Taylor & Francis
Taylor & Francis Group

Check for updates

Body condition of females during tadpole attendance and its potential costs in a Neotropical foam-nesting frog (*Leptodactylus podicipinus*)

Juan F. Cuestas Carrillo [1,*], Diego J. Santana [1] and Cynthia P.A. Prado [1,2]

[1]*Programa de Pós-Graduação Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Campo Grande 79070-900, Brazil*
[2]*Departamento de Morfologia e Universidade Estadual Paulista (UNESP), Professor Paulo Donato Castellane, 14884-900 Jaboticabal, São Paulo, Brazil*

Parental care increases offspring survival, but may be costly for parents, reducing future survival and reproduction. Offspring attendance may increase predation risk, or reduce food intake, mating opportunities, and reproductive rate of parents. Anurans exhibit a great diversity of parental care behaviours and offer an opportunity to investigate the costs and benefits of parenting. Here, we measured the body condition of females of the Neotropical frog *Leptodactylus podicipinus* during tadpole attendance. By comparing attending and non-attending females, we tested the hypothesis that females providing care will have reduced body condition and food ingestion. Although fat body mass did not differ, attending females had significantly lower body mass, ovary mass, and stomach volume after 6 days of care. Overall, attending females may lose up to 40% of body mass due to parental care, while non-attending females gain mass. Although 27% of attending females had empty stomachs, prey diversity was higher. Additionally, the higher proportion of aquatic hemipterans and spiders preyed on by attending females may be explained by different microhabitat use and active protection of offspring. Our results indicate that parental care has the potential to affect future reproduction of females via reduction in body condition and food intake. Studies have shown the benefits related to offspring survival in anurans, but few addressed the costs to parents. By evaluating the potential costs of parenting in a frog species with aquatic tadpoles for the first time, our study contributes to fill this gap. Studies investigating the costs and benefits of parental care in different lineages of frogs will improve our knowledge on the evolution of parenting in this highly diverse group.

KEY WORDS: Anura, Leptodactylidae, parental investment, maternal care, future reproduction, diet.

*Corresponding author: Juan Fernando Cuestas Carrillo, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul 79070-900, Brazil (E-mail: jfcuestas@gmail.com). This article has been republished with minor changes. These changes do not impact the academic content of the article.

INTRODUCTION

Parental investment can be defined as any investment performed by the parents to increase current offspring survival at the expense of the parents' future reproduction (Trivers 1972). Because individuals have limited energy and time to allocate among different activities (Gadgil & Bossert 1970), the benefits of parental care impose costs for the caregiver in terms of reduced future survival and reproductive investment (Williams 1966; Gross 2005; Klug et al. 2012). The trade-off between current versus future reproduction has long been considered important in shaping species´ life-history in different animal groups (Roff & Fairbairn 2007; Santos & Nakagawa 2012; Huang & Pike 2013). For species with parental care, the amount of investment in current offspring may lead to loss of energy due to less opportunity to forage, for instance, or to any other physiological stress for the caregiver (Townsend 1986; Alonso-Alvarez & Velando 2012; Saweki et al. 2019). Thus, because of this parent-offspring conflict (Trivers 1974), parental care may increase parents´ risk of mortality and/or reduce future fecundity, mainly in ectothermic females that typically present a positive correlation between body size and clutch/egg size (Gross 2005; Prado & Haddad 2005; Alonso-Alvarez & Velando 2012).

Anuran amphibians exhibit a great diversity of parental care behaviours that evolved mainly associated with terrestrial reproduction in the humid tropics, but is also observed in some aquatic breeders (Crump 1996; Gomez-Mestre et al. 2012; Vági et al. 2019). In the last years, the number of parental care modes in anurans doubled from 14 to 28 with the increase of studies on natural history (see Schulte et al. 2020). Nearly 10% of anuran species provide some form of parental care to their offspring after fertilization (Crump 2015; Schulte et al. 2020), which may be performed by the male, female, or even both parents (Rodrigues et al. 2011; Dugas et al. 2015; Delia et al. 2017). Parenting in frogs includes not only care of eggs (Valencia-Aguilar et al. 2020a), but also more complex behaviours, such as tadpole guidance, juvenile transportation, and tadpole feeding, with females providing unfertilized eggs (Prado et al. 2000; Bickford 2002; Brown et al. 2010; Dugas et al. 2015). Although many studies have investigated different aspects of parental care in anurans (Furness & Capellini 2019; Delia et al. 2020; Valencia-Aguilar et al. 2021), the costs and benefits remain under-explored (Townsend 1986; Hurme 2011; Dugas et al. 2015; Valencia-Aguilar et al. 2020a; Lange et al. 2021). Most studies using parent removal experiments focused on the adaptive benefits of egg attendance in a few frog lineages (reviewed by Schulte et al. 2020). Considering that the evolution of parental care depends not only on the benefits to offspring, but also the relative costs to parents (Schulte et al. 2020), studies on parenting costs including different types of parental care are needed if we want to understand the selective forces and mechanisms involved in the evolution of the great diversity of parental care in amphibians.

In the Neotropical frog family Leptodactylidae, tadpole schooling and attendance are restricted to species in the *L. melanonotus* and *L. latrans* groups (Prado et al. 2002; de Sá et al. 2014). *Leptodactylus podicipinus* belongs to the *L. melanonotus* species group and inhabits open formations in South America, from Brazil to Argentina (Frost 2021). Males of *L. podicipinus* construct basins covered with vegetation at the margins of ponds, where females deposit eggs embedded in foam nests (Prado et al. 2002). Females provide parental care for both eggs and tadpoles, guiding and guarding the tadpole schools from predators (Prado et al. 2000; Martins 2001). Females may also dig channels to connect temporary ponds when water level

decreases (Rodrigues et al. 2011). Studies on the body condition and foraging activity of caregivers have been published for anuran species that care for terrestrial eggs (e.g. Simon 1983; Townsend 1986); however, no studies have investigated such aspects in species with aquatic tadpole attendance. Herein, we evaluated the body condition and diet of females of *L. podicipinus* during parental care of aquatic tadpoles. Given the demand of parental care for this species in terms of energy (Prado et al. 2000; Martins 2001), we tested the hypothesis that tadpole attendance will decrease female body condition. In addition, due to time restrictions on foraging, we expect a more restricted diet and smaller stomach volume in attending females. By comparing body mass, fat body mass, ovary mass, and stomach content between attending females and females that were not caring for tadpoles, we discuss the potential costs of parental care for future reproduction in this species.

MATERIALS AND METHODS

Study site

Our study site is located in the Pantanal, in southern South America, considered one of the largest floodplains in the world, with an area of approximately 150,000 km^2 (Alho et al. 2019). Fieldwork was conducted at the research station Base de Estudos do Pantanal (BEP), Universidade Federal de Mato Grosso do Sul (19°34′36.4″S, 57°1′10.56″W), municipality of Corumbá, State of Mato Grosso do Sul, central-western Brazil. The climate in the Pantanal is markedly seasonal, with a dry season from May to September and a rainy season from October to April, with a mean annual rainfall of 1,215 mm at the study site (Prado et al. 2005). Floods are common between January and April, although duration and intensity of floods are unpredictable (Prado et al. 2005; Junk & da Cunha 2018). Observations were conducted from November 2015 to March 2016 and from September to November 2016, remaining in the field for 10–15 consecutive days. Observations and sampling were carried out during the day and night at the margins of ponds and flooded areas, where females remain with tadpoles.

Body condition measures

Females attend foam nests and tadpoles up to the final stages of the larval period (Prado et al. 2000; Martins 2001). The larval period of *L. podicipinus*, from hatching up to metamorphosis, lasts about 28 days, ranging from 25 to 32 days (Martins 2001). During female attendance of tadpoles, we measured daily variation in body mass during 6 consecutive days by weighing six attending and nine non-attending females every day. Non-attending females were considered those that had not spawned yet, thus they were not caring for offspring. To be able to follow and measure the same female in consecutive days, before data collection started, we collected and photographed many females. Each female was individually identified by photo-id of the dots present on the belly and inferior lip; images were analysed in the software Wild-ID 0.4.5 (Bolger et al. 2012). Body mass of individuals was measured with a digital balance (nearest 0.01 g). After measurements, females were released at the capture site.

To examine stomach contents and obtain measures of stomach volume, fat body mass and ovary mass, we collected 15 non-attending and 15 attending females of *L. podicipinus* (different individuals from those used above). Females were euthanized in situ with 5% lidocaine (cream) applied on the ventral and dorsal regions. Afterwards, they were fixed in 10% formalin, which was also injected into the cloaca to minimize the effects of digestion, and then preserved in 70% alcohol (McDiarmid 1994). In the laboratory, SVL (snout-vent length) of females was measured with a caliper (nearest 0.1 mm) and each female was dissected to remove fat bodies, ovaries and

stomachs. We weighed fat body mass and ovary mass (combining both ovaries) of attending and non-attending females in a digital balance Shimatzu AY 220 (nearest 0.01 g). To calculate the stomach content volume, we used a chamber made with microscopy slides and placed on a graph paper (nearest 1 mm^3), modified from Camera et al. (2014). Animals were deposited at the Coleção Zoológica de Referência of the Universidade Federal de Mato Grosso do Sul (vouchers ZUFMS-AMP 06473–06507).

Stomach content analyses

We identified insect prey items to Order level according to Triplehorn and Johnson (2011). To determine the importance of each prey item, we calculated the importance index following Kawakami and Vazzoler (1980), where: IAi = Fi * Vi/Σ(Fi * Vi) (Fi: Frequency of occurrence of i item, Vi: volume of i item). This index requires previous measurements of frequency and volume in relative percentages that were taken as follows. Prey frequency was calculated using Bowen's (1983) formula: Fi% = 100ni/n (Fi: frequency of i prey, ni: number of stomach where i item was found, n: stomach containing food); and volume was determined using Hynes' (1950) formula: V%i = Vi/(Vi + Vi$_2$ + Vi$_n$) (V%i: percent volume by item, Vi: volume by item). Although undetermined prey items were not included in the stomach content analysis, they were considered for the total volume analysis.

Statistical analyses

We used linear regressions to compare daily changes in body mass for both groups, attending and non-attending females, using only the mass difference between the 1st day and each of the consecutive days. Afterwards, we verified the difference between the two linear regressions using ANCOVA. To compare SVL, body mass, fat body mass, ovary mass and stomach volume content between attending and non-attending females, we used Student *t* tests or Mann-Whitney U tests (Welham et al. 2014), after checking for data normality and homoscedasticity.

We tested if the frequency of empty stomachs differed between non-attending and attending females using a chi-square test. Additionally, to assess stomach content variation between both groups, we performed a MANOVA analysis and a Principal Coordinates Analysis (PCoA) to check prey category contributions using the prey volume data. We performed the analyses in the R software v.3.5.1 (R Core Team 2020) using the *vegan* package (Oksanen et al. 2020).

RESULTS

Body condition

Attending and non-attending females of *L. podicipinus* did not differ in SVL ($t = 2.07$; $P = 0.24$; Table 1). However, we found that body mass of non-attending females increased ($R^2 = 0.55$; $P < 0.001$), while body mass of attending females decreased ($R^2 = 0.54$; $P < 0.001$) during the study period. Whereas non-attending females gained 0.10 g per day, attending females lost 0.09 g per day (Fig. 1). Additionally, linear regressions differed significantly from each other ($F = 65.95$; df = 1; $P < 0.001$). The difference in body mass between the last and 1st day of capture was significant ($U = 81$; $P < 0.001$; Fig. 2A); after 6 days, body mass of non-attending females increased on average 0.93 ± 1.03 g, while body mass of attending females decreased on average 0.49 ± 0.45 g (Table 1).

Table 1.

Measures of body traits compared between attending females and non-attending females of *Leptodactylus podicipinus* in the southern Pantanal, Brazil. Body mass difference (g) refers to the difference in female mass between the first and last day, after 6 days. SVL = snout-vent length.

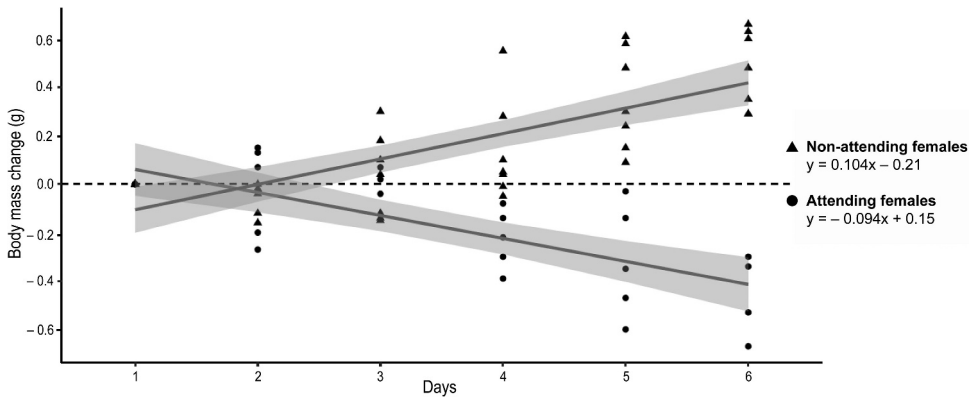|	Attending females			Non-attending females		
	Mean ± SD	Range	n	Mean ± SD	Range	n
SVL (mm)	38.2 ± 2.98	34.6–45.6	15	37.1 ± 1.71	34.1–40.0	14
Body mass (g)	5.69 ± 1.75	4.18–9.04	6	4.82 ± 0.98	3.64–6.84	9
Body mass difference (g)	– 0.49 ± 0.45	– 1.25–0.13	6	0.93 ± 1.03	0.29–3.46	9
Fat body mass (mg)	0.33 ± 0.53	0.0–1.5	15	1.37 ± 2.98	0.0–10.3	15
Ovary mass (g)	0.03 ± 0.02	0.01–0.08	15	0.38 ± 0.21	0.10–0.76	15
Stomach vol. content (mm³)	35.9 ± 42.9	0.0–135.0	15	105.5 ± 97.9	7.0–408	15

Fig. 1. — Daily changes in body mass of attending females and non-attending females of *Leptodactylus podicipinus* in the southern Pantanal, Brazil.

We did not find significant differences in fat body mass between attending and non-attending females (U = 121.5; P = 0.67; Fig. 2B). However, there were significant differences in ovary mass (U = 255; P < 0.001; Fig. 2C) and stomach volume content (U = 183; P < 0.01; Fig. 2D) between both groups. Ovary mass was higher in non-attending females when compared with attending females (Table 1). Likewise, stomach volume content was larger in non-attending females than in attending females (Table 1). Additionally, frequency of empty stomachs differed between the two groups (χ^2 = 4.61, df = 1, P = 0.031); a considerable percentage of attending females had empty stomachs (27%), while non-attending females had no empty stomachs.
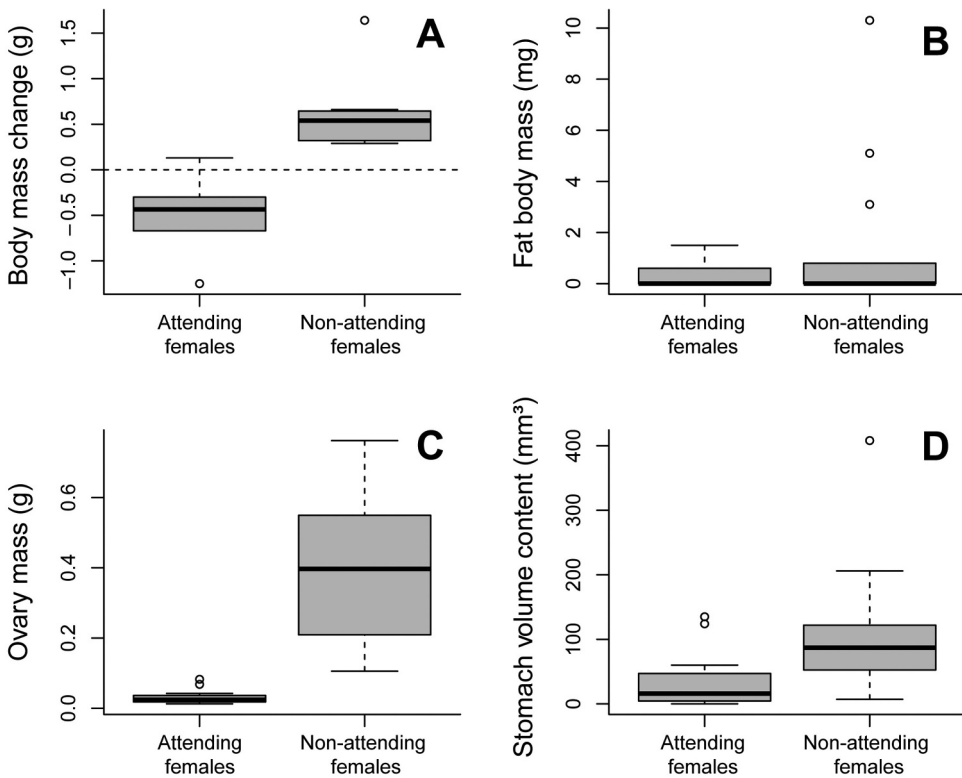
Fig. 2. — Comparison of body mass change (A), fat body mass (B), ovary mass (C), and stomach volume content (D), after 6 days, between attending females and non-attending females of *Leptodactylus podicipinus* in the southern Pantanal, Brazil.

Stomach content analysis

The stomach content composition of non-attending and attending females was significantly different (F = 9.24; df = 25; *P* = 0.001). Contrary to what we expected, attending females presented more diversified stomach content while in non-attending females it was more restricted (Fig. 3). Coleopterans were the most important item for non-attending females, according to Kawakami and Vazzoler (1980) feeding index calculation and PCoA ordinance (Fig. 4), while hemipterans were the most important item for attending females (Fig. 3A-B). We found spiders and crickets only in the stomachs of attending females and cockroaches occurred only in the stomachs of non-attending females (Fig. 3).

DISCUSSION

Studies assessing the costs of parental care in different vertebrates (e.g. Townsend 1986; Kenagy et al. 1990; Gillooly & Baylis 1999) and invertebrates (e.g. Cortez et al. 1995; Colancecco et al. 2007) have shown that offspring attendance may increase metabolic rate, predation risk, reduce food intake and body condition, negatively affecting future survival
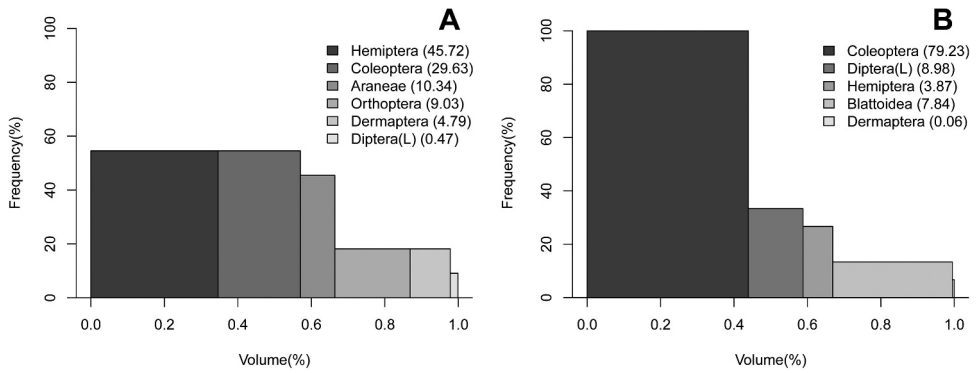
Fig. 3. — Frequency (%) and volume (%) of prey items in the stomachs of attending females (A) and non-attending females (B) of *Leptodactylus podicipinus* in the southern Pantanal, Brazil. Importance index (IAi) for each prey item is shown in parentheses. L = Larvae.
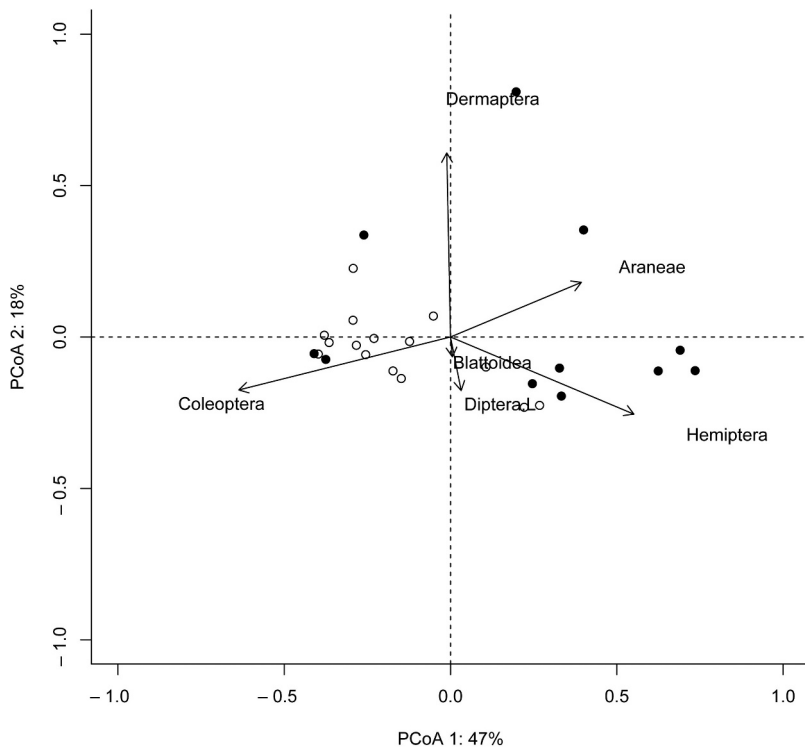

Fig. 4. — Principal coordinates analysis (PCoA) for attending females (black circles) and non-attending females (white circles) prey items.

and reproduction. As we have hypothesized, tadpole attendance was associated with reduced female body condition and food intake. We found that females of *L. podicipinus* guarding and leading tadpole schools to safe sites lost body mass compared to females that were not attending tadpoles. After 6 days, attending females lost, on average, 8.8% (≈ 0.5 g) of their body mass, while non-attending females increased, on average, 18.7% in mass (≈ 0.9 g; Table 1). If larval period lasts, on average, 28 days in this species (Martins 2001), *L. podicipinus* females may lose up to 40% of body mass during attendance of tadpoles. Egg brooding males of *Eleutherodactylus coqui* can lose nearly 20% of body mass along 20 days of parental care (Townsend 1986). Considering that *E. coqui* males remain stationary during parental care (Townsend 1986), our results indicate that maternal care in *L. podicipinus* may be more energetically demanding, probably because females perform displacements with tadpole schools (Martins 2001). According to parental investment trade-offs (Gross 2005; Klug et al. 2012; Huang & Pike 2013), because females of *L. podicipinus* are not able to feed properly during parental care (Rodrigues et al. 2004; this study), our study indicates that the investment in current offspring may reduce body condition and size, potentially decreasing future fecundity, because of the positive relationship between female body size and clutch size (Prado et al. 2000; Prado & Haddad 2005).

We found no differences in body size (SVL) between attending and non-attending females and ovary mass of non-attending females is within the range of that for mature females (Prado & Haddad 2005). As expected, attending females had lower ovary mass compared with non-attending females, which may result from the fact that attending females had recently emptied their ovaries. However, based on the presence of oocytes at different developmental stages in attending females (J.F. Cuestas Carrillo unpubl. data), it is feasible that females produce more than one clutch per year because *L. podicipinus* reproduces continuously throughout the year at the study area (Prado & Haddad 2005). Thus, parental care effort may have a short-term effect, reducing the number of oocytes produced in subsequent clutches given the reduction in food intake, which deserves to be confirmed.

In amphibians and reptiles, lipids are stored in special organs called fat bodies, serving as reserves during hibernation or proving energy for gametogenesis (Long 1987; Jorgensen 1992; Saidapur & Hoque 1996). Fat body mass was equally low for both groups of females and our results suggest that fat bodies are not involved in parental care in *L. podicipinus*. Fat bodies as a source of energy for parental care was observed in egg brooding frogs with direct development, such as *Cophixalus parkeri*, with a long parental care period of over 85 days (Simon 1983), and males of *Eleutherodactylus coqui* that exhibited lower fat bodies in the middle of the parental care period (Townsend 1986). At our study site in the Pantanal, a negative correlation between fat body and ovary mass was reported for females of *Leptodactylus macrosternum* and *L. fuscus*, which have seasonal reproduction, but not for *L. podicipinus*, which reproduces throughout the year (Prado & Haddad 2005). Hence, the continuous reproduction of *L. podicipinus* may explain the lack of differences in fat body mass we found between attending and non-attending females.

A total of 27% of attending females presented empty stomachs and mean stomach content volume was smaller compared to non-attending females, which may also decrease body condition and affect future reproduction, as mentioned above. High proportions of empty stomachs in caregivers and differences in diet composition between guarding and non-guarding parents were also reported for other frog species (Simon 1983; Townsend 1986). *Leptodactylus podicipinus* is considered a sit-and-wait generalist predator and its diet is mainly composed of arthropods (Rodrigues et al.

2004). We found that coleopterans were the main prey item in the stomachs of non-attending females, followed by dipterans and hemipterans, similar results found in other studies for *L. podicipinus* (e.g. Rodrigues et al. 2004; Ceron et al. 2018). On the other hand, attending females´ stomach content was more diverse, with more hemipterans, followed by coleopterans and spiders. Although we did not evaluate prey availability, attending and non-attending females were collected in the same study site and period of the year, thus we do believe that differences in stomach content may be related to differential use of microhabitat during parental care. Adults of *L. podicipinus* forage on the floor at the margins of water bodies, allowing the encounter of prey such as coleopterans (Rodrigues et al. 2004). In contrast, females performing parental care remain most of the time in shallow water close to the tadpoles (Martins 2001; Prado et al. 2002; Rodrigues et al. 2004). Similarly, attending females of *Leptodactylus insularum* showed differences in spatial occupation compared to males and non-attending females (Hurme 2015). This change in microhabitat due to parental care (Townsend 1986; Rodrigues et al. 2004) may explain differences in stomach content and the higher frequency of aquatic hemipterans in the stomachs of attending females in our study.

The diet of generalist anuran species varies according to seasonal changes in arthropod densities and availability (Toft 1981; Lieberman & Dock 1982), and diets follow the natural fluctuations of prey abundance (Whitfield & Donnelly 2006). However, the high frequency of ingestion of Araneae by attending females may not reflect opportunism or prey availability. 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 upon their eggs and tadpoles (Martins 2001; Santos & Amorim 2006). Therefore, the ingestion of spiders by attending females of *L. podicipinus* may be the result of active offspring protection separated from foraging per se.

In anurans, egg attendance is the most common form of parental care, occurring in 50% out of 56 frog families (Frost 2021), against 16% where tadpole guarding occurs, excluding cases of tadpole transport and feeding (sensu Schulte et al. 2020). However, in contrast to some *Leptodactylus* species, with protection and guidance of tadpole schools in open aquatic habitats (e.g. Wells & Bard 1988; Hoffmann 2006; Santos & Amorim 2006; Rodrigues et al. 2011), movements are limited in most species with tadpole care because attendance occurs in burrows or phytotelma (see Wells 2007). Recent studies point out that the evolution of egg care in amphibians requires only that parents remain at the oviposition site at a presumably low cost and that it drives the evolution of parenting at later stages (e.g. tadpoles, juveniles), which may evolve depending on the ecological opportunities and species´ life-history traits (Furness & Capellini 2019; Vági et al. 2019). For instance, the evolution of tadpole attendance requires that tadpoles remain restricted in space (e.g. underground nests, phytotelma) or form dense schools (Wells 2007; Furness & Capellini 2019). In this regard, tadpole schooling may have favoured parental care evolution in species of *Leptodactylus* of the *L. melanonotus* and *L. latrans* groups (de Sá et al. 2014), because it allows some foraging by the caregiver while providing protection and care (Furness & Capellini 2019; this study).

Costs and benefits of care, life-history traits, and ecological, environmental and evolutionary dynamics are suggested to affect the evolution of parental care (see Klug et al. 2012). Parental care will be favoured when the fitness benefits to the caregiver overcome the costs imposed by the care (e.g. decreased parent survival and/or future reproduction) (Klug et al. 2012). Offspring survival is the main benefit for the parents and

studies on different frog families have shown high offspring survival rates when parents provide care (e.g. Simon 1983; Burrowes 2000; Bickford 2004; Poo & Bickford 2013; Valencia-Aguilar et al. 2020b). However, intensity of parental care incurs in costs to caregivers, such as decrease in body condition, food intake, reduced mating opportunities, and adult survival, ultimately affecting lifetime reproductive success of parents (e.g. Simon 1983; Townsend 1986; Pröhl & Hödl 1999; Dugas et al. 2015). We found that females of *L. podicipinus* showed decrease in body mass and food intake during tadpole attendance, which have the potential to negatively impact future reproduction. Future long-term studies should evaluate the effect of maternal care on tadpole survival rates and investigate possible fecundity, survival, and mating costs for attending females. The exact extent and nature of parental costs remain to be measured in many frog lineages, thus the study of the costs and benefits of parenting in different taxa, with different types of care, is imperative to test hypotheses and advance our knowledge on the evolution of parental care in this highly diverse group (Schulte et al. 2020; Valencia-Aguilar et al. 2020a).

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

ETHICAL STANDARD

Animals were collected under licence # 52,245-1 (SISBIO) approved by the Brazilian environmental agency ICMBio. The study was conducted according to the Brazilian laws and approved by the Animal Use Ethical Committee (CEUA/UNESP Jaboticabal) under licence # 016549/09.

AUTHOR CONTRIBUTIONS

ORCID

Juan F. Cuestas Carrillo http://orcid.org/0000-0002-8881-568X
Diego J. Santana http://orcid.org/0000-0002-8789-3061
Cynthia P.A. Prado http://orcid.org/0000-0002-6105-4921

REFERENCES

Alho CJR, Mamede SB, Benites M, Andrade BS, Sepúlveda JJO. 2019. Threats to the biodiversity of the Brazilian Pantanal due to land use and occupation. Ambiente Soc. 22:e01891. doi:10.1590/1809-4422asoc201701891vu2019l3ao

Alonso-Alvarez C, Velando A. 2012. Benefits and costs of parental care. In: Royle NJ, et al., editors. The evolution of parental care. Oxford (UK): Oxford University Press; p. 40–61.

Bickford D. 2002. Male parenting of New Guinea froglets. Nature. 418:601–602. doi:10.1038/418601b

Bickford DP. 2004. Differential parental care behaviors of arboreal and terrestrial microhylid frogs from Papua New Guinea. Behav Ecol Sociobiol. 55:402–409. doi:10.1007/s00265-003-0717-x

Bolger DT, Morrison TA, Vance B, Lee D, Farid H. 2012. A computer-assisted system for photographic mark–recapture analysis. Methods Ecol Evol. 3:813–822. doi:10.1111/j.2041-210X.2012.00212.x

Bowen SH. 1983. Quantitative description of the diet. In: Nielsen LA, Johnson DL, editors. Fisheries techniques. Bethesda (MD): American Fisheries Society; p. 325–336.

Brown JL, Morales V, Summers K. 2010. A key ecological trait drove the evolution of biparental care and monogamy in an amphibian. Am Nat. 175:436–446. doi:10.1086/650727

Burrowes PA. 2000. Parental care and sexual selection in the Puerto Rican cave-dwelling frog, *Eleutherodactylus cooki*. Herpetologica. 56:375–386.

Camera BF, Krinski D, Calvo IA. 2014. Diet of the Neotropical frog *Leptodactylus mystaceus* (Anura: Leptodactylidae). Herpetol Notes. 7:31–36.

Ceron K, Moroti MT, Benício RA, Balboa ZP, Marçola Y, Pereira LB, Santana DJ. 2018. Diet and first report of batracophagy in *Leptodactylus podicipinus* (Anura: Leptodactylidae). Neotrop Biodivers. 4:70–74. doi:10.1080/23766808.2018.1467173

Colancecco M, Rypstra AL, Persons MH. 2007. Predation and foraging costs of carrying eggsacs of different mass in the wolf spider *Pardosa milvina*. Behaviour. 144:1003–1018. doi:10.1163/156853907781871888

Cortez T, Castro BG, Guerra A. 1995. Reproduction and condition of female *Octopus mimus* (Mollusca: Cephalopoda). Mar Biol. 123:505–510. doi:10.1007/BF00349229

Crump ML. 1996. Parental care among the Amphibia. In: Rosenblatt JS, Snowdon CT, editors. Parental care: evolution, mechanisms, and adaptive significance. Advances in the study of behavior. San Diego (CA): Academic Press; p. 109–144.

Crump ML. 2015. Anuran reproductive modes: evolving perspectives. J Herpetol. 49:1–16. doi:10.1670/14-097

Delia J, Bravo-Valencia L, Warkentin KM. 2017. Patterns of parental care in Neotropical glassfrogs: fieldwork alters hypotheses of sex-role evolution. J Evol Biol. 30:898–914. doi:10.1111/jeb.13059

Delia J, Bravo-Valencia L, Warkentin KM. 2020. The evolution of extended parental care in glassfrogs: do egg-clutch phenotypes mediate coevolution between the sexes? Ecol Monogr. 90:e01411. doi:10.1002/ecm.1411

de Sá RO, Grant T, Camargo A, Heyer WR, Ponssa ML, Stanley E. 2014. Systematics of the Neotropical genus *Leptodactylus* Fitzinger, 1826 (Anura: Leptodactylidae): phylogeny, the relevance of non-molecular evidence, and species accounts. S Am J Herpetol. 9:S1–S128. doi:10.2994/SAJH-D-13-00022.1

Dugas MB, Wamelink CN, Richards-Zawacki CL. 2015. Both sexes pay a cost of reproduction in a frog with biparental care. Biol J Linn Soc. 115:211–218. doi:10.1111/bij.12461

Frost DR. 2021. Amphibian species of the world: an online reference. Version 6.1. New York (NY): American Museum of Natural History. Available from: https://amphibiansoftheworld.amnh.org/index.php [Accessed 2021 Jul 7].

Furness AI, Capellini I. 2019. The evolution of parental care diversity in amphibians. Nat Commun. 10:4709. doi:10.1038/s41467-019-12608-5

Gadgil M, Bossert WH. 1970. Life history consequences of natural selection. Am Nat. 104:1–24. doi:10.1086/282637

Gillooly JF, Baylis JR. 1999. Reproductive success and the energetic cost of parental care in male smallmouth bass. J Fish Biol. 54:573–584. doi:10.1111/j.1095-8649.1999.tb00636.x

Gomez-Mestre I, Pyron RA, Wiens JJ. 2012. Phylogenetic analyses reveal unexpected patterns in the evolution of reproductive modes. Evolution. 66:3687–3700. doi:10.1111/j.1558-5646.2012.01715.x

Gross MR. 2005. The evolution of parental care. Q Rev Biol. 80:37–46. doi:10.1086/431023

Hoffmann H. 2006. Observations on behaviour and parental care of *Leptodactylus melanonotus* (Hallowell) in Costa Rica. Salamandra. 42:109–116.

Huang WS, Pike DA. 2013. Testing cost-benefit models of parental care evolution using lizard populations differing in the expression of maternal care. PLoS ONE. 8:e54065. doi:10.1371/journal.pone.0054065

Hurme KJ. 2011. Parental care and tadpole schooling in the neotropical frog, *Leptodactylus insularum* [Ph.D. Thesis]. Mansfield (CT): University of Connecticut.

Hurme KJ. 2015. Reproductive and spatial ecology of *Leptodactylus insularum* (Anura, Leptodactylidae) in Panama. J Herpetol. 49:36–45. doi:10.1670/13-214

Hynes HBN. 1950. The food of freshwater sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*), with a review of methods used in studies of the food of fishes. J Anim Ecol. 19:36–58. doi:10.2307/1570

Jorgensen CB. 1992. Growth and reproduction. In: Feder ME, Burggren WW, editors. Environmental physiology of the amphibians. Chicago (IL): The University of Chicago Press; p. 439–466.

Junk WJ, da Cunha CN. 2018. The Pantanal: a brief review of its ecology, biodiversity, and protection status. In: Finlayson CM, et al., editors. The wetland book. Dordrecht (The Netherlands): Springer; p. 797–811. doi:10.1007/978-94-007-4001-3_129

Kawakami E, Vazzoler G. 1980. Método gráfico e estimativa de índice alimentar aplicado no estudo de alimentação de peixes [Graphic method and food index estimation applied in the study of fish feeding]. Bol Inst Oceanogr. 29:205–207. Portuguese. doi:10.1590/S0373-55241980000200043

Kenagy GJ, Masman D, Sharbaugh SM, Nagy KA. 1990. Energy expenditure during lactation in relation to litter size in free-living golden-mantled ground squirrels. J Anim Ecol. 59:73–88. doi:10.2307/5159

Klug H, Alonzo SH, Bonsall MB. 2012. Theoretical foundations of parental care. In: Royle NJ, et al., editors. The evolution of parental care. Oxford (UK): Oxford University Press; p. 21–39.

Lange L, Bégué L, Brischoux F, Lourdais O. 2021. The costs of being a good dad: egg-carring and clutch size impair locomotor performance in male midwife toads (*Alytes obstetricans*). Biol J Linn Soc. 132:270–282. doi:10.1093/biolinnean/blaa185

Lieberman S, Dock CF. 1982. Analysis of the leaf litter arthropod fauna of a lowland tropical evergreen forest site (La Selva, Costa Rica). Rev Biol Trop. 30:27–34.

Long DR. 1987. A comparison of energy substrates and reproductive patterns of two anurans, *Acris crepitans* and *Bufo woodhousei*. Comp Biochem Physiol. 87:81–91. doi:10.1016/0300-9629(87)90429-4

Martins IA. 2001. Parental care behavior in *Leptodactylus podicipinus* (Cope, 1862) (Anura, Leptodactylidae). Herpetol J. 11:29–32. doi:10.1643/0045-8511

McDiarmid RW. 1994. Preparing amphibians as scientific specimens. In: Heyer WR, et al., editors. Measuring and monitoring biological diversity. Standard methods for amphibians. Washington (DC): Smithsonian; p. 289–297.

Menin M, Rodrigues DJ, de Azevedo CS. 2005. Predation on amphibians by spiders (Arachnida, Araneae) in the Neotropical region. Phyllomedusa. 4(1):39–47. doi:10.11606/.2316-9079.v4i1p39-47

Oksanen O, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, et al. 2020. vegan: an R package for community ecologists. Version 2.5-7. Available from: https://github.com/vegandevs/vegan [Accessed 2020 May 16].

Poo S, Bickford DP. 2013. The adaptive significance of egg attendance in a South-East Asian tree frog. Ethology. 119:671–679. doi:10.1111/eth.12108

Prado CPA, Haddad CFB. 2005. Size-fecundity relationships and reproductive investment in female frogs in the Pantanal, south-western Brazil. Herpetol J. 15:181–189.

Prado CPA, Uetanabaro M, Haddad CFB. 2002. Description of a new reproductive mode in *Leptodactylus* (Anura, Leptodactilydae), with a review of the reproductive specialization toward terrestriality in the genus. Copeia. (4):1128–1133. doi:10.1643/0045-8511

Prado CPA, Uetanabaro M, Haddad CFB. 2005. Breeding activity patterns, reproductive modes, and habitat use by anurans (Amphibia) in a seasonal environment in the Pantanal, Brazil. Amphibia-Reptilia. 26:211–221. doi:10.1163/1568538054253375

Prado CPA, Uetanabaro M, Lopes FS. 2000. Reproductive strategies of *Leptodactylus chaquensis* and *L. podicipinus* in the Pantanal. J Herpetol. 34:135–139. doi:10.2307/1565249

Pröhl H, Hödl W. 1999. Parental investment, potential reproductive rates, and mating system in the strawberry dart-poison frog, *Dendrobates pumilio*. Behav Ecol Sociobiol. 46:215–220. doi:10.1007/s002650050612

R Core Team. 2020. R: A language and environment for statistical computing. Available from: https://www.R-project.org/ [Accessed 2020 May 16].

Rodrigues AP, Giaretta AA, Da Silva DR, Facure KG. 2011. Reproductive features of three maternal-caring species of *Leptodactylus* (Anura: Leptodactylidae) with a report on allo-parental care in frogs. J Nat Hist. 45:33–34. doi:10.1080/00222933.2011.574799

Rodrigues DJ, Uetanabaro M, Prado CPA. 2004. Seasonal and ontogenetic variation in diet composition of *Leptodactylus podicipinus* (Anura, Leptodactylidae) in the southern Pantanal, Brazil. Rev Esp Herpetol. 18:19–28.

Roff DA, Fairbairn DJ. 2007. The evolution of trade-offs: where are we? J Evol Bio. 20:433–447. doi:10.1111/j.1420-9101.2006.01255.x

Saidapur SK, Hoque B. 1996. Long-term effects of ovariectomy on abdominal fat body and body masses in the frog *Rana tigrina* during the recrudescent phase. J Herpetol. 30:70–73. doi:10.2307/1564709

Santos EM, Amorim FO. 2006. Cuidado parental em *Leptodactylus natalensis* (Amphibia, Anura, Leptodactylidae) [Parental care in *Leptodactylus natalensis* (Amphibia, Anura, Leptodactylidae)]. Iheringia Sér Zool. 96:491–494. Portuguese. doi:10.1590/S0073-47212006000400015

Santos ESA, Nakagawa S. 2012. The costs of parental care: a meta-analysis of the trade-off between parental effort and survival in birds. J Evol Biol. 25:1911–1917. doi:10.1111/j.1420-9101.2012.02569.x

Saweki J, Miros E, Border SE, Dijkstra PD. 2019. Reproduction and maternal care increase oxidative stress in a mouthbrooding cichlid fish. Behav Ecol. 30(6):1662–1671. doi:10.1093/beheco/arz133

Schulte LM, Ringler E, Rojas B, Stynoski JL. 2020. Developments in amphibian parental care research: history, present advances, and future perspectives. Herpetol Monogr. 34:71–97. doi:10.1655/HERPMONOGRAPHS-D-19-00002.1

Simon MP. 1983. The ecology of parental care in a terrestrial breeding frog from New Guinea. Behav Ecol Sociobiol. 14:61–68. doi:10.1007/BF00366657

Toft CA. 1981. Feeding ecology of Panamanian litter anurans: patterns in diet and foraging mode. J Herpetol. 15:139–144. doi:10.2307/1563372

Townsend DS. 1986. The costs of male parental care and its evolution in a Neotropical frog. Behav Ecol Sociobiol. 19:187–195.

Triplehorn CA, Johnson NF. 2011. Estudo dos insetos [Study of insects]. São Paulo (Brazil): Cengage Learning. Portuguese.

Trivers RL. 1972. Parental investment and sexual selection. In: Campbell B, editor. Sexual selection and the descent of man. Chicago (IL): Aldine Publish Company; p. 136–207.

Trivers RL. 1974. Parent-offspring conflict. Am Zool. 14:249–264. doi:10.1093/icb/14.1.249

Vági B, Végvári Z, Liker A, Freckleton RP, Székely T. 2019. Parental care and the evolution of terrestriality in frogs. Proc Biol Sci. 286:20182737. doi:10.1098/rspb.2018.2737

Valencia-Aguilar A, Guayasamin JM, Prado CPA. 2020b. Male care status influences the risk-taking decisions in a glassfrog. Behav Ecol Sociobiol. 74:84. doi:10.1007/s00265-020-02869-2

Valencia-Aguilar A, Guayasamin JM, Prado CPA. 2021. Alloparental care in glassfrogs: males care for unrelated clutches only when associated with their own. Sci Rep. 11:1386. doi:10.1038/s41598-020-80771-7

Valencia-Aguilar A, Zamudio KR, Haddad CFB, Bogdanowicz SM, Prado CPA. 2020a. Show me you care: female mate choice based on egg attendance rather than male or territorial traits. Behav Ecol. 31:1054–1064. doi:10.1093/beheco/araa051

Welham SJ, Gezan SA, Clark SJ, Mead A. 2014. Statistical methods in biology: design and analysis of experiments and regression. New York (NY): CRC Press.

Wells KD. 2007. The ecology and behavior of amphibians. Chicago (IL): The University of Chicago Press.

Wells KD, Bard KM. 1988. Parental behavior of an aquatic-breeding tropical frog, *Leptodactylus bolivianus*. J Herpetol. 22:361–364. doi:10.2307/1564166

Whitfield SM, Donnelly MA. 2006. Ontogenetic and seasonal variation in the diet of Costa Rican leaf-litter herpetofauna. J Trop Ecol. 22:409–415. doi:10.1017/S0266467406003245

Williams GC. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. Am Nat. 100:687–690. doi:10.1086/282461