THE EFFECTS OF SEASONAL AND LUNAR CHANGES ON THE BREEDING PATTERN OF THE EDIBLE JAVANESE FROG, *RANA CANCRIVORA* GRAVENHORST [1]

by

GILBERT CHURCH

Department of Biological Sciences, Medical Faculty, University of
Indonesia, Djakarta [2]

INTRODUCTION

The Amphibia of the temperate zones have been shown to be controlled in their habits and behavior in large part by the changing seasonal cycles. Given an adequate moist habitat, of these varying conditions, that affecting them most is the variable temperature. Being poikilothermous, amphibian body temperatures are directly subjected to seasonal temperature variations, and these variations in turn directly or indirectly control metabolic and reproductive activity. Resultingly, seasonal changes have been shown to occur in the storage of muscle and liver glycogen, the morphology of the liver, the volume of the blood including the number of erythrocytes, the size of the fat bodies, the size and histology of the hypophysis, thyroid and adrenal glands, the ovaries and oviducts, and the testes. Minor secondary morphological changes take place in many species, especially in the males, which are apparently promoted hormonally and therefore can also be correlated with seasonal variations (see, for example, GADOW, '01; HOLMES, '27; NOBLE, '31; and a more recent review, GALLIEN, '58).

But in the wet tropics, including the island of Java, the temperature is almost constantly the same; the rainfall, although usually undergoing seasonal variations, is adequate to maintain amphibian populations throughout the year; and there is a constant supply of food available (CHURCH, '60). In spite of the fact that tropical conditions prevail which stand in high contrast to those of the temperate zones, few detailed studies have been made of the reproductive patterns of tropical amphibians. CHURCH and his co-workers ('58; '60) have recently published a study of the annual reproductive cycle of the Javanese toad, *Bufo melanostictus*. The present report

[2] Present address: Zoology Section, Institute of Technology, Bandung.

s concerned with a similar study of the changes that take place throughout
he year in the gonads, the pituitary gland and the liver glycogen concentra-
ion in the Javanese frog, *Rana cancrivora* Gravenhorst.

PART I. CHANGES IN THE OVARIES AND TESTES
MATERIALS AND METHODS

Rana cancrivora is distributed widely throughout the Indonesian Archi-
pelago. It is an aquatic nocturnal frog, and is found in large numbers in the
flooded rice paddies where it breeds. Unlike many Javanese amphibians,
which seem tame in comparison, *cancrivora* is extremely shy and, when
disturbed, will skitter rapidly over the surface of the water for a distance
of fifty feet or more. In the laboratory, if not kept in the dark, it will die
in a few days from injuries sustained in jumping against the walls of the
aquarium.

R. cancrivora is commonly sold in the markets of Java for food and
the animals used in this study were obtained from a local vendor in Dja-
karta who brought them into the city from the rice paddies just north
of Djakarta, and to the east at Krawang. In this area the average monthly
temperature throughout the year varies from about 26°C to 28°C; the ab-
solute maximum and minimum temperature range is from 18°C to 36°C.
The mean monthly relative humidity varies between 78 and 88 percent.
The rainfall is greatest from December to March, and in January averages
about 8 inches. The average rainfall for the month of September is about
2 inches (SCHMIDT and FERGUSON, '51). In spite of this variation in rainfall,
about 50 percent of the paddy is kept constantly under irrigation (SOEMAR-
TONO, '60) which makes possible a constant food supply and proper con-
ditions for breeding at all times.

1. *Female specimens.* Early on in the study it was found that females
in all stages of ovarian development were to be obtained at all times, but
unfortunately there were no external characteristics to indicate the stage
a particular animal might be in. As a result, seasonal variations could only
be detected by killing a large number of animals and examining the gonads.

From August, 1957 to August, 1958, 20 female specimens were obtained
one morning each week from the local market, brought directly to the
laboratory, killed and examined. Records were kept of the snout-vent
lengths, the gross body weights, the conditions and weights of each ovary,
the size of the oviducts, the presence of fat bodies, and the contents of the
stomachs.

The maturity of the ovarian eggs was determined by a consideration
of egg size and pigmentation. They were grouped as follows:

Group I: Small, unpigmented ova.

Group II: Ova small; pigmentation of ova variable.

Group III: Almost all ova pigmented completely, but no distinction between animal and vegetal hemispheres evident.

Group IV: A difference in pigmentation beginning to appear between the animal and vegetal hemispheres.

Group V: Large ova with a clearly distinguishable animal and vegetal hemisphere.

Group VI: Post-ovulation, distinguished by the condition of the ovary which usually contained some unovulated Group V ova.

2. *Male specimens.* The testes of a smaller number of males, obtained at weekly intervals from March to August, 1958 were studied. In a total of 227 animals, records were kept of the snout-vent lengths and the gross body weights. The lengths and widths of the testes of 150 specimens were determined, and the weights recorded. One of the testes of each of these males was crushed in water and examined for viable spermatozoa; the other, in some instances, was fixed, sectioned and stained with hematoxylin and eosin.

The approximate volumes of the testes were computed by considering them as cylindrical.

RESULTS

1. *Snout-vent measurements.* There was a marked sex dimorphism in the sizes of the two sexes. The range in snout-vent measurements of 1,098 females was 64 to 132 mm; that of 227 males, 61 to 93 mm. The monthly averages for both males and females remained more or less constant throughout the year, that of the females varying between 101 and 105 mm, and that of the males, from 74 to 82 mm.

When separated on the basis of snout-vent measurements, it soon became apparent that the animals used in this study were divided into two size groups. Collections made at Kemajoran, the area just north of Djakarta, showed the animals from that area to be decidedly smaller than those from Krawang, a town about 28 miles to the east. Figure 1 shows the range and distribution of the snout-vent measurements; the double peaks in the curves for both sexes indicate the difference between the two populations.

VAN KAMPEN ('23) gives 90 mm as the maximum snout-vent size of *R. cancrivora* in the Archipelago, BOULENGER ('20) records 88 mm as the maximum size of a female on the continent, and SMITH ('30) gives 75 mm as the size of the typical form from Java. The smaller specimens from Kemajoran compared favorably with these measurements.

Those from Krawang, however, attained a maximum snout-vent size of 132 mm and *R. cancrivora* of this general size have also been obtained in Central Java. It seemed curious that no previous record could be found of these larger animals on Java, and they were at first thought to be *R. tigrina*
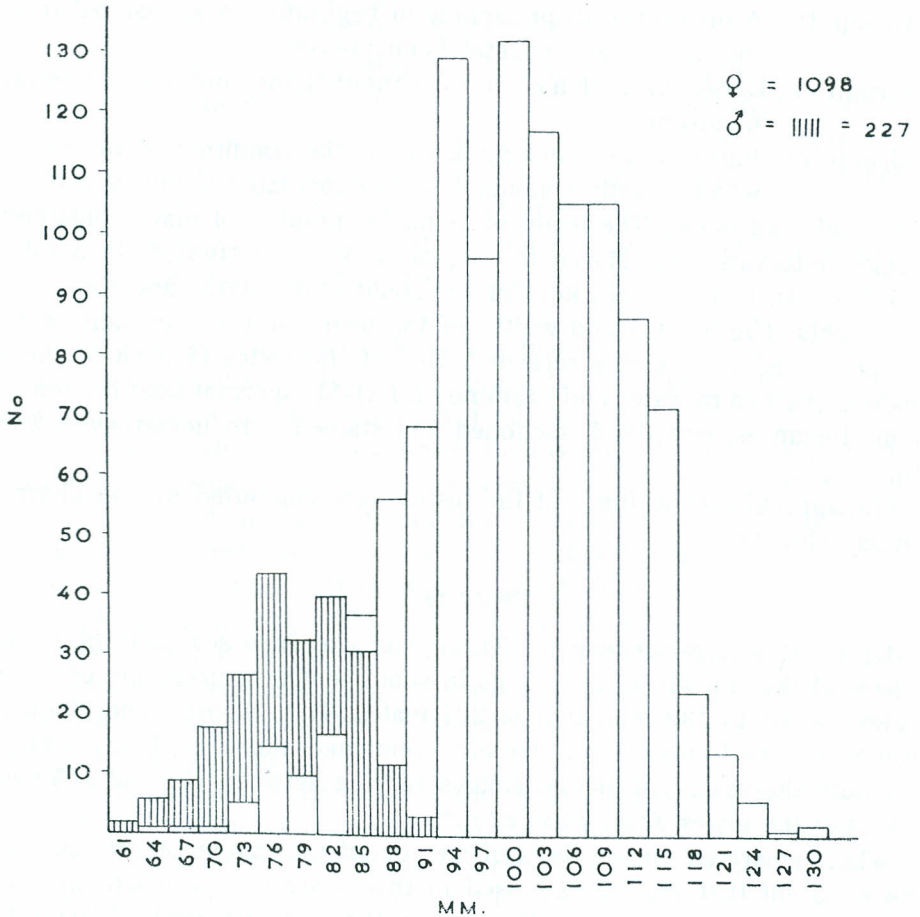

FIGURE 1.   Frequency distribution of the snout-vent lengths of 1098 female and 227 male *Rana cancrivora* from Djakarta and Krawang. Size differences between the two populations are indicated by double peaks in the two curves.

or one of its varieties — a large species closely related to *R. cancrivora* that is found on the continent — which might have been introduced into Java for its food value. But adult specimens were subsequently identified by MERTENS ('60) as *R. cancrivora*.

Interestingly, a similar population of large *R. cancrivora* has been recorded by SMITH ('30). He states that around Patani, a town in northern

Malaya, *R. cancrivora* grows to a much larger size than it does in other parts of its range, and he gives the snout-vent measurement of a female at 120 mm and of a male at 87 mm. It also differs from the smaller form in sometimes having a light vertebral stripe. SMITH gave this larger variety subspecies distinction and called it *R. cancrivora raja*. Perhaps the larger Javanese *cancrivora*, which are in the same size range and also frequently show a light vertebral stripe, should be included in this classification.

2. *Gross body weight*. The sex dimorphism noted in the snout-vent measurements was reflected in the weights of the two sexes. The gross body weights of 1,037 females ranged from 31 to 240 gm; those of 229 males, from 21 to 80 gm. The range and distribution of these measurements for both males and females are shown in figure 2. As noted in the curves
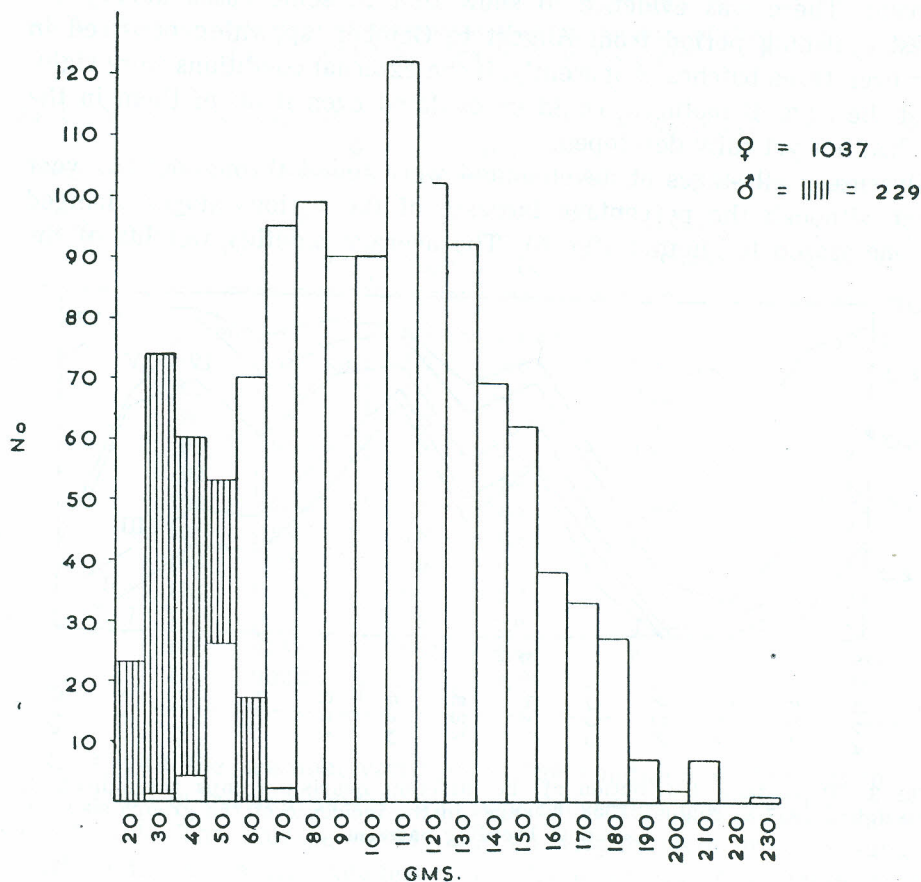

FIGURE 2.   Frequency distribution of the body weights of 1037 female and 229 male *Rana cancrivora* from Djakarta and Krawang. Size differences between the two populations are also indicated in these curves.

if the snout-vent distribution, two separate populations are indicated by a double peak in the curve of the female weights, and by skewness in that of the males.

· The average body weights of the females were greatest during September and October, 1957 after which they began to decline until January, 1958 when a low point was reached. From February until the following August there was a general increase. The average weights of the males dropped in March, and then increased until July.

3. *The ovaries*. In a total of 1,098 females a difference between the weights of the two ovaries was recorded, the left being heavier than the right almost without exception. The weights of the two ovaries together could equal as much as 25 percent of the gross body weight or as little as 1 percent. There was evidence to show that in some cases during the greatest spawning period from August to October, spawning occurred in two or even three batches. Apparently, if the external conditions were right, part of the eggs, if matured, could be ovulated even if all of them in the ovary had not yet fully developed.

Ovaries in all stages of development were found throughout the year studied, although the percentage increase of the various stages changed from one season to another (fig. 3). The average monthly weights of the
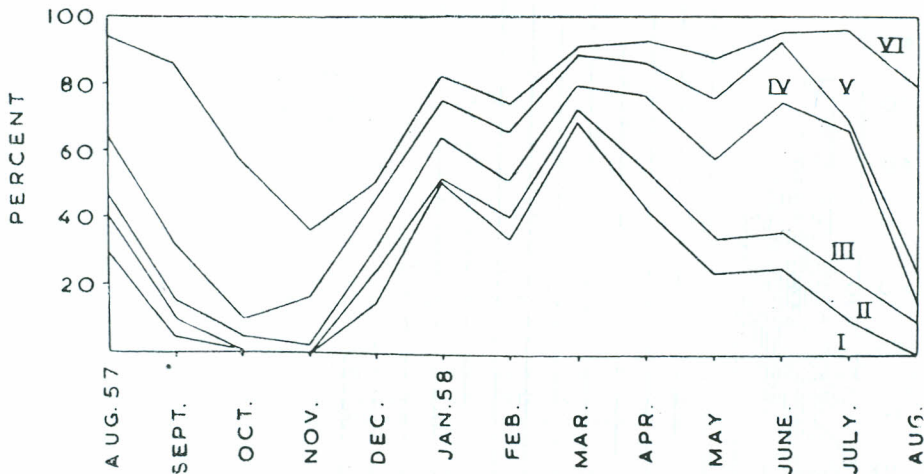

FIGURE 3. Percentage distribution of the different ovarian groups throughout the year August, 1957 to August, 1958. Changes in the incidence of the groups show the seasonal breeding pattern.

ovaries were greatest from September to November, after which they declined to a low point in March. Beginning in April, the ovarian weights

began to increase again, and they continued to grow larger until the end of the study in August (fig. 4). The monthly averages are tabulated in Table 1.
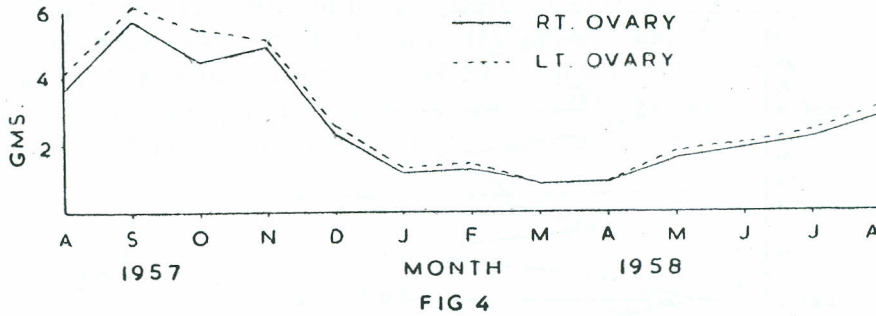

FIG 4

FIGURE 4.   Average monthly ovarian weights showing the peak of the breeding season. The left ovary was heavier than the right.

TABLE 1.   Monthly averages of measurements of female *Rana cancrivora*, August, 1957 to August, 1958.

Month	Snout-vent	Body Weight	Ovaries		Pituitary Volume	Glycogen mg/gm tissue
			Right	Left		
Aug., '57	90 mm	—	3.7 gm	4.1 gm	—	10.37
Sept.	101	138.3 gm	5.6	6.1	4.00 mm³	5.37
Oct.	101	138.5	4.4	5.4	3.29	5.03
Nov.	98	116.2	4.9	5.1	2.54	5.59
Dec.	98	94.9	2.3	2.5	4.18	4.50
Jan., '58	102	91.9	1.2	1.3	3.71	6.66
Feb.	106	109.9	1.3	1.4	4.60	7.19
Mar.	101	109.7	0.8	0.8	4.06	8.26
Apr.	102	102.1	0.9	0.9	3.90	9.55
May	103	106.6	1.6	1.8	4.40	7.39
June	107	145.5	1.9	2.0	3.63	5.81
July	105	135.4	2.2	2.4	4.57	—
Aug.	104	132.2	2.8	3.1	—	—

Along with the seasonal variation in the condition of the ovaries, a weekly rhythm in ovarian weights was recorded which was correlated with the lunar cycle. The average weekly ovarian weights were greatest during the dark of the moon and smallest when the moon was full. Attempts to correlate these data with rainfall, the one variable factor of any consequence, were unsuccessful. These results are shown graphically in fig. 5.
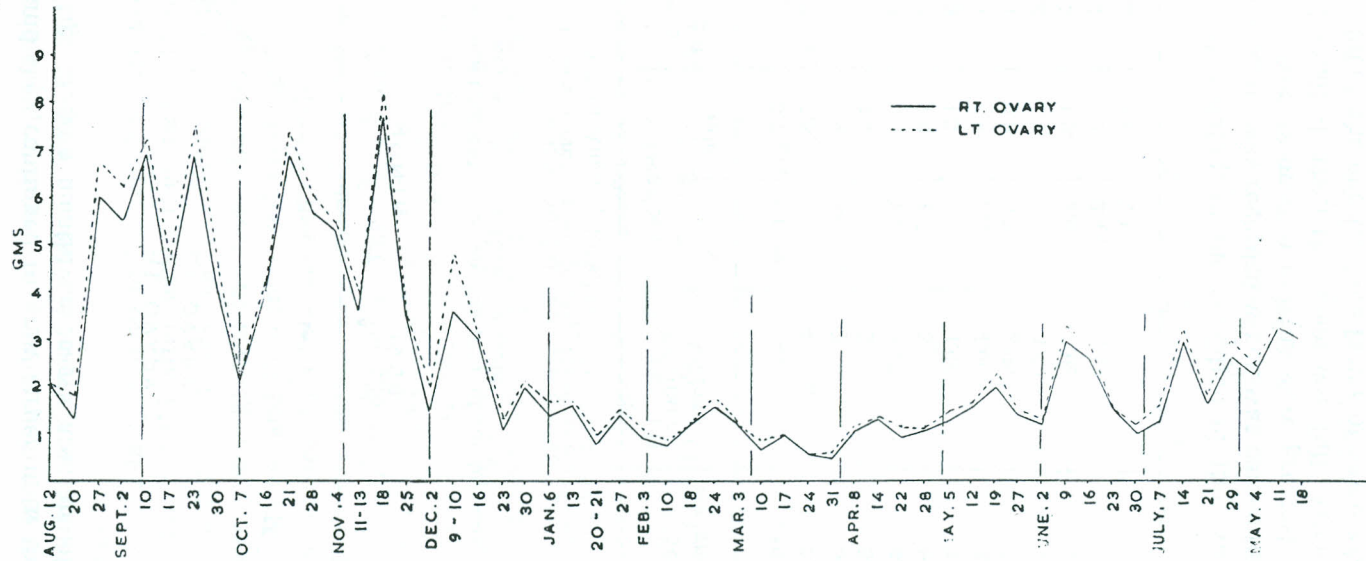
FIGURE 5. Average weekly weights of the right and left ovaries of 1098 female *Rana cancrivora* during the year August, 1957 to August, 1958. Vertical lines represent days of the full moon. The average weights were greatest during the dark of the moon.

The diameter and length of the oviducts invariably reflected the con-
lition of the ovaries. They were largest in animals ready to ovulate in Group
V, and smallest in Group I animals.

4. *The testes.* The testes of *R. cancrivora* are elongated and cylindrical
n form. As in the ovaries of the females, the left was almost always larger
:han the right, and this difference was also found in the weights of the
:wo (fig. 6). The ranges and means of the lengths, widths, weights and
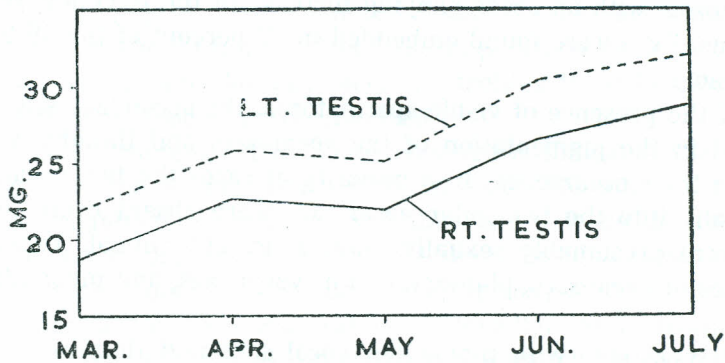approximate volumes of the left and right testes are given in Table 2.

FIG. 6

FIGURE 6. Average monthly testicular weights. The increase in weight paralleled the
increase in ovarian weights during the same time. As in the ovaries, the left testis was
heavier than the right.

TABLE 2. Monthly averages of measurements of male *Rana cancrivora*, August,
1957 to August, 1958.

| Month | Snout-vent | Body Weight | Testes | | | | Pit. Vol. mm³ | Glycogen mg/gm tissue |
| | | | Right | | Left | | | |
			vol.	wt.	vol.	wt.		
Febr., '58	78 mm	42 gm	—	—	—	—	—	—
Mar.	74	35	22.57 mm³	18.73 mg	29.72 mm³	22.14 mg	1.42	5.49
Apr.	79	44	31.76	22.70	37.68	25.70	2.20	5.68
May	82	48	31.24	21.97	34.07	25.10	2.79	4.89
June	82	51	37.89	26.70	43.52	30.30	1.80	—
July	78	47	42.99	28.89	54.05	32.16	—	—

Seasonally, the testes during the period from March through July,
1958 showed a continuous increase both in average monthly weight and in
average monthly volume which paralleled the increase in size of the ovaries
during this same period. Presumably, with the ovaries, the testes also reach

their maximum size in September, just prior to the period of greatest breeding activity.

Histologically, no morphological variations were found in the testes of *R. cancrivora* which might distinguish them from the typical ranid structure (RUGH, '51). Although no testis examined histologically was completely devoid of mature spermatozoa (all of the testes which were crushed were also found to contain viable mature spermatozoa), testes with tubules in all stages of spermatogenesis were found from March through July. During the same period there was an increase in the number of testicular tubules full of spermatozoa, with an accompanying increase in the thickness of the interstitial tissue. Eggs were found embedded in 57 percent of the 46 testes examined histologically.

In spite of the presence of viable spermatozoa, the secondary sex characters as noted in the pigmentation of the vocal sacs and thumbs, varied considerably in their occurrence. In a minority of cases the two vocal slits opening normally into the two gular vocal sacs were absent even though the animals were presumably sexually mature. In 112 animals examined for the presence of vocal sacs, pigmentation of vocal sacs, and pigmentation of thumbs:

61 had 2 vocal slits with pigmented vocal sacs and thumbs;

24 had 2 vocal slits with pigmented vocal sacs and unpigmented thumbs;

13 had 2 vocal slits with unpigmented vocal sacs and thumbs;

4 had 2 vocal slits with unpigmented vocal sacs and pigmented thumbs;

9 had no slits with unpigmented vocal sacs and thumbs;

1 had no vocal slits with unpigmented vocal sacs and pigmented thumbs.

5. *Fat bodies.* In a total of 838 female specimens examined, 394 (47 percent) had fat bodies of varying sizes. There was no correlation between the presence of fat bodies and the stage of the ovaries. Of the 394 females in which fat bodies were found:

28% of the animals in Group I had fat bodies;

58% of the animals in Group II had fat bodies;

59% of the animals in Group III had fat bodies;

39% of the animals in Group IV had fat bodies;

67% of the animals in Group V had fat bodies;

41% of the animals in Group VI had fat bodies.

There was a definite seasonal decline in the presence of fat bodies which followed the seasonal decline in ovarian weights from August to

February. The percentage of animals with fat bodies was highest in August, 1957, and lowest in February, 1958 after which the percentages began to increase again.

This finding differs from that on fat bodies in *Bufo melanostictus* from the same area. These toads in the more northern parts of their range on the continent show seasonal variation in fat body size (ALEXANDER, '33), but CHURCH ('60) has reported that few *melanostictus* possess fat bodies of any significance throughout the year in the Djakarta area. He concluded that the unending food supply rendered such food storage superfluous. Undoubtedly, a similarly continuous food supply was also available to R. *cancrivora* which should have reduced the importance of fat bodies to this species also if food were the only factor controlling their development. But factors other than food may be involved.

6. *Stomach contents*. The name *cancrivora* indicates that this frog eats crabs, and the examination of the stomach contents of 183 female specimens throughout the year showed this indeed to be the case. But its diet was by no means restricted to this item. Besides freshwater crabs, various other arthropods — including centipedes, spiders, grasshoppers, crickets, water beetles, roaches, crayfish, caterpillars and shrimp — snails, mice, birds, snakes (Typhlops), other frogs, vegetation (grass, leaves, buds) and mud were also eaten. Obviously, a constant supply of food was available at all times of the year and, although its main diet consisted of fresh water crabs, it appeared that this frog would eat anything it could get hold of.

PART II.   CHANGES IN THE VOLUME OF THE HYPOPHYSIS
(With GUSTI MOHAMMAD HUSSAINI)

The anterior lobe of the pituitary gland has been known for many years to influence the growth and liberation of sex products in adult amphibians in the temperate zone. The potency of the gland does not remain constant, however. The work of GRANT ('40) demonstrated the varying potency of the pituitary throughout the year in its ability to induce ovulation in *Rana pipiens;* and, supplementing this finding, it was shown by MILLER and ROBBINS ('55) that the volume of the hypophysis in the California newt, *Taricha torosa*, was smallest in size after the breeding season and that it gradually increased as the year progressed. There was also a concomittant change in the secretions of the acidophilic and basophilic cells during the course of the year. More recently it has been shown in *Bufo melanostictus* (CHURCH and HUSSAINI, '58) that here, too, in spite of the unchanging climatic factors, the volume and secretory condition of the

nterior hypophyseal lobe could be directly correlated with the breeding ondition of the animal. These results have since been confirmed in greater etail at the Technological Institute in Bandung by KWEE (unpublished).

MATERIALS AND METHODS

A varying number of female pituitaries, taken from the same animals which were used for the gonadal study (Part I), were measured every week rom September, 1957 to August, 1958. In all, 677 female animals were killed by decapitation and the whole pituitary glands removed by inserting he point of a fine pair of scissors through the foramen magnum, excising he brain case roof, lifting the roof and the brain out of the way, and dis-secting the pituitary free from the ventral surface of the brain. Similarly, the pituitaries of 43 males were measured from February through July, '58.

The long axis and the two short axes of the ellipsoidal glands were measured with an ocular micrometer. The approximate volumes were then calculated using the formula for finding the volume of an ellipsoid.

RESULTS

The volumes of the pituitaries taken from female animals were found to vary from 0.37 mm^3 in an animal with a snout-vent length of 108 mm, to 10.16 mm^3 in an animal with a snout-vent length of 112 mm. The average volume was 3.67 mm^3, and the average snout-vent length, 104 mm. (Table 1).

The volumes of the male pituitaries varied from 0.12 mm^3 in a spe-cimen with a snout-vent length of 78 mm, to 4.82 mm^3 in an animal with a snout-vent length of 86 mm. The average volume for the males was 2.00 mm^3; the average snout-vent length, 79 mm. (Table 2).

There was no correlation between the size of the hypophysis and the size of the animal, either in males or females. The size of the hypophysis varied widely regardless of the snout-vent size.

A definite correlation was found between the maturity of the ovaries and the size of the pituitary. As the ovaries matured and grew larger, the size of the pituitary increased (fig. 7).

Seasonally, there was a decrease in the volume of the female glands during October and November, the period of greatest breeding activity, after which the volumes increased until June and July when another de-crease occurred. The pituitaries of the males increased from February through May after which, like those of the females, they decreased during June and July. (fig. 8).
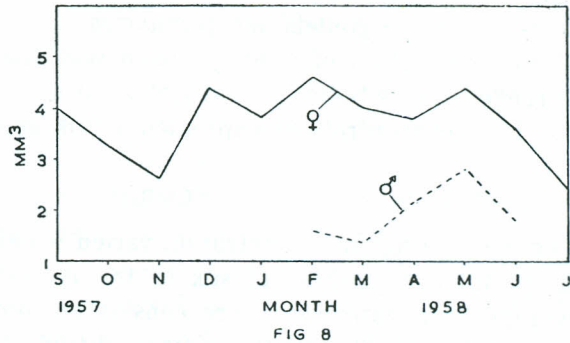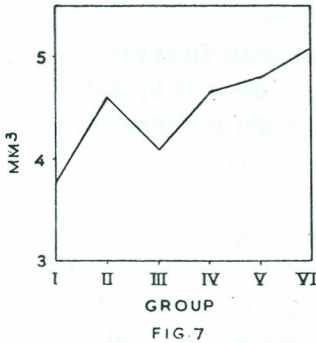
FIGURES 7 and 8. FIG. 7 shows the increase in volume of the pituitary as the ovary matures from Group I to Group VI. FIG. 8 indicates the decrease in pituitary volume after the peak of the breeding season, and the subsequent increase paralleling that of the testes and ovaries.

PART III. CHANGES IN THE LIVER GLYCOGEN CONCENTRATION
(With SIE KIAN TJONG)

The glycogen content of the amphibian liver has also been shown to vary at different times of the year in the temperate zone. During the breeding season in the spring, the amount of glycogen in the liver is at its minimum. After breeding, and after the frog begins to take food, glycogen is accumulated in the liver again, but because of the animal's active life during the summer months, it does not increase in any great quantity at that time. When the weather becomes cooler, and the animals less active, the glycogen increases considerably. It is stored during the hibernation period, only a small amount being used up at that time, but as the temperature rises on the approach of spring, the storage of glycogen is rapidly diminished in the maturation of sexual products and finally in mating.

It has been shown in the previously cited study of *B. melanostictus* (CHURCH and SIE, '58) that a correlation also exists between the maturity of the ovarian eggs and the concentration of liver glycogen in this tropical species even though the temperature and food are constant. Seasonal variations, however, were not detected. A similar study of the liver glycogen concentration in *R. cancrivora* is reported here.

MATERIALS AND METHODS

The liver glycogen concentrations were determined on approximately 20 female *R. cancrivora* each week from August, 1957 to January, 1958 after which the determinations were made bi-monthly until June, 1958. Determinations were made on a smaller number of males during March,

April and May, 1958. The same animals were used for the glycogen determinations as for the gonads and pituitaries.

The concentration of liver glycogen was analysed with Dreywood anthrone reagent using Seifer's modification of the method devised by MORRIS ('48), and the concentrations expressed as mg glycogen/gm of fresh tissue.

RESULTS

The liver glycogen concentration varied widely from individual to individual specimen regardless of sex or the maturity of the ovaries. When the average concentrations were considered, however, it was found in a total of 701 females that as the ovary matured, the glycogen concentration of the liver decreased until after ovulation when it began to rise again (fig. 9).
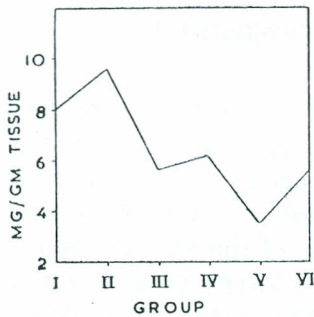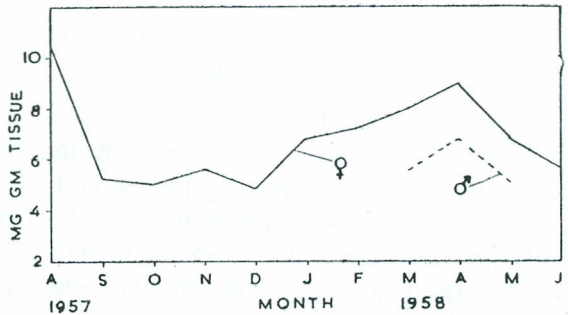

FIGURES 9 and 10. Figure 9 shows the decrease in liver glycogen as the ovaries mature from Groups I to V. Figure 10 shows the decline in liver glycogen during the peak of the breeding season and the subsequent increase, paralleling that of the ovaries. and testes through May.

Seasonally, the liver glycogen concentration followed the breeding curve (fig. 3). During the height of the breeding season the glycogen concentration was low, after which it began to rise again (fig. 10). The monthly average are given in Tables 1 and 2.

DISCUSSION

Rana cancrivora, like *Bufo melanostictus,* breeds throughout the year in West Java. But whereas *B. melanostictus* undergoes its peak spawning period with the onset of the northwest monsoon in November (CHURCH, '60), the peak breeding period for *R. cancrivora* takes place during the relatively dry months from August through October. Furthermore, since there is only one dry interval in the course of the year, the seasonal pattern is far more

pronounced and concentrated in *R. cancrivora* than in *B. melanostictus* which is apt to breed at any time.

A comparison of the pituitary findings and of the liver glycogen concentrations in the two species also shows *R. cancrivora* to have a more definite breeding period than *B. melanostictus*.

Why *R. cancrivora* prefers to spawn during the so-called dry season at a time when the rice paddies are under irrigation lacks complete explanation. Food cannot be a factor since the stomach contents indicate a continuing supply of food throughout the year. Nor can temperature or humidity, both of which remain almost constant, exert any appreciable effect.

There is, however, a definite yearly rhythm in the cultivation of the rice paddies where the animals are found. According to Mr. Soemartono, Acting Director of the Lembaga Penjelidikan Beras, Krawang ('60), the paddies around Djakarta depend solely on rain for their water and are planted only once a year in December during the rainy season. The rice is then cut in April and May. But in the Krawang region, where the largest frogs come from, half the area is sown as in Djakarta during the rainy season in December and harvested in April and May, after which it remains fallow if a secondary crop such as corn or beans is not planted; and the other half of the area is kept under constant irrigation, either by rainfall or artificially, and harvested in April and May; the other is sown in June after the rains have lessened, kept under irrigation and harvested in September and October.

It is during this period from June to October that the largest numbers of *R. cancrivora* breed (fig. 4). Apparently, this species, unlike *B. melanostictus* and many others, is not stimulated by, nor does it depend upon, heavy rainfall to bring about breeding (Marshall, '56).

After the harvest in September and October the irrigation system is shut off for inspection and repairs, and during this time the water in this constantly irrigated half of the paddy is at its yearly minimum. But by this time the peak has been passed and the number of breeding frogs has been reduced to a small fraction.

Since the paddies north of Djakarta remain fallow to a large extent from June to October when the paddies in Krawang are under irrigation, the smaller *R. cancrivora* in the Djakarta area must have to lay their eggs in drainage ditches. The unsuitable condition of the paddies for breeding during this half of the year may also restrict growth and be responsible for keeping these small frogs from ataining the large size shown by those in Krawang.

It should also be pointed out apropos of the seasonal breeding cycle of *R. cancrivora* that this frog undoubtedly migrated into the Indonesian Archipelago from a more northern habitat on the continent where it must have followed a typical seasonal breeding pattern as do other species in a changing yearly climate (NOBLE, '31; DARLINGTON, '57), and its reproductive behaviour on Java may be a physiological carryover from the continent. The continued development of fat bodies and the storage of liver glycogen in spite of the endless food supply which renders them no longer necessary would support this conclusion.

But if the assumption of BULLOUGH ('59), that the reproductive cycles of many vertebrates are under the dual control of an internal physiological rhythm and an external seasonal rhythm, is correct and applicable to amphibians, a less precise breeding period can be expected to develop in *R. cancrivora* on Java, since the external rhythm is here changed.

Having moved into an area with a relatively unchanging climate, presumably the mechanisms involved in its reproductive physiology have been only sightly modified. As pointed out by PROSSER ('58), time may be a factor in establishing physiological modifications in a species subjected to new conditions and it might be expected that *R. cancrivora*, like *B. melanostictus* which has also arrived from the continent but which has already acquired a more definite and advantageous adaptation to its new habitat, will ultimately develop a more modified seasonal breeding pattern.

No conclusive explanation has been found for the increase in ovarian weights which occurred during the dark of the moon. The local vendors, who captured the frogs at night by blinding them with a bright light, insisted that they were difficult or impossible to find when the moon was full. Giving support to their statements, on a number of occasions frogs were not available in the markets at this time of the month or, if they were available they were to be had only in small numbers. Restaurants, too, rarely offered fried frogs' legs during the time of the full moon.

BULLOUGH ('51) has pointed out that evidence of the effects of the lunar cycle on the reproductive rhythms of land vertebrates is singularly uncommon. But among the amphibians so influenced, FERGUSON ('60), in a study of *B. fowleri*, found that this species, like *R. cancrivora*, did not appear on moonlight nights, and CHURCH ('60) has reported that with *B. melanostictus* the opposite occurred, breeding females being more plentiful when the moon was full. At least one bird is affected by the lunar cycle. The nightjar, *Caprimulgus europaeus europaeus*, as reported by BULLOUGH ('51), is said by WYNNE-EDWARDS to lay its eggs during the last lunar quarter so the young will be born during a period when the moon is full, thereby making

possible for the hunt for food to continue throughout the night. And ARRISON ('52, '54) has postulated a "moonlight effect" to explain the reater number of conceptions that take place at one time of the lunar onth than another in four species of Malayan rat.

Unfortunately, it was not advisable for security reasons to go into the addies at night, especially in the Krawang area and, although on two ccasions trips were hazarded, a complete investigation of the effect of the unar cycle on *R. cancrivora* was not made. On these two moonless nights he guide made no attempt to capture frogs in thick rice growing in the looded paddies, but restricted his collecting to the built-up edges and neighboring ditches, a completely understandable procedure considering the shyness of the species and the impossibility of penetrating the paddy without ruining it.

This physical restriction on collecting may offer an explanation for the cyclic occurrence of lowered ovarian weights during the time of the full moon. Two possibilities present themselves. First, if vendors always collected only those frogs which could be caught along the edges of the paddies and, like *B. melanostictus*, there was a greater tendency for female *R. cancrivora* to breed during the time of the full moon, these animals would not be captured because mating would be taking place in the thickly planted, flooded paddies where the vendors do not venture. What animals there were to be collected along the edges would not be in breeding condition and would of course have small ovaries.

Considering the extreme shyness of this species, it may also be that these frogs are afraid of their own shadows, so to speak, and the bright light of the full moon, which is considerable in the tropics, may cause both breeding and nonbreeding females to leave the open areas of the dikes and banks to seek shelter in the dense cover of the growing rice. It is possible that a combination of both factors may be involved in cases where females are ready to spawn, but a complete answer to the problem must await a time when a thorough investigation can be made.

SUMMARY

1. There was a marked sex dimorphism in the snout-vent sizes of the two sexes; and a further marked difference in size was noted between the animals from Djakarta and those from Krawang.

2. Ovaries and testicular tubules in all stages of development were found throughout the year, but the peak of the breeding period occurred during the dry season in August and September.

3. The pituitary gland and the liver glycogen concentration varied with the condition of the gonads.

4. The average weekly ovarian weights were greatest during the dark of the moon and smallest when the moon was full.

5. Both the left ovaries and testes weighed more than the right.

6. Fat bodies were present in 47 percent of the females.

7. The stomach contents showed that a continuous and variable supply of food was always available.

REFERENCES

ALEXANDER, G., 1933. Secondary sexual characters of *Bufo melanostictus* Schneider. Copeia, 204.

BOULENGER, G. A., A Monograph of the South Asian, Papuan, Melanesian and Australian Frogs of the Genus *Rana*. Rec. Indian Mus., Calcutta, 1920.

BULLOUGH, W. S., Vertebrate Photostimulation. In Photoperiodism and Related Phenomena in Plants and Animals. AAAS. Washington, D. C., 1959.

————— Vertebrate Sexual Cycles. London, 1951.

CHURCH, G., 1958. The reproductive cycle of the Javanese toad, *Bufo melanostictus*. Part I. Ovulation in *B. melanostictus*. Indonesian Council for Sciences, Djakarta.

—————, 1960. Annual and lunar periodicity in the sexual cycle of the Javanese toad, *Bufo melanostictus* Schneider. Zoologica, in preparation.

—————, and THE DJIN TEK, 1958. The reproductive cycle of the Javanese toad, *Bufo melanostictus*. Part II. Cycle changes in the testes of *B. melanostictus*. Indonesian Council for Sciences, Djakarta.

—————, and SIE KIAN TJONG, 1958. The reproductive cycle of the Javanese toad, *Bufo melanostictus*. Part III. The glycogen concentration in the liver of *B. melanostictus* in relation to the maturity of the ovaries. Indonesian Council for Sciences, Djakarta.

—————, JANE A. KUSIN, and TIO KIAN LING, 1958. The reproductive cycle of the Javanese toad, *Bufo melanostictus*. Part IV. Cyclic changes in the hemoglobin concentration and the number of erythrocytes in *B. melanostictus*. Indonesian Council for Sciences, Djakarta.

—————, and GUSTI MOHAMMAD HUSSAINI, 1958. The reproductive cycle of the Javanese toad, *Bufo melanostictus*. Part V. The volume of the hypophysis in relation to the maturity of ovarian eggs in *Bufo melanostictus*. Indonesian Council for Sciences, Djakarta.

DARLINGTON, P. J., Zoogeography. New York, 1957.

FERGUSON, D., 1960. Observations on movements and behavior of *Bufo fowleri* in residential areas. Herpetologica, 16: 2.

GADOW, H., Amphibia and Reptiles. Cambridge, 1901.

GALLIEN, L., Endocrine basis for reproductive adaptations in Amphibia. In Comparative Endocrinology, Proc. of Columbia Univ. Symp. on Comp. Endocrinology, New York, 1959.

GRANT, R., 1940. Seasonal changes in the weight and ovulation-inducing potency of the glandular lobe of the pituitary in mature female *Rana pipiens*. Anat. Rec., 78: Suppl. 86.

HARRISON, J. L., 1952. Breeding rhythms of Selangor rodents. Bull. Raffles Mus., 24: 109.

————, 1954. The moonlight effect on rat breeding. Bull. Raffles Mus., 25: 166.

HOLMES, S. J., The Biology of the Frog. New York, 1927.

KAMPEN, P. H. VAN, The Amphibia of the Indo-Australian Archipelago. Leiden, 1923.

KWEE, Emmy, unpublished.

MARSHALL, F. H. A., Physiology of Reproduction, Vol. I. New York, 1956.

MERTENS, R., 1960. Personal communication.

MILLER, M. R., and M. E. ROBBINS, 1955. Cyclic changes in the pituitary gland of the urodele amphibian, *Taricha tarosa* (*Triturus torosus*). Anat. Rec., 122: 1.

MORRIS, D. L., 1948. Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Science, 107: 254.

NOBLE, G. K., The Biology of the Amphibia. New York, 1931.

PROSSER, C. L., Physiological Adaptations. Washington, 1958.

RUGH, R., The Frog. Philadelphia, 1951.

SCHMIDT, F. H. and J. H. A. FERGUSON, 1951. Rainfall types based on wet and dry period ratios for Indonesia with Western New Guinea. Kementerian Perhubungan Djawatan Meteorologi dan Geofisik, Djakarta.

SEIFER, S., 1950. The estimation of glycogen with the anthrone reagent. Arch. Bioch., 25: 191.

SMITH, M. A., 1930. The Reptilia and Amphibia of the Malay Peninsula. Bull. Raffles Mus., No. 3.

SOEMARTONO, 1960. Personal communication.