Paternal Care and Egg Survivorship in a Low Nest-Attendance Rhacophorid Frog

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Wei-Chun Cheng and Yeong-Choy Kam (2010) Paternal care and egg survivorship in a low nest-attendance rhacophorid frog. *Zoological Studies* **49**(3): 304-310. We conducted a field study in Mar.-June 2007 to evaluate the importance of egg attendance by male *Kurixalus eiffingeri* to egg survivorship. We found 38 egg clutches and visited them on a fixed scheme by checking all nests at night for 6 d, yielding 1368 visits of 10 min each. Egg attendance frequency was highly uneven among nests and was positively correlated with clutch size. On average, 6.8% ± 4.4% of eggs in an egg clutch died each day. Daily egg mortality was inversely correlated with the attendance frequency, suggesting that paternal care is important to egg survival even though this is a low nest-attendance species. Variances of egg mortality were low when paternal care effort was high, but opposite results were found when paternal care effort was low, suggesting the fate of eggs can also be affected by factors other than paternal care, such as stump conditions. Our results suggest that parental care effort varies via a cost-benefit relationship of investment in the present offspring vs. future reproductive chances. Male frogs spent more time attending eggs when the clutch sizes were large but sought additional mating opportunities if the egg clutches were small, which resulted in large variations in egg mortality among clutches. http://zoolstud.sinica.edu.tw/Journals/49.3/304.pdf

Key words: Anuran, Nest attendance, Egg brooding, Paternal care, Rhacophoridae.

Parental care is any form of parental behavior that increases an offspring's chance of survival (Clutton-Brock 1991). To understand the evolution of parental care, it is important to understand how reproductive success varies as a function of parental care. Parental care effort varies as individuals trade off between investment in the present offspring and future reproductive potential, with confidence in paternity (Werren et al. 1980, Winkler 1987, Whittingham et al. 1992, Xia 1992, Westneat and Sherman 1993, Houston 1995, Mauck and Grubb 1995), and with different mating systems (Maynard Smith 1977).

The occurrence of parental care in anuran amphibians is more common than originally thought (McDiarmid 1978, Duellman and Trueb 1986, Crump 1995, Lehtinen and Nussbaum 2003).

The forms of parental care are diverse; and it was found in about 10% of extant species, including 15 of 21 extant families (Crump 1995, Lehtinen and Nussbaum 2003). In total, 7 modes of parental care were found, including egg attendance, egg transport, tadpole attendance, tadpole transport, tadpole feeding, internal gestation, and froglet transport (Wells 1981 2007, Crump 1995 1996, Bickford 2001 2002).

One of the most common modes of parental care is egg attendance, i.e., a parent remains with an egg mass at a fixed location (Wells 1981 2007, Crump 1995, Lehtinen 2003). Earlier studies showed that many terrestrial and arboreal frogs exhibit an array of attending behaviors which include egg guarding, turning, brooding, and even oophagy to remove infected eggs (Simon 1983,

Townsend et al. 1984, Jacobson 1985, Hayes 1991, Crump 1995).

The degree of nest attendance varies among species. For species with high nest attendance, egg guardians stay with egg clutches for long periods of time; e.g., male *Eleuterodactylus coqui*, an aerial breeder, stayed at the nest 97.4% and 75.8% of the day and night time, respectively (Townsend et al. 1984). Nests from which male frogs were removed showed significantly lower embryo hatching success (Woodruff 1977, Simon 1983, Townsend et al. 1984, Junca 1996, Bickford 2004, Vockenhuber et al. 2009). In contrast, for species with low nest attendance, egg guardians spend a small portion of time with eggs; e.g., *Centrolenella fleischmanni*, an aerial breeder, stayed at the nest 12%-20% of the night (Hayes 1991). Nests from which male frogs were removed showed no difference in embryonic survivorship compared to a control group (Hayes 1991). This finding raises a question of whether paternal care serves any function in enhancing the survivorship of eggs in low nest-attendance species.

In this study, we used a Taiwanese frog (*Kurixalus eiffingeri*, Anura, Rhacophoridae) that breeds in water-filled bamboo stumps as a model animal to assess the function of paternal care to eggs. *Kurixalus eiffingeri* is a small-sized frog endemic to Taiwan and 2 of the adjacent Japanese Ryukyu Is., Iriomote and Ishigaki (Kuramoto 1973, Lue 1990). This is a prolonged breeder, the breeding season of which extends from Feb. to Aug., and an amplectant pair deposits its fertilized eggs on the inner wall of a water-filled bamboo stump or tree hole above the water line (Kam et al. 1996). Male frogs exhibited paternal care during the embryonic period but spent about 27% of time attending eggs, and egg attendance varied greatly among egg clutches at 0%-100% (Chen et al. 2007). *Kurixalus eiffingeri* eggs are subject to fungal infections, desiccation, predation, and flooding (Kam et al. 1996, Chen et al. 2007). Chen et al. (2007) reported egg brooding behavior in a field study where males lay on the clutches when brooding and often changed positions. Males frequently descended into the water while brooding, stayed for several minutes, and then climbed out of the water to lie on the clutch. Results from field observations clearly indicated the presence of nest attendance behavior, but its importance to embryonic survivorship has yet to be evaluated.

The aims of this study were to (1) evaluate the importance of egg attendance by *K. eiffingeri*

to egg survivorship given that it is a low nest-attendance species, (2) correlate the attendance frequency with clutch size, (3) quantify egg mortality of egg clutches with low and high levels of paternal care, and (4) elucidate the possible mechanism(s) underlying the paternal care effort.

MATERIALS AND METHODS

Study site

We conducted field observations in 2 bamboo forests at Chitou (120°48'10"E, 23°39'20"N), central Taiwan. Chitou, at an elevation of about 1,016 m, receives approximately 3,000 mm of rainfall annually, approximately 90% of which occurs during the wet season from Feb. to Sept. when local thunderstorms and typhoons bring copious amounts of precipitation. The mean annual temperature at the site is 18°C. The highest monthly temperature occurs in Aug. (20°C) and the lowest in Jan. (9°C). The bamboo, *Phyllostachys edulis*, was the most abundant species in the study site. The bamboo is cut periodically for commercial purposes, and the stumps remain for several years before collapsing. Bamboo stumps collect rain water and become calling, oviposition, and tadpole development sites for *K. eiffingeri* (Kam et al. 1996).

Field protocol and observations

A field study was conducted in Mar.-June 2007. We conducted weekly night surveys of empty bamboo stumps with standing water to search for frogs and newly laid egg clutches. Once a newly laid egg clutch was found, we recorded the clutch size and developmental stage (Gosner 1960) of the embryos. Because the developmental time for eggs to hatch is about 9-14 d, our weekly survey allowed us to locate all newly laid egg clutches.

After an egg clutch was found, we visited nests on a fixed scheme by checking all nests at night for 6 d. First, we divided the embryonic period into early and late phases according to whether any embryo in a clutch had reached Gosner stage 18 (Gosner 1960). For each phase, we conducted behavioral observations of frogs and visited egg clutches for 3 consecutive nights. Each night survey was conducted from 18:00 to 06:00 the next morning at intervals of 2 h, and each observation session lasted 10 min. At the end of

the study, each nest had been visited 6 times a night for 6 nights for a total of 36 visits of 10 min each. During each visit, we conducted behavioral observations of male frogs and recorded the duration of time male frogs were present in the stumps or brooding eggs. We used a digital video camera recorder with infrared night-shot mode (DCR-PC101, Sony, Tokyo Japan) to conduct observations at night without using white light. Chen et al. (2007) reported that attending males of *K. eiffingeri* spent 1/3 of their time brooding eggs. In this study, we scored egg attendance if a male frog exhibited egg brooding behavior. If brooding behavior was not observed, we considered that a male frog attended eggs if it was always close to the egg mass (i.e., < 20 mm away) between and during visits even when disturbed.

Male frogs were captured, weighed, and marked with a numbered waistband for individual identification. We used a soft cotton string to tie the band around the waist of the frog just in front of its hind limbs. The string was tied tightly enough to prevent tag loss, but loosely enough so it would not irritate the skin. A small, waterproof, numbered paper tag was attached to the string which was used for the behavioral observations (Donnelly et al. 1994). The waistband was on the frogs for about 9 d and was removed at the end of the behavioral observations. Throughout the study period, male frogs did not seem to be affected by the waistband. We observed that marked frogs moved as actively as unmarked frogs, and they brooded eggs and chased off intruding unmarked male frogs. Adult male frogs were also identified by the presence of vocal sacs and/or nuptial pads (Chen et al. 2007). During the daytime, we monitored the number, development, and conditions of the eggs. Eggs were 2-3 mm in diameter; fertilized eggs were characterized by having an animal and a vegetal hemisphere, whereas unfertilized eggs did not have the animal and vegetal hemispheres, and the egg yolk appeared cloudy. Eggs swell to as much as 4 mm in diameter if they obtain sufficient external water but shrink if they lose water via evaporation (Kam et al. 1998). Embryonic development and conditions can be monitored with the naked eye, and embryos after Gosner stage 18 responded to gentle tapping on the bamboo stumps. We considered an embryo dead when the jelly capsule became discolored and/or no embryonic movement was observed. We recorded the causes of mortality for the eggs as fungal infection, flooding, desiccation, predation, or unknown cause

(Kam et al. 1996, Chen et al. 2007). We recorded the 2 most common causes of mortality if eggs in a nest died of more than 1 cause. We considered that eggs had died of a fungal infection when we could clearly see hyphae covering the eggs, flooding when the eggs were below the water line and the embryos were dead, desiccation when the eggs had shrunk and become dehydrated, and predation when the eggs had disappeared between visits during embryonic development.

Data and statistical analyses

Attendance frequency was calculated by dividing the number of visits in which male frogs were attending eggs by the total number of visits (i.e., 36 visits). The daily egg mortality (percent of dead eggs/d) was calculated as follows: first, we divided the total number of dead eggs by the total days of the study, then divided that by the total egg number and expressed this as a percentage. For example, if 36 eggs died in a span of 6 d from an egg clutch originally with 100 eggs, then the daily egg mortality was 6%.

We performed statistical analyses using SAS software (SAS Institute 1996). We used Spearman correlation coefficients to assess correlations of attendance frequency with clutch size and daily egg mortality. We used the Wilcoxon two-sample and Chi-squared tests to compare the daily egg mortality and causes of egg mortality between low and high parental-care groups, respectively. Normality of the variance of egg mortality was analyzed by the Shapiro-Wilk test. A *p* value of < 0.05 was considered statistically significant.

RESULTS

We found 38 egg clutches and conducted 1368 visits of 10 min each to nests during the study period. We excluded data from 5 nests because 2 egg clutches were laid a few days apart, forming 2 cohorts that coexisted in a stump, and the increase in egg number prevented us from properly tracking the fate of the eggs and calculating daily egg mortality. The average clutch size was 59 ± 25 eggs (*n* = 33; range, 35-115 eggs; median, 53 eggs). Attendance frequency was highly uneven among nests, averaging 22.9% (SD = 22.4 %; *n* = 33; range, 0%-83%; median, 8%), and 1/2 of the nests had attendance frequencies of ≤ 10% (Fig. 1). Attendance frequency was significantly correlated with clutch size (r_s = 0.43; *n* = 33;

p = 0.012; Fig. 2). All attending frogs were male frogs, and most of them exhibited brooding behavior where they sat on the egg clutch and maintained body contact with the eggs. They often changed their posture during brooding and moved their legs in and out of the egg mass. If disturbed, males descended into the water, but had come out again by the time of our next visit.

On average, 6.8% ± 4.4% of eggs in an egg clutch died per day (range, 0.6%-14.3%; medium, 5.3%). Causes of mortality included desiccation (n = 10), predation (n = 9), fungal infection (n = 6), flooding (n = 5), and unknown causes (n = 27). Most of the unknown causes of mortality involved the loss of egg pigment, with the eggs turning whitish.

Daily egg mortality was inversely correlated with attendance frequency (r_s = 0.496, n = 33, p = 0.0033) (Fig. 3). It was noted that the variance in egg mortality with no or low paternal care (i.e., eggs were being attended at < 10% of total visits) greatly differed from that with high paternal care (i.e., eggs were being attended at > 40% of total visits). For example, 17 egg clutches that received no or low paternal care showed variations in daily egg mortality and a bimodal distribution that significantly deviated from normality (Shapiro-Wilk test, W = 0.819, p = 0.004, Fig. 4). In contrast, 10 egg clutches that received high paternal care showed less variation in daily egg mortality and had a normal unimodal distribution (W = 0.890, p = 0.172, Fig. 4). The daily egg mortality of the low paternal-care group (8.28% ± 5.01%, n = 17)

was significantly higher that of the high paternal-care group (3.93% ± 1.64%, n = 10, Wilcoxon two-sample test, Z = 2.135, p = 0.033). Furthermore, we found that desiccation (n = 6), predation (n = 5), fungal infection (n = 4), and unknown causes (n = 13) were the main reasons for egg mortality in the low paternal-care group, whereas those in the high paternal-care group included desiccation (n = 3), predation (n = 3), fungal infection (n = 1), flooding (n = 3), and unknown causes (n = 7). The patterns of causes of egg mortality did not statistically differ between the 2 groups (Chi-square test, X^2 = 5.97, $d.f.$ = 4, p = 0.201).

DISCUSSION

Our results showed that egg mortality was inversely correlated with attendance frequency, suggesting that paternal care of *K. eiffingeri* is important to egg survival even though it is a low nest-attendance species. The functions of egg attendance were demonstrated in many anuran species and include protecting eggs from predation, pathogen (e.g., fungal) infections, and desiccation (Simon 1983, Taigen et al. 1984, Townsend et al. 1984, Hayes 1991, Junca 1996, Bourne 1998, Burrowes 2000, Bickford 2004). Chen et al. (2007) used an instantaneous scan sampling to study paternal care of *K. eiffingeri* and found no significant correlation between attendance frequency and hatching success of embryos. There are 2 possible explanations for
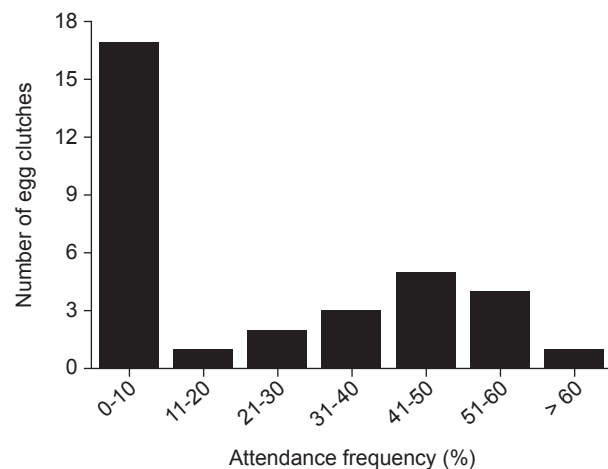

Fig. 1. Distribution of attendance frequency in *Kurixalus eiffingeri*. Attendance frequency (expressed as a percentage) of an egg clutch was calculated by dividing the number of visit in which eggs were being attended by the total number of visits (i.e., 36).
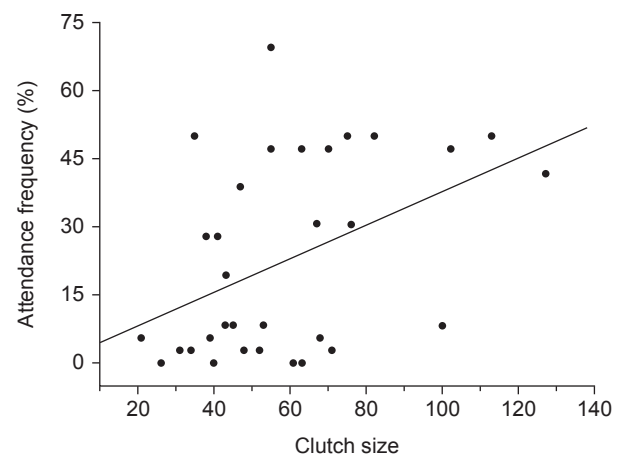
Fig. 2. Correlation between the attendance frequency (%) and clutch size. r_s = 0.43; n = 33; p = 0.012.

this discrepancy. First, nest attendance is time- and development-dependent (Chen et al. 2007), in that nest attendance frequency is higher during the nighttime and early developmental period compared to the daytime and late developmental period, respectively. Thus, more visits during the early development period and nighttime would overestimate the attendance frequency and vice versa. In this study, every nest was visited 6 times per night for 3 nights in each developmental period, but the number of visits by Chen et al. (2007) varied among time and developmental periods. In addition, we visited nests at night from 18:00 to 06:00 the next morning, but Chen et al. (2007) visited nests both during the day and at night with unequal numbers of samplings. We believe that the fixed scheme of nest visits in this study probably produced more consistent and reliable estimates of the attendance frequency compared to the instantaneous scan sampling observations employed by Chen et al. (2007).
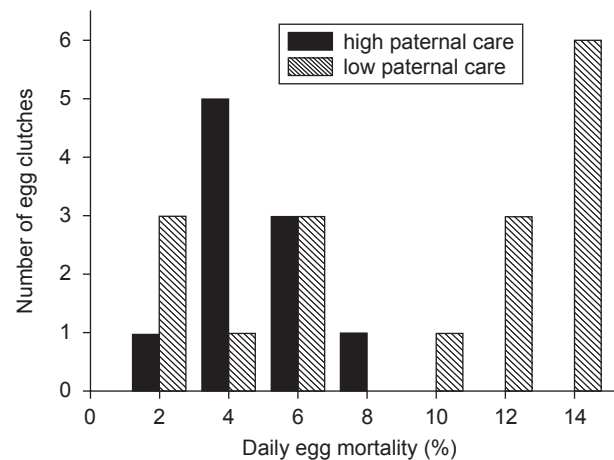
In the low paternal-care group, the variance of egg mortality was high among nests, i.e., some egg clutches suffered high mortality but others did not, suggesting that the fate of eggs can also be affected by factors other than paternal care. One of the most likely factors is the condition and quality of the bamboo stump (i.e., stump effects), which can best be illustrated by a scenario of egg desiccation. Bamboo stumps are unique and spatially isolated microhabitats which differ in height, diameter, cup depth, and water depth
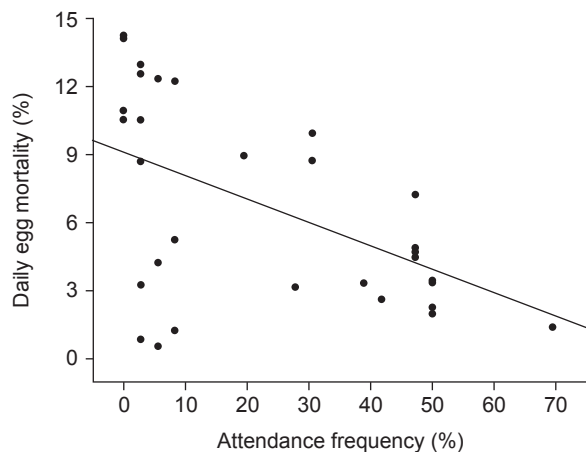
(Lin et al. 2008). In addition, the water level in a stump fluctuates through time, and the pattern and amount of water level fluctuations vary among stumps (Lin and Kam 2008). *Kurixalus eiffingeri* lays eggs on the inner wall of stumps above the water line. Like other amphibian eggs, *K. eiffingeri* eggs lose a significant amount of water via evaporation as a result of the high permeability of the jelly capsules to water vapor, and an external water source is needed for egg survival and development (Kam et al. 1998). Typically, *K. eiffingeri* eggs absorb water from the inner walls of the bamboo stumps, which are moist from water drawn up by capillary action from the water pool, forming a moisture gradient. Eggs that are near the water line will absorb more water and survive, whereas eggs that are laid far away will become dehydrated and die (Kam et al. 1998). However, eggs near the water line have a greater chance of being submerged and suffocating after a heavy rainfall (Kam et al. 1998, Lin and Kam 2008). We contend that *K. eiffingeri* eggs that are deposited in a stump with standing water probably have no immediate risk of desiccation. However, unpredictable rain patterns and varied stump conditions (i.e., water-holding capacity) during development dictate the availability of water to eggs, which could cause differential egg mortality between stumps which might explain the large variation in egg mortality.

In the high paternal-care group, the variance in egg mortality was low among nests, suggesting

Fig. 3. Correlation between the daily egg mortality (%) and attendance frequency (%). $r_s = 0.496$, $n = 33$, $p = 0.0033$. Daily egg mortality (percent of dead eggs/d) was calculated by dividing the total number of dead eggs by the total days of the study, then by the total egg number, and the result is expressed as a percentage.

Fig. 4. Distribution of daily egg mortality of egg clutches that received high (> 40 %, $n = 17$) and low attendance frequency (< 10 %, $n = 10$) levels of paternal care.

that eggs that are intensively attended by males survive better (i.e., paternal care effects). Desiccation is one of the major causes of egg mortality in *K. eiffingeri* (Kam et al. 1996, Chen et al. 2007). Chen et al. (2007) reported that attending male *K. eiffingeri* spent 1/3 of their time brooding eggs. Brooding behavior was demonstrated to serve the purpose of preventing egg desiccation, either through osmosis or through decreasing the exposed surface area of a clutch (Wells 1981, Forester 1984, Taigen et al. 1984, Townsend et al. 1984, Bickford 2004). Egg attendance by male frogs is likely to reduce the chance of eggs being eaten by small insects such as ants, earwigs, and aphids (Kam et al. 1996, Chen et al. 2007). Earlier studies on a salamander *Desmognathus* sp. in the eastern US suggested that attending males may remove infected eggs and/or move or jostle eggs to prevent fungal infection (Tilley 1972, Forester 1979).

If intense paternal care reduces egg mortality, why do *K. eiffingeri* not attend eggs more often? It is believed that the parental care effort varies via a cost-benefit relationship between investment in present offspring vs. future reproductive chances (Woodruff 1977, Townsend et al. 1984, Burrowes 2000). Our results suggest that male frogs spend more time attending eggs when the clutch size is large probably because the benefits of the present offspring outweigh the costs of losing additional mating opportunities. Consequently, intense egg attendance increases overall egg survivorship. In contrast, a male may prefer to seek additional mating opportunities if an egg clutch is small because the costs of losing the present offspring may be relatively low compared to the benefits gained by additional matings. Furthermore, the cost is even lower if eggs are laid in bamboo stumps where they have a high chance of surviving with little or no paternal care.

There is an apparent tradeoff between egg attendance and additional mating opportunities; thus, the modification of reproductive behavior of male frogs to either increase the benefits and/or lower the costs would in turn maximize the reproductive output. For example, if the water-holding capacity of a stump is critical to egg survivorship, we contend that it would be advantageous for male frogs to actively adjust the time spent with the present offspring and on future reproduction; that is to say, male frogs should spend less time attending eggs and more time seeking additional matings when eggs are laid in stumps with a high water level, and vice versa. On

the other hand, male frogs spend more time caring for the eggs when they have large egg clutches; by doing so, male frogs may lose additional mating opportunities. Our field survey showed that some *K. eiffingeri* male frogs called while attending eggs. This resulted in 2 egg clutches, that slightly differed in developmental stages, coexisting in the same stump (Kam, unpubl. data). Similar behavior was reported in earlier studies on Australian *Pseudophryne*, and Puerto Rican *Eleuterodactylus coqui* and *E. cooki* (Woodruff 1977, Townsend et al. 1984, Burrowes 2000). It is believed that male frogs can compensate for the loss of future opportunities by calling while attending eggs, and they might thus attract a female frog to mate and produce an additional egg clutch at the same site.

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