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Glassy Fathers Do Matter: Egg Attendance Enhances Embryonic Survivorship in the Glass Frog *Hyalinobatrachium valerioi*

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ABSTRACT.—**Males of the glass frog *Hyalinobatrachium valerioi* engage in diurnal and nocturnal attendance of egg clutches. To study the effect of parental care on embryonic survivorship, we conducted a male removal experiment and measured embryonic survival rates on day 4, 8, and 12 after oviposition in attended and unattended clutches. Embryonic survivorship was significantly higher in the control group than in the male removal group but decreased with time in both groups. Arthropod predation accounted for most of the mortality in both groups. Desiccation of clutches only occurred in unattended clutches. We hypothesize that egg attendance in *H. valerioi* increases embryonic survivorship by deterring egg predators and preventing desiccation.**

Parental care, the investment into offspring after fertilization, occurs in 17 of 27 frog families but only in 10–15% of extant anuran species (McDiarmid, 1978; Crump, 1996; Lehtinen and Nussbaum, 2003). In anurans, parental care is usually associated with terrestrial modes of reproduction (Crump, 1995; Lehtinen and Nussbaum, 2003). Egg attendance is the most common form of anuran parental care (Crump, 1995; Lehtinen and Nussbaum, 2003). Eggs represent a particularly vulnerable life-history stage (Duellman, 1992). Therefore, any behavioral pattern reducing embryonic mortality has a strong potential to increase reproductive success and consequently fitness in the attending parent. Various functions of egg attendance have been proposed, namely defense of clutches against heterospecific predators or conspecifics (Kluge, 1981; Juncá, 1996; Burrowes, 2000), prevention of desiccation (Taigen et al., 1984; Townsend et al., 1984; Hayes, 1991), egg jostling to avoid developmental abnormalities (Salthe and Mecham, 1974; Simon, 1983), and prevention of fungal infections (Green, 1999).

Although little is known about the reproductive behavior of most glass frog species (family Centrolenidae), parental care in the form of egg attendance is a recognized diagnostic character of the genus *Hyalinobatrachium* (Savage, 2002; Cisneros-Heredia and McDiarmid, 2007). *Hyalinobatrachium valerioi* is a small frog (snout–vent length 23–26 mm) occurring from Costa Rica to Colombia (Savage, 2002). This species is unique in its continuous (i.e., 24 h/day) egg attendance: Males remain both day and night at their oviposition sites, on the underside of leaves overhanging streams, often attending multiple clutches simultaneously (Kubicki, 2007; Vockenhuber et al., 2008).

The objective of this study was to determine the adaptive significance of parental care in *H. valerioi* by studying the effect of male egg attendance on embryonic survivorship. We predicted mortality would be higher in clutches where the attending male is removed compared to control clutches. In addition, we recorded the causes of egg mortality in attended and unattended clutches to assess how the presence of males may confer survival advantages to their offspring.

MATERIALS AND METHODS

Fieldwork was performed on a daily basis between 6 September and 26 October 2007 in the Parque Nacional Piedras Blancas on the southern Pacific slope of Costa Rica. The vegetation is classified as tropical wet forest (Holdridge et al., 1971). We studied a population of the Reticulated Glass Frog *H. valerioi* along a 650-m long section of the stream Quebrada Negra, located near the Tropical Research Station La Gamba (08°42′46″N, 83°12′90″W, 70 m.a.s.l.) in the Esquinas Forest. The stream is bordered by secondary forest on one shore and by the field station's gardens with a thin forest buffer on the other. Average annual precipitation is 6,000 mm, with the highest amount of rainfall occurring in October. Mean daily temperature is 25.2°C (Weber et al., 2001).

Males were found during nightly surveys by locating their calling sites and visually scanning the vegetation. We marked calling and oviposition sites of males with colored flags tied to leaves adjacent to the leaf holding the egg clutches. To test the effect of male egg attendance on clutch survival, 15 attending males were removed from their clutch in the morning after oviposition. The clutches of 25 attending males constituted the control group. Removed males were released by a stream at approximately 1-km distance from our study site because a previous study had shown that male *H. valerioi* returned to their oviposition sites if released within 450-m distance (Karpfen, 2006). Males with oviposition sites low enough to be monitored accurately were included in the experiment and assigned randomly to the control or experimental group. All clutches in the control group had males present at least until the final egg count on day 12 or until clutches were (mostly) destroyed by predators. If males attended multiple clutches, we included in the control group only the first clutch that could be monitored during its entire period of development.

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Many males that attended clutches in the control group continued to accumulate additional clutches during the time of the experiment, whereas all clutches except one in the experimental group were single clutches because males were removed right after the deposition of the first clutch. We did not remove additional clutches in the control group to avoid interfering with the egg attendance of males. The presence of multiple clutches might increase the likelihood of being located by a predator via olfactory or visual cues. However, this should lead to an increased predation rate in the control group and would make it less likely to detect higher embryo survival and, therefore, parental care in the control group.

Known egg clutches were revisited daily to inspect their condition and check for the presence of predators. The original egg number of each clutch was determined on the day following oviposition (day 0). We wanted to monitor clutch survival throughout embryonic development to detect the time when most clutch mortality occurs. The number of remaining eggs was recorded on day 4, day 8, and day 12. These times were chosen in accordance with distinctive developmental stages: On average, the eyes become visible shortly after day 4 (Gosner stage 17); heart and blood can be observed on day 8 (Gosner stage 18/19) (Karpfen, 2006). The last egg count (day 12) occurred shortly before the onset of hatching, because embryonic development lasts 13.9 days on average (Karpfen, 2006). We used the number of embryos present during this late developmental stage rather than the hatching rate because of the difficulty of distinguishing between hatched and eaten clutches. To test for differences in the percentage of surviving embryos between the control and the experimental group on day 4, 8, and 12, we used a repeated-measures ANOVA.

We considered four categories of embryonic mortality: (1) arthropod predation; (2) desiccation; (3) unfertilized eggs/developmental failure; and (4) unknown causes. Arthropod predation was recognized using direct observation of predation events or characteristic damage patterns of the remains of clutches. Desiccated clutches were characterized by a greatly reduced volume of egg jelly and a yellowish appearance. Unfertilized eggs or eggs failing to develop normally could be distinguished after the first few days of embryonic development by noting their lack of development compared to successful eggs in the same clutch.

Results

Clutch attendance was exclusively performed by males. During the night, attending males commonly engaged in brooding behavior by placing the ventral part of the body and thighs in close contact with the clutch. Clutch size ranged from 22–44 eggs. Mean clutch size did not differ significantly between the control and the male removal group ($\bar{x} \pm 1$ SD; 30.44 $\pm$ 5.21 eggs for the control group ($N = 25$), 31 $\pm$ 4.57 eggs for the experimental group ($N = 15$; Mann-Whitney U-test; $Z = -0.77$, $P = 0.44$)).

Embryo survivorship decreased with time (repeated-measures ANOVA; time: $F_{3,36} = 44.3$, $P < 0.001$), and this effect was significantly higher in male-deprived clutches than in attended control clutches (repeated-measures ANOVA; time $\times$ treatment: $F_{3,36} = 4.6$, $P = 0.008$) (Fig. 1). The disparity between the control and the experimental group is particularly noticeable on day 8 (compare medians for day 8 in Fig. 1A).

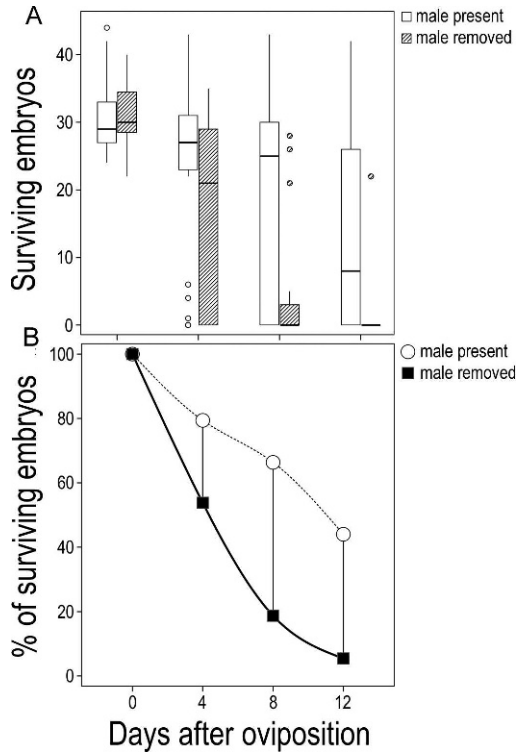

FIG. 1. (A) Decrease of embryo survival in arboreal clutches of *Hyalinobatrachium valerioi* as a function of time, and the presence or experimental removal of the guarding male; horizontal dark lines represent the median, boxes encompass the middle 50% of the data, and dots indicate outlier data points. (B) Profile plot obtained from the corresponding repeated-measures ANOVA on the percentage of surviving embryos. Dots represent averages for each corresponding category.

Arthropod predation was the dominant source of mortality (Fig. 2), causing 92.4% of total embryo mortality in control clutches and 81.7% in clutches where males had been removed. Unfertilized eggs or embryos failing to develop occurred in many clutches, but only a small number of individual eggs/embryos was affected. Desiccation as a source of mortality was exclusively found in unattended clutches. In two desiccated clutches, death of embryos was followed by fungal infection. Three unattended clutches that were destroyed by predation showed signs of desiccation beforehand.

Predation events were directly observed on six control clutches. Ants, copiphorid grasshoppers, and wasps (*Polybia* sp. and *Agelaia pallipes*) were each discovered feeding on two clutches. On one occasion, a wasp of the species *A. pallipes* approached an egg

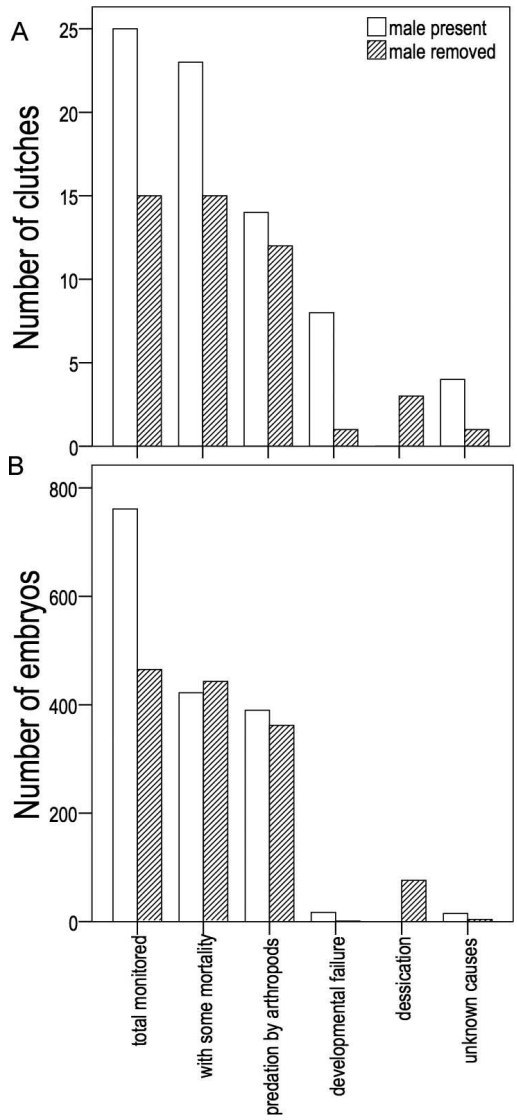

FIG. 2. The fate of experimental (male removed) and control (male present) clutches (A) and individual eggs (B) of *Hyalinobatrachium valerioi* according to mortality sources. As a reference, we first present the total number of monitored clutches and eggs, as well as the number that presented some kind of mortality. In the control group, one clutch did not hatch before the conclusion of the fieldwork. Three clutches in the control group and two clutches in the male removal group were affected by more than one type of mortality.

clutch but was deterred by the attending male through kicking with the forelimbs at the predator and positioning itself over the clutch. All predation events witnessed in the experimental group except one involved ants. A spider was observed preying upon an egg clutch that was afterward completely consumed by ants. Once, an opilionid acted as predator.

DISCUSSION

The results of our male removal experiment show that egg attendance in the glass frog *H. valerioi* enhances clutch survival to late prehatching stages and, therefore, can be considered as parental care. Previous studies experimentally confirmed the benefit of egg attendance because of increased offspring survivorship in various anuran species (Simon, 1983; Juncá, 1996; Burrowes, 2000; Bickford, 2004). Our study corroborates the findings of McDiarmid (1978), who attributed the higher survivorship of *H. valerioi* embryos in comparison to the sympatric *Hyalinobatrachium colymbiphyllum* to the continuous egg attendance in the former species. McDiarmid (1987) noted that the diurnal presence of males might deter predatory wasps.

Arthropod predation was the most important mortality factor for clutches with and without attending males. Comparable levels of insect predation have not been reported in glass frog populations thus far (compare McDiarmid, 1978; Jacobson, 1985; Hawley, 2006). Defense against egg predators is one possible function of egg attendance (Crump, 1995). In our study, arthropod predation on eggs was reduced in the presence of attending males. Similarly, clutch attendance led to lower predation rates in *Eleutherodactylus cooki* (Burrowes, 2000) and *Colostethus stepheni* (Juncá, 1996). Male *H. valerioi* occasionally engage in defensive behavior against wasps and ants by lunging or kicking at the predator (Vockenhuber et al., 2008). Although defensive behavior is infrequently observed, it appears to reduce arthropod predation. It seems likely that the small body size of males limits their effectiveness in deterring large predators such as wasps. However, the protection of egg masses against ants may be more feasible. The prevalence of ant predation on unattended eggs supports this idea.

Desiccation was a further cause of mortality in unattended clutches. However, only a few clutches were affected, contrary to comparable studies of tree-breeding frogs where the removal of the attending parent resulted in almost 100% clutch mortality caused by desiccation (H. *fleischmanni* in Hayes, 1991, *Oreophryne* sp. in Bickford, 2004). Attending males of *H. valerioi* engage in brooding behavior by bringing the ventral surface and thighs in close contact with the eggs. Brooding occurs more frequently on nights with lower humidity, and a hydrating function has been suggested (Hayes, 1991; Savage, 2002; Vockenhuber et al., 2008). Although predation is the prevalent mortality factor in our study population, desiccation does cause additional mortality in unattended clutches, and egg hydration can be considered as a function of parental care in *H. valerioi*.

The small number of embryos failing to develop agrees with similar findings on anuran embryonic mortality (Hayes, 1991; Lips, 2001; Hawley, 2006). Fungal infections only occurred in a few unattended clutches already affected by desiccation. The prevention of developmental abnormalities or fungal infections does not appear to be a function of egg attendance in *H. valerioi.*

Male reproductive success depends on the number of obtained matings (clutches), the number of eggs per clutch, and the number of surviving embryos (Townsend, 1989). Although parental care yields benefits to male fitness via a lowered embryonic mortality, costs may be incurred in the form of lost mating opportunities, higher risk of adult predation, or energetic costs resulting from reduced feeding (Townsend, 1986; Lehtinen, 2002). However, males of *H. valerioi*, as with males of other frog species, continue to call during egg attendance and often accumulate multiple clutches (Juncá, 1996; Burrowes, 2000; Vockenhuber et al., 2008). Consequently, lost mating opportunities appear to be minimal. An increased susceptibility to predation, for instance by bats, seems unlikely for attending males considering that males without clutches also engage in calling behavior and may move around more in the course of foraging for food. In *E. coqui*, males incur a small energetic cost by performing parental care on clutches caused by reduced food intake (Townsend, 1986). Further investigation might reveal a similar effect in *H. valerioi*.

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