WILEY, JOHN EDWIN. Environmental Effectors of the Reproductive Cycle and Breeding Behavior in the Male Upland Chorus Frog, Pseudacris triseriata feriarum. (1976)
Directed by Herbert T. Hendrickson. Pp. 58

This study attempts to define the spermatogenic cycle and its environmental effectors in the male upland chorus frog, Pseudacris triseriata feriarum (Wied) and to investigate the environmental stimulation of breeding behavior such as calling and mating. It was hypothesized that some environmental cue or cues might be operating to trigger reproductive behavior. It was also thought that spermatogenesis might be subject to environmental influences.

Frogs were collected monthly, preserved, and their testes removed. Some frogs were kept in outdoor cages and others were held in indoor aquaria. Caged frogs were killed and preserved in the same manner as wild frogs at times when wild frogs were not available. The testes were sectioned, stained, and examined microscopically. Counts were made of spermatogonia, spermatocytes, spermatids, and sperm. The cell counts were compared to environmental data using a computer-based multiple regression analysis. A computer program for discriminant function analysis was used to compare environmental data with mating call data.

The testicular cycle consists of two periods of spermatogenesis; one occurring concurrent with breeding which lasts from January to mid-March, and the other begins in late June and ends before November. "Resting periods" occur during the intervening months. P. t. feriarum may be a potentially continuous breeder exhibiting a discontinuous breeding behavior in Guilford County, N.C. Spermatogenesis and breeding behavior correlate strongly with environmental factors such as photoperiod,

temperature, and rainfall. Interstitial tissue exhibits a cycle characterized by proliferation of tissue before and during breeding and by degeneration of tissue during the remainder of the cycle. Keeping frogs at room temperature (20°C) causes degeneration of existing sperm-Sertoli cell complexes and initiates spermatogenesis. The cell counts of frogs kept in cages outdoors did not differ significantly (at the 0.05 level) from cell counts of frogs captured in the wild.

ENVIRONMENTAL EFFECTORS OF THE REPRODUCTIVE CYCLE AND BREEDING BEHAVIOR IN THE MALE UPLAND CHORUS FROG, PSEUDACRIS TRISERIATA FERIARUM

by

John E. Wiley
"

A Thesis Submitted to
the Faculty of the Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Master of Arts

Greensboro
1976

Approved by

H. T. Hendrickson
Thesis Advisor

APPROVAL PAGE

This thesis has been approved by the following committee of the Faculty of the Graduate School at the University of North Carolina at Greensboro.

Thesis Adviser H. T. Hendrickson

Committee Members Laura G. Anderton

Richard C. Schauer

Paul E. Lutz

April 5, 1996

Date of Acceptance by Committee

ACKNOWLEDGMENTS

I would like to thank Dr. Herbert T. Hendrickson, Dr. Laura G. Anderton, Dr. Paul Lutz, Dr. Richard C. Schauer, and the Biology Department of UNC-G for their time and their assistance in the preparation of this thesis. In addition, I would like to acknowledge the assistance of Gary S. Davis and Matt E. Stockard. I would like to thank Mr. W.W. Danielson, Superintendent of Guilford Battleground National Military Park for permission to work on park land. This work was supported by UNC-G Research Grant 756.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The experimental study of the environmental events influencing the reproductive cycle of anuran amphibians dates from 1924 when Witschi studied the influence of climatic conditions upon spermatogenesis and breeding in Rana temporaria (van Oordt, 1960). R. temporaria was found by Witschi to be a discontinuous breeder (one that breeds once a year and possesses a refractory period in which the germinal epithelium is unresponsive to gonadotropins) whose behavior and spermatogenic cycle were largely independent of environmental events. However, subsequent work has shown that raising the environmental temperature can induce spermatogenesis in R. temporaria (van Oordt, 1956), R. esculenta (van Oordt, 1960) and R. graeca (Lofts, 1974).

Leptodactylus ocellatus was discovered to have two breaks in the spermatogenic cycle; both dependent upon temperature (Lofts, 1974). It was suggested by Lofts (1961, 1964) and van Oordt and Lofts (1963) that temperature controls the sensitivity of the germinal epithelium to gonadotrophic hormones. Temperature has an influence on the life of mature sperm; in discontinuous breeders, sperm viability is limited to one spermatogenic cycle (van Oordt and Lofts, 1963). van Oordt (1960) stated that the spermatogenic cycle is genetically controlled, with low temperature acting only as a suppressor of meiotic activity and thus not a primary factor in the control of spermatogenesis.

Studies of the relationship between hormones and the spermatogenic cycle have been numerous. Glass and Rugh (1944) described the spermatogenic

cycle of R. pipiens and the effect of administration of pituitary hormones upon the cycle. Lofts (1961) investigated the effects of leutinizing hormone (LH) and follicle-stimulating hormone (FSH) upon hypophysectomized R. temporaria. Seasonal changes in the functional activity of the testes in R. esculenta were also documented by Lofts (1964). van Dongen et al. (1959, 1960) established the role of LH in spermiation (the release of mature sperm into the lumen of the seminiferous tubules) while Basu (1969) found that FSH and LH must be present in order for spermatogenesis to occur with LH alone inhibiting spermatogenesis. Chieffi (1972) investigated the effects of exogenous androgens on spermatogenesis. Hormonal feedback systems that influence mating calling have been described for many species of frogs by Schmidt (1966a, 1966b, 1968, 1973). Feedback systems controlling the release of gonadotropins have been described by Lisk (1960, 1962), Davidson and Sawyer (1961a, 1961b), Kaneumatsu and Sawyer (1963), and Davidson and Smith (1966).

Perception and mediation of environmental events by sensory and neural components and their effect on spermatogenesis and breeding behavior in anurans have been investigated by several workers. The role of the median eminence of the brain in controlling gonadotropin release has been investigated by Dierickx (1966) who found that extirpation of the nervous pathways from the median eminence to the hypothalamus resulted in sexual quiescence. The perception of photoperiod by extraoptic means (pineal body) has been described by Dodt and Morita (1967) and Adler (1970, 1971). The pineal body has been found to have a fine structure like that of the rods and cones of the eye and is sensitive to wavelength and intensity of light (Dodt and Heerd, 1962; Dodt and Jacobson, 1963). Albation

of the pineal brings about breeding readiness of the gonads. Photoperiod cues may cause the production of a gonadotropin inhibitor that may be responsible for the suppression of LH and FSH production and thus cause sexual quiescence (Thiebolt, 1965).

Ifft (1942) failed to show any relation between photoperiod and spermatogenesis in Triturus viridescens (now Notophthalmus), a urodele. Werner (1969) found that in Plethodon cinereus, also a urodele, photoperiod did cause advances in the spermatogenic cycle at certain stages of the cycle, but was a secondary factor with temperature being the primary controller of spermatogenesis. van Oordt (1960) states that photoperiod does not play a primary role in anurans but may have a secondary significance in the regulation of spermiation and breeding behavior. No study has shown a primary relationship between photoperiod and spermatogenesis.

Gosner and Black (1955) described the effects on the breeding behavior of Scaphiopus h. holbrooki of temperature and rainfall, stating that an inch of rain coupled with temperatures of 10°C or above will initiate spawning behavior. Noble (1931) lists Hyla andersoni and Bufo fowleri as having rain-controlled breeding behavior. Hock (1967) described the effects of temperature on the breeding behavior of Bufo variegatus in which an air temperature of 6°C is sufficient to initiate calling.

Most studies have been done on the larger ranid frogs, and few studies other than those on reproductive physiology and behavior have been performed on other families, such as the family Hylidae. Wright and Wright (1949) described reproductive behavior of many hylid frogs, but dealt little with the factors that induce the behavior. Rugh (1941) did a study on the reproductive physiology of Hyla crucifer in which the spermatogenic cycle

was described. Several works have dealt with the breeding behavior of the hylid genus commonly known as chorus frogs (Pseudacris). Bragg (1948), Livezay (1952), Martof and Thompson (1958) and Whitaker (1971) are the major investigators who have described breeding behavior in Pseudacris. No paper has described the spermatogenic cycle or dealt with possible environmental effectors of spermatogenesis in Pseudacris.

The genus Pseudacris, as a whole, ranges from Florida to the upper provinces of Canada and west to the Rocky Mountains (Conant, 1958). The southernmost representatives of the genus, Pseudacris nigrita verrucosa and P. clarki, may breed any time of year with breeding activity associated with heavy rains (Conant, 1958). Generally, however, the representatives of the genus Pseudacris breed once a year with the time of year dependent upon climate. The subject of the present study, Pseudacris triseriata feriarum (Wied), occupies piedmont and mountain habitats and ranges north into Pennsylvania and west to Texas (Conant, 1958). P. t. feriarum breeds from late January through early March in Guilford County, N.C. Breeding habitat consists of temporary rain water pools or ditches usually containing various grasses and sedges, although breeding can occur in pools devoid of vegetation.

Since this frog breeds only once a year throughout its range, in a fairly limited and predictable period, it was hypothesized that some environmental cue or cues may be operating to trigger reproductive behavior. The unusual time of breeding (for a poikilothermic animal) implies that certain reproductive advantages are inherent in this behavior. The temporal occurrence of breeding behavior, however, has to be related to the life cycle as a whole; therefore, the reproductive cycle, as well as possible

environmental effectors, needed to be described since this information could not be obtained from the literature. The present study is an attempt to define the spermatogenic cycle and its environmental effectors in the male upland chorus frog, P. t. feriarum and to investigate the environmental stimulation of breeding behavior such as calling and mating.

MATERIALS AND METHODS

Male Pseudacris triseriata feriarum were collected and calling data were gathered from five areas of Guilford County, N.C. The main collection and study site was in Guilford Counthouse National Memorial approximately 11 km NNW of Greensboro, N.C. The study area was formerly a drive-in theatre, but was acquired in 1969 by the Federal Government. The present study was initiated four years after abandonment of the drive-in site. The study ponds were actually rain pools that were adequately supplied with water during the winter rains. All but one of the ponds completely evaporated during the summer. Before construction in the area by the Federal Government in 1975, four ponds were utilized for collection and study; three ponds remained after construction had ceased, and were designated ponds 1, 2, and 3. Pond 1 was approximately 25 m long, with the width depending upon the amount of rainfall, but within the range of 2-3 m. Pond 2 was about 30 m in length, with the approximate width falling within the same range as pond 1. Both of these ponds usually contained water to the depth of 45 cm except when drought occurred and the ponds evaporated. Pond 3 was 40 m long and 5 m at its widest point. Water in this pond approached 1 m in depth until alteration by bulldozer decreased this to 0.75 m at the deepest point. This pond decreased sharply in size during drought, but some water ramined in the deepest portions even when ponds 1 and 2 were completely dry. All three ponds contained stable populations of Typha latifolia (cattails), Cyperus sp. (sedges), and Zizania aquatica (wild rice). No fishes were seen at any time in the

ponds, but a large assortment of aquatic invertebrates (insects and arachnids) were present at all times. The other four collection areas were within an 11 km radius of the center of Greensboro, N.C. and were principally sites utilized in order that no one population would endure intense collection pressures.

During each of two breeding seasons, collections were made at approximately two-week intervals (when possible) in order to obtain a representative sample of breeding males. During the first year of collection (1974), some of the collected males were placed in wood and wire cages 60 cm by 90 cm by 60 cm with a 15 cm layer of sand and humus in the bottom. These were sampled beginning at the end of March, and every month thereafter until the supply of frogs was exhausted. In the second year (1975) wild frogs were collected in the same manner as in the previous year, and a portion of these was also kept in outdoor cages.

In order to determine what effect captivity had upon the testicular cycle, an indoor population was also established from frogs collected at the same time as the wild sample and outdoor cage frogs. These frogs were kept in ten gallon aquaria at a mean temperature of 22.5^{o}C and were watered daily with 10 ml of distilled water. The room in which the frogs were kept had windows facing west across the length of the wall. The indoor population was thus maintained at a constant temperature with constant moisture, but was subject to an altered photoperiod, as no control over the use of the fluorescent lights in the room was attempted. Open cultures of Drosophila were placed in the enclosures of both captive populations.

Wild frogs were obtained during the breeding season, but were difficult to procure after the season ended due to the secretive nature of

these frogs. A male Pseudacris was captured in November, 1975 on a wet road after a hard rain. Indoor samples were taken at the same time as wild and outdoor cage specimens except during February when an indoor sample was taken two weeks after being placed in the aquaria, and after April, when wild frogs could no longer be obtained.

The sample frogs were preserved in neutral buffered formalin. Stomachs were dissected and their contents noted. Testes were removed, measured by ocular micrometer and fixed in Bouin's fixative. They were later bulk stained with Erlich's hematoxylin and counterstained with eosin, sectioned at 2-5 micrometers and placed on microscope slides for examination. The sections were examined microscopically at 400X magnification and spermatogonia, primary and secondary spermatocytes, spermatids and sperm were counted. Sperm numbers were estimated by using an ocular grid; counts were made in one square, and this number was multiplied by the number of squares that contained sperm. Cell types were recognized by using the following diameters: spermatogonia, 4 micrometers; primary and secondary spermatocytes, 10-12 and 6-8 micrometers respectively; and spermatids, 2-4 micrometers. In addition, primary and secondary spermatocytes were identified by their meiotic activity. In the absence of such activity, primary spermatocytes were identified by their eosinophilic appearance and secondary spermatocytes were recognized by their basophilic appearance. Sperm were easily identified and needed no definition by size. Seminiferous tubule diameter was measured also. Counts and measurements were made using five tubules selected at random, and the counts and measurements were averaged. Representative tubules were also photographed. The cell type counts, testis width and length, and seminiferous tubule diameter were

analyzed with environmental data using a computer program for multiple regression analysis (described in Nie et al., 1975). The three populations, wild, outdoor cage, and indoor cage, were compared using analysis of variance for the two months during which the three sample populations were taken; March and April. Environmental data were obtained from the U.S. Weather Service office at the Greensboro-High Point-Winston-Salem Airport. Photoperiodic data were obtained from the American Ephemeris and Nautical Almanac; 1972-1975. Environmental data were recorded at the breeding site, but were not complete enough for statistical treatment. However, the U.S. Weather Service data did not differ from those recorded at the study area, and thus were used instead.

From December, 1972 until November, 1975, the study areas were checked for the presence of calling males. During the breeding season, including one month prior to and one month after calling had ceased, there was intensive monitoring of the study areas for mating calls with observations being made daily during this time. Thereafter, spot checks were made in an attempt to collect wild samples out of season, but which were successful in only one case, that of the November frog mentioned above. Calling and environmental data were analyzed using a computer program for discriminant analysis (Nie et al., 1975). Slides and sample frogs are in my private collection.

RESULTS

Breeding Behavior

The breeding period for Pseudacris in Guilford County ran roughly from late January into early March. Synchronous and sympatric breeders included Rana pipiens, Hyla crucifer (which began to breed during February but peaked in March), and Bufo americanus (which began breeding around March). Male Pseudacris were observed several times calling from holes in the ice covering the ponds when water temperature was 4°C and air temperature was 1°C. Evidence of a chorus structure (cohesive groups of calling individuals within the total chorus) was found. If the chorus ceased calling which it often did especially during the waxing and waning of the breeding period, it was usually initiated again by the same male. Trios and duets could also be discerned. It was noted with some interest that if jet planes crossed over the field, it could initiate calling of a silent chorus. Conversely, if a plane passed over when a chorus was calling, it would immediately become silent. This confirms observations by Bellis (1957) and Hardy (1959).

Gastro-Intestinal Contents

Numerous insects were found around the breeding area, so food during this period was not an apparent limiting factor. Checks of the stomachs and intestines showed that 23 breeding males had gastro-intestinal tracts full (out of a total of 47 captured while breeding); 3 had traces of material in their stomachs, but with full intestines; and 12 had empty gastro-intestinal tracts. Therefore, 55.3% contained some material in their

stomachs (Table I). Since 12 had completely empty gastro-intestinal tracts, 80.9% contained some material in the tracts (Table I). The remains found most often were those of arthropods, with arachnids found more often than any other form. Mud and plant remains were also found, and in one case a sprouting seed was discovered. These observations agree with those of Whitaker (1971) who also found arachnids to be present more than any other group, and who also found mud and vegetation in the stomachs of Pseudacris triseriata triseriata. Whitaker's figure of 64.9% for the percentage of frogs with gastro-intestinal contents also agrees generally with the 80.9% figure noted above.

Both captive populations, indoor and outdoor, were observed capturing food items. Of a total of 13 frogs caged outdoors, 4 had full gastro-intestinal tracts, and 4 had empty gastro-intestinal tracts, while 5 had empty stomachs with material in the intestines (Table I). Contents consisted of Drosophila, various arachnids, and some isopods. A total of 30.7% had full gastro-intestinal tracts, and 69.3% had some gastro-intestinal contents (Table I). The indoor population consisted of 16 frogs, of which 4 had full gastro-intestinal tracts, 8 had empty gastro-intestinal tracts, and 4 had empty stomachs with full intestines (Table I). All contents were Drosophila remains. A total of 25% had a full gastro-intestinal tract, whereas 50% had some gastro-intestinal contents (Table 1). Any "captivity effect" (Zwarenstein and Shapiro, 1933) therefore, is probably not due to starvation.

Calling

One such "captivity effect" is cessation of calling. Frogs placed in outdoor cages continued to call for approximately two weeks after capture,

TABLE I. Gastro-intestinal contents of sample frogs

	N	% of total
Breeding frogs		
Gastro-intestinal tract full	23	48.9%
Intestines full (but stomach empty)	9	19.2%
Gastro-intestinal tract empty	12	25.5%
Trace in stomach (intestines full)	3	6.4%
Total	47	100.0%
Non-breeding frogs (outdoor cage)		
Gastro-intestinal tract full	4	30.7%
Intestines full (but stomach empty)	5	38.6%
Gastro-intestinal tract empty	4	30.7%
Trace in stomach (intestines full)	0	0.0%
Total	13	100.0%
Non-breeding frogs (indoor cage)		
Gastro-intestinal tract full	4	25.0%
Intestines full (but stomach empty)	4	25.0%
Gastro-intestinal tract empty	8	50.0%
Trace in stomach (intestines full)	0	0.0%
Total	16	100.0%

whereas the indoor population ceased calling within three days except for periodic release calls and a few aberrant mating calls. All calling in the indoor population ceased after a week of captivity.

Discriminant analysis of calling data is summarized in Table II.

TABLE II. Significant (at a level below 0.001) discriminant function coefficients of independent environmental variables determining calling

Month	-0.34222
Photoperiod (length of daylight)	-0.31743
Daily low temperature	0.16680
Daily rainfall	0.03223

The higher the absolute value of the number, the greater is the importance of the factor. All other factors were discarded by the computer as being not significant (at a level below 0.001) as coefficients in the equation used to predict calling. The month, of course, would be important, as these frogs usually call only during the breeding season, though calling in October and November occurred during 1975. Late calling has also been noted by Evenden (1946), Blair (1951), and Whitaker (1971). Photoperiod (length of daylight) is the most important environmental factor in determining calling having a high negative discrimination coefficient. As photoperiod increases, calling decreases (Table II). Daily low temperature is the second important environmental variable (Table II) having a positive value. The higher the low temperature of the day, the greater the possibility of frogs calling. Pseudacris was rarely heard calling in the

field if the temperature dropped below freezing. Of the factors studied, the least important of the significant environmental variables was rainfall which had a positive effect on calling (Table II). As rainfall amounts increase, so does calling. On the basis of the values in Table II, the computer attempted to predict whether or not, on any given day, calling or no calling would be observed. Table III shows the accuracy of the predictions. The computer predicted correctly that on any given day, no calling would occur on 860 out of 1007 actual days that no calling was observed for an 85.4% correct record. It also predicted correctly that calling would occur on 111 of 119 actual days that calling was observed for a 93.3% correct record. The total per cent of correctly classified cases was 86.23%. The discrimination coefficients, therefore, are highly accurate when used to predict calling activity.

TABLE III. Calling or no calling predication results based on discrimination function coefficients (Table II)

	No. of cases	Predicted Group Membership	
		No Call	Call
No call	1007	85.4% (860)	14.6% (147)
Call	119	6.7% (8)	93.3% (111)

Percentage of cases correctly classified: 86.23%

Cell Counts

In addition to calling data analysis, cell counts were examined and analyzed with environmental data. Table IV shows the mean, standard deviation, variance, number, and range for the cell type counts of wild, outdoor cage, and indoor cage samples. These cell counts are graphed in Figure 1. An analysis of variance was performed on the cell counts for the months of March and April at the 0.05 level of significance. No significant difference was found between the three sample populations, except in spermatid numbers, where March outdoor cage specimens differed from wild specimens in March.

Figure 1 shows the relationships of the three sample populations with respect to cell counts. In Figure 1A, the graph of spermatogonia counts shows that there is not much difference in the curves of the three sample populations. The wild population hit a low at the end of the breeding season. The outdoor cage population fluctuated in monthly pattern while indoor cage numbers remained constant. The November wild sample showed a spermatogonia count lower than January's which probably indicates that new sperm wave formation began in January, the start of the breeding season for Pseudacris. The count is almost the same as in April when sperm wave formation had ceased.

In Figure 1B, combined primary and secondary counts are graphed. The count for the wild population increased slightly as the season progressed, and fell off in April to correspond with cessation of breeding. Indoor and outdoor samples paralleled one another closely. Peaks occurred in March, and lowest counts occurred in May as sperm replenishment ceased and spermatocytes were being reabsorbed. Peaks were noted again in July

TABLE IV. A. Mean, standard deviation, variance, number and range for spermatogonia counts

Month and Year	Type	Mean	Standard Deviation	Variance	N	Range
1/74	wild	13.077	7.000	50.410	13	4-28
1/75	wild	5.800	1.330	1.067	10	3.6-7.4
2/74	wild	8.800	1.304	1.700	5	6.6-10.2
2/75	wild	5.000	1.054	1.111	10	3.4-5.8
2/75	indoor	5.222	1.641	2.694	9	3-7.6
3/74	wild	4.000	0.000	0.000	2	3.6-4.4
3/75	wild	3.800	1.643	2.700	5	2.2-6
3/75	outdoor	5.500	1.915	3.667	4	4-8.4
3/75	indoor	4.750	0.957	0.917	4	4-6.2
4/74	wild	5.000	0.000	0.000	1	5
4/75	outdoor	5.000	1.320	3.000	3	3.6-7.2
5/74	outdoor	15.000	12.738	162.000	2	6.4-23.8
5/75	outdoor	4.000	0.000	0.000	1	4
5/75	indoor	4.600	0.000	0.000	2	4.6
6/74	outdoor	5.600	0.707	0.500	2	5.2-6
7/74	outdoor	7.800	0.000	0.000	1	7.8
7/75	indoor	2.600	0.000	0.000	1	2.6
11/75	wild	4.400	0.000	0.000	1	4.4

TABLE IV. B. Mean, standard deviation, variance, number and range for spermatocyte counts

Month and Year	Type	Mean	Standard Deviation	Variance	N	Range
1/74	wild	22.615	10.959	120.090	13	8-41.8
1/75	wild	12.900	5.384	28.989	10	4.2-24
2/74	wild	14.600	7.537	56.800	5	5.6-23
2/75	wild	21.800	10.789	116.401	10	4.8-41.6
2/75	indoor	44.889	20.019	437.611	9	18.8-88
3/74	wild	22.000	5.657	32.000	2	17.6-26
3/75	wild	18.800	11.649	135.700	5	4.2-35.6
3/75	outdoor	42.000	26.013	676.667	4	19.6-73
3/75	indoor	32.000	12.247	150.000	4	20.4-49
4/74	wild	15.400	0.000	0.000	1	15.4
4/75	outdoor	34.000	35.595	1267.000	3	11.4-75
5/74	outdoor	2.000	2.828	8.000	2	0.4-4.4
5/75	outdoor	7.000	0.000	0.000	1	6.6
5/75	indoor	13.000	11.314	128.000	2	5.4-21.4
6/74	outdoor	10.300	14.849	220.500	2	0-20.6
7/74	outdoor	45.200	0.000	0.000	1	45.2
7/75	indoor	55.600	0.000	0.000	1	54.6
11/75	wild	4.000	0.000	0.000	1	4

TABLE IV. C. Mean, standard deviation, variance, number and range for spermatid counts

Month and Year	Type	Mean	Standard Deviation	Variance	N	Range
1/74	wild	10.846	10.383	107.808	13	0-31
1/75	wild	17.300	17.945	322.011	10	0-62
2/74	wild	16.000	8.515	72.500	5	7.2-26.4
2/75	wild	14.900	10.630	112.989	10	3-34
2/75	indoor	18.667	10.909	119.000	9	4-37
3/74	wild	15.500	16.263	264.500	2	4.2-27
3/75	wild	41.600	23.923	572.301	5	14.6-78
3/75	outdoor	10.750	6.076	36.917	4	3.8-18.2
3/75	indoor	20.750	12.038	144.917	4	5.4-32.2
4/74	wild	7.000	0.000	0.000	1	7
4/75	outdoor	4.666	1.528	2.333	3	3.4-6.2
5/74	outdoor	5.000	7.071	50.000	2	0-9.8
5/75	outdoor	20.000	0.000	0.000	1	20
5/75	indoor	30.000	32.527	1058.000	2	7-53
6/74	outdoor	6.500	9.192	84.500	2	0-13
7/74	outdoor	0.000	0.000	0.000	1	0
7/75	indoor	20.000	0.000	0.000	1	20
11/75	wild	5.400	0.000	0.000	1	5.4

TABLE IV. D. Mean, standard deviation, variance, number, and range for sperm counts

Month and Year	Type	Mean	Standard Deviation	Variance	N	Range
1/74	wild	235.693	44.973	2022.568	13	181.4-298
1/75	wild	215.700	66.625	4438.902	10	120.6-351.8
2/74	wild	259.000	30.257	915.500	5	216.2-297
2/75	wild	229.600	46.261	2140.049	10	136-312
2/75	indoor	91.889	41.787	1746.117	9	25-162.4
3/74	wild	170.000	41.012	1682.000	2	140.8-199.2
3/75	wild	142.200	67.703	4556.703	5	39.4-208.3
3/75	outdoor	162.000	41.545	1726.000	4	126.8-221.8
3/75	indoor	117.750	39.178	1534.917	4	79.4-169.2
4/74	wild	154.000	0.000	0.000	1	154
4/75	outdoor	168.667	115.820	13414.344	3	40.6-267.4
5/74	outdoor	76.500	28.991	840.500	2	56.2-96.6
5/75	outdoor	173.000	0.000	0.000	1	173
5/75	indoor	153.500	4.950	24.500	2	150.2-156.8
6/74	outdoor	118.500	6.364	40.500	2	114.2-122.8
7/74	outdoor	84.000	0.000	0.000	1	84
7/75	indoor	69.000	0.000	0.000	1	69
11/75	wild	307.400	0.000	0.000	1	307.4

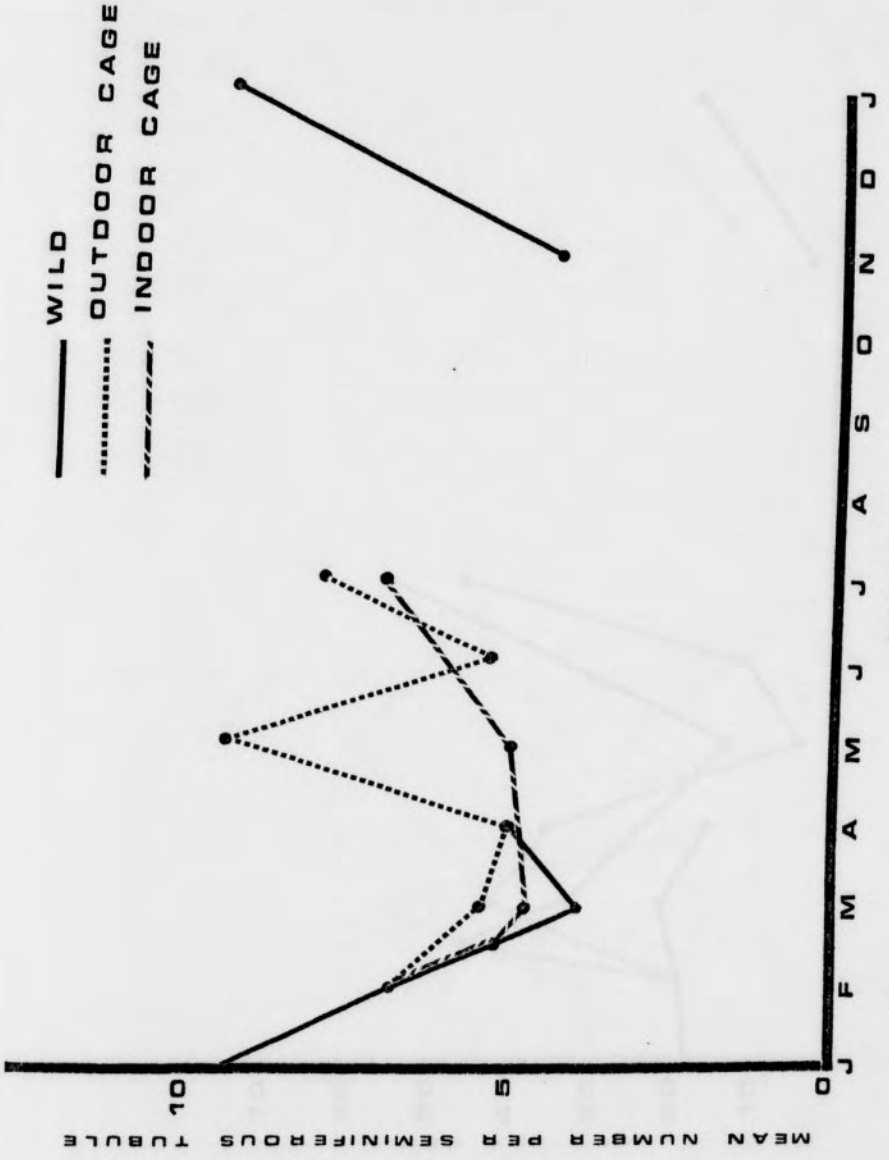

FIGURE 1A. NUMBER OF SPERMATOGONIA

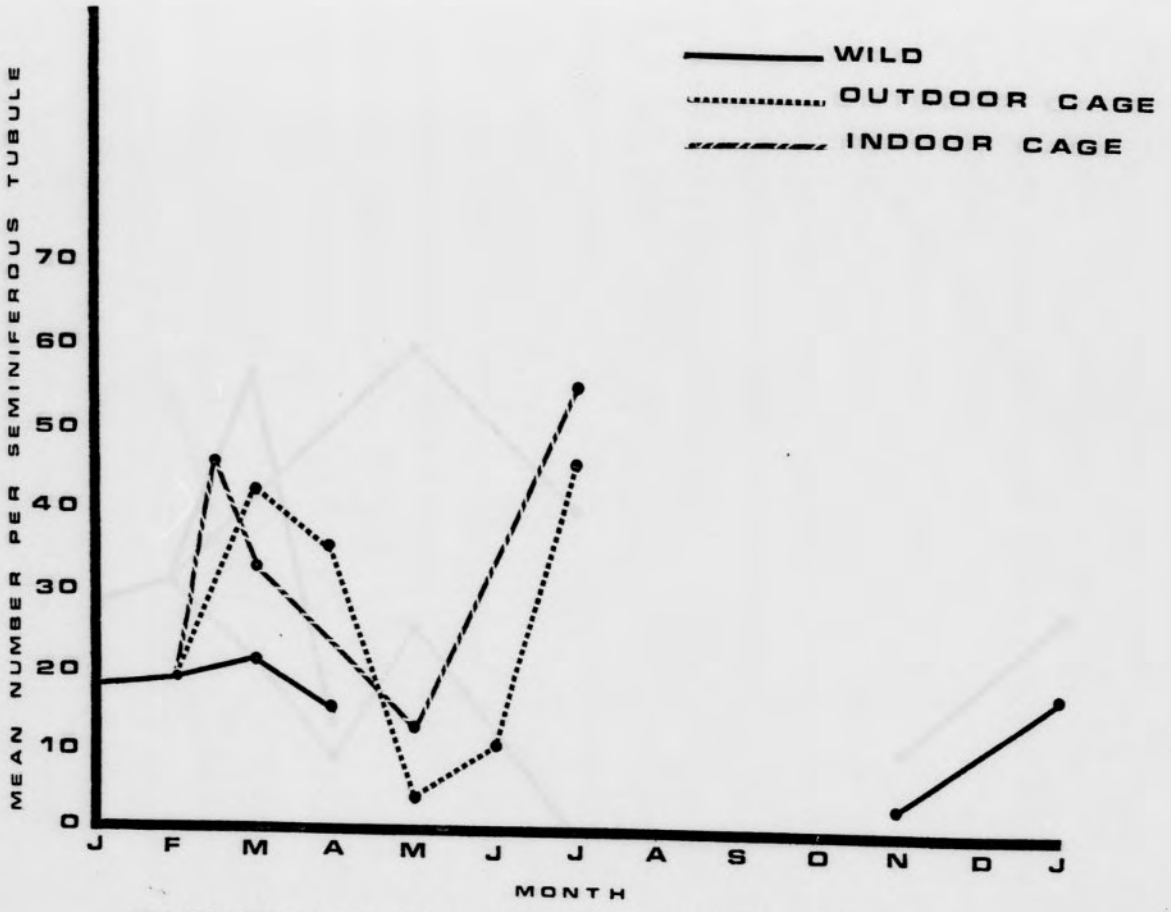

FIGURE 1B. NUMBER OF 1° AND 2° SPERMATOCYTES

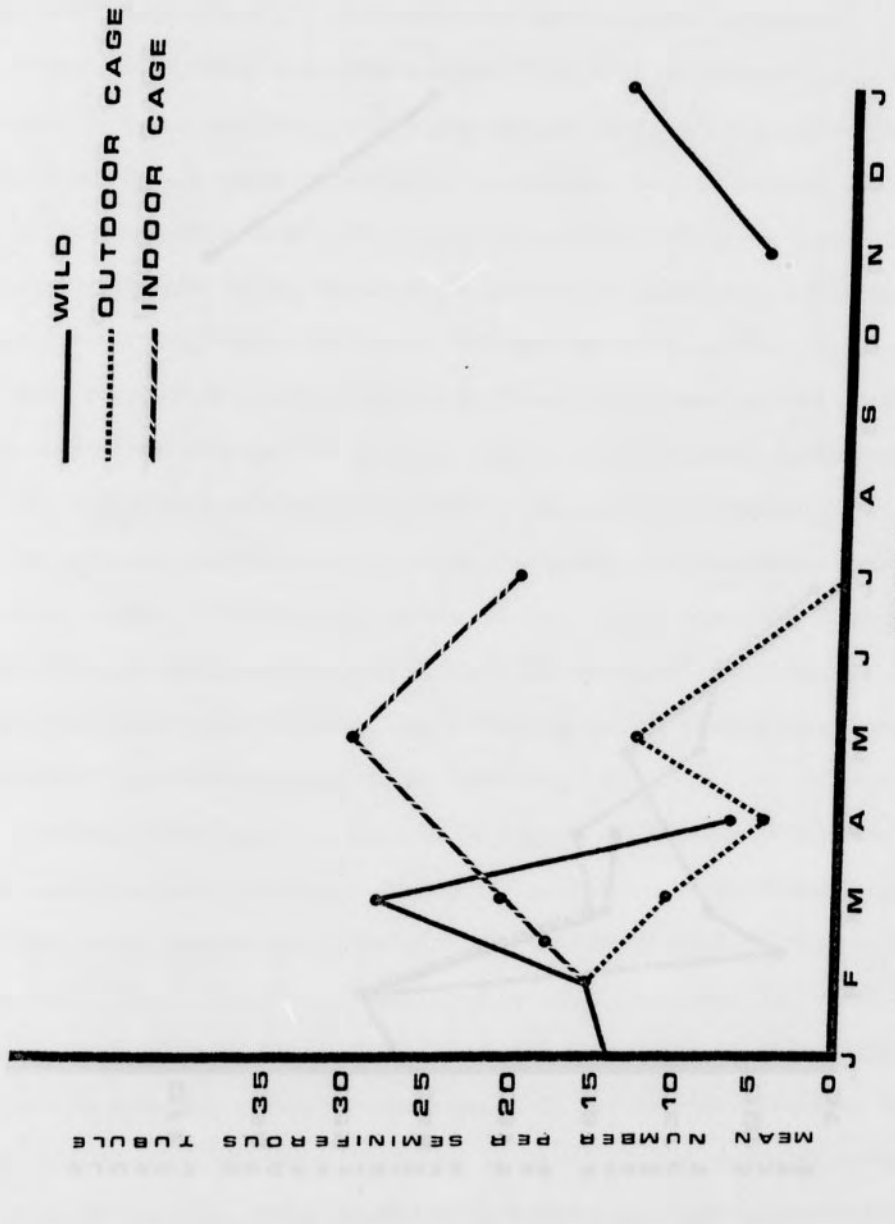

FIGURE 1C. NUMBER OF SPERMATIDS

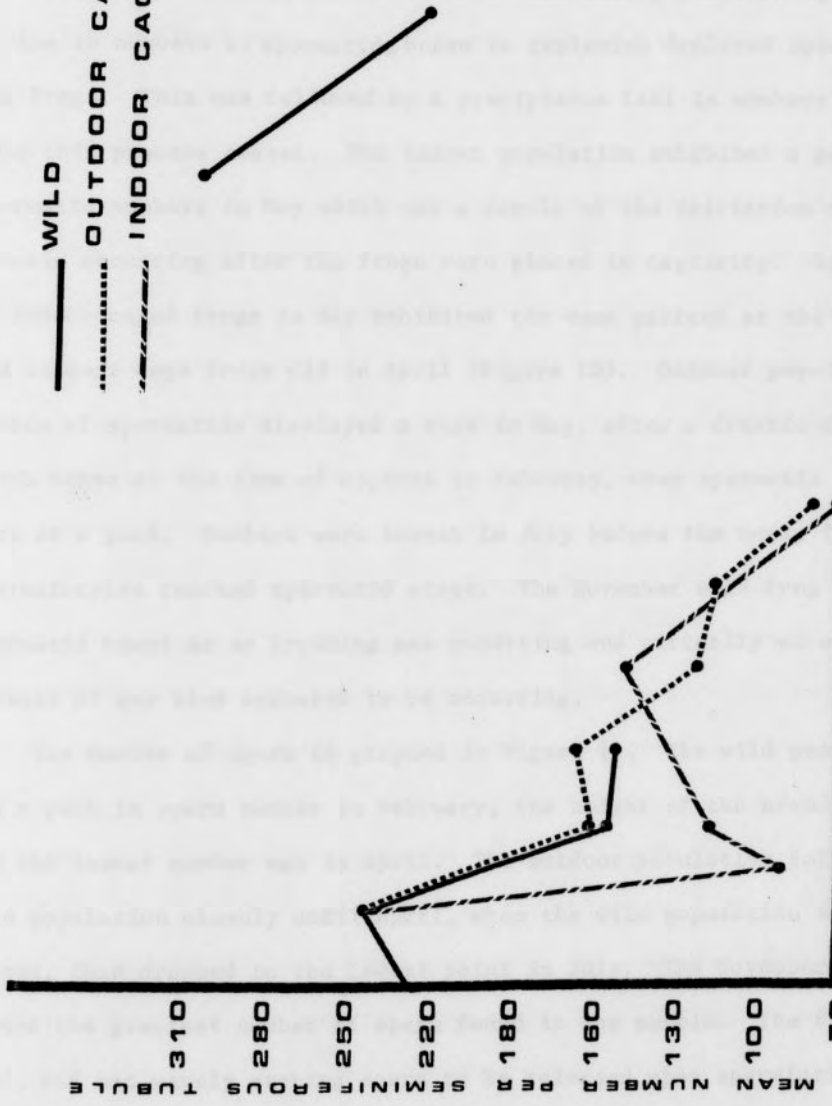

FIGURE 10. NUMBER OF SPERM

as spermatogenesis began in both populations. The November wild frog, having completed spermatogenesis, had a low spermatocyte count, which was different from January's when waves of sperm were being formed.

Figure 1C shows spermatid counts. The wild population again displayed a rise in numbers as spermatids began to replenish depleted sperm in breeding frogs. This was followed by a precipitous fall in numbers by April when this process ceased. The indoor population exhibited a peak in spermatid numbers in May which was a result of the initiation of spermatogenesis occurring after the frogs were placed in captivity. Sperm numbers of indoor caged frogs in May exhibited the same pattern as the wild frogs and outdoor cage frogs did in April (Figure 1D). Outdoor population counts of spermatids displayed a rise in May, after a drastic decline which began at the time of capture in February, when spermatid numbers were at a peak. Numbers were lowest in July before the newly formed spermatocytes reached spermatid stage. The November wild frog had a low spermatid count as no breeding was occurring and virtually no spermatogenesis of any kind appeared to be occurring.

The number of sperm is graphed in Figure 1D. The wild population had a peak in sperm number in February, the height of the breeding season, and the lowest number was in April. The outdoor population followed the wild population closely until April, when the wild population samples ceased, then dropped to the lowest point in July. The November wild frog showed the greatest number of sperm found in any sample. The frog had not bred, and was merely storing sperm to be released upon spermiation which occurred in January. Frogs captured in January may have already bred once, which was evident in the counts from January, as no breeding has ever been

observed by me in the Guilford County area from November to January (and there is no reference to such activity in the literature) thus no apparent spermiation occurred during this time. This is also evident from the photographs (Figure 2) in which released sperm was evident in the January frog (Figure 2B) whereas all sperm were embedded in the November wild frog (Figure 2C).

Environmental Factors

The influences of environmental factors on the various stages of spermatogenesis and related variables were examined using a multiple regression from which a correlation table and "R Square" values were derived. This is shown in Tables V and VI. Only significant values are shown in Table VI. In Table V, an absolute value of 0.5000 or better was considered significant. In summary, significant relationships with respect to spermatogenesis are as follows: the date had a strong correlation with the number of days in the month the frogs called and with testis width; mean monthly temperature had a negative correlation with sperm number; mean monthly photoperiod was negatively correlated with the number of days of calling and testis width (photoperiod is, of course, strongly correlated with the date); and number of primary and secondary spermatocytes had a negative correlation with the number of sperm (Table V).

These data, in turn, correlate to the graphs in Figures 1A-1D. As the months progressed the temperature rose, and photoperiod increased. Calling fell off as the breeding season ended as did sperm number. Because new sperm were formed from the preceding stages, one would expect spermatocyte numbers to fall as sperm numbers increased. The reverse was also true. The decrease in testis width was interesting as it seemed to indicate

TABLE V. Correlation coefficients

	01	02	03	04	05	06	07	08	09	10	11	12
01	1.000											
02	-0.044	1.000										
03	0.666*	-0.513*	1.000									
04	0.986*	-0.025	0.657*	1.000								
05	-0.516*	-0.157	-0.400	-0.554*	1.000							
06	-0.265	-0.234	-0.116	-0.254	0.086	1.000						
07	-0.523*	-0.337	-0.148	-0.532*	0.368	0.443	1.000					
08	-0.225	0.211	-0.345	-0.216	0.081	-0.128	-0.083	1.000				
09	0.070	-0.478	0.312	0.021	0.057	0.228	0.268	-0.209	1.000			
10	-0.012	-0.161	0.087	-0.004	-0.152	0.115	0.099	-0.179	0.071	1.000		
11	-0.489	0.361	-0.732*	-0.479	0.237	0.174	0.140	0.240	-0.511*	-0.302	1.000	
12	-0.372	-0.075	-0.232	-0.356	0.188	0.332	0.427	-0.276	0.013	0.085	0.340	1.000

*Significant values (any coefficient over 0.5000 in absolute value).

01 Date
02 Total Monthly Rainfall
03 Mean Monthly Temperature
04 Mean Monthly Photoperiod
05 # of Days Calling
06 Testis Length
07 Testis Width
08 # of Spermatogonia
09 # of Primary and Secondary Spermatocytes
10 # of Spermatids
11 # of Sperm
12 Seminiferous Tubule Diameter

TABLE VI. Relationship of environmental factors to spermatogenic stages and related variables as shown by R Square values

Dependent variable: # of days in month frog called

	R Square Value
Mean monthly photoperiod	0.30742
Date	0.34229
Total monthly rainfall	0.36509
Mean monthly temperature	0.41715

Above factors account for 42% of the variability in the data

Dependent variable: Mean testis width

	R Square Value
Mean monthly photoperiod	0.28311
Total monthly rainfall	0.40586
Mean monthly temperature	0.40827
Date	0.40944

Above factors account for 41% of the variability in the data

Dependent variable: # of sperm

	R Square Value
Mean monthly temperature	0.53646
Total monthly rainfall	0.53674
Mean monthly photoperiod	0.53690
Date	0.53739

Above factors account for 54% of the variability in the data

that the tubules were no longer packed with sperm and thus had shrunk to some extent.

Table VI shows the relationship of environmental factors to spermatogenic stages and related variables (such as testis width). In summary, mean monthly photoperiod (a function of the date), the date, total monthly rainfall, and mean monthly temperature accounted for a high percentage of variability and were significant in the following: the number of days per month that the frog called, 42% of the variability; mean testis width, 41% of the variability; and number of sperm, 54% of the variability. The order in which these factors are placed in Table VI indicates their degree of importance in determining variability of data. Mean monthly photoperiod was of greatest importance in determining the number of sperm. The remaining unaccounted for variability was due to factors which could not be ascertained from the data given.

Histology of the Testes

Figure 2 is a series of photographs depicting representative tubules from each month and population type. Figure 2A shows representatives of each cell type. Figure 2B depicts a testis from a frog captured wild in January. There was well-developed interstitial tissue, and most sperm were embedded in Sertoli cells. Very few other cell types were evident. A February testis showed signs of breeding activity. More sperm were loose in the lumen and meiotic figures could be seen as well as primary and secondary spermatocytes, as sperm formation had begun (Figure 2C). A February indoor cage sample is shown in Figure 2D. This specimen had been in captivity for two weeks. No sperm were embedded and many of the early cell stages of spermatogenesis were present. Interstitial tissue showed

signs of decline compared to that of the wild February sample (Figures 2C and 2D). March wild frog testes show the last wave of sperm of the breeding season (Figure 2E). Interstitial tissue was not as well developed as in previous months, but blood vessels were still large indicating a generous blood supply to the testes at that time. Testes from frogs taken in March from outdoor cages showed good interstitial cell development, with all sperm loose in the lumen of the tubule, and sperm wave formation occurring (Figure 2F). Testes from frogs kept in indoor cages in March showed very little interstitial tissue development, but meiotic figures and all stages of spermatogenesis were seen (Figure 2G). Testes from wild frogs taken in April showed the lumen full of loose sperm (some degenerating), and very few interstitial cells, though connective tissue capsules for each tubule had begun to thicken (Figure 2H). There was very little evidence of maturation of spermatocytes, with only a few spermatids present (Figure 2H). Testes from outdoor cage frogs sampled in April resembled the testes from wild frogs taken in April except interstitial cells were present and spermatocytes indicated sperm formation had been occurring (Figure 2I). Testes from outdoor cage frogs taken in May showed thick connective tissue capsules, few interstitial cells, loose and degenerating sperm, and degenerating spermatocytes (Figure 2J). Testes from frogs kept in indoor cages and sampled in May contained all stages of spermatogenesis, including embedded sperm (Figure 2K). Interstitial tissue was still thick and fibrous, with very few interstitial cells. Testes from samples taken in June from the outdoor cages showed spermatogenesis beginning (Figure 2L). Though degenerate sperm were still present, new sperm could be seen embedded in Sertoli cell cytoplasm and many primary and

secondary spermatocytes could be seen, though the interstitium was still thick and fibrous (Figure 2L). Figure 2M shows the process of spermatogenesis beginning in a testis taken from an outdoor cage frog in July, which indicates individual variability. Some frogs begin spermatogenesis earlier than others. The same features noted in Figure 2L apply to this testis. Testes from frogs taken from indoor cages in July showed spermatogenesis occurring, with all cell types present (Figure 2N). The testes from the November wild frog showed the culmination of spermatogenesis (Figure 2O). All sperm were embedded, Sertoli cell cytoplasm was thick, interstitial tissue was developing, and only residual spermatogenic stages were evident. As mentioned before, this was probably the state of the testis until late January, when spermiation occurred, releasing sperm from Sertoli cell "nests" and initiating a new wave of sperm.

The drop in sperm number from November to January and the subsequent rise in numbers in February indicates new sperm formation. Figure 2P shows diplotene of meiosis in a wild frog taken in February. Chromocenters and the double stranded nature of the chromosome of this stage is evident. Figure 2Q depicts several groups of tetrads of meiosis also in a wild frog taken in February. Pseudacris possesses 24 chromosomes. Twelve groups of tetrads are visible in the photographs. Inspection of the serial sections revealed that the chromosomes did lie in the same plane and that twelve was an accurate count of the number of tetrads. Meiotic stages were observed in a number of breeding frogs indicating spermatogenesis was occurring at this time.

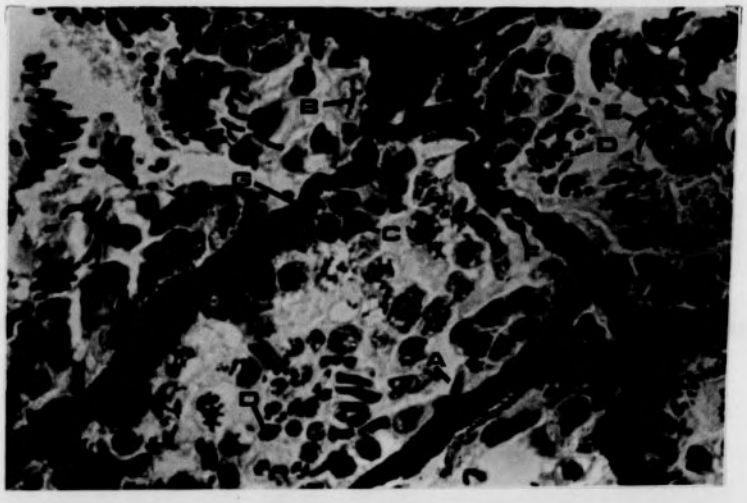

Figure 2A. Testis tubule showing spermatogonia (A), primary and secondary spermatocytes (B and C respectively), spermatids (D), sperm (E), Sertoli cell nucleus (F), and interstitium (G). Magnification X 400.

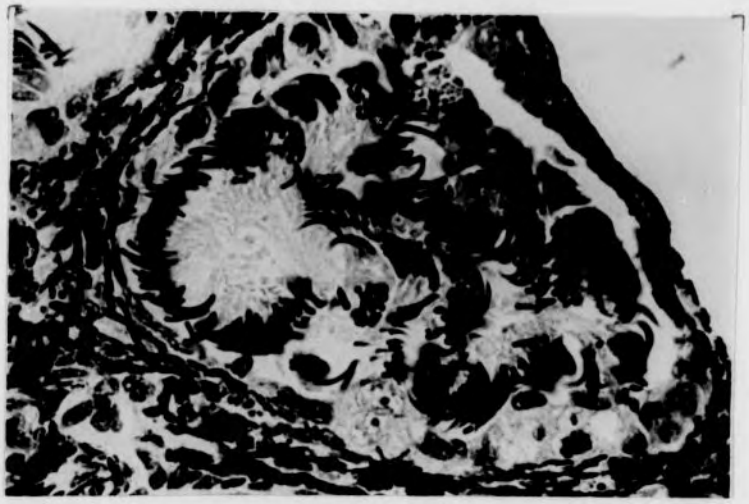

Figure 2B. Testis tubule of a wild frog captured in January. X400.

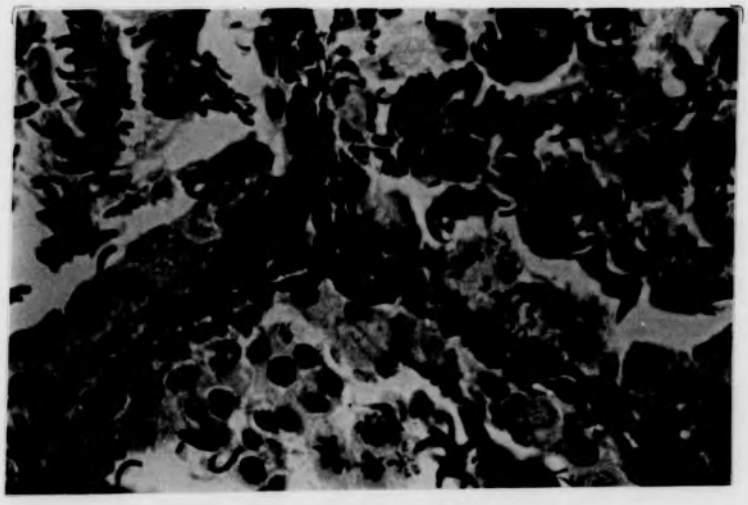

Figure 2C. Testis tubule of a wild frog captured in February. X400.

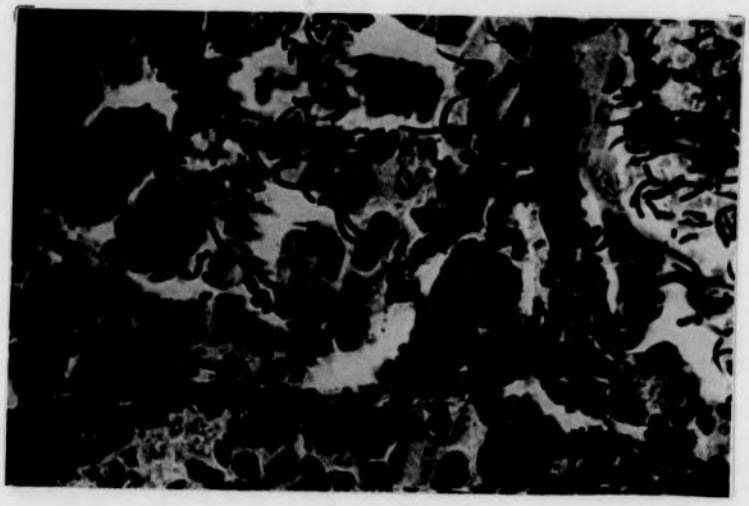

Figure 2D. Testis tubule of a frog taken from the indoor cage population in February. X400.

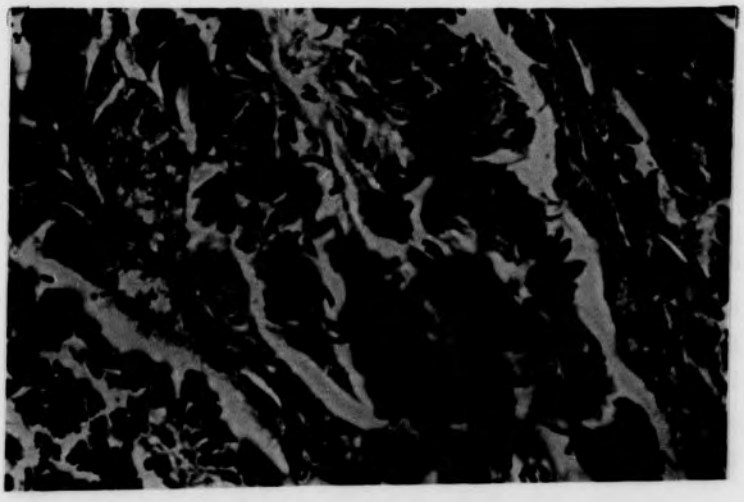

Figure 2E. Testis tubule of a wild frog captured in March. X400.

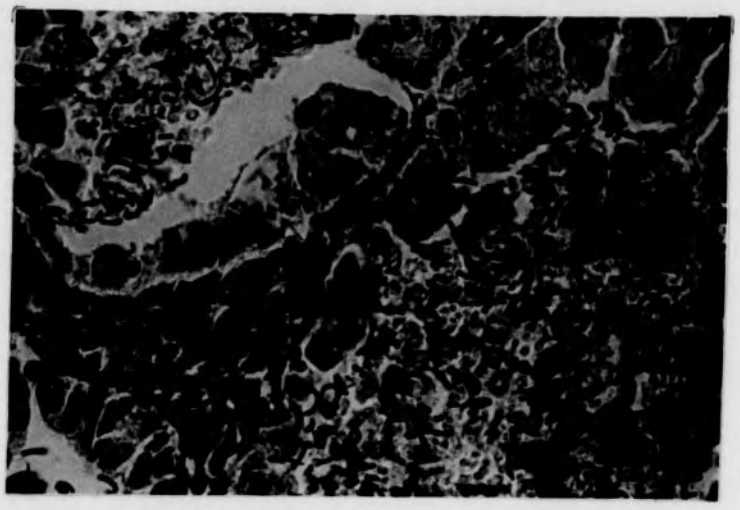

Figure 2F. Testis tubule of a frog taken from the outdoor cage population in March. X400.

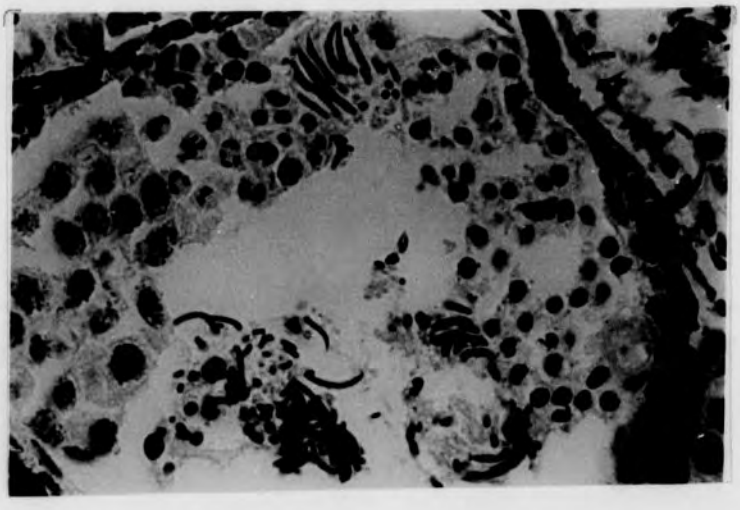

Figure 2G. Testis tubule of a frog taken from the indoor cage population in March. X400.

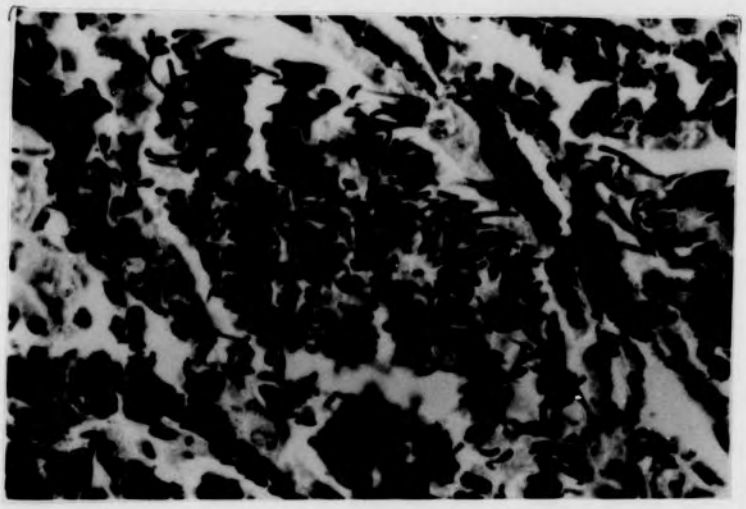

Figure 2H. Testis tubule of a wild frog captured in April. X400.

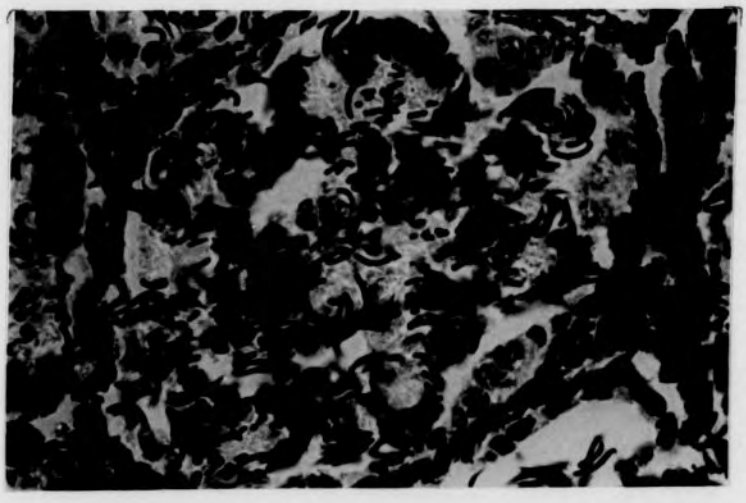

Figure 2I. Testis tubule of a frog taken from the outdoor cage population in April. X400.

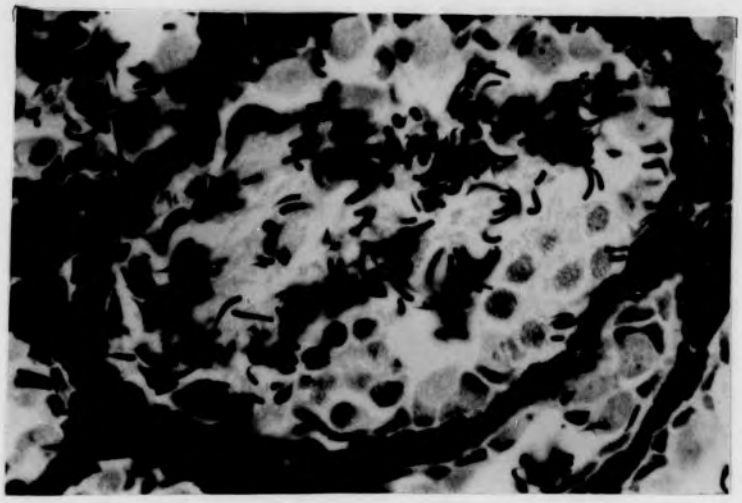

Figure 2J. Testis tubule of a frog taken from the outdoor cage population in May. X400.

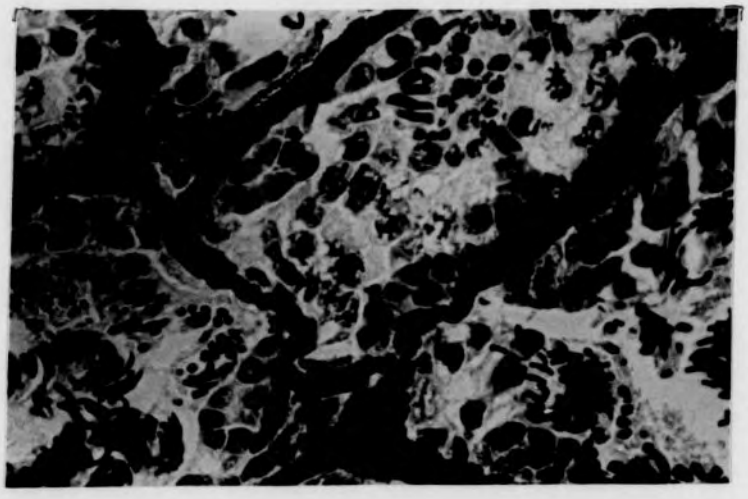

Figure 2K. Testis tubule of a frog taken from the indoor cage population in May. X400.

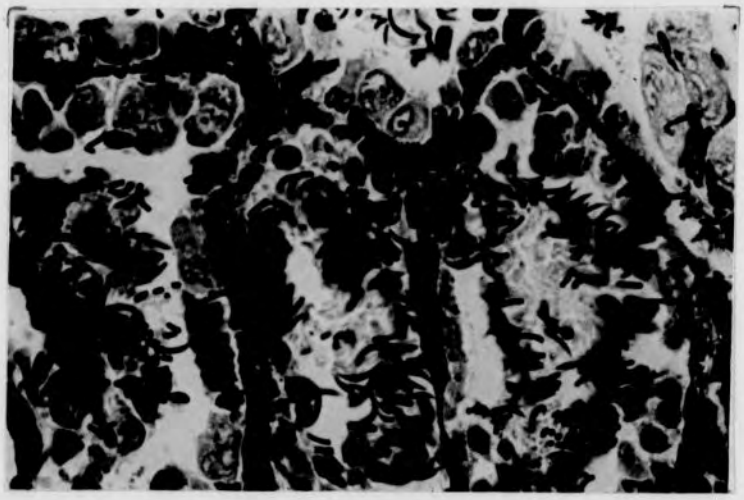

Figure 2L. Testis tubule of a frog taken from the outdoor cage population in June. X400.

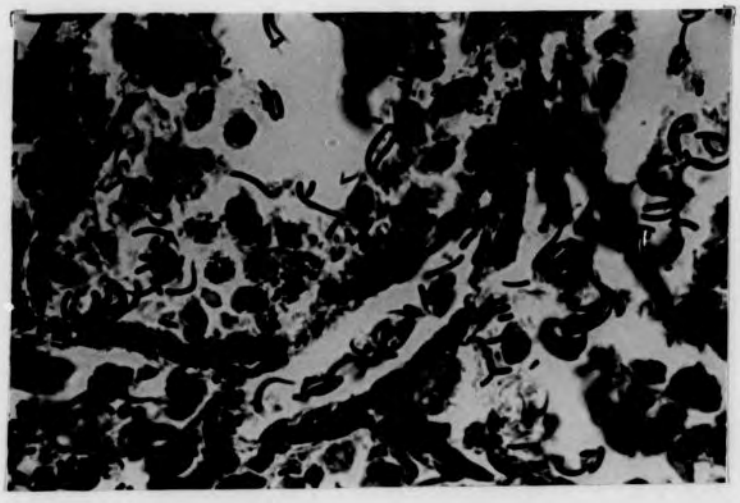

Figure 2M. Testis tubule of a frog taken from the outdoor cage population in July. X400.

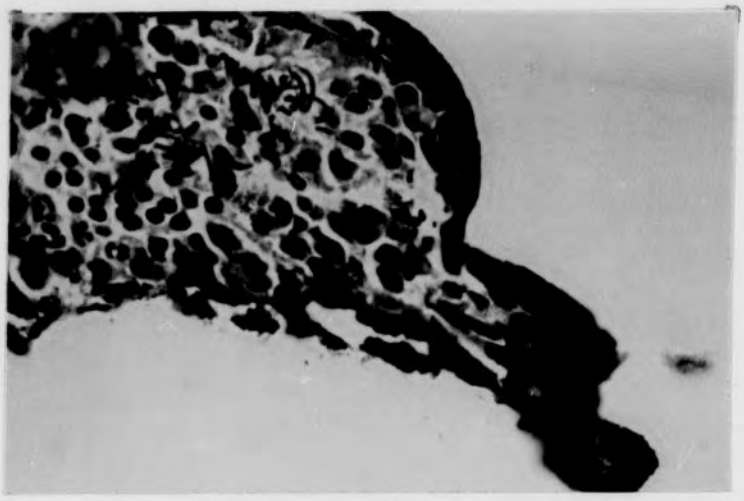

Figure 2N. Testis tubule of a frog taken from the indoor cage population in July. X400.

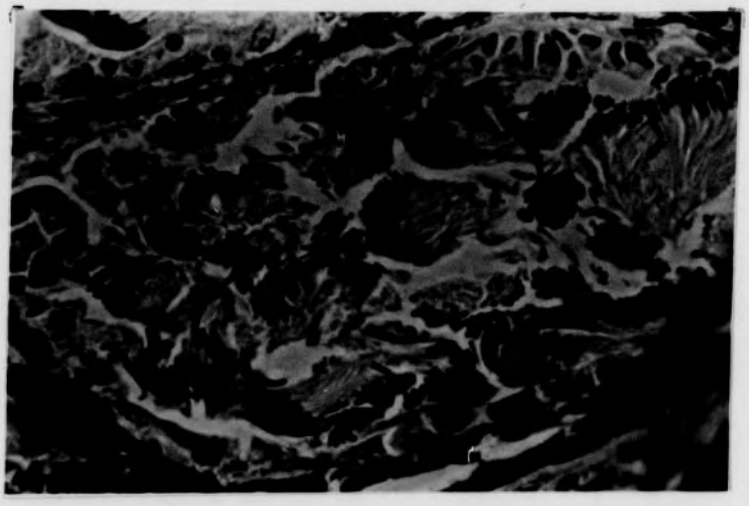

Figure 2O. Testis tubule of a wild frog captured in November. X400.

Figure 2P. Diplotene of meiosis in the testis of a wild frog taken in February. X1000.

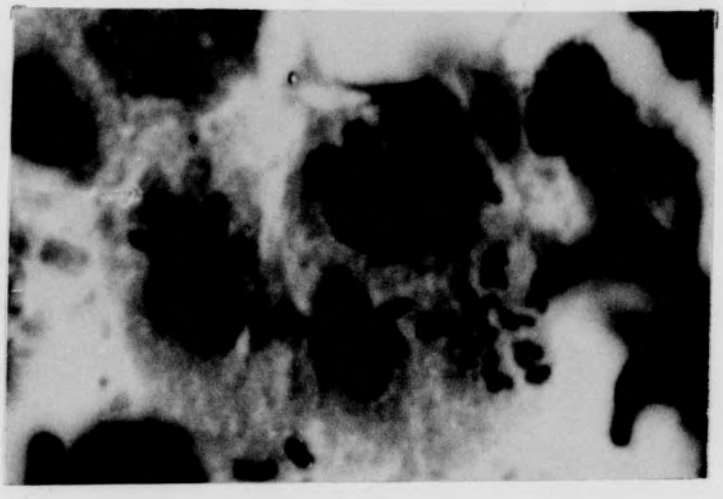

Figure 2Q. Meiotic figures in the testis of a wild frog taken in February. X1000.

DISCUSSION AND CONCLUSIONS

The breeding behavior of Pseudacris triseriata feriarum is like that of an anuran with a discontinuous testicular cycle. However, P.t. feriarum exhibits at least two periods of spermatogenesis: one occurring during the period from July to November, and the other occurring simultaneously with spermiation, which occurs from January through March. There is no spermatogenesis in April, and the rise of spermatid numbers during March is probably a reflection of the last meiotic products of breeding season spermatogenesis. There is a "resting period" (period of no spermatogenesis) extending from April until June, but it was not determined whether or not it is a true resting period characteristic of a discontinuous testicular cycle, in which the germinal epithelium is refractory to gonadotropins. There is possibly another "resting period" from November until January. Again, exogenous gonadotropins were not administered. However, there are striking histological similarities between the testes of the wild November frog and the description of the testes and photographs of testes from Rana temporaria (van Oordt, 1960). Environmental events relating to the spermatogenic cycle accounted for 54% of the variability in the sperm number data (Table VI). What about the remaining unaccounted for variability the 46% that's left over in the R Square for sperm numbers? Environmental factors cannot account for this, so perhaps it is an endogenous rhythm of the frog, genetically encoded, that determines the rest of the cycle. In experiments on Amphibia in general and R. temporaria in particular (van Oordt, 1960; van Oordt and Lofts, 1963), it was found

that at certain times of the cycle, such as in winter, when only sperm bundles and spermatogonia are present, exogenous gonadotropins failed to initiate spermatogenesis. This was explained by lack of sensitivity of the germinal epithelium to gonadotropins (van Oordt, 1960; van Oordt and Lofts, 1963). This implies either chemical inhibition of the germinal epithelium or a genetic "block" preventing cell division from taking place, and subsequently preventing spermatogenesis from occurring. Thus, the period of gonadal inactivity in P. t. feriarum may correspond with the resting period described by van Oordt (1960) for R. temporaria. The "resting period" occurring from April until June does not in any way resemble that described by van Oordt (1960). Old sperm are cleared from the tubules, and the germinal epithelium appears to begin the process of spermatogenesis again very slowly. The fact that frogs kept in indoor cages underwent spermatogenesis without a "resting period" indicates that the germinal epithelium may not be refractory to gonadotropins at this time. The cell numbers in breeding P. t. feriarum corresponded roughly with those reported for breeding R. pipiens (a discontinuous breeder) by Glass and Rugh (1944). However, the cell counts of P. t. feriarum differed markedly from those reported for R. pipiens during the remainder of the cycle by Glass and Rugh (1944). P. nigrita verrucosa and P. clarki exhibit continuous breeding behavior (Conant, 1958), and thus probably have a continuous testicular cycle. P. t. feriarum, a closely related species, has what appears to be a potentially continuous testicular cycle, and appears to be a potentially continuous breeder, though it exhibits discontinuous behavior. Given the correct environmental cues, P. t. feriarum might be capable of breeding at any time of year.

An alternate hypothesis for bimodal spermatogenesis is that a sizable proportion of wild sample frogs were first year frogs that were undergoing delayed spermatogenesis. This is true for the great-tailed grackle (Quiscalus mexicanus) in which first year males exhibit breeding behavior, and in the population as a whole there is bimodal spermatogenesis (Selander and Hauser, 1965). However, no reliable method of determining age of frogs captured in the field exists (except for recapture of previously marked specimens) and thus this hypothesis was not tested in this study. In addition, the number of sample frogs exhibiting spermatogenesis in February greatly exceeded the number of first year male frogs that would be expected in a random sample according to Green's (1957) figures on longevity of P. brachyphona.

The causitive agent of spermiation is luteinizing hormone (LH), which is secreted by the alpha cells of the pars distalis of the pituitary (van Dongen and DeKort, 1959; van Dongen et al., 1959; and van Dongen et al., 1960). In conjunction with follicle-stimulating hormone (FSH) spermatogenesis continues, while LH alone inhibits spermatogenesis (Basu, 1969). LH also stimulates the Leydig cells to produce androgens, which are responsible for secondary sexual characters (throat pigmentation, calling, etc.). Chieffi (1972) found that exogenous androgens inhibit spermatogenesis, but FSH can ameliorate the effects of both LH and androgen and allow the process of spermatogenesis to continue (Basu, 1969). As long as FSH is at high enough levels to counter the effects of LH and androgen, spermatogenesis will continue. The rise in spermatid numbers from November to January is indicative of the process of spermatogenesis which probably begins at the end of January. A new wave, however, cannot

start until the tubules are cleared of sperm through spermiation, as these sperm and their Sertoli cells are target sites for FSH and use most of the available hormone, thus inhibiting spermatogenesis (van Oordt and Lofts, 1963). This may explain the lack of spermatogenic activity in November (and probably December), but the increase in such activity in January, when sperm are released from Sertoli cells.

After April, no captures were made of wild frogs, but some conclusions can be drawn from the evidence presented by samples from the outdoor cage. No difference was found between cell counts of the three sample populations except in regard to spermatid counts of the March outdoor cage frogs and the spermatid counts from wild frogs captured in March. It is conceivable that the last spermatogenic wave had already been completed in the majority of the outdoor cage specimens, while wild frogs had not done so, and thus the two populations diverged in spermatid numbers. Since this is the only difference between cell counts found in the three sample populations, it can be assumed that differences between the three populations are not quantitative but qualitative. Numbers of cells do not reflect the condition of the cells (degenerating as opposed to viable).

Assuming this relationship is valid, some conclusions about the testicular cycle can be made from the testicular cell counts of outdoor cage frogs for the months during which no wild population frogs could be procured. The second period of spermatogenesis appears to begin in June. Apparently, maturation of secondary spermatocytes was not fast enough to be reflected in spermatid numbers in July (Figure 1C). As no spermatids had matured, sperm numbers, that had steadily dropped since February, also did not exhibit an increase in July, but fell to a low point. Between

July and November, however, this maturation of intermediate stages was probably occurring, as the November cell counts showed a peak in sperm numbers, but a low point for the cell counts of all other stages (Figure 1). Thus, between July and November, spermatogenesis had apparently occurred and by November, the testes were full of sperm embedded in Sertoli cells with no sign of additional spermatogenesis being evident (Figure 20).

The spermatogenic cycle of frogs kept in cages indoors differed greatly from those of the wild population frogs and frogs kept in cages outdoors. The frogs were taken from a fluctuating outdoor environment, put at a relatively high constant temperature, and put on a regular "rain" cycle, and probably a different photoperiod. In noting the effects of high temperature on R. temporaria, a discontinuous breeder, van Oordt and Lofts (1963) observed that high temperature (in the 20 to 24°C range) caused breakdown of Sertoli cells and degeneration of sperm and sperm bundles. As the indoor cage frogs were maintained at approximately 20°C, they exhibited a fall in sperm numbers beginning in May. Presumably, this cycle of spermatogenesis, alternating with a "resting period", would persist as long as the frog was maintained at a high temperature. The indoor cage frogs may be exhibiting what occurs in the wild and outdoor cage frogs much later. That is, the indoor cage frogs, induced by high temperature to begin spermatogenesis, may be manifesting the same characteristics of wild and outdoor cage frogs subjected to the higher (in relation to the breeding season temperatures) environmental temperatures in May, June, and July. The alpha cells of the pituitary are also affected by high temperature with LH production suppressed and FSH production stimulated (van Oordt and Lofts, 1963); thus the spermatogonia are stimulated

by FSH to divide and proliferate into primary and then to secondary spermatocytes. Figure 1C reflects the results of this spermatocyte "boom" in the rise in numbers of spermatids occurring in May in indoor cage frogs. Calling in the indoor population ceased after 3 days. Calling is induced by androgen acting on a "calling center", the preoptic nucleus in the brain (Schmidt, 1966a, 1966b, 1968, 1973). When androgen is no longer produced, then calling ceases. The accumulation of cholesterol-positive lipid (cholesterol being a constituent of androgen), indicates that the Leydig cells are no longer secreting androgen. This buildup was found in R. esculenta exposed to high temperatures, indicating high temperatures inhibit androgen production (van Oordt and Lofts, 1963). The interstitium in Pseudacris degenerates rapidly in frogs kept indoors (Figures 2D, 2G, 2K, and 2N). This may explain why calling ceases in Pseudacris kept at room temperature within a short period of time after capture. In staining the indoor sample testes for sectioning, it was noticed that staining time was longer and the sections were harder to cut due to the thickness of the interstitial connective tissue. There also exists a feedback system of hormonal regulation where high levels of androgen inhibit gonadotropin production (Lisk, 1960, 1962; Davidson and Sawyer, 1961a, 1961b; Kaneumatsu and Sawyer, 1963; Davidson and Smith, 1966). If androgen secretion is cut off, then gonadotropins are produced, which could easily account for proliferation of spermatocytes and spermatids in Pseudacris kept indoors.

Basu and Nandi (1965) found high temperature stimulated spermatogenesis in R. pipiens. Thus, it appears that in at least two discontinuous breeders, R. esculenta and R. pipiens, room temperature induces spermatogenesis. Since P. t. feriarum may have the potential for continuous

breeding, a shift of the indoor cage frogs to the proper temperature, photoperiod, and humidity, could possibly induce breeding behavior. Thus, the "captivity effect" that has prevented study of breeding behavior and testicular cycles in a laboratory situation without the aid of exogenous gonadotropins might be avoided at least in P. t. feriarum. There was a problem with die-off of indoor and outdoor caged specimens. A survival rate of only 30% was managed the first year of the study and only 50% the second year. This is the main reason for the small sample size. The outdoor cage method is probably valuable in determining what the state of the breeding cycle is if several requirements are met. Large collections must be made of wild specimens that are to be caged to insure against die-off, and fewer frogs must be put in each cage to avoid territorial and feeding problems due to crowding. Larger numbers will give larger sample sizes and decreased variances in statistical treatments. No difference was found between cell counts of the wild population and the outdoor caged frogs (except in spermatid numbers, which has already been discussed) either qualitatively or quantitatively. The frogs in the two populations are subject to the same environmental events. Therefore, it seems reasonable to assume that outdoor cage specimens reflect what is happening in wild specimens. The evidence of the November wild frog, showing a lumen full of embedded sperm (Figure 1D) implies a spermatogenic peak between April and November. Since the outdoor cell counts roughly follow the extrapolated course of wild sample cell counts, outdoor cage study should be valuable in documenting monthly occurrences in the spermatogenic cycle of Pseudacris and other frogs displaying discontinuous behavior.

van Oordt (1960) dismisses the role of all environmental factors except temperature in regulation of the spermatogenic cycle, and states that the spermatogenic cycle in R. temporaria is largely dependent upon intrinsic factors. This study of Pseudacris supports the conclusion that environmental factors may regulate the cycle, though intrinsic factors probably do play a role in determining behavioral responses to environmental cues as the unexplained variability in Table IV attests.

Photoperiod is the most influential environmental factors in determining calling (Table II). The main photoperiodic event near the breeding period is the winter solstice in December when photoperiod begins to lengthen. Increasing photoperiod, then, may be the environmental cue for breeding behavior. However, it will be remembered from the previous section that calling in November was heard. It is probable that photoperiod of the right length triggers the physiological events that induce calling, and not increasing photoperiod since photoperiod in November is about the same as in January.

The preoptic center of the brain in the hypothalamus is the relay center for impulses to the trigemino-esthmic tegmentum, the direct calling control center (Schmidt, 1966a, 1966b, 1968, 1973). This is true in hylid frogs, among which are Hyla crucifer, H. cinerea, and Pseudacris (Schmidt, 1966a, 1966b, 1968, 1973). The preoptic nucleus is a target organ for androgen, and when this hormone is in sufficiently high concentrations in the system, calling results (Schmidt, 1966a, 1966b, 1968, 1973). High levels of androgen are indicative of interstitial cell development (Leydig cells) and thus of high LH levels. It is the hypothalamus that secretes gonadotropin-releasing factor (GRF) to stimulate the pituitary to release

both FSH and LH. The area responsible for these secretions is the hypophysiotrophic area of the hypothalamus, also a target organ for androgens, and in this way androgens can cause the inhibition of gonadotropin secretion that results in the cessation of spermatogenesis and Leydig cell secretion and hence ends breeding behavior (List, 1960, 1962). If the mechanism of regulation of the breeding cycle were wholly internal, the hypothalamus would be expected to regularly secrete GRF in accordance with the internal rhythm. However, in R. temporaria, when nervous pathways to the median eminence in the hypothalamus were cut, gametogenesis and seasonal development of the gonads were absent (Dierickx, 1966). Thus, neurosecretory fibers ending in the median eminence in the hypothalamus could not receive impulses from optic centers, no GRF was produced, the pituitary released no gonadotropins, and sexual quiescence resulted. Therefore, in Dierickx's study, environmental cues in the form of photoperiod changes could not be responded to by the hypothalamus. It has been shown in this study that photoperiod is a highly significant environmental correlate of breeding behavior, and the evidence of Dierickx (1966) supports the conclusion that breeding behavior in some anurans is photoperiodically controlled. It is probable that the same nervous pathways exist in P. t. feriarum and operate in the same fashion.

Environmental events may play a role in the termination of gonadotropin secretion. The pineal body, located on the roof of the diencephalon, is photo-sensory (Dodt and Morita, 1967; Adler, 1971). Its fine structure is like that of rods and cones of the eyes and it is sensitive both to wavelength and intensity of light (Dodt and Heerd, 1962; Dodt and Jacobson, 1963). Adler (1970) states that it is important in gonadal development

and in the establishment of internal rhythms. Ablation of the pineal brings about breeding readiness of the gonads (Thiebolt, 1965). Thus, photoperiodic cues may cause production of a gonadotropin inhibitor that would result in suppression of FSH and LH production (Thiebolt, 1965). Such an inhibitor has been identified but not in pure form (Thiebolt, 1965). It has been shown in this study that photoperiod plays a significant role in both breeding behavior (Table II) and in spermatogenesis (Table VI). Thus, photoperiod may not only be an environmental effector of the onset of spermatogenesis and breeding but may also cue the suppression of these events.

The final question that must be dealt with is that of why these frogs breed at such a seemingly hostile time of the year for a poikilothermic animal. Possible limiting factors at this time of year include scarcity of food, freezing temperatures, and extremely low humidity (a hazard for frogs traveling to the breeding ponds). These factors (except for low humidity) apply to all stages of the life cycle from egg to adult. Whitaker (1971) concluded in his study of Pseudacris triseriata triseriata that the frogs feed at the breeding sites during the breeding season. Table I shows that food is not a limiting factor for wild frogs as 80.9% of the wild sample frogs contained some material in their gastro-intestinal tracts. Flying insects, spiders, and numerous aquatic insects were seen at the ponds during this study at times coinciding with the peak of breeding activity. It would appear that food, then, is not a limiting factor.

Low temperatures seem to have been the greatest risk to tadpoles and adults. But, as noted in the previous section, frogs were seen calling as long as open water was present, even if the ponds contained ice.

Seldom were the ponds frozen over completely, and on only three occasions were there noted any anuran mortality. A newly formed rain puddle was used as a laying site and egg masses within 5 cm of the surface were frozen. On two occasions, adults were found dead after a particularly cold period, and both were found on the ground at the periphery of the ponds.

When the temperature fell below freezing, frogs were found in grass clumps in the ponds, partly submerged, or totally submerged and clinging to the grass roots. Whitaker's (1971) observations on P. t. triseriata parallel these findings. Water around the grass clumps rarely froze completely, and on several occasions, frogs known to call from specific grass clumps were calling again from these same clumps after the ponds had frozen over. During cold periods the frog could immerse itself entirely in water and use the bottom silt or the grass roots as insulation. Whitaker (1971) found frogs under the surface of the water with some submerged as deep as 20 cm. Thus, temperature does not seem to pose a great threat to the breeding frogs or the eggs and tadpoles. Pseudacris has behaviorally adapted to survive cold temperatures.

Low humidity is no great problem to Pseudacris as migration rarely takes place until after dark when the humidity is highest, or when appreciable rainfall drives the frogs from their over-wintering habitat. Low himidity in Guilford County generally is accompanied by very cold temperatures in winter; thus, the frogs do not emerge and no migration to the ponds takes place when these conditions prevail.

What are the advantages of breeding during the winter months? The advantages are manifold. In Guilford County, the wettest months of the year usually are January and February. This creates and maintains the

kind of habitat suitable for breeding in Pseudacris. Closely allied with this is the fact that potential predators are less likely to be active at this time of year. Breckenridge (1944) found the snakes Thamnophis radix and T. sirtalis to be predators of Pseudacris. Whitaker (1971) took 7 Pseudacris from the stomachs of the snake Natrix sipedon, and when fishes were introduced into his study ponds, mortality of tadpoles hit 100%. The ponds in the present study contained no fishes. No reptilian predators of any kind were seen or anticipated, since January and February are the coldest months of the year in Guilford County. The only possible predators noted were grackles (Quiscalus quiscula) seen during the day at the ponds, but the greatest amount of breeding activity, and also the time when Pseudacris is most conspicuous, is at night, when grackles are not active. Grackles could, however, pose a threat to tadpoles, but the healthy population of froglets in the spring indicates that predation pressure from grackles is not very heavy. Pseudacris is probably not found in more permanent aquatic situations because of predation upon its tadpoles, and at least one reason for early breeding behavior may be the lack of predators on adults.

Another consideration is food supply for the tadpoles. R. pipiens, H. crucifer, and B. americanus breed sympatrically in this area with Pseudacris. However, the earliest species of frog to emerge as froglets is Pseudacris, whose breeding activity peaks earlier than any sympatric breeder. Only isolated individuals of R. pipiens were noted breeding, and the peak of breeding in H. crucifer is in March. B. americanus begins to breed only at the very end of Pseudacris activity. The reasons, then, for the evolution of early breeding behavior in Pseudacris are probably the

lack of predators of either adults or tadpoles, reduced competition among tadpoles for food, and availability of suitable breeding habitat.

SUMMARY

Pseudacris triseriata feriarum may be a potentially continuous breeder exhibiting a discontinuous breeding behavior in Guilford County, N.C. The testicular cycle consists of two periods of spermatogenesis; one occurring during spermiation (breeding) and the other beginning in late June and ending before November. Spermiation occurs from late January to mid-March. "Resting periods", or periods of no spermatogenic activity, occur from mid-March until May and from November through late January.

Spermatogenesis and breeding behavior correlate strongly with environmental factors such as photoperiod, temperature, and rainfall. These factors are perceived through sensory means and stimulate hormone production through the mediation of the hypothalamus. Photoperiod, the low temperature of the day, and rainfall amounts can be highly significant in determining whether or not mating calling will occur on any given day, with photoperiod being the most important environmental effector. Mean monthly temperature, rainfall, and photoperiod are highly correlated with the number of sperm present in the tubules each month, with temperature having the highest correlation coefficient. A negative correlation exists between temperature and sperm number, with high temperatures causing degeneration of sperm bundles and initiating spermatogenesis. Interstitial tissue also exhibits a cycle, with Leydig cells degenerating as the breeding season ends, and connective tissue becoming

increasingly prevalent. Build up of interstitial tissue parallels the summer spermatogenic period and is complete by November.

Keeping frogs in indoor aquaria at high temperatures initiates spermatogenesis. Frogs in outdoor cages probably reflect testicular conditions found in wild frogs. The degeneration of sperm and initiation of spermatogenesis in frogs kept in indoor aquaria is due to high temperatures.

Lack of food, freezing temperatures, and low humidity during the winter months do not seem to be limiting factors for Pseudacris in Guilford County, N.C. Frogs do feed while breeding, and are behaviorally adapted to avoid freezing and dessication. The reasons for the evolution of early breeding in Pseudacris probably include lack of predators of either tadpoles or adults, reduced competition with the tadpoles of sympatric breeders, and the availability of suitable habitat.

LITERATURE CITED

Adler, K. 1970. The role of extraoptic photoreceptors in amphibian rhythms and orientation: a review. J. Herpetol. 4(3-4): 99-112.

Adler, K. 1971. Pineal endorgan: role in extraoptic entrainment of circadian locomotor rhythm in frogs. In: M. Menaker (ed.), Biochronometry. Washington, D.C. United States National Academy of Sciences. pp. 342-350.

Basu, S.L. 1969. Effect of hormones on the salientian spermatogenesis in vivo and in vitro. Gen. Comp. Endocrinol. Suppl. 3: 203-213.

Basu, S.L. and J. Nandi. 1965. Effects of testosterone and gonadotrophins on spermatogenesis in Rana pipiens Schreber. J. Exp. Zool. 159: 93-112.

Bellis, E.D. 1957. The effects of temperature on salientian breeding calls. Copeia 1957: 85-89.

Blair, A.P. 1951. Winter activity in Oklahoma frogs. Copeia 1951: 85-89.

Bragg, A.N. 1948. Observations on the life history of Pseudacris triseriata (Wied) in Oklahoma. Wasmann Collector 7: 149-168.

Breckenridge, W.J. 1944. The reptiles and amphibians of Minnesota. University of Minnesota Press. Minneapolis. 202 pp.

Chieffi, Giovani. 1972. Comparative endocrinology of the vertebrate testis. Amer. Zool. 12: 207-211.

Conant, R.M. 1958. A field guide to reptiles and amphibians. Houghton Mifflin Co. Boston. 366 pp.

Davidson, J.M. and C.H. Sawyer. 1961a. Effects of localized intracerebral implantation of oestrogen on reproductive functions in the female rabbit. Acta Endocrinol. 37: 385-393.

Davidson, J.M. and C.H. Sawyer. 1961b. Evidence for a hypothalamic focus of inhibition of gonadotrophins by androgen in the male. Proc. Soc. Exp. Biol. (N.Y.) 107: 4-7.

Davidson, J.M. and E.R. Smith. 1966. Testosterone feedback in the control of somatic and behavioral aspects of male reproduction. 2nd Int. Cong. Horm. Ster. Int. Congr. Ser. 111. Excerpta Med. Found. Amsterdam, New York, London, Milan, Tokyo, Buenos Aires. p. 77.

Dierickx, K. 1966. Experimental identification of a hypothalamic gonadotrophic center. Z. Zelforsch. 74: 53-79.

Dodt, E. and E. Heerd. 1962. Mode of action of pineal nerve fibers in frogs. J. Neurophysiol. 25: 405-429.

Dodt, E. and M. Jacobson. 1963. Photosensitivity of a localized region of the frog diencephalon. J. Neurophysiol. 26: 752-758.

Dodt, E. and Y. Morita. 1967. Conduction of nerve impulses within the pineal system of the frog. Pflugers Arch. Ges. Physiol. 293: 184-192.

Evenden, F.G. 1946. Notes on the herpetology of Elmore Co. Idaho. Copeia 1946: 257.

Glass, F.M. and R. Rugh. 1944. Seasonal study of the normal and pituitary stimulated frog (Rana pipiens). I. Testis and thumb-pad. J. Morphology 74: 409.

Gosner, K.L. and I.H. Black. 1955. The effects of temperature and moisture on the reproductive cycle of Scaphiopus h. holbrooki. Amer. Midl. Nat. 54(1): 192-203.

Green, N.B. 1957. A study of the life history of Pseudacris brachyphona (Cope) in West Virginia with special reference to behavior and growth of marked individuals. Diss. Abstr. 17: 23692.

Hardy, D.F. 1959. Chorus structure in the Striped Chorus Frog, Pseudacris nigrita. Herpetologica 15: 14-16.

Hock, R.J. 1967. Temperature effect on breeding of the toad, Bufo variegatus, in southern Chile. Copeia 1967: 227-230.

Ifft, J.D. 1942. The effect of environmental factors on the sperm cycle of Triturus viridescens. Biol. Bull. 33: 111-128.

Kaneumatsu, S. and C.H. Sawyer. 1963. Effects of hypothalamic estrogen implants on pituitary LH and prolactin in rabbits. Amer. J. Physiol. 205: 1073-1076.

Lisk, R.D. 1960. Estrogen sensitive centers in the hypothalamus of the cat. Proc. 1st Intern. Congr. Horm. Ster. Milan. 2: 457-469.

Lisk, R.D. 1962. Testerone sensitive center in the hypothalamus of the rat. Acta Endocrinol. 41: 195-204.

Livezay, R.L. 1952. Some observations on Pseudacris nigrita triseriata (Wied) in Texas. Amer. Midl. Nat. 47: 372-381.

Lofts, B. 1961. The effects of follicle stimulating hormone and luteinizing hormone on the testis of hypophysectomized frogs (Rana temporaria). Gen. Comp. Endocrinol. 1: 179-189.

Lofts, B. 1964. Seasonal changes in the functional activity of the interstitial spermatogenic tissues of the green frog, Rana esculenta. Gen. Comp. Endocrinol. 4: 550-562.

Lofts, B. 1974. Reproduction. In: B. Lofts (ed.), Physiology of the Amphibia. Academic Press. New York and London. Vol. II: 107-218.

Martof, B.S. and E.F. Thompson Jr. 1958. Reproductive behavior of the chorus frog, Pseudacris nigrita. Behavior 13: 243-257.

Nie, N., C.H. Hull, J.G. Jenkins, K. Steinbrenner, D.H. Brent. 1975. Multiple regression analysis. Introduction to discriminant analysis. Pages 320-323, 435-440 in Nie, et al., Statistical package for the social sciences, 2nd ed. McGraw-Hill Inc., New York.

Noble, G.K. 1931. The biology of the Amphibia. McGraw-Hill, New York. 577 pp.

Rugh, R. 1941. Experimental studies on the reproductive physiology of the male spring peeper, Hyla crucifer. Proc. Amer. Phil. Soc., 84: 617-627.

Schmidt, R.S. 1966a. Central mechanisms of frog calling. Behavior 26: 251-285.

Schmidt, R.S. 1966b. Hormonal mechanisms of frog mating calling. Copeia 1966: 637-644.

Schmidt, R.S. 1968. Preoptic activation of frog mating behavior. Behavior 30: 239-257.

Schmidt, R.S. 1973. Central mechanisms of frog calling. Amer. Zool. 13: 1169-1177.

Selander, R.K. and R.J. Hauser. 1965. Gonadal and behavioral cycles in the Great-Tailed Grackle. The Condor 67(2): 157-182.

Thiebolt, L. 1965. Physiology of the pineal body. In: Structure and function of the epiphysis cerebri. J. Ariens Kappers and J.P. Schade (eds.) The Netherlands Central Institute for Brain Research. Amsterdam. pp. 479-488.

U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Environmental Data Service, Greensboro-High Point-Winston-Salem Airport, N.C. 1972-1975. Local climatological data.

U.S. Governmental Printing Office. The American ephemeris and nautical almanac; 1972-1975.

van Dongen, W.J. and E.M.J. DeKort. 1959. Spermiation in the common frog (Rana temporaria). I. Experiments on the isolated testis. Proc. Kon. Nederl. Acad. Wetensch. 62C: 327-332.

van Dongen, W.J., R.E. Draisma, and E.J.M. DeKort. 1959. Spermiation in the common frog (Rana temporaria). II. Experiments in vitro. Proc. Kon. Nederl. Acad. Wetensch. 62C: 327-332.

van Dongen, W.J., R.E. Ballieux, H.J. Geursen, and T. Offermans. 1960. Spermiation in the common frog (Rana temporaria). III. Histological and chemical investigations. Proc. Kon. Nederl. Akad. Wetensch. 63C: 257-263.

van Oordt, P.G.W.J. 1956. Regulation of the spermatogenetic cycle in the common frog (Rana temporaria). van Der Wiel, Arnhem. 116 pp.

van Oordt, P.G.W.J. 1960. The influence of internal and external factors in the regulation of the spermatogenic cycle in Amphibia. Symp. Zool. Soc. London 2: 29-52.

van Oordt, P.G.W.J. and B. Lofts. 1963. The effects of high temperature on gonadotrophin secretion in the male common frog (Rana temporaria) during autumn. J. Endocrinol. 27: 137-146.

Werner, J.K. 1969. Temperature, photoperiod effects on spermatogenesis in the salamander Plethodon cinereus. Copeia 1969: 592-602.

Whitaker, J.O. 1971. A study of the western chorus frog, Pseudacris triseriata, in Vigo County, Indiana. J. Herpetol. 5(3-4): 127-150.

Wright, A.H. and A.A. Wright. 1949. Handbook of frogs and toads of the United States and Canada. Ithaca, N.Y. 640 pp.

Zwarenstein, H. and H.A. Shapiro. 1933. Metabolic changes associated with endocrine activity and the reproductive cycle in Xenopus laevis: III. Changes in the calcium content of the serum associated with captivity and the normal reproductive cycle. J. Exp. Biol. 10: 372.