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Original Research Article

Reproductive parameters of *Ingerophrynus parvus* (Boulenger, 1887) (Anura: Bufonidae) from Peninsular Malaysia

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A b s t r a c t

Ingerophrynus parvus is a small-sized of toad that inhabits the forests of Peninsular Malaysia. It has a generalized reproductive mode, breeding in stagnant or slow-moving water. Between March 1998 and February 1999, several egg clutches of this species were collected from temporary pools and puddles at the edge of Junjong River, Kedah. The mean ± SD (range, N) of cluth size and egg diameter were 1874.1 ± 614.11 (997-2892, 20) eggs and 1.19 ± 0.11 (1.03-1.47, 10) mm respectively. Subsequently the egg clutches were raised in the laboratory until hatching and complete metamorphosis. The hatching rates under laboratory conditions were very high [97.77 ± 1.23 (95.40-99.57, 10) percent] while the metamorphosis rates were very low [3.26 ± 0.98 (2.12-5.10, 10) percent].

K e y w o r d s

Clutch
Egg diameter
Hatching
Metamorphosis
Spawn
Toad

Introduction

Four toad species of the genus *Ingerophrynus*, namely *I. parvus*, *I. kumquat*, *I. quadriporcatus* and *I. gollum* are native to Peninsular Malaysia. *Ingerophrynus parvus* is a small toad with snout-vent length (SVL) up to 35 mm for males and 39 mm for females (Taylor, 1962). This bufonid is widely distributed in Peninsular Thailand, Peninsular Malaysia, Southern Myanmar, Cambodia, Sumatra and Java and can be found up to 520 m asl (IUCN, 2014). In Peninsular Malaysia this species inhabits forest floors, including along jungle footpaths, under twigs and dead leaves and near streams (Berry, 1975; Ibrahim et al., 2008). Several forested areas such as Janda Baik Pahang, Templer Park Selangor, Gunung Tapis Pahang (Berry, 1975), Teluk Bahang Penang (Ibrahim et al., 2008), Bukit Hijau Kedah (Shahriza et al., 2011), Peta Endau-Rompin Johor (Shahriza et al., 2012a) and Ulu Paip Kedah (Shahriza and Ibrahim, 2014) are known to house this species.

Toads belonging to family Bufonidae breeds in stagnant or slow moving water (Inger, 1966). *I. parvus* breeds in temporary pools that connect to streams, rainpool puddles (Taylor, 1962), swampy areas (Arak, 1984), forest pools and slow-moving streams (IUCN, 2014). This species also prefered pools alongside small streams and rocky pools beside small creeks to breed (Inger et al., 1974).

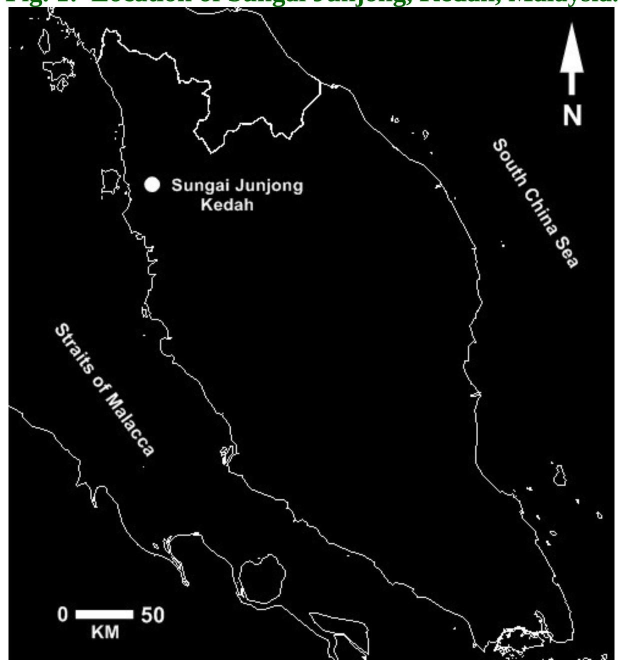
Data on the reproductive biology of Asian bufonids are scarce and only a few studies have been made, most of which focussed on a single species, the Asian Common Toad, *Duttaphrynus melanostictus*. Early studies were done by Church (1960) on the sexual cycle of *D. melanostictus* in Java. Four years later, Berry (1964) studied the breeding pattern of *D. melanostictus* in Singapore. Subsequently, Inger and Bacon (1968) documented the reproduction and clutch size of *Phrynoidis aspera* from Sarawak, Saidapur and Girish (2001) reported the growth and metamorphosis of *D. melanostictus* tadpoles in India, Shahriza et al. (2012b) studied the breeding activities of *I. parvus* in Peninsular Malaysia and Ngo and Ngo (2013) documented the reproductive activity of *D. melanostictus* in Vietnam. The purpose of the present study is to provide more information on the reproductive biology of *I. parvus* from Kedah, Malaysia.

Materials and methods

Sampling on egg clutches of *I. parvus* was done along Junjong River, (5^0 17'N, 100^0 33' E; elevation < 200 m asl) (Fig. 1) Kedah, Malaysia between March 1998 and February 1999. Junjong River originates from the hilly areas of Junjong, flows into Jawi River and empties into the Straits of Malacca. Old secondary forests, orchards and old rubber plantations surround the sampling area. The understorey is dominated by ferns, herbs, epiphytes and shrubs. Our sampling took place from the car park area and upstream about 300-500 m. Within the sampling area, the width of the river is about 3-6 m. The crystal clear water runs through rocks and boulders along many riffles, torrents and pools. There were many temporary puddles, rain pools and rock pools along the river banks. These shallow pools are varied in sizes (approx. 10-30 cm depth), mostly full with dead leaves and twigs.

Most of the egg clutches of *I. parvus* were collected from these shallow pools but sometimes in the river, in places with slow moving currents. All the clutches were collected early in the morning and usually after a night shower. Each egg clutch was placed into a plastic bag that contained river water and brought to the laboratory for further examination.

Fig. 1: Location of Sungai Junjong, Kedah, Malaysia.



In the laboratory, every egg clutch was deposited into a glass aquarium (60 × 30 × 30 cm), containing tap water and an aerator to supply oxygen. The number of eggs in every clutch was counted. In each clutch, two eggs were randomly selected and their diameter measured using a microscope with an ocular micrometer. All the 10 egg clutches were raised in the laboratory under 25-30°C until hatching (Gosner's stage 19). The hatching rates were calculated when all the eggs had hatched and become larvae. At stage 19, the heart of the larvae began to beat and the emerging of external gill (Gosner, 1960).

The larvae were continuously raised in the laboratory until they metamorphosed and became froglets (Gosner's stage 46). Subsequently, the larvae that successfully transformed into froglets were counted. During metamorphosed they are several physiological and morphological transformation occurred such as tail become shorter, mouth development and the emerging of forelimb (Gosner, 1960). While raised, the larvae were fed with fish pellets twice a day. Aquatic plants such as *Cabomba sp.* or *Hydrilla sp.*, twigs, drift woods and dead leaves were put in every aquarium as hiding places for the larvae. Some of the drift woods are located above water level and provided a shelter placed for newly froglets. The water in each aquarium was changed (50%) twice a week to make sure it was free from any pollution.

Results and discussion

Mean ± SD (range, *N*) of clutch size, egg diameter, hatching and metamorphosis rate of *I. parvus* were 1874.1 ± 614.11 (997-2892, 10) eggs, 1.19 ± 0.11 (1.03-1.47, 20) mm, 97.77 ± 1.23 (95.40-99.57, 10) and 3.26 ± 0.98 (2.12-5.10, 10) percent respectively (Table 1). An adult specimen of *I. parvus* is shown in Fig. 2.

Recently, 39 reproductive modes have been reported in Anura (Haddad and Prado, 2005). Spawning in standing (Mode 1) or flowing (Mode 2) water are the most common and phylogenetically widespread oviposition modes (Duellman and Trueb, 1986). During our survey, most of the egg clutches were collected from temporary pools, puddles and rock pools along the river banks and sometimes in the river with slow moving currents. Thus, we suggest modes 1 and 2 as the reproductive modes for *I. parvus*. It is comparable with previous observations on *I. parvus* breeding in stagnant or slow-moving water (Taylor, 1962; Inger et al., 1974; Arak, 1984; Shahriza et al., 2012a; IUCN, 2014).

Table 1. Clutch size, egg diameter, hatching and metamorphosis rate of *I. parvus*.

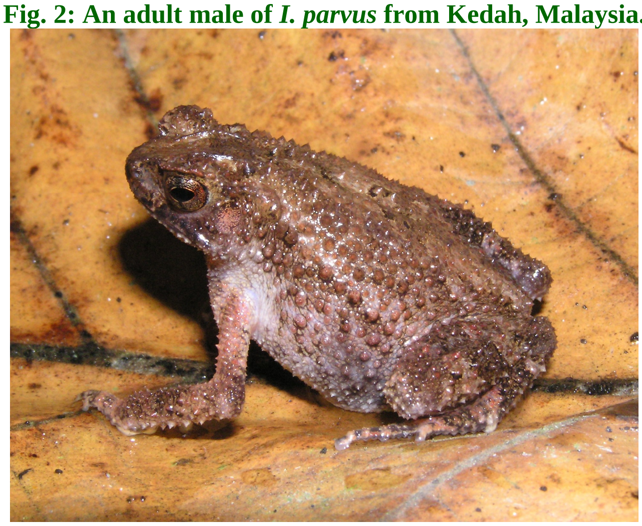
Number	Egg diameter (mm)	Clutch size (eggs)	Hatching rate (%)	Metamorphosis rate (%)
1	1.08	1678	96.25	3.27
2	1.21	997	95.40	4.50
3	1.18	2764	98.75	5.10
4	1.32	1456	98.60	2.17
5	1.08	2453	97.24	3.25
6	1.21	2892	99.57	2.12
7	1.14	1240	98.64	4.10
8	1.03	1574	97.20	2.75
9	1.18	2100	97.48	3.20
10	1.22	1587	98.64	2.14
11	1.47			
12	1.06			
13	1.38			
14	1.12			
15	1.25			
16	1.17			
17	1.05			
18	1.36			
19	1.13			
20	1.18			
Total	23.82	18741	977.78	32.60
Mean	1.19	1874.1	97.77	3.26
SD	0.11	614.11	1.23	0.98
Range	1.03-1.47	997-2892	95.40-99.57	2.12-5.10
N	20	10	10	10

The reproductive mode influences the clutch size of amphibian species (Kuramoto, 1978; Kaplan, 1980). Species that have generalized reproductive modes have larger clutches than those with specialized modes (Duellman and Trueb, 1986). Additionally, larger species produce more eggs compared to smaller ones and species depositing their eggs in water have much larger clutches (Duellman and Trueb, 1986). Our observations show that *I. parvus* has a generalized reproductive mode, depositing its eggs in stagnant or slow-moving water and producing 1874.1 ± 614.11 (997-2892, 10) eggs.

The medium-sized toad, *D. melanostictus* is known to produce 2,090 (45-8,200) (Berry, 1964, data for Singapore) to 8,447 ± 928 (7,125-9,547, 30) eggs (Ngo and Ngo, 2013, data for Vietnam). The bigger toad, *P. aspera* is known to produce 12,792 (9,557-16,027) eggs (Inger and Bacon, 1968, data for Sarawak).

In anurans, egg diameter varies from species to species and several factors such as reproductive mode (Kuramoto, 1978; Kaplan, 1980), female age, size and genetics contribute to this variation (Berven, 1982; Rafinska, 1991). In our study, the egg diameter of *I. parvus* was 1.19 ± 0.11 (1.03-1.47, 20) mm. This value is comparable to the values observed in other tropical Asian bufonids such as *P. aspera* from Sarawak and *D. melanostictus* from Vietnam which were 0.46-1.23 mm (Inger and Bacon, 1968) and 1.5-1.8 mm (Ngo and Ngo, 2013) respectively.

Fig. 2: An adult male of *I. parvus* from Kedah, Malaysia.



All the egg clutches were raised in the laboratory with temperature between 25-30^{0}C and given adequate oxygen supply to provide optimum conditions for the embryos to develop. As a result the hatching rate was high, 97.77 ± 1.23 (95.40-99.57, 10) percent. Additionally, laboratory condition allowed avoiding predators, extreme temperatures, pollution and pond dessication.

In contrast, only 3.26 ± 0.98 (2.12-5.10, 10) percent of *I. parvus* larvae successfully metamorphosed and became froglets. This number is very low and most of the larvae were dead in the second week after hatching although the larvae were raised in the laboratory. Several factors such as unsuitable environment, unadaptable larvae, unsuitable food, limited space and movement area may have affected the high mortality rate of the larvae.

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