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The Correlation between Total Rainfall and Breeding Parameters of White-Lipped Frog, *Rana labialis* (Anura: Ranidae) in Kedah, Malaysia

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ABSTRACT.– The correlation between total rainfall and breeding parameters of White-Lipped frog, *Rana labialis* Schlegel was investigated at Junjong River, Kulim, Kedah, Malaysia for a period of 12 months. A number of breeding activities such as calling, amplexus, spawning and the presence of larvae were observed every week in each month. Observations showed that the breeding of these frog occurred throughout the year without any specific season and rain seems to be the major extrinsic factor that increased the breeding activities of the frog. Pearson correlation showed a positive relationship between total rainfall and a number of breeding parameters such as calling activities (r=0.85), amplexus (r=0.68) and number of egg clutches (r=0.82).

KEY WORDS: breeding, correlation, *Rana labialis*, Junjong River, Kedah, Malaysia

INTRODUCTION

The wet tropical rainforest in Peninsular Malaysia provides various suitable habitats for amphibians to live and breed, such as streams, rivers, waterfalls, swamps, ponds, rain pools and puddles. Amphibians breed in a variety of water bodies and almost half of all frogs species in Malaysia breeds in the streams and rivers (Inger, pers. comm.). The kind of breeding sites used by frogs is peculiar to each species. Some frogs lay eggs in small rain pools, others in large ponds and still others in small creeks or rivers (Inger and Stuebing, 1989). Temporary ponds, rainpools and puddles provide suitable breeding sites for several commensal species like *Microhyla heymonsi, Microhyla butleri* and *Kaloula pulchra*. These species have short development periods that allow them to use any evanescent pools. There are also fewer potential predators and competitors in the small temporary pools (Inger, pers. comm.). Other species such as *Phrynoides aspera*, *Limnonectes blythii, Odorrana hosii* and *Megophrys nasuta* choose rivers and swampy areas in the forest as their breeding sites. Meanwhile, tree frogs species such as *Rhacophorus nigropalmatus* and *Rhacophorus pardalis* that lived high in the tree canopy and lay their eggs in pool or wild animal (Sumatran Rhinoceros and Wild Pigs) wallows on the forest floor, which are their main breeding sites (Inger and Stuebing, 1989).

Rana chalconota is a widespread species in the Sundaland and recent molecular analyses showed that it consists of at least seven species ranging from Thailand, Peninsular Malaysia through Borneo and Java (Inger et al., 2009). The three species are *Rana chalconota* (type locality Java),

Rana raniceps (type locality Sarawak) and *Rana labialis* (type locality Malacca) while the other four are described as a new species namely *Rana eschatia* (Thailand morphotype), *Rana megalonesa* (Borneo large morphotype), *Rana rufipes* (Padang large morphotype) and *Rana parvaccola* (Padang small morphotype) (Inger et al., 2009). Iskandar and Colijn (2000) also mentioned that *R. chalconota* species complex is widely distributed from southern Thailand to Java including Borneo and Sumatra.

From the recent molecular analysis it has been shown that the type of *R. chalconota* species complex in Peninsular Malaysia is referred to as *R. labialis* (type locality Malacca). In Peninsular Malaysia, *R. labialis* is a common forest frog and can easily be found along the river banks and swampy areas. This species inhabits forest streams with clear and moderate water current (Berry, 1975). In Sundaland, *R. chalconota* species complex breeds along streams of various sizes in lowland forests of various types, from lowland primary rain forest to swamp forest to secondary forest (Inger, 1969).

Although Malaysia is rich with amphibian species, information on their breeding and development are lacking and for forest frogs there are almost no data available. Only a few scientists have done research on breeding and development of tropical frogs. For example Church (1960a,b) studied the breeding of *Duttaphrynus melanostictus* and *Fejervarya cancrivora* in Java, Inger and Greenberg (1963) studied the breeding of *Hylarana erythraea* in Sarawak, Berry (1964) studied the breeding of seven anuran species (*D. melanostictus, Polypedates leucomystax, Fejervarya limnocharis, Leptobrachium nigrops, Kaloula pulchra, Microhyla butleri*

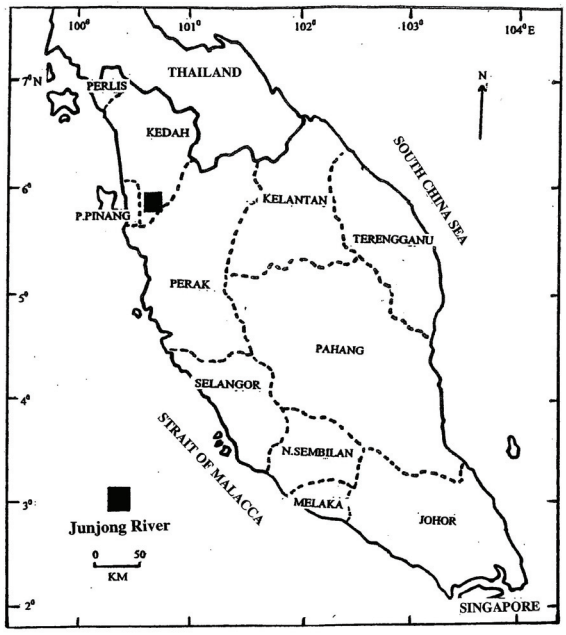

FIGURE 1. Location of Junjong River, Kedah.

and *M. heymonsi*) in Singapore, Inger and Bacon (1968) studied the breeding of six forest frogs species (*P. aspera, L. blythii*, *Limnonectes ibanorum*, *Limnonectes macrodon, O. hosii* and *Amolops jerboa*) in Sarawak, Ibrahim et al. (1999) studied the breeding of *F. cancrivora* and *F. limnocharis* in Peninsular Malaysia and Sheridan (2008) studied the breeding of *P. leucomystax* in Thailand. In other tropic regions such as India, scientists such as Kanamadi et al. (1993; 1994; 2002), Meren and Sabitry (2000), Girish and Kanamadi (2000) and Savitha et al. (2004) studied the reproductive behavior of several species of Indian frogs. However, to the best of our knowledge no data on the breeding of *R. labialis* exist in the literature. The main objectives of this study is to investigate the correlation between total rainfall and a number of breeding parameters (calling, amplexus and spawning) of *R. labialis*

TABLE 1. Monthly rainfall, relative humidity, minimum and maximum temperature for Kulim station.

Month	Number of raining days	Rainfall (mm)	Humidity (%)	Min. temp. (°C)	Max. temp. (°C)
Mar 98	3	35.1	95	23.3	36.4
Apr 98	10	99	94	24.3	36
May 98	18	357.8	95	24.7	34.4
Jun 98	13	140.3	95	23.8	33.1
Jul 98	18	213.5	93	23.3	33.3
Aug 98	20	412.7	95	22.9	32.5
Sep 98	13	274.4	95	23.2	32.7
Oct 98	22	553.3	93	23.2	32.4
Nov98	21	427.9	95	23.1	32.6
Dec 98	16	224.9	96	22.8	32.3
Jan 99	12	227.9	95	22.4	33.3
Feb 99	14	124.9	93	22.8	33.7
Mar99	13	182.1	94	23	33.6
Total	**193**	**3273.8**	**1228**	**302.8**	**436.3**
Mean	**14.8**	**251.83**	**94.5**	**23.3**	**33.56**
SD	**4.96**	**143.05**	**0.93**	**0.61**	**1.27**
Range	**3.0-22.0**	**35.1-553.3**	**93-96**	**22.4-24.7**	**32.3-36.4**

Note: Elevation 32 m above sea level (Source: Meteorology Department, Petaling Jaya, Selangor)

under natural conditions at Junjong River, Kedah, Malaysia.

MATERIALS AND METHODS

The breeding activities of *R. labialis* was investigated under natural conditions at Junjong River (5° 17' N/ 100° 33' E; < 300 m asl), Kedah (Fig. 1) for a period of 12 months starting from March 1998 until February 1999. The study area around Junjong River is mainly surrounded by old secondary forest, fruit orchards and rubber plantations. Junjong River was choosen as the study site because early observations showed a high population of *R. labialis* in the area, where suitable breeding sites can be easily found.

Physicochemical characteristics such as water temperature, pH, dissolved oxygen and conductivity were measured using pH meter (Jenway model 3071), D.O. meter (YSI model 55/25 FT) and SCT meter (HACH model 44600). The measurement of each parameter was taken once a month starting from March 1998 until February 1999. Data on rainfall, relative humidity, minimum and maximum temperature for Kulim station, about 15 km from the study site were provided by Meteorology Department, Petaling Jaya, Selangor.

To determine the size of male and female frogs, 50 individuals from each sex were captured at night (21:00 until 24:00 hours) around Junjong River by hand and sweep net. Battery powered torch lights and head lamps were used to locate the frogs. Subsequently the captured frogs were put into black plastic bag and taken to laboratory for morphological measurements. The snout-vent length (SVL) and head width (HW) of each frog were measured using Vernier calipers (± 0.05 mm) while their weight (W) were measured using

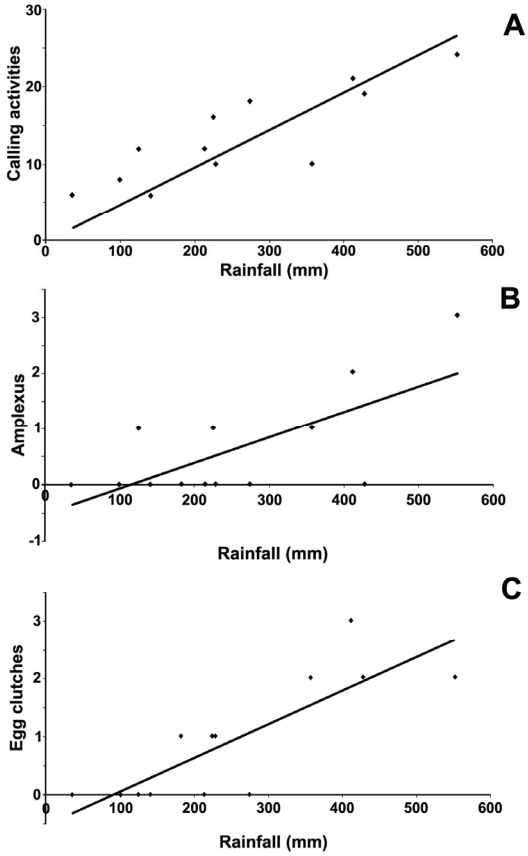

FIGURE 2. A. The correlation between calling activities and rainfall for *R. labialis* (r=0.85). **B.** The correlation between amplexus activities and rainfall for *R. labialis* (r=0.68). **C.** The correlation between egg clutches and rainfall for *R. labialis* (r=0.82).

Mettler Toledo electronic balance (max=310 g, d=0.001 g). Using the SPSS 13 software, independent t-test was carried out to determine if there is a significant difference for SVL, HW and W between male and female frogs.

The breeding activities such as calling, amplexus, spawning and the presence of larvae were observed every week in each month (4 times per month) starting from March 1998 until February 1999. The total number of field work observations for the entire project (12 months) were 48 times. Observations for each parameter were done for a distance of 300 meters along the stream. For calling and amplexus activities, observations were done at night while for spawning and the presence of larvae, observations were done in a day time. We recorded the number of calling males and the number of amplecant pairs found. For spawning activities, we recorded the number of egg clutches found while larvae were recorded as present or absent. Finally using the SPSS 13 software, Pearson correlation was carried out to determine the relationship between total rainfall and all the observed and recorded breeding parameters.

RESULTS

The data for monthly rainfall, relative humidity, minimum and maximum temperature for Kulim station was provided by Meteorology Department, Petaling Jaya, Selangor (Table 1). The reading of water temperature, pH, conductivity and dissolved oxygen for Junjong River from March 1998 until February 1999 is presented in Table 2. The measurements of weight (W), snout-vent length (SVL) and head width (HW) is shown in Table 3.

Independent t-test showed there is a significant difference for weight ($t=-15.31$, $df=98$, $p<0.01$), snout-vent length ($t=-32.11$, $df=98$, $p<0.01$) and head width ($t=-23.16$, $df=98$, $p<0.01$) between male and female *R. labialis.*

The calling activities of male *R. labialis* occurred in every month from March 1998 until February 1999. These activities were less frequent in March 1998 (six times) and Jun 1998 (six times) but becoming more active in October 1998 (24 times) when the total rainfall increased to 553.3 mm. Only a few amplecant pairs of *R. labialis* were found during the observations, and a high number of the egg clutches were recorded in

TABLE 2. Water physico-chemical characteristics of Junjong River, Kulim, Kedah.

Month	Temp. (°C)	Con. (ms/cm)	DO(mg/L)	TDS(g/L)	pH
Mar 98	25	0.028	8.8	0.014	7.4
Apr 98	25	0.03	8.84	0.012	7.5
May 98	24	0.026	8.86	0.015	7.2
Jun 98	25	0.027	7.85	0.013	7.05
Jul 98	24	0.025	7.94	0.012	6.95
Aug 98	23	0.032	8.25	0.013	7.4
Sep 98	25	0.03	9	0.011	6.85
Oct 98	26	0.027	8.28	0.015	7.2
Nov98	24	0.034	8.36	0.014	7.6
Dec 98	24	0.02	8.17	0.011	7.3
Jan 99	24	0.027	8.45	0.012	7.25
Feb 99	25	0.027	8.25	0.014	7.4
Mar99	25	0.028	8.3	0.013	7.3
Total	**319**	**0.361**	**109.35**	**0.169**	**94.4**
Mean	**24.54**	**0.0278**	**8.412**	**0.013**	**7.262**
SD	**0.78**	**0.0034**	**0.361**	**0.00135**	**0.214**
Range	**23-26**	**0.020-0.034**	**7.85-9.0**	**0.011-0.015**	**6.85-7.60**

Note: Temp. = Temperature Con. = Conductivity DO = Dissolved Oxygen TDS = Total Dissolved Solids

August 1998 (three clutches) when the total rainfall increased to 412.7 mm. Tadpoles of this species in various stages of development were found in abundance in every month. All the breeding activities data are represented in Table 4.

We used Pearson correlation to determine the relationship between total rainfall and all the breeding parameters. The results showed a positive correlation between total rainfall and calling activities ($r=0.85$, $p<0.01$) (Fig. 2A), total rainfall and amplexus activities ($r=0.68$, $p<0.01$) (Fig. 2B) and between total rainfall and egg depositions ($r=0.82$, $p<0.01$) (Fig. 2C).

DISCUSSION

From the morphological measurements, we found that the values of snout-vent length (SVL), head width (HW) and weight (W) of males *R. labialis* are smaller compared to females. Earlier observations by several scientists also showed the same results (Berry, 1975; Inger and Stuebing, 1989; Inger and Iskandar, 2005). Shine (1979) mentioned that 90% of females frog have bigger size compared to males while Crump and Kaplan (1979) reported that the producing of eggs during breeding period by females frog is the reason why their size are bigger compared to males. The size of amphibians is also different between places although they are from the same species. Several factors such as habitat types, food sources and food quality influence the size of the amphibians (Banks and Beebee, 1986).

During the breeding period the male frogs started their calling activities to attract the females. Calling activity was the most important communication method made by anuran to attract mature female during reproductive period (Schwartz, 1994;

TABLE 3. Mean ± SD (Range, n=50) values of SVL (mm), HW (mm) and W (g) for *R. labialis*

Sex	SVL (mm)	HW (mm)	W (g)
Male *R. labialis*	43.38 ± 0.98	15.02 ± 0.71	8.76 ± 1.30
	(41.7-45.4)	(13.7-16.1)	(6.37-11.10)
Female *R. labialis*	57.25 ± 2.90	19.38 ± 1.14	13.18 ± 1.55
	(52.1-64.2)	(17.4-22.1)	(9.86-15.72)

Toledo and Haddad, 2005). Advertisement calls are important determinants of reproductive success and could have evolved in a common ancestor. Vocalizations in amphibians are also related to territory defense and competition for the calling sites (Bastos and Haddad, 2002). From this study, we found that the calling activities of *R. labialis* occurred every month and the presence of higher rainfall increased these activities. In March 1998, when the total rainfall was 35.1 mm, six calling activities were recorded. But in October 1998, when the total rainfall increased to 553.3 mm, 24 calling activities were recorded (4 times more). Pearson correlation between these two parameters showed a positive relationship and it indicated that the rainfall influences the calling activities of *R. labialis* in Junjong River. Several other scientists such as Girish and Kanamadi (2000), Savitha et al. (2004), Toledo and Haddad (2005) and Aichinger (1987) also reported the influence of rainfall on the calling activities of frogs in their studies. In some situations, a few calling males (not all) will stop their activities when they detected movements of observers and these became one of the weaknesses while observing this kind of parameters.

When the male frogs successfully attracted the females, amplexus will take place, in which the males clasp the axillary of females. They will stay in this position for a few hours until the females spawn their eggs in the water and the males fertilize the clutch. From our observations only a few amplecant pairs were found in certain months. However the correlation between the number of amplecant pairs and rainfall shows a positive relationship and this indicates that rainfall has an influence on amplexus activities. More egg clutches of *R. labialis* were found in months with higher rainfall. The highest being in August 1998 with three clutches when the monthly rainfall was 412.7 mm. Pearson correlation showed a strong positive relationship between the number of egg clutches and rainfall which again indicates the influence of rainfall on the spawning activities of this species in Kedah. The tadpoles of *R. labialis* in various stages of development were easily detected at their breeding sites every month and this showed that their breeding activities occurred throughout the year. In Singapore, the breeding activities of seven species of anura occurred throughout the year and the larvae for all the species were found almost every month (Berry, 1964).

The three breeding parameters (calling, amplexus and spawning) showed a positive correlation with the rainfall which indicated that rainfall plays an important role in stimulating the breeding activities of the frogs. Rainfall provides water for the puddles, rainpools and temporary ponds that become the breeding sites for the frogs (Zina and Haddad, 2005; Prado et al., 2005; Gascon, 1991). Several scientists such as Inger (1954), Church (1960a), Berry (1964) and Kanamadi et al. (1989) also reported the positive relationship between rainfall and the breeding activities.

TABLE 4. The breeding activities of *R. labialis* at Junjong River, Kulim, Kedah.

Month	Rainfall (mm)	Calling	Amplexus	Egg clutches	Larvae
Mar 98	35.1	6	0	0	Present
Apr 98	99	8	0	0	Present
May 98	357.8	10	1	2	Present
Jun 98	140.3	6	0	0	Present
Jul 98	213.5	12	0	0	Present
Aug 98	412.7	21	2	3	Present
Sep 98	274.4	18	0	0	Present
Oct 98	553.3	24	3	2	Present
Nov98	427.9	19	0	2	Present
Dec 98	224.9	16	1	1	Present
Jan 99	227.9	10	0	1	Present
Feb 99	124.9	12	1	0	Present
Mar99	182.1	8	0	1	Present
Total	**3273.8**	**170**	**8**	**12**	
Mean	**251.83**	**13.08**	**0.62**	**0.92**	
SD	**143.05**	**5.7**	**0**	**0**	
Range	**35-553**	**6.0-24**	**0-3**	**0-3**	

In Junjong River, the calling activities of male frogs were heard every month and the larvae of the frogs were present in every month eventhough there is light or heavy rainfall. This indicates that the breeding activities of *R. labialis* occurred throught out the year without any specific season. The increase of rainfall will increase the breeding activities and it indicates the role of rainfall as an extrinsic factor that activated and stimulated the breeding activities of these frogs.

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