

SHORT COMMUNICATION

Notes on paternal behavior in *Hyalinobatrachium* glassfrogs (Anura: Centrolenidae)

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Egg attendance is a widespread form of parental care in animals (Smiseth *et al.* 2012). Among amphibians, it has evolved many times and involves a diversity of parental behaviors that protect embryos from ecological and environmental risks (Wells 2007). Egg attendance is common in Neotropical glassfrogs (Centrolenidae), whereby parents hydrate and guard arboreal eggs over streams. A study by McDiarmid (1978) was the first to highlight the diversity of parental strategies in centrolenids, comparing the relative advantages of different forms of attendance between syntopic species of *Hyalinobatrachium* ('*Centrolenella*'). Recent research has further uncovered a diversity of parental behaviors in glassfrogs, and field experiments have identified the functional benefits of female-only and male-only egg attendance in 4

of 12 genera (Vockenhuber *et al.* 2009, Delia *et al.* 2013, 2017, Lehtinen *et al.* 2014, Bravo-Valencia and Delia 2016). Here we present field observations of undocumented paternal behaviors in *Hyalinobatrachium colymbiphyllum* (Taylor, 1949) and *Hyalinobatrachium fleischmanni* (Boettger, 1893), indicating that males are responsive to uncommon and complex problems for embryonic development.

We made field observations at two sites in Central America: during June–October 2010 near San Gabriel Mixtepec in Oaxaca, Mexico (*H. fleischmanni*), and during June–August in 2012 and 2013 in Parque Nacional Soberania, Colon, Panama (*H. colymbiphyllum* and *H. fleischmanni*).

Egg Brooding in H. colymbiphyllum

Egg brooding in glassfrogs involves a specific form of ventral-contact where the parent positions its body over the egg clutch. This behavior largely functions to hydrate embryos.

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Prior to brooding, parents appear to ‘collect’ dew forming on vegetation using their pelvic patch and deliver it to clutches using a bladder structure (presumably their urinary bladder; Delia *et al.* 2017).

We observed a male *H. colymbiphyllum* using a heterospecific egg clutch of *Teratohyla pulverata* (Peters, 1873) as a water-source to hydrate his embryos. During a dry period in July 2013, this male repeatedly visited a well-hydrated *T. pulverata* clutch in Gosner (1960) Stage ~20, that was being monitored for another experiment located approximately 26 cm away from leaf where his three clutches were developing. Once on top of the *T. pulverata* clutch, the male assumed an appressed position with his pelvic patch well seated in the egg jelly for extended periods (20–40 min; Figure 1A). After leaving the *T. pulverata* clutch, his water-filled bladder was evident through the skin in the side/groin region. The bladder appeared depleted after he brooded his clutches (see Figure 1 for the general sequence of hydration behavior). This male used the *T. pulverata* clutch as a water-source to hydrate his own eggs over multiple nights (at least seven), and often several times on some nights (up to three), until his clutches were hatching competent. This male also used the *T. pulverata* clutch as a call site on several dry evenings (Figure 1A)—in our experience, *Hyalinobatrachium* males often locate and call from patches of dew on drier evenings.

In addition to *T. pulverata*, many co-occurring species lay egg clutches that can take on and retain larger amounts of water compared to those of *Hyalinobatrachium* (e.g., *Cochranella*, *Espadarana*, *Nymphargus*, *Vitreorana*; Delia and Bravo-Valencia *unpublished data*). Species with these globular clutches require an initial bout of maternal brooding and subsequent rainfall to establish hydration levels to ensure embryo survival (Delia *et al.* 2017). In contrast, *Hyalinobatrachium* eggs require repeated bouts of brooding during embryonic development (Delia *et al.* 2013, Lehtinen *et al.* 2014).

Heterospecific clutches could be an effective source of arboreal water for *Hyalinobatrachium*, potentially facilitating embryo survival and vocal activity during low humidity. If so, there may be negative consequence if egg hydration is ‘parasitized’ by heterospecific parents (although we did not observe any obvious detrimental effects during this rare event).

Context-dependent Behaviors in H. fleischmanni

The first observation of context-dependent behavior was made in Mexico (2010) while tracking males to quantify nightly attendance frequencies over 8-hr sampling periods (for details see Delia *et al.* 2013). On the 27th of June, a male repeatedly brooded a single egg that had fallen from his clutch (Figure 2). During oviposition on June 26th, one of the eggs was stacked on top of another, and fell from the clutch shortly after deposition landing ca. 130 cm below on the upper surface of another leaf. At 22:28 h the following night the male was found sitting next to that egg and appeared to be looking at it (Figure 2A). After ca. one minute he moved towards the egg, contacted it with his snout and remained in that position for ca. two minutes, then moved on top of the egg (Figure 2B, C). He began brooding the egg, performing rotations and undulations interspersed by periods without movement. These movements would occasionally move the egg from under the male and he would then use his limbs to reposition it back under his venter (Figure 2D, E). This brooding bout lasted for 43 min and ended at 23:19 h. Following this observation, the male was seen brooding the main clutch three different times totaling 44 min (ranging from 12–17 min for each bout). In between each brooding bout, he moved to and sat in patches of dew on the leaf surfaces (presumably re-hydrating). This male was again observed brooding the single egg for 43 min (Stage 15/16; Gosner 1960) on the night of 28th of June. Over the eight hour sampling period the male brooded the main clutch only once for 15 min. The following day

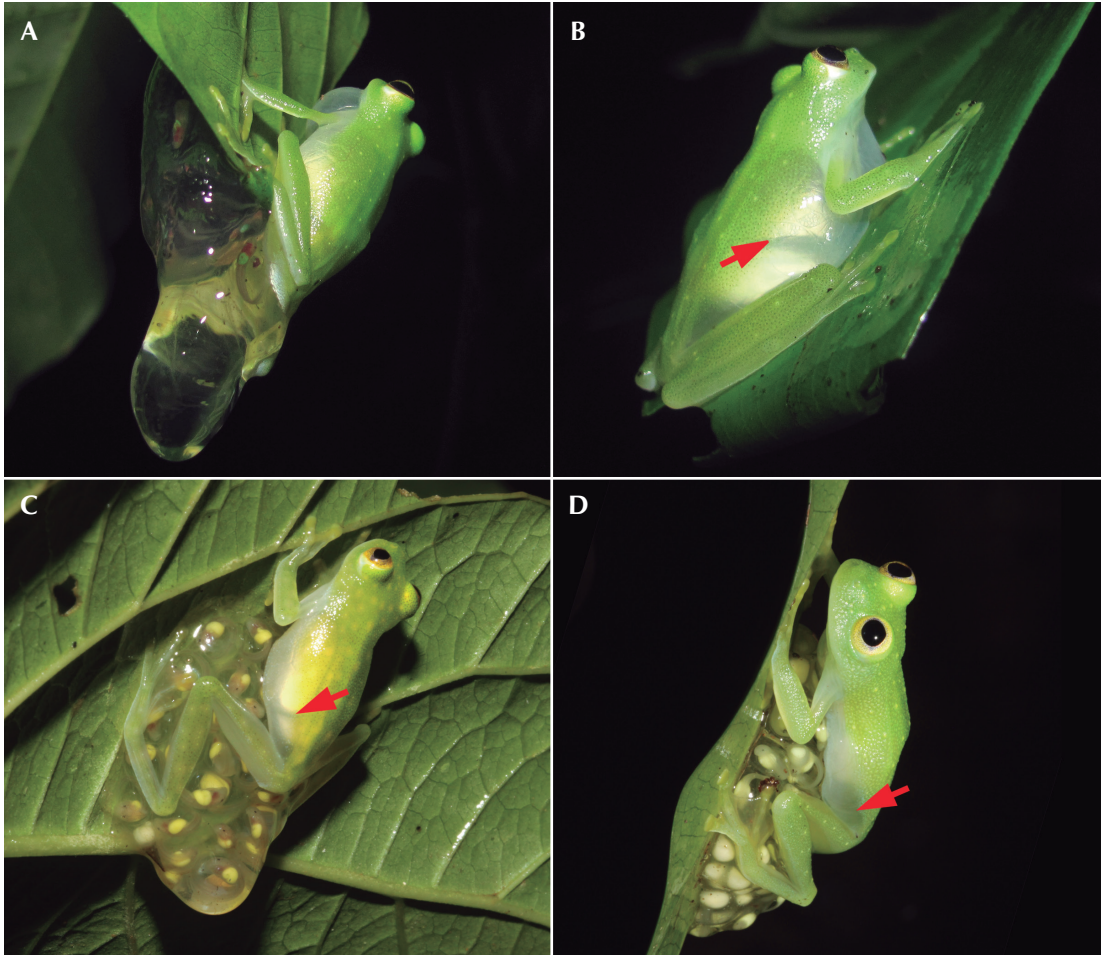


Figure 1. Egg hydration behavior in *Hyalinobatrachium colymbiphyllum* (A, B and D) and *H. fleischmanni* (C). (A) A male *H. colymbiphyllum* calling while sitting on a *Teratohyla pulverata* clutch during a dry evening. After sitting on the *T. pulverata* clutch, a water bladder was visible through the lateral-ventral surface of the male. (B–D) The sequence of hydration behaviors observed in both species: red arrows indicate the anterior edge of the water-filled bladder, which swells anteriorly while males sit in dew on leaves (B) and then depletes after brooding eggs (C, D). Note difference in hydration state of the bladder; *H. fleischmanni* is just beginning to brood in (C), whereas *H. colymbiphyllum* is terminating a brooding bout in (D).

a rainstorm washed the single egg from the leaf into the stream and ended our observation.

The second set of observations involves an undocumented attendance behavior for glassfrogs. In Panama on 7 August 2013 a monitored clutch (Stage 23–24) was found

partially consumed, presumably by an invertebrate. The predation resulted in six viable eggs and four capsule-less embryos scattered on the leaf around the main clutch, which now contained only six eggs. At 23:36 h the male moved onto the main clutch and began brooding.

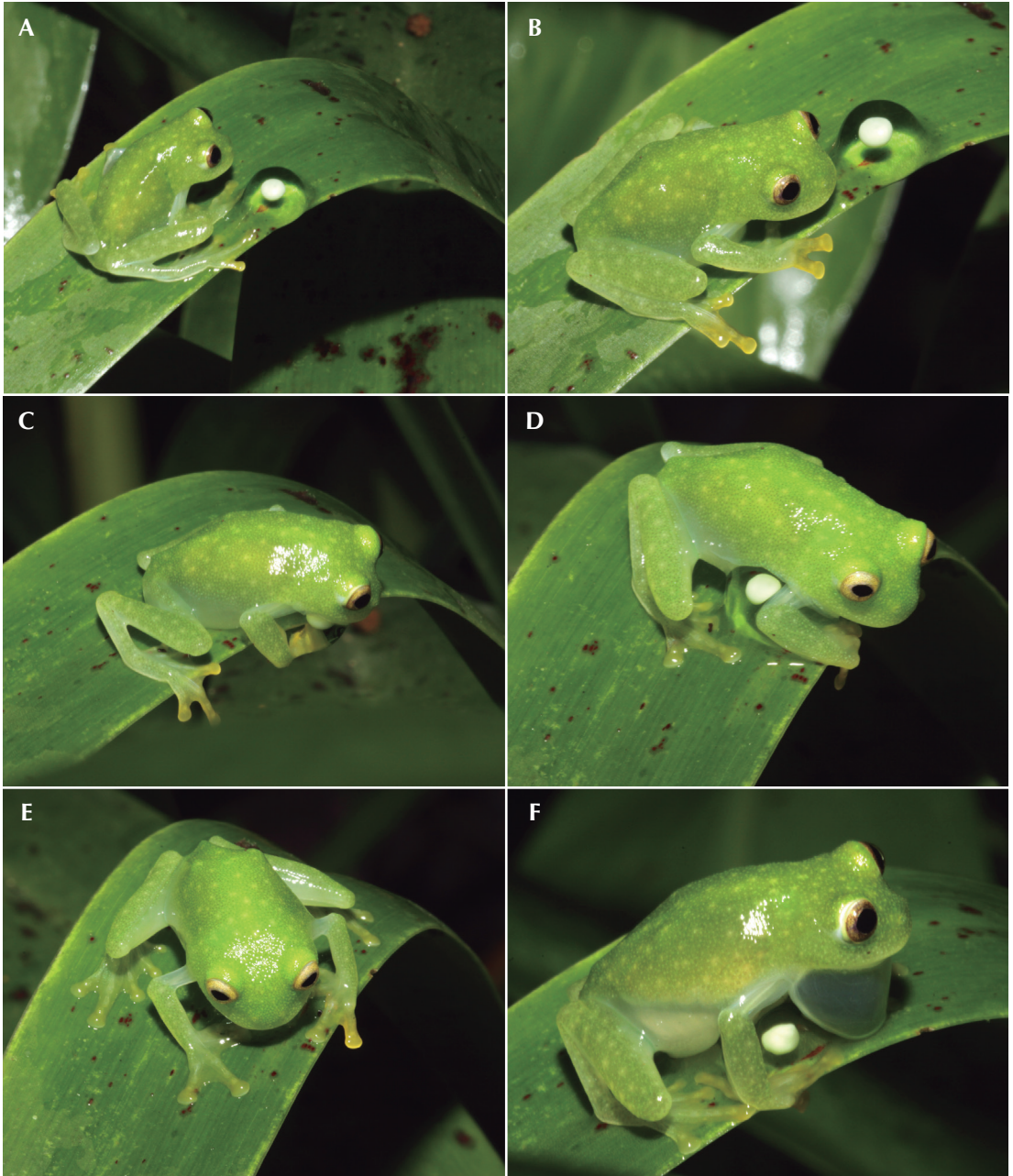


Figure 2. Sequence of a male *H. fleischmanni* brooding a single egg that fell from his clutch. (A) The male moved towards the egg, (B) contacted it with his snout, and (C) moved on top to brood it. (D, E) While brooding he would use his limbs to reposition the egg under his venter. (F) On the third night the male called while brooding the egg (Gosner Stage 16/17).

After brooding, he moved from the main clutch to visit each stray embryo. Upon encountering each egg/capsule-less embryo the male made physical contact with his snout (arching his back), then moved on top and began to brood (as indicated by rotations, undulations, and an evident moisture trace following this activity). During this time the male brooded embryos in capsules and those without them. After ca. 30 min the male moved off the oviposition-site leaf but returned after about one hour. He then proceeded to remove the capsule-less embryos from the oviposition leaf. He first moved next to a capsule-less embryo, pushed his snout up against it (with his back arched), and began to manipulate the embryo with his hand. Occasionally he would stop to press his snout up against the embryo. This was repeated several times until the male picked up the embryo in his mouth. He then began to paw at it with his hand, ultimately flinging the embryo from his mouth and off the leaf into the stream below. He performed this same sequence of behaviors on all four capsule-less embryos, twice using his mouth and hand, and the other times using only his hands. Stray embryos still in egg capsules and those in the main clutch were again brooded and hatched on their own over the following evenings. While it appeared that all the embryos were transported off the leaf, the height of the nest site made it difficult to determine whether some were eaten. Accordingly, this male was collected and euthanized the same night of the observation and prepared as a voucher for research at the site (CH 7891, Círculo Herpetológico de Panamá, Panama City). The following morning the male was dissected and his stomach contents examined. No embryos were found; only parts of unidentified invertebrates.

Male *H. fleischmanni* appear to regulate brooding behavior in response to weather and egg-hydration levels of individual clutches (Delia *et al.* 2013). The observations presented here indicate that males are attentive to individual embryo needs. This species often cares for

several clutches concurrently, which may be located on different leaves throughout their territory (pers. obs.). Villa (1984) noted that the vegetation structure of male territories changes considerably over short periods, due to their preferences for fast-growing herbaceous plants (e.g., Araceae, Heliconiaceae, Zingiberaceae) that frequently move during rainstorms and can fall into streams. This likely creates a dynamic environment, and could potential favor cognitive capacities to recall changing offspring locations. The fact that the Mexican male tended a 'lost' egg over several nights demonstrates that fathers are capable of discovering and recalling new locations of eggs. It would be interesting to test whether males recognize their individual offspring or simply provide care to any eggs in their territory—in the latter case male territoriality would be a component of both mating and parental effort. The observation of the Panamanian male further indicates that males modify their behavior in response to the conditions of individual embryos. It is also possible that they make parental decisions regarding life-stage requirements. Capsule-less embryos are at risk of dehydration on leaves, but they may lack the strength to wiggle off vegetation. This clutch was about 24 h from reaching hatching competence (Stage 25); assisting these capsule-less embryos to the stream could have been a rescue strategy to avoid dehydration (centrolenid embryos can successfully develop submerged in water; J. Delia and K. M. Warkentin, unpubl. data). Alternatively, this male may have removed capsule-less embryos due to infection risk that might jeopardize other viable eggs. We have conducted research on both species of *Hyalinobatrachium* over six field seasons at this site. However, we have never observed males removing eggs infected with mold nor did this male ingest his embryos (selective oophagy of infected eggs occurs in other families of amphibians, reviewed by Wells 2007).

Diurnal Attendance

McDiarmid (1978) described diel patterns of paternal care in *H. colymbiphyllum* and *H. valerioi* (Dunn, 1931). He noted that the former species exhibits strictly nocturnal attendance and retreats to secluded locations away from clutches to sleep during the day, while the latter usually attends clutches 24 hr a day. *H. fleischmanni* was also thought to exhibit strictly nocturnal attendance (e.g., Savage 2002). In Panama, however, we observed that most male *H. colymbiphyllum* sleep next to eggs and are sometimes active around clutches during daylight hours (Figure 3A). In Mexico, we observed male *H. fleischmanni* sometimes sleeping next to,

active around, and occasionally brooding clutches during the day (Figure 3B–D).

In summary, we document previously unknown paternal behaviors in *Hyalinobatrachium*. While our observations are anecdotal, they offer ‘proof of concept’ demonstrating that males can cope with unforeseen and uncommon problems for their embryos. Glassfrogs offer opportunities to test questions concerning cognitive capacities and proximate mechanisms that guide parental behavior.

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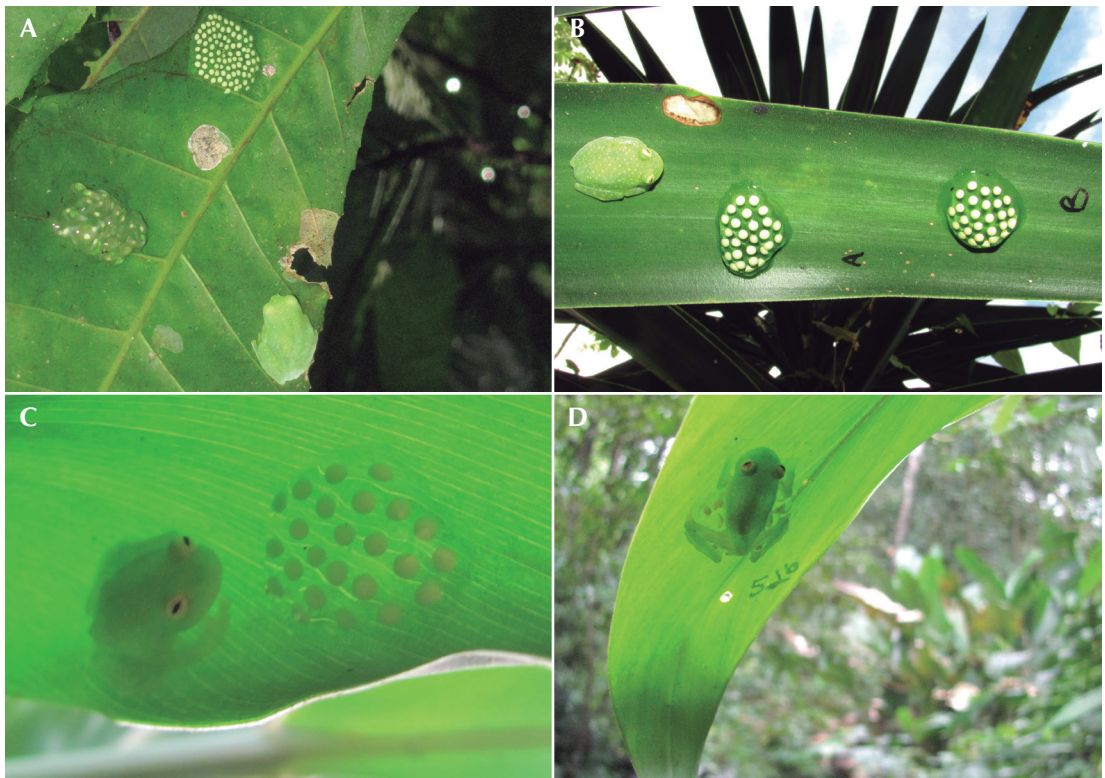


Figure 3. Diurnal attendance in *H. colymbiphyllum* (A) and *H. fleischmanni* (B–D). (A–C) Both species were thought to exhibit strictly nocturnal attendance; however, we found males frequently sleeping next to and active around egg clutches during the day. (D) Male *H. fleischmanni* from Mexico brooding a clutch at ~ 09:30 h.

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