Reproductive behaviour of the glass frog

Hyalinobatrachium valerioi (Anura: Centrolenidae)

at the tropical stream Quebrada Negra

(La Gamba, Costa Rica)

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Abstract: The glass frog Hyalinobatrachium valerioi is commonly found along the stream Quebrada Negra close to the research station La Gamba. Males perform parental care by attending egg clutches 24 hours a day. In order to collect data on the reproductive behaviour of this little-known species, we marked oviposition sites and carried out daily and nightly checks on attending males. Most male H. valerioi successfully obtained at least one mating, and more than half of males attended multiple clutches. Arthropod predation was the main cause of clutch mortality. Males were observed to engage in ventral brooding behaviour of clutches. There was no relationship between height or leaf area of the oviposition site and the mating success of males. The number of males calling or mating successfully increased on nights with more rainfall, while the number of brooding males decreased. Leaves with a larger surface area were preferentially chosen as oviposition sites.

Key words: parental care, egg attendance, amphibians, Centrolenidae.

Introduction

The development of terrestrial reproductive modes constitutes a major trend in the evolution of anurans (Duellman 1992, McDiarmid 1978). Eggs are a particularly vulnerable life history stage; getting them out of the water can decrease mortality caused by habitat unpredictability or aquatic predators, which can be as high as 100% during the embryonic stage (Duellman 1992). The adoption of a more terrestrial lifestyle, however, also comes with its share of problems, such as desiccation of egg clutches or their exposure to terrestrial predators (McDiarmid 1978). This in turn creates selective pressure for the development of parental care, which Wells (1981) defined as non-gametic parental investment resulting in increased offspring survivorship. Parental care is most common among anurans with terrestrial or semi-terrestrial development (Lehtinen & Nussbaum 2003, Crump 1995). While it is a phylogenetically widespread phenomenon, parental care is also relatively rare, occurring only in 10-20% of anuran species (Lehtinen & Nussbaum 2003, McDiarmid 1978). Egg attendance, which describes the remaining of a parent with an egg mass at a fixed lo-
cation, is the most common form of anuran parental care. It is found among 14 families, including glass frogs (Crump 1995, Leitinen & Nussbaum 2003).

Members of the neotropically endemic family Centrolenidae, commonly known as glass frogs, are small, nocturnal frogs which typically deposit their egg clutches on leaves overhanging or near rivers and streams. Their common name refers to the semi-transparent nature of their skin and the transparent ventral surface of some species. In Costa Rica, 13 centrolenid species have been recorded (Kubicki 2007). Parental care occurs most notably in the genus Hyalinobatrachium, which predominantly includes species in which males exhibit egg attendance for various periods of time (Hayes 1991, Kubicki 2007, Savage 2002). After hatching, tadpoles drop into the stream below. Glass frog species commonly show territorial behaviour, including aggressive interactions among males (Greer & Wells 1980, McDiarmid & Adler 1974, Ryan 2006).

Several previous studies on centrolenid reproductive behaviour focused on the importance of clutch attendance on embryonic survivorship. Egg attendance in the common glass frog Hyalinobatrachium fleischmanni reduced embryonic mortality caused by desiccation, especially in early stages of larval development and during periods of low humidity (Hayes 1991). However, brooding behaviour, which consisted of close ventral contact with clutches, appeared to be linked to increased fungal growth and ensuing predation by drosophilid fly larvae. The infrequent occurrence of brooding observed by Hayes (1991) might be related to this phenomenon.

In a study comparing the reproductive behaviour of H. fleischmanni and Centrolenella prosoblepon at Monteverde, Costa Rica, no relationship between egg attendance and increased embryonic survivorship was observed (Jacobson 1985). Centrolenella prosoblepon showed no parental care, whereas males of H. fleischmanni were observed brooding eggs late in the evening when the chance of obtaining additional matings is low, which might minimise the cost of lost mating opportunities. Greer & Wells (1980), however, did not observe parental care in a H. fleischmanni population on Barro Colorado Island, Panama.

While some studies on centrolenids reported relatively high levels of embryonic survivorship (Greer & Wells 1980, Hawley 2006, McDiarmid 1978), others found low survival rates, mostly caused by predation (Jacobson 1985, Hayes 1991, Villa 1984). Drosophilid fly larvae were identified as important agents of clutch mortality (Hayes 1991, Villa 1977, 1984). In addition, grapsid crabs (Sesarma roberti), phalangids (Prionostemma frontale), gryllids (Paroecanthus tibialis), and hymenopterans (including Polybia sp.) were noted as predators of centrolenid eggs (Drake & Ranvestel 2005, Hayes 1983, McDiarmid 1978).

The influence of the height of oviposition sites on male mating success and clutch survival was the subject of several studies. Higher sites are selected more frequently by males of H. fleischmanni, and males at high sites receive new clutches at a faster rate (Greer & Wells 1980). No difference in embryonic survivorship was found between low and high sites (Greer & Wells 1980, Jacobson 1985). High calling sites on large leaves are best suited for sound propagation of calls in H. fleischmanni, but there is no evidence that males choose calling sites because of their acoustic properties (Wells & Schwartz 1982).

Levels of rainfall, or relative humidity, may influence the reproductive behaviour of glass frogs (Hawley 2006, Hayes 1991): The rate of brooding behaviour is higher in dry periods and a drastic drop in calling activity occurs on nights with less than 93% relative humidity (Hayes 1991). Conversely, Greer & Wells (1980) did not report a correlation between number of male H. fleischmanni calling each night and the amount of rainfall. No relationship was found between number of clutches deposited each night and rainfall in H. fleischmanni or C. prosoblepon (Jacobson 1985). Clutches starting to hatch in Hyalinobatrachium pulveratum and nocturnal rainfall are positively correlated, while maximum embryonic duration and mean daily rainfall showed a negative correlation (Hawley 2006).

Of all glass frog species recorded at the stream Quebrada Negra (La Gamba, Costa Rica), the reticulated glass frog Hyalinobatrachium valerioi is most commonly observed. Its reproductive behaviour is unusual: contrary to most other glass frogs, it remains with its egg clutches also during daylight hours, performing 24-hour egg attendance (Kubicki 2007). In a comparative study between H. valerioi and H. colymbiphyllum, McDiarmid (1978) argued that the higher embryonic survivorship in H. valerioi might be due to its diurnal attendance behaviour, which could be more effective in deterring egg predators than the solely nocturnal attendance of H. colymbiphyllum. Hayes (1991) and Cisneros-Heredia & McDiarmid (2007) report attending male H. valerioi sitting on egg clutches in a posture that Hayes (1991) views as suggestive of hydrative brooding. Male removal experiments performed with H. valerioi at Quebrada Negra revealed a significantly higher rate of embryo survival when the attending male was present (Vockenhuber, in prep.).

The scarcity of reproductive data available on H. valerioi in combination with the easily accessible popu-
lation of this glass frog species prompted our study. Our aims were to:

- collect descriptive data concerning the reproduction of *H. valerioi* and describe the species’ reproductive behaviour,
- document egg/embryo predation,
- test how the number of clutches attended by males influences the likelihood of receiving additional clutches,
- investigate the spatial distribution of oviposition/calling sites, preferred oviposition site characteristics and their influence on a male’s reproductive success,
- study the relationship between amount of rainfall and reproductive activities.

**Materials and methods**

We studied a population of the reticulated glass frog *Hyalinobatrachium valerioi* along the Quebrada Negra, a small stream situated just beyond the botanical garden of the tropical research station La Gamba. On the right shore, the stream is bordered by secondary forest, on the left side by gardens of the field station and the nearby Esquinas rainforest lodge with a thin forest buffer. Field work was performed by E. Köck from July 13th 1999 to November 26th 1999 (study period 1 = SP1), by U. Karpfen from July 8th 2002 to December 6th 2002 and from November 7th 2003 to January 13th 2004 (SP2 and SP3), and by E. Vockenhuber from August 1st 2007 to October 26th 2007 (SP4). Study sections of the stream (1999: 500m, 2002: 800m, 2003: 500m, 2007: 650m) were delineated with coloured, numbered flags tied to trees at 2m or 5m (SP4) intervals in order to allow mapping of oviposition and calling sites by estimating the distance to the nearest flag. The course of the stream was mapped using GPS (see Fig. 15).

We performed daily and nightly surveys of the population, except when high water levels after strong rainfall did not allow safe passage in the stream. During data collection, we walked in the middle of the stream in order not to disturb the vegetation. At night, males were found by locating their calls and scanning the vegetation visually, particularly the underside of leaves. On encountering a new male, we registered the position along the stream, number of attended clutches, eggs per clutch, developmental stage of clutches (after Gosner 1960), and its position inside or outside the stream bed. Plants used as oviposition site were identified to the species whenever possible. We identified males by calling activity and proximity to clutches, whereas approaching females could be distinguished by the presence of eggs in the oviducts visible through the skin (Fig. 1). Clutches located too high up to allow direct counting of eggs were digitally photographed and their egg number was determined on the computer image.

We marked each new calling and oviposition site with a coloured tag bearing an identification number. To avoid disturbing males or approaching females, the tag was placed on a leaf or branch adjacent to the leaf used as oviposition site. Attending males were not marked or measured as this might have modified their behaviour during the rest of the study (McCARTHY & PARRIS 2004, MAY 2004). Males showed high site fidelity. Thus marked oviposition sites usually allowed continuous controls of reproductively active males.

Known calling and oviposition sites were revisited every night between 7 pm and 3 am. The male’s presence at the oviposition site, behaviour, calling activity, position on the upper or lower side of the leaf and position in regard to the clutches (next to clutches, touching clutches, sitting on clutches) were noted, as well as the presence of females or predators. We avoided placing the beam of the head lamp directly on frogs to minimise altering their behaviour. If the vocal sac was not inflated and the male did not call during an observation time of at least 30 seconds, we regarded it as vocally inactive during that night. The condition of clutches was checked and any disturbance of egg jelly, missing embryos, discoloration or presence of mould was registered. In cases of direct contact with one or more clutches, we determined which clutch was touched by the male if multiple clutches were present. Nocturnal surveys took place during 368 out of 447 nights of all study periods (SP 1-4).

In the course of diurnal surveys, we collected data on the males’ presence, behaviour and position on the leaf. Additionally, the number of clutches, presence of females or predators and condition of all clutches were recorded. If new clutches had been laid during the night, we noted their position in regard to any previously present clutches and counted the number of eggs per clutch. Oviposition sites were monitored until all clutches hatched or disappeared and the male left.
We assumed larvae of a clutch to have hatched successfully when the outer capsule jelly was empty and did not show any signs of damage. A hatching rate of ≥ 70% was considered clutch survival (JACOBSON 1985). Certain clutches could not be clearly identified as hatched or eaten because there were no clear traces of predation, but the clutches appeared to be too young to have hatched already. Therefore, a minimum survival rate (clutches considered as eaten in case of doubt) and a maximum clutch survival rate (clutches considered as hatched) is given for SP 4.

We recorded instances of predation through direct observations or characteristic traces such as missing embryos not yet developed enough to hatch, damaged egg jelly and pieces of egg jelly strewn around.

To study total time budgets of reproductively active males, we conducted seven 24-hour observations of instantaneous sampling in 15 min intervals (ALTMAN 1974) on three males. Time from onset of amplexus until oviposition was measured for three mating couples.

A clutch removal experiment was performed from August 2nd to October 11th 2007 (SP 4) in order to determine the influence of attended clutches on a male's chance to receive a new clutch in the subsequent night. Males with oviposition sites at accessible heights were included in the experiment and assigned randomly to the control or experimental group. The control group received no treatment and all new clutches added during a 14 day period were recorded. The experimental group likewise received no treatment for the first set of seven days. During the second set of seven days, all clutches except one were removed every morning, so each frog would start into the night attending only a single clutch. In order to test for negative effect of not possessing any clutches, a third group was originally selected to lose all clutches every morning. This element, however, was abandoned as the first 5 individuals lost their oviposition sites after total clutch removal within 0 and 5 days (mean 1.2 days). We compared the average rate of clutch addition between the first and second week within both the control and experimental group using a Student’s t-test for paired samples.

Clutches were removed in the course of diurnal observations. We carefully disengaged clutches from the leaf using a blunt plastic knife and transferred them into Petri dishes lined with moistened filter paper discs. Attending males were never observed to leave oviposition sites during the careful removal procedure. Clutches were kept in the biological station until hatching. We put a lid on the Petri dish and occasionally moistened the filter paper to prevent desiccation. Hatched larvae were periodically released into a turbulent section of the stream to decrease the risk of immediate predation.

To determine whether males differentially used leaves as oviposition sites with reference to leaf area or surface texture, we measured these characteristics both in leaves holding egg clutches and in all leaves up to 3 m in height at 21 transects with 25 m intervals. Surface texture was recorded as smooth or rough. Leaf area was estimated as an ellipse based on the length of the leaf’s orthogonal axes in all cases except for leaves of Carludovica drupei, where individual sections of leaves were treated as triangles. We estimated length and width of leaves located at inaccessible heights by measuring comparable leaves at lower levels. To test whether sites for calling and oviposition are chosen according to the leaf area, we compared used and available leaves with a Mann-Whitney U-Test. We tested with a chi-squared test for preferences in surface texture. Height of oviposition sites was measured as well. Spearman’s rho correlation coefficients were calculated to test for relationships between height/area of oviposition site and total number of clutches/average number of new clutches received per night.

During the 2007 study period (SP 4), rainfall data was measured using a rain gauge located at the research...
station La Gamba. It was emptied daily at 7 am, revealing the amount of rain during the previous 24 hours. We used Spearman's rho correlation coefficients to examine the relationships between amount of rainfall and the nightly percentage of males that (1) receive a new clutch, (2) have ventral contact with a clutch and (3) are calling.

Descriptive statistics are presented as ± 1 SD. Unless otherwise noted, analyses are given for data from the study period 2007 (SP 4).

Results

Presence at oviposition sites and clutch attendance

Males, which exclusively attended the clutches, remained on average 13.9 ± 8.6 days at oviposition sites (range 1-37 days, n = 106). Time males spent at the calling site without obtaining a successful mating subsequently averaged 2.9 ± 2.0 days (range 1-9 days, n = 17). Males found before receiving clutches were observed at their future oviposition sites for a period of 3.4 ± 3.3 days (range 1-19, n = 58). Of all attending males, 53.4% remained with their clutches until all larvae hatched or were eaten, while 46.6% abandoned their clutches beforehand for a variety of reasons (n = 118) (Fig. 2).

Before definitely leaving the oviposition site, attendance levels were high: on average, males were present 96.5 ± 12.1% (n = 112) of days and 96.1 ± 10.7% (n = 119) of nights with their clutches.

Males performed brooding behaviour similar to ventral hydric brooding in H. fleischmanni (see Hayes 1991) on 29.7 ± 21.7% (n = 119) of nights. During bouts of brooding, males sat on clutches, typically with the ventral part of the body and thighs in close contact with the clutch (Fig. 3). The area of the leaf surrounding clutches frequently appeared moist. It could not be determined if males emptied their bladder over clutches. In 9.7 ± 17.9% (n = 119) of nights males touched clutches, but did not have ventral contact with them. When more than one clutch was present, males were most frequently observed brooding the youngest clutch (Fig. 4). Occasionally males were sitting on more than one clutch at the same time.

During the day, males were typically found resting next to their clutches, with forelimbs, hind limbs, or head touching the clutch in 50.8 ± 36.9% (n = 108) of days (Fig. 5). The oldest clutch was touched most commonly during the day (Fig. 4). Short distances between clutches occasionally led to simultaneous contact with up to three clutches.

Fig. 4: Mean percentage (± 1 SD) of nights/days male H. valerioi perform nocturnal brooding/diurnal contact with clutches of different ages. Males attending ≥2 clutches were analysed. n = 62 males (nocturnal brooding) and 64 males (diurnal touching). Quebrada Negra, La Gamba (Costa Rica), study period 4.

Fig. 5: Male Hyalinobatrachium valerioi assuming the diurnal resting position, with head and forelimb touching a clutch. Notice the different developmental stages and arrangement of clutches, with the most recent clutch in the highest position and the oldest, lowest clutch already hatched. Photo: E. Vockenhuber.

Fig. 6: Female Hyalinobatrachium valerioi (right) approaching a calling male (left). Photo: E. Vockenhuber.
Sequence of reproductive behaviour and clutch characteristics

Males generally emitted advertisement calls from the undersides of leaves (95.8%), seldom from the upper side (4.2%, n = 527 observations of calling males). The percentage of nights a male was recorded as vocally active amounted to 46.4 ± 29.3% (n = 140). Calling males were observed between 16:45 and 05:00. Seven continuous 24-hour observations of three males showed that the male frogs spent between 5 and 8 hours advertising and 13.5 to 19 hours resting (SP 2).

Females approached calling males (Fig. 6, Table 1), who responded to their presence by increasing the call frequency. Males initiated amplexus (Fig. 7), which lasted 5 h 45 min to 7 h 15 min (n = 3, SP 2). Amplectant couples frequently moved around on the leaf. Out of 164 recorded amplexi, 21 were observed on the upper side of leaves. Oviposition occurred between 23:40 and 02:45 (n = 6, SP 2 and 4). After egg deposition, the male fertilised the clutch and manipulated it with the hind legs, spreading it out into a single layer. The female then left the oviposition site, whereas the male performed slow rotational movements on the new clutch prior to calling again.

Out of 164 observed amplexi, 153 (93.3%) led to a successful oviposition. The greenish-white clutches were positioned on the underside of leaves. In the few cases where eggs were found on the upper side, the leaf was turned or folded in a way to allow the oviposition site more protection, or another leaf was covering the area used for oviposition. New clutches were placed on the leaf at a higher or equal level in relation to existing clutches in 612 (95.8%) out of 639 additions (SP 1-4), whereas 21 clutches (3.3%) were deposited at a lower level. Only 6 clutches (0.9%) were laid on a neighbouring leaf.

The mean number of eggs per clutch was 29.2 ± 5.0 eggs (n = 393) (Fig. 8). Tadpoles hatched at Gosner stage 24/25 (Table 2). The duration until hatching averaged 13.9 ± 2.6 days (range 9-19 days, n = 14, SP 2,3). Typically, the majority of larvae hatched at once, but a few larvae remained in the clutch for several more days.

On 5.5 ± 15.1% of days that a male was present at the oviposition/calling site, females were already found there during daytime (n = 125), both assuming a resting position (Fig. 9). When a female was present during the day, amplexus occurred in 87.3% of the subsequent nights (n = 55).

Male mating success

The majority of calling males obtained clutches (Fig. 10). Out of 128 successful individuals, 53 (73.3%) received multiple clutches. The total number of clutches acquired during the continuous presence of a single male at one oviposition site ranged from 1 to 9 clutches ( = 2.7 ± 1.6, n = 329; SP 1-4) (Fig. 11). Males simultaneously attended up to 7 unhatched clutches at various stages of development ( = 2.6 ± 1.3 clutches, n = 128) (Fig. 12). During the continuous presence at their oviposition sites, successful males acquired 0.19 ± 0.17 clutches per night (n = 121).

Clutch mortality and predation

Arthropod predation mainly led to low clutch survival (Table 3). We witnessed 63 predation events (Fig. 13). Two wasp species, *Agelais pallipes* and *Polybia* sp., were observed feeding on clutches during daytime (Fig. 13).
14 A-C), at least three different ant species (Fig. 14 D-E), including Camponotus atriceps and Paratrechina guatemalensis, during day and night time, and an unidentified earwig at night. Instances of grasshopper predation, often by copiphorids (Fig. 14 F), were observed both during day and night. Predators commonly remained at or returned to oviposition sites for several days, until all clutches were consumed. Parasitizing frogfly larvae as described by Villa (1977) were not observed.

Typically, attending males left the oviposition site when predation occurred. In six cases, however, we observed the attending male engaging in diurnal defensive behaviour (SP 4): three times against a wasp, twice against an ant, and once against an unidentified arthropod eight millimetres in size. The defensive behaviour consisted of movement towards the predator, kicking with fore- and hind limbs and positioning of the body above the clutches. Defence against ants and the unidentified arthropod was successful. Two of the three attacking wasps were deterred by the male, one, however, only after the nearly complete consumption of all clutches. In the course of our observations, 6 males released a sharp stream of urine when an observer came close to an oviposition site. Twice during wasp predation, embryos in the clutch under attack were observed to move around violently until they hatched.

Clutch removal experiment

Both in the control group, which received no treatment during both weeks of the experiment, and in the experimental group, where males lost all clutches but

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<td>1 – 3</td>
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Fig. 8: Number of eggs per clutch in H. valerioi. Mean ± 1 SD = 29.2 ± 5.0. n = 393 clutches. Min. = 12 eggs, max. = 44 eggs. Quebrada Negra, La Gamba (Costa Rica), study period 4.

Table 3: Rate of clutches hatching successfully (%) and causes of clutch mortality (%) in H. valerioi. Clutches with > 70% of larvae hatching are considered as hatching successfully. Minimum – maximum survival rate and minimum – maximum percentage of each mortality factor are given for study period 4. n = total number of clutches analysed for hatching rate and number of destroyed clutches (minimum and maximum number for study period 4) for mortality factors. Quebrada Negra, La Gamba (Costa Rica).

Fig. 9: Female Hyalinobatrachium valerioi (above) present at a male’s oviposition site in the daytime. Photo: E. Vockenhuber.

Fig. 10: Percentage of male H. valerioi receiving at least 1 clutch (cyan section of left circle). Right circle shows percentage of males with multiple clutches. n = 145 males. Quebrada Negra, La Gamba (Costa Rica), study period 4.
one every day during the second week, the average rate of clutch addition was lower in the second week than in the first week. However, the difference was only significant in the control group (Student’s t-test with paired samples, $t = 3.16, p = 0.006, n = 16$), but not in the experimental group (Student’s t-test with paired samples, $t = 1.75, p = 0.10, n = 14$).

Spatial distribution

The spatial distribution of oviposition sites along the stream was clumped (Fig. 15). Parts of the study area contained aggregations of reproductively active frogs, whereas in 24 (37%) out of 65 10m sections analysed no calling/oviposition sites were recorded. On average, three calling/oviposition sites were found in every section, with a peak of 27 sites between 100 and 110m. Average height of recorded oviposition sites was 268 ± 112cm (range 20-600cm, $n = 182$). Most oviposition sites were located within the streambed (90%, $n = 182$), many (80%) within the stream banks, positioned over water or gravel banks indicating the flow of water after strong rainfall (Fig. 16). Most frequently, clutches were attached to large lanceolate leaves with a smooth surface ending in a tip. *Heliconia* sp. and *Carludovica drudei* were the most common species used as oviposition sites (Fig. 17).

No relationships were found between total number of clutches/average number of clutches received per night and height of oviposition site (Spearman’s rho = -0.11/-0.02, $n = 142/129$, $p = 0.20/0.86$) or leaf area (Spearman’s rho = 0.08/0.12, $n = 141/129$, $p = 0.34/0.19$). A comparison of surface area between used leaves and all leaves counted at the 25 m transects revealed that leaves chosen as calling and oviposition sites by male *H. valerioi* had a significantly larger surface area (Mann-Whitney U-Test; $Z = 13.68, p <0.001$) (Fig. 18) and featured a smooth surface more frequently ($\chi^2 = 40.50, p <0.001$).

Effect of rainfall on reproductive activity

We found a negative correlation between the percentage of males performing nocturnal brooding and the amount of daily rainfall (Fig. 19), while the percentage of males calling and amount of rainfall were positively correlated (Fig. 20). The nightly percentage of males mating successfully and the amount of rainfall also showed a positive correlation (Fig. 21).

Discussion

The high level of diurnal and nocturnal egg attendance in males of *H. valerioi* corroborates findings of previous research on the parental care in this species. However, slightly more than half of the attending males
Fig. 14: Arthropod predation on eggs of *Hyalinobatrachium valerioi*. (a) The wasps *Polybia* sp. (left) and *Agelaia pallipes* (right), Photo: E. Vockenhuber; (b) *Agelaia pallipes* grasping an embryo, Photo: U. Karpfen; (c) 3 individuals of *Agelaia pallipes*, Photo: U. Karpfen; (d) *Camponotus atriceps*, Photo: U. Karpfen; (e) unidentified ants, Photo: E. Vockenhuber; (f) copiphorinid grasshopper, Photo: U. Karpfen.
Fig. 15: Spatial distribution of calling and oviposition sites of *H. valerioi* along the studied stream section. Stream colours correspond to the number of oviposition sites found in each of 65 10m-sections. A: Starting point of mapped study area (0m): Bridge on road from La Gamba to field station. B: End point of mapped study area (650m). Numbers along the stream indicate distance from starting point (m). Gardens include the grounds of the field station La Gamba and of the Esquinas Rainforest Lodge. Large arrow indicates flowing direction of the stream. Quebrada Negra, La Gamba (Costa Rica), study period 4.

Fig. 16: Location of oviposition sites of *H. valerioi* in relation to the stream bed profile and height above ground/water. *n* = 182 oviposition sites. Quebrada Negra, La Gamba (Costa Rica), study period 4.

Fig. 17: Plants most commonly used as oviposition sites by male *H. valerioi*. Ten plant species used only once are not included. *n* = 182 oviposition sites. Quebrada Negra, La Gamba (Costa Rica), study period 4.

Fig. 18: Surface area (cm²) of leaves used as calling and oviposition sites by males of *H. valerioi* compared with randomly sampled leaves. Leaves used as calling/oviposition sites: median = 590.7cm², *n* = 201, randomly sampled leaves: median = 106.8cm², *n* = 747. Difference between groups significant at a level of *p* <0.001 (Mann-Whitney U-Test; *Z* = 13.68). Quebrada Negra, La Gamba (Costa Rica), study period 4.

Fig. 19: Percentage of male *H. valerioi* performing nocturnal brooding behaviour as a function of daily rainfall (mm). *Rs* = Spearman’s correlation coefficient. Quebrada Negra, La Gamba (Costa Rica), study period 4.
abandoned their oviposition sites before all clutches hatched or were totally consumed by predators. Partial predation of egg masses, one of the main reasons for clutch abandonment, may induce males to leave. It is likely that predators return until all clutches are gone, making further presence of the male futile. Furthermore, some males may become victims of predation themselves, for instance by ctenid spiders (HAYES 1983) or bats (HAYES 1991). Males which leave one or two days before hatching of a clutch may have perceived that hatching was imminent.

Males performed nocturnal brooding by adopting a posture in which ventral surface and thighs were in close contact with the clutch. A hydrative function was attributed to this behaviour in *H. valerioi* and the closely related *H. fleischmanni* (HAYES 1991) and *Eleutherodactylus coqui* (TAIGEN et al. 1984). Moistening may be achieved by movement of water across the ventral surface of the brooding male and into the eggs at places of close physical contact, caused by a difference in water potential between eggs and the bodily fluids of the male (TAIGEN et al. 1984). The higher rate of brooding in drier conditions supports the hydrative function of the males’ position on top of the clutch (see also HAYES 1991). The rate of nights in which brooding occurred was 30% in comparison to only 12-20% in a similar study at Monteverde, Costa Rica (HAYES 1991). The increased brooding behaviour at Quebrada Negra may be linked to the apparent lack of clutch infestations by frog-fly larvae at our study site. Frog-fly larvae seem to create a higher amount of clutch mortality in *H. fleischmanni* when high brooding frequencies led to over-watering (HAYES 1991).

The percentage of males mating successfully and the percentage of males obtaining multiple clutches was high. The average number of clutches obtained by a male at one oviposition site closely resembles the value recorded by MCDIARMID (1978), but the percentage of males with at least one clutch was 21% higher. The presence of multiple clutches at oviposition sites indicate that males are able to attract additional females while already attending clutches. As a consequence, lost mating opportunities, which can be a cost of parental care (KLUGE 1981, TOWNSEND 1986), appear to be min-

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**Fig. 20:** Percentage of male *H. valerioi* calling at night as a function of daily rainfall (mm). Rs = Spearman’s correlation coefficient. Quebrada Negra, La Gamba (Costa Rica), study period 4.

**Fig. 21:** Percentage of male *H. valerioi* obtaining a new clutch as a function of daily rainfall (mm). Rs = Spearman’s correlation coefficient. Quebrada Negra, La Gamba (Costa Rica), study period 4.

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**Fig. 22:** (a) *Cochranella granulosa* (?), Photo: E. Vockenhuber; (b) *Cochranella albomaculata*, Photo: U. Karpfen; (c) *Hyalinobatrachium pulveratum*, Photo: E. Vockenhuber.
Fig. 23: Egg clutch of Cochranella granulosa (?). Photo: Walter Hödl.

ima. This is further supported by the fact that males continue to call while attending clutches. Although calling activity usually ceases during actual brooding of clutches, brooding most often occurs in nights with little or no preceding rainfall which leads to low levels of general calling activity. Similarly, daily rainfall can act as a predictor of nightly calling activity in H. fleischmanni (Hayes 1991), Hyla parviceps (Hödl & Amézquita 2004), and leaf-breeding members of neotropical anuran communities as a whole (Gottsbürg & Gruber 2004). In addition, the percentage of males receiving a new clutch increases with the amount of rainfall (cf. Fig. 21). As females orientate themselves towards calling males acoustically, the number of ovipositions increases with rising calling activity. Glass frogs easily reach lethal dehydration states due to lower humidity and increased wind associated with their arboreal habitat, which may be avoided by reduced activity levels in times of lower humidity (Kubičkí 2007).

Time of embryonic development varied between clutches and within clutches, similar to other glass frog species (Hawley 2006, Hayes 1991). Commonly, a small number of embryos remained in a clutch after the rest had already hatched. The variation in embryonic duration between clutches may be caused by climatic conditions, especially rainfall. Numbers of clutches initiating hatching and nocturnal rainfall show a positive correlation in H. pulzeratum, whereas maximum embryonic duration and mean daily rainfall are negatively correlated (Hawley 2006). It appears that clutches prolong the embryonic duration in order to enable hatching during heavy rainfall. Approximately 20% of clutches were deposited above the stream banks or outside the stream bed, where hatching larvae apparently reach the water when washed into the stream by heavy rain. Moreover, higher levels of turbidity and surface disruption in streams during strong rainfall decrease the probability that larvae dropping into the water will be immediately detected by predatory fish (McDiarmid 1983). In fact, we observed several instances of immediate fish predation when releasing tadpoles into calm sections of the stream.

Clutch survival was low (37% – 50%) in comparison to the 86% survival rate presented by McDiarmid (1978). Studies on other glass frog species reported lower (32%, H. fleischmanni, Jacobson 1985), similar (47%, C. prosoblepon, Jacobson 1985) or higher (61% – 71%, H. fleischmanni, Greer & Wells 1980) estimates of whole clutch survival. Arthropod predation was by far the most important cause of clutch mortality. Conversely, Hayes (1991) found very low levels of insect predation other than infection by frog-fly larvae in H. fleischmanni. In a study on H. pulzeratum, only 3% of clutches were affected by arthropod predation (Hawley 2006). McDiarmid (1978) asserted that the higher percentage of clutch predation in H. colymbiphyllum (44% in 1971, 26% in 1973) compared to that of H. valerioi (10% in 1971, 14% in 1973) was due to the diurnal egg attendance of H. valerioi. The “alert” day posture described by this author, with the attending male often touching the edge of a clutch, was adopted frequently by frogs in our study population, with males placing head or limbs on clutches on approximately half of the total days present. Male H. valerioi may adopt a quiet resting posture during the day, but move when ants or flies disturb the egg clutches (Cisneros-Heredia & McDiarmid 2007).

Defensive behaviour by male H. valerioi against egg predators was rarely observed. A male removal experiment to test the effect of egg attendance in H. valerioi on embryonic survival demonstrated a higher mortality due to predation in unattended clutches (Vockenhuber, in prep.). Thus, attendance of males seems to cause a decrease in arthropod predation. Egg mass defence performed by male H. colymbiphyllum against a wasp of the genus Polybia (Drake & Ranvestel 2005) shows great similarity to corresponding behaviour witnessed in H. valerioi. Another example of clutch defence against arthropods occurs in the microhylid Hylolophorus rufescens, which defends eggs by eating approaching ants (Bickford 2004). Hylnobatrachium valerioi, however, was not observed feeding on ants or other clutch predators during our study.

Wasps are important egg predators of leaf-breeding frogs (McDiarmid 1978, Warkentin 1995, 2000). Wasp-induced hatching of embryos as documented for Agalychnis callidryas (Warkentin 2000) may also occur in glass frogs. However, we could not determine if the two instances in which we saw embryos falling from clutches under wasp attack were caused by self-determined early hatching of the embryos or by the wasp rup-
turing the outer jelly of eggs. Glass frog embryos in late stages of development do hatch out in response to physical contact with the clutch or the leaf on which it is attached (KUBICKI 2007). Wasps of the genus Agelaia were observed to prey upon living, undamaged clutches and thus do not appear to be restricted to dead anuran eggs, as suggested in WARKENTIN (2000).

Aggressive interactions between males of H. valerioi were never observed in the course of our study, although McDIARMID & ADLER (1974) reported territorial encounters between males resulting in amplexus-like clasps and KUBICKI (2007) mentions changes in the calling pattern during male-male interactions, but no physical combat.

The results of the clutch-removal experiment suggest that males guarding more clutches do not obtain matings at a significantly higher rate. In the control group, the average rate of clutch addition decreased in the second week although most males attended more clutches during the second week than during the first. Possibly, a high number of clutches limits the calling activity in males. The average rate of clutch addition of the experimental group did not decline significantly in the second week, when males lost all clutches except one every day. Females do not appear to preferentially choose males attending a high number of clutches.

Male H. valerioi preferred smooth leaves with a surface area larger than that of generally available leaves as calling and oviposition sites. A smooth surface may be beneficial for attaching egg clutches to the leaf. Larger leaves improve advertisement call propagation in glass frogs (WELLS & SCHWARTZ 1982) and may offer more space for additional egg clutches. We did not find a relationship between the average clutch number obtained per night or the total number of clutches and the surface area of the leaf acting as oviposition site. The two measured parameters of male mating success were not correlated with the height of oviposition sites, either. However, GREER & WELLS (1980) discovered a positive relationship between the rate of obtaining matings and the height of oviposition sites in H. fleischmanni. Similarly, PROHL & HOHL (1999) reported a positive correlation between number of obtained matings and average perch height in Dendrobates pumilio. It is possible that other oviposition site characteristics, such as density of surrounding vegetation, are more important for the reproductive success in H. valerioi. Alternatively, certain qualities in males, for instance in regard to their calling behaviour, might make them more attractive to females (WELLS 2007).

Different species of the genus Heliconia were most commonly used as oviposition sites, followed by Carlucivia drudei (Cyclanthaceae) and the neophyte Edlingera elatior, which is native to South-east Asia. The genera Heliconia, Calathea, Anthurium, Philodendron and Syngonium, the first four of which were used at a high rate by male H. valerioi in our study, were also chosen most frequently as oviposition sites in a H. fleischmanni population studied in Matagalpa, Nicaragua (VILLA 1984).

Male H. valerioi are known to form groups ranging from 1-10 calling individuals along sections of their habitat (KUBICKI 2007). Calling and oviposition sites of H. valerioi were not evenly distributed along the studied sections of the stream, suggesting a spacing separation mechanism among the local glass frog species.

Addendum

Apart from the egg-attending Hyalinobatrachium valerioi, we recorded three glass frog species which do not perform parental care (SAVAGE 2002). During the nightly surveys along Quebrada Negra, Cochranella granulosa, Cochranella albomaculata and Hyalinobatrachium pulveratum were occasionally observed (Fig. 22). Out of these three species, males of C. granulosa were most frequently encountered. Their preferred calling sites are the upper side of leaves several metres above the stream, where they deposit brown-pigmented egg clutches at the tips of leaves (Fig. 23). Hyalinobatrachium pulveratum was frequently heard calling (three rapidly repeated “tik”-like notes) from positions higher than H. valerioi. As calling and egg laying in H. pulveratum occurs on the upper side of the leaves and up to 10 m in height (KUBICKI 2007), visual encounters were scarce. Cochranella albomaculata was infrequently encountered and calling and oviposition behaviour was not observed.

According to the distribution maps published in SAVAGE (2002) and KUBICKI (2007), H. colymbiphyllum and C. prosoblepon may also occur within the region.

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