ORIGINAL ARTICLE

Maternal care in a glassfrog: care function and commitment to offspring in *Ikakogi tayrona*

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Abstract Parental care is important for offspring success for many animals. Both male-only and female-only care occur equally often among frogs, creating opportunities to examine ecological factors that may favor sex-specific patterns of care. Experimental tests of care function can elucidate such factors and identify benefits of parental behavior to offspring. In glassfrogs (Centrolenidae), only male egg attendance has been documented and male care is thought to have evolved repeatedly from a state of no-care. We provide experimental evidence demonstrating female care in the glassfrog *Ikakogi tayrona*—a species sister to all other members of the family. We used repeated observations to quantify egg attendance behaviors, and a removal experiment to evaluate the benefits of maternal care and test whether care function changes with embryonic development. Given the importance of care to female fitness, we examined maternal commitment to providing care in risky situations, using a simple behavioral challenge. We found that egg attendance reduces embryo dehydration and predation. Moreover, the specific benefits of care changed across embryo development, suggesting that different threats to embryos select for egg attendance at different stages. The maternal commitment assay revealed that caring females are more risk-tolerant than non-caring females. Our finding of maternal care in *I. tayrona* reveals previously unknown diversity in glassfrog parental care and contributes to emerging evidence that care functions are similar among frogs with female-only and male-only egg attendance. This suggests that a lack of sex-specific constraints may have facilitated evolutionary changes in sex roles.

Keywords Parental care · Offspring benefits · Maternal commitment · Centrolenidae · Frog

Introduction

Parental care is fundamental to offspring survival in many animals. It can be defined as any form of post-fertilization parental behavior that enhances offspring survival or growth (Clutton-Brock 1991). Care patterns vary widely among vertebrates, and considerable theoretical research has focused on understanding the conditions that favor care and which sex will provide it (reviewed by Klug et al. 2012). This theory predicts that parental behavior will be favored when the benefits to the sex providing care outweigh the cost (Trivers 1972; reviewed by Klug et al. 2012). The benefits of care can be accurately measured by using parent removal experiments to quantify the impact of care on offspring growth and survival. Furthermore, analysis of the specific fates of experimentally orphaned young can provide insights into the environmental conditions that favor and maintain parental behavior.

Parental care patterns among vertebrates include male-only, female-only, and biparental care, and the contributions of each sex vary among taxonomic groups (Kokko and Jennions 2008). Female care predominates among mammals (Clutton-Brock 1991). Biparental care is the most common form among birds, although females tend to invest more than males (Cockburn 2006). In reptiles, only uniparental female

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and biparental care has been documented (Shine 1988; Reynolds et al. 2002). Fishes and amphibians exhibit all patterns of care (Reynolds et al. 2002; Balshine 2012). Unlike teleost fishes, in which male care predominates, both uniparental male and female care appear to occur equally among frogs (Reynolds et al. 2002; Wells 2007; Summers and Tumulty 2014). This "intermediate" pattern offers ideal opportunities to examine the ecological factors that favor sex-specific forms of parenting.

Egg attendance is the most common form of parental care in frogs that lay terrestrial eggs, having evolved multiple times in approximately 14 families (Wells 2007; Gomez-Mestre et al. 2012). This form of care involves a variety of parental behaviors performed to combat constraints on embryonic development in terrestrial eggs. In many species, parents guard eggs from predators via aggressive behaviors during prolonged attendance (Simon 1983; Townsend et al. 1984; Martins et al. 1998; Cook et al. 2001; Bickford 2004; Vockenhuber et al. 2009). To prevent embryo dehydration, parents hydrate eggs by brooding them with their ventral region (Townsend et al. 1984; Bickford 2004; Delia et al. 2013; Poo and Bickford 2013). Egg brooding may also alter microbial communities and reduce fungal infections of eggs (Simon 1983; Walke et al. 2011). Some species have been observed to periodically jostle eggs, which may oxygenate embryos, distribute moisture, and/or prevent yolk stratification (Wells 2007). These different threats to embryonic survival and development have likely influenced the evolution of diverse attendance behaviors. However, empirical research on anuran parental care is lacking in comparison to other groups of vertebrates (Balshine 2012; Bee et al. 2013).

In neotropical glassfrogs (Centrolenidae), only male egg attendance has been documented. It is thought that male care evolved independently in the genera *Centrolene* and *Hyalinobatrachium* (McDiarmid 1978; Guayasamin et al. 2009; Delia et al. 2013; Vargas-Salinas et al. 2014), presumably from an ancestral state of no-care. However, experimental data are limited to just three species from *Hyalinobatrachium*, 1 of 12 genera in this family (Vockenhuber et al. 2009; Delia et al. 2013; Lehtinen et al. 2014). An anecdotal observation of a female near an egg clutch in *Ikakogi tayrona* (M. Rada, personal communication in Guayasamin et al. 2009)—a monotypic taxon sister to all other glassfrogs—suggests that sex-specific roles of care may be more diverse than currently thought. Given *I. tayrona*'s phylogenetic position, determining whether care is present and which sex provides it is a critical step toward understanding the evolutionary patterns of parenting among glassfrogs.

We studied a wild population of *I. tayrona* to determine the existence of parental care and which sex provides it, and to test hypothesized care functions. Aside from its initial description, phylogenetic relationships, and call description (Ruiz-Carranza and Lynch 1991; Guayasamin et al. 2009;

Vargas-Salinas et al. 2015), no additional information on the behavior and ecology of this species exists to date. In all other studied glassfrogs, eggs are deposited on vegetation and/or rocks above forested streams and, at hatching, larva continue development in the water (McDiarmid 1978; Guayasamin et al. 2009). To detect and quantify egg attendance in *I. tayrona*, we made repeated observations of oviposition sites. We then tested whether maternal attendance benefits embryo survival by conducting a female removal experiment. We removed mothers at different stages of embryonic development to evaluate whether the importance of care to embryo survival changes during development. Furthermore, testing whether the functional benefits of care change during embryonic development, allowed us to examine how different threats to embryos influence the duration of maternal care. Lastly, we used a simple behavioral challenge to experimentally assess maternal commitment to attending eggs. If maternal attendance is important to embryo survival, and thus female fitness, caring females should tolerate higher threat levels compared to non-caring females. We discuss our results in regard to factors affecting the evolution of egg attendance in frogs.

Methods

This study was conducted during the rainy seasons of May–June 2013 and 2014 near the village of La Tagua at 1580 m in the Sierra Nevada de Santa Marta (SNSM), an isolated mountain range on the Caribbean coast of Colombia. *I. tayrona* is endemic to the cloud forests of the SNSM between 800 and 2200 m and the only glassfrog known from this isolated mountain range (Ruiz-Carranza and Lynch 1991; Vargas-Salinas et al. 2015). The SNSM experiences a bimodal rainy season, with two periods of rain between April–June and September–November. Like other glassfrogs, *I. tayrona* is nocturnal and arboreal and breeds on vegetation alongside streams (LBV personal observation; Vargas-Salinas et al. 2015). It was not possible to record data blind because our study involved focal animals in the field.

Field observations of maternal attendance behaviors

To first determine whether *I. tayrona* exhibits parental care and which sex provides it, we conducted repeated observations of egg clutches to quantify egg attendance behaviors. The locations of calling males along stream transects were marked with flagging tape and checked nightly to monitor mating activity. Once adults mated, we conducted repeated observations of egg clutches to detect parental behaviors and assess their nature and frequency. We conducted diurnal and nocturnal surveys on different groups of clutches, due to the time constraints of nocturnal experiments. Eight clutches in different stages of development were checked every 30 min

over 7-h sampling periods during seven nights (between 20:30 and 03:30). At each observation, we recorded whether a presumed parent was present at the oviposition site, its sex, and its position with respect to the egg clutch. Parental behaviors were categorized as (a) absent from the oviposition site leaf, (b) present at the oviposition site leaf but not in contact with eggs, (c) in non-brooding contact with eggs (hand or head touching the clutch), and (d) ventral contact egg brooding (parent positioned on top of the clutch). The sex of the attending parent was determined by examining individuals for sex-specific external characters, such as humeral spines and vocal sacs, which only occur in males (Ruiz-Carranza and Lynch, 1991). The transparent venter of these frogs also allowed us to see immature ovules developing in females. Daytime observations were made on 13 clutches. Each clutch was checked a minimum of twice each day between 8:00 and 17:00 h, for a period of 1–4 days. A subset of five males were uniquely marked with toe clips and followed nightly over 4 weeks to examine territoriality.

Female removal experiment

To test the functional benefits of maternal attendance, we conducted a female removal experiment. Newly deposited clutches were randomly assigned to either removal or control treatments ($n=29$ and 25, respectively); clutch size did not differ between treatments ($t_{44.9}=-0.038$, $p=0.96$, $n=54$). Control clutches were allowed to receive maternal care during embryonic development, whereas removal clutches had their mother removed between 0–6 days post-oviposition (dpo). A minimum of three removals were conducted for each clutch age, which allowed us to examine how the duration of care (measured as the dpo of removal) affects different rates and sources of embryonic mortality. If embryo requirements change during development, egg fates should differ with their stage at maternal removal. Removed females were released ~50 m upstream or downstream from their clutch; none subsequently returned to their developing clutches. Hatching success and time were monitored using 9-oz plastic cups positioned below clutches with thin metal wire (sensu Hayes 1983a). Hatchlings were counted and released daily to quantify egg stage survival. We used daily observations and photographs of clutches to determine specific causes of mortality, according to established characteristics used to discern embryonic mortality in terrestrial frog eggs (sensu Warkentin 2000; Hawley 2006). We examined clutches for all possible sources of embryo mortality, namely: dehydration, predation, fungal infection, developmental abnormalities (i.e., eggs that partially developed and then died), and eggs removed by heavy rainfall (rain-stripped). Five control females abandoned their clutches within 2 days after oviposition. To test the effects of prolonged parental care, these abandoned clutches were removed from analyses comparing survival between treatments.

However, these naturally abandoned clutches were included in analyses examining the influence of care duration on particular embryo fates.

Maternal commitment assay

We used a simple behavioral challenge to experimentally assess maternal commitment to attending eggs. A standardized series of graded threat stimuli were applied to females in two behavioral contexts: caring and not caring for eggs. Most non-caring females were gravid and found approaching male territories prior to mating. The stimulus series consisted of (1) slowly approaching the frog with a finger to ~1-cm distance, and then waiting 10 s for a response; (2) touching the frog softly with a finger, up to five times at 10-s intervals; (3) a slightly stronger touch, up to five times; (4) pinching the frog between two fingers, up to five times; and finally (5) pushing the frog laterally until it was physically displaced from its location. Such generalized threat stimuli provide a simple means to create tradeoffs between avoiding perceived risks and continuing parental care or another behavior (Frid and Dill, 2002). We measured the level of disturbance at which females stopped what they were doing and fled. Comparisons were made between behavioral contexts to evaluate whether females tolerate higher threat levels when caring for embryos.

Statistical analyses

Statistical analyses were conducted with R version 3.0.2 (R Development Core Team 2013) and JMP Pro version 11. To describe parental attendance patterns, we calculated the frequency of egg attendance and specific behaviors for each individual female, during daytime and during nighttime observations, and then present summary statistics across females (mean±SD). We used generalized linear models with a beta-binomial error distribution (betabin) and a logit link function (using the "aod" package; Lesnoff and Lancelot 2015), to test whether the proportion of embryo fates differed between control and removal treatments. P values were computed with likelihood ratio tests comparing nested models with and without the treatment predictor. Comparisons between treatments were made separately for each type of mortality to identify the specific function of maternal care. Because the accuracy of confidence intervals depends on parametric assumptions, which proportional data do not meet, bootstrap analyses (1000 times) were used to calculate more robust non-parametric estimates of 95 % confidence intervals of the mean for each cause of mortality (Davison and Hinkley 1997). Determining whether the function of care changes during embryonic development can provide insights on the factors that maintain the duration of egg attendance. We modeled the influence of maternal care duration, measured as the dpo of removal, on both overall embryonic mortality and particular

embryo fates associated with care function (dehydration and predation) using beta-binomial GLMs. These analyses allowed us to evaluate whether the importance of care changes during embryonic development, and whether predation and dehydration differentially favor care at different developmental stages. To assess threat tolerance of females, the threat stimulus level at which females responded was compared between behavioral contexts using chi-square tests.

Results

Natural history and maternal behaviors

I. tayrona exhibits parental care in the form of prolonged attendance of eggs. All attending individuals were females, indicated by a lack of humeral spines and the presence of immature eggs seen through the venter of adult frogs ($n=30$ individuals, Fig. 1a–c). Females only cared for one clutch at a time. In most cases, oviposition sites were located in the same places (same leaves) where males were seen calling on the previous evening. Repeated observations of marked males revealed that they exhibit call site fidelity, calling from the same leaf or nearby leaves (within 1 m) night after night. We observed ten combat events between males with neighboring territories (Fig. 1d). No females were observed during combat, suggesting that combat may occur over territory disputes. Some males left their territories after mating but returned to the same call site on subsequent nights (Fig. 1e). Oviposition sites were more frequently located on the underside of leaves above the stream (81 %, $\chi^2=30.31$, df=1, $p<0.0003$, $n=76$). The height of oviposition sites ranged from 51 to 200 cm ($\bar{x}=151$ cm, SD=63, $n=35$), although we were unable to monitor males calling from higher sites.

While females attended eggs during both the day and the night, diel patterns of attendance varied. Attendance was more constant at night and consisted of prolonged periods of ventral contact egg brooding, where females covered most of the clutch with their bodies throughout the night (Fig. 1a). During brooding, females performed active movements on top of the clutch, including undulations and rotations, followed by long periods without movement. Just following oviposition, females moved around on leaves near oviposition sites and stopped in wet spots, presumably absorbing water to hydrate egg clutches. We performed 26–95 observations per clutch over seven nights (eight clutches, 491 nocturnal checks in total). Individual females were present at the oviposition site during 65.4–100 % ($\bar{x}=87.9\pm10.6$ %) of observations. When present, they were engaged in brooding during 63.4–96.8 % ($\bar{x}=78.5\pm12.7$ %) of observations, and in non-brooding contact during 0–34.1 % ($\bar{x}=9.48\pm11.2$ %) of observations. When present at night, females were always in contact with their clutch; they were never seen in non-contact attendance (category b). Females were absent from oviposition sites on average of 11.8 ± 10.5 % of nocturnal observations. Interestingly, 90 % of observed absences occurred either immediately after oviposition (when they were absorbing water, as described above) or after females had completely


Fig. 1 Maternal attendance and mating behaviors. **a** Throughout the night, females remained in egg brooding contact with their clutch and performed active movements on eggs. **b** During the day, egg attendance was less frequent but, when attending, females often rested in brooding contact with their clutch. **c** Close-up of the posterior region of a female brooding an egg clutch, with immature ova visible through the transparent venter. **d** Males in combat during territory defense. **e** A male (left) calling next to a previous mate, who is caring for their clutch. Dead *Heliconia* leaves above streams were a common site for male territories
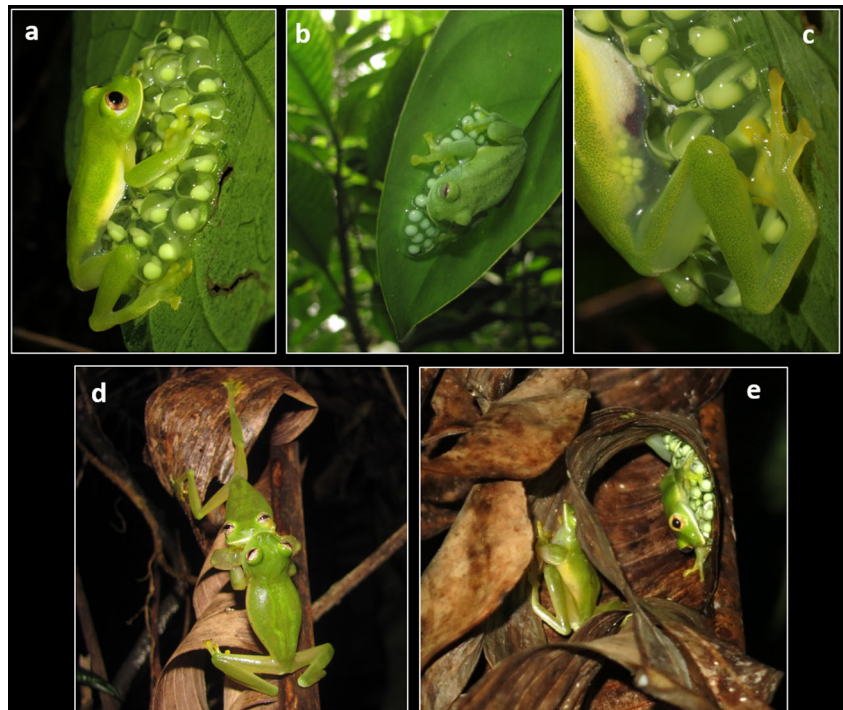
abandoned clutches (indicated by continued absence during all subsequent checks). Diurnal attendance frequency was calculated from an average of 4.6 checks for each of 13 females (58 diurnal observations in total). While our diurnal observations were limited, attendance did appear more variable during the day than at night. Females were found at the oviposition site during 0–100 % ($\overline{x}$ =30.1±41.8 %) of daytime observations. When present, they were always in contact with the clutch, sometimes in a brooding posture (20.8 %±25.9). Daytime absence did not indicate abandonment, as many females absent from oviposition sites during the day were later seen attending eggs at night. Females also moved to and from egg clutches during the day, being absent during one or more daytime checks, and then observed attending during others. This contrasts with nocturnal attendance frequencies, where females were seen in contact with eggs during all observations, with the exception of hydration behavior just following oviposition and abandonment. Nightly observations to monitor embryo mortality in control clutches (from the removal experiment) found a similar pattern; females usually brooded eggs throughout the night until completely abandoning clutches (n=25). Hence, nighttime absence accurately indicates female abandonment. Embryos became competent to hatch from 16 to 18 days post-oviposition. The duration of care was variable among females, and on average, females terminated care before embryos were capable of hatching (Table 1).

Female removal experiment

The removal experiment clearly revealed that female attendance benefits embryo survival, as removal clutches experienced significantly higher rates of egg mortality (χ^2=40.75, df=1, p<0.0001). On average, embryo mortality for the removal treatment was 85 %, compared to 21.8 % in control clutches (Fig. 2). We observed several causes of mortality in clutches of both treatments: dehydration, predation, fungal infection, rain stripped, and developmental abnormalities. Maternal attendance significantly reduced mortality caused by dehydration (χ^2=23.57, df=1, p<0.0001) and predation (χ^2=7.26, df=1, p<0.006). Dehydration was the primary cause of mortality (41.2 %) of embryos in the removal treatment, and among all naturally abandoned clutches (50 %, n=
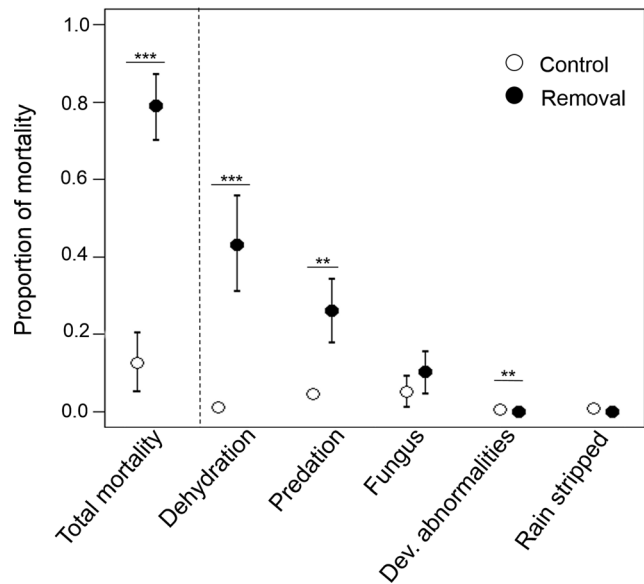

Fig. 2 The effect of female removal on the proportion of total embryo mortality and specific sources of mortality among individual clutches for control and removal treatments (n=25 and 29, respectively). Female care primarily reduced mortality due to dehydration and predation. *Circles* indicate the means and *bars* represent 95 % confidence intervals from bootstrap analyses. ***p< 0.0001; **p< 0.001

5). Developmental abnormalities occurred in very low proportions in both treatments but were significantly higher in control clutches (0.48 % control vs. 0.042 % removals, χ^2=−8.67, df= 1, p=0.007); however, this could be because other sources of mortality more often killed removal clutches prior to our ability to detect developmental abnormalities. Pathogenic fungal infections also occurred infrequently and did not differ between treatments (χ^2=−0.49, df= 1, p=0.48, Fig. 2), although saprophytic fungus occurred more often in unattended clutches. Spiders of the family Anyphaenidae, harvestmen (order Opiliones), and whip scorpions (order Arachnida) were observed eating embryos from removal clutches at night. Predation also occurred in some control clutches, but it appeared to occur more frequently during the day when females were absent from oviposition sites. Small coleopterans and crickets were seen on three occasions eating dehydrated embryos from unattended clutches.

Analyses of care durations (dpo of female removal) revealed that the benefit of care to embryo survival declined with embryonic age (χ^2=10.35, df= 1, p=0.001, n=33). The duration of care appeared to be differentially associated with the two primary causes of mortality. Specifically, embryonic dehydration was higher for earlier removals (χ^2=13.27, df= 1, p=0.0002), indicating that hydration-related care is the most important during the first few days of embryonic development (Fig. 3a). The same analyses on predation rates found no such effect

Table 1 Summary of reproductive traits

| Trait	Number	Mean±SD	Min	Max
Clutch size	59	76.2±15.2	45	119
Care duration (days)	26	11.65±6.29	1	22
Embryonic period (days)	19	25.3±4.2	16	33
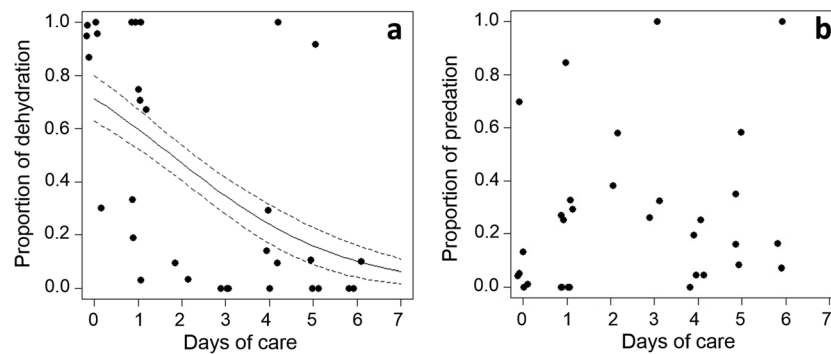
Fig. 3 Embryo mortality in individual clutches across care durations, measured as embryo age when mothers were removed. **a** The proportion of mortality caused by dehydration declined with embryos' age at removal. The line is predicted from the generalized linear model; *dashed lines* represent 95 % confidence intervals. **b** In contrast, the proportion of mortality caused by predation was consistent across age at removal

($\chi^2 = 3.19.66$, df = 1, $p = 0.074$), indicating that risk of predation continues throughout embryonic development (Fig. 3b).

Maternal commitment

The assay of maternal commitment revealed that caring females were significantly more threat-tolerant and required higher levels of threat stimuli to induce a flight response, compared to non-caring females (chi-square test $\chi^2 = 26$, df = 3, $p < 0.0001$, $n = 30$, Fig. 4). Females approaching calling males fled after comparatively little disturbance, whereas females attending eggs were more reluctant to flee and frequently continued brooding until they were physically dislodged from their clutch (see Online Resource 1 for a video). These females often remained on the same leaf as their clutch and returned to their eggs a few minutes after being pushed off.
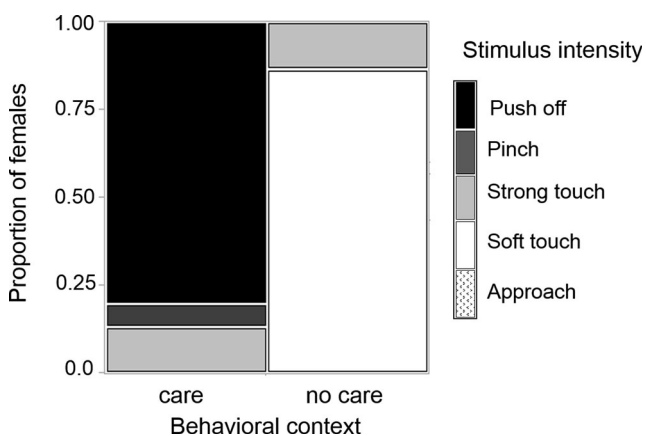
Fig. 4 The proportion of females that fled at different levels of a graded series of threating stimuli, for females caring and not caring for eggs (for a video see Online Resource 1). The *darkness* of the *colored bar* indicates the increasing levels of disturbance applied to females until they ceased their current activity

Discussion

We document the first case of female care in glassfrogs. We found that female *I. tayrona* exclusively attended eggs and removing mothers dramatically increased rates of embryonic mortality. The benefits of care changed during embryonic development, suggesting that multiple threats to embryos differentially select for care at different stages. Our behavioral assay of maternal commitment revealed that females tolerate greater threats while providing care than they do in other contexts. The existence of maternal care in a lineage sister to all other glassfrogs calls into question assumptions about the ancestral care state for the family. In combination with emerging research, this work indicates that evolutionary transitions in care patterns among arboreal-breeding frogs are likely more diverse than currently thought.

Maternal care in *I. tayrona* improved offspring survival by protecting embryos from dehydration and predation, serving similar functions as male care in *Hyalinobatrachium* (Vockenhuber et al. 2009; Delia et al. 2013; Lehtinen et al. 2014). These care functions have been documented in other terrestrial-breeding anurans, suggesting that dehydration and predation are driving the evolution of parental behavior across frogs with independent origins of terrestrial egg deposition (e.g., Simon 1983; Townsend et al. 1984; Bickford 2004). Exclusive female care is less common than male care among arboreal-breeding frogs (Wells 2007), but a recent study on the rhacophorid frog *Chiromantis hansenae* found that females attend and brood eggs to prevent embryonic dehydration (Poo and Bickford 2013). This emerging evidence on the functional similarity of female and male egg attendance reveals a lack of sex-specific constraints, which may have facilitated evolutionary transitions in anuran parental care. This contrasts with other sex-limited forms of care in anurans, such as larval provisioning with trophic eggs, which is necessarily sex-specific (Brown et al. 2010; Summers and Tumulty 2014).

We found that the importance of maternal care to embryo survival declined with embryonic age. Moreover, the functional role of care changed during embryonic development. Under the study conditions, the importance of care to egg hydration greatly declined after 48 h past oviposition, whereas predation occurred at similar rates throughout embryonic development. Thus, it appears that egg predation selects for prolonged attendance, whereas hydration-related attendance is most critical in a relatively brief period following oviposition. Nonetheless, prolonged brooding may be important to maintain egg water balance under more variable weather conditions or at lower elevations. This result is consistent with studies of male care in *Hyalinobatrachium fleischmanni*, where dehydration mortality for early removal clutches was higher than for those conducted 48 h after oviposition (Delia et al. 2013). Research on *Hyalinobatrachium orientale* found that sources of embryo mortality changed across seasons, with higher rates of predation occurring in removals conducted during the rainy season (Lehtinen et al. 2014). These studies provide evidence that temporal variation in risks to offspring impacts the function of parental care across glassfrogs.

Both the relative duration of care and the nightly amount of egg brooding appear to differ between *I. tayrona* and male *Hyalinobatrachium*. Female *I. tayrona* continuously brood clutches throughout the night but usually abandon embryos before they are competent to hatch. The presence of immature eggs in *I. tayrona* during care suggests that they lay more than one clutch within a reproductive season. It seems possible that the intense form of care provided by *I. tayrona* imposes a cost in terms of energy allocation to future clutches, favoring relatively shorter care periods. In contrast, male *Hyalinobatrachium* brood clutches infrequently during multiple short bouts at night and often provide care well past hatching competence (Vockenhuber et al. 2009; Delia et al. 2013, 2014). Many *Hyalinobatrachium* also guard multiple clutches while continuing mating activity (Vockenhuber et al. 2009; Valencia-Aguilar et al. 2012; Lehtinen et al. 2014). Reduced conflict between paternal behavior and mating opportunities may have permitted longer care durations in *Hyalinobatrachium* (Williams 1975). More research is needed to examine how reproductive effort influences sex-specific levels of parental care in arboreal-breeding frogs.

Parental care is often associated with costs to parents in terms of resource allocation, decreased mating opportunities, and physical risks from predators (reviewed by Alonso-Alvarez and Velando 2012). Our behavioral assay of maternal commitment indicates that females are more willing to remain in risky situations when providing care, and guarding eggs from predators probably increases their risk of injury or mortality. Predation risk to parents has been documented in several other frogs (reviewed by Wells 2007), and a variety of predators—including snakes, spiders, and katydids—eat both adult glassfrogs and eggs (Hayes 1983b; LBV and JD personal observation). Mothers of some leptodactylid frogs also exhibit extremely aggressive behavior during offspring defense (Hurme 2011; Rodrigues et al. 2011). Our study provides a simple method to quantify parental commitment and assess parental risk and decisions about offspring abandonment.

While females tend to invest more in care than do males across the animal kingdom (Kokko and Jennions 2008), exclusive male care predominates among frogs with semi-terrestrial reproduction (Wells 2007). In glassfrogs, it was thought that male care evolved repeatedly from a state of no care. However, our study reveals that both uniparental male and female care exist, and raises questions concerning the ancestral state of care in the family. It is possible that maternal care also occurs in other lineages. Jacobson (1985) observed female *Espadarana* (*Centrolenella*) *prosoblepon* sitting on clutches for brief periods immediately after oviposition, but parents never returned to clutches on subsequent nights. More research is needed among glassfrogs and other arboreal-breeding anurans to determine the sex-specific patterns of parental care. Understanding the diversity and evolutionary history of parental care in frogs will likely impact our broader understanding of, and assumptions about, care patterns among tetrapods.

Compliance with ethical standards

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

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the Universidad de Los Andes and the Colombian Government; research was approved and permits were provided by the Autoridad Nacional de Licencias Ambientales (Expediente IDB 0337).

References

Alonso-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 40–61

Balshine S (2012) Patterns of parental care in vertebrates. In: Royle NJ, Smiseth PT, Kölliker M (eds) The evolution of parental care. Oxford University Press, Oxford, pp 40–61

Bee MA, Schwartz JJ, Summers K (2013) All's well that begins Wells: celebrating 60 years of Animal Behaviour and 36 years of research on anuran social behaviour. Anim Behav 85:5–18

Bickford DP (2004) Differential parental care behaviors of arboreal and terrestrial microhylid frogs from Papua New Guinea. Behav Ecol Sociobiol 55:402–409

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

Cockburn A (2006) Prevalence of different modes of parental care in birds. Proc R Soc Lond B 273:1375–1383

Cook CL, Ferguson JWH, Telford SR (2001) Adaptive male parental care in the giant bullfrog, *Pyxicephalus adspersus*. J Herpetol 35: 310–315

Clutton-Brock T (1991) The evolution of parental care. Princeton University Press, Princeton

Davison AC, Hinkley DV (1997) Bootstrap methods and their applications. Cambridge University Press, New York

Delia JR, Ramírez-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 JR, Ramírez-Bautista A, Summers K (2014) Glassfrog embryos hatch early after parental desertion. Proc R Soc B 281:2013–3237

Frid A, Dill L (2002) Human-caused disturbance stimuli as a form of predation risk. Conserv Ecol 6:11

Gomez-Mestre I, Pyron RA, Wiens JJ (2012) Phylogenetic analyses reveal unexpected patterns in the evolution of reproductive modes in frogs. Evolution 66:3687–3700

Guayasamin JM, Castroviejo-Fisher S, Trueb L, Ayarzagüena J, Rada M, Vilà C (2009) Phylogenetic systematics of glassfrogs (Amphibia: Centrolenidae) and their sister taxon *Allophryne ruthveni*. Zootaxa 2100:1–97

Hawley TJ (2006) Embryonic development and mortality in *Hyalinobatrachium pulveratum* (Anura: Centrolenidae) of southwestern Costa Rica. J Trop Ecol 22:731–734

Hayes MP (1983a) A technique for partitioning hatching and mortality estimates in leaf-breeding frogs. Herpetol Rev 14:115–116

Hayes MP (1983b) Predation on the adults and prehatching stages of glass frogs (Centrolenidae). Biotropica 15:74–76

Hurme K (2011) Tadpole schooling and parental care in the neotropical frog, *Leptodactylus insularum*. PhD Dissertation, University of Connecticut, Connecticut, USA

Jacobson SK (1985) Reproductive behavior and male mating success in two species of glass frogs (Centrolenidae). Herpetologica 41: 396–404

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

Kokko H, Jennions MD (2008) Parental investment, sexual selection and sex ratios. J Evol Biol 21:919–948

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

Lesnoff M, Lancelot R (2015) aod: analysis of overdispersed data. R package, version 1.3, http://cran.r-project.org/package=aod

Martins M, Pombal JP, Haddad CF (1998) Escalated aggressive behaviour and facultative parental care in the nest building gladiator frog, *Hyla faber*. Amphibia-Reptilia 19:65–73

McDiarmid RW (1978) Evolution of parental care in frogs. In: Burghardt GM, Bekoff M (eds) The development of behavior: comparative and evolutionary aspects. Garland, New York, pp 127–147

Poo S, Bickford DP (2013) The adaptive significance of egg attendance in a south-east Asian tree frog. Ethology 119:671–679

R Development Core Team (2013) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, https://www.r-project.org/

Reynolds JD, Goodwin NB, Freckleton RP (2002) Evolutionary transitions in parental care and live bearing in vertebrates. Philos T Roy Soc B 357:269–281

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

Ruiz-Carranza PM, Lynch JD (1991) Ranas Centrolenidae de Colombia II. Nuevas especies de Centrolene de la Cordillera Oriental y Sierra Nevada de Santa Marta. Lozania 58:1–26

Shine R (1988) Parental care in reptiles. In: Gans C, Huey RB (eds) Biology of the Reptilia, vol 16, Ecology B: Defense and Life History. AR Liss, New York, pp 275–329

Simon MP (1983) The ecology of parental care in a terrestrial breeding frog from New Guinea. Behav Ecol Sociobiol 14:61–67

Summers K, Tumulty J (2014) Parental care, sexual selection, and mating systems in neotropical poison frogs. In: Macedo HR, Machado G (eds) Sexual selection: perspectives and models from the neotropics. Academic Press, New York, pp 289–320

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. Aldine-Atherton, Chicago, pp 136–179

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

Vargas-Salinas F, Bravo-Valencia L, Amézquita A (2015) The advertisement call and calling site of the early diverging glassfrog *Ikakogi tayrona* (Anura: Centrolenidae). South Am J Herpetol 10:65–70

Vargas-Salinas F, Quintero-Ángel A, Osorio-Domínguez D, Rojas-Morales JA, Escobar-Lasso S, Gutiérrez-Cárdenas PDA, Amézquita A (2014) Breeding and parental behaviour in the glass frog *Centrolene savagei* (Anura: Centrolenidae). J Nat His 48:1689–1705

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

Walke JB, Harris RN, Reinert LK, Rollins-Smith LA, Woodhams DC (2011) Social immunity in amphibians: evidence for vertical transmission of innate defenses. Biotropica 43:396–400

Warkentin KM (2000) Wasp predation and wasp-induced hatching of red-eyed treefrog eggs. Anim Behav 60:503–510

Wells KD (2007) The Ecology and Behavior of Amphibians. University of Chicago Press, Chicago, Illinois

Williams CG (1975) Sex and Evolution. Princeton University Press, Princeton