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The Male Reproductive Cycle of the Toad, *Bufo bankorensis*, in Taiwan

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Wen-San Huang, Jun-Yi Lin and John Yuh-Lin Yu (1996) The male reproductive cycle of the toad, *Bufo bankorensis*, in Taiwan. *Zoological Studies* **35**(2): 128-137. Studies of the male reproductive cycle in the toad, *Bufo bankorensis* were conducted on spermatogenetic activity, direct measurements of plasma androgen, and changes in the weights of testes, liver and fatbody. A total of 110 male *Bufo bankorensis* were collected from March 1990 to March 1991 in central Taiwan. Histological evidence indicates that the spermatogenetic cycle of this toad is of a potentially continuous type. Although cell nests of all spermatogenic types were present every month of the year, the greatest intensity of spermatogenetic activity (sperm bundles and mature sperm) occurred during August to November. Plasma androgen levels exhibited a significant difference in level between September and June (mean values = 93 ng/ml and 2.5 ng/ml, respectively). The weights of fat bodies peaked during the months of May, June and July, coincident with the beginning of breeding, and they had regressed in December by the end of the period of intense spermatogenetic activity. Testicular weight increased slowly in August and peaked in September. Combined data from spermatogenetic activity, plasma androgen, and changes in weights of testes, fat bodies, and livers showed that *B. bankorensis* is a potentially continuous breeder. However, its annual reproductive cycle could be clearly divided into these periods: 1) breeding period (August to November); 2) postbreeding period (December to February); 3) reproductive energy preservation period (March to May); and 4) torpid period (June to July).

Key words: Reproduction, Bufonidae, Spermatogenesis, Androgen, Taiwan.

Bufo bankorensis is an endemic species of toad in Taiwan (Zhao and Adler 1993). In the past, *B. bankorensis* was commonly treated as a synonym of *Bufo bufo gargarizans* (Lue 1990), but was regarded by Matsui (1986), as a distinct species. It is one of only 2 species of Bufonidae in Taiwan. *B. bankorensis* and *B. melanostictus* are distributed, respectively, in high elevation mountains and lowland plains. Few investigations on *B. bankorensis* have been conducted, but they include a study by Lue and Chen (1982) dealing with morphology and distribution.

Amphibians exhibit remarkable diversity in reproductive patterns (Duellman and Trueb 1986). The annual spermatogenetic cycles in the anurans are grouped into 3 categories: discontinuous, potentially continuous and continuous types (Lofts 1974). Temperate-zone species are of the discontinuous type. They generally have discrete seasonal cycles of reproduction with pronounced changes in gonadal size, gamete production and sexual accessory structure, (e. g., *Rana esculenta*, Lofts 1964, and *R. temporaria*, Lofts et al. 1972). In the potentially continuous type there is a partial cessation of spermatogenetic activity during some months, but primary spermatogonia remain sensitive to gonadotrophic stimulation, (e. g., *R. tigrina*, Saidapur and Nadkarni 1975). Those species inhabiting tropical areas, where climatic conditions do not show appreciable fluctuation, have evolved a continuous type of spermatogenetic cycle, (e. g., *R. cyanophlyctis*, Saidapur and Nadkarni 1973,

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B. melanostictus, Kanamadi et al. 1983, and *R. limnocharis*, Alexander et al. 1979, Sun 1979).

Studies of plasma hormone levels in wild animal species provide useful information concerning endocrine control mechanisms. Characterization of the annual cycle of plasma sex steroid levels is especially important in the study of reproduction in seasonally breeding species. Seasonal changes in plasma steroid levels have been reported in a number of amphibian species: *R. esculenta* (d'Istria et al. 1974), *R. pipiens* (Basu and Nandi 1965, Wada et al. 1976), *B. mauritanicus* (Siboulet 1981), *R. catesbeiana* (Licht et al. 1983), *Dicroglossus occipitalis* (Kuhn et al. 1987), *R. perezi* (Delgado et al. 1989) and *B. japonicus* (Itoh and Ishii 1990a,b), and *R. rugulosa* (Kao et al. 1993). However, plasma steroid levels have been studied in only 2 species of Bufonidae, *B. mauritanicus* (Siboulet 1981) and *B. japonicus* (Itoh and Ishii 1990a).

This paper reports: 1) the circennial changes in the weights of body, testis, liver and fat body; 2) the spermatogenic cycle; 3) plasma androgen levels; and 4) the correlations of plasma androgen levels with seasonal environmental factors (rainfall and temperature), with spermatogenesis and with other reproductive activities.

MATERIALS AND METHODS

Study site

The toads in this study were collected from 5 sites along the Honken River in DehKen (24°10'N, 120°43'E), Taichung City, central Taiwan. *Bufo bankorensis* generally inhabits cultivated bamboo fields, road and river sides, and bodies of water, where several of the collected toads were found during the breeding period (Fig. 1). The temperature patterns in 1990-1991 are summarized in Fig. 2. The mean temperatures in March and July, 1990 were 19 °C and 28 °C, respectively, and decreased to a minimum of about 17 °C in January, 1991.

Collection of blood and tissue

The toads were collected at night from the 5 sites on the 5th, 15th and 25th of days each month from March, 1990 through March 1991. Numbers of toads collected each month are shown in Table 1. In the torpid period, it was difficult to find toads, especially in July. The collection of blood and tissue were done in the laboratory on the next day following capture of the toads. All toads were weighed, and measured before being killed in the laboratory; the weights of testes, fat bodies, and liver were recorded. To minimize body weight differences of individual toads, weights of organs were expressed as an organosomatic index as

Table 1. Changes in the mean weights of body, testis, liver, and fatbody during an annual reproductive cycle of *Bufo bankorensis*[a]

Month	N[b]	Body		Organ weights		
		length (cm)	weight (g)	testes (mg)	liver (g)	fat body (g)
1990						
Mar	2	63.8 ± 6.1	23.7 ± 6.1	44.3 ± 6.1	0.82 ± 0.07	0.18 ± 0.02
Apr	8	66.8 ± 6.0	32.3 ± 6.5	54.3 ± 5.5	1.12 ± 0.08	0.23 ± 0.04
May	1	69.0	36.6	71.0	2.10	1.40
Jun	1	55.0	14.6	30.0	0.50	0.30
Aug	14	62.7 ± 9.2	26.4 ± 7.2	53.4 ± 6.7	0.90 ± 0.04	0.75 ± 0.08
Sep	12	71.5 ± 7.8	36.1 ± 4.4	80.2 ± 8.1	1.00 ± 0.06	0.65 ± 0.04
Oct	13	65.2 ± 7.9	29.0 ± 5.6	73.2 ± 5.5	0.70 ± 0.04	0.32 ± 0.01
Nov	14	68.5 ± 6.3	32.6 ± 3.2	50.3 ± 4.1	0.73 ± 0.04	0.13 ± 0.00
Dec	11	65.0 ± 9.5	29.6 ± 4.6	45.3 ± 6.1	0.63 ± 0.05	0.00 ± 0.00
1991						
Jan	11	68.9 ± 6.0	34.9 ± 6.4	63.9 ± 7.8	0.70 ± 0.08	0.16 ± 0.01
Feb	10	60.3 ± 2.4	19.7 ± 3.0	30.0 ± 3.5	0.30 ± 0.03	0.00 ± 0.00
Mar	10	65.2 ± 7.1	29.6 ± 7.7	43.8 ± 5.6	0.90 ± 0.10	0.12 ± 0.00

[a]Data are expressed as the means ± SE.
[b]indicates the monthly sample sizes.

(gram organ weight/gram body weight) × 100%.

Blood was collected in heparinized microtubes from the conus arteriosus after the toads were anesthesized with diethyl ether. The blood sampling was usually completed within 10 min for each individual. The blood was centrifuged at 1 000 g for 10 min and plasma (0.5 to 1.2 ml) was collected, and then stored at −20 °C until hormone measurements are performed.

Histological examination

The right testis of each animal was fixed in Bouin's solution, embedded in paraffin, serially sectioned at 6 μm, and stained with hematoxylin and eosin. Spermatogenetic activity was assessed as described by Saidapur et al. (1983): stage 0, primary spermatogonia; stage I, secondary spermatogonia; stage II, primary spermatocytes; stage III, secondary spermatocytes; stage IV, numbers of sperm bundles in seminiferous tubule; and stage V, numbers of mature sperm in the seminiferous tubule lumen. The intensity of spermatogenetic activity was expressed by the number of sperm bundles and free sperm in the seminiferous tubule. Sperm bundles were counted from 20 random sections of each testis. Numbers of sperm in the seminiferous tubules were calculated as the area of free sperm in the seminiferous tubule lumen divided by the total area of the seminiferous tubule lumen; values were expressed as abundant (> 50%), median (30-50%), few (< 30%), and none (0%). The proliferation of Leydig cells in the interstitial tissue was recorded.

Fig. 1. A photograph shows a male *Bufo bankorensis* appearing at night on a rock in a stream and waiting for female toads, during the breeding season (the photo was taken in September of 1990 in the Dehken area, Taichung, Taiwan).

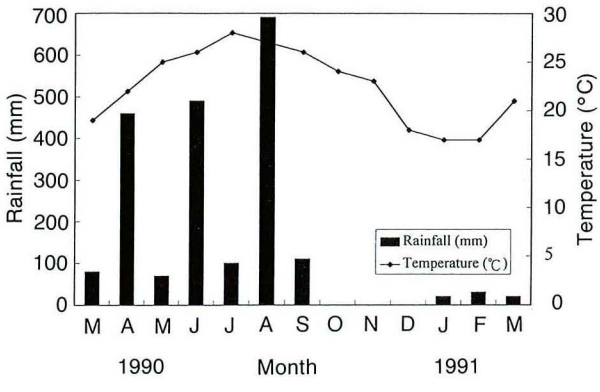

Fig. 2. Monthly changes in rainfall and air temperature in Taichung, Taiwan.

Radioimmunoassay of androgen levels in plasma

Plasma androgen was measured by a radioimmunoassay described previously by Kao et al. (1993). Androgens in the samples were extracted with diethyl ether; the average recovery of added testosterone was 75.3 ± 4.3%. The cross-reactivities of the antiserum with steroids were tested for testosterone (100%), dihydrotestosterone (74%), androstenedione (1.23%), and androstenediol (0.59%). Thus, the data are expressed as "androgen" which mainly represents testosterone and dihydrotestosterone present in the plasma. The coefficient of variation (CV) of the interassay was 12.9% (N = 4) and that of the intraassay was 6.7% (N = 4) for plasma androgen.

Statistical analysis

General linear models procedure and Turkey's studentized range test were used to examine differences among the numerous means. Linear correlations were performed for all variables. Probability levels of 0.05, 0.01, and 0.001 were taken to indicate significance in comparisons of means and correlations.

RESULTS

Histological observations of testes

Histological examination of *Bufo bankorensis* testes collected in April 1990 revealed that the most advanced stage was in stage 3 of spermatogenic development. Those testes collected the rest of the year, from January 1990 to March 1991,

were in stage 5 (Table 2). The numbers of sperm bundles increased from August 1990 to a peak in October and then slowly decreased to April 1991 when there were no sperm bundles found in the seminiferous tubules (Table 3). In the 4 annual periods, August to November, December to February, March to May and June to July, the mean numbers of sperm bundles for each period were 10, 8, 4 and 3, respectively. Spermatogenic intensity was moderate in August-November and in December-February, but was relatively low in March-May and June-July. Notwithstanding, spermatogenic intensity in the August-November period was the highest of the 4 periods (Table 3). The number of free sperm in the seminiferous tubules of *B. bankorensis* increased to a peak in October (Fig. 3A) and then gradually declined (Fig. 3B); no free sperm was discernible in April (Fig. 3C). Proliferation of Leydig cells also changed with season; being relatively abundant in August-November (Fig. 3A) and becoming less numerous in December-February (Fig. 3B). In June-July, Leydig cells were sparse and difficult to distinguish from connective tissue (Fig. 3C).

Annual changes in the weights of testis (TW), liver somatic index (LSI), and fat body somatic index (FBSI)

As indicated in Table 4, the mean TW showed no significant monthly trend ($F_{1,12} = 1.76$, $p = 0.23$) and had no correlation with sperm bundles ($r = 0.5$, $p = 0.1$). However, TW was significantly correlated with plasma androgen ($r = 0.72$, $p < 0.01$). The mean LSIs were 4.0%, 2.8% and 2.1% for the periods March-May, August-November and December-February, respectively (Fig. 4A). The mean FBSIs were 2.8%, 0.4% and 0.2% for the periods March-May, August-November and December-February, respectively (Fig. 4B). Both LSI and FBSI had significant differences, respectively, between March-May and December-February ($F_{2,5} = 5.82$, $p < 0.05$, for LSI; $F_{2,5} = 6.23$, $p < 0.05$, for FBSI). High correlation was also observed between LSI and FBSI ($r = 0.86$, $p < 0.001$).

Annual cycle of plasma androgen levels

Plasma androgen levels of male *B. bankorensis* peaked in September (93 ng/ml), and decreased gradually to a low in June (2.6 ng/ml) (Fig. 5). A significant monthly trend in plasma androgen levels was recorded ($F_{1,11} = 4.8$, $p < 0.05$).

Correlations of sperm bundles, FBSI, LSI, level of plasma androgen, TW, temperature and rainfall are summarized in Table 4. As indicated, FBSI and LSI, FBSI and TEM, SB and AL, AL and TW,

Table 2. Most advanced stage of spermatogenesis found in testis of *Bufo bankorensis* in different months of the year

	1990									1991		
SS[a]	M	A	M	J	A	S	O	N	D	J	F	M
III[b]		100%										
IV		0%										
V	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

[a]SS denotes the stage of spermatogenesis.
[b]Stages III, IV, and V represent the status of the seminiferous tubules, containing secondary spermatocytes, sperm bundles, and mature sperm, respectively.

Table 3. Monthly changes of spermatogenetic intensity (number of sperm bundles and number of sperm in seminiferous tubules) of *Bufo bankorensis*

	1990									1991		
	M	A	M	J*	A	S	O	N	D	J	F	M
SB	4.7	0	8.0	3.5	6.2	8.6	12.8	11.6	10.6	8.2	4.6	4.2
SN	M	N	M	F	F	M	A	M	M	M	M	F

SB: number of sperm bundles; SN: number of sperm in seminiferous tubules; A: abundant (> 50%), M: median (30%-50%), F: few (< 30%), N: none (see Materials and Methods). J*: June.

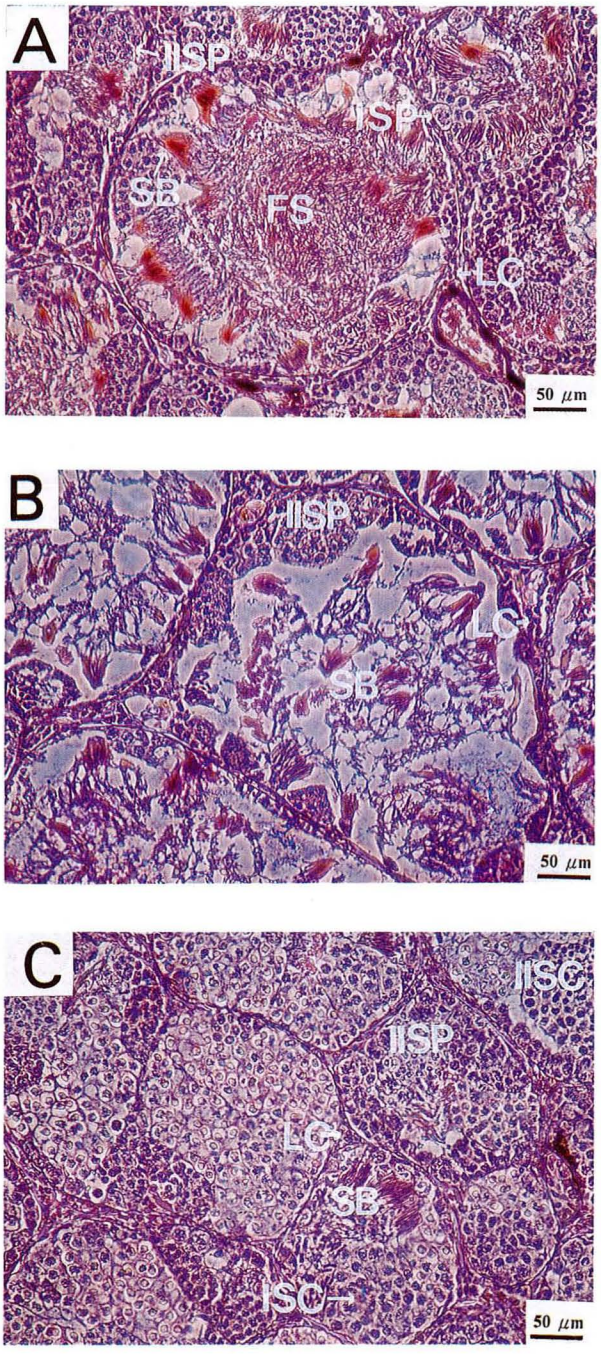

Fig. 3. Seasonal changes in the spermatogenetic cycle of *Bufo bankorensis*. A. Breeding season (October). Numberous sperm bundles and free sperm were present during this period. Leydig cells were abundant in the intertubular spaces. B. Post-breeding season (January). Most cell nests had disappeared. Few free sperm occurred in the lumen in this period. Leydig cells were greatly reduced in number in the intertubuler areas. C. Torpid season (June). There are few sperm bundles in this period. Leydig cells were relatively few between the seminiferous tubules. ISP: primary spematogonia; IISP: secondary spermatogonia; ISC: primary spermatocyte; IISC: secondary spermatocyte; FS: free sperm; LS: leydig cells; SB: sperm bundle.

and SB and RF were significantly correlated.

Observations of breeding behavior

Observations of breeding behavior in *B. bankorensis* were conducted from March 1990 to March 1991. Amplexus was observed in August, and tadpoles appeared in October. The breeding season continued to November; the numbers of adults and tadpoles decreased after December and tadpoles disappeared in the middle of January. In June-July, the temperatures reached 28 °C, and it was difficult to find and collect toads.

Reproductive cycle

Although the spermatogenic cycle of *Bufo bankorensis* appears to be of the potentially continuous type, the number of sperm bundles in the seminiferous tubules (N = 10), the numbers of free sperm (moderate) and plasma androgen levels (93 ng/ml) in August-November all were higher than during the other 3 periods. Field observations also showed that a high level of breeding behaviors occurred in August-November. Thus, August-November was considered to be the breeding period. In December-February, the numbers of sperm bundles (N = 8), free sperm (moderate) and adult toads were lower, and thus this was considered the post-breeding period. In May-March, the number of sperm bundles and the numbers of free sperm were low, but the values of LSI (= 4.0%) and of FBSI (= 2.8 %) were higher than during the other 3 periods. In June-July, the

Table 4. Correlation coefficients (r) of sperm bundle (SB), fatbody somatic index (FBSI), liver somatic index (LSI), level of plasma androgen (AL), testis weight (TW), temperature (TEM), and rainfall (RF) of male *Bufo bankorensis* during an annual reproductive cycle

	SB	FBSI	LSI	AL	TW	TEM	RF
SB		0.04	−0.21	0.7*	0.5+	0.01	−0.55+
FBSI			0.86***	0.06	0.43	0.64*	0.33
LSI				−0.21	0.29	0.42	0.24
AL					0.72**	0.28	0.40
TW						0.31	0.20
TEM							—

Correlation coefficients (r) were analyzed from the monthly means of those parameters. +: $0.1 < p < 0.05$; *: $p < 0.05$, **: $p < 0.01$; ***: $p < 0.001$.

number of sperm bundles (N = 3) and the number of free sperms were lowest; in the meantime, adult toads were difficult to find in the wild. This period was thus considered the torpid period. The reproductive cycle of male *B. bankorensis* accordingly could be divided into 4 periods: 1) active breeding period, August-November; 2) postbreeding period, December-February; 3) reproductive energy preservation period, March-May; 4) torpid period, June-July.

DISCUSSION

Testicular activity

Amphibians generally are seasonal breeders. Those inhabiting tropical areas, where climatic conditions show no appreciable fluctuations, often have evolved a continuous type of spermatogenic cycle; whereas other species in temperate regions have evolved a discontinuous or potentially continuous type of spermatogenic cycle (Basu and Nandi 1965, Lofts 1974). In species with continuous cycles, spermatozoa are generally produced throughout the year and the testes always contain spermatic cell nests as well as a complete spectrum of spermatogenic stages (Sun 1979, Saidapur and Kanamadi 1982, Saidapur 1983). Spermatogenic activity of species living in temperate regions is restricted to late spring and summer (Lofts 1974, Saidapur and Kanamadi 1982). This discontinuous production of spermatozoa by temperate zone species has been attributed, at least in part, to a seasonal lowering of environmental temperatures and an accompanying decline of gonadotropin output (Lofts 1974). Mondal and Basu (1960), Lofts (1974) and Saidapur (1983) considered that the spermatogenic cycle of species which have free sperm in the seminiferous tubules belongs to the continuous type. In other words, the classification of spermatogenic cycles of amphibian species belonging to continuous or discontinuous types depends up on the presence or absence of free sperm in the seminiferous tubules. In the present study, *Bufo bankorensis* had some free sperm in the seminiferous tubules the entire year except April. According to the categorizations by Lofts (1974) and Saidapur (1983), the spermato-

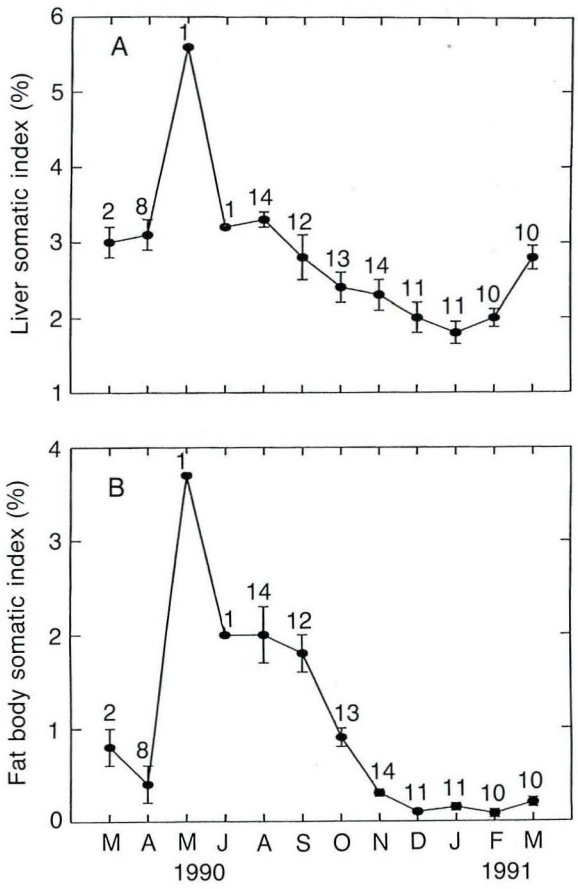

Fig. 4. Monthly changes of the liver somatic index (A), and fat body somatic index (B). The data are expressed as means ± SE, and the numbers above the dots indicate monthly sample sizes.

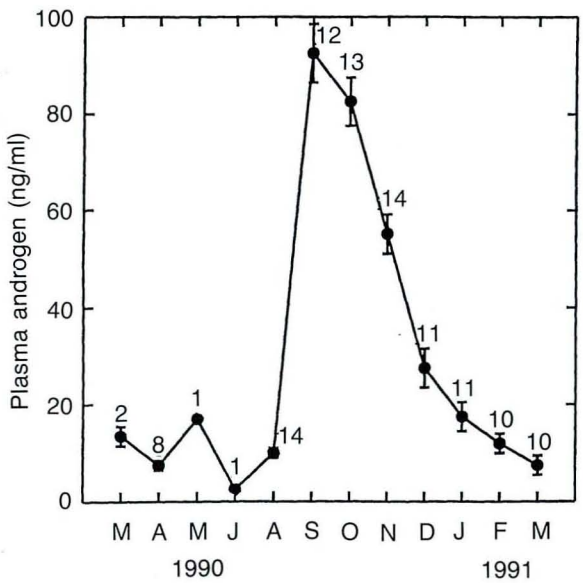

Fig. 5. Monthly changes of plasma androgen levels during an annual reproductive cycle of *Bufo bankorensis*.

genic cycle of *B. bankorensis* belongs to the continuous type. However, comparison of spermatogenic intensities showed that they were significantly higher in the breeding period than during any of the other 3 periods. Such observations reveal that these toads belong to the potentially continuous breeder group, but they do have a distinct breeding period. Accordingly, it is inappropriate to determine the nature of the reproductive cycle of toads by depending solely on the presence of free sperm in the seminiferous tubules.

In the past, the changes of the weight of testis also have been used as a basis to determine the reproductive cycle of amphibians (Lofts et al. 1972, Rastogi et al. 1986). However, the results of the present study reveal that the annual variations of testicular weight of *B. bankorensis* are not well correlated with spermatogenic activity. The findings are in agreement with previous observations in other amphibians, such as *Rana esculenta* (Rastogi et al. 1975), *R. catesbeiana* (Licht et al. 1983), *Ambystoma tigrinum* (Norris et al. 1985) and *R. perezi* (Delgado et al. 1989), that testicular weight is not correlated with spermatogenic activity. Thus, seasonal variations in testicular gametogenesis are not reflected in gonadal weight. This pattern is in sharp contrast to species with a discontinuous type of testicular cycle, such as *R. temporaia*, in which testicular weight is an appropriate index of testicular activity (Lofts et al. 1972).

Androgen patterns and correlation with reproductive cycle

Duellman and Trueb (1986) considered that the reproductive cycles are controlled by hormones. The present study shows that *B. bankorensis* males exhibit pronounced seasonal changes in plasma androgen levels. The patterns of seasonal plasma androgen levels of *B. bankorensis* observed in the present study are very similar to those previously reported by Kuhn et al. (1987) for *Dicroglossus occipitalis*, and by Itoh et al. (1990a) for *B. japonicus*. It is usually accepted that androgen is important for the initiation of breeding activity and the expression of sexual behavior. Although the exact biological functions of androgen in *B. bankorensis* are unknown at present, the fact that plasma androgen concentrations are maximal in the prebreeding and breeding periods, when pairing of the two sexes occurs in nature, suggests that this mating behavior is likely androgen dependent. In this regard, Siboulet (1981) has also demonstrated that circulating androgen levels in male *B. mauritanicus* are higher during periods of amplexus.

In many amphibians investigated, a notable decrease in androgen levels and an increase in spermatogenetic activity can be observed after the breeding period (*Bufo mauritanicus* by Siboulet 1981, and *Pachymedusa dacnicolor* by Rastogi et al. 1986). A possible androgen negative feedback mechanism at the hypothalamus-hypophysis level was proposed (Rastogi et al. 1976, Delgado et al. 1989, Itoh and Ishii 1990a). It is generally believed that, in adult amphibians in the early phases of the spermatogenic cycle, the mitotic proliferation of primary and secondary spermatogonia is gonadotrophin independent (Lofts 1974) and the meiotic progression of secondary spermatocytes, formation of sperm bundles and presence of free sperm are androgen dependent (Saidapur 1983). The present study on *B. bankorensis* reveals that the high levels of plasma androgen during the breeding period from August to December were coincident with the increased numbers of cell cysts of ScI, ScII (except May), and free sperm in the seminiferous tubule lumen. Such observations are consistent with those of other anurans reported by Rastogi et al. (1976), Kobayashi and Iwasawa (1986), and Saidapur (1983).

Relations of changes of fatbody and liver weights to the reproductive cycle

The strategy employed by a species in allocating stored energy is an important consideration for studying the evolution of reproductive tactics in ectothermic vertebrates. Lipids are the most efficient storage form of the 3 potential energy sources (lipids, glucose, and proteins) (Long 1987). Patterns of annual lipid variation in anurans reflect body lipid reduction for metabolic use during dormancy, for vitellogenesis, and possibly for steroid production during the active reproductive period (Ting and Boring 1940, Lofts et al. 1972, Chieffi et al. 1975, Rastogi et al. 1978, Brackin 1979, Morton 1981, Pramoda and Saidapur 1984, Kanamadi and Saidapur 1988). Liver glycogen also serves as a metabolic substrate in anurans (Brenner 1969). As observed in the present study, both LSI and FBSI exhibited annual variations, suggesting that fat and glycogen serve as metabolic substrates during both torpid and breeding periods. Such findings are similar to those reported by Schlaghecke and Blum (1978) and Morton (1981). We also found that the annual

changes of FBSI and LSI were positively correlated ($r = 0.86$, $p < 0.001$). Such a finding is similar to the report for *R. rugulosa* (Kao et al. 1993).

The present study has demonstrated that the spermatogenetic cycle of male *B. bankorensis*, inhabiting subtropical areas of Taiwan, belongs to a potentially continuous type, but this species exhibits clear and definite maxima and minima in the annual reproductive cycle. Based on such observations, modifications of the methods of characterizing the spermotogenic and reproductive cycles by Basu and Mondal (1961), Lofts (1964), and Saidapur (1983) are needed. In order to formulate the reproductive cycle accurately, it is necessary to consider the timing of spermatogenic activity, as well as changes in testicular weight, sperm bundles, free sperm, androgen, liver, fat body and other tissue.

Association of environmental factors with the reproductive cycle

Three major environmental factors implicated in the amphibian breeding cycle: rainfall, photoperiod and temperature. Rainfall has been shown to influence the breeding behavior in various urodeles, frogs and toads (Lofts 1974). Rastogi et al. (1976), however, demonstrated that photoperiod potentially modifies testicular activity in *R. esculenta*. Temperature, on the other hand, has been shown to play an important role in regulating plasma androgen levels and synchronizing the different phases of the seasonal testicular cycle in several amphibians (Rastogi et al. 1976 1978, Iela et al. 1980, Pancak and Taylor 1983, Pierantoni et al. 1985). In late spring and early summer, under high ambient temperatures, the testis of *R. esculenta* shows active spermatogenesis and contains a very large number of primary spermatocytes (Delgado et al. 1989). Rastogi et al. (1978) affirmed that temperature was the most important factor and that light played only a "permissive" role in the mediation of the temperature influence on gametogenic and endocrine activity of *R. esculenta*. Our study of the subtropical anuran species, *B. bankorensis*, reveals that there is no apparent correlation between temperature and plasma androgen level ($r = 0.42$, $p > 0.05$) in males. Most anurans of temperate climates have a fairly definite breeding season that tends to occur in the spring or early summer months. In regions with tropical and subtropical climates, temperature is rarely a limiting factor, and breeding in most anuran species is closely related to rainfall (Saidapur and Kanamadi 1982). However, the results from the present study indicate that rainfall is not correlated with testicular activity or plasma androgen levels of male *B. bankorensis*.

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臺灣盤谷蟾蜍之雄性生殖週期型態

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本研究探討臺灣中部雄性盤谷蟾蜍之生殖週期型態；根據其生精活動，血漿雄性素，睪丸重，肝重和脂肪體重之變化，定出其生殖週期。從1990年 3 月至1991年 3 月共捕獲一百一十隻雄蟾。睪丸組織切片顯示盤谷蟾蜍屬於潛藏型全年生精作用。雖然整年每個月份其細精管內具有各型細胞束，但8－9月的生精作用（精子束和成熟精子）最強。血漿雄性素濃度於 9 月最高($\bar{X}$=93 ng/ml)，6 月最低($\bar{X}$=2.5 ng/ml)；兩者呈顯著差異($p<0.05$)。脂肪體重量於5－7月達到高峰，與生殖期的開始時間一致，12月時生精作用減弱，此時脂肪體重量亦降至最低。8 月，睪丸重量漸次增加；9 月達到高峰。統合考量生精作用，血漿雄性素，睪丸重，肝重和脂肪體重，顯示臺灣中部之雄性盤谷蟾蜍屬潛藏型全年生殖週期。然而，其生殖週期可區分爲 4 期：1)生殖期，8－11月；2)後生殖期，12－2月；3)生殖能量貯存期，3－5月；4)休眠期，6－7月。

關鍵詞：生殖，蟾蜍科，生精作用，雄性素，臺灣。

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