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Reproductive ecology of Sichuan digging frogs (Microhylidae: *Kaloula rugifera*)

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Abstract. We investigated the reproductive ecology of Sichuan digging frog (Microhylidae: *Kaloula rugifera*) in Mianyang, China during the wet season (from May to Septemper) of 2013. Male *K. rugifera* first appeared at temporary ponds following the first heavy rain of the wet season and initiated calling. Male frogs formed choruses throughout the wet season during the evenings and nights after rainstorms. Females arrived at ponds shortly after males start calling and leaved the pond once they lay egg masses. Amplexus lasted up to three hours. Females were larger than males in terms of body size, but we found no evidence of size-assortative mating. Clutch size varied from 920 to 2200 eggs, with egg diameter ranging from 1.33 to 1.93 mm. Larger females laid more eggs, and there was no correlation between egg number and egg size. Embryos hatched from eggs within 18-20 hours of oviposition, and grew for 25-40 days before complete metamorphosis occurred. Growth was fastest immediately after hatching, and declined asymptotically with increasing tadpole body size. Overall, *K. rugifera* have a breeding biology characterized by strong male-male competition with prolonged breeding coinciding with the annual wet season.

Keywords. Breeding ecology, *Kaloula rugifera*, life history, mating system.

Microhylidae Günther, 1858 (Fei et al., 2009; Frost, 2013) is a geographically widespread family of frogs, that includes 426 species (Fei et al., 2009), five (Mo et al., 2013) of which occur in China (*Kaloula borealis*; *K. nonggangensis*; *K. pulchra*; *K. rugifera*; and *K. verrucosa*). The reproductive ecology of Chinese Microhylidae is largely unknown and only the reproductive ecology of *K. verrucosa* has been reported (Fei et al., 2009). In the present study, some aspects of the breeding ecology of Sichuan digging frogs (Microhylidae: *K. rugifera*) are described, providing basic information about the breeding season, reproductive behavior, duration of the larval period, and tradeoffs between egg number/size and female size.

Kaloula rugifera is distributed in the northeastern Sichuan and southernmost Gansu provinces in south-western China (Fei et al., 2009; Fei and Xie, 2004). This small species (adults snout to vent length range from 35-55 mm; Fei et al., 2009) is often found in hilly areas near villages, and has been recorded sheltering in tree-holes. This species breeds in rain-filled, temporary pools and small ponds (Fei and Ye, 1983; Fei and Xie, 2004).

Fieldwork was conducted at a temporary pond (104°46″E and 31°29″N; 459 m a.s.l. elevation) in Mianyang, Sichuan, China from May to September 2013. This area is characterized by annual average temperature of 16.3°C, and total annual precipitation averages of 865.5 mm (Fig. 1). During the field season, the max-min range of temperature was 21.4-25.7°C. The temporary pond was located in dense woodland situated on Mianyang Normal University campus. The pond had an area of 7 m^2, a max-

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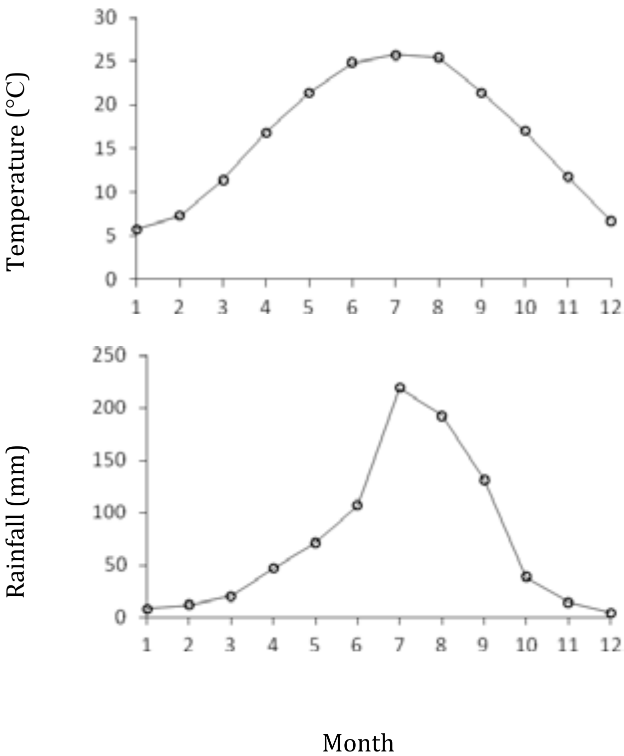

Fig. 1. Monthly mean air temperatures and mean rainfall during the reproductive season of Sichuan digging frogs (*Kaloula rugifera*) at a seasonal pool at Mianyang Normal University campus (Mianyang, Sichuan, China).

imum seasonal depth of 10 cm and some wilted leaves in the water. The study site was visited after sudden rainstorm during all the breeding season.

During the breeding season (May-September), we hand-collected 36 frogs (14 pairs found in amplexus, and four males and four females not in amplexus). We measured the snout-vent length (SVL, to the nearest 0.1 cm) of all the collected frogs (Fig. 1) and we transported each pairs to our laboratory (50 m away) using buckets containing pond water. After laying eggs, we determined the number of eggs per clutch, and photographed the eggs of eight clutches using a canon camera with macro lens (EOS 550) with a scale ruler in order to measure egg size (Chen et al., 2013a). We quantified egg size by measuring 10-30 eggs per clutch from digital photos, and averaged the minimum and maximum diameter of each egg (to the nearest 0.01 mm) to obtain a measure of egg size (Chen et al. 2013b).

We haphazardly selected two egg clutches from females with different size to study time to reach hatching and tadpole growth rates, and released all other egg clutches and frogs back into the study pond after data collection. The first was collected in late May, while the second in late June. Tadpoles were reared in groups of 20-30 individuals in plastic buckets (10 liter/capacity) containing a 2 cm substrate of mud and 10 cm of water, both collected from our study pond. The buckets were maintained at room temperature in our laboratory. We fed tadpoles with 2-2.5 g of crushed goldfish feed daily (Shangdong Dongpinghu feed Co., Ltd, China: 32% crude protein, 5% crude fat, 3% crude fibre, 10% moisture, and 12% crude ash). A single investigator measured the tadpoles' body size (SVL) at 9:00-10:00 am of every day, until metamorphosis occurred. Body size measures were used to estimate the tadpole growth rates using Von Bertalanffy's (1957) equation. The equation takes the form $S_t = S_{\max} (1 - e^{-kt+b})$, where S_t is body size at age t (time, in days), $S_{\max}$ is the estimated asymptotic maximum body size that tadpoles can reach before metamorphosis occurred, k is a growth coefficient, and b is a constant. Then, growth rate can be calculated as $R = dS/dt = k (S_{\max} - St)$.

Due to the small sample size, we used non parametric Spearman's correlation to explore the relationship between female body size and reproductive output (egg number and size), as well as between clutch size and egg size. We used SPSS software (Version 16.0. Chicago, SPSS Inc) for statistical analysis, all statistics are two-tailed and summary statistics are presented as means ± standard deviation (SD).

K. rugifera initiated breeding just after the first sudden rainstorm at the outset of the annual rainy season (25 May 2013; the rainy season lasted from late May-early September; Fig. 1), when the rain filled the study pond. Breeding continued throughout most of the rainy season due to sudden rainstorm, but became less frequent in September (we found 13 pairs in May-August, but just one pair in September), when temperatures became cooler and rainfall less frequent (Fig. 1). These changes in weather are associated with gradual drying of temporary ponds (5-8 cm in depth) and, thus, with the end of the reproductive season of *K. rugifera*.

In the evening following the first major rainstorm of the season, male frogs arrived at the temporary pool and began to chorus strongly. We observed females arriving at the pool two to three hours after males began advertising, then females engaged in amplexus within the first minutes of arriving at ponds (5.6 ± 2.3 min, n = 8; range: 3-9 min, Fig. 2b). Females generally laid eggs within three hours of initiating amplexus (116 ± 42 mins, n = 6, range: 60-180 mins). After fertilization occurred, the pairs separated and females left the breeding pond after mating. Males remained at the pond and called unless there were no females at the breeding site.

Adult males were significantly smaller than adult females in terms of body size ($Z = -5.004$, $p < 0.001$;

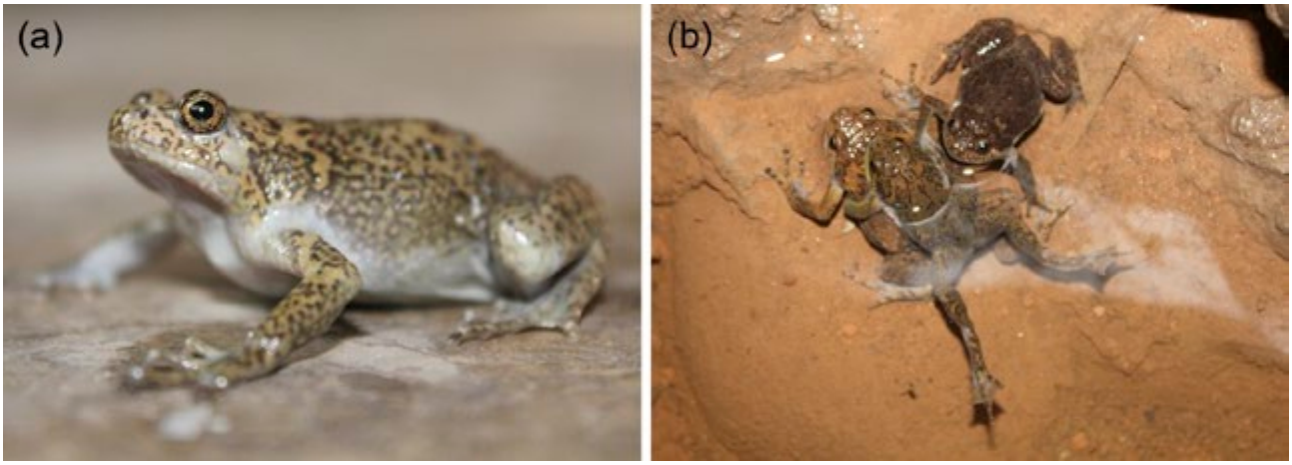

Fig. 2. Sichuan digging frogs (*Kaloula rugifera*): a male (a) and a couple in amplexus (b) being approached by another male (top). Photographs by Wei Chen.

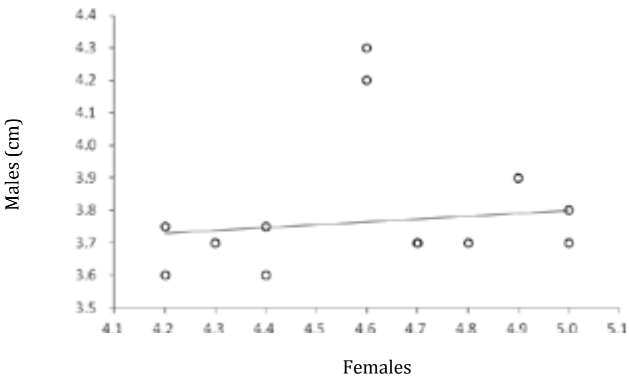

Fig. 3. Body size relationship of amplexing males and females (SVL measurements) of Sichuan digging frog (*Kaloula rugifera*; n = 14).

males SVL = 3.77 ± 0.23 cm; range: 3.3-4.3 cm, n = 19; females SVL= 4.57 ± 0.30 cm; range: 4.1-5.0 cm, n = 18; Fig. 2a). Despite the wide range of body sizes of both males and females, the body lengths of males and females found in amplexus were not significantly correlated (r_s = 0.157, p = 0.592, n = 14; Fig. 3). Thus, no evidence of size-assortative mating (a significant positive relationship between male and female body lengths of pairs in amplexus) was detected, as both large and small males successfully amplexed and mated with both large and small females.

The number of eggs per egg clutch ranged from 920-2200 eggs (1430 ± 456 eggs, n = 18 clutches), and egg diameter ranged from 1.33-1.93 mm (1.62 ± 0.12 mm, n = 355 eggs from eight clutches). We found significant positive correlations between female body size (SVL) and clutch size (r_s = 0.772, p < 0.001, n = 18; Fig. 4a), however, we did not find a significant correlation between female body size and egg size (r_s = 0.429, p = 0.289, n = 8; Fig. 4b) as well as between the number of eggs per clutch and egg size (r_s = 0.5, p = 0.207, n = 8; Fig 4c).

Eggs developed and hatched within 18-20 hours, tadpoles grew for 25-40 days before complete metamorphosis occurred. The initial body length of tadpoles ranged from 2-3 mm (Fig. 5a). The individual growth rate of tadpoles was fastest immediately after hatching, and declined asymptotically with increasing body size (Figs. 5a, b).The tadpoles hatching from eggs laid during late June had a larger initial body size and grew faster than tadpoles hatching on late May (K_{May} = 0.035; K_{June} = 0.046, Fig. 5).

K. rugifera breed along the entire rainy season, which lasts approximately four months, making this species a prolonged breeder (Wells, 1977). Breeding was initiated in late May, after the first heavy rainfall of the season, suggesting that the species' reproductive phenology is driven by precipitation and ambient temperature (Fei and Ye, 1983), similar to con-generic species *K. verrucosa* (Verrucous Digging Frog He et al., 2006). Breeding aggregations at a single pond are relatively small in both species (18-20 males; He et al. 2006) Similarly to *K. verrucosa*, also *K. rugifera* lay free-floating eggs, probably as a reproductive strategy to speed embryo development by exposing the entire egg surface to the high water temperatures (Warkentin et al., 2005).

Larger and older female amphibians with larger abdominal cavities are often able to allocate more energy to reproduction (Wells, 2007) by laying more eggs than smaller and younger conspecifics (Dziminski and Alford, 2005; Chen et al., 2011). Many amphibian females invest a lot of energy into body growth, enabling the incubation of a larger number of eggs and thus increasing fecundity (Czarnoleski and Kozlowski, 1998). In our study, larger

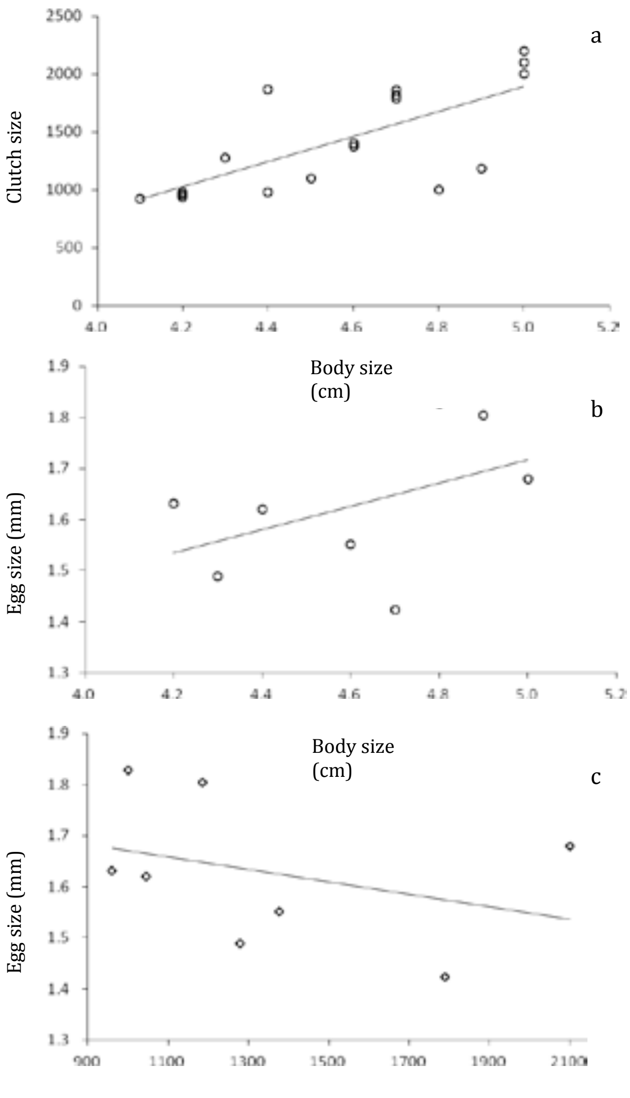

Fig. 4. Relationship between (a) female body size and clutch size (n = 18,); (b) female body size and egg size (n = 8), and (c) reproductive parameters such as clutch size (egg number) and egg size (n = 8) in Sichuan digging frogs (*Kaloula rugifera*).

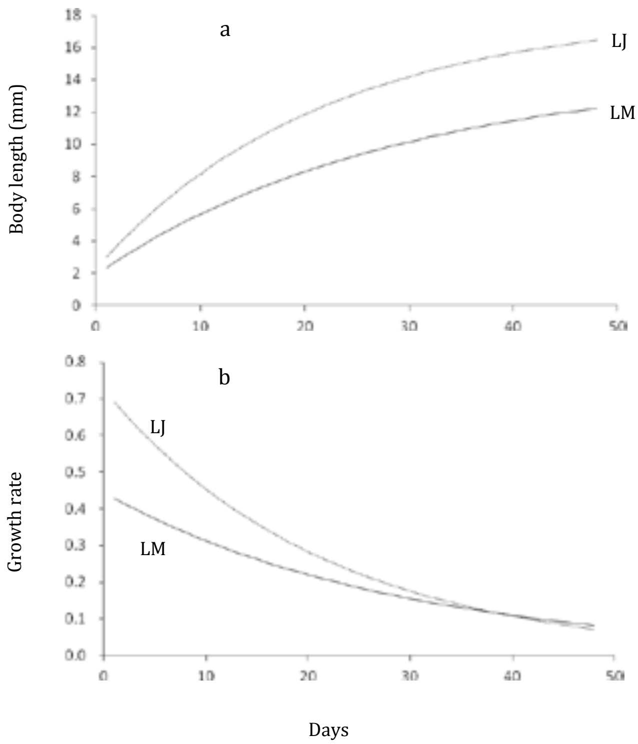

Fig. 5. Growth curve (a) and growth rate (b) of Sichuan Digging Frog (*Kaloula rugifera*) tadpoles hatching in late May (LM, solid lines) and late June (LJ, dotted lines).

female *K. rugifera*laid eggs with identical size in comparison with the little ones. Two possible explanations for this pattern are that: 1) larger females can invest more energy into egg production, which results into larger clutch size (Duellman and Trueb, 1986; Roff, 1992); and/or 2) the females in this population are below maximum physical constraint and egg size is mainly constrained by proximate factors prior to breeding, such as energy availability during vitellogenesis (Kaplan and Salthe, 1979).

Many amphibians have tradeoff between clutch size and egg size (Wells. 2007), but *K. rugifera* shows no correlations between egg number and size. This can be explained by the fact that limited physical clutch holding capacity cannot increase simultaneously the clutch size and egg size (Jorgensen, 1981), which leading to the fact that females do not trade off clutch size with egg size.

In our study, tadpoles finished metamorphosis within 25-40 days, much slower than tadpoles from Chengdu, which matured with 20-23 days (Fei and Ye, 1983). These differences on developmental time may be due to the dependence of tadpole growth on temperature or density (Chen et al. 2013c). Von Bertalanffy's (1957) equation provided a realistic simulation of tadpole growth during development and showed that tadpoles raised during late June show faster growth rates than those individuals raised during late May. In late June, the faster growth strategy of tadpoles may be adaptive to the fast dry-offs of the ponds. This still need further study to understand the cause of differentiated development rates.

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