OBSERVATIONS ON THE REPRODUCTIVE BEHAVIOR OF A NEOTROPICAL GLASSFROG, *HYALINOBATRACHIUM FLEISCHMANNI* (ANURA: CENTROLENIDAE)

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ABSTRACT. This study investigated geographic variation in a suite of reproductive behavioral traits in *Hyalinobatrachium fleischmanni* across several sites in Mexico and Ecuador. We made observations on the frequency of egg-mass brooding behavior and compared brooding encounter rates between wet and dry forest types. Brooding encounter rates were significantly higher in the drier region, suggesting a potential relationship between paternal care and climatic conditions. We also compared calling and oviposition sites between geographic regions and tested for associations with the distributional range of the frog-eating bat, *Trachops cirrhosus*. In regions where *H. fleischmanni* and *T. cirrhosus* co-occur, both calling males and egg masses were more frequently found on 'less exposed' lower surfaces of leaves, where risk of attack from aerial predators may be reduced. We also provide novel information on brooding behavior and clutch guarding, and discuss the implications of our results in the framework of existing information on the reproductive biology of this species.

KEYWORDS. parental care; geographic variation; behavioral plasticity; reproductive behavior; Amphibia; Centrolenidae; *Hyalinobatrachium fleischmanni; Trachops cirrhosus.*

INTRODUCTION

Throughout the humid Neotropics from southern Mexico to northwestern Ecuador, *Hyalinobatrachium fleischmanni* (Boettger, 1893) is a commonly-encountered arboreal anuran in many riparian habitats (McDiarmid, 1983; Villa, 1984; Hayes, 1991; Savage, 2002). Like other species of centrolenid frogs, *H. fleischmanni* frequents stream-side vegetation during their reproductive season, where territorial males can be observed calling for mates and caring for eggs deposited on leaves above streams (McDiarmid and Alder, 1974; Clark, 1981; Jacobson, 1983; Hayes, 1991). This species is noted for occurring in high densities during reproductive periods, reaching 1 male territory per 1-2 m of stream-side habitat in Central America (Greer and Wells, 1980; Villa, 1984; Jacobson, 1985; Hayes, 1991; Pounds *et al.,* 1997). These high densities, along with easily-observable reproductive behaviors, have led to several studies on the reproductive ecology of this species (see Villa, 1977, 1978, 1984, Greer and Wells, 1980; Clark, 1981; Wells and Schwartz, 1982; Jacobson, 1983, 1985; Hayes, 1991).

Parental care behavior is an interesting aspect of the Centrolenid reproductive biology. In *H. fleischmanni* parental care in the form of egg-mass brooding behavior predominantly functions to hydrate embryos exposed to moisture-limited conditions of arboreal microhabitat (Hayes, 1991). This behavior appears to be stimulated by temporal weather patterns, with a noted increase of care on dry evenings (Hayes, 1991; in *H. valerioi* see Vockenhuber *et al.,* 2009). Considering that parental care in this species should theoretically serve to maintain optimal conditions for developing embryos in a variable environment, it seems logical that the frequency of care would respond to environmental heterogeneity on a larger geographic scale. However, there is currently little information on this topic. Another interesting feature of this species' reproductive biology, as well as other species of *Hyalinobatrachium,* is the use of the undersides of leaves for calling and oviposition sites. As these locations may be low quality for sound propagation and embryonic survivorship (Wells and Schwartz, 1982; Hayes, 1991), it has been suggested that males may select such sites to avoid potential predation (Greer and Wells, 1980; Wells and Schwartz. 1982; Wells, 2007).

Herein we examine geographic variation of both parental care and site-use, considering environmental and predator-prey interactions. The unique

biogeography of montane southern Mexico, along with the distinct semi-isolated ranges of the study species, provides an interesting situation to compare behavior between adjacent sites that vary greatly in ecology. We analyzed data from several independent investigations in southern Mexico to compare: (1) brooding encounter rates between wet and dry forest types, and (2) calling and oviposition site use between populations outside and within the distributional range of the frog-eating bat, *Trachops cirrhosus*. We provide supporting observational data from additional sites in Ecuador, and contribute novel information on parental care function in this species. The extensive distributional range of *H. fleischmanni* has led many authors to suggest that it likely represents multiple species (Goin, 1964; Starrett and Savage, 1973; Kubicki, 2007), a hypothesis which has

received some support in recent phylogenetic studies (Guayasamin *et al.,* 2008; Castroviejo-Fisher *et al.,* 2009). Although our observations of variation may be attributed to multiple species, we use this opportunity to speculate on possible relationships of evolutionary interest.

MATERIAL AND METHODS

Fieldwork was carried out independently by each author in their region of study. All observations were made during visual encounter surveys (VES) along riverine transects (Heyer *et al.,* 1994) in several regions of Mexico and Ecuador. Depending on the investigator, objectives varied from site to site: Rene Murrieta-Galindo (RM) compared herpetofaunal
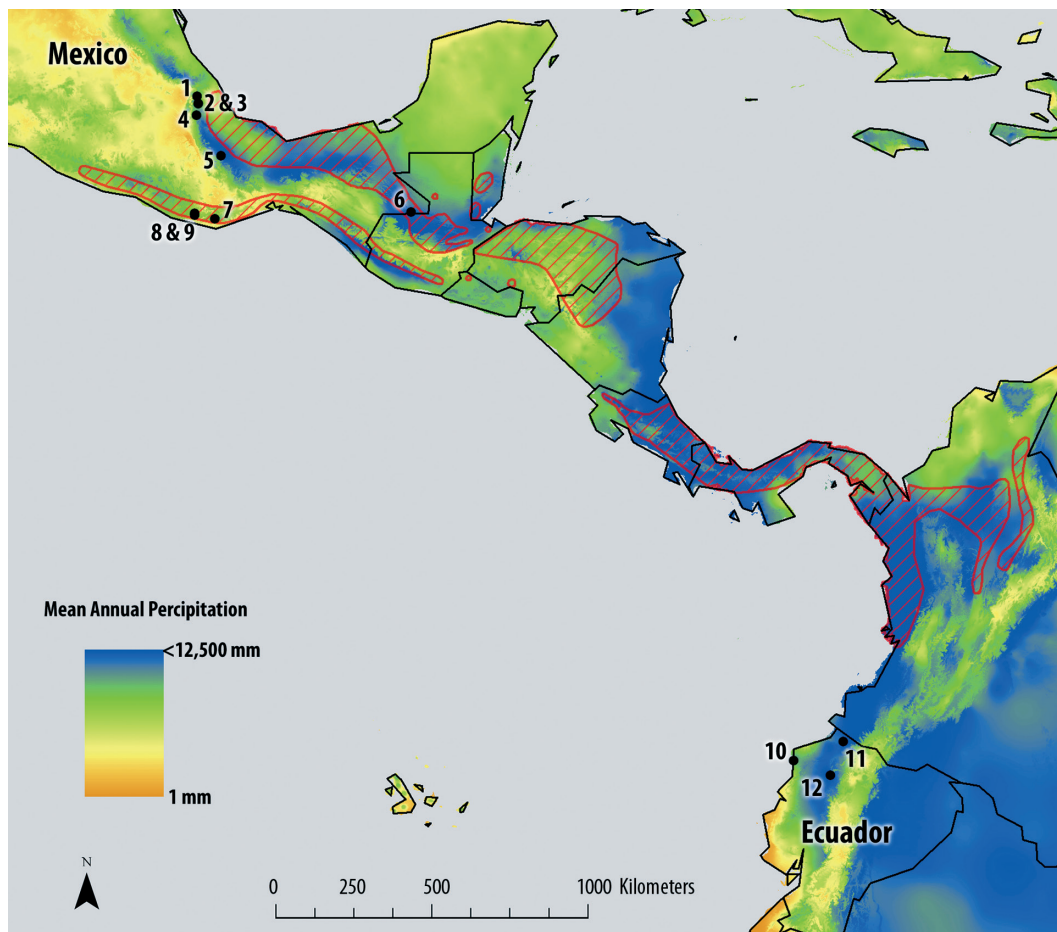

FIGURE 1. Map of the known range of *Hyalinobatrachium fleischmanni,* showing our study sites, and mean annual precipitation, which emphasize differences in forest type. Mean annual precipitation values were extracted from WORLDCLIM 1.4 database (Hijmans *et al.,* 2005; www.worldclim.org) and used to make the mean annual precipitation layer. The red polygons join known localities, based on specimens in collection, and indicate this species extent of occurrence (shapefiles downloaded from IUCN, 2009; www.iucnredlist.org). Black dots denote our sample localities that occur in: region 1 of Mexico (1) Xico, (2) and (3) Huatusco, (4) Cuautlapan, (5) Vista Hermosa, and (6) Chajul; region 2 of Mexico (7) Candelaria, (8) San Gabriel Mixtepec, and (9) Arroyo Camaron; and in Ecuador (10) Rio Mompiche, (11) Rio Bogotá, and (12) Hacienda La Joya.

TABLE 1. A list of study sites of *Hyalinobatrachium fleischmanni* and corresponding regions, with GPS coordinates, mean annual precipitation (extracted from the WORLDCLIM 1.4 database), and forest-type classification (according to Holdridge, 1967).

	Site	Geographic coordinates	Annual precipitation	Forest type
Mexico Region 1	1. Xico, Sierra Madre Oriental, Veracruz	19°25'22"N, 97°00'35"W; 1300 m	1901 mm	LWF
	2 and 3. Huatusco, Sierra Madre Oriental, Veracruz	19°13'02"N, 96°58'18"W; 1300 m	2004 mm	LWF
	4. Cuautlalpan, Sierra Madre Oriental, Veracruz	18°52'22"N, 97°01'23"W; 1100 m	2281 mm	LWF
	5. Vista Hermosa, Sierra de Juarez, Oaxaca	17°43'63"N, 96°19'57"W; 700 m	3535 mm	PWF
	6. Chajul, Reserva de la Biosfera Lacantun, Chiapas	16°07'00"N, 90°55'00"W; 548 m	3080 mm	TMF
Mexico Region 2	7. Candelaria Loxica, Sierra de Miahuatlan, Oaxaca	15°55'30"N, 96°28'50"W; 450 m	1291 mm	PMF
	8. San Gabriel Mixtepec, Sierra de Miahuatlan, Oaxaca	16°05'10"N, 97°04'46"W; 685 m	1435 mm	PMF
	9. Arroyo Camaron, Sierra de Miahuatlan, Oaxaca	16°02'16"N, 97°04'00"W; 554 m	1482 mm	PMF
Ecuador	10. Rio Mompiche, Esmeraldas	00°30'20"N, 80°01'22"W; 25 m	2010 mm	TMF
	11. Rio Bogota, Esmeraldas	01°03'N, 78°37'W; 300 m	3222 mm	TMF
	12. Hacienda La Joya, Pichincha	00°05'N, 78°59'W; 70-800 m	3047 mm	TMF

assemblages between undisturbed and altered forests (coffee plantations) at three sites in central Veracruz, Mexico (Fig. 1, localities 1-3), during June and July 2005, 2006, and 2008; Jesse Delia (JD) and Jonathan Whitney (JW) investigated the occurrence and natural history of montane stream-breeding frogs in Veracruz and Oaxaca, Mexico (Fig. 1, localities 4, 5, and 7), during June and July 2007; in July and August 2009, JD was investigating the behavioral ecology of this species at two streams near San Gabriel Mixtepec, Oaxaca, and Chajul, Chiapas, Mexico (Fig. 1, localities 6, 8, and 9); and Diego F. Cisneros-Heredia (DC) observed the species at three sites in western Ecuador (Fig. 1, localities 10-12), some of which he has visited since 1998. Geographic coordinates were obtained using GPS devices. Sites were classified according to criteria defined by Holdridge (1967) using bioclim data from WORLDCLIM 1.4 database (Hijmans *et al.,* 2005) extracted from GPS points in an ARC GIS environment, along with other published information (Stuart, 1966; Caldwell, 1974; Breedlove, 1981; Janzen, 1983; Lips *et al.,* 2004).

In southern Mexico, this species occurs in distinct semi-isolated ranges that correspond exclusively with the Gulf or Pacific slopes and lowlands of southern Mexico's principle mountain ranges. These distinct ranges are separated from each other by arid interior valleys and mountain ranges. All analyses consider this distributional pattern and we group study sites accordingly into two distinct regions in southern Mexico: 6 sites in region 1, which occurs along the Gulf lowlands, slopes of the northern escarpment of Oaxaca and Chiapas, and the Sierra Madre Oriental of Veracruz (Fig. 1, Tab. 1, localities 1-6). Sites in this region are primarily mildly seasonal broadleaf evergreen forests classified as Tropical Moist (TMF), Premontane Wet (PWF), and Lower Montane Wet Forest (LWF) (Stuart, 1966; Caldwell, 1974; Breedlove, 1981). Region 2 includes three sites along the Pacific slopes of the Sierra Madre del Sur of Oaxaca (Fig. 1, Tab. 1, localities 7-9). In this region, the forest is comparatively drier, strongly seasonal, 30% deciduous, and classified as Premontane Moist Forest (PMF) (Stuart, 1966; Caldwell, 1974). The region 1 sites occur within the distributional range of a frog-specialist bat, *Trachops cirrhosus;* whereas region 2 sites fall outside of its range, which terminates east/southeast of region 2 at the Isthmus de Tehuantepec (Emmons, 1997; Medellín *et al.,* 2008).

To develop preliminary insights on care intensity we recorded brooding encounter rates as the number of males encountered brooding along a transect. In each region, we calculated brooding encounter rates (no. of males found brooding / no. of males with

calling sites observed) and compared them between regions using a chi-square test of independence. To examine microhabitat use we recorded the location of egg deposition and calling sites, as on the upper or lower leaf surfaces, along transects. Any calling or oviposition sites on vertical surfaces where excluded from analyses. We used a chi-square test of independence to examine differences in the ratio of leaf surface use (lower: upper) for both oviposition and calling site locations between region 1 and region 2 study sites. Due to inconsistencies in personal objectives, we lack data for some parameters (specifically calling site location and brooding encounter rates) at certain sites. Considering that behavioral observations were opportunistic and infrequent, we present descriptive data on particular observations.

RESULTS

Egg Mass Tending Behavior

In Mexico we observed differences in encounter rates of brooding males between wet sites in region 1 (1.4-1.6) and dry sites in region 2 (1.7-1.8). In region 1, we observed a total of 36 males with calling sites (= territorial), of which only two were found brooding eggs. In region 2, we observed a total of 34 territorial males, of which 12 were found brooding eggs (Table 2). Brooding encounter rates in the drier region 2 (35.3%) were significantly higher than in region 1 (5.6%; $\chi^2 = 9.67$; df = 1; n = 70; p = 0.002).

Though comparative data are lacking, in Ecuador we observed a similar pattern. At Mompiche, the driest of our Ecuadorian sites, we occasionally monitored three egg masses over a period of two weeks. During this time, males were consistently encountered next to eggs throughout the course of the evening (observations terminated at 01:00) and on successive nights. In comparison, at Rio Bogotá, the wettest Ecuadorian site, we monitored two egg masses for one week in August 2000, four egg masses during eight days in January 2002, and two egg masses over two weeks in January 2004, and only occasionally found caring males (neither throughout the night nor on successive nights).

In addition to increased encounter rates of brooding males, in San Gabriel Mixtepec (region 2) we also noted a marked increase in parental care intensity. During the nights of June 16 and 17, 2009, we observed four males returning to brood eggs multiple times in a single evening. These males brooded a given egg mass three to five times during the same night. In

between brooding bouts males were observed moving through the vegetation, and appeared to be searching for wet spots on leaves. Once a wet spot was located, males would assume an adpressed position with their arms and legs slightly spread and their entire ventral surface pressed against the leaf. In several cases it was evident that males were absorbing moisture, as we could observe a noticeable decrease in the surface area of the wet spot. Males were then seen returning to the egg mass and again performing brooding behavior. This pattern was observed for males tending several egg masses, thus the vast majority of the evening was spent brooding, locating and absorbing moisture, and again returning to brood eggs.

Calling sites

We observed a total of 61 calling males among five sites in southern Mexico (region 1: n = 40; region 2: n = 21). Within region 1 (1.1-1.3, 1.6), calling males were encountered using the lower surface of leaves (77.5%; 31/40) more frequently than the upper surface (22.5%; 9/40); and this difference was found to be highly significant ($\chi^2 = 11.03$; df = 1; n = 40; p < 0.001). In region 2 (1.8-1.9), males called from both sides of leaves with more or less equal frequency: 52.4% (11/21) on lower surfaces and 47.6% (10/21) on upper surfaces ($\chi^2 = 0.00$; df = 1; n = 21; p = 1.0). Between regions, the ratio of calling sites on the upper leaf surface to lower leaf surface are significantly higher in region 2 ($\chi^2 = 4.05$; df = 1; n = 61; p = 0.044). This indicates that calling behavior in *H. fleischmanni* is altered in different geographic regions (Fig. 2). In addition, we noted temporal variation of calling site use at Rio Bogotá in Ecuador. At dusk, multiple male *H. fleischmanni* at this site were encountered calling from the upper surfaces of leaves, however, shortly after dark they retreated to the undersides and continued calling.

Egg Mass Locations

We observed a total of 88 egg masses among all of our sites in southern Mexico (region 1: n = 48; region 2: n = 40). We compared oviposition site use (upper vs. lower surface of leaves) between both regions (Fig. 3). In region 1, egg masses were almost exclusively located on the lower surfaces of leaves (97.9%; 47/48), with only one egg mass (2.1%; 1/48) located on the upper surface of a leaf. On the Pacific slope

TABLE 2. Encounter rates of egg mass brooding behavior of *Hyalinobatrachium fleischmanni* at various sites throughout two regions of southern Mexico. Encounter rates are described as proportion of all males observed that were found brooding egg masses.

Region	Site	Total number of male territories observed		Number of males encountered brooding		Brooding Encounter Rate	
1	Vista Hermosa	7		1		14.3%	
1	Cuautlapan	23		1		4.3%	
1	Chajul	6		0		0.0%	
2	Candelaria	10		3		30.0%	
2	San Gabriel	24		9		37.5%	
TOTALS	Region 1	36		2		5.6%	
	Region 2	34		12		35.3%	
MEANS	Region 1	12.0	± 9.5	0.67	± 0.58	6.2%	± 7.3%
(± sd)	Region 2	17.0	± 9.9	6.0	± 4.2	33.8%	± 5.3%
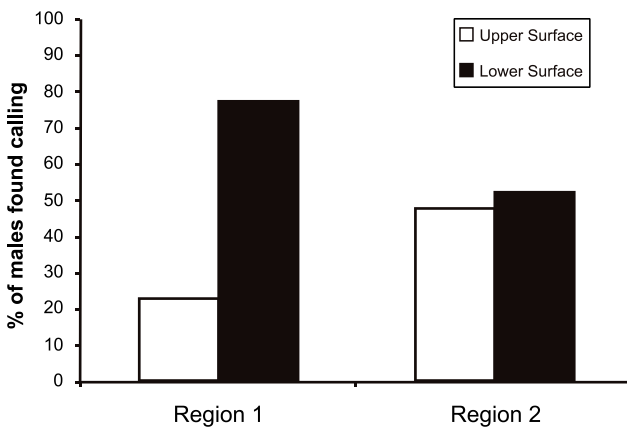
FIGURE 2. Comparisons of calling sites (upper vs. lower surfaces of leaves) of *Hyalinobatrachium fleischmanni* observed in two regions of Mexico. Data are presented as the percentage of males observed calling on upper versus lower surfaces of leaves in each region. Region 1 (n = 40) includes Chajul, Huatusco, and Xico, and is within the range of the frog-eating bat *Trachops cirrhosus* in TMF/LWF; Region 2 (n = 21) includes San Gabriel Mixtepec and Arroyo Camarón on the Pacific slope of Oaxaca in PMF and is outside the range of *T. cirrhosus.*
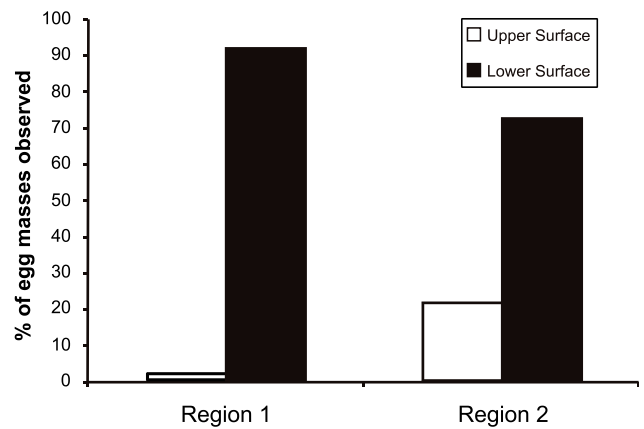

FIGURE 3. Comparisons of oviposition sites (upper vs. lower leaf surface) of *Hyalinobatrachium fleischmanni* egg masses observed in two regions of Mexico. Data are presented as the percentage of total egg masses observed per region. Region 1 (n = 48) includes 6 sites from the Gulf slopes of Chiapas, Oaxaca, and Veracruz (see Fig. 1, localities 1-6), and is within the range of the frog-eating bat, *Trachops cirrhosus*, in TMF/PWF/LWF; Region 2 (n = 40) includes 3 sites from the Pacific slope of Oaxaca (see Fig. 1, localities 7-9) and is outside the range of *T. cirrhosus* in PMF.

(region 2), oviposition sites were also more frequently located on the lower sides of leaves (77.5%; 31/40), however upper sides were used more often than in region 1 (22.5% vs. 2.1%). Differences in the frequency of upper leaf surface use were significantly higher in region 2 (χ^2 = 9.84; df = 1; n = 88; p < 0.002).

Egg Mass Defense Behavior

At Mompiche, two males were observed defending their eggs from predatory insects during nighttime surveys: a katydid and a predatory wasp. The first male was brooding an egg mass, while the second male was next to the eggs on the same leaf. During each encounter, when the insect predators came into contact with the jelly of the eggs, the male kicked the intruder with his hind legs knocking the insect off of the leaf. The egg mass attacked by the katydid was observed on subsequent days in a healthy state. However, the eggs attacked by the wasp were predated the following day, presumably by a wasp since the egg jellies were intact and only the developing embryos had been pulled out (see McDiarmid, 1978).

DISCUSSION

Egg-attendance behavior has been reported in numerous species from several centrolenid genera (*i.e.,*

Centrolene, Ikakogi, and members of the Hyalinobatrachinae), yet there is little published information confirming the benefit of this behavior to offspring survivorship. In some cases, it is possible that the presence of attending males may be nothing more than site fidelity, resulting from male occupancy of high-quality calling/oviposition sites. However, in other cases egg mass tending behavior has been directly examined, and is thought to serve two primary functions: (1) guarding behavior or active defense by the parent against possible egg-predators (Fig. 4D), and/or (2) egg-brooding behavior to hydrate eggs and alleviate desiccation-related mortality (Fig. 6).

To date, evidence demonstrating the beneficial function of parental care in this family exists for just three species (McDiarmid, 1978; Jacobson, 1985; Hayes, 1991; Vockenhuber *et al.,* 2008, 2009), with only two investigations utilizing a rigorous experimental approach (Hayes, 1991; Vockenhuber *et al.,* 2009). McDiarmid (1978) attributed higher offspring survivorship in *Hyalinobatrachium valerioi* compared to *H. colymbiphyllum* to increased tending behavior in the former, in which males spend 24 hrs next to egg masses compared to only nocturnal attendance in *H. colymbiphyllum.* Though experimental evidence was lacking, he speculated that guarding had a specific anti-predator function, because diurnal predators accounted for the higher rates of embryonic mortality in *H. colymbiphyllum.* Through comparing embryonic survivorship in a male removal experiment, Vockenhuber *et al.* (2009) addressed parental care function in *H. valerioi,* finding that guarding behavior enhances embryonic survivorship. Their results support McDiarmid (1978), as they found a significantly higher mortality rate in egg masses where the guarding male was removed and noted that arthropod predation accounted for the majority of mortality in this treatment. Active defense against embryonic predators has been reported in *H. colymbiphyllum* (Drake and Ranvestel, 2005), *H. valerioi* (Vockenhuber *et al.,* 2008), and *H. fleischmanni* (this work), thus guarding behavior appears widespread within the Hyalinobatrachinae.

Egg-brooding behavior in *Hyalinobatrachium* has been studied by Hayes (1991) in *H. fleischmanni* and by Vockenhuber *et al.* (2008, 2009) in *H. valerioi.* Hayes (1991) implemented an experimental approach and clearly demonstrated that ventral-contact-brooding behavior functions primarily in the hydration of the egg masses to limit desiccation-related embryonic mortality. Brooding behavior in *H. valerioi* also
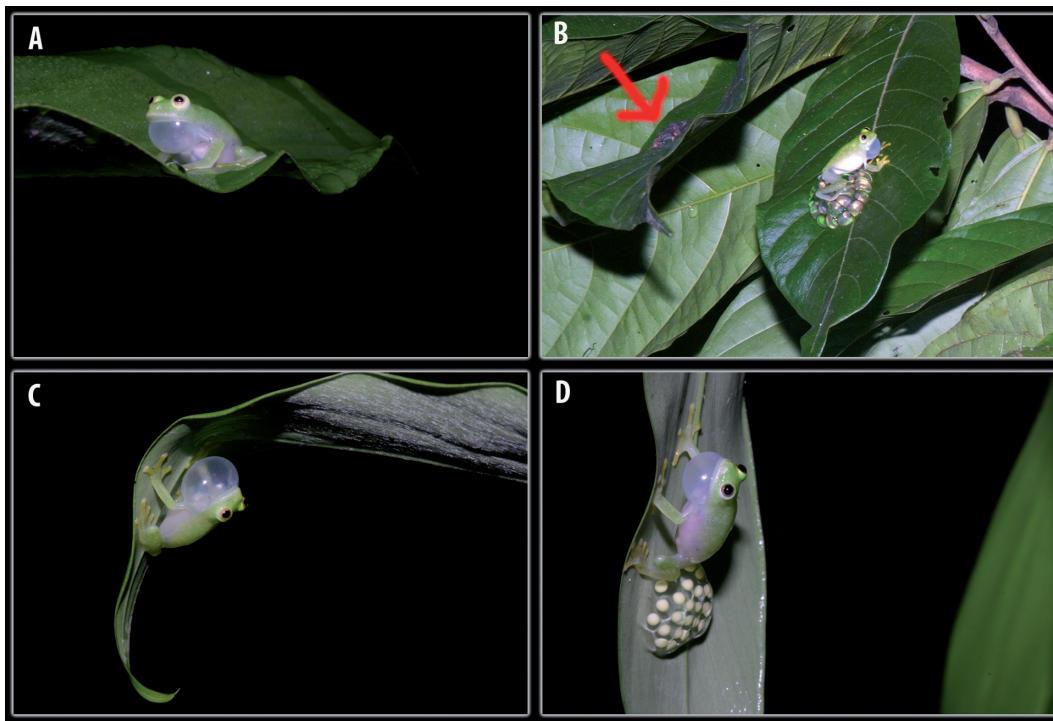

FIGURE 4. Examples of calling and oviposition sites of *Hyalinobatrachium fleischmanni:* upper (A and B) and lower (C and D) leaf surfaces from Candelaria (B) in 2007 and San Gabriel (A, C, and D) in 2009. Red arrow in (B) indicates the location of a second egg mass attached to the upper surface of a leaf just above a male calling while brooding. The male in (D) was often seen calling next to his egg throughout development, which may provide some guarding function.

appears to provide a hydrating function as embryonic-desiccation related mortalities were only observed in egg masses where the caring male was removed (Vockenhuber *et al.,* 2009).

In *H. fleischmanni,* males brood eggs immediately following oviposition (Fig. 7C) and during occasional nocturnal bouts throughout development (Clark, 1981; Jacobson, 1983, 1985; Hayes, 1991). Hayes (1991) concluded that this behavior is an infrequent form of parental care, with males brooding eggs only 12-20% of the nights throughout development. This degree of care appears intermediate in comparison to those species that, throughout embryonic development, guard eggs for up to 24 hours a day and perform brooding behavior (*e.g., H. valerioi*). Though guarding behavior does not seem as intense in *H. fleischmanni,* periods marked with frequent bouts of brooding are certainly energy demanding with males searching for and providing offspring with an important resource that maintains the embryonic environment. We observed males returning to brood the same

egg masses up to 5 times in a single evening. Between brooding bouts males where seen searching for and absorbing moisture, then returning to again brood the egg mass. This pattern was observed in multiple males, each of which was tending several egg masses. In these instances, males were spending the majority of the evening caring for offspring.

It is difficult to determine a relative degree of parental care without considering environmental relationships. As egg mass brooding appears to alleviate the risk of embryo desiccation, climatic differences between sites should affect the frequency of brooding behavior. Hayes (1991) demonstrated that weather can influence brooding activity, with a significant increase in brooding on drier and/or windier nights and during drier intervals. In addition, Vockenhuber *et al.* (2008) observed a higher rate of brooding behavior in *H. valerioi* during drier evenings. Our observations on variation of brooding-frequency in *H. fleischmanni* support Hayes (1991), and suggest a geographic pattern that could potentially be related to climatic
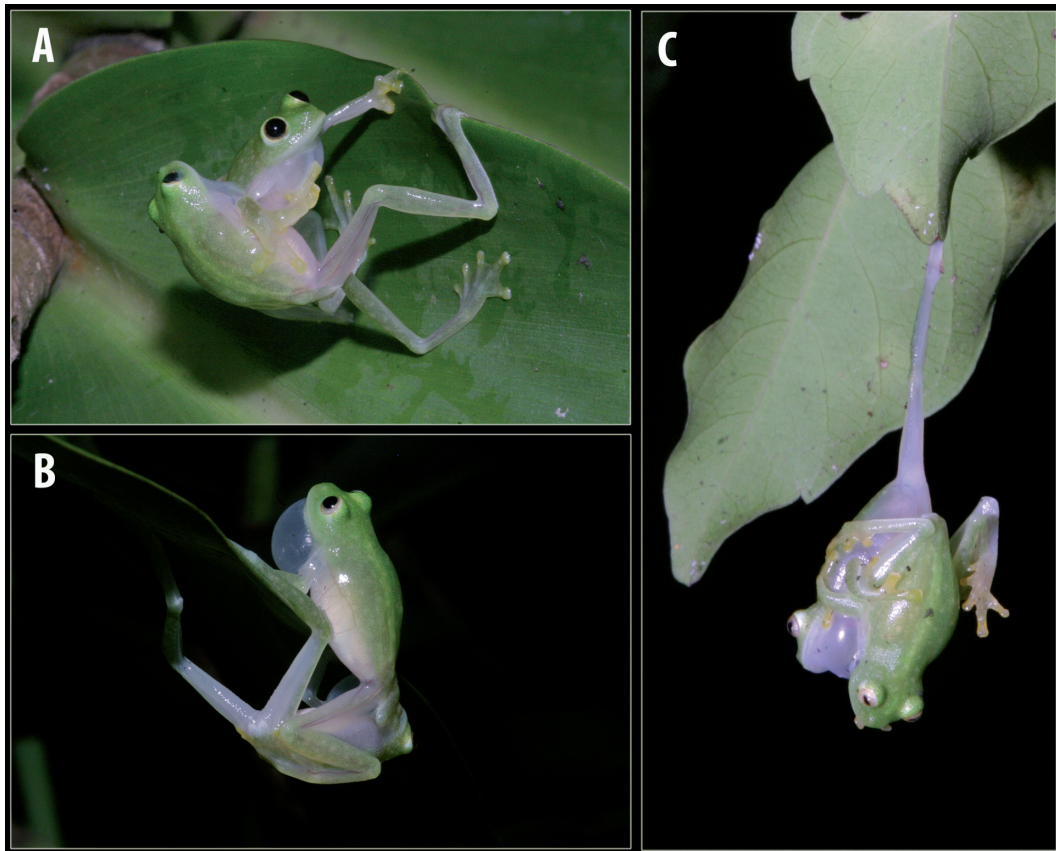

FIGURE 5. Male-male combat behavior in *Hyalinobatrachium fleischmanni* from San Gabriel in 2009, where males appeared to try and pin (A) and/or remove (B and C) the other male from the leaf. Note venter-to-venter combat in (C), which started with both males dangling upside down while holding vegetation with their hind limbs (see Bolivar *et al.,* 1999; Guayasamin and Barrio-Amoros, 2005) and lasting for ca. 20 min till one frog was knocked from the leaf.

regimes. Our preliminary results suggest increased brooding behavior in populations from comparatively drier forest types. However, our interpretations are limited to brief sample periods that did not consider current weather conditions nor the intense male-monitoring needed to determine if and when eggs are
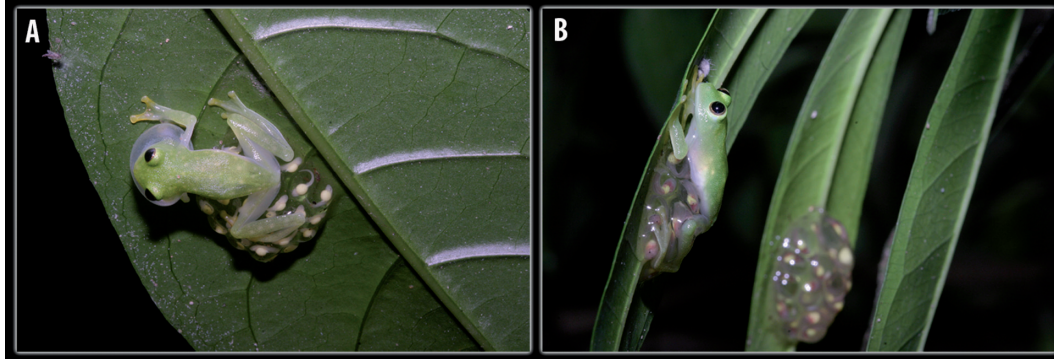

FIGURE 6. Brooding behavior (A and B) in male *Hyalinobatrachium fleischmanni* from San Gabriel Mixtepec in 2009.
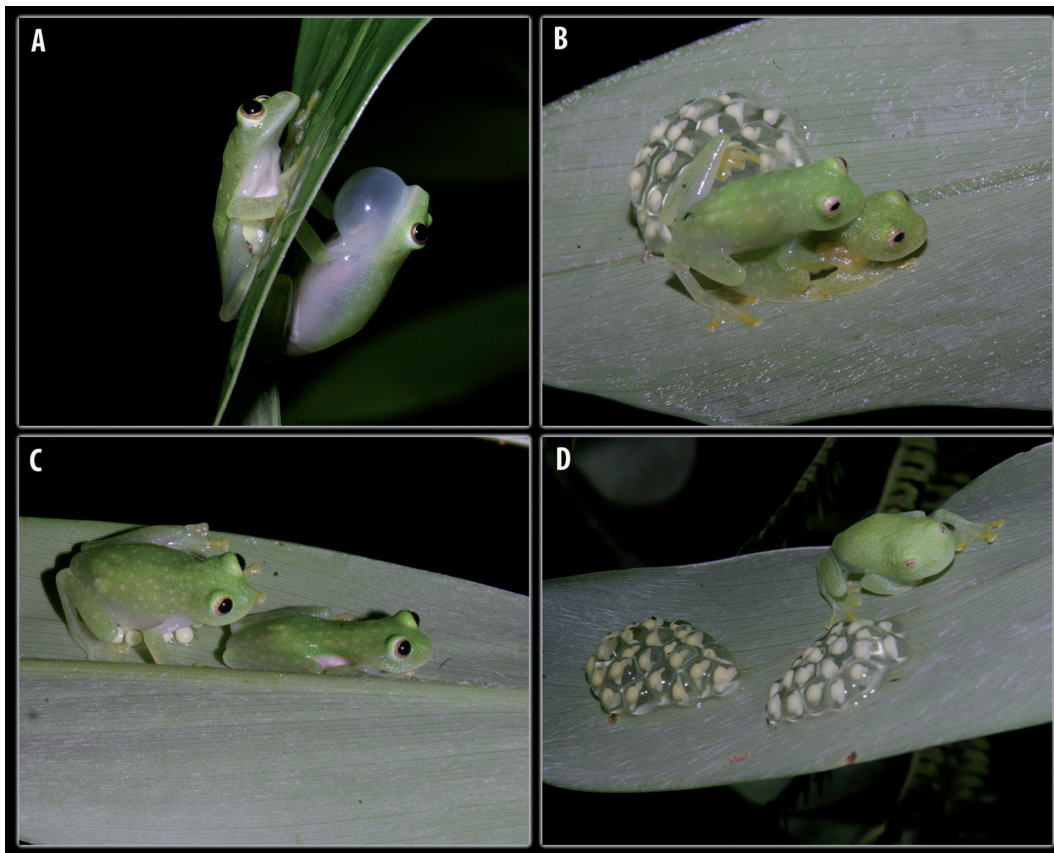

FIGURE 7. Examples of reproductive behavior in *Hyalinobatrachium fleischmanni* from San Gabriel in 2009: Male-female interactions (A), where females appear to "inspect" male territories (see Greer and Wells, 1980). A pair in amplexus (B), this male successfully attracted a female while brooding an egg mass, during which time the female initiated amplexus, removing the caring male from his eggs. Oviposition brooding behavior (C), where just following oviposition the female moves forward while the male remains on the eggs to perform an obligatory bout of brooding (see Hayes, 1991). And (D), a male sleeping next to eggs during the daytime at San Gabriel. Reports of diurnal bouts of care in *H. fleischmanni* are extremely rare and limited to heavy overcast days (specifically brooding behavior; see Hayes, 1991). Note that in this photo the male is sleeping and not in an alert position as described in *H. valerioi* by McDiarmid (1978) and Vockenhuber *et al.* (2008). Though an infrequent event during our studies, this behavior of sleeping next to clutches may have led to diurnal forms of guarding behavior we see in other *Hyalinobatrachium*.

brooded. Considering that Hayes (1991) documented a significant increase of brooding frequency at his drier site during the drier year, compared to the wetter site during the wetter year, it is likely that males would spend more time watering eggs if forest types had comparatively lower levels of humidity and rainfall. Detailed research is currently in progress to further evaluate this hypothesis.

Throughout its range, male *H. fleischmanni* predominantly call from the underside of leaves (Jacobson, 1985; Hayes, 1991; Kubicki, 2007). Results from study regions in Mexico (region 1) add support, as 77.5% of all calling sites observed were located on the undersides of leaves (Fig. 4C). The most frequently observed oviposition site is also on the undersides of leaves (Greer and Wells, 1980; Jacobson, 1983; Villa, 1984; Hayes, 1991; Kubicki, 2007). For example, during several years of intense fieldwork monitoring 409 egg masses, Hayes (1991) found that 99% were attached to the undersides of leaves. Egg mass location in populations from the Gulf side of southern Mexico in region 1 strongly support this trend, as 97.9% of observed egg masses were located on the underside of leaves (Fig. 4D). Such reproductive characters appear to be derived in certain lineages of centrolenids (Cisneros-Heredia and McDiarmid, 2007).

Considering mating success and offspring survivorship, undersides of leaves could be low-quality locations. Wells and Schwartz (1982) demonstrated that *H. fleischmanni* calling from the undersides of leaves may be subject to a broadcasting disadvantage, since vegetation increases attenuation and reflects much of the sound energy towards the ground. However, this disadvantage may be balanced by calling from high sites as Greer and Wells (1980) found significantly higher mating success for those males that called from higher sites. Hayes (1991) concluded that eggs located on lower surfaces are subject to significantly higher risk of desiccation-induced embryonic mortality when the caring parent is removed, because eggs are shielded from mist and rain. If males respond to hydration conditions of the egg mass, than it is possible that eggs deposited on the lower surfaces instigate brooding behavior due to their inability of directly acquiring environmental moisture.

Several authors have speculated that this species' bizarre habit of using the underside of leaves may serve as an anti-predator behavior, by concealing males during periods of pronounced vulnerability, such as when they are calling or attending eggs (Greer and Wells, 1980; Wells and Schwartz, 1982;

Wells, 2007). Tuttle and Ryan (1981) found that the bat *Trachops cirrhosus* is readily attracted to *H. fleischmanni* calls, and Hayes (1991) listed an unidentified bat as one of two of the most prominent predators of adult *H. fleischmanni* at his sites in Costa Rica. Wells and Schwartz (1982) consider that the presence of this frog predator has prompted the use of lower leaf surfaces, despite placing males at a signaling disadvantage. It is clear that *T. cirrhosus* is less attracted to reduced calling rates, call complexity, and call synchronization (*e.g.,* Tuttle and Ryan, 1981; Tuttle and Ryan, 1982). Although males calling from the undersides of leaves suffer a broadcasting disadvantage, it is possible that *H. fleischmanni* may be able to maintain optimum calling rates in the presence of hunting bats by using these concealed sites. Such adaptive strategies in signaling behavior may affect other reproductive traits. For example, guarding behavior functions to protect embryos from potential predators (McDiarmid, 1978; Drake and Ranvestel, 2005; Vockenhuber *et al.,* 2008; this work). Therefore, maintaining calling and oviposition sites in close proximity, on the same surfaces, would be advantageous for attracting mates while also caring for offspring. Yet, eggs located on the undersides are at a greater risk of desiccation, potentially requiring increased brooding behavior by males. In this theoretical situation, there is a tradeoff in maintaining calling rate by altering signal sites to avoid aerial predation, which may instigate increased parent care to combat the resulting increased risk of embryonic desiccation.

Comparisons of calling and oviposition sites from both populations inside and outside the range of *Trachops cirrhosus* suggest an interesting geographic association. *Trachops cirrhosus* is widely distributed throughout Central and South America and occurs in sympatry with *H. fleischmanni,* except along the Pacific versant of Guerrero and Oaxaca, Mexico, in our region 2 (Fig. 1, localities 7-9; Emmons, 1997; Medellín *et al.,* 2008). At sites within the bat's range, *H. fleischmanni* predominantly call from and almost exclusively deposit eggs on the undersides of leaves (Fig. 4C and D). However, outside of the range of *T. cirrhosus* (in region 2), we found that calling and oviposition sites were more frequently located on the upper leaf surfaces (Fig. 4A and B). Though there may be any number of factors contributing to site-use variation, it is possible that male *H. fleischmanni* take advantage of high-profile calling sites on the upper surfaces of leaves in regions lacking aerial predation pressures from *T. cirrhosus*. Further support comes from observations at Rio Bogotá (within

range of *T. cirrhosus*), where male *H. fleischmanni* were encountered calling from the upper leaf surfaces at dusk, but shortly after dark they moved to the undersides of leaves and resumed calling. It is possible that males are using the upper leaf surface for better broadcasting just prior to this predator's activity period, and subsequently move to safer sites during periods of hunting activity, or when visual predator detection by the frog may be more difficult. Eggs deposited on upper surfaces may be subjected to greater opportunities for environmental hydration, which would minimize the risk of embryonic desiccation, and may ultimately reduce the benefit of brooding. However, lack of pigmentation of the ovum may also put early developing embryos at risk of solar radiation (see Hayes, 1991), resulting in deposition sites on the inferior surfaces.

Though more detailed experiments are needed to test such interactions, these results illustrate how environmental factors may influence the frequency and function of parental care behaviors in centrolenid frogs. We encourage further study on the subject, as this group likely offers important information on behavioral ecology and evolution. With some initiative, data on several important reproductive parameters can be easily gathered during even brief visits to streams occupied by centrolenids. We refer the reader to Cisneros-Heredia and McDiarmid (2007) for important criteria needed on species' reproductive biology. In many species, parental care is easily observed, yet some species do exhibit different frequencies, kinds, and combinations of behaviors that may require more than a single evening of observation to detect. Any specific details of clutch attendance, such as the parent sitting on or touching eggs, are particularly relevant. Reporting the absence of parental care along with the amount of time spent making observations is extremely useful, as many authors tend to report only when care is detected. Only through such efforts will we gain some understanding of the ecological and behavioral diversity in this important and interesting group of stream breeding frogs.

RESUMEN

Investigamos la variación geográfica de varias características comportamentales reproductivas en *Hyalinobatrachium fleischmanni* en diferentes sitios en México y Ecuador. Realizamos observaciones sobre la frecuencia de cuidado parental directo sobre las masas de huevos y comparamos las tasas de este tipo de cuidado parental entre bosques húmedos y secos. Las tasas de cuidado parental directo fueron significativamente más altas en la región más seca, sugiriendo una potencial relación entre el cuidado parental y las condiciones climáticas. También comparamos los sitios de llamada y oviposición entre regiones geográficas y evaluamos su relación con el rango de distribución del murciélago verrugoso come-ranas, *Trachops cirrhosus*. En regiones donde *H. fleischmanni* y *T. cirrhosus* son simpátricas, tanto los machos cantores como las masas de huevos fueron más frecuentes en el envés de las hojas, donde el riesgo de ataque por un predador aéreo puede ser reducido. También presentamos nueva información sobre el comportamiento de cuidado parental directo sobre las masas de huevos, discutiendo las implicaciones de nuestros resultados en el marco de la información existente sobre la biología reproductiva de esta especie.

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