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The costs of parental care and skin-feeding in *Leptodactylus podicipinus* (Anura: Leptodactylidae)

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Dissertação apresentada como requisito para a obtenção do título de Mestre em Ecologia, pelo Programa de Pós Graduação em Ecologia e Conservação, Universidade Federal de Mato Grosso do Sul

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Dedicated to my family:
Albana Carrillo, Alcibiades Cuestas,
Eduardo Cuestas and Lucrecia Ramos.
“Tómalo con calma, la cosa es así. Ya
se hace de noche, me tengo que ir”
Charly García.

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General abstract

Parental care is very diverse among anuran families. Species in the genus *Leptodactylus* exhibit many different reproductive modes, including parental care. This additional investment generates a cost that parents have to face. We used *Leptodactylus podicipinus*, a member of the *L. melanonotus* group, as a model species to investigate costs of maternal care and mother-offspring interaction in the south Pantanal. *Leptodactylus podicipinus* females care for eggs and larvae up to metamorphosis. This Master´s dissertation is presented in two chapters. In chapter 1, we measured the costs of maternal care for *L. podicipinus* using capture-recapture data to compare energetic variables between attending and non-attending females (i.e. body mass, fat body mass, ovary mass and stomach volume content). In chapter 2, we studied mother-offspring relationship by examining mothers´ skin traits, tadpoles´ guts, and behavior to investigate the occurrence of offspring skin-feeding (dermatotrophy) in *L. podicipinus*. Skin traits were compared among attending, non-attending females, and males. We found that attending females lost mass after a week of recapture compared to non-attending females. Ovary mass and stomach volume were also smaller in attending females and empty stomachs occurred only in these females guarding tadpoles. However, we found no differences in fat bodies. Our results indicate that benefits of parental care in *L. podicipinus* may impose some costs to females, as reduction in food intake and ovary mass may decrease body size and future fecundity. Attending females had thicker epidermis and stratum corneum. Furthermore, concentration of lipids in the epidermis was higher in attending females and we found epithelial cells in the tadpoles' guts, suggesting dermatotrophy in *L. podicipinus*, the first record of this type of parental care for Anura.

Resumo geral

O cuidado parental é muito diversificado entre as famílias de anuros. As espécies do gênero *Leptodactylus* exibem diferentes modos reprodutivos, incluindo cuidado parental. Este investimento adicional gera custos que os parentais devem enfrentar. Nós usamos *Leptodactylus podicipinus*, um membro do grupo de *L. melanonotus*, como modelo para investigar os custos do cuidado maternal e a interação mãe-prole no Pantanal sul. Fêmeas de *L. podicipinus* cuidam dos ovos e dos girinos até a metamorfose. Esta dissertação de mestrado é apresentada em dois capítulos. No capítulo 1, medimos os custos do cuidado maternal usando dados de captura-recaptura para comparar variáveis energéticas entre fêmeas cuidadoras e não-cuidadoras (i.e. massa do corpo, massa do corpo adiposo, dos ovários e do volume do conteúdo estomacal). No capítulo 2, estudamos a relação mãe-prole, examinando as características da pele, o trato digestório dos girinos e o comportamento para investigar a ocorrência de dermatotrofia em *L. podicipinus*. Características da pele foram comparadas entre fêmeas cuidadoras, não-cuidadoras e machos. Fêmeas cuidadoras perderam massa após uma semana, comparadas às fêmeas sem girinos. A massa dos ovários e o volume dos estômagos também foram menores em fêmeas cuidadoras e estômagos vazios ocorreram apenas nessas fêmeas com girinos. Porém, não houve diferença na massa dos corpos gordurosos. Nossos resultados indicam que os benefícios do cuidado parental em *L. podicipinus* podem impor alguns custos às fêmeas, pois a redução na tomada de alimento e na massa dos ovários podem reduzir o tamanho do corpo e a fecundidade futura. Fêmeas cuidando de girinos exibiram epiderme e estrato esponjoso mais espesso. Além disso, a concentração de lipídeos foi maior na epiderme de fêmeas cuidadoras e encontramos células epiteliais no trato digestório de girinos, sugerindo dermatotrofia em *L. podicipinus*, o primeiro registro deste tipo de cuidado em Anura.

General Introduction

Brazil harbors almost 15% of anuran species of the world and in spite of this great diversity and behaviors little is known about life history of most of them (Haddad & Prado 2005, Segalla et al. 2014, Frost 2017). Besides the lack of knowledge, global evaluations about conservation on amphibians indicate that about 30% of species are in danger of extinction and 43% of populations are declining rapidly (Stuart et al. 2004, Stuart et al. 2008). In order to face these threats, it is extremely important to develop behavioral and ecological studies of anurans to apply in conservation and species management (Heyer et al. 1994, Eterovick et al. 2005).

Anurans show the highest diversity of reproductive modes among tetrapods, with more than 39 variations described in the literature, including different types of parental care behavior (Duellman & Trueb 1994, Haddad & Prado 2005, Wells 2007, Crump 2015). Parental behavior occurs in only about 10% of all anuran species and in 20% of salamanders (Summers et al. 2006, Wells 2007, Crump 2015). In anurans, parental care is most frequent in terrestrial breeding species from the humid tropics, although it may occur in some aquatic breeders (Prado et al. 2002, Gomez-Mestre et al. 2012, Zamudio et al. 2016). Care may be provided by the male, female, or even by both sexes (Wells 1981, Bickford 2002, Wells 2007). Parental care behaviors in anurans include not only care of eggs and tadpoles but also feeding, usually by means of unfertilized eggs deposited to nourish tadpoles during development (Brust 1993, Heying 2001, Vassilieva et al. 2013). Categories of parental care were suggested based on oviposition site (aquatic or terrestrial), development form (free-swimming larvae or direct development), sex role and transportation of larvae (McDiarmid 1978, Bickford 2002, Lehtinen & Nussbaum 2003).

Trivers (1972) introduced the term “parental investment” and defined it as “any investment by the parent in an individual offspring that increases the offspring’s chance of surviving (and hence reproductive success) at the cost of the parent’s ability to invest in other offspring”. The term parental care in amphibians has been used as a subset of parental investment, then including a cost for the parents, and is restricted to include only post-ovipositional behavior (Crump 1995). The evolution of parental care in anurans has being discussed in many studies at the light of the costs and benefits for both males and females (e. g., Wells 1981, Crump 1995, Beck 1998, Summers & Earn 1999). The great diversity in parental care and reproductive modes in anurans provide an excellent opportunity to test hypotheses on the costs and benefits involved, as well as processes and mechanisms leading to the evolution and maintenance of parental care behavior (Zamudio et al. 2016).

Based on oviposition site, tadpole development site, and form of larvae nourishment, frog species in the Neotropical genus *Leptodactylus* may exhibit six different reproductive modes (Prado et al 2002, Gibson & Buley 2004). Our focal species is *Leptodactylus podicipinus* (Cope, 1862), a common frog in the Pantanal, one of the largest floodplains in the world (Uetanabaro et al. 2008). The species is widely distributed in South America, in the open formations of Paraguay, Uruguay, Argentina, Bolivia, and in central Brazil. It also occurs in the Madeira and Amazon rivers of Brazil up to 500 m a.s.l. (Frost 2017). Females are larger and heavier than males, which present spines in their hands (Prado et al. 2000). In the south Pantanal, although individuals of *L. podicipinus* may reproduce through the whole year, males are more active and reproduction more intense during the rainy season between October and March (Prado et al. 2005a). Males construct basins containing water where eggs are deposited and females care for eggs and tadpoles up to metamorphosis (Prado et al. 2000, Martins 2001, Prado et al. 2002).

Taking all considerations above, the aim of this work was to evaluate the costs of parental care for female of *Leptodactylus podicipinus* in the Pantanal, examining variations in body mass, fat body mass and stomach volume content during the period of parental care. Furthermore, we also studied mother-offspring interaction, examining skin traits, tadpole guts, and behavior to investigate the occurrence of offspring skin-feeding (dermatotrophy) in this species.

1. The costs of parental care in *Leptodactylus podicipinus* (Anura: Leptodactylidae)

Abstract

To increase offspring survival, amphibians exhibit a great diversity of parental care behaviors. The costs of offspring attendance have important implications in the origin and evolution of parental care, however, lack of studies precludes us from advancing our knowledge on this issue. Herein we evaluated the costs of maternal care in the frog *Leptodactylus podicipinus* in the Brazilian Pantanal. We measured variation in body mass in attending females (N = 6) and non-attending females (N = 9) during six consecutive days and compared total differences. We also collected 15 attending and 15 non-attending females to measured and compare fat body mass, ovary mass, and stomach volume content and prey items. A total of 27% of attending females had empty stomachs, whereas none of non-attending females had empty stomachs. Hemiptera was the most important food item for attending females and Coleoptera for non-attending females. Attending females lost 0.09 g per day while caring for tadpoles and non-attending females gained 0.10 g per day. Mean body mass, ovary mass, and stomach volume content of non-attending females were significantly higher compared to attending females. However, there was no difference in fat body mass. Changes in diet are possibly related to different microhabitat use, as attending females remain most of the time in the water with the tadpoles. Our results indicate that benefits of parental care in *L. podicipinus* may impose some costs to females, as reduction in food intake and ovary mass may decrease body size and future fecundity. Species in the genus *Leptodactylus* exhibit a great diversity of reproductive modes and parental care behaviors, providing an excellent opportunity to investigate parental care evolution.

Resumo

Para aumentar a sobrevivência da prole, os anfíbios exibem uma grande diversidade de comportamentos de cuidado parental. Os custos associados ao cuidado com a prole tem importantes implicações para a origem e evolução do cuidado parental. Porém, a falta de estudos impede avanços no conhecimento sobre essa questão. Neste trabalho, avaliamos os custos do cuidado maternal na rã *Leptodactylus podicipinus* no Pantanal brasileiro. Medimos a variação em massa do corpo em fêmeas cuidando de girinos (N = 6) e em fêmeas sem girinos (N = 9) durante seis dias consecutivos e comparamos as diferenças. Também coletamos 15 fêmeas que cuidavam de girinos e 15 fêmeas sem girinos para comparar a massa dos corpos de gordura, massa dos ovários e o volume do conteúdo estomacal e itens da dieta. Um total de 27% das fêmeas cuidadoras tinha os estômagos vazios, enquanto nenhum estômago vazio ocorreu nas fêmeas sem girinos. Hemiptera foi o item mais importante na dieta de fêmeas cuidadoras e Coleoptera foi o mais importante para fêmeas sem girinos. Fêmeas cuidando de girinos perderam 0,09 g por dia, enquanto que fêmeas sem girinos ganharam 0,10 g por dia. As médias de massa do corpo, massa de ovários e volume do conteúdo estomacal foram significativamente maiores nas fêmeas sem girinos. Porém, não houve diferença na massa média dos corpos de gordura. As mudanças na dieta estão, possivelmente, relacionadas ao uso de diferentes microhabitats, já que fêmeas com girinos permanecem mais tempo na água. Nossos resultados indicam que os benefícios do cuidado parental em *L. podicipinus* podem impor alguns custos às fêmeas, pois a redução na tomada de alimento e na massa dos ovários podem reduzir o tamanho do corpo e a fecundidade futura. As espécies de *Leptodactylus* exibem uma grande diversidade de modos reprodutivos e comportamentos de cuidado parental, oferecendo uma excelente oportunidade para estudos sobre a evolução do cuidado parental.

Introduction

Amphibians exhibit elaborate and unique forms of parental care to increase offspring survival and consequently their fitness (Crump 1996, Wells 2007). However, this additional investment in the offspring often comes at the cost of future reproductive opportunities (Trivers 1972, Clutton-Brock 1991). Parental care in anurans can be provided by male, female or both, and may include different types of investment, such as in nest construction, attendance, brooding and transport of egg and tadpoles, tadpole feeding, and viviparity, which may have evolved to avoid dehydration, fungal infection, and offspring protection against predators (Crump 1996, Lehtinen & Nussbaum 2003, Wells 2007).

Particularly, nest construction and tadpole attendance have been documented in several anuran families (e.g. Bufonidae, Dendrobatidae, Leptodactylidae, Microhylidae, Myobatrachidae, Ranidae) (Lehtinen & Nussbaum 2003, Wells 2007). However, both nest construction with tadpole attendance have been observed in relatively few species, because although either the female or male could build the nest, they do not necessarily remain with the eggs after female spawn (Martins et al. 1998). Especially, offspring attendance may increase mortality risk and reduce future fecundity of the care-giver beyond the cost of producing gametes (Trivers 1972, Crump 1996, Alonso-Alvarez & Valendo 2012). Females typically invest more per gamete than do males (Trivers 1972, Clutton-Brock 1991), therefore it is expected that females care more for their offspring (Queller 1997). Besides, parental care role of females and males are addressed by differential selection on the sexes (Queller 1997, Monroe & Alonzo 2014).

Study of costs and benefits is important for the origin and evolution of parental care as a tool to understand why some parents care more than others. Based on cost-benefit theory, only when benefits are higher than costs, parental care is selected

(Crump 1996). This theory explains why some parents invest more in larger clutches. For example, males of *Kurixalus eiffingeri* provide more care to large size clutches because more benefits are presumed (Cheng & Kam 2010).

The evolution of parental care in anurans has being discussed in many studies at the light of the costs and benefits for both males and females (e. g. Wells 1981, Beck 1998, Summers et al. 1999, Brown et al. 2008). Studies about costs of maternal care have focused on the members of the families Dendrobatidae (Prohl & Hold 1999, Dugas et al. 2016) and Rhacophoridae (Kam et al. 1997). However, species of the genus *Leptodactylus* exhibit singular reproductive traits, such as different reproductive modes and parental care behaviors, making them an excellent model to study the costs and benefits of parental care (Prado et al. 2002, Pereira et al. 2015, Zamudio et al. 2016).

Males of *L. podicipinus* build basins at the margins of lentic water bodies, where females spawn eggs embedded in foam nests (Prado et al. 2002). Attending females care for eggs and tadpoles until metamorphosis is complete, guiding and guarding the tadpole school from predators (Martins 2001, Rodrigues et al. 2011). Thus, herein, we evaluated the cost of maternal care in *Leptodactylus podicipinus*. Because females of *L. podicipinus* attend their offspring during larval development, their performance could be reduced during the breeding season (Rodrigues et al. 2004), therefore we expect to find a negative correlation between attendance frequency and female body mass, fat body mass, and ovary mass. Besides, we also expect that attending females will have smaller stomach volumes due to time restrictions to feed while attending offspring, with more frequency of empty stomachs and with a more restricted diet.

Methods

Study site

The Pantanal is a region characterized by a savanna-like vegetation with an average temperature of 25.1 ºC (Mateus & Petrete 2004, Prado & Haddad 2005). The Pantanal has a seasonal climate with a wet and warm season from October to March and dry and cold season from May to September (“Aw” Köppen clasification) (Prado & Haddad 2005, Alvares et al. 2013). Fieldwork was conducted at the Base de Estudos do Pantanal, Federal University of Mato Grosso do Sul (19°34' S - 57°00' W), municipality of Corumbá, Mato Grosso do Sul state. This place is located in the Miranda river sub region, south Pantanal, where floods are common between January and April, but duration and intensity of floods are unpredictable (Prado et al. 2000, Prado et al. 2005a). Observations were conducted from November 2015 to March 2016 and from September 2016 to November 2016, remaining in the field for 10-15 consecutive days. We observed and collected data during the day and night at the margins of ponds and flooded areas, where females remain with tadpoles.

Energetic costs

We measured energetic costs for maternal care as follow: variation in mass of attending and non-attending females was measured during six consecutive days by weighing six attending and nine non-attending females every day during the breeding period. Each female was identified using a ventral photographic register of the dots present on the face and lips with a camera (Sony nex3) and body mass was measured with a digital balance (nearest 0.01 g). After measurements, females were released at the capture site. Using the first and last day of recapture, we calculated the total difference in mass and compared the differences between attending females and non-attending females.

In the field, we collected 15 attending and 15 non-attending females of *Leptodactylus podicipinus*. Females were euthanized in situ with 5% xylocaine (cream) applied on the ventral and dorsal regions. Afterwards, they were fixed in 10% formalin (we also injected in the cloaca to minimize the effects of digestion) and preserved in 70% alcohol. In the laboratory, each female was dissected to remove fat bodies, ovaries and stomachs. We weighted fat body mass and ovary mass (combining both ovaries) using an electronic balance Shimatzu AY 220 to compare attending and non-attending females. To calculate the stomach content volume, we used the sum of each prey volume, which were measured using a chamber made by microscopy slides and placed on a graph paper, modified from Camera et al. (2014).

Prey items found in each stomach were identified to order level using Triplehorn & Johnson (2011). To determine the importance of each prey in the diet of attending females and non-attending females, we calculated feeding index with Kawakami & Vazzoler (1980) formula: $Ii= Fi*Vi/\Sigma(Fi*Vi)$ (Fi: Frequency of occurrence of i item, Vi: volume of i item). This index requires previous measurements of frequency and volume that were taken as follows. Prey frequency was calculated using Bowen (1983) formula: $F\%=100ni/n$ (Fi: frequency of i prey, ni: number of stomach where i item was found, n: stomach containing food); and volume using Hynes (1950) formula: $V\%i=Vi/(Vi+Vi_2+Vin)$ (V%i: percent volume, Vi: volume by item). Although undetermined prey items were not included in the diet analysis, they were used for the total volume analysis.

Statistical analysis

We divided data between two categories to compare the effect of parental care in females and we did the same analyses for both (attending females and non-attenging females). To compare between categories we made a Mann-Whitney test to verify differences in body mass between first and last day of recapture, fat body mass, ovary mass and stomach volume content (Sokal & Rohlf 1995). We used PAST statistic program to conduct the analyses (Hammer et al. 2001).

Results

Food intake

Only attending females presented empty stomachs (27%), compared to non-attending females. In general, attending and non-attending females consumed the same prey items (Table 1). However, prey frequency varied between female categories. Coleopterans were the most important prey item for non-attending females (Figure 1), while attending females consumed hemipterans and arachnids more frequently (Figure 2). Besides, we found spiders and crickets only in the stomachs of attending female and cockroaches occurred only in the stomachs of non-attending females (Table 1).

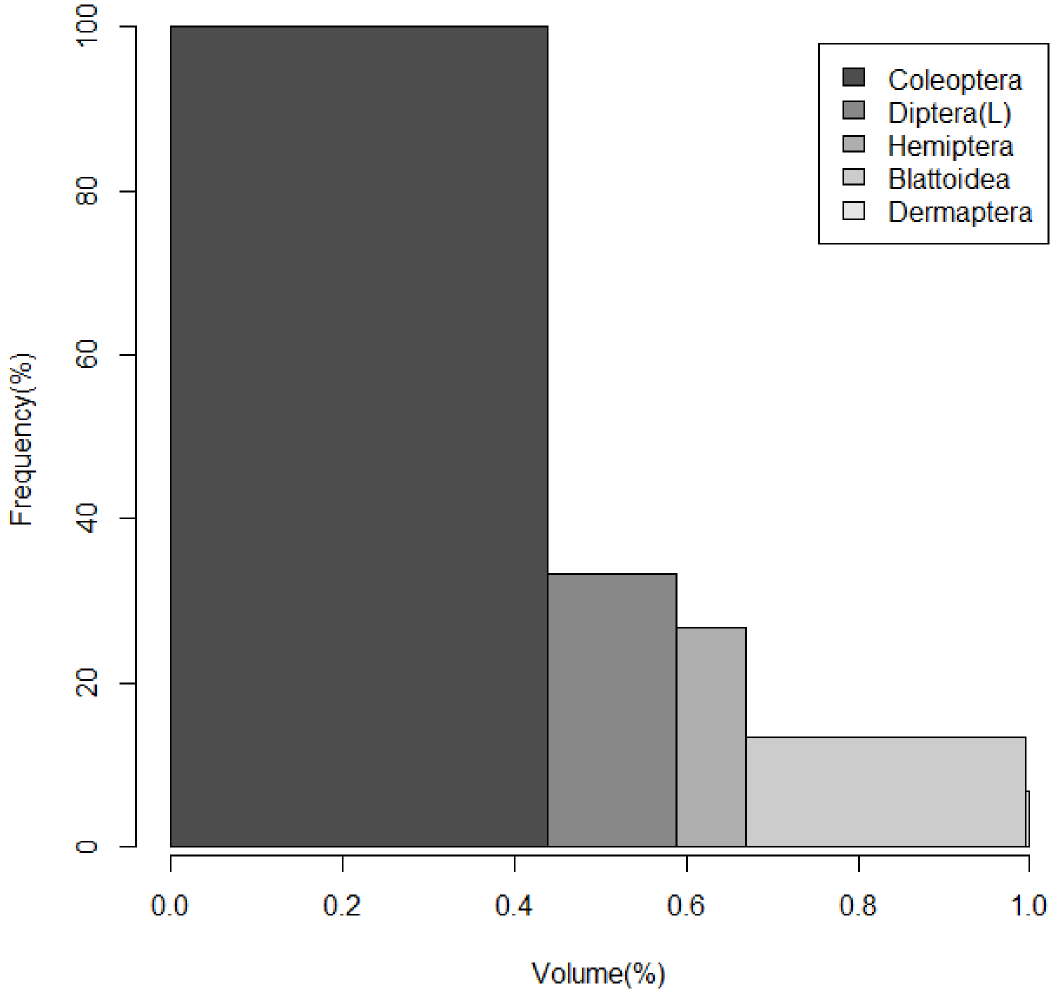

Figure 1. Frequency (%) and volume (%) of prey items for non-attending females of *Leptodactylus podicipinus* in the south Pantanal, Brazil. L = Larvae.

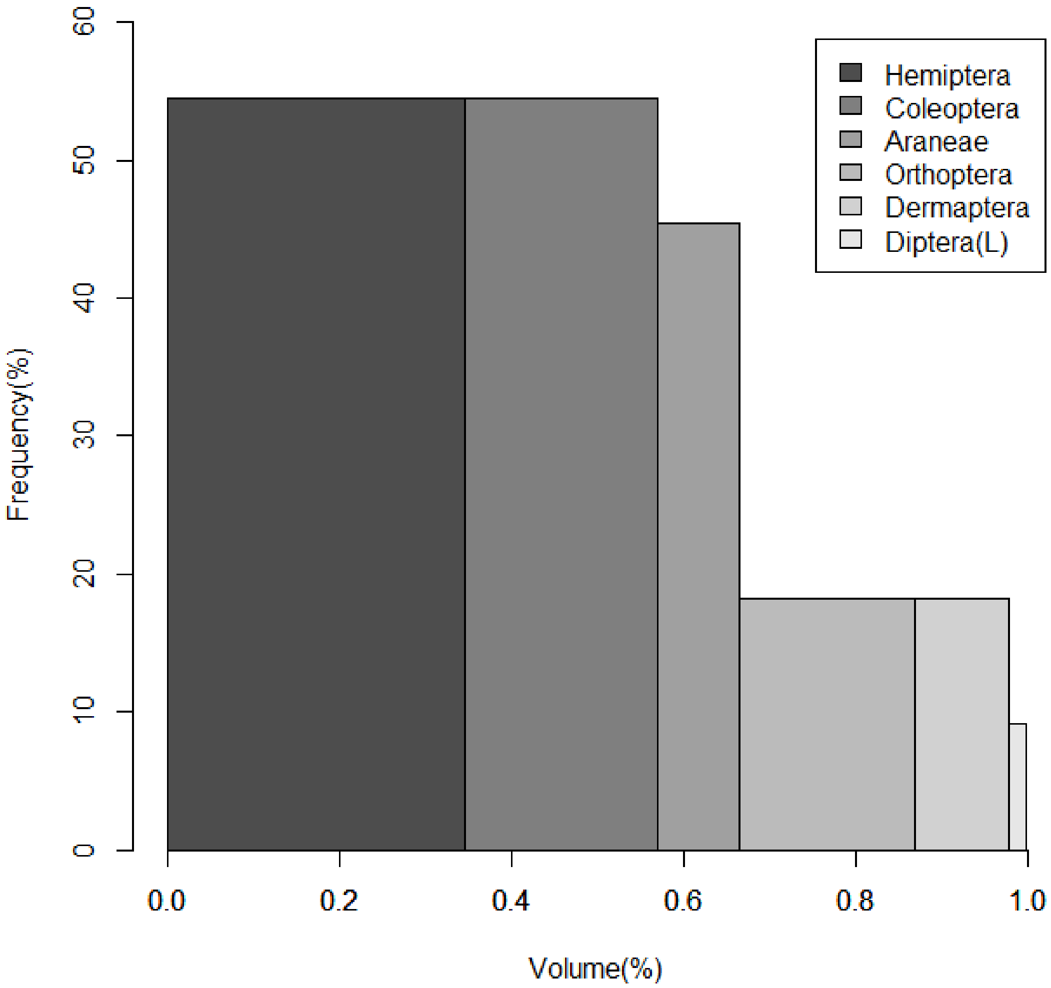

Figure 2. Frequency (%) and volume (%) of prey items for attending females of *Leptodactylus podicipinus* in the south Pantanal, Brazil. L = Larvae.

Table 1. Frequency (Fi%), volume (Vi%), and feeding index (Iai) for prey items found in the stomachs of attending and non-attending females of *Leptodactylus podicipinus* in the south Pantanal, Brazil. L = Larvae.

	Attending Females			**Non-attending Females**		
Order	**Fi%**	**Vi%**	**Iai**	**Fi%**	**Vi%**	**Iai**
Coleoptera	54.545	22.436	29.633	100	43.904	79.234
Dermaptera	18.182	10.897	4.798	6.667	0.503	0.061
Diptera (L)	9.091	2.137	0.470	33.333	14.933	8.983
Hemiptera	54.545	34.615	45.720	26.667	8.054	3.876
Blattoidea	-	-	-	13.333	32.606	7.846
Orthoptera	18.182	20.513	9.031	-	-	-
Araneae	45.455	9.402	10.348	-	-	-

Energetic costs

Regarding body mass, attending females lost, on average, 0.09 g per day, whereas non-attending females gained 0.10 g per day (Figure 3A and 3B). After six days of capture-recapture, attending females lost, on average, 0.49 g (± 0.45) and non-attending females gained, on average, 0.93 g (± 1.03). Female body mass, ovary mass, and stomach volume content of attending and non-attending females were significantly different after six days ($p = 0.002$; $p < 0.001$; $p = 0.003$, respectively; Figure 4A, 4C and 4D). Ovary mass weighted less in attending females ($\overline{X} = 0.03$ g ± 0.02) compared to non-attending females ($\overline{X} = 0.3$ g ± 0.2). Similarly, stomach volume content was always smaller in attending females ($\overline{X} = 35.86$ mm^3 ± 42.86) than in of non-attending ($\overline{X} = 105.46$ mm^3 ± 42.86). However, fat body mass did not show significant differences between non-attending females and attending females ($p = 0.72$; Figure 4B).

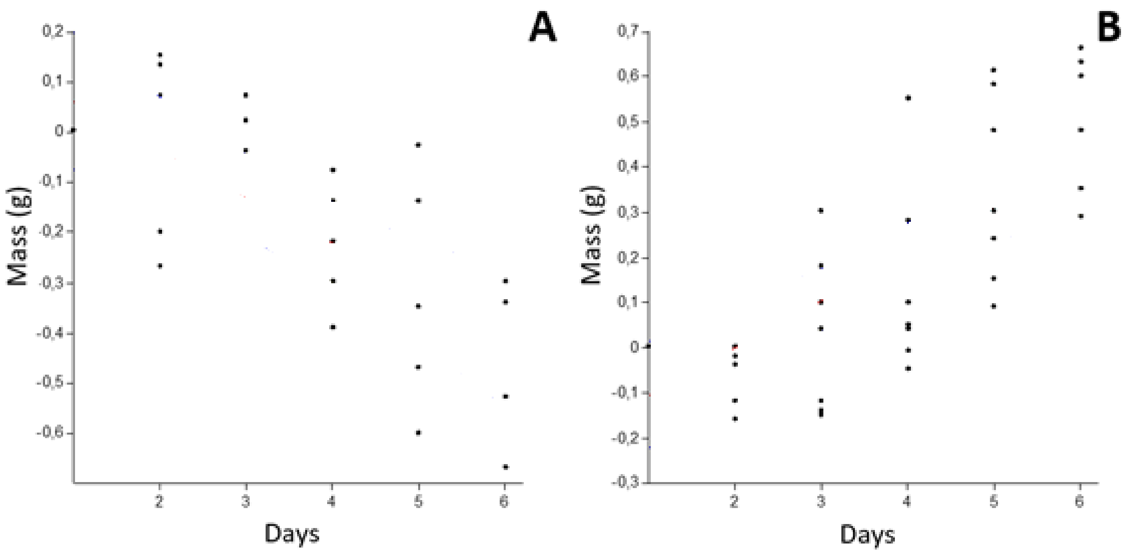

Figure 3. Body mass (initial and final measurements) for attending females (A) and non-attending females (B) of *Leptodactylus podicipinus*, measured within a six-day period, in the south Pantanal, Brazil.

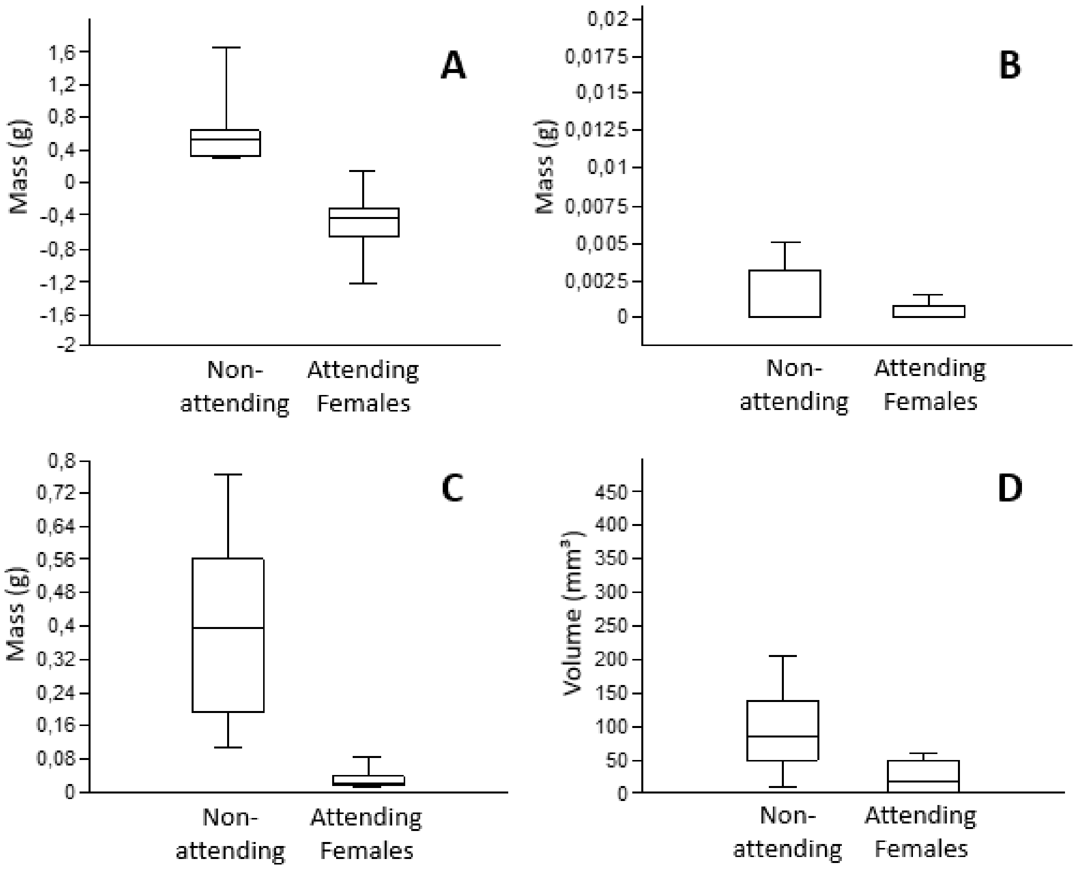

Figure 4. Comparison of mean body mass (A), fat body mass (B), ovary mass (C), and stomach volume content (D) between non-attending females and attending females of *Leptodactylus podicipinus*, measured within a six-day period, in the south Pantanal, Brazil.

Discussion

The frog *Leptodactylus podicipinus* is considered a sit-and-wait generalist predator and its diet is mainly composed of arthropods, especially Coleoptera, one of the most abundant preys in many different habitats (Borror & Delong 1962, Pough et al. 1998, Rodrigues et al. 2004, this study). We found that although coleopterans were the main prey item in the stomachs of non-attending females, followed by dipterans and hemipterans, attending females preyed more on hemipterans, coleopterans and spiders. These differences in prey category frequencies detected in our study could be due to (1) the use of a different microhabitat during parental care and/or (2) the availability of preys in the environment. In general, *L. podicipinus* forage at the margins of water bodies, allowing the encounter of some preys such as coleopterans (Rodrigues et al. 2004). However, females performing parental care remain most of the time in the water close to the tadpoles (Prado et al. 2002, Rodrigues et al. 2004). This change in microhabitat due to parental care behavior (Townsend 1986, Rodrigues et al. 2004) may explain the higher frequency of aquatic hemipterans in their diet.

Diet of generalist species, such as many anurans, may vary according to seasonal variations in arthropod densities and availability (Toft 1981, Lieberman & Dock 1982). As a result, diet may follow the natural fluctuations of prey abundancies (Whitfield & Donnelly 2006). Unfortunately, we did not evaluate prey availability, but as attending and non-attending females were collected in the same study site and in the same period of the year, we do believe that differences in diet may be related to microhabitat use due to parental care behavior of attending females.

As behavior changes during parental care because of the offspring attendance, parental care may have different requirements that encourage females to change prey selection. In our study, the high frequency of ingestion of Araneae by attending females may not reflect opportunism. Spiders are recognized as opportunistic tadpole predators (Menin et al. 2005), and attending females of *L. podicipinus* and *L. natalensis* have been reported preying on insects and spiders, specifically on spiders approaching to prey eggs and tadpoles (Martins 2001, Santos & Amorim 2006). Thus, we suggest that attending females may benefit preying on spiders, obtaining some energy while increasing tadpole survival.

In amphibians fat is stored in special organs, the fat bodies, associated to gonads, and their mass are used as indicator of organism body condition (Jorgensen 1992). Fat bodies may serve as reserves for unfavorable periods, but also may provide energy for gametogenesis during the reproductive activity (e.g. Long 1987, Saidapur & Hoque 1996). In the south Pantanal, a negative correlation between fat body mass and ovary mass has been found for females of some anuran species, but not for *L. podicipinus* (Prado & Haddad 2005). Furthermore, decrease of fat bodies has been shown for species with long periods of parental care, such as *Cophixalus parkeri* (Microhylidae), where males stay with their offspring for 85 to 100 days. During this time, they consume less food and remain long time with empty stomachs (Simon 1983). We found no differences in fat body masses between attending and non-attending females of *L. podicipinus* (Figure 2B). It seems that attending females did not use fat bodies as a source of energy to compensate for the costs of parental care. Alternatively, we may not have detected differences because of the short period of our study, not as long as in *C. parkeri* (Simon 1983).

In the evolution of life histories, five trade-offs have received much attention: (1) current reproduction vs. survival; (2) current vs. future reproduction; (3) current reproduction vs. parental growth; (4) current reproduction vs. parental condition; and (5) number vs. size of offspring (Stearns 1989). Many trade-offs, i.e., a beneficial change in one trait linked to a detrimental change in another, are related to the costs of parental care, which may increase offspring survival at the expenses of increasing parental vulnerability to predation, decreasing food intake, or reducing parental mating opportunities or fecundity (Stearns 1989, Crump 1996, Huang & Pike 2013). In the present study, only attending females presented empty stomachs (nearly 30%) and mean stomach content volume was smaller compared to non-attending females. This result might be due to foraging time restriction, possibly associated to parental care (Rodrigues et al. 2004), which may have contributed to loss of body mass in attending females. Similarly, attending females invested less in ovary mass compared to non-attending females. However, some immature oocytes were present in attending females´ ovaries, evidencing that they are able to invest some energy in future reproduction. We were unable to measure offspring survival or performance (benefits), nor mother survival to future reproduction or body growth (costs), but our results indicate that benefits of parental care in *L. podicipinus* may impose some costs to females, as reduction in food intake and ovary mass may decrease body size and future fecundity, two variables highly positively correlated in anurans (Prado & Haddad 2005, Wang et al. 2009).

Parental care is expected to evolve when fitness benefits overcome the costs (Kvarnemo 2010). Costs paid by males and females have important selective consequences (Trivers 1972), and because females are more often in charge of parental care (Queller 1997), costs may play a more important role in female selection. Some authors suggest that as initial investment is high in females (egg production), they tend to care more, resulting in multiple types of care behaviors (e.g. Downie et al. 2005). Queller (1997) proposed that a pre-mating asymmetry in investment encourages a similar post-pairing asymmetry in parental investment. Furthermore, as males, in general, experience more intense sexual selection (male competition; female choice), what leads to a greater variance in male reproductive success, males are less likely to provide care after mating than females (Queller 1997). Another explanation for why females care more is the "certainty of paternity hypothesis" (Trivers 1972, Queller 1997), which argues that when females have clutches sired by multiple males, each male has lower expected relatedness to the offspring, making him less likely to provide care than female. Females of *Leptodactylus* in the groups *L. melanonotus* and *L. latrans* exhibit a complex parental care behavior, attending eggs and tadpole schools (Prado et al. 2002, de Sá et al. 2014) and multiple-male spawning has been described for *L. podicipinus* (*L. melanonotus* group) and *L. chaquensis* (*L. latrans* group) in the Pantanal (Prado & Haddad 2003). Thus, future studies should investigate the fitness costs and benefits for males and females and the role of polyandrous mating in the evolution of maternal care in the genus *Leptodactylus*.

Females can adjust their parental care expenditure in relation to the variation in costs to themselves and the benefits to their offspring to maximize fitness (Winkler 1987). Levels of care can increase when the cost to the parent is low and decrease when the cost is high (Crump 1996). Species in the genus *Leptodactylus* show a gradient of different parental care behaviors, from lack of parental care to complex behaviors, such as tadpole feeding: females of *L. bufonius* close the entrance of the subterranean chambers where eggs are deposited (Reading & Jofré 2003), females of many species in the *L. latrans* and *L. melanonotus* groups, including *L. podicipinus*, care for eggs and tadpoles with complex associated behaviors (Heyer 1969, Prado et al. 2002, de Sá et al. 2014), and some species in the *L. pentadactylus* group feed offspring with unfertilized eggs (Crump 1995, Gibson & Buley 2004, Wells 2007). Moreover, *Leptodactylus* is a genus with six different reproductive modes, from aquatic to terrestrial ones (Prado et al. 2002, Gibson & Buley 2004). Considering the strong relationship between parental care, terrestrial reproduction, and reproductive modes (Gomez-Mestre et al. 2012), the genus *Leptodactylus* provide a great opportunity to test whether gradient of costs have led to the evolution of gradients of parental care.

2. First evidence of offspring skin-feeding by females of *Leptodactylus podicipinus* (Anura: Leptodactylidae)

Abstract

In some anuran species, parental care includes not only attending offspring but also feeding, usually with females providing unfertilized eggs to nourish tadpoles. Dermatotrophy is a very unusual mode of parental care in tetrapods, only known among caecilians. In the frog *Leptodactylus podicipinus*, parental care is provided by females that care for eggs and tadpole schools, which browse the back and legs of attending females, suggesting dermatotrophy or chemical communication. We investigated skin traits of attending females of *L. podicipinus*, trying to identify glands/secretions involved with offspring skin-feeding or chemical communication. We compared skin traits of attending females with those of non-attending females and males. Individuals were collected from January to March 2016, in temporary ponds in the south Pantanal, Brazil (19°34' S - 57°00' W). We used light microscopy and histochemical analyses to characterize individuals´ skin features and measured epidermis and stratum corneum thickness from different parts of the body (anterior back, arm, posterior back and leg). We also examined variation in body mass of attending females within an interval of six days and analyzed tadpole esophagus searching for epithelial cells. Attending females exhibited thicker skin compared to non-attending females and males, and the epithelial cells of attending females were slightly more reactive to total lipids despite the absence of lipid droplets. Moreover, attending females lost body mass after six days of parental care and we found epithelial cells in the esophagus of tadpoles in different stages of development. Although different glands were not found in females skin, chemical communication between female and tadpoles is not disregarded. However, our results

suggest the occurrence of dermatotrophy in *L. podicipinus*, being the first record of this type of parental care for Anura.

Resumo

Em algumas espécies de anuros o cuidado parental inclui não apenas o cuidado com a prole, mas também o fornecimento de alimento, em geral na forma de ovócitos tróficos. Dermatotrofia é uma forma muito rara de cuidado parental em tetrápodes, conhecida apenas para cecílias. Na rã *Leptodactylus podicipinus* o cuidado parental é exercido pela fêmea, que cuida dos ovos e do cardume de girinos, os quais raspam a pele das patas traseiras e do dorso das fêmeas, sugerindo dermatotrofia ou comunicação química. Nós investigamos as características da pele das fêmeas de *L. podicipinus* exercendo cuidado parental na tentativa de identificar glândulas/secreções relacionadas à alimentação dos girinos por dermatotrofia ou comunicação química. Comparamos as características da pele de fêmeas cuidadoras com aquelas de fêmeas sem girinos e machos. Os indivíduos foram coletados de janeiro a março de 2016, em alagados temporários no Pantanal sul, Brasil (19°34' S - 57°00' O). Por meio de microscopia de luz e análises histoquímicas, caracterizamos a pele dos indivíduos e medimos a espessura da epiderme e do estrato esponjoso de diferentes partes do corpo (pata traseira, braço, região dorsal anterior e posterior). Também comparamos a massa do corpo de fêmeas exercendo o cuidado parental recapturadas em um intervalo de seis dias e analisamos o trato digestório de girinos, buscando por células epiteliais. Fêmeas com cuidado parental apresentaram pele mais espessa na comparação com fêmeas sem girinos e machos, e as células epiteliais das fêmeas com girinos foram mais reativas para lipídios, apesar da ausência de gotas de lípidos. Além disso, fêmeas com cuidado parental perderam massa corpórea após seis dias de cuidado e encontramos células epiteliais no trato digestório de girinos em diferentes estágios de desenvolvimento. Apesar de não termos encontrado glândulas diferenciadas na pele das fêmeas, não descartarmos a ocorrência de comunicação química entre fêmeas e girinos. Porém, nossos resultados sugerem a ocorrência de

dermatotrofia in *L. podicipinus*, sendo este o primeiro relato deste tipo de cuidado parental para Anura.

Introduction

Anurans are the most diverse among terrestrial vertebrates in number of reproductive modes (Haddad & Prado 2005), which includes parental care as an important component (Crump 2015, Zamudio et al. 2016). Percentage of species known to exhibit parental care among anurans is around 10% of all species (Crump 2015), but such proportion may be underestimated due to lack of field studies for many species. Parental care is most frequent in terrestrial breeding anurans from the humid tropics, although it is reported for some aquatic breeders (Wells 1981, Prado et al. 2002, Gomez-Mestre et al. 2012). Care may be provided by males, females, or even by both sexes (Wells 1981, Bickford 2002, Wells 2007). In some species of anurans parental care include not only care of eggs and tadpoles but also feeding, usually by means of unfertilized eggs deposited to nourish tadpoles during development (Brust 1993). This behavior occurs in five families of anurans, Dendrobatidae (Weygoldt 1980), Hylidae (Lourenço-De-Morais et al. 2009), Leptodactylidae (Gibson & Buley 2004), Mantellidae (Heying 2001), and Rhacophoridae (Vassilieva et al. 2013).

Dermatotrophy is a very uncommon form of parental care among tetrapods (Kupfer et al. 2006). Among amphibians, offspring feeding by dermatotrophy has been observed in direct-developing oviparous caecilians (Kupfer et al. 2006, Kouete et al. 2012). Females attend offspring and eventually the hatchlings bite and take pieces of mother’s skin with special “fetal-like” teeth (Kupfer et al. 2006). During parental care, attending females of caecilians change skin color and loose body mass, reflecting a significant energetic cost and physiological changes in skin and lipids concentrations (Kupfer et al. 2006, Kouete et al. 2012). One of the hormones always related with physiological changes and parental care behavior is prolactin (Schradin & Anzenberger

1999). In frogs, it is usually related with skin osmoregulation, permeability, and adaptation to environmental conditions (Inceli et al. 2010).

In the Neotropical leptodactylid *Leptodactylus podicipinus*, parental care is provide only by females (Martins 2001, Prado et al. 2002). Females attend eggs deposited in foam nests and maternal care extends to the end of the larval development (Martins 2001). Besides guarding eggs and tadpoles, females guide the school of tadpoles using “pumping” behavior, they present aggressive behavior against potential predators, and guide tadpoles to safe sites (Prado et al. 2000, Martins 2001, Prado et al. 2002). Water movements caused by “pumping” behavior mark the route for tadpoles, being considered a form of mechanical communication between female and offspring (Martins 2001, Rodrigues et al. 2011).

Female orientation of tadpoles may also include chemical signs (Wells & Bard 1988, Kam & Yang 2002; Brunetti et al. 2015). However, the mechanisms and adaptations related to this female-tadpole bimodal communication are unclear (Brunetti et al. 2015). Besides mechanical communication and guidance (Prado et al. 2000, Martins 2001, Prado et al. 2002), previous behavioral observations of schooling tadpoles of *L. podicipinus* browsing the back and legs of attending females indicated a possible chemical communication or trophic interaction by means of dermatotrophy between attending females and schooling tadpoles (C.P.A. Prado, pers. comm.), similar to the description of tadpoles scraping the dorsal skin of attending females of *Leptodactylus latrans* (Vaz-Ferreira & Gehrau 1975). Although dermatophagy, when an animal eats epidermis shed from itself or from conspecifics, is considered a common behavior in anurans, including Leptodactylidae, it has not been related with parental care yet (Weldon et al. 1993). Thus, here we studied the relationship between attending females and tadpoles, trying to investigate chemical communication and/or possible trophic interaction by dermatotrophy by examining skin features of *L. podicipinus*. We present for the first time evidences of offspring skin-feeding as part of the parental care behavior in anurans.

Methods

Study species

The frog *Leptodactylus podicipinus* (Cope 1862) is a Neotropical leptodactylid member of the *Leptodactylus melanonotus* group, one of the four recognized groups for the genus, which also includes species in the *L. fuscus*, *L. pentadactylus* and *L. latrans* groups (Heyer 1969, de Sá et al. 2014, Pereira et al. 2015). The species *L. podicipinus* is widely distributed mainly throughout open formations in South America, including Paraguay, adjacent Argentina, Bolivia, northwestern Uruguay, and central Brazil, extending along the Rio Madeira and Rio Amazonas in the Amazon Basin (Frost 2017).

In the south Pantanal, Brazil, the species reproduces throughout the year, mainly in the hot and rainy months, between November and March, at the margins of ponds and flooded areas (Prado et al. 2000). Males call from the edges of water bodies, where they construct basins containing water; eggs embedded in foam nest are deposited by the pair inside the basins (Prado et al. 2002). Females attend nests, guide and protect schooling till the end of metamorphosis (Martins 2001, Prado et al. 2002).

Data collection

We collected individuals of *Leptodactylus podicipinus* during January and March 2016 in multiple temporary ponds (reference point 19°34' S - 57°00' W) at Base de Estudos do Pantanal (BEP), Universidade Federal de Mato Grosso do Sul, municipality of Corumbá, Mato Grosso do Sul state, southwestern Brazil. We categorized each collected individual as belonging to one of the three groups: attending females (i.e.

females attending tadpoles), non-attending females (i.e. females with no parental care), and males. We collected seven attending females, seven non-attending females, and seven males. All individuals were anesthetized with xylocaine 5% and died by overdose. The skin samples of the arm, leg (right), anterior and posterior back of each individual were dissected and fixed in paraformaldehyde 10%.

Histology and histochemistry

Some samples of each group were fixed in formaldehyde 10% to test for lipidic substances; the remaining parts were dehydrated in alcohol 70%. The skin samples were washed twice in 0.1M phosphate buffer (pH 7.4) for one hour. The dehydration procedure was carried out in ascending alcohol series (70-95%) and the samples were embedded in glyco-metacrylate resin Leica. The polymerized blocks were cut on a rotatory microtome and the cuts of 4um were mounted in slides. To general histological description, the slides were stained with hematoxilin and eosin (HE), according to Junqueira & Junqueira (1983).

For the histochemical analyses, slides were stained with xylidine ponceau (Mello & Vidal 1980) for proteins, periodic acid-Schiff (PAS) and Alcian blue (pH 2.5) for neutral and acid polysaccharides, respectively (Junqueira & Junqueira 1983). For lipid demonstration, some skin samples were not dehydrated and directly embedded in Leica resin. The cuts were stained with Sudan black B (Junqueira & Junqueira 1983).

Skin thickness was measured using transversal sections from the different body regions. We measured the whole epidermis and also only the stratum corneum. For each body region, we measured the thickness in 30 points separated by 160 µm in three random parts of the sample, totalizing 90 values for each body part of each individual. We calculated means for all measures. We measured samples using a Leica DM2000 light microscope at 40X objective lens and the skin measurements were taken using Leica IM50 software.

We also collected five tadpoles from five different schools, totaling 25 tadpoles. Individuals were fixed in formaldehyde 10%. From each tadpole, we separated 5 mm of esophagus as close as possible of the mouth, and we included the content in 1 ml of distilled water. Then, we took a 0.1 ml sample to count number of cells over a slide covering the entire visual camp of the slide. We stained with Hematoxylin-Eosin to confirm if epithelial cells could be part of the diet.

Statistical analyses

We tested measures for normality using the test Shapiro-Wilk in the software Past (Hammer et al. 2001). The thickness of the epidermis and stratum corneum for each group (attending females, non-attending females, males) and each body part were compared by a MANOVA (Sokal & Rohlf 1995). We reduced measurements using average.

We made a separate analysis for posterior back with n = 7. After normality test with a negative result, we performed a Kruskall-Wallis test to compare thickness of epidermis and stratum corneum between groups (attending females, non-attending females, males), and a Post Hoc analysis for all. We also compared the body mass of attending females in two times of capture with six days of difference using a t paired test (n = 6 females).

For number of epithelial cells in tadpole esophagus, we used ANOVA test to compare number of epithelial cells among five stages of development, according to Gosner (1960): stage 28, 29, 30, 37, and 38.

Results

Histology

Among the three groups compared, attending females presented thicker epidermis and stratum corneum compared to non-attending females and males (Figure 1A). Additionally, non-attending females and males showed very similar thickness in these two tissue layers (Figure 1B, 1C; see statistical analyses in Measurements below).

We found granules of melanin migrating to the top of epidermis in all samples. Skin of attending females and non-attending females presented serous gland with the same structure and granulated content, sometimes with thick acidophil walls (Figure 1D), sometimes with thin walls (Figure 1E). Male serous glands presented the same structure, but they were larger and located in groups (Figure 1F).

Among the three groups, the mucous glands were similar in size and shape with the major axis always parallel to the epidermis (Figure 1G, 1H, 1I). However, mucous glands in attending females had a longer duct as a result of the thicker epidermis (Figure 1G).

Histochemical analyses

We only found histochemical differences among groups for lipidic concentrations. Attending females´ epidermis was slightly more reactive with lack of droplets (Figure 1M). On the other hand, non-attending females and males presented similar concentration of lipids (Figure 1N and 1O).

We present the remaining histochemical results by skin parts. Epidermis and serous gland were positive for proteins, stratum corneum and mucous glands were strongly positive for proteins (Figure 2A, 2B). Only mucous gland were strongly positive for neutral polysaccharides, and serous glands and epidermis were negative

(Figure 2C, 2D). All skin parts were negative for acidic polysaccharides (Figure 2E, 2F).

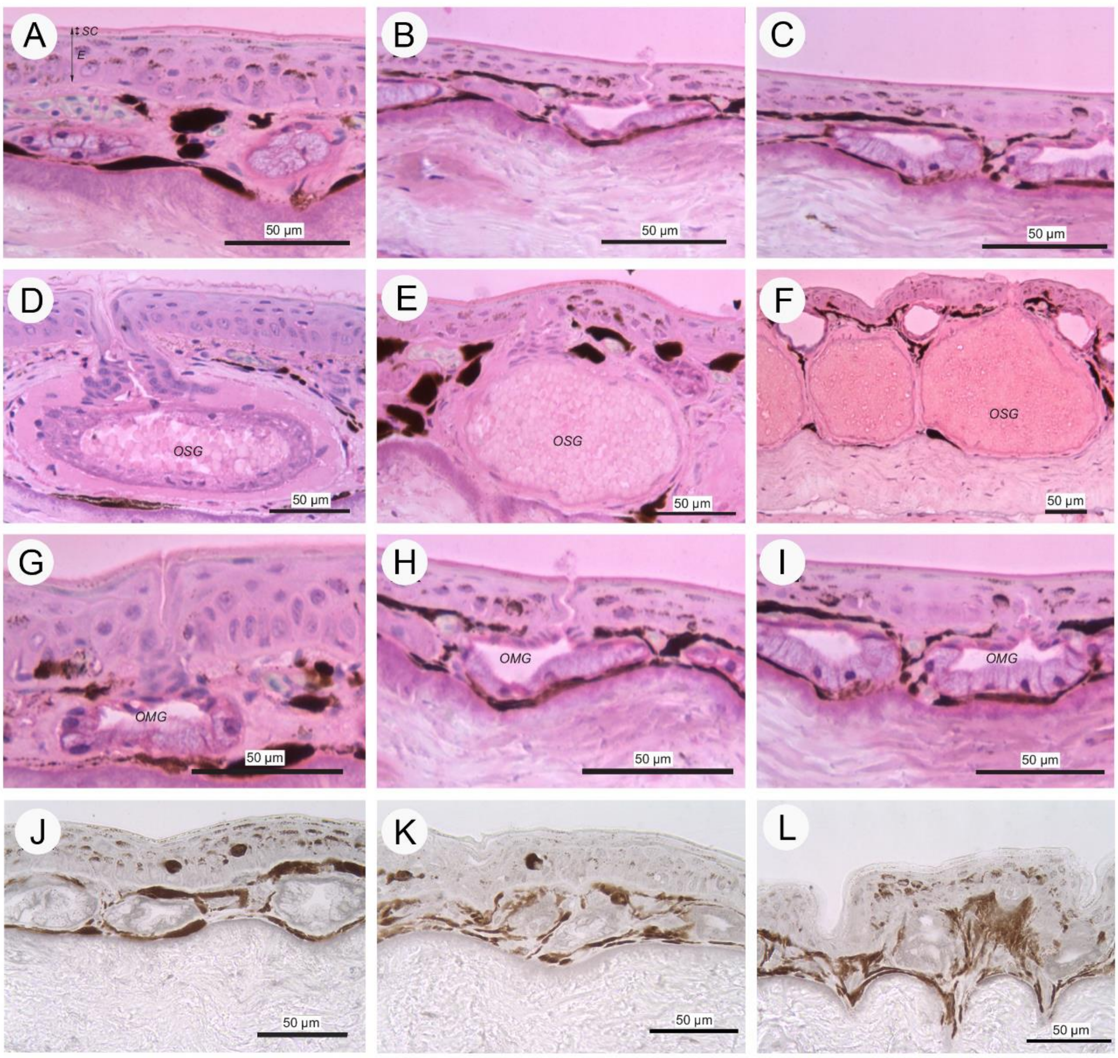

Figure 1. Comparison of skin from posterior back of attending females, non-attending females, and males of *Leptodactylus podicipinus*. Epidermis of attending females (A), non-attending females (B), and males (C). Notice the difference in the epidermis thickness of attending females and the other groups. Ordinary serous gland in attending females (D), non-attending females (E), and males (F). These glands are very similar, being more abundant in males. Absence of histological differences in the ordinary mucous gland of attending females (G), non-attending females (H), and males (I). Epidermis of attending females slightly more positive for lipids when compared to (J) non-attending females (K) and males (L). Sudan black stain. *E*= epidermis, *OMG*= Ordinary mucous gland, *OSM*= Ordinary serous gland, *SC*= stratum corneum.

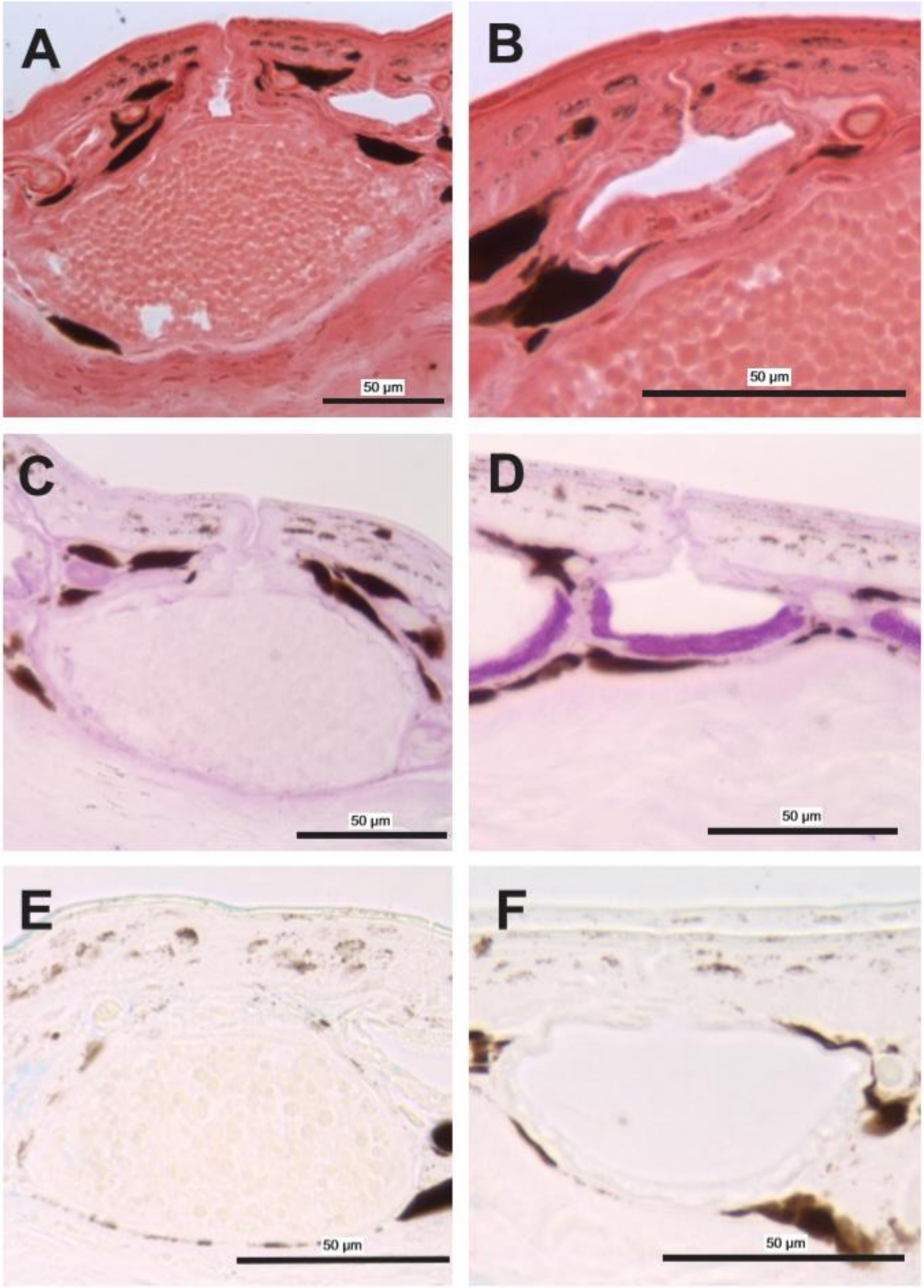

Figure 2. Histochemical analyses for *Leptodactylus podicipinus* skin. (A) Ordinary serous gland showing the reservoir filled with granular secretion strong reactive for proteins and (B) ordinary mucous gland stained with xylindine poanceau; (C) ordinary serous gland depicting absence of neutral polysaccharides at the reservoir granule; (D) ordinary mucous gland stained with PAS; (E) ordinary serous gland and (F) ordinary mucous gland stained with Alcian blue pH 2.5.

Measurements

Differences between animal groups where significant for epidermis and stratum corneum (p = 0.007; p = 0.005; respectively). Epidermis of attending females was thicker and significant different from non-attending females and males (Table 1, Table 2 and Figure 3A). On the other hand, stratum corneum of attending females was significantly different and thicker from non-attending females and males only in the posterior back and anterior back (Table 1, Table 2 and Figure 3B).

Analysis of the posterior back skin thickness indicated a significant difference among attending females (n = 7), non-attending females (n = 7), and males (n = 7) in both the epidermis (p<0.001; Figure 4A) and stratum corneum (p<0.001; Figure 4B). All animal groups were different in-between in epidermis (p<0.004) and stratum corneum (p<0.002). Attending females´ posterior back epidermis was thicker ($\bar{X}$ =34.62 µm ± 5.6), followed by females ($\bar{X}$ =20.61 µm ± 2.14), and males ($\bar{X}$ =15.86 µm ± 2.19). For stratum corneum, attending females also exhibited the highest thickness ($\bar{X}$ =6.62 µm ±1.23), followed by females ($\bar{X}$ =3.08 µm ± 0.37), and males ($\bar{X}$ =2.53 µm ± 0.18). Regarding attending female body mass, we found a difference in a six-day period of recapture (p = 0.046; n = 6). Attending females lost 0.49 g (± 0.45) after six days of parental care (Figure 5).

Table 1. Post hoc test between attending females, non-attending and males by body parts for epidermis and stratum corneum.

	Epidermis	Stratum Corneum
Anterior		
Attending Female vs. Non-attending	<0.0001	0.0202
Attending Female vs. Male	<0.0001	0.0003
Non-attending vs. Male	0.9776	0.2228
Arm		
Attending Female vs. Non-attending	0.0166	0.1024
Attending Female vs. Male	0.0004	0.3315
Non-attending vs. Male	0.3089	0.7711
Leg		
Attending Female vs. Non-attending	0.0458	0.51
Attending Female vs. Male	<0.0001	0.2822
Non-attending vs. Male	0.0678	0.9005
Posterior		
Attending Female vs. Non-attending	0.0001	<0.0001
Attending Female vs. Male	<0.0001	<0.0001
Non-attending vs. Male	0.5937	0.5386

Table 2. Thickness of epidermis and stratum corneum of attending females, non-attending females and males by body parts. Av: Average (µm); SD: Standard deviation.

		Anterior		Arm		Posterior		Leg	
		Av	SD	Av	SD	Av	SD	Av	SD
Epidermis	Attending Female	30.47	2.46	32.89	4.36	29.55	1.69	32.46	1.01
	Non- Attending Female	17.99	3.48	25.69	0.65	23.45	1.33	20.33	0.90
	Male	17.50	6.09	22.09	1.01	17.31	4.52	17.96	1.13
Strarum Corneum	Attending Female	4.49	0.43	4.18	0.72	5.65	1.22	3.76	0.64
	Non- Attending Female	3.09	0.43	3.14	0.02	3.00	0.64	3.21	0.59
	Male	2.26	0.66	3.47	0.29	2.48	0.09	3.00	0.34

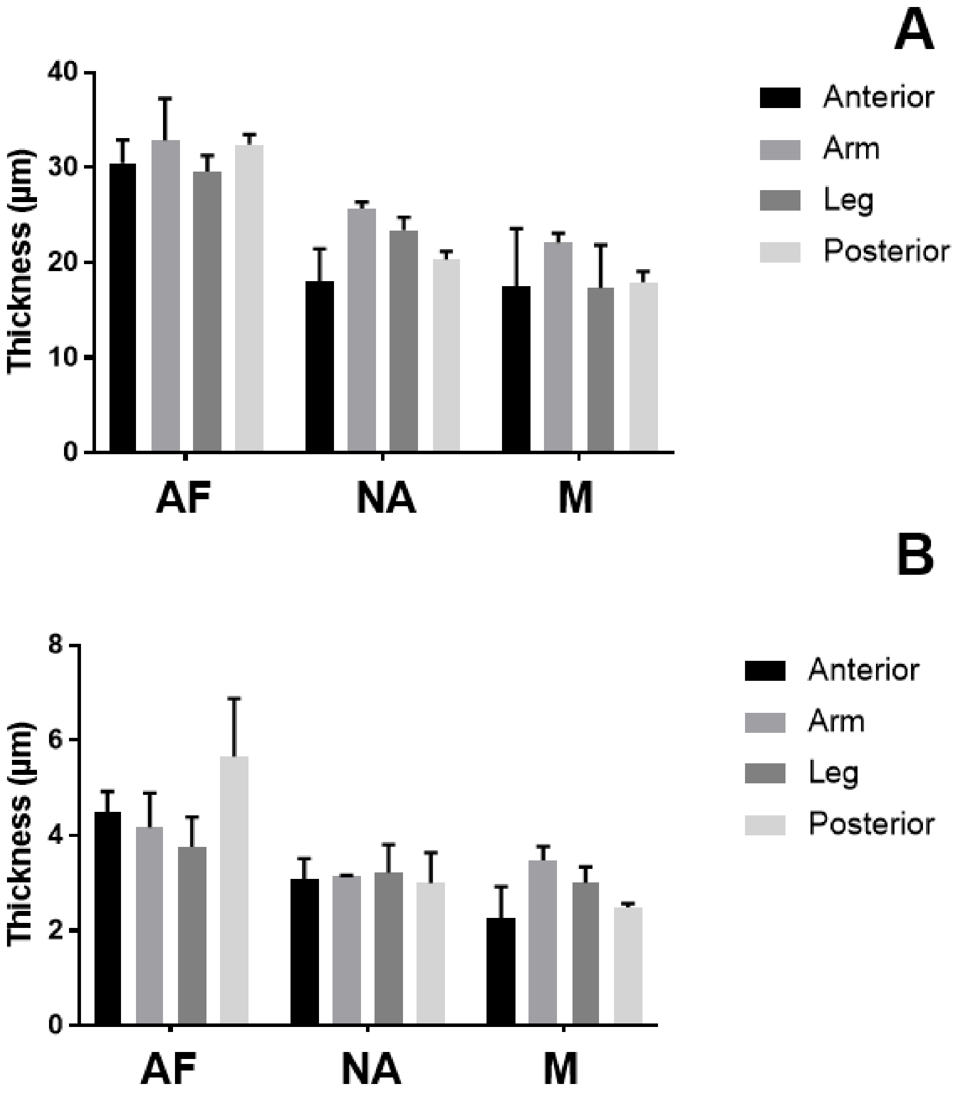

Figure 3. Comparison of epidermis (A) and stratum corneum (B) thickness of different body parts among attending females (AF), non-attending females (NA), and males (M) of *Leptodactylus podicipinus*.

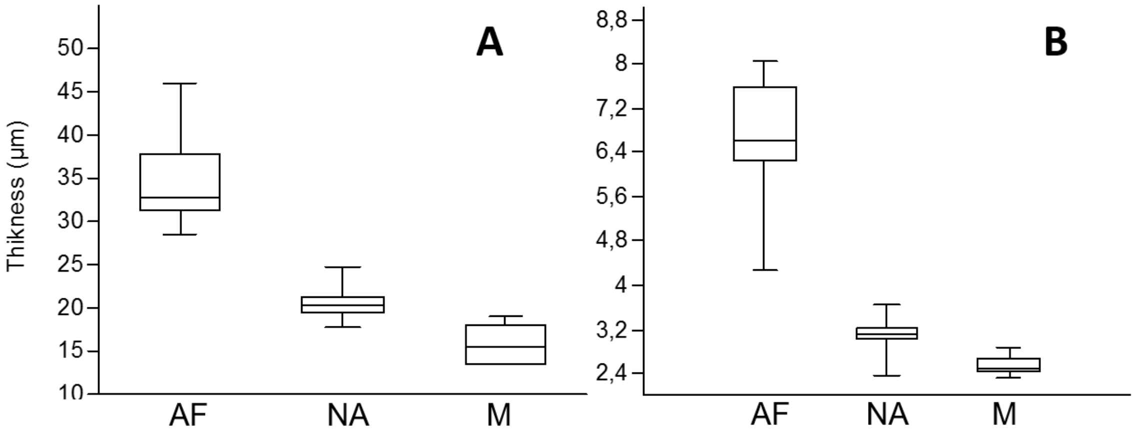

Figure 4. Thickness comparison of epidermis (A) and stratum corneum (B) from the posterior back skin in attending females (AF), non-attending females (NA) and males (M) of *Leptodactylus podicipinus*.

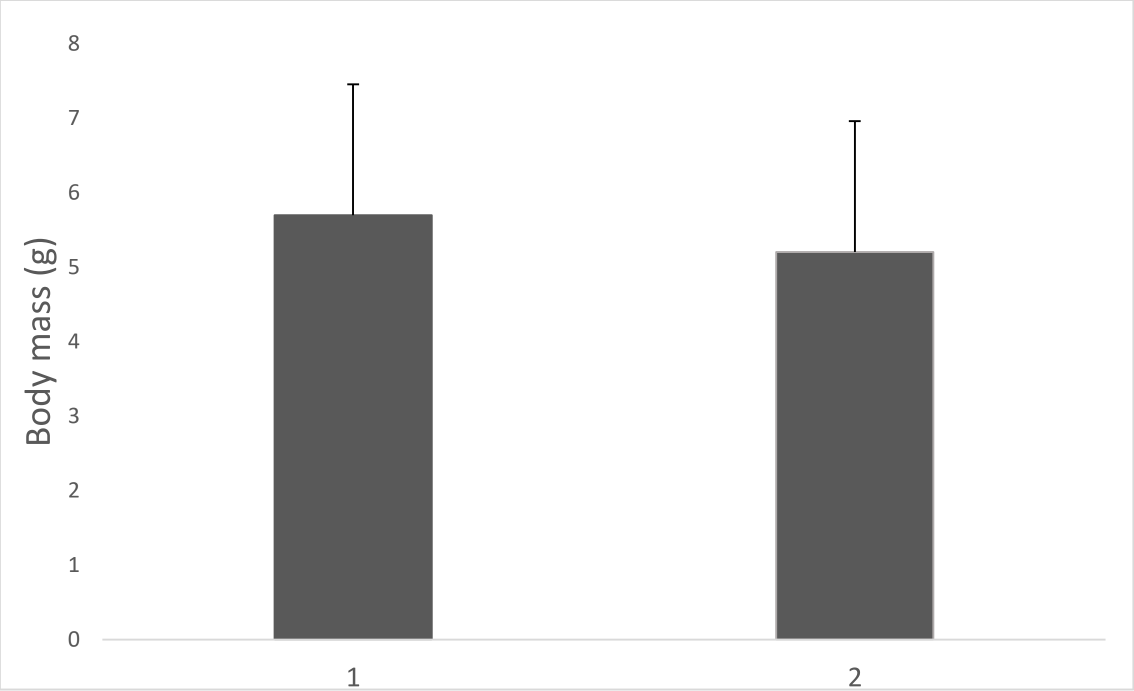

Figure 5. Mean body mass of attending females of *Leptodactylus podicipinus* between a first (1) and a second (2) measurement with a difference of six days in-between.

Tadpoles

We found epithelial cells in the esophagus of tadpoles in all stages of development, but not in all tadpoles; 16% of tadpoles did not have epithelial cells. We found squamous epithelial cells with irregular shape and central nucleus (Figure 6). Only tadpoles in stage 38 differed in number of cells in the esophagus from tadpoles in stage 28 ($p = 0.02$), and stage 29 ($p = 0.03$). Tadpoles in stage 38 had less epithelial cells ($\overline{X} = 0.6 \pm 0.86$; $n = 5$) than tadpoles in stages 28 ($\overline{X} = 3.2 \pm 1.78$; $n = 5$) and 29 ($\overline{X} = 2.6 \pm 1.14$; $n = 5$).

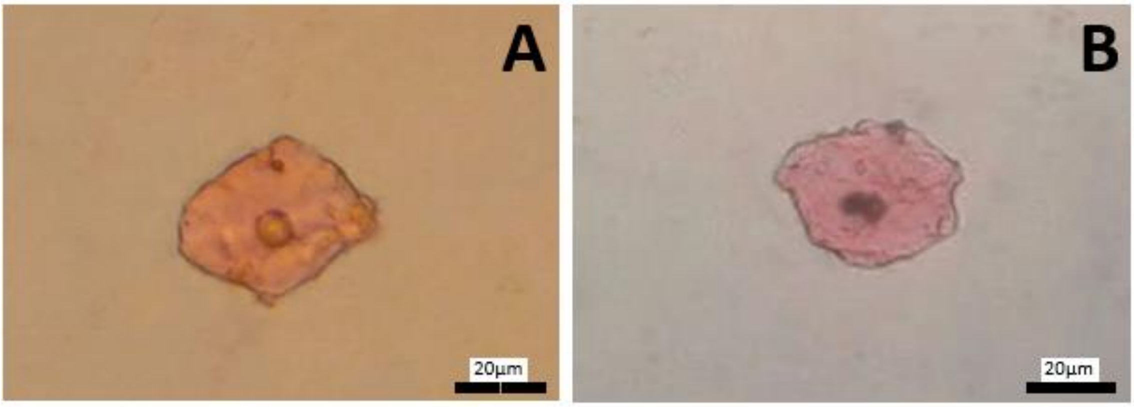

Figure 6. Epithelial cells from the stratum corneum found in the esophagus of tadpoles of *L. podicipinus*; stained with eosin (A) and hematoxylin-eosin (B).

Discussion

Considering amphibians as a group between aquatic and terrestrial life that fill up the evolutionary gap between both environments, skin plays important functions in survivorship besides the usual (Huang et al. 2016), such as protection, osmoregulation or permeability (Inceli et al. 2010). Generally, frog skin is thin, especially the stratum corneum, and protection is more provided by gland secretions than from epidermis itself (Li et al. 2006, Lillywhite 2006). Two substances have been related with osmoregulation and permeability in frog skin, prolactin and glycoconjugates (Inceli et al. 2010). Prolactin receptors were found in the skin of *Pelophylax ridibundus*, especially in the back skin (Inceli et al. 2010), and we have several reports of prolactin involved in parenting for other groups, such as mammals and birds (Schradin & Anzenberger 1999). Besides, prolactin has a significant role in proliferation of cells in amphibians (Hessler & Landesman 1981). Thus, this hormone might be involved in the proliferation of cells we observed in the epidermis and stratum corneum of attending females of *L. podicipinus*, which needs further investigation.

Parent-offspring chemical communication has been suggested for species with mechanical communication, such as *L. podicipinus* (Wells & Bard 1988). As parental care in *L. podicipinus* is exclusively performed by females, we expected to find special glands in female skin, which could be related to secretion of offspring aggregative or feeding substances. Instead, we observed that females and males have the same glandular structures, preventing us to associate glands and potential contents with chemical communication between mother and offspring. However, for attending females of *L. podicipinus* we found that epidermis was always thicker than epidermis from non-attending females and males, and if protection is not the main function of skin

(Chuong et al. 2002), alternative functions may be involved with skin thickness, such as offspring feeding.

We did not find any pattern of skin color change when comparing non-attending females with attending females of *L. podicipinus*. Different from caecilians that exhibit offspring skin-feeding (dermatotrophy; Kupfer et al. 2006), attending females of *L. podicipinus* did not change color skin during parental care, probably because lipidic concentration is not enough to produce the change. Besides, melanin granules could interfere with skin color of attending females of *L. podicipinus*. Caecilians cells are specialized and produce and secrete a compound lipid-rich to feed offspring (Kupfer et al. 2006). Although strongly reactive for lipids, we did not find lipidic droplets in the skin of attending females of *L. podicipinus*, such as those observed in caecilians (Kupfer et al. 2006).

In amphibians, there are two types of offspring feeding, providing unfertilized eggs or by skin-feeding (dermatotrophy). Providing unfertilized eggs to offspring is most common in anurans (Crump 1996, Wells 2007) and dermatotrophy has been described for some direct-developing oviparous caecilians (Kupfer et al. 2006). Because tadpoles of many species are opportunists and may consume eggs from other species, oophagy can be erroneously interpreted as parental care. In the case of parental care behavior, however, mothers are who offer unfertilized eggs to their own offspring (Brust 1993, Perry & Roitberg 2006). Oophagy is a common behavior found in phytotelmata species, such as *Oophaga pumilo* (Dendrobatidae) and *Aparasphenodon arapapa* (Hylidae), but it is also observed in *Leptodactylus fallax* and *L. labyrinthicus* (Leptodactylidae), species that deposit eggs in foam nests (Weygoldt 1980, Gibson & Buley 2004, Prado et al. 2005b, Lourenço-De-Morais et al. 2009).

On the other hand, dermatophagy frequently occurs in adults of reptiles, salamanders, and frogs as a way to reclaim the loss of epidermal proteins (Frye 1991, Gunzburger 1999). However, such behavior has never being reported as a way to provide nutrition to the young in these vertebrate groups. In amphibians, dermatophagy occurs in many anuran families, including Leptodactylidae, but offspring skin-feeding (dermatotrophy) was only described as a way of parental care in caecilians (Weldon et al. 1993, Kupfer et al. 2006). It was first registered in *Boulengerula taitanus* (Caecillidae), and later extended to *Siphonops annulatus* (Siphonopidae), *Microcaecilia dermatophaga* (Siphonopidae), and *Herpeles squalostoma* (Caecillidae), all oviparous caecilian species with direct development (Kupfer et al. 2006, Wilkinson et al. 2008, Kouete et al. 2012, Wilkinson et al. 2013).

In the case of *L. podicipinus* tadpoles, after absorbing the yolk during the first developmental stages, they may receive additional food supply from attending females by dermatotrophy. Caecilians hatchlings with skin-feeding are altricial (unable to move), making mothers entirely responsible for supplying food (Kouete et al. 2012). *Leptodactylus podicipinus* larvae are free swimming, which allows them to explore and feed from different items besides attending female skin. Food items include microalgae, fungus, Rotifera, Nematoda, Crustacea, among other itens (Rossa-Feres et al. 2004). Thus, skin-feeding may be only complementary to *L. podicipinus* and possibily it is more important for tadpoles in early stages of development, as suggested by our findings of more epithelial cells in the esophagus of early stages.

Feeding offspring with skin may represent an advantage over providing them only with yolk (Kupfer et al. 2006). This way, part of female´s energetic investment that would be used to produce yolk can be divided to be invested later on parental care (Kupfer et al. 2006). Furthermore, females might allocate less energy in yolk and more in number of eggs in current reproduction (Kupfer et al. 2006), then replenishing the energy during parental care, using it to feed tadpoles. Therefore, number of offspring may increase and energy per tadpole may keep stable. Future studies should investigate this hypothesis.

Attending females of *L. podicipinus* “pump” the body against the water apparently to show their position to schooling tadpoles and guide them through the pond (Wells & Bard 1988, Prado et al. 2002, Rodrigues et al. 2011). Observations of this parental care behavior also registered tadpoles swimming around attending females and scratching their legs and posterior back (da Silva 2009, C.P.A. Prado pers. comm.), indicating a possible additional interaction between them besides the mechanical communication by the usual “pumping” behavior (Wells & Bard 1988, Prado et al. 2002). Tadpoles biting posterior back of attending females was also registered for *L. latrans*, *L. melanonotus* and *L. natalensis* (Gallardo 1964, Vaz-Ferreira & Gehrau 1975, Hoffmann 2006, Santos & Amorim 2006).

Although we do not discard some form of chemical communication, such previous behavioral observations, summed to our findings of epithelial thicken-up in the posterior back of attending females, the loose of body mass of mothers during parental care, and the presence of epithelial cells in tadpoles´ esophagus, suggest a trophic interaction between attending females and tadpoles by means of dermatotrophy for *L. podicipinus*, being the first record of this type of parental care for Anura.

Parental care of *Leptodactylus podicipinus* include maternal care of eggs and exotrophic free-swimming tadpoles (Prado et al. 2002). We propose to include skin-feeding as part of *L. podicipinus* parental care behavior, as an alternative type of parental care behavior in Anura. Furthermore, we suggest that dermatotrophy might occur in other *Leptodactylus* species from the *L. melanonotus* and *L. latrans* groups, with maternal care of eggs and tadpoles (Heyer 1969, Prado et al. 2002).

General conclusion

Although some studies have been conducted on the parental care behavior in the genus *Lepodactylus* (e.g., Wells & Bard 1988, Prado et al. 2002, Prado et al. 2005b, Rodrigues et al. 2011, Pereira et al. 2015), there are many aspects that we still do not know, including forms of communication between mother and offspring, costs and benefits of parental care in terms of fitness consequences for females and offspring, and factors and processes leading to the evolution and maintenance of parental care. Besides the variety of reproductive modes, our study showed that the complex relationship between mother and offspring makes this genus unique for the study of parental care evolution.

We found that *L. podicipinus* females do lose weight during the period of parental care, however no differences in fat body mass occurred and they could still forage while caring for the tadpoles. Although not conclusive, we consider our results a step further in the comprehension of the parental care behavior in *L. podicipinus*, mainly because measures were taken in the field. We are aware, though, that the energetic cost of parental care in this species cannot be interpreted in terms of fitness costs, which implies measuring offspring performance/survivorship and energy expenditure effects on female future reproduction. However, our findings indicate that parental care in *L. podicipinus* may impose some energetic costs to females, as reduction in food intake and ovary mass, which may affect female fitness by decreasing body size and future fecundity.

The measure of costs and benefits of parental care are needed to understand not only how parental care evolved, but why it is provided by the male, female or both (Kvarnemo 2010). Many hypotheses have been proposed to explain which sex will provide care (see Trivers 1972, Gross & Shine 1981, Kvarnemo 2010). In general, parental care evolves from no care to uniparental care, when offspring survival or quality is increased, i.e., offspring fitness is increased (Kvarnemo 2010). In this case, both mother and father will benefit, regardless of which sex provides de care, and care will be provided by the sex with a positive balance between costs and benefits (see Kvarnemo 2010). The evolution of female care is rare among externally fertilizing vertebrates (Gross & Shine 1981) and the "certainty of paternity" hypothesis has been suggested to explain maternal care when males do not have assurance of paternity of all offspring in a clutch (Trivers 1972). This hypothesis could explain maternal care in *L. podicipinus* because males exhibit large testes and polyandry has been reported for the species in the Pantanal (Prado & Haddad 2003). Besides costs and benefits of parental care in terms of fitness consequences, future studies should investigate the influence of polyandry on the evolution of maternal care in the genus *Leptodactylus*.

Up to now, dermatotrophy was only known to occur in Caecilians. Our observation of offspring skin-feeding in *L. podicipinus* is the first record for Anura. This is an additional trait in the parental care behavior of *L. podicipinus* that we were able to relate with physiological changes in skin (lipid concentration) and epithelial cells in tadpoles´ guts. Attending females not only protect and guide tadpoles during larval development (Martins 2001, Prado et al. 2002), but can also provide additional nutrition. As tadpoles of *L. podicipinus* have an extended diet (Rossa-Feres et al. 2004), it is necessary to investigate the importance of the skin-feeding for the offspring, and whether it is most important in early developmental stages. Tadpoles biting the female dorsum has been described for other *Leptodactylus* species (Hoffmann 2006, Santos & Amorim 2006), thus we suggest that dermatotrophy might be present in other species of the genus that exhibit maternal care of tadpoles.

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