Correlations of Reproductive Parameters of Two Tropical Frogs from Malaysia

Ibrahim H. Jaafar[1], Ahmad Ismail[2], and Abd-rahman Kurais[2]

[1]*Biological Sciences Program, School Of Distance Education, Universiti Sains Malaysia, 11800, Minden, Penang, Malaysia.* [2]*Department of Biology, Faculty of Science and Environmental Studies, Universiti Putra Malaysia, 43360,UPM, Selangor, Malaysia.*

Abstract.- A study on the relationships of reproductive parameters such as fecundity, egg size, clutch weight and body size of two frogs, *Rana cancrivora* (Gravenhorst) and *R. limnocharis (*Boie), from Malaysia was carried out in February 1992. The results showed that all the parameters quantified were greater in *R.cancrivora* than in *R. limnocharis.* No correlation was found between the spawned clutch size and egg size for both species. However, correlation analyses of unspawned clutch showed that there exist positive relationships between female size and clutch size, female size and clutch weight, female size and egg weight, clutch size and clutch weight, and clutch weight and egg weight in *R. cancrivora.* For *R. limnocharis* positive correlations were found between clutch size and clutch weight, and clutch weight and egg weight for the unspawned complement. The advantages, non-advantages, correlation and non-correlations of these parameters with respect to their reproductive strategies and success of these frogs are discussed and compared with the current information from the literature.

Key words.- Anura, Ranidae, *Rana cancrivora, R. limnocharis,* Malaysia, reproductive parameters, female size, clutch size, egg size, clutch weight, egg weight.

Introduction

The fecundity or clutch size in amphibians is highly variable depending on body size and reproductive mode (Salthe, 1969; Salthe and Duellman, 1973; Kaplan, 1980). As a general rule, large species produce more eggs than smaller ones and species that have generalized reproductive modes have larger clutches than those with specialised modes (Duellman and Trueb, 1986). The fecundity and hatching success of an amphibian population play an important role in the propagation and future survival of the population (Ibrahim and Ahmad, 1992). Reproductive success of a female depends upon the number of eggs produced and their quality, but are constrained by physiological capacities of the female and the trophic quality of the environment (Rafinska, 1991). The number of eggs deposited by amphibians varies greatly from species to species and this large disparity in fecundities is explained by differences among amphibians in sizes of eggs, patterns of development, sizes of females and reproductive behaviour (Porter, 1972; Salthe and Duellman, 1973; Kuramoto, 1978; Kaplan, 1980). Studies on the fecundity of temperate frogs have generated considerable data on this facet of amphibian reproduction (Collins, 1975; Kuramoto, 1978; Duellman and Trueb, 1986), but information on tropical oriental species are few and far between. Berry (1964) reported that seven anuran species in Singapore exhibited difference in fecundities between and within species, while four species of frogs from Malaysia also showed differences in fecundities both between and within species (Ibrahim and Ahmad, 1992). Inger and Bacon (1968) found that four large ranids in Borneo have clutch sizes in excess of a thousand eggs, while Yorke (1983) reported that the average clutch size for a rhacophorid in Malaysia to be 225 eggs.

Ovum size is another important aspect of anuran reproductive biology. Large ovum size is generally associated with large female body size and thus directly hints of better fitness and survival value. Egg size variability in amphibians has been interpreted as adaptive since, where breeding habitats vary unpredictably, production of a wide range of sizes may enhance adult fitness more than does the output of a single 'optimal' egg size (Tejedo and Reques, 1992). The egg size of numerous temperate anurans are well documented in the literature (e.g. Salthe, 1969; Salthe and Duellman, 1973; Bell and Lawton, 1975; Collins, 1975; Kuramoto, 1978; Jorgensen, 1981; Duellman and Trueb, 1986; Reading, 1986; Rafinska, 1991; Tejedo and Reques, 1992), but again however, only a few reports concerning this parameter are available for tropical species (e.g. Liem, 1959; Alcala, 1962; Inger and Bacon, 1968; Uchiyama et al., 1990).

Rana cancrivora (Gravenhorst) and *R. limnocharis* (Boie) are two species of anura that have a wide distribution across the oriental zoogeographical realm (Church, 1960; Alcala, 1962; Berry 1964; Inger, 1966; Kuramoto, 1978). In spite of this only a handful of information have been published concerning the fecundity and size of their eggs (Alcala, 1962; Berry, 1964; Kuramoto, 1978). It is also evident from the literature that there exist considerable variations in the relationships of body size, egg size and fecundity within the Amphibia (Salthe, 1969; Collins, 1975; Kuramoto, 1978; Jorgensen, 1981; Reading, 1986; Duellman and Trueb, 1986). This paper therefore determines the fecundity and egg size of both *R. cancrivora* and *R. limnocharis* and then examines the existing relationships in the reproductive parameters of both frogs.

The Study Site

The place chosen for the study is a rice growing region near the small town of Tanjung Karang (3° 20'N, 101° 10'E), in the state of Selangor, elevation about 3 m above mean sea level. It is situated about 100 km to the northwest of Kuala Lumpur, the Malaysian capital. Previously this area was a freshwater peat swamp forest which was opened up and converted to paddy fields by settlers in the 1920's. Presently due to the improved and upgraded irrigation and drainage system, the farmers are planting two rice crops a year.

Material and Methods

To determine the fecundity and egg size of spawned eggs, newly deposited egg clutches of both species were collected from recently innundated rice fields. Breeding choruses were visited, amplecant pairs of frogs were located and their positions marked with wooden pegs. Male frogs usually start calling as soon as dusk sets and individuals of both species usually initiated mating as soon as the males begin clasping the females. This usually occurs at around 0100hrs or 0200 hrs. The pairs will then move into the flooded rice paddy and egg laying will occur around 0500 hrs. The number of eggs in each clutch were then recorded and the eggs collected and preserved in 4% formalin and kept in labeled, airtight plastic 40 dram bottles. In the laboratory, 30 to 50 eggs from each clutch were then randomly chosen and their diameters measured under a stereo microscope with an ocular micrometer to determine the mean size of eggs of each species. All eggs measured were between Stages 3 and 9 of Gosner (1960).

To investigate the relationship between female body size and, (a) the number of unspawned eggs, (b) weight of unspawned egg clutch and (c) the weight of unspawned eggs, the snout-urostyle length of gravid females with large pigmented ova in the oviducts were measured, the unspawned egg complement were weighed (clutch weight) and counted (clutch size or fecundity). This is a modified method of Kuramoto (1978). Inger and Bacon (1968) reported that in most Indomalayan frogs the eggs in the ovary are essentially in two size classes : an enlarged or developing set and a much smaller, white 'undeveloped set' ; the latter contributes no significant portion to the total volume of the ovary.

Results

Forty-two spawned batches of *R. cancrivora* eggs and thirty-four of *R. limnocharis* were collected from the field in February 1992. These were investigated for fecundity and size of spawned eggs. In addition thirty-two batches of *R. cancrivora* eggs and thirty of *R. limnocharis* were obtained from sacrificed gravid females.

The results showed that the average number of eggs laid by *R. cancrivora* females from Tanjung Karang is 1077.9 (sd = ± 238.97, range 662 to1677). That for *R. limnocharis* 405.5 (sd = ± 92.45, range 233 to 657). Weighted t-test for unequal variance (Cochran and Cox, 1957) showed that the number of eggs oviposited by *R. cancrivora* is significantly greater than that laid by *R. limnocharis* ($t = 16.751$, $p < 0.001$, $df = 74$).

The mean diameter of *R. cancrivora* eggs is 1.35 mm (sd = ± 0.091, range 1.16 to 1.53) while the mean diameter of *R. limnocharis* eggs is 1.15 mm (sd = ± 0.027, range 1.10 to 1.21). Again a weighted t-test for unequal variance showed that the egg size for both species is significantly different ($t = 12.980$, $p < 0.001$, $df = 74$). However, no correlation was found between the spawned clutch size and egg size for both species.

For clutch complement of unspawned *R. cancrivora* eggs, the average clutch size was 2904.6 (sd = ± 1600.6, range 889 - 7573), the average clutch weight was 2.25 g (sd = ± 1.726, range 0.37 - 5.71) and the average egg weight was 0.75 mg (sd = ± 0.257, range 0.28 - 1.35). The same parameters for *R. limnocharis* was 702.6 (sd = ± 306.49, range 312 - 1659), 0.40 g (sd = ± 0.264, range 0.074 - 1.04) and 0.55 mg (sd = ± 0.249, range 0.237 - 1.01) respectively. Weighted t-tests for unequal variance on the clutch size, clutch weight and egg weight between the two species showed that these parameters were significantly different ($t = 7.634$, $p < 0.001$, $df = 60$; $t = 6.799$, $p < 0.001$, $df = 60$; and $t = 3.019$, $p < 0.05$, $df = 60$ respectively).

Correlation analyses carried out on the reproductive parameters of unspawned eggs for each species showed that for *R. cancrivora* there is a positive correlation between female size and clutch size ($p < 0.001$, $r = 0.6333$), female size and clutch weight ($p< 0.001$, $r = 0.6867$), female size and egg weight ($p < 0.05$, $r = 0.4073$), clutch size and clutch weight ($p < 0.001$, $r = 0.8870$) and clutch weight and egg weight ($p < 0.001$, $r = 0.5983$). There was no statiscally significant correlation between clutch size and egg weight. For *R. limnocharis* however, there exists positive correlation between clutch size and clutch weight ($p < 0.001$, $r = 0.7501$) and clutch weight and egg weight ($p < 0.001$, $r = 0.6818$) only, while no correlation was found between female size and clutch size, female size and clutch weight, female saize and egg weight, and clutch size and egg weight.

Discussion

The number of eggs oviposited by breeding females of both species were lower than that found in their ovaries. This is not unexpected because presumably the breeding females were depositing the complement of eggs that were mature and retaining the unripe ones for later oviposition (Inger and Bacon, 1968; Telford and Dyson, 1990). It is likely that both females of *R. cancrivora* and *R. limnocharis* in Tanjung Karang may breed more than once during the breeding season. Wells (1976) concluded that one of the major advantages of a prolonged breeding period may be the capacity for females to breed twice or more in a single season. Hence it is possible that as a security measure the females only release a portion of eggs from their ovarian complement. The incidence of multiple clutches is not uncommon in amphibians and have been reported in anumber of anuran species (Collins, 1975; Howard, 1978; Perril and Danial, 1983; Telford and Dyson, 1990; Rafinska, 1991).

Alcala (1962) and Uchiyama (1990) reported that the clutch size for *R. cancrivora* was probably more than 2000 and 1800 eggs respectively. Our study found a lower clutch size than these two. Berry (1964) presented data on the number of ovarian eggs in Singaporean *R. limnocharis*. Calculations based on her data gave the average clutch size for her samples as 576.4 eggs (range 266-1318). This is comparable to the results of this study. However, Kuramoto (1978) reported larger clutch size, clutch weight and egg weight for *R. limnocharis* from Japan. This discrepancy could be due to the clinal effect within the species (Duellman and Trueb, 1986) or even geographical differences, since the Japanese frog would be at the northernmost edge of its distribution.

The difference in egg size between different different populations of amphibian species have been attributed to adaptation of the population to their particular environment (Pettus and Angleton, 1967; Salthe and Duellman, 1973; Jorgensen, 1981). There exists a positive correlation between egg size and hatchling size in frogs (Salthe and Duellman, 1973) and a large metamorphic size is important because of the interrelationship of body size, time of metamorphosis, age at first reproduction and fecundity (Wilbur, 1972; Wilbur and Collins, 1973; Doty, 1978). Thus the observed larger egg size of *R. cancrivora* over *R. limnocharis* could probabaly be assumed to confer reproductive advantage with respect to fecundity, and could probably also be assumed to do the same with respect to metamorphic size, post metamorphic growth and age at first reproduction.

The interspecific differences in the sizes of eggs in amphibians is well documented in the literature (Inger and Bacon, 1968; Collins, 1975; Kuramoto, 1978; Duellman and Trueb, 1986). For example, Collins (1975) found that there is significant difference in average egg diameter between *Hyla versicolor, R. sylvatica, Bufo americanus* and *R. catesbeiana*, while for *H. crucifer* and *Psuedoacris triseriata* there was no significant difference. Generally this reflects the great variety in amphibian species with respect to egg production, reproductive modes and other demographic parameters (Salthe and Duellman, 1973; Kuramoto, 1978; Kaplan, 1980; Duellman and Trueb, 1986) as well as the diversity of adaptations to the different array of environmental selection and pressure. Phenotypic plasticity related to female age, size, trophic conditions and genetic factors also contribute to the observed egg size variation in amphibians (Berven, 1982; Rafinska, 1991). Hence it is not surprising that there exists significant difference in egg size between *R. cancrivora* and *R. limnocharis* in this study since , as mentioned earlier, a number of workers have concluded that within a reproductive mode, the greater the female size, the larger will be the ovum size (Salthe and Duellman, 1973; Kuramoto, 1978; Kaplan, 1980).

For *R. cancrivora* in this study, there exist positive correlations between female size and clutch size, female size and clutch weight, clutch size and clutch weight, and clutch weight and egg weight. Since body size is an indication of body condition, the high correlation between body size and clutch size is expected (Reading, 1986). Other workers also found that there is a positive relationship between clutch size and female body size (Pettus and Angleton, 1967; Salthe and Duellman, 1973; Collins, 1975; Howard, 1978; Kuramoto, 1978; Banks and Beebee, 1986; Gibbons and McCarthy, 1986; Reading, 1986). The positive relationship between clutch size and clutch weight, and clutch weight and egg weight in *R. cancrivora* is also anticipated because generally speaking, the greater the egg number, the greater will be the clutch weight, and the greater the egg weight, the higher the clutch weight. Kuramoto (1978) also found the same phenomenon in some of his Japanese anurans and similarly Reading (1986) found that there was a trend as a whole in the population of British *B. bufo* for egg weight, egg number and ovary weight to increase with female size.

Collins (1975) reported that there was positive correlation between egg diameter and female body size in *R. sylvatica, H. versicolor* and *B. americanus* from Michigan. Banks and Beebee (1986) found that snout-vent lenght is correlated with egg size in *B. calamita* from England and suggested that this was, in part, an adaptation to survive in habitats with erratic pond provisions. However, in this study no correlation could be found between female body lenght and egg size (as quantified by egg weight) in both *R. cancrivora* and *R. limnocharis*, nor could Kuramoto (1978) find a relationship between female body weight and egg weight in *R. limnocharis, R. tagoi, R. brevipoda, H. japonica* and *Rhacophorus schlegelii*. He attributed this event to the fact that all these species are lentic water-breeding frogs that generally produced their egg output, not in smaller number of large eggs but in larger number of small eggs. Thus no obvious advantage is procured in producing large eggs by large females. Also Jorgensen (1981) reported that egg size is not directly related to body size in *R. temporaria* in Denmark, while Howard (1978) wrote that the average egg size for *R. catesbeiana* from Michigan is not correlated to female size. Rafinska (1991) found no correlation between egg size and snout-vent lenght in European *Bombina bombina*, and Collins (1975) also found no statistically significant correlation between egg diameter and female body size in *P. triseriata, H. crucifer* and *R. catesbieana*. Why some species exhibited correlation between female size and egg size while others do not lack complete explaination at this point, and even Howard (1978) was convinced that the underlying reasons for such relationships remain unclear.

It is thus evident that variations occuring in the relationships of reproductive parameters in various amphibian species are results of selective forces and environmental constraints that shape their life history traits such that a species will optimize reproductive energy into producing the optimum number of offsprings to ensure future survival and continuance of the species. And since anuran species occupy various and diverse types of habitats and have different types of reproductive strategies, they are thus subjected to various differences in environmental pressures to which they must adapt in order to survive and prosper. This then manifests into the huge variability of amphibian reproductive demography as what we are witness to today.

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