



Male care status influences the risk-taking decisions in a glassfrog

Anyelet Valencia-Aguilar¹ · Domingos de Jesus Rodrigues² · Cynthia P.A. Prado^{1,3}

Received: 29 January 2020 / Revised: 4 June 2020 / Accepted: 5 June 2020
© Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

Parental care increases offspring survival, but may impose costs by decreasing adult survival or mating opportunities. Because individuals have limited resources, time allocation and risk decisions are expected to vary according to the care status and threat faced by parents. Herein, we evaluated the sources of mortality of clutches of the glassfrog *Hyalinobatrachium cappellei* and the importance of paternal care for offspring survival. We also compared time allocation patterns and antipredator behavior between attending and non-attending males. Using naturalistic observations and field experiments with different dummy predators, we hypothesized that (1) offspring survival would be positively correlated with paternal care; (2) attending males would spend most of the time caring for the embryos, while non-attending males would spend more time foraging and calling; (3) attending males would prioritize their own survival when facing a high risk of predation, even with negative impacts on offspring survival. Main sources of embryo mortality included predation and dehydration, and offspring survivorship was almost totally dependent on paternal care. Although non-attending males spent more time calling and foraging, attending males also called and were able to attract females and increase their mating success. However, contrary to our prediction, we found that attending males were more risk-tolerant, increasing not only offspring survival but also their mortality risk. Our findings highlight the importance of the predation risk level on parents' decisions and that the reproductive status may play a significant role in determining antipredator behavior and mating success in glassfrog males.

Significance statement

In species with parental care, parents face a trade-off between investment in current offspring survival and chances of future reproduction. Hence, it is expected that natural selection will favor parents that attend offspring without incurring in higher risk of predation. We investigated time allocation patterns and behavioral responses to different levels of threat in a glassfrog with paternal care. Using field observations and predation experiments, we found that attending and non-attending males of *Hyalinobatrachium cappellei* behaved differently depending on their care status and level of threat. Males were more likely to tolerate high risks only when they were caring for clutches. We also found that the commitment of males to continue caring resulted in higher offspring survival. Parental care behavior increased not only larvae hatching success, but also mating success of attending males, suggesting that paternal care might be under sexual selection.

Keywords Anura · *Hyalinobatrachium cappellei* · Time allocation · Paternal care · Predation risk · Offspring survival

Communicated by K. Summers

✉ Anyelet Valencia-Aguilar
anyelet@gmail.com

¹ Pós-Graduação em Ciências Biológicas (Zoologia), Instituto de Biociências, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Rio Claro, São Paulo, Brazil

² Acervo Biológico da Amazônia Meridional, Universidade Federal do Mato Grosso, Sinop, Mato Grosso, Brazil

³ Faculdade de Ciências Agrárias e Veterinárias, Departamento de Morfologia e Fisiologia Animal, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Jaboticabal, São Paulo, Brazil

Introduction

Trade-offs related to the cost of reproduction play important roles in life-history evolution (Williams 1966; Stearns 1989). Because time and energy are limited resources, any investment in current reproduction may impose costs paid in terms of reduced survival and future reproduction (Gadgil and Bossert 1970; Stearns 1989; Gross 2005). This is especially evident in species with parental care, where the amount of parental effort may expose adults to a higher predation risk and decrease mating opportunities (Gross 2005; Klug et al.

2012), and any variation in parental care effort can affect both offspring and parents' fitness (Clutton-Brock 1991; Klug and Bonsall 2014). Given this parent-offspring conflict (Trivers 1974; Klug et al. 2012), previous studies have shown that parents are able to assess and adjust their parental investment according to brood size, remating opportunities, and level of threat, for instance (e.g., Coleman et al. 1985; Székely and Cuthill 2000; Sih et al. 2004; Tilgar and Kikas 2009). When only the offspring faces a threat, parents can display diverse behaviors to defend current offspring against predators or stressful abiotic conditions, which may include attack or avoidance of potential predators (Magnhagen 1992; Rodrigues et al. 2011; Toledo et al. 2011; Ghalambor et al. 2013), or adjustment of care frequency according to climate condition (Lissåker and Kvarnemo 2006; Consolmagno et al. 2016). On the other hand, when the threat is high to both offspring and adults, parents have to decide between offspring and their own survival and future reproduction (Trivers 1972; Lima and Dill 1990; Coleman and Gross 1991). Hence, based on the type and threat intensity, parents will probably prioritize their own survival, taking fewer risks to protect their offspring (Andersson et al. 1980; Consolmagno et al. 2016). Risk-taking decisions can also vary according to predators' traits (Lima and Dill 1990), such as size and density; attending males can attack more often adult predators instead of younger ones (Poo et al. 2016), or escape when predator size exceeds their own size (Chuang et al. 2017).

Many species in the Centrolenidae family, known as glassfrogs, exhibit male parental care and studies have shown that embryo survival is positively correlated with parents' guard behavior (Vockenhuber et al. 2009; Delia et al. 2013, 2017). Main causes of embryo mortality include fungal infection, dehydration, predation, and developmental failure, and alterations in both parental behavior and offspring survival have been related to predator-parent interaction (McDiarmid 1978; Delia et al. 2017). Although clutch predation has been reported for many glassfrog species (Valencia-Aguilar et al. 2012; Delia et al. 2017; Noronha and Rodrigues 2018), antipredator behavior or male-predator interactions remain poorly studied in this family (Delia et al. 2017; Ospina-L et al. 2019). The glassfrog *Hyalinobatrachium cappellei* occurs in the Amazon basin (Frost 2020) and individuals reproduce during the rainy season (November to April) in central-north Brazil, along streams or flooded areas (Noronha and Rodrigues 2018). During the breeding season, males vocalize from leaves to attract females and clutches containing ca. 17 eggs are deposited on the underside of leaves above the water (Noronha and Rodrigues 2018). Males care for the clutches during the embryonic development (approximately 15–20 days), actively hydrating the embryos to prevent dehydration, and larvae complete development in the water (Noronha and Rodrigues 2018; Valencia-Aguilar et al. 2020).

As males of *H. cappellei* occupy a fixed territory before and after oviposition (Valencia-Aguilar et al. 2020) and can attend multiple clutches for a couple of weeks (Noronha and Rodrigues 2018), this species is an excellent model to evaluate parents' time allocation patterns and how threat level posed by different predators modify males' behavior. Herein, we used field observations and conducted experiments with different dummy predators to test whether risk-taking decisions are associated with male care status in *H. cappellei*. We also compared how attending and non-attending males distribute their time among different activities (e.g., calling, mating, foraging, guarding), and tested the effect of care on embryos' survival with controlled parent removal experiments. Although attending males might be more risk-tolerant because of the benefits associated with embryo protection, we predicted that they will abandon the clutches and prioritize their own survival when facing a high risk of predation. Moreover, we predicted that attending males will devote more time to care activities, while non-attending males will spend more time calling to attract females, and that embryo survival and hatching success will be higher in those clutches cared for their parents.

Methods

Study area

Field observations of *H. cappellei* were carried out at the São Nicolau farm (9° 51' 16.9" S; 58° 14' 57.7" W), from January to February in two consecutive years, 2016 and 2017, in the municipality of Cotriguaçu, Mato Grosso state, north-western Brazil. The locality is close to the Juruena River, with 7000 ha of Amazon forest (Rodrigues et al. 2010). The vegetation is classified as dense humid forest, located in an agriculture expansion area named as "Arch of Deforestation" (Rodrigues et al. 2010; Blaser et al. 2011). According to Köppen classification, monsoon climate predominates in the region (Rodrigues et al. 2010), with a rainy season between September and March (rainfall 1300–1700 mm; air humidity 80%) and a dry season between June and August (rainfall 20–50 mm; air humidity 40%). Air temperature varies between 24 and 36 °C in the rainy season and 32 and 38 °C in the dry season (Bittencourt-Rosa et al. 2013).

Activities of attending and non-attending males

We monitored 65 individuals ($N = 20$ attending and $N = 5$ non-attending males in 2016; $N = 25$ attending and $N = 15$ non-attending males in 2017) and 85 clutches ($N = 31$ in 2016 and $N = 54$ in 2017) by diurnal and nocturnal sampling with headlamps through visual and acoustic searches. Individuals and clutches were monitored from 15 to 20 days, which varied according to time spent by males in their territories and

embryos' developmental rate. We observed each male and their clutches at a distance of 1.0 m and recorded for 20–40 min (once in the morning and twice or three times at night) until larvae hatching. To minimize male disturbance, we used a digital video camera recorder in infrared night-shot mode (Sony Handycam AVCHD) during nocturnal observations and behavioral experiments. For individual recognition, we took digital photographs of the dorsum spot patterns of adults (Van Lidth de Jeude 1904; Kenyon et al. 2010). For sex identification, we used behavioral and/or morphological traits; males were identified by their calls and vocal sacs, and females were identified after oviposition or by the presence of oocytes visible through their transparent skin. We measured the snout-vent length (SVL) of all monitored males using a digital caliper (0.01 mm). During focal observations, we recorded general male activities (calling, mating, foraging), caring activities of attending males (sitting close to or on the eggs, hydrating, handling, behaving aggressively towards potential predators), clutch attendance frequency (proportion of time that male provided care during the day, between 08:00 and 13:00, and at night, between 20:00 and 02:00), and egg/embryo clutch condition (parasitism, predation, embryonic stage of development). Here, we considered clutch attendance any passive or active caring behavior performed by males to prevent embryos' mortality, such as sitting close and guarding the eggs/embryos, hydrating the embryos, embryo handling to prevent fungus infection, or active defense against potential predators (Simon 1983; Townsend et al. 1984). To compare time devoted to different activities between attending and non-attending males, we calculated the proportion of time that each male spent in each activity (calling, foraging, mating, attending) relative to the total hours of observation in 1 day (08:00–13:00, and 20:00–02:00). Then, we estimated time budget for each male by summing occurrences of a given activity across days and divided by the total days of observation. We considered attending males those individuals caring for the clutches and non-attending males those without clutches. It was not possible to record data blind because our study involved focal animals in the field.

The adaptive significance of egg attendance: embryo development and survivorship

To characterize and quantify egg care duration in *H. cappellei* and assess its adaptive significance, we compared larvae hatching success between unattended egg clutches (experimental group; $N=15$ attending males removed; five males in 2016 and 10 in 2017) and attended egg clutches (control group; $N=15$ attending males not removed; 10 males in 2016 and five in 2017). Number of eggs per clutch and embryo developmental stage were not different between treatments (t test $t_{14}=3.09$, $P=0.75$, t test $t_{14}=1.92$, $P=0.52$, respectively). We classified embryonic stage in two categories: early-

stage embryos (stages 1–19, Gosner 1960) and late-stage embryos (stages 20–25, Gosner 1960) (following McDiarmid and Altig 1999). For the experimental group, we removed the attending male to prevent clutch assistance and in the control group, attended egg clutches received paternal care during embryonic development. Removed males were released ~10 m upstream or downstream from their clutches and none returned to their territories. We examined and photographed each clutch daily (once in the morning and twice or three times at night) to quantify and record egg/embryo mortality source. We registered embryo mortality by predation, dehydration, fungal infection, or developmental failure. Following oviposition, time required for embryos to hatch is about 15–20 days (Valencia-Aguilar et al. 2020). When larvae were about to hatch, we positioned plastic bags beneath the clutches to collect the hatchlings and determine larval survival. Larvae hatching success was the proportion of hatchlings relative to the number of eggs per clutch.

Predation risk and behavioral response of attending and non-attending males

To understand if male's responses to threat vary according to care status, we simulated different levels of risk, using non-toxic rubber models of a katydid (25 mm) and a spider (50 mm), which were presented to attending and non-attending males. Katydid are common predators of both adults and clutches of some glassfrogs (*Cochranella granulosa*, Delia et al. 2017). However, based on previous observations of predation events on clutches of *H. cappellei* (Noronha and Rodrigues 2018; AVA pers. obs.), we considered katydids (Tettigoniidae) as a threat only for eggs/embryos. Moreover, we observed a spider preying on clutches of *H. cappellei* and a male leaving his territory after noticing the spider, a common escape response to avoid predation (Toledo et al. 2011; Ortega-Andrade et al. 2013). For these reasons, we used a katydid and a spider as predator models in our experiments, representing different levels of threat: predation risk only for offspring (katydid) and predation risk for male and offspring (spider). We tested our rubber models using a dark resin ball as a control model, which were presented to males of *H. cappellei* ($N=10$) and three sympatric anuran species: *Allophryne ruthveni* ($N=5$), *Boana cf. fasciata* ($N=10$), and *Dendropsophus brevifrons* ($N=10$). All individuals used in the validation tests responded similarly to the three models, fled from predators' proximity, and ignored resin ball models. After validation, we exposed two groups of attending ($N=5$ in 2016 and $N=5$ in 2017) and non-attending males ($N=5$ in 2016 and $N=5$ in 2017) to the following threats: (a) physical disturbance to simulate a medium-risk predator attack; we used a katydid dummy that represented a threat only for the clutches, and (b) physical disturbance to simulate a high-risk predator attack; we used

a spider dummy that represented a threat for both males and clutches. In each treatment, we approached the dummy predators to males and clutches using a wooden stick. We used the dummies to touch and squish males and clutches and recorded if males remained in their initial position (pre-stimulus), faced the predator or left the territory. We also registered males' responses, as follows: body-raising (male stretches legs and arms, lifting his body to increase the apparent body size; sensu Toledo et al. 2011), remaining alert (male remains in the same posture without calling or moving), fleeing (active escape), and cloacal discharge (male releases a transparent liquid during escape).

To test the effect of stimuli variation on males' responses, we exposed each group of males to the following treatments: (1) random stimuli; for the first group of attending ($N = 5$ in 2016) and non-attending males ($N = 5$ in 2016), we presented each dummy during 1 min, in a random sequence in consecutive nights; (2) non-random stimuli; for the second group of attending ($N = 5$ in 2017) and non-attending males ($N = 5$ in 2017), we presented each dummy during 1 min in the same sequence (katydid, spider) in the same night, with an interval of 5 min between each stimulus. When a non-attending male abandoned his territory, we monitored the site for five consecutive nights to determine if the male would return to the same place or to a nearby leaf. Likewise, when an attending male abandoned his territory with clutches, we monitored the clutches until all embryos hatched or died. All predation risk experiments were conducted by the same researcher (AVA), from the same distance (1.0 m) and position (under) in relation to males, moving carefully and slowly to avoid additional disturbance to males.

Statistical analyses

Proportion of time that attending and non-attending males spent in each activity was compared using the Kruskal-Wallis test, because behavioral variables were not normally distributed. We compared embryo survival between attended and unattended clutches using a generalized linear model (GLM), with a quasibinomial distribution. In this model, we considered larvae hatching success as the response variable, and mortality causes (predation, dehydration, and developmental failure) and male presence (present or removed) as explanatory variables. We also built models considering the additive effect and interaction between the explanatory variables. Moreover, we used a linear regression to evaluate whether an increase in the time that males spent attending the clutches led to an increase of hatching success. We found that males' responses were not affected by stimuli variation ($P = 0.99$, Fisher's exact test medium risk; $P = 0.99$, Fisher's exact test high risk), i.e., males' responses were similar during predation experiments regardless of presentation order of dummies (in sequence or randomly) or presentation time

interval (the same night or consecutive nights). Hence, data were combined for the subsequent analysis. To determine whether care status (attending and non-attending) influenced males' behavior when facing a medium (katydid) and high (spider) risk situation, we used logistic regression models, with males' responses as the response variable. We categorized males' responses as passive if males raised the body and remained alert or active if males fled with or without cloacal discharge. First, we tested whether isolated threat type (medium or high risk) influenced males' behavior change. Second, we included males' body size (SVL) and developmental stage of embryos (early or late stages) as predictors to test whether each males' responses (passive or active) could be attributed to body size and male care status. For visual representation of the results, bar plots were used. All analyses were conducted using the R software version 3.4.3 (R Core Team 2017).

Results

Activities of attending and non-attending males

Attending and non-attending males exhibited site fidelity, but time allocation patterns differed among them (Fig. 1a). During the day, attending males remained inactive close to their clutches and hydrated the embryos only on dark, cloudy, and rainy days for few hours (08:00–09:00 h). At night, they distributed their time among calling (45%) or sitting close to the clutches in an alert posture (30%), foraging (10%), hydrating or handling the eggs (10%), and in amplexus (5%; Fig. 1a, b). Non-attending males were inactive during the day and remained in a resting position on the same leaf used as calling site. At night, non-attending males distributed their time among calling (68%) from the same area but from different leaves, foraging (30%), and mating (2%, Fig. 1a). Non-attending males ($N = 20$) spent much more time foraging ($P < 0.001$) and calling ($P = 0.002$) than attending males ($N = 45$); however, attending males were more frequently observed in amplexus than non-attending males ($P = 0.001$). Considering the first day of male encounter, non-attending males obtained their first clutch after a week ($X \pm SD = 7.16 \pm 1.72$ days, $N = 6$ males) while attending males obtained more clutches after a couple of days (second clutch $X \pm SD = 3.05 \pm 1.07$ days, $N = 19$ males; third clutch $X \pm SD = 2.77 \pm 1.20$ days, $N = 9$ males; fourth clutch $X \pm SD = 2.00 \pm 0.89$ days, $N = 6$ males; fifth clutch $X \pm SD = 2.00 \pm 1.41$ days, $N = 2$ males; sixth clutch 1 day, $N = 1$ male).

The adaptive significance of egg attendance: embryo development and survivorship

Mean number of eggs per clutch was 19.91 ± 4.19 ($N = 85$ clutches) and attending males simultaneously cared for one

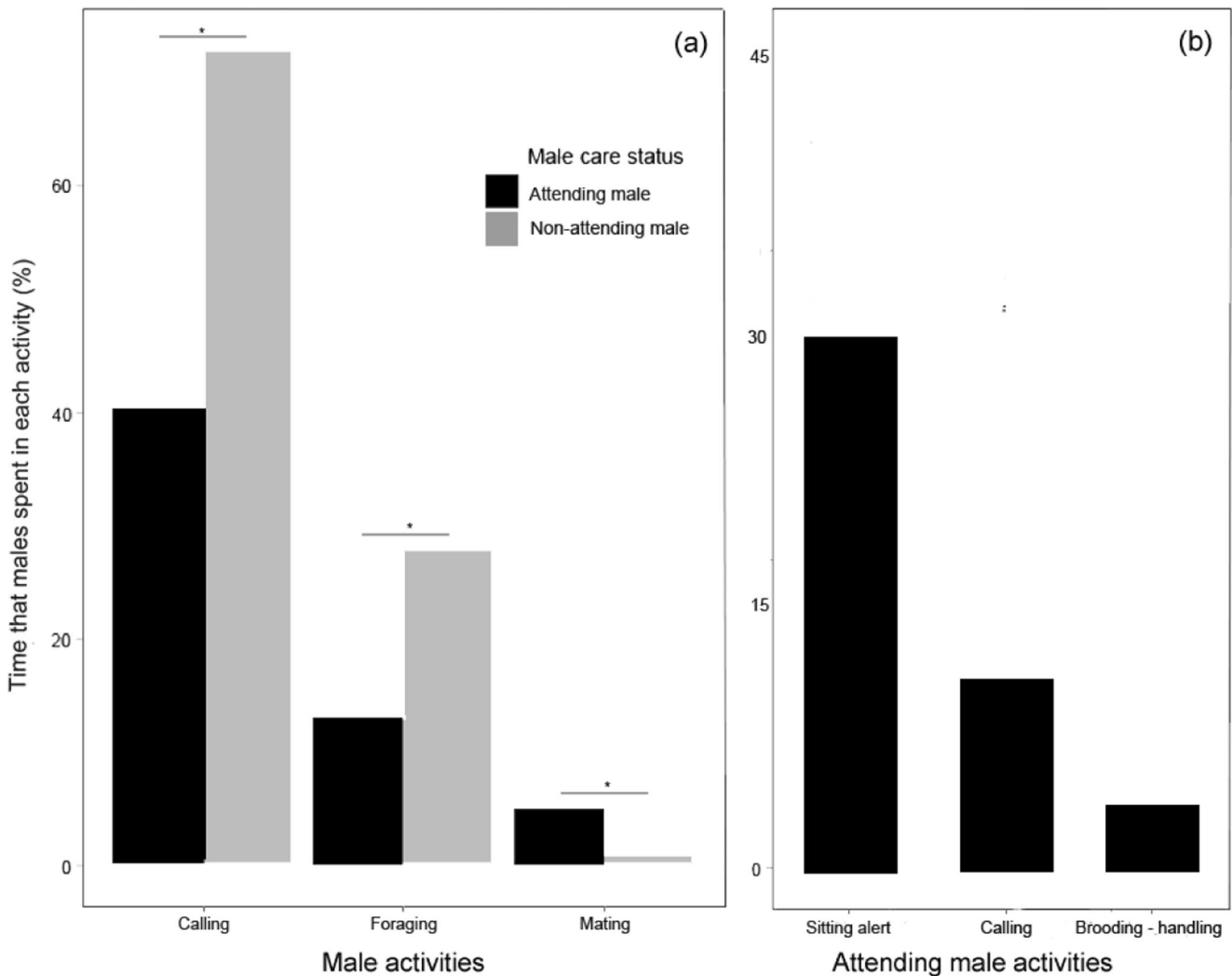


Fig. 1 Percentage of time spent by males of *Hyalinobatrachium cappellei* in different activities during two breeding seasons (January–February 2016 and January–February 2017) at São Nicolau Farm, municipality of Cotriguaçu, Mato Grosso state, north-western Brazil. **a** Time spent in

each activity by attending and non-attending males. **b** Proportion of time spent by attending males in different activities while assisting the clutches. Single asterisk indicates significant difference between bars

to six clutches ($X \pm SD = 1.83 \pm 0.99$), during the day and at night. Clutches were deposited on the underside of leaves and hydrated by males after heavy rains or during drizzling rain. Because rains do not reach the clutches on the underside of the leaves, on these occasions, males jumped and sat on wet areas on the upper side of leaves to absorb and store water in the bladder. After 10–20 min, males returned and covered the clutches with their moistened bodies, making up and down movements, with their bodies and hind limbs over the eggs, changing their position and starting over again until hydrating the entire clutch. We also observed males using their hands and feet to rotate or clean the eggs/embryos. Embryo survival in attended clutches was much higher ($X \pm SD = 86\% \pm 27.25$) when compared with that of unattended clutches ($X \pm SD = 19.5\% \pm 35.33$) (ANOVA $F_{2,23} = 11.28$, $P = 0.0003$; Fig. 2a). Main causes of embryo mortality in unattended clutches were predation ($X \pm SD = 50.0\% \pm 44.0$) and dehydration ($X \pm$

$SD = 40.0\% \pm 37.3$) and in attended clutches were predation ($X \pm SD = 14.0\% \pm 9.0$) and developmental failure ($X \pm SD = 7.5\% \pm 4.2$) (Fig. 2b). Thus, male's presence reduced clutch predation ($P = 0.0008$) and dehydration ($P = 0.019$). Although developmental failure occurred in clutches of both treatments (Fig. 2b), it was not a major cause of embryo mortality ($P = 0.99$).

Regarding care duration, we found a significant positive correlation between the time male remains with the offspring and larvae hatching success ($F_{2,30} = 64.94$, $R^2 = 0.79$, $P < 0.001$, $N = 30$ males (15 males in 2016 and 15 males in 2017), 63 clutches (26 in 2016 and 37 in 2017)). On average, males spent 13.5 days (range 10–19 days, $N = 30$ males) taking care of a single clutch, and when taking care for multiple clutches (more than one), offspring hatching success increased 3.54 times for each extra day that males spent with them. During fieldwork, we observed spiders, katydids, ants, a

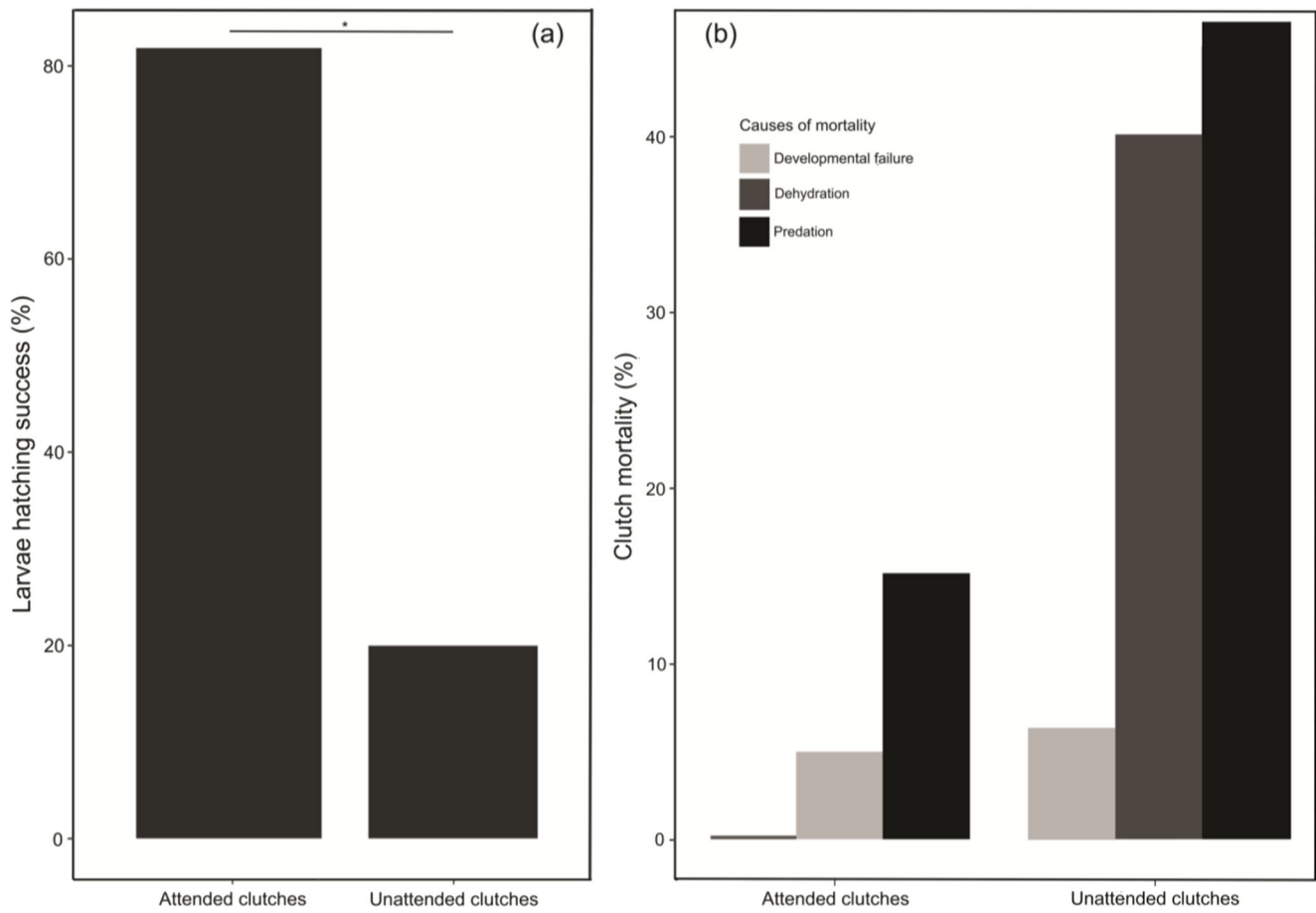


Fig. 2 **a** Larvae hatching success of attended (control group) and non-attended egg clutches (experimental group) of the glassfrog *Hyalinobatrachium cappeliei*. **b** Causes of embryo mortality identified as predation, dehydration, and developmental failure for both attended

($N = 30$) and unattended clutches ($N = 20$) monitored during our study in north-western Mato Grosso state, Brazil. Single asterisk indicates significant difference between bars

cockroach, and a snake (juvenile *Leptodeira annulata*) preying on embryos of attended and unattended clutches during the day and at night. Moreover, we observed an attending male caring for two clutches on separate leaves, one in front of the other, exhibiting passive defensive behavior to prevent predation of one of his clutches. When a cockroach began to eat the eggs, the male from a different leaf started to raise his body until the cockroach stopped and left the leaf. Although we observed the snake *L. annulata* preying on some egg clutches, we could not observe if the male fled from the snake or if he had already left the territory when the snake found the clutches. In any case, probably the male would not have been able to defend the embryos from the snake.

Predation risk and behavioral response of attending and non-attending males

Our results showed that males' responses to threat varied as a function of care status. During the medium-risk experiment (katydid dummy), attending and non-attending males remained in their territories, without significant changes in

their behaviors (Table 1). While attending males raised their bodies a couple of times ($N = 2$ males in 2016, $N = 1$ males in 2017, Fig. 3a) or remained in the same position ($N = 3$ males in 2016, $N = 4$ males in 2017), non-attending males stopped calling and remained in the same position ($N = 5$ males in 2016, $N = 5$ males in 2017, Fig. 3b). On the other hand, during the high-risk experiment (spider dummy), behavioral responses of attending and non-attending males changed considerably (Table 1). Attending males crouched down and remained motionless (Fig. 3c). Unlike non-attending males, attending males only fled after we touched or squished their dorsum with the spider ($N = 5$ males in 2016, $N = 5$ males in 2017), releasing a colorless liquid through their cloacae ($N = 2$ males in 2016, $N = 3$ males in 2017) while escaping (Fig. 3d). Besides, all attending males remained on the same leaf or nearby and returned close to the clutches few minutes after perturbation ($N = 5$ males in 2016, $N = 5$ males in 2017). By contrast, non-attending males fled when we started approaching the spider and never returned to the same leaf, but remained in the same area ($N = 4$ males in 2016, $N = 5$ males in 2017, Fig. 3e, f). Attending and non-attending males did not

Table 1 Logistic regression for factors associated with changes in the responses of attending and non-attending males of *Hyalinobatrachium cappellei* submitted to two levels of predation risk (medium and high risk) during a fieldwork experiment conducted in the municipality of Cotriguaçu, Mato Grosso state, north-western Brazil. SVL snout-vent length

	df	t	P	Coefficient
Threat type				
Medium risk	18	-0.007	0.99	-18.72
High risk	18	-108.50	<0.001	-51.132
Attending males				
Threat	15	-99.02	<0.01	<0.001
Embryo development	15	<0.001	1.00	0.00
SVL	15	<0.001	1.00	<0.001
Clutches development*SVL	15	<0.001	1.00	<0.001
Non-attending males				
Threat	16	<0.01	0.003	-3.38
SVL	16	<0.001	1.00	0.00
Threat*SVL	16	<0.001	1.00	0.00

exhibit aggressive behaviors (calls or fighting attempts) towards the dummies. Finally, our logistic regression models showed that males' responses were affected by threat type rather than males' size or developmental stage of embryos (Table 1).

Discussion

Our study showed that the reproductive status of males influences time allocation patterns and risk-taking decisions in a species of glassfrog with paternal care, with possible implications for mating success. As expected, we found that non-attending males of *H. cappellei* spent more time calling and foraging compared with attending males. However, although non-attending males called more, attending males continued calling, mated more frequently, and obtained more clutches. Calling and caring activities are energy-demanding; thus, it is expected that males allocate certain amount of time or energy in one activity at a time (Lissåker and Kvarnemo 2006; Bleu et al. 2016). Furthermore, paternal care may reduce food intake, increase mortality risk, and/or decrease mating opportunities of males (Simon 1983; Steinhart et al. 2004; Requena et al. 2012). Nonetheless, we found that parental care not only increased offspring survival, but also may benefit attending males of *H. cappellei* by increasing their chances of gaining both new mates and clutches. Evidences of parental care and mating advantages in *H. cappellei* have been reported before for a small sample size (Noronha and Rodrigues 2018), which we confirmed here. Similar results have also been reported for other species of anurans (Chen et al. 2011; Mangold et al.

2015), fishes (Coleman et al. 1985; Matsumoto et al. 2011), and arthropods (Requena and Machado 2015), where males continue to mate while caring for clutches in their territories. Hence, our results showed that in *H. cappellei*, both care investment and mating are non-mutually exclusive activities and that future studies should investigate parental care in glassfrogs in the context of sexual selection.

As we predicted, embryos were almost totally dependent on male care for survival. Only, 19.5% of the embryos of the unattended clutches survived, compared with 86% of survivorship in the attended clutches. High rates of embryo survival were also found in other glassfrog species with parental care (*Hyalinobatrachium aureoguttatum* 96.5%, *Hyalinobatrachium fleischmanni* 82%, *Hyalinobatrachium orientale* 79.8%, *Hyalinobatrachium valerioi* 50%, *Ikakogi tayrona* 78.2%, *Centrolene peristicta* 78.89%), as well as in some terrestrial (*Eleutherodactylus cooki* 85%, *Cophixalus parkeri* 95%, *Hylophorbus rufescens* 78.2%) and arboreal frogs (*Chiromantis hansenae* 75%, *Oreophryne* sp., 100%) when parents (male or female) remained attending clutches (Simon 1983; Burrowes 2000; Bickford 2004; Vockenhuber et al. 2009; Valencia-Aguilar et al. 2012; Delia et al. 2013; Poo and Bickford 2013; Lehtinen et al. 2014; Bravo and Delia 2015; Salgado and Guayasamin 2018). Desiccation and predation are the main causes of mortality of eggs and embryos in many animal groups (Andersson et al. 1980; Alonzo-Alvarez and Velando 2012). Particularly in glassfrogs, parental care seems to be mainly associated with reduction of embryo dehydration rather than active defense against predation (Valencia-Aguilar et al. 2012; Delia et al. 2013). Similarly, our results also suggest that, although parental care reduced clutch predation, prevention of desiccation is more likely to be the primary driver of parental care in *H. cappellei*. We also found that males of *H. cappellei* with multiple clutches increased hatching success for each additional day they spent providing care. By extending the duration of care, parents may incur in energetic and survival costs, as well as decrease mating opportunities (Clutton-Brock 1991; Gross 2005; Klug et al. 2012). However, as we mentioned above, our results suggest that males of *H. cappellei* do not pay high energetic and mating costs by providing prolonged parental care. On the contrary, by increasing offspring survival and mating success, prolonged parental care in *H. cappellei* seems to increase males' fitness. Furthermore, although survival costs were not directly addressed here, we hypothesize that mortality risks in males of *H. cappellei* do not increase as a consequence of care, similar to what was found in *Eleutherodactylus coqui* (Townsend 1986).

Anurans exhibit diverse and complex parental care behaviors (Furness and Capellini 2019; Vági et al. 2019). Some species, for example, are active caregivers that constantly hydrate their clutches (e.g., *Kurixalus eiffingeri*, Chen et al.

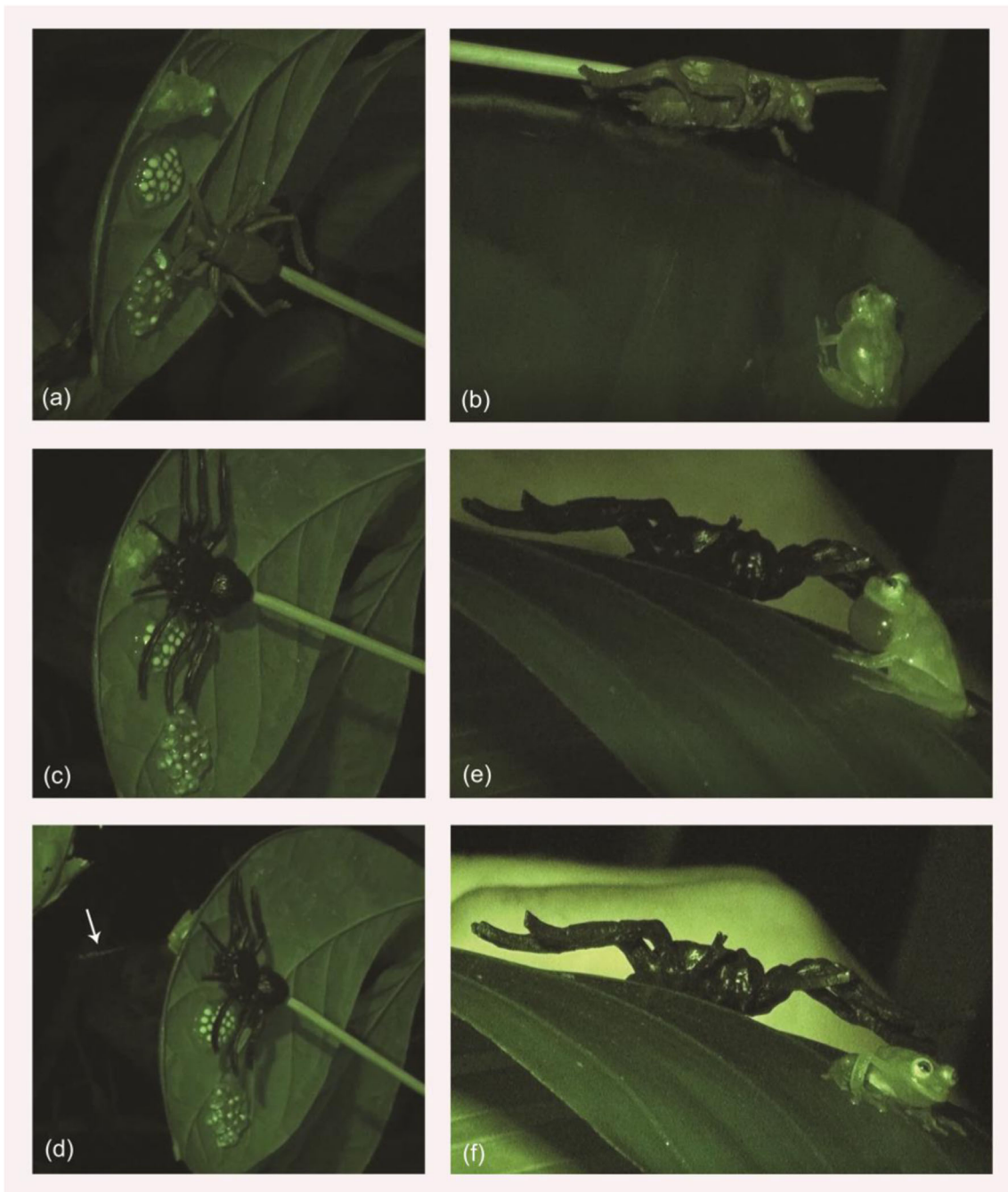


Fig. 3 Behavioral responses of males of *Hyalinobatrachium cappellei* to two levels of disturbance. **a** Attending and **b** non-attending males' responses to medium-risk predation (katydid dummy). **c**, **d** Attending and

e non-attending males' responses to high-risk predation (spider dummy). In **d**, the arrow indicates attending male cloacal discharge after being touched by the spider

2007) or cover long distances looking for suitable water bodies for larval deposition (e.g., *Allobates femoralis*, Ringler et al. 2013), whereas in glassfrogs (*Hyalinobatrachium* genus), parents are mainly passive caregivers (Vockenhuber et al. 2008; Valencia-Aguilar et al. 2012; Bravo and Delia 2015; this study). In *H. cappellei*, we observed that males care for the clutches during the embryonic development (approximately 10–19 days; this study), remaining most of the time close to clutches, even when emitting calls, but also hydrating

or handling the eggs/embryos. Brooding behavior to hydrate the clutches is typical of glassfrogs with parental care, where parents, after absorbing water accumulated on leaves, position their bodies on the eggs/embryos and release the collected water on them (Valencia-Aguilar et al. 2012; Delia et al. 2017). Glassfrog parents also manipulate the eggs and embryos not only to prevent fungal infection and abnormal development, but also to facilitate oxygen exchange between the environment and embryos by performing embryo-turning

movements (Simon 1983; Valencia-Aguilar et al. 2012; Delia et al. 2013).

Our last hypothesis was that attending males would prioritize their own survival when facing a high predation risk, even with negative effects on offspring survival. Indeed, we found that attending and non-attending males of *H. cappellei* were able to discriminate between a medium and a high risk of predation, adjusting their responses accordingly. However, contrary to our prediction, attending males were more risk-tolerant than expected. Theoretical models assume that by behaving more cautiously, caregivers will be able to lower predation costs (Andersson et al. 1980). Likewise, experimental studies have shown that parental risk decisions varied according to parents' chance of survival, but not offspring risk, i.e., when the threat is too high, parents will abandon their current offspring to avoid being preyed and increase their chances of future reproduction (Shaffer and Formanowicz 1996; Mahr et al. 2015; Chuang et al. 2017). Nonetheless, our results showed that attending males of *H. cappellei* are willing to risk their own lives to protect their offspring. Similarly, attending males of *Centrolene savagei* (Ospina-L et al. 2019) and attending females of *Ikakogi tayrona* (Bravo and Delia 2015) are more risk-tolerant and required higher levels of threat stimuli to abandon their clutches increasing their risk of mortality. Attending males of *H. cappellei* did not actively protect their clutches against the spider dummies. However, despite of the spider dummy being twice males' size, they did not abandon their clutches, risking their own survival. In *Hyalinobatrachium aureoguttatum* (Valencia-Aguilar et al. 2012), an attending male was observed trying to attract the attention of a harvestmen that was preying on his clutches by moving close to the predator. We could hypothesize that males of *H. cappellei* remain in their territories in an attempt to attract the attention of larger predators, such as spiders, escaping following predator approach. Nonetheless, contrary to this prediction, males only fled after we touched their dorsum with the spider a couple of times, a behavior that in natural conditions could have cost male's life (Ortega-Andrade et al. 2013). Alternatively, this tolerance of males to a high predation risk could be a context-dependent behavior, exhibited only under certain circumstances (e.g., when larvae are ready to hatch). In fact, in glassfrogs, embryos are capable of hatching prematurely following parent desertion or to escape from predation, but only after a period of obligate care (ca. 5 days) (Delia et al. 2014, 2018). However, hatching plasticity is unknown in our study species. Furthermore, we found no effect of embryo developmental stage on males' responses. The tolerance of *H. cappellei* males to high predation risk could be explained by the advantages in terms of offspring survival and mating success we found, offsetting the mortality risk.

Similarly to what has been described for other anurans (Poo et al. 2016; Chuang et al. 2017), fishes (Magnhagen 1992), and birds (Mahr et al. 2015), our results suggest that males of

H. cappellei use visual cues to detect the presence of predators, as our predator models did not emit chemical signs. Males of *H. cappellei* did not actively or aggressively defend their clutches from predators, contrary to what was reported for other glassfrog species (*H. valerioi*, *Hyalinobatrachium colymbiphylum*, and *H. fleischmanni*), in which males defend their offspring by kicking wasps away (Drake and Ranvestel 2005; Vockenhuber et al. 2008; Delia et al. 2010). Wasps can be considered a low threat for males, which can be kicked off or consumed by males (McDiarmid 1978). However, katydids can be a threat not only for the clutches but also for males by preying on them or causing injuries (e.g., *Feihyla hansena*, Poo et al. 2016; *Cochranella granulosa*, Delia et al. 2017). Although we did not observe any active behavior defense against katydids preying on embryos of *H. cappellei*, we did observe a male trying to deter a similar predator, a cockroach, when attacking the embryos by performing body-raising. Body-raising is a common defense behavior of anurans (Toledo et al. 2011); hence, males of *H. cappellei* may effectively protect their clutches against medium-size predators, such as cockroaches, minimizing energy expenditure and mortality risk by avoiding physical contact. Although not aggressively protecting the clutches, our study demonstrated that male parental care is crucial for offspring survival, not only by preventing desiccation, but also by reducing embryo predation.

In conclusion, we found that offspring survival is extremely dependent on parental care, which seems to be more related to dehydration avoidance than predator defense in *H. cappellei*. Although, not exhibiting active defense, attending and non-attending males were able to identify and respond accordingly to the threat level imposed by the predator (i.e., predator type and behavior). But, contrary to our expectations, attending males remained close to their clutches even when facing a high predation threat, increasing their mortality risk to protect their offspring. Although time allocation patterns differed between attending and non-attending males, we found that care activities did not affect male's mating opportunities; attending males exhibited higher mating success and continued obtaining clutches. We suggest that differences in reproductive status may play a significant role in the antipredator behavior pattern exhibited by glassfrogs, which needs further investigation, and that future studies should evaluate the role of paternal care for female choice in centrolenids.

Acknowledgments We thank the staff members of the Fazenda São Nicolau and the Acervo Biológico da Amazônia Meridional – ABAM, for the logistic support in the field. We are also grateful to Célio F.B. Haddad and Nadya C. Pupin, from the Laboratório de Herpetologia, UNESP-Rio Claro, São Paulo, for providing us the necessary equipment used during the fieldwork. To Roy W. McDiarmid for valuable discussions and two anonymous reviewers provided suggestions that improved the manuscript.

Code availability Not applicable.

Author contributions AVA and CPAP designed the original research questions. AVA collected field data, performed all statistical analyses, and drafted the manuscript. All authors substantially edited and approved the final manuscript.

Funding information This research was funded by the São Paulo Research Foundation, FAPESP (grant #2016/05070-5).

Data availability All data generated or analyzed during this study are included in this published article.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Field observations and behavioral experiments were carried out in accordance with the Association for the Study of Animal Behaviour guidelines and the Ethics Committee on Animal Use of the Universidade Estadual Paulista (UNESP), Rio Claro, São Paulo state, Brazil (protocol number 9457) and the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio, license number 51479-4).

Consent to participate Not applicable.

Consent for publication Not applicable.

References

- Alonzo-Alvarez C, Velando A (2012) Benefits and costs of parental care. In: Royle NJ, Smiseth PT, Kölliker M (eds) The evolution of parental care. Oxford University Press, Oxford, pp 1–17
- Andersson M, Wiklund CG, Rundgren H (1980) Parental defence of offspring: a model and an example. *Anim Behav* 28:536–542
- Bickford DP (2004) Differential parental care behaviors of arboreal and terrestrial microhylid frogs from Papua New Guinea. *Behav Ecol Sociobiol* 55:402–409
- Bittencourt-Rosa D, Nascimento LA, Dubreuil V, Figueiredo LF, Cassiano JE (2013) Novos dados acerca dos aspectos geográficos e geológicos da área da fazenda São Nicolau e circunvizinhanças-município de Cotriguaçu-MT. XIV EGAL—Encontro dos Geógrafos da América Latina 04:08–11. Lima, Perú.
- Blaser J, Sarre A, Poore D, Johnson S (2011) Status of Tropical Forest Management 2011. ITTO Technical Series No 38. International Tropical Timber Organization, Yokohama, Japan
- Bleu J, Gamelon M, Sæther BE (2016) Reproductive costs in terrestrial male vertebrates: insights from bird studies. *Proc R Soc B* 283:20152600
- Bravo LV, Delia J (2015) Maternal care in a glassfrog: care function and commitment to offspring in *Ikakogi tayrona*. *Behav Ecol Sociobiol* 70:41–44
- Burrows PA (2000) Parental care and sexual selection in the Puerto Rican cave-dwelling frog, *Eleutherodactylus cooki*. *Herpetologica* 56:375–386
- Chen YH, Yu HT, Kam YC (2007) The ecology of male egg attendance in an arboreal breeding frog, *Chirixalus eiffingeri* (Anura: Rhacophoridae), from Taiwan. *Zool Sci* 24:434–440
- Chen YH, Cheng WC, Yu HT, Kam YC (2011) Genetic relationship between offspring and guardian adults of a rhacophorid frog and its care effort in response to paternal share. *Behav Ecol Sociobiol* 65:2329–2339
- Chuang MF, Lee WH, Sun JS, You CH, Kam YC, Poo S (2017) Predation risk and breeding site value determine male behavior and indirectly affect survivorship of their offspring. *Behav Ecol Sociobiol* 71:122
- Clutton-Brock TH (1991) The evolution of parental care. Princeton University Press, Princeton
- Coleman RM, Gross MR (1991) Parental investment theory: the role of past investment. *Trends Ecol Evol* 6:404–406
- Coleman RM, Gross MR, Sargent RC (1985) Parental investment decision rules: a test in the bluegill sunfish. *Behav Ecol Sociobiol* 18:59–66
- Consolmagno RC, Requena GS, Machado G, Brasileiro CA (2016) Costs and benefits of temporary egg desertion in a rocky shore frog with male-only care. *Behav Ecol Sociobiol* 70:785–795
- Delia J, Cisneros-Heredia DF, Whitney J, Murrieta-Galindo R (2010) Observations on the reproductive behavior of a Neotropical Glassfrog, *Hyalinobatrachium fleischmanni* (Anura: Centrolenidae). *S Am J Herpetol* 5:1–12
- Delia J, Ramirez-Bautista A, Summers K (2013) Parents adjust care in response to weather conditions and egg dehydration in a neotropical glassfrog. *Behav Ecol Sociobiol* 67:557–569
- Delia J, Ramirez-Bautista A, Summers K (2014) Glassfrog embryos hatch early after parental desertion. *Proc R Soc B* 281:20133237
- Delia J, Bravo LV, Warkentin KM (2017) Patterns of parental care in Neotropical glassfrogs: fieldwork alters hypotheses of sex-role evolution. *J Evol Biol* 30:898–914
- Delia J, Rivera-Ordóñez JM, Salazar-Nicholls MJ, Warkentin KM (2018) Hatching plasticity and the adaptive benefits of extended embryonic development in glassfrogs. *Evol Ecol* 33:37–53
- Drake DL, Ranvestel AW (2005) *Hyalinobatrachium colymbiophyllum* (glass frog). Egg mass defense. *Herpetol Rev* 36:434
- Frost DR (2020) Amphibian species of the world: an online reference, version 6.0. American Museum of Natural History, New York, USA. <https://doi.org/10.5531/db.vz.0001http://research.amnh.org/herpetology/amphibia/index.html>. Accessed 20 Jan 2020
- Furness AI, Capellini I (2019) The evolution of parental care diversity in amphibians. *Nat Commun* 10:4709
- Gadgil M, Bossert WH (1970) Life history consequences of natural selection. *Am Nat* 104:1–24
- Ghalambor CK, Peluc SI, Martin TE (2013) Plasticity of parental care under the risk of predation: how much should parents reduce care? *Biol Lett* 9:20130154
- Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183–190
- Gross MR (2005) The evolution of parental care. *Q Rev Biol* 80:37–46
- Kenyon N, Phillott AD, Alford R (2010) Temporal variation in dorsal patterns of juvenile greeneyed tree frogs, *Litoria genimaculata* (Anura: Hylidae). *Herpetol Conserv Biol* 5:126–131
- Klug H, Bonsall MB (2014) What are the benefits of parental care? The importance of parental effects on developmental rate. *Ecol Evol* 4:2330–2351
- Klug H, Alonzo SH, Bonsall MB (2012) Theoretical foundations of parental care. In: Royle NJ, Smiseth PT, Kölliker M (eds) The evolution of parental care. Oxford University Press, Oxford, pp 21–39
- Lehtinen RM, Green SE, Pringle JL (2014) Impacts of paternal care and seasonal change on offspring survival: a multiseason experimental study of a caribbean frog. *Ethology* 120:400–409
- Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. *Can J Zool* 68:619–640
- Lissåker M, Kvamemo C (2006) Ventilation or nest defense parental care trade-offs in a fish with male care. *Behav Ecol Sociobiol* 60:864–873
- Magnhagen C (1992) Parental care and predation risk in fish. *Ann Zool Fenn* 29:227–232
- Mahr K, Riegler G, Hoi H (2015) Parental risk management in relation to offspring defence bad news for kids. *Proc R Soc B* 282:20141670

- Mangold A, Trenkwalder K, Ringler M, Hödl W, Ringler E (2015) Low reproductive skew despite high male-biased operational sex ratio in a glass frog with paternal care. *BMC Evol Biol* 15:181
- Matsumoto Y, Tawa A, Takegaki T (2011) Female mate choice in a paternal brooding blenny: the process and benefits of mating with males tending young eggs. *Ethology* 117:227–235
- McDiarmid RW (1978) Evolution of parental care in frogs. In: Burghardt GM, Bekoff M (eds) *The development of behavior: comparative and evolutionary aspects*. Garland STPM Press, New York, pp 127–147
- McDiarmid RW, Altig R (1999) Research: materials and techniques. In: McDiarmid RW, Altig R (eds) *Tadpoles: the biology of anuran larvae*. The University of Chicago Press, Chicago, pp 7–23
- Noronha JC, Rodrigues DJ (2018) Reproductive behaviour of the glass frog *Hyalinobatrachium cappellei* (Anura: Centrolenidae) in the Southern Amazon. *J Nat Hist* 52:207–224
- Ortega-Andrade HM, Rojas-Soto O, Paucar C (2013) Novel data on the ecology of *Cochranella mache* (Anura: Centrolenidae) and the importance of protected areas for this critically endangered glassfrog in the Neotropics. *PLoS One* 8:e8183
- Orpina-L AM, Navarro-Salcedo P, Rios-Soto JA, Duarte-Marín S, Vargas-Salinas F (2019) Temporal patterns, benefits, and defensive behaviors associated with male parental care in the glassfrog *Centrolene savagei*. *Ethol Ecol Evol* (published online. <https://doi.org/10.1080/03949370.2019.1682056>)
- Poo S, Bickford DP (2013) The adaptive significance of egg attendance in a south-east Asian tree frog. *Ethology* 119:1–9
- Poo S, Evans TA, Tan MK, Bickford DP (2016) Dynamic switching in predator attack and maternal defence of prey. *Biol J Linn Soc* 118: 901–910
- R Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria <http://www.R-project.org/>
- Requena GS, Machado G (2015) Effects of egg attendance on male mating success in a harvestman with exclusive parental care. *Behav Ecol* 26:926–935
- Requena GS, Buzatto BA, Martins EG, Machado G (2012) Paternal care decreases foraging activity and body condition, but does not impose survival costs to caring males in a Neotropical arachnid. *PLoS One* 7:e46701
- Ringler E, Pašukonis A, Hödl W, Ringler M (2013) Tadpole transport logistics in a Neotropical poison frog: indications for strategic planning and adaptive plasticity in anuran parental care. *Front Zool* 10:67
- Rodrigues DJ, Izzo TJ, Battirola LD (2010) Descobrimos a Amazônia Meridional: Biodiversidade da Fazenda São Nicolau. Pau e Prosa Comunicação Ltda, Mato Grosso, Brazil
- 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 alloparental care in frogs. *J Nat Hist* 45:2037–2047
- Salgado AL, Guayasamin JM (2018) Parental care and reproductive behavior of the minute dappled glassfrog (Centrolenidae: *Centrolene peristictum*). *S Am J Herpetol* 13:211–219
- Shaffer LR, Formanowicz DR (1996) A cost of viviparity and parental care in scorpions: reduced sprint speed and behavioural compensation. *Anim Behav* 51:1017–1024
- Sih A, Bell A, Johnson JC (2004) Behavioral syndromes: an ecological and evolutionary overview. *Q Rev Biol* 79:241–277
- Simon MP (1983) The ecology of parental care in a terrestrial breeding frog from New Guinea. *Behav Ecol Sociobiol* 14:61–67
- Stearns SC (1989) Trade-offs in life-history evolution. *Funct Ecol* 3:259–268
- Steinhart GB, Sandrene ME, Weaver S, Stein RA, Marschal EA (2004) Increased parental care cost for nest-guarding fish in a lake with hyperabundant nest predators. *Behav Ecol* 16:427–434
- Székely T, Cuthill IC (2000) Trade-off between mating opportunities and parental care: brood desertion by female Kentish plovers. *Proc R Soc Lond B* 267:2087–2092
- Tilgar V, Kikas K (2009) Is parental risk taking negatively related to the level of brood reduction? An experiment with pied flycatchers. *Anim Behav* 77:43–47
- Toledo LF, Sazima I, Haddad CFB (2011) Behavioural defences of anurans: an overview. *Ethol Ecol Evol* 23:1–25
- Townsend DS (1986) The costs of male parental care and its evolution in a Neotropical frog. *Behav Ecol Sociobiol* 19:187–195
- Townsend DS, Stewart MM, Pough FH (1984) Male parental care and its adaptive significance in a neotropical frog. *Anim Behav* 32:421–431
- Trivers RL (1972) Parental investment and sexual selection. In: Campbell BG (ed) *Sexual selection and the descent of man 1871–1971*. Aldine, Chicago, pp 136–179
- Trivers RL (1974) Parent-offspring conflict. *Am Zool* 14:249–264
- Vági B, Végvári Z, Liker A, Freckleton RP, Székely T (2019) Parental care and the evolution of terrestriality in frogs. *Proc R Soc B* 286:20182737
- Valencia-Aguilar A, Castro-Herrera F, Ramírez-Pinilla MP (2012) Microhabitats for oviposition and male clutch attendance in *Hyalinobatrachium aureoguttatum* (Anura: Centrolenidae). *Copeia* 2012:722–731
- Valencia-Aguilar A, Zamudio KR, Haddad CFB, Bogdanowicz SM, Prado CPA (2020) Show me you care: female mate choice based on egg attendance rather than male or territorial traits. *Behav Ecol*. <https://doi.org/10.1093/beheco/araa051>
- Van Lidth de Jeude TW (1904) Reptiles and batrachians from Surinam. *Notes Leyden Museum* 25:83–94
- Vockenhuber EA, Hödl W, Karpfen U (2008) Reproductive behaviour of the glassfrog *Hyalinobatrachium valerioi* (Anura: Centrolenidae) at the tropical stream Quebrada Negra (La Gamba, Costa Rica). *Stapfia* 88:335–348
- Vockenhuber EA, Hödl W, Amézquita A (2009) Glassy fathers do matter: egg attendance enhances embryonic survivorship in the glass frog *Hyalinobatrachium valerioi*. *J Herpetol* 43:340–344
- Williams GC (1966) Natural selection, the cost of reproduction, and a refinement of Lack's principle. *Am Nat* 100:687–690