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Reproductive Mode of *Fejervarya limnocharis* (Anura: Ranidae) Caught from Mae Sot, Thailand Based on Its Gonadosomatic Indices

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Abstract Amphibians show a variety of reproductive modes and strategies. The cyclicity and continuity of reproduction can often be predicted from the annual gonadosomatic index trends in a species specific manner. This research aims to document the female and male gonadosomatic index profiles and to ascertain the reproductive cyclicity or continuity of *Fejervarya limnocharis* (Anura: Ranidae) caught in Mae Sot, Tak Province, Thailand. Frogs were collected monthly between November 2007 and October 2009 in rice fields and their surrounding areas in the study site. For each frog, total weight and ovarian/testicular weight were measured to obtain the female and male gonadosomatic index. The number of female individuals with mature eggs (postvitellogenic eggs) was also counted. The results showed that female frogs had two main surges of increased GSI in March and September of 2008. Alternatively, male frogs showed a more gradual increase and decrease in gonadosomatic index, thus the index remaining high throughout the year. This study concluded that while *F. limnocharis* in this area is essentially a continuous breeder, it is more optimized for a cyclic reproduction mode with two breeding cycles during the rainy season.

Keywords rice frog, cyclic reproduction, egg deposition, GSI profile

1. Introduction

Amphibians show highly variable reproductive modes and strategies. According to Duellman and Trueb (1994), the diversity of reproductive modes for amphibians, especially frogs, is much greater than that observed in other groups of vertebrates, especially amniotes. Most tropical frogs exhibit seasonality in reproduction. According to Zug *et al*. (2001) rainfall is one of the major determinants for timing of reproduction. Timing and intensity of rainfall play a major role in determining when breeding should occur. Frogs in non-seasonal tropical environments often breed year round. These frogs often show a cyclical reproductive pattern. On the other hand, in seasonal tropical areas, breeding is usually timed to coincide with the wet season. Some species show explosive patterns while others exhibit a cyclical/continuous breeding mode throughout the wet season. The employment of continuous (acyclic) or discontinuous (cyclic) reproduction is developed accordance to egg incubation and the resulting hatchlings/neonates. These stages are fragile, and, therefore, need hospitable condition, maximum survivorship, minimum physiological stress, few predators and maximum food.

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Hence, timing reproduction with the optimal environmental condition (e.g., rain) is crucial in ensuring the survival of the eggs and neonates.

In tropical species, continuous reproduction is less widespread because much of the tropics, including Thailand, are seasonal (wet/dry or wet/wetter). Females are often cyclic because of the high energetic demand for oogenesis and vitellogenesis (Zug *et al*., 2001). Cyclic reproduction involves alternating reproduction readiness and quiescence. Tropical anurans are commonly cyclic, they have a breeding period that may begin just prior to the onset of the wet season. While most of tropical amphibians are cyclic, the acyclic ones have some levels of periodicity (i.e., population acyclic but not all members). In most tropical species cyclic reproduction is also complemented with synchronization with periods of high rainfall. Most cyclic amphibians are monoestrous with female producing one set of ova during each reproductive cycle, while some are polyestrous by producing multiple egg clutches (Duellman and Trueb, 1994). Multiple clutches within one reproductive cycle are common among tropical anurans. Species with continuous reproduction may reproduce all year round or reproduce cyclically but asynchronically. However, even with the knowledge that a species breeds cyclically, there is no knowing whether breeding occurs in a synchrony or asynchrony fashion.

Zug *et al*. (2001) stated that most frogs lay clutches of eggs comprising a large portion of their body mass. Therefore, egg development would constitute a large portion of their overall energy budget. The high energy demand is used for oogenesis and vitellogenesis. Data regarding ovary size during oogenesis and vitellogenesis on frogs are very limited. However, in rainbow trout (*Onchorhynchus mykiss*), during pre-vitellogenesis, the female gonadosomatic index remains a minimum 0.5% (Bon *et al*., 1997). The next period, the endogenous vitellogenesis showed an increase in gonadosomatic index along with the increase in vitellogenin level. There is a significant correlation between the two parameters. Gonadosomatic index would then continue to increase up to a maximum of 18%. Olmstead *et al*. (2008) reported that in *Xenopus tropicalis*, oocytes that are released from the ovaries pass through the oviducts while acquiring several protective jelly coats before being released for fertilization. The oviducts also become thickened and convoluted. At this stage (Stage VI), the size of the oocyte was the largest and they are postvitellogenic and ready for ovulation (Dumont, 1972)

In many cases, reproductive development and timing of reproduction can be predicted from gonadosomatic indices. Often, gonadosomatic indices are the highest just prior to mating. However, references on the fluctuation of gonadosomatic index of amphibians are quite scarce and data related gonadosomatic indices of *Fejervarya limnocharis* are even more limited. Therefore, this paper aims to document the female and male gonadosomatic indices profiles of *F. limnocharis* caught from Mae Sot, Tak Province, Thailand for future reference. The gonadosomatic indices profile is then used to predict the breeding pattern of *F. limnocharis* in the study area in accordance to its rainfall pattern (Figure 1).

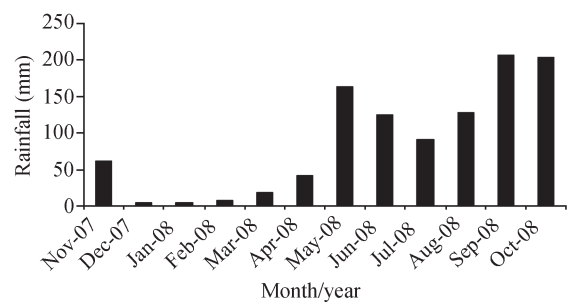

Figure 1 Monthly total rainfall in Mae Sot, Tak, Thailand

2. Materials and Methods

2.1 Study sites Frogs were collected on a monthly basis from November 2007 to October 2008 in rice fields and their surrounding areas in Mae Pa and Mae Tao in Mae Sot District, Tak Province. Mae Pa is located at 16°40′43″N and 98°35′36″E, and the area is irrigated by Huay Luek Creek. Mae Tao is located 8.4 km south of the Mae Pa at 16°45′13″N and 98°35′25″E, and is irrigated by the Mae Tao Creek. Both areas are highly used for agriculture, hence their dependence on irrigation.

2.2 Methods One hundred and ninety-seven frogs were caught live (hand-caught and net-caught) at night during visual encounter surveys (Crump and Scott, 1994), and then they were placed in a plastic aquarium. The frogs were then transported live to the lab. The frogs were individually subjected to cold anesthesia procedure before sacrificing by double-pith at brain and spinal cord (National Research Council, 1974; Suckow *et al.*, 1999; Tharp and Woodman, 2002). Specimens were then weighed and their snout-vent length (SVL) measured before they were dissected. Testis and ovary were removed and weighed. Gonadosomatic indices (Goodwin *et al*., 1992; Tilton *et al.,* 2003; Maitra *et al*., 2007) were calculated for each frog. These indices were based on the weight ratio of each gonad to the body weight. The numbers of females bearing postvitellogenic eggs were also counted. The authors assure that all portions of this research project involving animal subjects had been conducted in accordance with the National Code of Ethics for Laboratory Use by the National Research Council of Thailand and the Wild and Protected Animal Act of Thailand 1992. The sampling methods and animal handling techniques had been approved by the Graduate School Committee of Chulalongkorn University, Thailand.

2.3 Statistical analysis All data were statistically analyzed either with one-way ANOVA followed by Student-Newman-Keuls test (for female GSI) or Kruskal-Wallis one-way ANOVA on Ranks followed by Dunn's test (for male GSI) using the SigmaStat 2.0 program.

3. Results and Discussion

Figure 2 shows the monthly average of female gonadosomatic index (GSI) of *F. limnocharis* caught from Mae Sot, Tak, Thailand. The gonadosomatic indices ranged from 1.151 to 12.367. The graph showed that the female GSI indices presented two bottoms during the months of November 2007 (GSI= 1.890) and December 2007 (GSI= 1.151), and two tops in March, 2008 (GSI= 12.367) and in September, 2008 (GSI = 11.313).

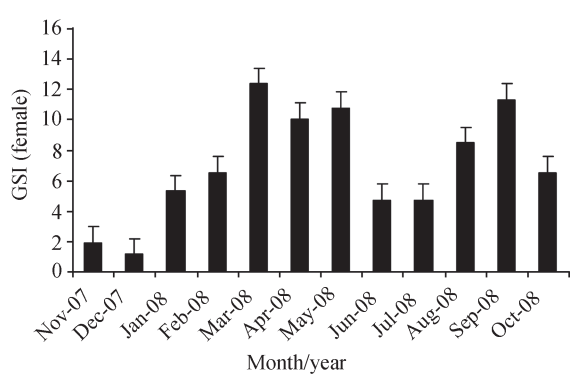

Figure 2 Monthly average of female gonadosomatic index of *F. limnocharis* caught from Mae Sot, Tak. The differences between the months are statistically significant ($P < 0.001$) .

From Figure 3, it is shown that the percentage of females bearing postvitellogenic eggs ranged from 16.7% to 33.3% during the months of November 2007, December 2007 and January 2008. This number increased to 50.0% in February 2008 and then plateaud during the months of March, April and May 2008. All of the female frogs caught during these three months were bearing mature eggs. The percentage of gravid females then fluctuated during the months of July 2008 to October 2008.

Male GSI was the lowest in November 2007 (GSI= 0.095). GSI gradually increased to its highest value of 0.300 in April 2008. It then showed a slow decline during the months of May 2008 to September 2008 before dropping to 0.137 in October 2008.

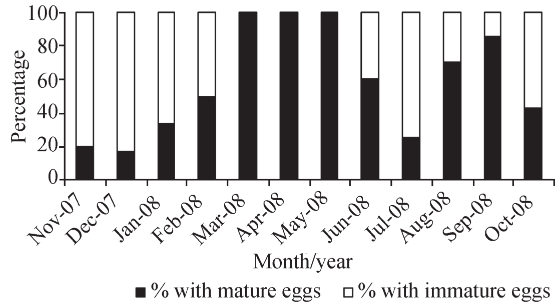

Figure 3 Monthly percentage of females bearing mature/immature eggs of *F. limnocharis* caught from Mae Sot, Tak

Our results showed that there was a gradual increase of female GSI between November 2007 and April 2008. The same pattern was also observed in the male GSI of the rice frog. This may indicate that during this period, the female frogs were at the preparation stage to produce mature eggs, while sperms were developing and increasing in number. This was to coincide with time of the expected start of rainy season during the months of May and June. This was complemented by an increase in the percentage of females bearing mature eggs. At the same time, the percentage of females with immature eggs was also falling. This means that the majority of the females were ready for breeding. The months of March, April and May showed that all the females sampled had mature eggs, which indicated their readiness for the egg deposition process. The breeding of *F. limnocharis* is triggered by rain (AmphibiaWeb 2008) and it is usually the first species to come to the calling sites. Therefore, in order for the rice frogs to be the first species to come to the calling sites during the early wet season, their ovaries and testes will have to start developing and maturing before the start of rainy season.

Beginning May 2008, an increase in rainfall signified the beginning of the rainy season. The increase in rainfall was followed by a drop in female GSI during the months of June and July. The drop in female GSI may be justified by the egg deposition by some of the females. During these months, we could also observe the reduction in the percentage of females bearing mature eggs and the resurgence of the females bearing immature eggs.

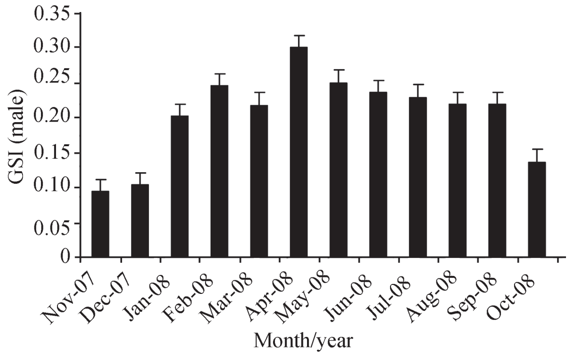

Figure 4 Monthly average of male gonadosomatic index of *F. limnocharis*. The differences between the months are statistically significant ($P < 0.001$).

This could be interpreted as these females having had deposited their eggs and were preparing for the next cycle of egg development. The male GSI remained high during this period, suggesting a high reproductive development, followed by high reproductive activities.

After that, an increase in rainfall during the months of August and September was followed by an increase in female GSI again, insinuating that a second cycle of egg development and egg deposition was under way. Then, during the month of October, both the female GSI and the percentage of females bearing mature eggs dropped, indicating a second wave of egg deposition had occurred. All this while, the male GSI remained high.

Looking at the trend, it is quite apparent that there were two main waves of increased female GSI. The first surge occurred just before the beginning of rainy season and the second surge occurred towards the end of the season. This is in line with the suggestions that *F. limnocharis* in Tanjung Karang, Malaysia may breed more than once during the breeding season (Jaafar *et al*., 1999). However, our study could not determine whether this species lays multiple clutches of eggs during the season. Similar trend was observed with the percentage of gravid females. However, it was also obvious that even during the drier months, females with mature eggs still existed. This may suggest that *F. limnocharis* is essentially a continuous breeder. However, because they live in a wet/dry seasonal area, they are optimized to the cyclic reproduction mode which is synchronized with rainfall.

According to Kang e*t al*. (1995), testosterone levels in male *X. tropicalis* have been reported to vary widely between summer and winter months, while Olmstead *et al*. (2008) reported that there was evidence of cyclicity in testosterone levels in the same species. Since testicular development and reproductive maturation often follow testosterone level, it could be insinuated that that in *X. tropicalis*, there would be cyclicity in the male gonadosomatic index as well. Our results seemed to agree with this notion. In our study, we found that cyclicity in male gonadosomatic index of *F. limnocharis* was quite apparent, where the index was lower during the drier months and gradually increased in the months just prior to rainy season. However, our result was not consistent with the trend showed in *Rana dybowskii* (Ko *et al*., 1998), where a single and obvious peak of male GSI was observed characterized by a sharp increase prior to and a sharp decrease after the peak. Instead, our result showed that there was a gradual increase and decrease of gonadosomatic indices throughout the year. When we compared with the female frogs, the male GSI, however, did not show similar trend with the female gonadosomatic index where two main waves of increased gonadosomatic index were observed. The male gonadosomatic index remained high during most months of the year. This showed that for male, there was a continuous readiness to mate throughout the mating season. However, additional observations are required to justify this notion. It is imperative to state that this study was done for a period of one year in a specific location in Thailand.

4. Conclusion

To date, there are very limited data regarding the gonadosomatic index fluctuation in amphibians and for *F. limnocharis*, and the data are almost non-existent. This paper aims to document that fluctuation. In Mae Sot, Thailand, we found out that while this species is essentially a continuous breeder, the fluctuation in its female GSI suggests this species is more optimized for a cyclic reproduction mode with two cycles of reproduction during the rainy season. The highest female gonadosomatic index is recorded during the months just prior to the rainy season and towards the end of the rainy season. The fluctuations in the male gonadosomatic index are less enhanced, suggesting that the male is sexually ready throughout the year.

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