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SEASONAL CHANGES IN PLASMA GONADAL STEROIDS *IN RANA TIGERINA RUGULOSA* AND *RANA CATESBEIANA*

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ABSTRACT

Annual plasma gonadal steroids, estradiol 17β (E_2) and estosterone (T) were determined by RIA to demonstrate their seasonal variations. Plasma were collected from the adult male and female *Rana tigerina rugulosa* and *Rana catesbeiana* of the age between 12-18 months. Plasma E2 and T concentrations in the female *Rana tigerina rugulosa* peaked at 2,000 pmol/L and 1,000-2,000 pmol/L, respectively, between April and August. The lowest levels of both hormones (100-200 pmol/L) were observed between November and February. Similar pattern was found in the plasma T profile of the male *Rana tigerina rugulosa* which exhibited the highest value between May and October. Unlike the *Rana tigerina rugulosa*, female *Rana catesbeiana* exhibited higher values of E_2 (1,000 -5,000 pmol/L) and T (1,000 -2,500 pmol/L) all year round. The plasma T levels in male *Rana catesbeiana* were about 1,000-2,500 pmol/L with the lowest value (700 pmol/L) observed in January. Gonadosomatic index percentage (GSI %)of both sexes in both species showed correlation with the plasma gonadal steroids. Results obtained suggest that the *Rana tigerina rugulosa* exhibited breeding seasonality while the *Rana catesbeiana*

tended to have a longer breeding activities when both species were reared and kept in the similar environmental conditions.

Key words : Rana tigerina rugulosa, Rana catesbeiana, estradiol 17β, testosterone

INTRODUCTION

Reproductive behavior in most vertebrates is widely understood to be affected by the hypothalamo-pituitary gonadal axis. The relationship between hormonal secretion and gonadal function has long been specifically recognized in frogs. Previous studies have found plasma gonadotropins and gonadal steroids at high concentrations during breeding seasons in bull frogs, *Rana catesbeiana*, (Licht et al., 1983) and in green frogs, *Rana esculenta*, (Pierantoni et al., 1984). Observed incrementation of androgen secretion in male *Rana catesbeiana* has therefore been interpreted as an indicator of sexual capability at the time of breeding season (Licht et al., 1983). Preovulatory surges of estradiol -17 (, progesterone and testosterone , in female *Rana catesbeiana* have also been documented (McCreery and Licht, 1983).

It has been proposed that *Rana tigerina rugulosa*, (Frost, 1985) a frog in parts of Southeast Asia including Thailand, breeds exclusively during the rainy season (between May and October) and estivates during the dry season (between November and February) (Pariyanonth et al., 1985). But to our knowledge there have been no studies to document hormone secretion patterns in population of *Rana tigerina rugulosa*. The initiation and duration of seasonal breeding cycles in *Rana catesbeiana* have been shown to be affected, through a mechanism which is not thoroughly understood, by lattitude and temperature. (Byrne and White 1975; Licht et al., 1983). Humidity may also be an important factor as the breeding season of certain species of tropical frogs has been shown to be initiated by an increase in humidity (Whitter and Crew, 1987).

Frog farming in Thailand has become a commercial success due to the availability of synthesized frog chow and the use of hormonally-induced breeding. *Rana tigerina rugulosa*, local frog, and introduced *Rana catesbeiana* are now widely bred and reared in frog farms in Thailand as an agricultural product. Even though some of these farms induce breeding artificially with gonadotropin

releasing hormone, no data on the seasonal hormone secretion patterns of *Rana catesbeiana* or *Rana tigerina rugulosa* in Thailand have previously been published. Bearing these things in mind, we have recorded adult male and female *Rana tigerina rugulosa* and *Rana catesbeiana* for their body weight, gonadal weight, and plasma gonadal steroid concentrations with the expectation of demonstrating relationships between season, reproductive behavior pattern, and endocrinological profile.

This information will be of immediate practical use in frog farms in Thailand. If it can be shown that one or more species of *Rana* are capable of reproduction throughout the year in Thailand, then stock may be bred continuously for commercial or academic use rather than exclusively in the wet season. If present or subsequent studies indentify optimal environment or physiological conditions in which Rana tigerina rugulosa and *Rana catesbeiana* reproduce, then this information will also be of practical value for the regulation of such conditions, such as is possible, or the appropriate location of future frog farms to take advantage of existing ideal or nearly ideal environmental conditions.

MATERIALS AND METHODS

Animals

Adult male and female frogs, Rana tigerina rugulosa and Rana catesbeiana between 12 and 18 months of age were used. Frogs of both species were bred and reared in concrete tank 2.0 x 2.5 x 1.0 m3, with constant water level of 10 cm. depth which was changed every two days. Animals were fed twice daily (5% body weight / kg) with frog chow and were kept in natural environment in Pechaburi Province. The average ambient temperature ranged from 25.0-29.6° C and relative humidity was about 69-81 % throughout the year (Chaitiamwong, 1995). The natural daily light and dark cycle were approximately 12:12 hours.

Ten animals of each sex and each species were obtained every month from January to December 1994. Frogs were moved to the laboratory and kept in ba skets one day prior to the sample collection. Animals were weighed and killed by decapitation. Trunk blood was collected in heparinized tubes. Ovaries in the female and testes in the male were dissected out and weighed. Plasma samples were kept at -20° C until assayed.

Hormone assay

Plasma estradiol -17β (E_2) and testosterone (T) were determined by radioimmunoassay. The validity of the assay using polyclonal antibodies against E2 and T for frog's plasma was done as described previously (Chaitiamwong, 1995). The assay was performed as follows: Duplication of 200 µl of plasma from each sample were extracted in 5 ml diethyl ether. After drying, each extract was dissolved in 500 µl phosphate buffer saline (0.05 M, pH 7.4). The 100 µl of hormone tracer and 100 µl of antiserum were added. The mixture was incubated at 4 °C for 18-20 hours. Bound and free steroid were separated by dextran-coated charcoal.

RESULTS

Plasma gonadal steroid concentrations in male and female Rana tigerina rugulosa

As shown in figure 1, Plasma E_2 concentration in female *Rana tigerina rugulosa* peaked at approximately 2,000 pmol/L between March and May. E2 concentrations were lower (approximately 1,000 pmol/L) from June to September before reaching their lowest levels (between 100 and 200 pmol/L) between November and February. Plasma T levels in female *Rana tigerina rugulosa* (figure 1) followed a similar pattern. The highest levels were recorded between April and August (1,000 and 2,000 pmol/L) and the lowest levels (100 and 200 pmol/L) were observed between November and February. Plasma T levels in male *Rana tigerina rugulosa* (figure 1) peaked noticeably in May (at approximately 3,000 pmol/L) and remained comparatively high (at approximately 1,500 pmol/L) until October, and were at their lowest (approximately 100-200 pmol/L) between November and February.

Gonadosomatic index percentage in Rana tigerina rugulosa

Figure 2 depicts the pattern of gonadosomatic index percentage (GSI%) or gonadal weight as percentage of body mass, in female Rana tigerina rugulosa. GSI% was lowest (approximately 0.1) between November and January (data for February are not available) and highest in April and May (more than 0.7), steadily decreasing between May and December. Figure 3 shows male Rana tigerina rugulosa GSI% to be comparatively low (< 0.1) between November and January and at high levels (between 0.2 and 0.25) between March and September.

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Plasma gonadal steroid concentrations in male and female Rana catesbeiana

E_2 levels in the female *Rana catesbeiana* (figure 3) fluctuated throughout the year, peaking once in February at approximately 4,000 pmol/L, then decreased to their lowest levels in May (< 1,000 pmol/L) before irregularly increasing to their highest levels in September (at approximately 5,000 pmol/L) and falling to approximately 2,500 pmol/L in November. T levels in female Rana catesbeiana (figure 3) were comparatively stable, reaching comparatively low levels in April and November (although still more than 1,000 pmol/L) and their highest levels in June (approximately 2,500 pmol/L). Plasma T concentrations (figure 3) in the male *Rana catesbeiana* peaked in April (at approximately 2,500 pmol/L), then decreased steadily until January. The lowest plasma T level observed was approximately 700 pmol/L.

Gonadosomatic index percentage in Rana catesbeiana

GSI% in female *Rana catesbeiana* is depicted in figure 4. Values fluctuated throughout the year but comparatively high values were found in January, April and June (between 5 and 6.5) with other months ranging in value between 2.5 and 5. GSI % in male Rana catesbeiana remained comparatively constant throughout the year, reaching a minimum of about 0.1 in June and a maximum of 0.17 in February (figure 4).

DISCUSSION

Our results of plasma gonadal steroid concentrations in the male and female *Rana tigerina rugulosa* seem to confirm the breeding season of this frog in nature (Pariyanonth et al., 1985). The lowest values, however, of all steroids studied in both sexes between November and February are consistent with an estivation status. The high values of gonadal steroids found in between March and October, peaking in May or June indicates sexual activities in this animal as were found in Rana catesbeiana (Licht et al., 1983) and Rana esculenta (Pierantoni et al., 1984). The high value of GSI% in this female frog between April and August also indicates the mating possibility (McCreery and Licht, 1983). The readiness of mating in the female is concomittant with the period of spermatogenesis in the male frog when the GSI % value is high (Yoneyama and Iwasawa, 1985). The GSI % and plasma testosterone concentrations was found to be highly correlated in male *Rana tigerina rugulosa* (Chaitiamwong, 1995).

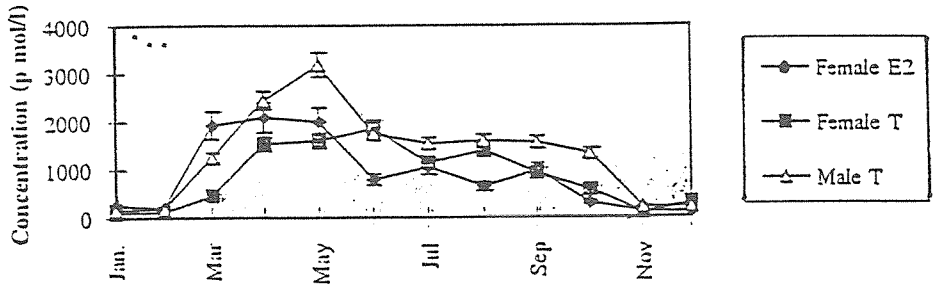

Figure 1. Annual profiles of plasma gonadal steroid concentration (pmol/L) in male and female *Rana tigerina rugulosa* collected during January-December 1994.
(—O— female E2, —□— female T, —Δ— male T)
Each point represents the mean ± SEM (n = 10)

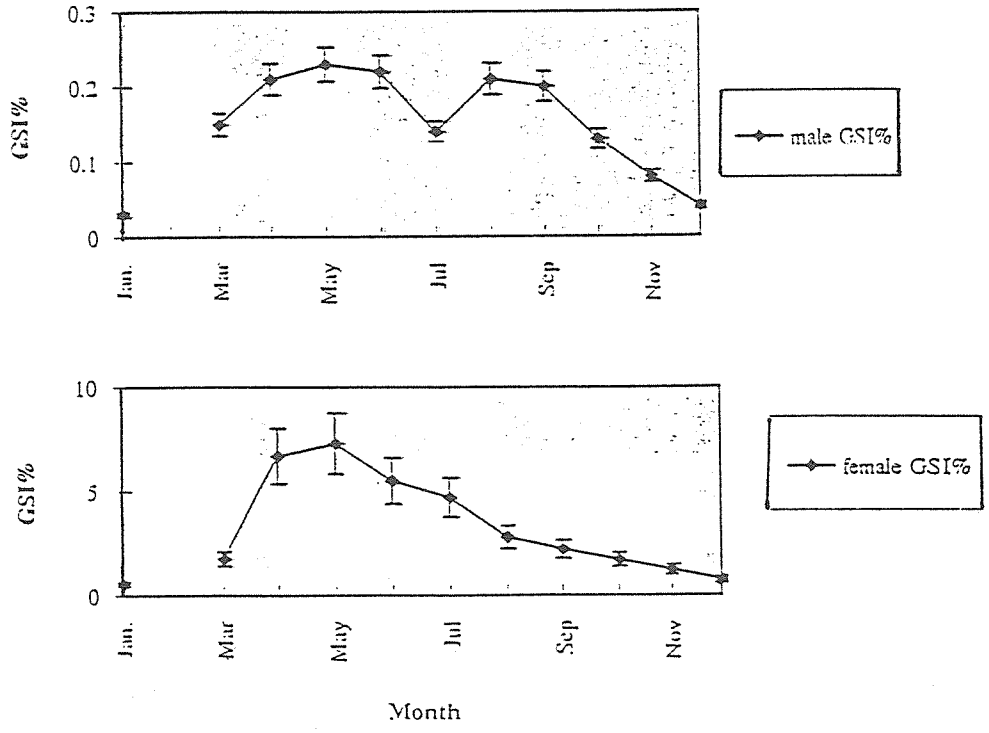

Figure 2. Annual gonadosomatic index percentage (GSI%) in male and female *Rana tigerina rugulosa* collected during January-December 1994.

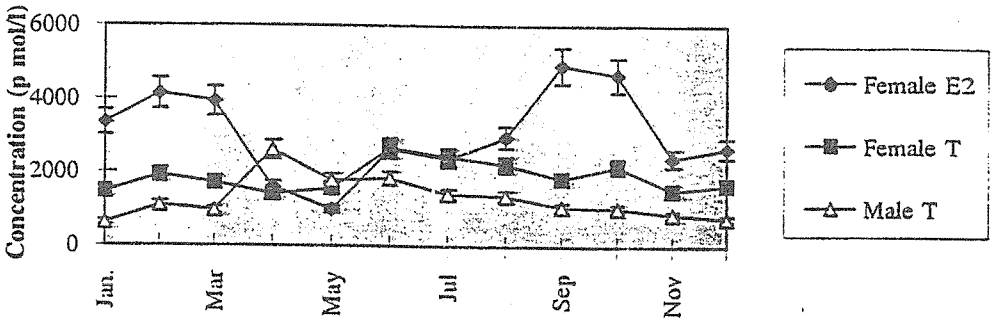

Figure 3. Annual profiles of plasma gonadal steriod concentration (pmol/L) in male and female *Rana catesbeiana* collected during January-December 1994.
(—●— female E2, —■— female T, —△— male T)
Each point represents the mean ± SEM (n = 10)

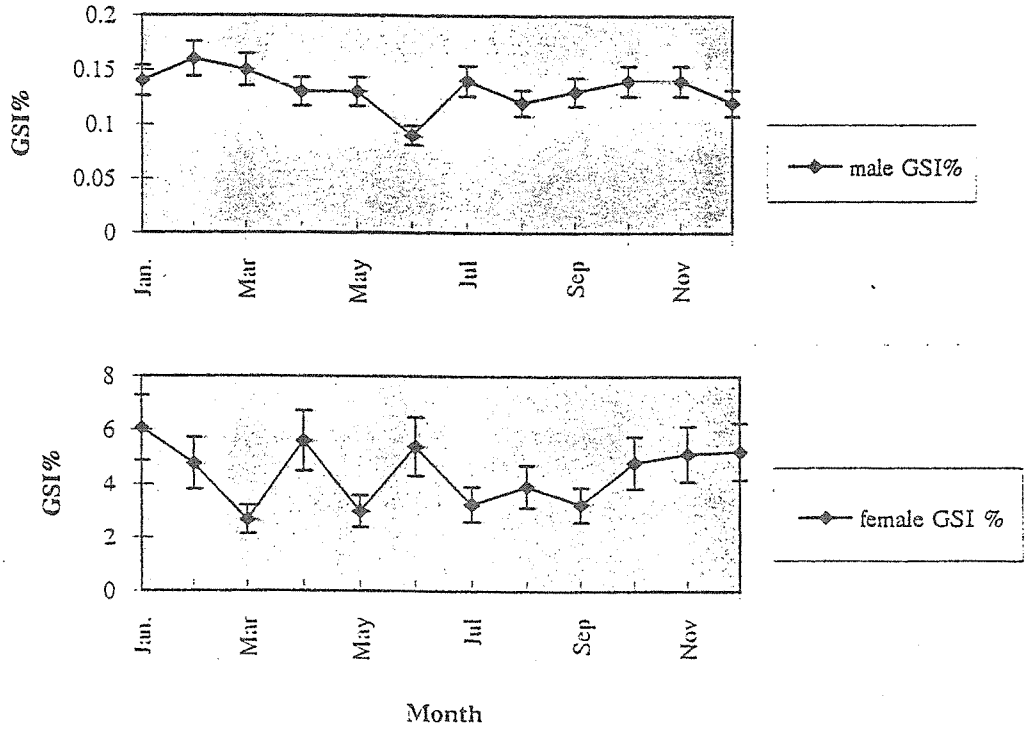

Figure 4. Annual gonadosomatic index percentage (GSI%) in male and female *Rana catesbeiana* collected during January-December 1994.

The fluctuation of plasma testosterone concentration as well as the GSI % in male Rana catesbeiana were not remarkable throughout the year. The testosterone values were at their lowest in December and January and peaked in April before slightly and steadily decreasing till December and reached the lowest in January. The lowest plasma T level observed (approximately 700 pmol/L) were much higher than those of minimum T levels in the male Rana tigerina rugulosa (100-200 pmol/L). The unremarkable drop in male GSI% to 0.1 during June is not significantly different from other values found during the rest of the year. Thus, its seem that high concentration of gonadal stroids in the male frog could indicate its sexual activity which may occurred in almost every month of the year (Mendoca et al., 1985). The consistent high level of E_2 in the female *Rana catesbeiana* which reach their peaks in February and September (figure 3) and the high value of GSI % indicates the sexual ability in this female frog (McCreery and Licht, 1983 ;Yoneyama and Iwasawa, 1985) The duration of sexual ability in both sexes of this frog reared in Thailand seems to happen all year round. This may confirm the finding of Byrne and White (1975) that the breeding cycle of the *Rana catesbeiana* in lower lattitude of North America, where the temperature is warmer, begins earlier and last longer than those live in the higher lattitude. Therefore the frog reared in Thailand which located in lower lattitude tend to have a longer breeding activity. There is also an evidence that *Rana erythraea*, found in Borneo, breed all year round under the constant environmental conditions (Inger and Greenberg, 1963). However, the relationship between ganadal steroids and actual mating capability in both species reared in Thailand needs to be more investigated.

Results obtained indicated that the *Rana tigerina rugulosa* exhibits breeding seasonality irrespective of no food and water restriction. The *Rana catesbeiana*, however, tend to exhibit longer sexual activity periods than the *Rana tigerina rugulosa* under similar environmental conditions. Since *Rana catesbeiana* is the one of many temperate amphibian species which should be able to change their breeding behaviour as temperature of breeding ponds become warmer (Caldwell, 1986; Seale, 1982). From this results it can be shown that these two species of frogs should be reared together in the farm, therefore the product of the frog meat can be produced throughout the year.

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บทคัดย่อ

ได้ทำการวัดปริมาณฮอร์โมนเอสตราไดออล 17 บีต้า และเทสโทสเตอโรนในพลาสมาของกบนา และกบบูลฟรอกโตเต็มวัยที่มีอายุระหว่าง 12 ถึง 18 เดือน โดยวิธีเรดิโออิมมูโนเอสเสย์ พบว่ากบนาเพศเมียมีปริมาณของฮอร์โมนเอสตราไดออล 17 บีต้า ถึง 2,000 พิโกโมลต่อลิตร และเทสโทสเตอโรนสูง 1,000 ถึง 2,000 พิโกโมลต่อลิตร ระหว่างเดือนเมษายนถึงเดือน สิงหาคม และมีระดับต่ำสุดระหว่าง 100 ถึง 200 พิโกโมลต่อลิตร ในช่วงเดือนพฤศจิกายนถึง เดือนกุมภาพันธ์ และปริมาณของเทสโทสเทอโรน-ในพลาสมาของกบนาเพศผู้สูงสุดในช่วงเดือน พฤษภาคมถึงเดือนตุลาคม ส่วนในกบบูลฟรอกพบว่าในกบเพศเมียจะมีปริมาณฮอร์โมนเอสตราไดออล 17 บีต้า สูง 1,000 ถึง 5,000 พิโกโมลต่อลิตร และเทสโทส-เตอโรนมีปริมาณสูง 1,000 ถึง 2,500 พิโกโมลต่อลิตรตลอดทั้งปี และปริมาณเทสโทสเทอโรนในกบเพศผู้จะอยู่ในปริมาณ 1,000 ถึง 2,500 พิโกโมลต่อลิตรเกือบตลอดทั้งปี ยกเว้นในเดือนมกราคมวัดได้เพียง 700 พิโกโมลต่อลิตร ทั้งนี้ค่าของฮอร์โมนเอสตราไดออล 17 บีต้า และเทสโทสเตอโรนในพลาสมากบทั้งสองชนิดจะสอดคล้องกับน้ำหนักของอวัยวะสืบพันธุ์ จากผลการศึกษานี้แสดงให้เห็นว่ากบนาเป็นกบพื้นเมืองและกบบูลฟรอกเมื่อนำมาเลี้ยงในสภาพแวดล้อมที่เหมือนกัน กบนามีการสืบพันธุ์เป็นฤดูกาล ส่วนกบบูลฟรอกมีแนวโน้มที่จะมีการสืบพันธุ์ได้ในช่วงที่ยาวนานกว่ากบนา

คำสำคัญ : กบนา, กบบูลฟรอก, เอสตราไดออล 17 บีต้า, เทสโทสเตอโรน