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Reproductive Parameters of *Chalcorana labialis* (Anura: Ranidae) from Peninsular Malaysia

(Parameter Pembiakan *Chalcorana labialis* (Anura: Ranidae) dari Semenanjung Malaysia)

S. SHAHRIZA*, J. IBRAHIM & M.S. SHAHRUL ANUAR

ABSTRACT

Egg clutches of Chalcorana labialis *were collected from their natural habitat at Sungai Junjong, Kedah, between March 1998 and February 1999. Egg clutches were typically encountered in temporary pools, rock pools and isolated pools at the edge of the river. In the laboratory several reproductive parameters including clutch size, egg diameter, hatching and metamorphosis rates were measured. The mean ± SD (range, N) of clutch size, egg diameter, hatching and metamorphosis rates of* C. labialis *were 1365.4 ± 421.1 (787-2100, 10) eggs, 1.36 ± 0.15 (1.12-1.68, 20) mm, 97.30 ± 0.99% (95.87-98.86, 10) and 4.15 ± 0.74% (3.1-5.8, 10), respectively. These results indicated that the successfully rate of larvae to become froglets is relatively low, although it was reared in the laboratory. To keep survived, almost all species of frogs produced eggs in a large quantity.*

Keywords: Clutch size; egg diameter; frog; hatching; metamorphosis

ABSTRAK

Kelompok telur Chalcorana labialis *telah dikutip dari habitat semulajadinya di Sungai Junjong, Kedah, antara Mac 1998 dan Februari 1999. Kebanyakan daripada kelompok telur tersebut ditemui di lopak sementara, lopak batu dan lopak-lopak terpisah yang berhampiran dengan sungai. Di makmal beberapa parameter pembiakan termasuklah saiz kelompok telur, diameter telur, peratus menetas dan metamorfosis telah diukur. Min ± SD (julat, N) bagi saiz kelompok telur, diameter telur, peratus menetas dan metamorfosis bagi* C. labialis *masing-masing ialah 1365.4 ± 421.1 (787–2100, 10) telur, 1.36 ± 0.15 (1.12–1.68, 20) mm, 97.30 ± 0.99% (95.87–98.86, 10) and 4.15 ± 0.74% (3.1–5.8, 10). Hasil kajian menunjukkan peratus larva yang berjaya menjadi anak katak adalah amat rendah, walaupun telah dipelihara di dalam makmal. Untuk meneruskan kemandirian, hampir kesemua spesies katak menghasilkan telur dalam kuantiti yang besar.*

Kata kunci: Diameter telur; katak; kelompok telur; menetas; metamorfosis

INTRODUCTION

The White-lipped frog, *Chalcorana labialis* (Boulenger 1887), is a moderate-sized frog that inhabits in the forest of Peninsular Malaysia. This cryptic species is a member of the *Rana chalconota* complex, and consists of at least seven species, ranging from southern Thailand, Peninsular Malaysia, Borneo, Sumatra and Java (Inger et al. 2009). In Peninsular Malaysia, *C. labialis* can be found as inhabitant of forest streams, frequenting mainly the vegetation on stream banks. It prefers more shaded surrounding areas with clear and moving water but could also be found in swampy areas (Berry 1975). The size of the male individual (32-44 mm) is smaller compared to female (46-60 mm) (Berry 1975).

Different species of frogs prefer different types of environment to live and breed. Some species of frogs lay eggs in small rain pools, others in large ponds and others in small creeks or rivers (Inger & Stuebing 1989). For example, the Mahogany frog, *Abavorana luctuosa*, breeds in the rain pools on the forest floor while the Spotted Stream frog, *Pulchrana picturata* can be found in lowland areas and breeds among leaf drifts in riparian habitats (Das 2007). The commensal species, *Hylarana erythraea* breeds in paddy fields and man-made ponds (Sheridan 2009). Other species such as *Pulchrana glandulosa* can be found near swampy areas or slow flowing rivers and breeds in stagnant water (IUCN 2014). As for *C. labialis*, this species prefer side pools of medium-sized streams and the edges of ponds to breeds (Inger & Stuebing 1989).

In Peninsular Malaysia, studies on frogs are more focussed on biodiversity compared to other biological aspect such as breeding, feeding and behaviour. Several studies on breeding and reproductive biology of frogs were carried out a long time ago. Fifty years ago, Inger and Greenberg (1963) documented the breeding activities of *H. erythraea* from Sarawak. Later, Berry (1964) reported the breeding patterns of seven anuran species from Singapore. Four years later, Inger and Bacon (1968) works on the breeding activities of six forest frog species from Sarawak. Subsequently, Ibrahim et al. (1999) investigated the breeding biology of *Fejervarya cancrivora* and *F. limnocharis*, Sheridan (2008) reported the breeding ecology of *Polypedates leucomystax*, Sheridan (2009) studied the reproductive variation of three tropical frog

species and Shahriza et al. (2012, 2010) documented the breeding activities of *C. labialis* and *Ingerophrynus parvus*. Hence, this study was carried out to provide more information on reproductive parameters of White-lipped frog, *C. labialis* from Peninsular Malaysia.

Materials and Methods

The sampling site, Sungai Junjong (5°17'N, 100°33'E; elevation < 200 m asl) (Figure 1) is located approximately 15 km southward from Kulim town, in the district of Kulim Bandar Baru, Kedah. This river, originated from the hilly areas of Junjong, flows into Sungai Jawi and empties into Straits of Malacca. Our surveys began from the car park and upstream about 500 m. Within the sampling areas, the width of the river is about 3-6 m. The water is crystal clear, flows through rocks and boulders with gravel and sandy substrates. Many riffles and cascades were encountered within the sampling sites. At the edges of the river, there were many puddles, rain pools, rock pools and isolated pools providing suitable sites for several species of frogs to breed. Both banks of the river are surrounded by lowland dipterocarp forest, orchard and old rubber plantation.

Egg clutches of *C. labialis* were collected from the rock pools, isolated pools and occasionally in the river with slow moving current. The size of these pools were varied but mostly around 1 × 1.5 m and the water was approximately 10-30 cm in depth. Some of the pools were filled with dead leaves and twigs. Usually the egg clutches were collected in the morning after night shower. The collected egg clutches were places immediately into plastic bags containing water, and then brought to the laboratory for further inspections.

In the laboratory, all the egg clutches (ten clutches) were places separately into a glass aquarium, containing tapped water and an aerator to supply oxygen. Subsequently, the number of egg in every clutch was counted. Two eggs from each clutch (total = 20 eggs) were randomly selected and measured their diameter using a microscope with an ocular micrometer. All egg clutches were raised in the laboratory (temperature between 25-30°C) until hatching. When the egg hatched and became larvae (Stage 19), the percentage of hatching was calculated.

The larvae were continuously raised until metamorphose and became froglets (stage 46). Subsequently, the percentage of larvae that successfully became froglets was calculated. During raising period, all the larvae were feed on fish pallets twice a day. Aquatic plants such as *Lemna minor* or *Cabomba* sp. and dead leaves were put in every aquarium as a shelter or hiding places for the larvae. The water in each aquarium was changed twice a week to make sure it was clean and free from debris. All the stages for larval development follow Gosner (1960) while taxonomic nomenclature of the frog species follows Oliver et al. (2015).

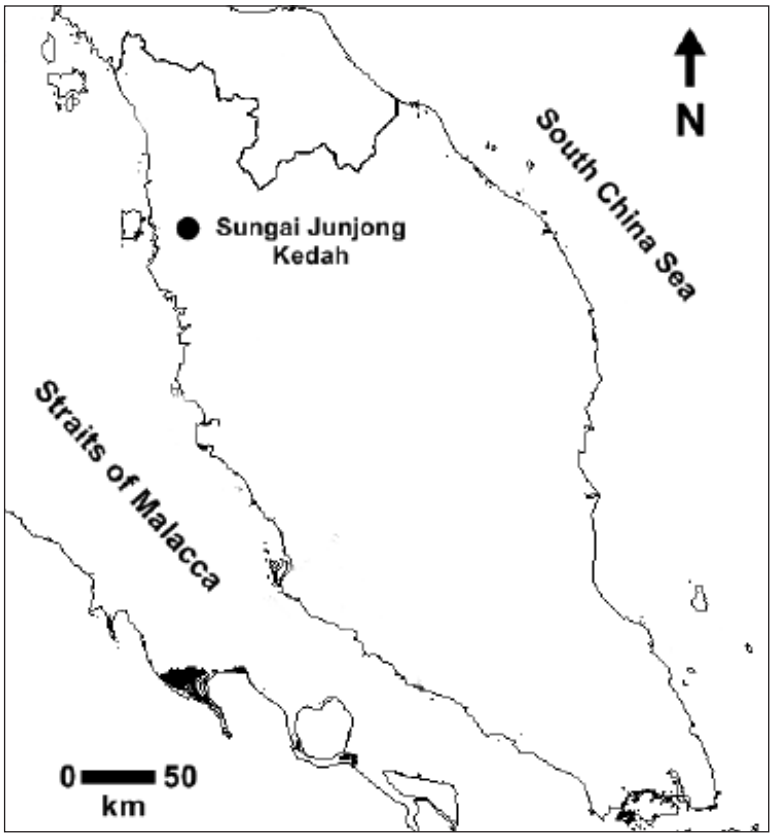

FIGURE 1. Location of Sungai Junjong, Kedah

Results

Mean ± SD (range, N) of clutch size, egg diameter, hatching and metamorphosis rate of *C. labialis* were 1365.4 ± 421.1 (787-2100, 10) eggs, 1.36 ± 0.15 (1.12-1.68, 20) mm, 97.30 ± 0.99 (95.87-98.86, 10) and 4.15 ± 0.74% (3.1-5.8, 10), respectively. Table 1 shows the clutch size, egg diameter, hatching and metamorphosis rate and Figure 2 shows an adult specimen of *C. labialis*.

Discussion

Recent record documented 107 species of amphibians, including two species of caecilians inhabit the forest of Peninsular Malaysia (Chan et al. 2010). Different species of frogs inhabit in a different type of environment and produce different mode of reproduction. Twenty-nine reproductive modes have been recognized in anuran (Duellman & Trueb 1986) and 10 new modes were added additionally (Haddad & Prado 2005), totalling 39 reproductive modes. Mode of reproduction is a combination of ovipositional and developmental factors, including oviposition site, ovum and clutch characteristics, rate and duration of development, stage and size of hatching and type of parental care, if any (Salthe & Duellman 1973). The most common and phylogenetically widespread site of oviposition is in standing (Mode 1) or flowing (Mode 2) water bodies, permanent or temporary. Aquatic eggs and tadpoles are characteristic of all megophryids, bufonids, ranids, most discroglossids and some microhylids and rhacophorids (Duellman & Trueb 1986). From our observations, *C. labialis* were usually breeds in the side pools or rock pools that can be found at the edge of the river. However, sometimes their tadpoles were also encountered in the river with slow moving current. Therefore, we suggest modes 1 and 2 as reproductive modes for this species. Mode 1 defines the eggs and feeding tadpoles in lentic water while mode 2 defines eggs and feeding tadpoles in lotic water (Duellman & Trueb 1986).

In amphibian the clutch size is highly variable, depending on body size and reproductive mode (Kaplan 1980; Kuramoto 1978). Larger species produced more eggs than smaller ones and species that have generalized reproductive modes have larger clutches than those with specialized modes (Duellman & Trueb 1986). Additionally, species depositing their eggs in water have much larger clutches (Duellman & Trueb 1986). *Chalcorana labialis* is a medium-sized of frog, with total length of female (46-60 mm) is larger compared to male (32-44 mm) (Berry 1975). This species has generalized reproductive mode, which deposits its eggs in standing or slow moving water. From this study, *C. labialis* from Sungai Junjong, Kedah produced 1365.4 ± 421.1 (787-2100, 10) eggs. This number is larger when compared to eggs produced by *F. cancrivora* and *F. limnochris* from Selangor, which were 1077.9 ± 238.97 (662-1677) and 405.5 ± 92.45 (233-657)

TABLE 1. Clutch size, egg diameter, hatching and metamorphosis rate of *Chalcorana labialis*

Number	Egg diameter (mm)	Clutch size (egg)	Hatching rate (%)	Metamorphosis rate (%)
1	1.12	1550	95.87	3.10
2	1.27	988	96.42	3.55
3	1.27	1369	97.55	3.74
4	1.29	1875	96.84	4.10
5	1.54	874	95.78	4.22
6	1.49	1520	97.84	5.10
7	1.48	1225	98.10	5.80
8	1.21	2100	97.56	3.76
9	1.17	787	98.30	3.94
10	1.24	1355	98.86	4.23
11	1.42			
12	1.32			
13	1.32			
14	1.37			
15	1.54			
16	1.23			
17	1.24			
18	1.33			
19	1.68			
20	1.58			
Total	27.11	13654	973.12	41.54
Mean	1.36	1365.4	97.31	4.15
SD	0.15	421.08	0.99	0.74
Range	1.12-1.68	787-2100	95.78-98.86	3.10-5.80
N	20	10	10	10

FIGURE 2. An adult *Chalcorana labialis* from Sungai Junjong, Kedah

eggs, respectively (Ibrahim et al. 1999), larger than *F. limnocharis* from Singapore, which produced 576 (266-1318) eggs (Berry 1964), larger than *L. ibanorum* from Sarawak, which generated 1122 (811-1433) eggs (Inger & Bacon 1968) and larger than *H. erythraea* from Thailand, which generated 1074 ± 73.0 eggs (Sheridan 2009). On the other hand, clutch size produced by *C. labialis* is much smaller when compared to *L. blythii, O. hosii* and *Meristogenys jerboa* from Sarawak, which produced 1852 (1232-2472), 2320 (2004-2636) and 2462 (2162-2762) eggs, respectively (Inger & Bacon 1968).

Egg diameter of frog varies from species to species. Various factors such as reproductive modes (Kaplan 1980; Kuramoto 1978), female age, size and genetics contribute to the variation of egg size in amphibians (Berven 1982; Rafinska 1991). In this study, the mean ± SD (range, N) egg diameter of *C. labialis* was 1.36 ± 0.15 (1.12-1.68, 20) mm. This number is slightly larger when compared to egg diameters of *F. cancrivora* and *F. limnocharis,* which were 1.35 ± 0.091 (1.16-1.53) mm and 1.15 ± 0.027 (1.10-1.21) mm, respectively (Ibrahim et al. 1999). Meanwhile, the range of egg diameter of *L. blythii, L. ibanorum, O. hosii* and *Meristogenys jerboa* from Sarawak were 1.02-2.24, 1.33-2.14, 0.53-2.28 and 1.12-1.51 mm, respectively (Inger & Bacon 1968).

The hatching rate of *C. labialis* was high at 97.30 ± 0.99% (95.87-98.86, 10). Rearing in the laboratory can provide constant temperature and adequate oxygen supply that generates optimal condition for the embryo to develop. Additionally, factors such as predator, extreme temperature and pond desiccation potentially affect the development of the eggs and can be avoided.

On the other hand, the rate of *C. labialis* larvae to become a froglet was relatively low and only 4.15 ± 0.74 (3.1-5.8, 10) percent of the tadpoles successfully metamorphosed and became froglets. However, these data are comparable with other studies on larval survivorship in Peninsular Malaysia. For examples, the survivorship of *F. cancrivora* and *F. limnocharis* larvae were 13.1 and 11.0%, respectively (Ibrahim 1994), while the survivorships of *P. leucomystax* larvae in two different ponds were 3.4 and 2.8%, respectively (Yorke 1983). According to Wilbur (1972), the high mortality rate is typical characteristic for amphibian larvae, therefore they deposit eggs in large quantity.

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S. Shahriza
School of Pharmaceutical Sciences
Universiti Sains Malaysia
11800 Penang, Pulau Pinang
Malaysia

J. Ibrahim
School of Distances Education
Universiti Sains Malaysia
11800 Penang, Pulau Pinang
Malaysia

M.S. Shahrul Anuar
School of Biological Sciences
Universiti Sains Malaysia
11800 Penang, Pulau Pinang
Malaysia

*Corresponding author; email: shahriza20@yahoo.com

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