na literatura, assumimos que a espécie apresenta o modo acima descrito como 4.

- O modo reprodutivo onde ovos são depositados em ambiente lêntico e os girinos desenvolvem-se nesse mesmo local, ocorreu em maior proporção na comunidade estudada (62,5%), sendo seguido pelos modos onde os ovos são depositados em ninhos de espuma (33,3%).

- As espécies de anuros exibiram diferenças quanto à utilização dos ambientes ao longo do ano. Além disso, em relação às espécies que se reproduziram no mesmo período e local, os machos exibiram segregação em relação aos sítios utilizados para a emissão de cantos de anúncio.

- A grande ocorrência de espécies com padrão reprodutivo explosivo e com atividade reprodutiva concentrada no início da estação chuvosa é esperada para ambientes abertos e de clima marcadamente sazonal em relação às chuvas, como o Pantanal.

- Na maior parte das espécies estudadas as fêmeas foram maiores que os machos, tanto em comprimento rostro-cloacal (CRC), quanto em massa. Dimorfismo sexual em tamanho não foi observado para *Scinax fuscomarginatus*, *Leptodactylus chaquensis*, *L* cf. *macrosternum* e *Physalaemus albonotatus*.

- Os tamanhos de machos e fêmeas amplectados foram analisados para três espécies: *Scinax acuminatus*, *Lysapsus limellus* e *Physalaemus albonotatus*. Houve correlação positiva entre o CRC de machos e fêmeas em amplexo apenas em *L. limellus*. Os machos em amplexo foram menores que as fêmeas em *S. acuminatus* e *L. limellus*. Porém, em *P. albonotatus*, os machos em amplexo foram um pouco maiores que as fêmeas, tanto em CRC quanto em massa. Dentre as três espécies estudadas, machos não amplectados foram significativamente menores que machos amplectados apenas em *P. albonotatus*.

- Ainda em relação aos casais amplectados, houve uma correlação negativa significativa entre a razão do tamanho do macho em relação à fêmea e o tamanho da fêmea em *S. acuminatus* e *P. albonotatus*, i. e., fêmeas maiores tenderam a se acasalar com machos proporcionalmente menores quando comparadas às fêmeas menores. Diferenças relacionadas à variância nos tamanhos de machos e fêmeas podem ter causado tal resultado.

- Tanto o CRC quanto a massa da fêmea correlacionaram-se positivamente com o tamanho da desova (número de ovos por desova) em sete das oito espécies examinadas. No entanto, o coeficiente de regressão linear (r^2) foi muito variável nas diferentes espécies. Diferenças sazonais na produção de ovos parecem explicar tais variações.

- *Leptodactylus podicipinus*, uma espécie de reprodução contínua, apresentou diferenças em relação à produção de óvulos nas estações úmida e seca. Fêmeas desta espécie produziram, em média, mais óvulos na estação úmida.

- Quanto às relações de tamanho-fecundidade interespecíficas, houve correlação positiva entre o CRC da fêmea e o número de ovos depositados e o diâmetro dos ovos. Porém, espécies com modos reprodutivos terrestres, *P. hypochondrialis*, *L. fuscus* e *A.* cf. *diptyx*, apresentaram desovas menores e ovos maiores quando comparados às espécies com modos aquáticos (desova aquática e desova em ninho de espuma sobre a água).

- O investimento reprodutivo (IR), medido como a porcentagem da massa do ovário em relação à massa do corpo da fêmea, variou de 5,5 a 18% em 11 espécies analisadas. O IR foi comparado entre espécies que exibem modo reprodutivo aquático, desovas em ninho de espuma sobre a água e modos terrestres. Não houve diferença significativa no IR entre essas três categorias de modos reprodutivos.

- Houve correlação negativa entre o IR e a massa das fêmeas; proporcionalmente, fêmeas de espécies maiores investiram menos em gônadas comparadas às fêmeas de espécies menores. *Leptodactylus chaquensis* exibiu um IR maior que o esperado, e *L. fuscus* e *P. paradoxa* apresentaram investimentos muito menores que o esperado.

- De três espécies de *Leptodactylus* analisadas, houve correlação negativa entre a massa do corpo gorduroso e a massa do ovário em *L. chaquensis* e *L. fuscus*. Para *L. podicipinus*, não houve correlação significativa entre essas variáveis.

- As variações nas estratégias reprodutivas observadas para as espécies do presente estudo, principalmente em relação à produção de óvulos, deposição de gordura, investimento reprodutivo, estão relacionadas aos padrões de atividade e modos reprodutivos das espécies, mas também parecem ser controladas por fatores ambientais, principalmente aqueles relacionados à sazonalidade climática do Pantanal.

- Foi observado o comportamento de desova múltipla para as espécies *L. chaquensis* e *L. podicipinus* na área de estudo, onde mais de um macho participou da oviposição envolvendo uma única fêmea. Também foram observados combates físicos entre os machos de *L. chaquensis* que participaram das desovas múltiplas.

- A massa relativa dos testículos de *L. chaquensis* e *L. podicipinus* foi maior quando comparada à de outras espécies do gênero e outros leptodactilídeos.

- A massa relativa dos testículos de *L. chaquensis*, *L. podicipinus* e de outras espécies da família Rhacophoridae, que também exibem o comportamento de desova múltipla, foi maior comparada à de outras espécies onde esse comportamento não é conhecido.

- O tamanho relativo dos testículos das espécies poliândricas aqui investigadas sugere que a competição de esperma deve exercer uma pressão para o aumento do tamanho dos testículos nessas espécies. Além disso, o comportamento de desova envolvendo vários machos, e a ocorrência de paternidade múltipla, devem ser fenômenos mais comuns entre anuros do que se acredita.

- Um novo modo reprodutivo para o gênero *Leptodactylus* foi descrito no presente trabalho. O novo modo reprodutivo, o qual foi observado para a espécie *Leptodactylus podicipinus*, pode ser descrito da seguinte forma: ovos pigmentados em ninho de espuma são depositados em depressões contendo água, as quais são construídas pelos machos nas margens de corpos d'água; os girinos exotróficos desenvolvem-se na água; a fêmea exerce cuidado parental em relação aos ovos e girinos. As depressões, normalmente, estão recobertas por vegetação, as quais funcionam como um teto.

- Para o gênero *Leptodactylus*, este novo modo reprodutivo representa um estágio intermediário na evolução dos modos reprodutivos, desde o mais aquático até o mais terrestre.

- Com a descrição deste novo modo reprodutivo, e com base em informações da literatura, mais dois novos modos reprodutivos são propostos para o gênero *Leptodactylus*, o qual passa a apresentar cinco modos reprodutivos.