REPRODUCTIVE VARIATION IN THE FEMALE RICE FROG RANA LIMNOCHARIS DURING THE SPRING SEASON IN TAIWAN

Paul S. Alexander, Cheng-ming Chang, and Ching-hua Yang

Certain periods of reproductive development or breeding in female vertebrates are characterized by great individual variation in ovary and oviduct sizes. The high degree of variation in reproductive condition during these periods of rapid change obscures critical appraisal of the timing of significant changes in the rate of secretion of the several reproductive hormones and thus of their interactions. Experimental studies require large numbers of animals for uncertain results. This problem has been solved successfully for studies on mammals and some lower vertebrates by the use of simple external diagnostic observations such as the vaginal smear, skin or feather coloration, etc. Alexander (1959) demonstrated the value of defining maturation stages for the rapidly maturing pullet based on morphological changes in the ovary and oviduct making possible a more critical analysis of pituitary-ovarian interactions (1). The present report is a prelude to similar studies using the rice frog.

A growing body of literature on anuran reproductive patterns is gradually replacing the common stereotyped conceptions based on a few temperate zone species with a clearer picture of the wide variation in reproductive patterns among individuals in the same species under different conditions as well as among species in the same genus (2~8). The rice frog is an excellent species for comprehensive studies of this kind. It is abundant and suitable for both field observations and laboratory experiments.

Rana limnocharis ranges from India across Southeast Asia to the Philippines and northward to Japan (9). It is probably the most common amphibian in all Central and South China, following the culture of rice everywhere over a wide range in altitude(10, 11). References to studies on reproductive variation in this species, however, have not come to our attention. Inger and Greenberg (1963) have recently reported on the annual reproductive pattern of the medium-sized frog *Rana erythraea* in Sarawak (8). This tropical species has a habitat similar to that of *limnocharis* and may resemble it also in the potential for continuous breeding throughout most of the year.

MATERIALS AND METHODS

Nearly 2000 *Rana limnocharis* in a ratio of two males to one female were collected on 22 evenings throughout March and April of 1963. Flooded rice fields were visited within two miles downhill from the Tunghai University campus in central Taiwan. Most collections were made by the authors working usually from 7:00 to 8:30 P.M.

A serious drought beginning in mid-April affected the supply of irrigation water near the campus so that by the end of April collections from the rice fields were discontinued. Four small collections totaling 39 frogs, however, were made from a large irrigation ditch on campus during the first half of May, and one collection of 6 clasping pairs was made from the same ditch in mid-June. Collections from rice fields were resumed in mid-July and are continuing at present.

Frogs were captured by hand with the aid of a flashlight along the small paths between rice fields. Single frogs and clasping pairs were placed in separate plastic bags for immediate transfer to the laboratory where each clasping pair was placed in a finger bowl containing about 50 ml of tap water. Single frogs of both sexes were put together into a large sink filled with water from which males and females were separated into battery jars. Clasping pairs formed in the sink were also separated into finger bowls.

Complete autopsy data on body, ovary and oviduct weights as well as descriptions of ovarian morphology were recorded for a total of 325 frogs. Data on reproductive variation reported in this paper refer to females collected from April 5 to 23 and autopsied within 6 days after collection unless stated otherwise.

Frogs were weighed on a pan balance sensitive to the nearest 0.1 gram. Body length from snout to tip of urostyle was measured along the dorsal surface with a millimeter ruler. Ovaries and oviducts were removed and weighed immediately on a triple-beam balance sensitive to the nearest 0.01 gram.

Recipient female frogs for testing induced ovulation were isolated from males after collection the previous night and were injected with pituitary suspensions about 20 hours later. Fresh female pituitaries for injection were collected in Ringer's solution, and a fine suspension was made by forcing the pituitaries several times through a 25-gauge syringe needle. Pituitary suspensions were injected immediately in 0.5 ml doses intraperitoneally. Injected frogs were kept in separate finger bowls for observation of ovulatory response.

RESULTS AND DISCUSSION

Body length and age estimates: It is important to have some idea of the age characteristics of the population of frogs sampled in this study. The preliminary considerations presented here suggest the direction of the more comprehensive studies which are in progress.

Body size is the only external character of the female rice frog which may be correlated with age and sexual maturation. Fig. 1 presents the frequency distribution of body lengths of 398 females collected during March and April. The earliest collection on March 1, made within one week after the first rice planting in the area and two weeks after the first break in the cold season, includes immature females with unpigmented ovaries ranging from 23~34 mm in body length and mature females with ripe ova ranging from 34~50 mm in body length. Throughout March no female longer than 32 mm had unpigmented ovaries and none shorter than 28 mm had pigmented ovaries. Numerous frogs of 33 mm length had fully developed ovaries. The April collections of small females follow the pattern described for March with the exception of three 21~22 mm females collected at the same time which show early ovarian pigmentation.

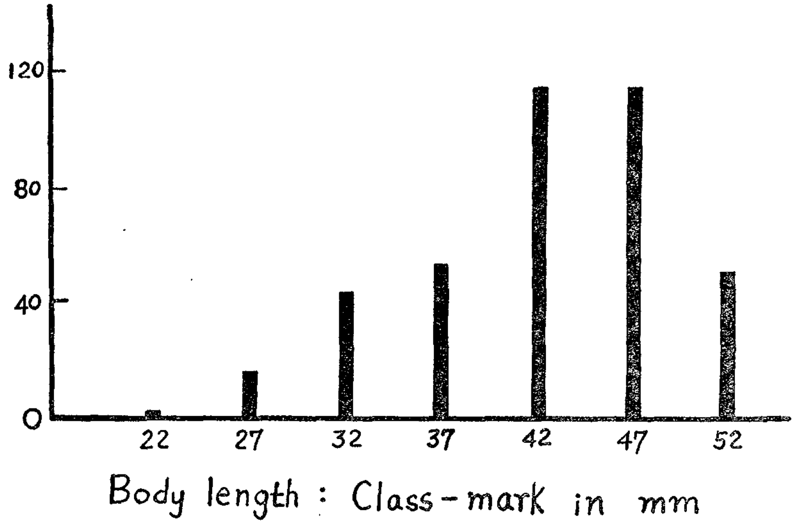

Fig. 1. Frequency distribution of body lengths of 398 female rice frogs collected in March and April.

Estimates of the actual ages of these rice frogs from the time of fertilization are based on the following observations.

a) The larval period is relatively short, probably less than two months under favorable conditions. Large numbers of metamorphosing frogs are found in April in rice fields planted 6~8 weeks previously. Again in August equal or greater numbers of metamorphosing frogs are found in rice fields flooded in early July.

b) A pause in breeding activity probably extends from September through part of February. Meager data supporting this conclusion are provided by three 50~52 mm females collected in early October which have oocytes mostly ranging in diameter from 0.1~0.2 mm but reaching a maximum diameter of 0.3 mm without pigment deposition. Three 42~45 mm females collected in early November also have immature ovaries, but the smallest of these has a few oocytes with diameter as large as 0.6 mm in the initial stage of pigment deposition. (Mature oocytes have a diameter of approximately 1.0 mm.) These frogs all have large fat bodies and thus might be expected to achieve ovarian maturation during December. Hibernation probably continues during the coldest weather in January and February when temperatures usually range from 10~20°C, occasionally dropping down to 5°C but rarely to freezing.

Yuan has reported that the breeding season of *R. limnocharis* in north

Taiwan extends from March through July(12). We have observed during the past two years that this species breeds in central Taiwan from late February through August. We have not found metamorphosing frogs by extensive collecting during March. The smallest frogs collected during March with body lengths from 20~25 mm presumably metamorphosed in the late fall and then hibernated for two or three months.

c) The rapid rate of growth of newly metamorphosed frogs fed red worms(*Tubifex*) in our laboratory during late summer confirms field observations suggesting that sexual maturation may require less than four months after metamorphosis(13). An average body growth increment of 2 mm per week is characteristic of these frogs throughout the range in body length from 15 mm at metamorphosis to at least 34 mm at which length the males all show mature secondary sexual characters. Considerable variation in growth rate, however, is noted among frogs of the same age during the first month of postmetamorphic life. Sampling field populations at weekly intervals and toe-marking studies indicate a similar growth potential in the field(13).

In July and August 175 female frogs ranging in body length from 25~44 mm were autopsied soon after collection from the field(13). Two-thirds of these frogs have mature or maturing ovaries with pigmented oocytes. Eleven of these frogs with body lengths from 40~44 mm, however, have unpigmented ovaries and only 6 frogs with body lengths from 29~34 mm show pigmented ovaries. Thus ovarian maturation under these summer conditions occurs usually in frogs with body lengths from 35~44 mm. These maturing frogs obviously represent the same frogs found metamorphosing in this area in April and do attain full ovarian development in July and August.

Based upon the above considerations, an analysis of the frequency distribution of body lengths presented in Fig. 1 may now be attempted. The large numbers of females found in the 42 mm and 47 mm classes seem to represent those frogs produced 8~14 months previously. Frogs shorter than 40 mm in body length probably represent those produced 6~8 months previously in late summer. Frogs in the 52 mm class are at least 18 months of age or older. Genetic and environmental influences may obscure age differences among larger frogs. The frequency distribution tallied for 1 mm intervals of body length offers some additional points of interest. The following actual body lengths are represented by frequencies of 25~35 frogs: 40 mm, 42 mm, 43 mm, 45 mm, 47mm, and 50 mm. Only the 47 mm length includes more than 28 frogs. The most distinct cleavage in frequencies for these lengths lies between 47 mm and 50 mm, representing a time interval of at least 6 months. The spring breeding population of females, therefore, falls predominantly into the 8~12-month age range. Considerable potential still exists for breeding among older frogs, but the survival of females apparently falls off rapidly during the second and third years of life.

Body length and reproductive variation: Critical studies of physiological mechanisms in reproduction require careful definition of the reproductive status of experimental animals. The lack of other external characters diagnostic for the reproductive status in the female rice frog led us to test the correlation of body size with ovary and oviduct sizes. Fig. 2 presents the reproductive variation among 73 female frogs within samples selected for uniform length at 5 mm intervals from 22 mm to 52 mm. The numerical data for this graph are also presented in Table 1. Means, standard deviations and ranges for body, ovary and oviduct weights are given for each sample.

All frogs 35 mm or larger have mature ovaries and oviducts in varying phases of the ovulatory cycle. The wide range in relative frequency of mature ova compared to ova of smaller sizes shows that partial ovulation is the common experience of the rice frog. The individual frog, therefore, may ovulate several times during a period of breeding activity.

Positive correlations between body length and body, ovary and oviduct weights are apparent from Fig. 2. The wide overlap in ranges for these variables at different body lengths, however, suggests a limited usefulness of body length as a criterion for the selection of samples having uniform reproductive characters. The overlap among sample ranges for ovary and oviduct weights is much greater than that for body weights. The ovulating history of the mature frog is obviously a more important determinant for the weights of these reproductive organs than is the body length or age. For both ovary and oviduct the overlap of ranges extends from 37 mm to 52 mm samples, and the overlap of standard deviations extends from 37 mm to 47 mm and from 42 mm to 52 mm samples. Such great variation means that experimental treatment must produce large effects upon the weights of these organs in order to permit statistically significant conclusions to be drawn based on weight changes.

Variation in ovarian morphology: During the spring breeding season four stages in the ovarian cycle of the mature frog may be defined on the basis of morphological generalizations about follicle sizes and the surface appearance of the ovary. Throughout the season many females are found with ovaries completely covered by follicles which are slightly larger than 1 mm diameter: a ***pre-ovulatory*** stage. Many frogs are found with several hundred of these large follicles situated among numerous small follicles: evidence of ***partial ovulation***. Some mature frogs have smaller, darkly-pigmented ovaries predominantly covered by small follicles which obscure from surface view the next crop of larger maturing follicles: a ***post-ovulatory*** stage. Other frogs have ovaries covered by maturing follicles not quite 1 mm diameter: a ***mid-ovulatory*** stage.

Table 2 presents the variation in ovarian morphology among 112 mature frogs collected on April 12, 17 and 23 from a few adjacent rice fields planted from April 8 to 18. The total number of frogs in Table 2 is distributed rather uniformly with 21% in each of three ovulatory classes and 37% in the mid-ovulatory stage. The average distributions of larger and smaller frogs among these ovulatory classes are somewhat similar. The greatest difference is in the percentage of frogs showing partial ovu-

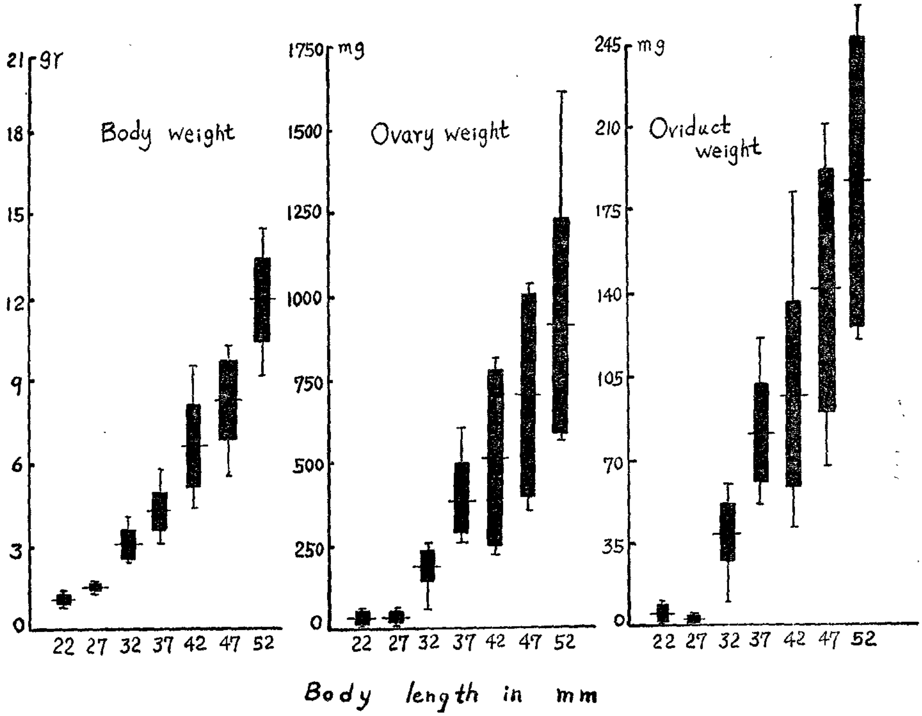

Fig. 2. Reproductive variation among 73 female frogs within samples selected for uniform body length at 5 mm intervals from 22 to 52 mm. Same data presented in Table 1.

Table 1. Reproductive variation related to body length. (Same data presented in Figure 2)

No. of frogs	Body length mm	Body weight g	Ovary weight mg	Oviduct weight mg
13	52 (50～53)	12.8±1.7 (9.1～14.3)	899±317 (560～1600)	185±60 (120～270)
13	47	8.3±1.3 (5.5～10.1)	694±300 (350～1030)	140±51 (50～210)
13	42	6.5±1.5 (4.3～9.4)	500±210 (220～800)	96±39 (40～180)
13	37 (36～38)	4.2±0.7 (3.1～5.7)	370±90 (250～590)	80±20 (50～120)
13	32 (31～33)	3.0±0.5 (2.3～4.0)	180±61 (60～250)	39±15 (10～60)
5	27 (26～28)	1.5±0.1 (1.4～1.6)	29±17 (10～50)	2±1.5 (1～4)
3	22 (21～22)	1.1±0.2 (0.9～1.3)	28±21 (10～50)	4±4 (1～10)

lation: 27% of the larger frogs compared with 14% of the smaller frogs. The numbers of frogs in the various subgroups are too small to permit conclusive generalization about their differences. Some suggestions, however, may be noted.

Table 2. Variation in ovarian morphology among 112 mature frogs collected from a few adjacent rice fields.

Date collected	Body length mm	Total no. of frogs	% in different ovulatory stages* Pre-ovulat.	Partial ovulat.	Post-ovulat.	Mid-ovulat.
April 12	45~52	34	35%	15%	24%	26%
	33~44	19	47	16	5	32
April 17	45~52	16	6	6	25	63
	33~44	19	11	16	37	37
April 23	45~53	12	0	92	0	8
	33~44	12	0	8	25	67
TOTALS	45~53	62	21	27	19	32
	33~44	50	22	14	22	42
	33~53	112	21%	21%	21%	37%

* Stages:

Pre-ovulatory- ovaries covered by follicles slightly larger than 1 mm diameter.

Partial ovulatory- ovaries with many large follicles among numerous small follicles.

Post-ovulatory-smaller, darkly pigmented ovaries predominantly covered by small follicles which obscure from surface view the next crop of maturing follicles.

Mid-ovulatory- ovaries covered by maturing follicles not quite 1 mm diameter.

A marked decrease in percentage of pre-ovulatory frogs is observed after April 12 for both larger (45~52 mm) and smaller (33~44 mm) frogs. The percentage of larger pre-ovulatory frogs falls from 35% on April 12 to 6% on April 17 and then to zero on April 23 whereas the corresponding percentages for the smaller frogs are 47, 11 and 0%. The data suggest that these three samples may represent the same population of frogs which began to breed in this newly-planted area during the second week in April.

A striking increase in percentage of larger mid-ovulatory frogs from 26% on April 12 to 63% on April 17 and a subsequent fall to 8% on April 23 are noted. These observations suggest that the transition from post-ovulatory to mid-ovulatory stage may require only a few days and that these females may very soon ovulate again. This short period of transition in ovarian morphology involves regression of ovulated follicles exposing to surface view the next crop of large oocytes undergoing the final phase of rapid yolk deposition. An increase in oocyte diameter from 0.8 mm to 1.0 mm approximately doubles the volume. The absence of larger pre-ovulatory and post-ovulatory frogs in the April 23 collection suggests that ovulation of the second crop of follicles begins before maximum follicle size is attained and does not proceed rapidly to completion.

A lag in ovulatory response of smaller frogs is suggested by the higher percentage of these frogs in the pre-ovulatory stage and the very low percentage in the post-ovulatory stage on April 12. Furthermore, the distribution of smaller frogs on April 23 with a high percentage in the mid-ovulatory stage is almost identical to the distribution of larger frogs among ovulatory classes on April 17.

Follicles of all sizes are found in the ovary of the mature frog during the spring. The smaller follicles may be crowded out of surface view by the larger follicles, or the larger follicles may be obscured in the post-ovulatory stage by the thickened ovarian surface tissue with its many immature and recently ovulated follicles. If the ovarian sac of the post-ovulatory frog is torn open, the inner surface of the ovarian wall is found covered with protruding follicles of various sizes approaching 1 mm diameter. Some frogs, which presumably take a longer period of time to complete ovulation of one crop of mature ova, have many large follicles as well as many follicles approaching mature size clearly visible on the external ovarian surface. Intermittent ovulation of a few hundred ova each time may allow the ovary to be converted from the condition of partial ovulation to that of the mid-ovulatory stage. When conditions are not suitable for breeding, increasingly large numbers of follicles are brought to maturity and maximum size. For example, in early May several females collected from a large irrigation ditch on campus had ovaries and oviducts 50-100% larger than the largest recorded during the previous two months. One clasping female collected on June 11 from the same ditch spawned 60% more eggs than the maximum number recorded for 46 clasping females from early March to late April. One female collected from the same ditch on July 4 prior to the summer rice-planting season had ovaries weighing twice as much as the largest found in early May and four times the largest found in March and April.

Variation in ovulatory responses to clasping or pituitary injection: A total of 74 clasping pairs was observed for ovulation in the laboratory during March and April. Approximately half of these frogs were collected from the field in the clasping condition. The remaining pairs were found clasping in the laboratory when the evening catch of frogs was emptied into a sink filled with water. Each clasping pair was placed in a separate finger bowl for observation of spawning which was completed usually before 8:00 A. M. and always before noon.

The percentage of these females ovulating in the laboratory is about 60% for both those caught in the clasping condition and those which formed clasping pairs after collection. Great variation among samples, however, is observed. For example, on April 12 only 2 out of 7 frogs caught clasping in the field ovulated whereas one week later all of 4 frogs collected singly from the same area but found clasping in the laboratory sink ovulated. From another area on April 19 all of 5 frogs caught clasping ovulated and 2 out of 3 frogs found clasping in the laboratory sink ovulated. One week later from the same area no clasping frogs were collected and only 1 out 7 frogs found clasping in the laboratory sink ovulated. Ovulation never was observed later in those frogs which failed to ovulate on the first morning after collection even though some intermittent clasping activity was noted for two or three days in some cases.

The question may be raised as to whether clasping is the normal stimulus required for inducing ovulation in this species. Only 1 of more than 500 females collected singly in March and April and separated from males upon return to the laboratory was observed to ovulate. In contrast the total number of mated females which ovulated is about 8% of the total number of mature females collected. It is possible, however, that the hormonal balance which is favorable for ovulation also produces a behavioral change in the female which favors mating. Single males and females were collected in the same plastic bag and were placed together in a sink filled with water prior to their separation, allowing time for such a selective factor for mating to operate. On the other hand, ovulation occurred in little more than half of the clasping females which suggests some random activity in selection of mates by the males.

It is interesting to note a few observations about the interval of time between mating and ovulation or spawning. We have observed considerably greater movement of frogs in the early evening as compared with late evening and perhaps most mating occurs in early evening. On only three occasions in two years have females caught clasping in the field been found to have uterine eggs by 11:00 P. M. One clasping female without uterine eggs was accidentally separated from its mate when caught at 11:00 P. M. on April 19 but was remated an hour later with another male. This frog spawned about 1300 eggs of which more than 90% were naturally fertilized from 2~3:00 A. M. Three other frogs caught clasping at the same time spawned 550~650 eggs about 6:00 A. M. and another similar frog spawned 350 eggs at 8:00 A. M. Two females caught singly at 11:00 P. M. on the same night and mated at midnight spawned 500~650 eggs from 5:30~6:00 A. M. Another female collected singly from the same field on April 25 at 11:30 P. M. mated at midnight and spawned approximately 1000 eggs at about 4:00 A. M. These data suggest that, if clasping provides the normal stimulus which triggers the release of ovulation-inducing hormone from the pituitary, the ovarian response may require as little as 4 hours before ovulation begins. Ovulation may proceed rapidly to completion and spawning may follow in a few minutes or may be delayed a few hours. Alternative explanations are possible.

Table 3 presents the spawning and autopsy data for frogs collected on April 19 and 23 and on June 11. The April 19 sample is the same as that for which spawning records are described above and is from a different area with a poorer water supply than the April 23 sample. Four of the five frogs in the April 23 sample were mated immediately after collection and include both the highest and the lowest ovulatory responses. The June 11 sample was collected from a large irrigation ditch on campus about six weeks after regular breeding in the rice fields near the campus had ended. Three of these 6 females had uterine eggs present at 11:00 P. M. The mean number of

Table 3. Reproductive variation related to ovulatory response to clasping.

Date collected	No. of frogs	No. of eggs laid	Body length mm	Body weight g	Ovary weight mg	Oviduct weight mg
April 19	6	658 (350～1300)	42 (37～44)	6.6±1.2	610±170	122±40
April 23	5	468 (30～1300)	43 (41～45)	6.8±1.5	443±180	135±31
June 11	6	1875 (550～2400)	50 (48～51)	9.8±1.1	951±160	230±17

			% of above frogs in different ovulatory stages			
			Pre-ovulat.	Partial ovulat.	Post-ovulat.	Mid-ovulat.
April 19	6	658	0%	50%	0	50%
April 23	5	468	0	40	0	60
June 11	6	1875	0	100	0	0

Table 4. Reproductive variation related to ovulatory response to single injections of female pituitaries.

No. of pit.	No. of frogs	No. of eggs laid	Body length mm	Body weight g	Ovary weight mg	Oviduct weight mg
Experiment A.						
Control	7	0	48	8.2±2.6	774±394	139±76
2	7	0	47	9.3±3.3	763±364	120±73
2	7	276 (60～800)	46	8.3±1.2	603±220	123±64
Experiment B.						
Control	12	0	48	9.2±1.8	850±223	173±47
2–4	7	0	47	9.5±1.4	727±331	160±48
2–4	11	338	47	8.7±1.9	605±287	121±51
2	(6)	353 (20～650)	47	9.2±1.6	727±312	123±39
4	(5)	320 (50～500)	47	8.0±1.1	458±183	118±67

			% of frogs from Expt. B in different ovulatory stages			
			Pre-ovulat.	Partial ovulat.	Post-ovulat.	Mid-ovulat.
Control	12	0	33%	0%	8%	58%
2–4	7	0	28	0	28	43
2–4	11	338	0	18	64	18

eggs spawned by the June frogs is more than three times the mean number spawned by the two April samples combined.

Table 4 presents the spawning and autopsy data for frogs collected on April 10 and 12 and injected with two or four female pituitaries each on the day following collection. The autopsy data for control frogs and for frogs which did not respond to pituitary injection are also included. In experiment B no significant difference is noted in the ranges or the mean numbers of eggs spawned in response to injections of two or four pituitaries although 83% of 6 frogs injected with four pituitaries ovulated in contrast to only 50% of 12 frogs injected with two pituitaries.

A comparison of weight data for controls and frogs which gave positive or negative ovulatory responses to pituitary injection fails to reveal any striking differences in weights of the body, ovary or oviduct which might be correlated with selective factors influencing the ovulatory response. Ovulation and spawning are expected to reduce the ovary and oviduct weights in proportion to the number of eggs released. In experiment A of Table 4 spawning frogs have mean weights of both ovary and oviduct which are smaller than those for controls or for pituitary-injected frogs which failed to ovulate, but none of the differences between groups is significant by the "t" test. In experiment B spawning frogs have mean ovary and oviduct weights which are smaller than those for controls with differences significant at the 3% and 1% levels, respectively.

It is interesting to compare the ovulatory response to clasping with the response to pituitary injection. The maximum response to clasping during March and April was 1800 eggs spawned whereas the maximum response to pituitary injection during the same time was 800 eggs spawned. The minimum responses were 30 and 20 eggs spawned, respectively. Two out of 7 frogs caught clasping on April 12 spawned totals of 150 and 1300 eggs compared with a mean of 338 eggs(range: 20~650 eggs)spawned by 11 pituitary-injected frogs collected at the same time. The finding of pre-ovulatory frogs which failed to ovulate in response to clasping or to injections of two or four pituitaries suggests that the presence of some inhibitory factor may account for both the unresponsiveness of these frogs and also for the larger follicles which thereby have had a longer period of time for growth. Evidence in Tables 2 and 4 as well as from other pituitary injection experiments shows that some "mid-ovulatory" frogs are responsive to ovulation-inducing stimuli.

Wright (1945) showed in vitro inhibition of ovulation in ovarian fragments of ***Rana pipiens*** by concentrations of female pituitary suspensions greater than the optimal ovulation-inducing concentrations of 1/8 to 1/16 pituitary per 10 ml Holtfreter's solution(14). In his in vitro experiments ovulatory responses were obtained with concentrations of 1/512 to 2 pituitaries, but the response to one or two pituitaries was greatly reduced in comparison with the response to 1/8 pituitary. Several attempts to repeat these results using the rice frog in our laboratory have essentially failed in inducing ovulation in vitro with 1/320 to 1 female pituitary per 5 or 10 ml of Holtfreter's or Ringer's solutions. The relative proportion of ovulation-inducing hormone and any inhibitory factor stored in the pituitary would be expected to vary among species and even within a species under different conditions. Injections of pituitary suspensions must provide a different balance of these factors than that provided by neural control of pituitary release mechanisms.

Experimental control of reproductive variation: The lack of an external character diagnostic for the reproductive condition of the female frog led us to try surgical inspection of the ovary and oviduct as a method for selecting experimental samples with well-defined reproductive characteristics. The results of this study will be reported in a later paper, but we may note considerable success with this approach. After urethane anesthesia an incision about 15 mm long is made lateral and parallel to the ventral abdominal vein. One ovary is completely exposed on the body surface so that the oviduct may also be examined. The ovulatory stage and estimates of the weights of the ovary and oviduct within limits of 5~20% error are recorded for each frog. Surgical recovery is excellent. Injection of pituitary suspension into the dorsal lymph sac immediately following surgery results in spawning of the usual number of eggs from 12~24 hours later. Ovulatory responses vary in a manner consistent with predictions based on ovulatory stages described above. This approach can be applied to the study of the rapidly maturing female frog as well as to the breeding frog.

SUMMARY

Mature female rice frogs collected during March and April 1963 from flooded rice fields in central Taiwan range from 33~53mm in body length, 2.3~14.3 g in body weight, 0.22~1.60 g in ovary weight and 0.04~0.27 g in oviduct weight. The frequency distribution of body lengths of 398 mature and immature females along with evidence of a pause in breeding activity during fall and winter suggests that frogs may reach sexual maturity at 6~8 months of age. Four ovulatory stages based on rapid inspection of follicle sizes and ovarian surface appearance are defined. Changes in ovarian morphology in a population of frogs sampled during April suggest that partial ovulation is the common experience and that the same frogs may ovulate repeatedly during this period. Many pigmented follicles from 0.4 mm to 0.8 mm in diameter are always present even after heavy ovulation of the ripe 1.0~1.1 mm follicles. Soon after the passing of optimal conditions for breeding, females are found with unusually enlarged ovaries and oviducts, indicating that yolk deposition continues to produce increasing numbers of ripe follicles. Half of all females collected in the clasping condition as well as half of those mated immediately after collection were observed to ovulate. The highly variable ovulatory responses during March and April to clasping (30~1800 eggs) and to pituitary injection (20~800 eggs) are compared. A method based on surgical inspection of the ovary and oviduct is proposed for selecting experimental samples of females with well-defined reproductive characteristics.

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臺灣雌性澤蛙在春季之生殖變化現象

本文研究臺灣雌性澤蛙（*Rana limnocharis*）之生殖變異。著者在民國五十二年三、四兩月自臺灣中部東海大學附近之滿水稻田採集澤蛙。成體雌蛙其體長、體重、卵巢、輸卵管重量之變域，彼此對照，體長爲 3.3～5.3 公分，體重 2.3～14.3克，卵巢重 0.22～1.60克，輸卵管重 0.04～0.27克。根據 398 隻雌蛙體重分佈之頻率和秋冬兩季排卵中止之現象來看，澤蛙（雌）之性成熟年齡約爲生後 6～8 個月。吾人依據卵泡之大小和卵巢之外表形態將生殖週期分爲四個階段。當最佳之產卵環境過去後，於五月初在校園內灌漑稻田之水渠所採之雌蛙，其卵巢輸卵管重量均較三、四月所採之最大者大 50～100 %。在成對雌蛙中（包括在稻田中及在實驗室內成對者）有半數在第二天早晨產卵。交配行爲刺激產卵（30～1800 個卵）及腦下垂體注射產卵（20～800 個卵）曾比較其顯著之差異性。腹部切開檢視卵巢及輸卵管提供目的選樣方法俾作實驗之用。