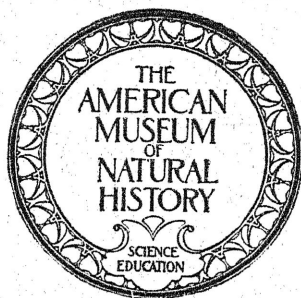


THE SEXUAL BEHAVIOR OF ANURA

1. THE NORMAL MATING PATTERN OF *RANA PIFIENS*

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Article V.—THE SEXUAL BEHAVIOR OF ANURA

1. THE NORMAL MATING PATTERN OF *RANA PAPIENS*^{1,2}

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PLATE I AND TABLES I-IV

Anura have been used as common laboratory animals for many years; *Rana pipiens* has been particularly favored in this country. Nevertheless, many phases of the sexual behavior of Anura in general and of the leopard frog in particular are little understood. Most of the earlier work on *Rana pipiens* was done in the field, where observational difficulties are so great that little can be seen. Unfortunately when most Anura are brought into the laboratory, their sexual activity rapidly recedes. The discovery of Wolf (1929) and Houssay, Guisti and Lascano-Gonzalez (1929) that implantation of the

anterior lobe of the hypophysis will induce amplexus in the male and ovulation in the female, and the standardization of this method by Rugh (1935a, 1937) made possible a laboratory study of the sexual behavior of *Rana pipiens*. With this method we have been able to investigate and clarify many of the features of the sexual behavior of this species. Those aspects of the social behavior which do not lend themselves readily to laboratory investigation were not included in this study. Thus, migrations to and from the ponds, congregations in the ponds, the land drive of the female after oviposition and related topics are not considered.

MATERIAL AND METHODS³

Leopard frogs were obtained from the region around Alburgh, Vermont, during the months of September to April, 1939 to 1941. During the spring, summer and autumn, they are found in large numbers in the fields. About the beginning of October, according to the Vermont frog dealers, the frogs are collected and placed in large wire-framed crates, which are sunk in about four feet of water in Lake Champlain. A cable leading from the box to shore marks the location of the crate. During the winter, if frogs are needed, the cable is followed by chopping through

the ice, and the crate is recovered. To prevent the frogs from freezing, the crates are immediately wrapped in heavy blankets and are taken to a heated shack where they are assorted and shipped.

The artificially hibernated frogs just described were used both as experimental animals and as donors of pituitaries. Because it is more difficult to induce ovulation in the females kept in the laboratory for several months (Robinson and Hill, 1940), they were always used within a month of their arrival. For many of the experiments, fresh frogs were obtained every week. They were stored in the laboratory in large stone tanks sprayed on one side by ice water. Since most of the experiments were of short duration, feeding was seldom necessary.

The frogs used for experimental purposes were kept in stone tanks, 30 cm. × 30 cm. × 75 cm., covered by wire screens. A damp moss floor and a pan of water completed the habitat. Those frogs kept for three or more weeks were fed meal

¹ This is the first in a series of studies of the sexual behavior of Anura which was initiated in the latter months of 1939. Following the death of Dr. Noble in December, 1940, the junior author undertook the task of completing the work.

The junior author wishes to express his appreciation to Mr. Charles M. Bogert and Dr. Roberts Rugh for reading and criticizing the manuscript.

² Aided by grants from the Committee for Research in Problems of Sex, National Research Council, made to the senior author and to Dr. Frank A. Beach.

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worms (*Tenebrio*) and blow flies (*Calliphora*).

Ovulated females were obtained by the method of Rugh (1935a, 1937). Tests for ovulation were made by carefully squeezing the abdominal wall to force a few eggs out of the cloaca. If the females still croaked, the ovulation was considered incomplete (see page 134, line 28) and they were left until the next day. Since fully ovulated frogs lose their eggs within a few days, or become egg-bound due to the swelling of the jelly within the uteri, those ovulated females not needed immediately were stored in a refrigerator at 4° C. in tanks containing wet moss. In this way the

females' usefulness was often extended five or six days.

Sexually excited males were obtained by injecting stock males twenty-four hours before needed with one female or two male pituitaries. Males injected daily were used for five days and then discarded. The identification of individuals was made possible by cutting the webs between the toes.

Many phases of the behavior were illustrated by means of a 16-mm. motion picture film.

Further details on methods will be given in the description of the individual experiments.

DESCRIPTION OF THE MATING PATTERN

VOICE

The male leopard frog emits two kinds of croaks: a sex call and a warning croak. With a little experience these can be readily distinguished, but at times they merge into each other, resulting in a combination sex-warning croak. The female voice is limited to the warning croak. The fright cry described by Boulenger (1897) was seldom heard in *Rana pipiens*, although in the southern leopard frog, *Rana sphenoccephala*, it was readily elicited by pinching the animal's leg.

MALE SEX CALL.—The sex call of the male can be represented phonetically as an "ir-a-a-a—a-a-h" lasting about three seconds. It is a quavering sound which starts softly and grows louder as the vocal sacs become completely inflated. Often, particularly when the male is about to pursue a female, three or more short "rah-rah-rah's" follow the initial vocalization. Males call most readily when floating with outstretched legs, but a highly excited male will often call when out of the water. Wright and Wright (1933) described the sex call as "a long low guttural croak, three or more seconds long followed by 3-6 short notes each a second or less in length." From their field observations they noted that males will also call from the bottom of the pond.

The sex call is heard during the period in which *pipiens* breed. Afterwards it is

seldom heard. Males brought into the laboratory during the breeding season called only occasionally, even when they were kept in the best habitats. Pituitary injections during this season often caused laboratory males to call, but similar treatment during the fall and early winter induced sexual activity, but the sex call seldom occurred.

In many *Anura* (*Bufo*, *Hyla*, etc.) the chief function of the sex call is to attract the female (Courtis, 1907; Miller, 1909; Wellman 1917; Noble and Noble, 1923; Noble, 1931; Locher, 1939; and Bragg, 1940). No one has demonstrated this function of the sex call for *Rana*. On the other hand, Cummins (1920) and Savage (1935) have shown that voice does not direct the migration to the breeding ponds. Our extended laboratory observations gave no indication whatever that either the male or female was attracted by the sex call. The sex croak showed, however, that the frog was sexually excited and served as an excellent indicator for experimental purposes.

MALE WARNING CROAK.—The warning croak, which is readily elicited when the back or sides of the male are touched, can be described as "ir-a-a-a-h—ir-a-a-h—ir-a-a-h— etc." This croak will continue until the stimulus is removed. If the male frog is held for many minutes, it may stop croaking for a few seconds,

apparently to rest. Then it will start croaking again. At first the "ir-a-a-h's" are soft, and the vocal sacs are slightly inflated, but as the "annoyance" continues the croak becomes louder, and the sacs become fully inflated.

If the water is disturbed while the male is clasping a female, or if she moves about, the male will often croak in a manner similar to the warning croak but at a slightly higher pitch. This change in pitch is probably due to the pressure of the female's back upon the male's throat.

The warning croak of the male is cyclical, similar to the sex call. It is loudest and most easily evoked during the breeding season. During the summer a stronger stimulus is necessary to elicit it. At this time some males will not croak even when their backs are firmly held. When they do croak, the vocal sacs are not inflated, and the sound is very much like that of a female. During the winter males taken out of hibernation have a strong, easily evoked warning croak.

MALE SEX-WARNING CROAK.—When the male is dashing around excitedly, attempting to clasp a female, a croak is often heard which combines the crescendo of the sex croak with short "ira-a-h's" of the warning croak. This is termed the sex-warning croak.

FEMALE WARNING CROAK.—The croak of the female is similar to the warning croak of the male but not so loud. This is due to the female's lack of vocal sacs, which in the male act as resonators. The warning croak prevents the male or non-ovulated female from being clasped. Its function will be considered more fully in the section on sex recognition.

AMPLEXUS

The sexually active male attempts to clasp a female or object resembling a female (see page 132, line 60). If an ovulated female is clasped, amplexus will be maintained until oviposition is completed.

Normal amplexus is pectoral. The male's back is slightly arched convexly, and his ventral surface closely adheres to the back of the female. His hind legs remain tightly flexed, regardless of the

female's activity. He clasps the female just behind the axillae, his fingers touching on the ventral side. The enlarged thumb pads press into the body of the female, and the palms of the hands face ventrally (Pl. 1, fig. 1). The male remains in this position until the egg-laying commences.

OVIPOSITION

Oviposition in *Rana pipiens* has been outlined only by Wright (1914) who described one case seen in the field. As our observations have shown, and as Wright suspected, he witnessed an atypical case. Short descriptions of ovipositions in other species of *Rana* have been published by Wright (1914), Liu (1931), Savage (1934) and others. Waring, Landgrebe and Neill (1941) described the muscular and skeletal mechanism involved in the retention and extrusion of eggs in *Rana*.

The description of an oviposition of *Rana pipiens* which follows is based on the observation of forty-one cases among which considerable variation was noted. Attempts have been made to standardize quantitatively certain phases of the activity, so that comparisons with atypical or experimental cases could be made (Table I). By placing some of the pairs in a large concrete tank with a sloping floor, it was found that some pairs laid on the bottom at various depths; others laid in deep water, clinging to grass and sticks just below the surface, while still others picked shallow spots where both male and female could readily breathe. Aquaria with slate bottoms, 30 cm. × 40 cm. × 25 cm. with 10 cm. of water were found most convenient for clear, accurate observations. Deep water was not satisfactory unless grass was placed in the water for the frogs to support themselves. Since the grass tended to mar the observations, shallow water was used.

The procedure was to select a female that had ovulated sufficiently, and to place her in a tank with an active male. The male soon clasped the female, and since she had ovulated he retained his clasp (see page 134, line 100).

TABLE I.—OVIPOSITION AND PSEUDO-OVIPOSITION

	No. of Cases Witnessed	Average Time of Oviposition or Pseudo-Oviposition (in min.)	Average No. of Pumps	No. of Cases Releasing Within 6 Min.	Per Cent Releasing Within 6 Min.	Per Cent Pre-release Motions*	Average Release Time (in min.)	Median Release Time (in min.)
Normal ovipositions	41	4	16	36	88	80†	6	1
Ovipositions of ♂ <i>R. clamitans</i> and ♀ <i>R. pipiens</i>	3	3	..	0	0
Pseudo-ovipositions with saline	38	2	3	19	50	90	..	1
Pseudo-ovipositions with sesame oil	3	2.3	6	2	67	100	..	1
Pseudo-ovipositions of females spent less than 1 day	9	3	5	8	89	88	..	1
Pseudo-ovipositions of females spent 1-13 days	7	2.8	4	7	100	86	1	1
Ovipositions substituting weights for males	2	3.5	18

* Only cases where release occurred.

† Only 30 cases observed for pre-release motions.

BACKWARD SHUFFLING.—After a length of time, which varied from several minutes to a full day, the clasped female started to perform a peculiar movement which we have termed "backward shuffling." In this movement the female shuffled her hind legs in such a manner as to cause her to move backward. Alternate periods of backward shuffling, complete rest and normal swimming occurred. As the time for depositing the eggs approached, the backward shuffling began to resemble the female's egg-laying posture. Sometimes oviposition occurred rather suddenly; at other times the backward shuffling changed to the egg-laying posture and then returned to the backward shuffling movement. This shifting of movements occasionally lasted an hour or more.

OVIPOSITION POSTURE.—In oviposition posture (Pl. 1, figs. 2-5), the female's thighs were extended backward and laterally at approximately a 45° angle; her hind legs were turned inward at the same angle, thus forming a diamond-shaped enclosure; the knees were pointed downward and in shallow water rested on the substratum. The feet rested upon the bottom with the toes pointing cephalad and approximating the midline. The

distance of the toes from the midline varied considerably in different individuals. The tarsi were pointed caudally and inward and were raised above the substratum.

As the female moved into the posture just described, the male moved forward very slightly and rotated his hind legs somewhat downward and inward. The posture assumed by the male always followed the posturing of the female and appeared to be caused by the latter.

EJACULATORY PUMPS.—Shortly after the pair assumed the egg-laying position, the female started the oviposition by a sharp contraction of the abdominal walls followed by a concave arching of her back. The male responded to the female's abdominal contraction by spreading his hind legs slightly and arching his back convexly, thus drawing his cloaca forward (upstroke). He responded to the female's arching back by straightening out the arch of the upstroke which brought his cloaca caudally past the female's, and by pressing his legs against the female's abdominal wall (downstroke).

The complex of movements described in the preceding paragraph we have termed an ejaculatory pump. Following each

pump, a cluster of eggs was ejected from the female's cloaca, and sperm were emitted from the male's. The number of pumps per egg-laying varied from ten to twenty-three, with an average of sixteen. The duration of oviposition, calculated as the length of time the pair remained in the egg-laying posture, varied from two to eight minutes, with an average of four (Table I).

EGG-BOUND FEMALES.—Since ovulated females were often kept many hours or days before they were used, they often became egg-bound. This was due to a partial swelling of the egg capsules within the cloaca, making normal expulsion impossible. In these cases, when no eggs or only a few eggs appeared after the third or fourth pump, the egg-laying would cease. In other cases the female would lay a small cluster of eggs, go out of position, lay another small cluster, etc. Such a procedure often took several hours. It is apparently this latter type of egg-laying which Wright (1914) witnessed in the field. Since it is believed that these cases were abnormal, we have excluded them from the statistical summary of the oviposition.

RELEASE AFTER OVIPOSITION

We have studied the male's release of the female following forty-one ovipositions. The release time was recorded in each case as the length of time between the female's moving out of the egg-laying posture and the male's release. If the release occurred before or within a minute of the time the female moved out of oviposition posture, it was recorded as one minute. It will be seen (Table I) that the average release time was six minutes; the median for the release time was one

minute. Delayed releases, i.e., those above the six-minute average, constituted 12 per cent of the cases. The male released before the female moved out of egg-laying posture in 31 per cent of the cases; in 29 per cent, after the female moved from the posture, while in 40 per cent, movement out of posture and release occurred simultaneously.

In the case of male 39, who was paired with three different females on succeeding days, a delayed release followed the first oviposition, but the succeeding two releases were normal. Similarly with male 20, the first release was normal, but when paired on the following day, a delayed release occurred after oviposition. The other males having delayed releases were only paired once.

PRE-RELEASE MOTIONS.—Just prior to the release, in 80 per cent of the cases observed, definite pre-release motions of the male were witnessed. These consisted of short kicking movements of the male's hind legs, which caused the male to swing from side to side and finally to release from one side of the female (Pl. I, fig. 5). Thus release after oviposition is considerably different from the release of a non-ovulated female or a male, in that the latter do not exhibit any such pre-release movements.

The most careful scrutiny of the pair just prior to release did not reveal any action of the female which might be construed as a release signal. The movement out of the egg-laying posture might be considered such a sign, except that 31 per cent of the males released before the female moved. In three of the five cases of delayed release definite pre-release motions were observed immediately following the ovipositions. In the remaining two cases no such movements were noted.

EXPERIMENTAL INVESTIGATION

RESPONSE OF A MALE TO A CLASP OBJECT

By "response of a male to a clasp object" we mean that reaction of the sexually excited male indicated by his swimming towards the female or to an object resembling a female and attempting to clasp.

At a distance this is a visual process, as blinded males never swam over to a female if she was more than an inch or two away. When the female passed close by a blinded male, however, the latter often attempted to clasp the female; and if not successful,

he sometimes followed the female for a considerable distance. Hinsche (1926) noted that blinded male toads will clasp females when the latter are close by.

It has been noted by many authors, Miller (1909), Banta (1914), Hinsche (1926), Noble and Farris (1929) and Liu (1931), that a sexually excited male frog or toad will swim over and try to clasp any object that roughly resembles a female. Banta (1914), however, observing wood frogs in the field, thought that males recognized the peculiar swimming movements of the female from a distance. Hinsche (1926) claimed the same for the toad, *Bufo vulgaris*. Savage (1934), observing the common toad, *Bufo bufo*, credited the males of this species with the capacity of recognizing a pair at a distance.

PROCEDURE.—To test the possibility in *Rana pipiens* of partial recognition of the male, female or of a pair at a distance, the following experiment was set up. In a large aquarium tank, 110 cm. \times 50 cm. \times 31 cm., filled with 10 cm. of water at 23° C., the following leopard frogs were introduced: four pairs, four gravid females,

observation periods. In those cases where males successfully clasped and did not release immediately, they were forcibly separated from the clasped animal. It should be noted that the important criterion in this experiment was the attempt to clasp, and that in summarizing the data no distinction was made between successful and unsuccessful attempts. The observers had little difficulty in distinguishing random movements from attempts at clasping, since the latter usually involved a chase after the clasp object, and were often accompanied by the sex croak or the sex-warning croak.

RESULTS.—The results of the experiment are summarized in Table II. It is seen that there were no marked differences between the number of attempts to clasp males, females or pairs. Hence, within the limits of the experimental situation, there was no recognition or discrimination of a female or pair at a distance. From our observations it appeared that the daily variations depended entirely on the swimming activity of the frogs themselves. None of the 126 attempts of the males to clasp pairs was directed at the female of

TABLE II.—DISTANCE DISCRIMINATION OF PAIRS, MALES OR FEMALES BY SEXUALLY EXCITED MALES, AS SHOWN BY ATTEMPTS AT CLASPING

Date	Total No. of Clasps	Percentage of Times Males Tried to Clasp		
		Pairs	Males	Females
2/13/41	82	23.2	41.5	35.3
2/14/41	69	27.5	30.4	42.1
2/17/41	24	37.5	37.5	25.0
2/18/41	76	38.2	32.9	28.9
2/19/41	102	21.6	32.4	46.0
2/26/41	100	28.0	55.0	17.0
		M = 27.8	M = 39.3	M = 32.9

two males not sexually excited, three sexually active males.

The three active males were selected from a group that had received one female pituitary on the previous day. They were marked by sewing a piece of thread into the skin of their backs so that they could readily be distinguished even at a distance.

Each active marked male had a choice of four pairs, four females or four other males. The number of times that these frogs clasped or attempted to clasp pairs, males or females was recorded in half hour

the pair. Either the active male was kicked away before he got near enough to the pair, or else he landed on the back of the male. In no case did he wedge himself between the male and female of the pair, although this behavior was seen rarely in other laboratory experiments.

THE CLASP REFLEX

The clasp reflex has aroused considerable attention for many years. In recent times it has been studied by Koppanyi and Pearcy (1924) and Smith (1936). The

former workers, using *Rana pipiens*, noted that the clasp reflex is exactly the same in the male and female, while the latter, using *Rana temporaria* and *Bufo vulgaris*, claimed that there is no clasp action at all in the female.

In an attempt to investigate these opposing results, we have noted the following:

1.—Mechanical stimulation of the sternal skin and ventral surface of forelimbs elicited the clasp reflex in both male and female *Rana pipiens*.

2.—The reflex was considerably stronger in the male than in the female *Rana pipiens*, except for a few months after the breeding season when the reflex became almost equal in both sexes.

3.—Occasionally, *Rana catesbeiana* and *Rana clamitans* females have been seen clasping other females (Pl. I, fig. 6). Liu (1931) noted the same for *Rana nigromaculata*.

4.—As noted by Smith (1936), electrical stimulation of the sternal skin with a faradic current (Harvard Inductarium) causes a direct action on the muscles and not a nervous reflex. At low currents (coil 8 to 12 cm. from primary; initial input, 6 volts) a clasp action was obtained from both male and female *Rana pipiens*. The female's clasp was considerably weaker but nevertheless quite definite. At high currents (secondary 0 to 6 cm. from primary) a strong clasp action was induced in the male but caused rigid extension of the arms in the female. High current applied to males after the breeding season caused a partial extension, and in castrate males it caused complete extension of the forelimbs as in normal females.

5.—Intraperitoneal injection of strychnine sulphate (1 cc. of 1 per cent sol.) into *Rana pipiens* caused clasping in the male and extension in the female. In out-of-season males, strychnine caused a partial extension.

6.—Electrical stimulation of the sternal skin after intraperitoneal injection of curare (2 mg., aqueous sol.) caused clasp action in the male at all current strengths. In the female, low currents caused a clasp action similar to that in normals, while high currents caused a partial clasp action.

It should be noted that electrical stimulation does not give a true picture of the clasp reflex. As the flexor muscles are situated directly under the sternum and on the ventral surface of the arm, it is evident that they will be stimulated more strongly than the dorsally situated extensor muscles. Weak currents stimulate the flexor muscles first and therefore induce the clasp in both male and female. Strong currents stimulate the flexors and extensors maximally and equally; the result is antagonistic, rigid extension in the female. In the male, however, as Smith (1936) correctly noted, the greater strength of the flexor muscles accounts for the continued clasp when all the limb muscles are maximally stimulated. This is verified by our results with strychnine injections which caused maximal reflex stimulation and resulted in a clasp of the male and in rigid extension in the female. Why curare should cause a partial clasp in the female when she is stimulated by the strong faradic current, we cannot explain at present.

Koppanyi and Percy (1924) did their work in April which is after the breeding season. This explains why they failed to note the sex differences in the clasp. Smith (1936) possibly used currents which were too strong and which resulted in extension in the female.

The results of our investigations indicate that there is a fundamental clasp pattern common to both male and female, which is subject to seasonal modifications in the male.

SEX RECOGNITION

Voice, girth and repulsive action have been postulated as the major factors governing sex recognition in *Rana* after the male has attempted amplexus. Banta (1914), observing wood frogs in the field, hypothesized a chemical sense controlling sex recognition, but even his own experiments contradicted this idea. Cummins (1920) stated that sex recognition depended upon two factors, namely, (1) the croak of the clasped male and the silence of the female, and (2) the weak resistance of the

female to being clasped, as compared to the strong resistance of the male.

Noble and Farris (1929) demonstrated the importance of girth as a factor in sex recognition in *Rana sylvatica*. They considered voice secondary and listed as minor factors fatigue, degree of sexual ardor and retreat of the pair below the surface. Liu (1931), observing *Rana nigromaculata* in the field and laboratory, minimized the importance of girth and emphasized voice and repulsive action as the major factors in sex recognition. Savage (1934), working with *Rana temporaria*, considered the peculiar rough skin of the female as the major factor controlling sex recognition in this species. Voice and girth were believed to be secondary.

In *Rana pipiens*, the skin in both sexes appears exactly the same, but the female is somewhat larger than the male, and when full of eggs she is considerably stouter. As the eggs pass down the oviduct into the uteri, they receive a coating of jelly (Rugh, 1935b; Waring, Landgrebe and Neill, 1941), so that the egg mass is now much larger, and the female is fatter than just prior to ovulation. The presence of the enlarged egg mass in the uteri apparently causes an intra-abdominal pressure, which is a major factor in bringing about the cessation of the warning croak. (If a non-ovulated female is injected intraperitoneally with sufficient normal saline solution or sesame oil to create an abdominal rigidity, the female will not croak when clasped by a male.)

While we could not measure repulsive action quantitatively in the male and female, our observations led us to conclude that it is the same in both sexes. Fully ovulated silent females were often observed to struggle violently for a minute or two when clasped by males, and in some cases, by hooking their toes under the male, they were able to dislodge him.

As we have shown in the section on the response of a male to a clasp object, such factors as appearance, swimming manners, etc., did not materially influence sex recognition. Thus the remaining differences are the voice and girth of the female and the sexual excitement of the male.

PROCEDURE.—In the experiment, the sexual ardor was kept relatively constant by selecting only highly excited males from the group which had been injected with a pituitary on the previous day. Girth, voice and the croaking mechanism of the sex object were varied by using the following animals:

- 1.—A normal male, thin and croaking.
- 2.—Muted male (vocal cords cut), thin and silent, but croaking motions present; i.e., extension and contraction of the abdominal walls.
- 3.—Anaesthetized male (3 per cent urethane sol.), thin and silent; no croaking motions.
- 4.—Spent female, not so thin as a male and having a weaker croak.
- 5.—Full female before ovulation, fat and croaking.
- 6.—Ovulated female, fat and silent.

The sexually excited males were marked and placed in an aquarium, 110 cm. × 50 cm. × 31 cm., with 10 cm. of water, and frogs belonging to each of the above categories were placed with the males, one group at a time. The length of time the marked males clasped was recorded in seconds. If they did not release after five minutes, they were forcibly separated, and the record was marked "no release." This arbitrary designation of five minutes was made for experimental convenience; it does not mean that males clasping for more than five minutes will never release. Preliminary tests showed that this did happen occasionally, but most of the releases took place well within the first half minute.

The above experiment was repeated, except that the sexually excited males were placed upon the females. The purpose of this procedure was to see if this method could be used in testing the discriminatory reaction in brain-operated males where the ability to swim towards a female was lost.

RESULTS.—The results of these experiments (Table III) show clearly that, as the croak was reduced or eliminated and as the girth was increased, there was a rise in (1) the average clasp time, (2) the median of the clasp time and (3) the per cent of males not releasing. The experiments also show that placing the male on the test frogs yielded results

similar to those obtained when the males were free swimming. While the record shows that an anaesthetized male was not held for more than five minutes, our experience, as well as that of other investigators, has shown that occasionally a very highly excited male held a sick (not croaking) or a dead male for many hours or days. Never, in all our observations of hundreds of clasping males, did we see a normal male clasp another normal croaking male for more than twenty seconds, no matter how excited the clasping male was. It should be noted that we are referring here to true, tight, pectoral clasps, and not to the loose, floating, pelvic grips which sometimes occur. To induce a speedy release, the clasped male must croak continuously as healthy males will do.¹

male. In the above experiments this factor was controlled by selecting only highly excited males.

Attempts to control sexual excitability by pituitary implantations were not successful. Rugh (1935a) correctly concluded that male or female pituitaries implanted in males will increase their excitability to the point where they will clasp. However, it cannot be assumed that untreated laboratory males never clasp. Table IV shows that approximately 35 per cent of the stock males clasped after being kept in the laboratory for periods varying from one day to two weeks. Some males kept in the laboratory for eight months still coupled with ovulated females when placed with them in an aquarium. On the other hand, after one or more treatments with pituitaries, some

TABLE III.—DISCRIMINATORY REACTION OF MALE *Rana pipiens* TO VARIOUS CLASP OBJECTS

Clasp Object	Sexually Excited Males Swam Over to Clasp Object				Sexually Excited Males Placed on Clasp Object			
	No. of Trials	Average Time of Clasp (in sec.)	Median of Duration of Clasp (in sec.)	Per Cent Which Did Not Release	No. of Trials	Average Time of Clasp (in sec.)	Median of Duration of Clasp (in sec.)	Per Cent Which Did Not Release
Normal croaking male (thin + loud croak)	160	1.4	1	0	50	1.2	1	0
Muted male (thin + silent; croaking motions present)	101	5.9	2	0	78	3.2	1	0
Urethanized male (thin + silent)	90	14.0	2	0	31	5.3	1	0
Spent females (thin + dull croak)	67	..*	3	5.8	50	3.1	2	0
Fat non-ovulated fe- males (fat + croak)	77	..*	20	21.3	52	...*	7	13.4
Ovulated females (fat + silent; estrous)	57	..*	No re- lease	89.1	50	...*	No re- lease	88.0

* As some of the males did not release no average clasp time can be calculated.

SEXUAL EXCITABILITY OF LABORATORY MALES.—The third major factor affecting sex recognition is the sexual ardor of the

males still did not clasp.

Canturivili (1936) noted that male *Bombina bombina* which ordinarily do not clasp in the laboratory would often do so when taken out of cold water and placed with a female in warm water.

SUMMARY.—In summarizing the data on sex recognition it can be said that continued croaking plus thinness of the clasped frog tends to cause release, while increasing sexual excitement of the male causes re-

¹ For the purpose of comparing normal males with brain-operated males (Aronson and Noble, 1942) we are able to set a standard for testing the discriminatory reaction to an ovulating female of an experimental male *Rana pipiens*, regardless of the degree of sexual excitement, as follows: if the frog being tested clasps a normal croaking male with a true tight pectoral grip for more than one minute, we can safely conclude that the discriminatory reaction (sex recognition) mechanism has been disturbed.

tention of the clasp. Likewise, silence plus increased girth of the clasped frog causes retention of the clasp, while decreasing sexual ardor of the male tends to cause release.

OVIPOSITION WITHOUT A MALE

It is generally assumed in the literature that ovulating females of various species of *Anura* will occasionally lay their eggs without the presence of a male. However, no actual descriptions of such egg-layings exist. Among the hundreds of ovulated *Rana pipiens* females which we observed in the last two years, we never saw an unclasped female lay her eggs. The eggs often oozed out, sometimes slowly, other times rapidly, but never did the female

egg-laying. From their descriptions it appears that these authors have not made the distinction between oviposition, a specific pattern of behavior for the mediation of fertilization, and the loss of eggs held too long in the uteri.

SIMULATING THE MALE.—If the clasping male is simulated by various mechanical methods, normal ovipositions may follow. When brass weights $2 \times 5 \times .4$ cm., weighing 28 gr. (the weight of an average male), were tied on the back of ovulating females just behind the axillae, the string squeezed the female in a manner similar to a male's clasp. Although this crude representation of a male irritated the females considerably, we succeeded in causing three of these females to exhibit

TABLE IV.—CLASPING BEHAVIOR OF STOCK MALES

Date	No. of Males Tested	Time Males Were in Laboratory (in days)	Time Allowed for Test (in hours)	Per Cent Which Clasped*
1/16/41	17	7	6	41
1/17/41	13	7	5	23
1/21/41	15	1	4	33
3/11/41	40	14	1	35
3/11/41	20	14	1½	25
3/12/41	17	14	2¾	52
	$\Sigma = 122$			$M = 35$

* Clasped ovulated females a minimum of 5 minutes.

assume the egg-laying posture and never did we see the arching of the back and abdominal contractions characteristic of normal oviposition. Only where the clasping male was simulated by elastic bands, strings and weights, did the female lay alone.

The same results were obtained in the other *Anura* examined. We watched, for example, a group of ovulated *Hyla cinerea* for a full day as their eggs oozed from their cloacas and ran down the side wall of the tank. Not once did any of the oviposition movements appear. Similarly, *Xenopus* females injected with gonadotropic hormones lost their eggs without posturing, arching the back or attempting to deposit the eggs on objects on the substratum.

Waring, Landgrebe and Neill (1941) state that the male is not essential for oviposition, but that his presence is advantageous for a complete and undelayed

backward shuffling and oviposition. In one case the female was egg-bound, but in the other two, the females went through all the phases of a normal egg-laying (Table I).

In one instance we also succeeded in obtaining an oviposition by slipping an elastic band about the pectoral region of an ovulating female. In another case, we tied a castrate male to an ovulating female. The latter went through an entire normal egg-laying without any response from the male.

In an experiment involving brain-operated males (Aronson and Noble, 1942), in two cases the release occurred just as the females assumed the egg-laying posture. The females continued the oviposition in these instances in the normal fashion. Thus it appears that once the oviposition has started, the male may not be necessary for its consummation.

PSEUDO-OVIPOSITION

GRAVID NON-OVULATED FEMALES.—

When fat non-ovulated females were injected intraperitoneally with 10 to 20 cc. of normal saline solution, they no longer croaked when clasped by males. Hence the males retained the clasp. After a period ranging from a few minutes to a few hours, many of the females of these pairs started the backward shuffling movement just as ovulated females do. Following a variable amount of backward shuffling the females assumed the normal oviposition posture. The characteristic pumps followed; however, no eggs appeared since none was in the uteri. Other differences from the normal behavior are now noted. The number of pumps was considerably reduced and the percentage of cases with delayed releases was higher (Table I).

After a small number of pumps, the female moved out of the egg-laying posture and away from the non-existent egg mass. In two cases, the females' pumps were abnormally short and irregular. Use of sesame oil yielded results similar to those obtained by saline injections. Post-mortem examination of the twenty-four females studied revealed no ovulation whatsoever.

In order to ascertain whether the males were actually ejaculating during the pseudo-oviposition, a pipette was used to draw some water from the region of the male's cloaca during the ejaculatory pump. Microscopic examination of this water revealed large numbers of spermatozoa. A similar procedure carried out during amplexus but prior to the pseudo-oviposition revealed no sperm.

SPENT FEMALES.—Since the warning croak of recently spent females generally did not return until about twelve hours post-oviposition, highly excited males coupled with them. Such pairs pseudo-oviposited. This behavior differed from that exhibited by the saline injected non-ovulated females, in that release by the males occurred in all cases. Occasionally a few eggs were laid.

Females, spent a few days, were very thin and croaked vigorously. Even the most excited males did not maintain their

clasp on such frogs. When 15 to 30 cc. of normal saline solution were injected intraperitoneally into the females, their girth was increased and their croak reduced or inhibited. The males now clasped. As in the preceding experiment pseudo-oviposition followed (Table I). No eggs were laid in any of the cases witnessed.

Pseudo-ovipositions were thus obtainable in spent females up to thirteen days after oviposition. We were not successful in obtaining pseudo-ovipositions in females spent fourteen to twenty-three days previously even after many hours of amplexus. However, the number of such cases tested was too few to permit detailed analysis.

The data that are available are of extreme interest in a consideration of the factors responsible for the cessation of the warning croak, for oviposition and for pseudo-oviposition. That abdominal distention and clasp are highly important we have already indicated. But the decreasing efficacy of saline injection in inhibiting the warning croak of females after oviposition and the fact that pseudo-oviposition could not be induced in females spent more than thirteen days suggest that a hormonal factor is also involved. Further work on this problem is now in progress.

Waring, Landgrebe and Neill (1941) removed (a) the ovaries, (b) the complete pituitary and (c) the anterior pituitary of ovulating female *Rana* and found no marked interference with oviposition. They conclude that the "more likely excitant substances" are eliminated as agencies in oviposition. The question as to whether these authors witnessed true ovipositions is again raised.

STIMULUS FOR EJACULATION

Savage (1934) demonstrated in *Rana temporaria* that the ejaculations were initiated by the outcoming eggs stimulating the male's cloacal region. That the mechanism is different in *pipiens* can be demonstrated by pseudo-ovipositions where no eggs were present. From our observations, we are inclined to believe that the ventral surface of the male's body, particularly the pectoral region, and the

ventral surface of the forelimbs are areas sensitive to the female's oviposition movements. We feel that it is these movements which, by stimulating the sensitive skin areas of the male, cause the ejaculatory pumps. It is of interest that stimulation of this same region causes the clasp reflex. Three successful cross-ovipositions between female *Rana pipiens* and male *Rana clamitans* further substantiate our view of this subject.

OVIPOSITION OF *Rana clamitans*.—The female green frog assumes the oviposition posture by markedly arching her back concavely so that her cloaca points dorsally and is raised just above the surface of the water. Her legs are fully extended and point caudally and downward.

The male green frog, *Rana clamitans*, responds to the female's egg-laying posture by sliding far forward, so that the male's tympani are on a level with the tip of the female's head. The male's hind limbs are brought up, the knees point outward, while the thighs make an angle of approximately 60° with the longitudinal axis of the body. The tarsi and ankles almost touch along the midline, the toes pointing laterally. Ejaculatory pumps consist of

caudal and lateral rotations of the legs, pushing the eggs away from the upturned female cloaca which protrudes above the surface of the water.

CROSS-OVIPOSITION.—In the cross amplexus, the male green frogs, which were somewhat heavier than the *pipiens* males, clasped laterally, below the axillae instead of ventrally as *pipiens* do. These two factors accounted for some of the difficulty of properly stimulating the females to oviposit. Moreover, the caudal half of the male's body floated instead of closely adhering to the female, so that the male's cloaca was at least 1 cm. from that of the female. As the female went through the backward shuffling movements, the male dangled considerably but nevertheless held tightly. In the oviposition which followed, the female went through all the movements characteristic of a normal *Rana pipiens* oviposition, while the male ejaculated in a manner characteristic of *Rana clamitans*. Throughout the oviposition, only the ventral pectoral region and ventral surface of the male's arms touched the female. As the cloacae at no time approximated each other, it was not surprising that the eggs were sterile.

DISCUSSION

ESTROUS BEHAVIOR

Estrus or the period of female receptivity (Young, 1941) associated with ovarian maturation, ovulation and histological changes of the genital tract has not been generally recognized in lower vertebrates (fish, amphibia and reptiles). Noble and Greenberg (1940, 1941) were the first to describe estrous behavior in reptiles. These authors (1942) demonstrated that copulation in *Anolis carolinensis* can normally take place only with a receptive female, successful mating depending largely upon the estrogen level in the female. They compared the periods of estrus in reptiles with the periods of receptivity in birds and mammals, as to ovarian maturation, behavior and histological modifications of the genital tracts.

Although the sexual behavior of *Anura* differs considerably from that of other

vertebrates, estrous behavior can be recognized. Estrus is the period in *Rana* when the recently ovulated female, swollen with eggs and silent, allows the male to retain his clasp until the egg-laying is completed. The behavior pattern includes cessation of the warning croak, backward shuffling and oviposition movements. In *Bufo* and *Hyla* the pattern probably includes the approach of the female towards the male in response to the latter's sex call. Phases of estrous behavior can be induced in *pipiens* females by intraperitoneal injections of normal saline solution and by mechanical stimulation. (See section on pseudo-ovipositions.) Berk (1938) described the mating season as the estrous stage in the sexual cycle of *Xenopus laevis*, but no mention is made of the receptive behavior of the female.

From Table III it is seen that while the percentage of persistent clasps rises rapidly with ovulation, a small number of couplings will take place with non-ovulated females, or even with spent females. Here we have another comparison with reptilian estrus, namely, that the possibility of matings varies directly with the maturation of the ovaries.

The recognition of estrous behavior in *Anura* is of considerable importance in interpreting the results of endocrinological investigations of sexual behavior. The measurement of the sexual excitability of males by their clasping activity is valid only when the estrous behavior of the females is known. Shapiro (1935, 1936a, 1936b) noted that when *Xenopus laevis* females were injected with pregnancy urine extract (gonadotropic) and were placed with an equal number of untreated males, 30 per cent of the males coupled. (Untreated *Xenopus* never clasp in the laboratory.) He suggested that "some stimulus from the female activates the male." A more likely interpretation of this phenomenon than that offered by Shapiro is suggested by our findings on *Rana pipiens*. As noted before, 35 per cent of untreated laboratory males will exhibit the clasping reaction. We feel that the males which Shapiro refers to as "activated" by the females were normally active but were unable to couple because none of the females was receptive.

Xenopus oviposits shortly after ovulation, and there is no accumulation of the eggs in the lower third of the oviduct as in *Rana pipiens*. Hence, mechanical distention of the abdomen cannot be considered an essential factor for inducing estrous behavior, although we have found that saline injections will cause cessation of the "tick" and allow clasping by the sexually active male. (When a non-ovulated female is touched by a male, or by the observer, a very soft ticking sound comparable to the *pipiens* warning croak is often elicited.) In this species Shapiro (1936a) has produced evidence indicating that an ovarian mechanism of the female is necessary for "evoking the mating reflex in the males." Our opinion is that if such

a hormonal mechanism is present, its function is to induce female receptivity.

Rugh (1935a, 1937) noted that "the male cannot accomplish amplexus without the cooperation of the sexually activated female." He observed (Rugh, 1937, p. 99, Exp. A-15) that a pituitary implanted male, given a choice of three females which had ovulated 0.0 per cent, 2.63 per cent and 89.4 per cent, respectively, was found clasping the last female. The first two females undoubtedly croaked when clasped, while the third was silent. We can elaborate on Rugh's conclusion by saying that males will generally retain their clasp only on estrous females.

RELEASE MECHANISM

The release mechanism after oviposition has not been extensively studied. Noble and Farris (1929) advanced the theory that the loss of girth after egg-laying was the responsible factor in *Rana sylvatica*. Liu (1931) noted a peculiar stretching of the spent female's body which he believed caused the male *Rana nigromaculata* to release. Savage (1934), studying the mechanism in *Bufo bufo*, noted that since the toads lay their eggs gradually, reduction in girth would not be a factor. He advanced the hypothesis that the break in the egg chain was the signal for the male to release.

From the examination of our release data on the various types of pseudo-ovipositions, the mechanism of release can be better understood. In the pseudo-ovipositions of the non-ovulated females, there was no reduction of girth, yet 50 per cent of the males released normally. The same is true for the pseudo-ovipositions of the spent females. In all of these cases a large percentage of the males released without any change in the females' girth. Hence girth reduction cannot be the only factor controlling release. Since in none of the pseudo-ovipositions were eggs laid, the presence or absence of eggs could not materially affect release.

The major difference between these pseudo-ovipositions and normal egg-layings was the reduction in the number of pumps. Apparently, the inability of the female to

lay any eggs caused the sequence of normal events to be broken. It is probable that release after oviposition in *Rana pipiens* is due to a number of factors including (1) ejaculations of the male, (2) reduction in girth of the female, (3) cessation of the female pumps, and (4) movement of the female from the egg-laying posture.

SEQUENCE OF EVENTS IN THE BREEDING PATTERN

It is now well established that the breeding cycle of all vertebrates is controlled by the anterior pituitary. However, the nature of such hormonal control in Anura is not well known.

Rugh (1935a, 1937) believed that the anterior pituitary hormone which causes ovulation in the female and amplexus in the male is bound in some manner and is released only during the breeding season. Temperature, humidity and rain have been suggested as factors causing pituitary activity which in turn starts the breeding cycle. Hinsche (1926) pointed out the importance of the activity of the frogs. Savage (1934) suggested the algae content of the breeding pools as a factor starting the breeding cycle. Bragg (1941) believed that rain and other environmental conditions during the proper season stimulate the male pituitary. The sexually excited male, Bragg said, now clasps the female;

amplexus in turn stimulates the female pituitary and ovulation ensues. This sequence is untenable for *Rana* where the female will not permit the male to retain his clasp unless ovulation is almost complete.

It is our belief that a complex of favorable environmental factors stimulates the anterior pituitaries of both sexes of *Rana pipiens* simultaneously, shortly after their emergence from hibernation. As the male is far more sensitive to gonad stimulation than the female (Creaser and Gorbman, 1939), sexually active males are found in the ponds before the females are in estrus. In *pipiens*, this interval may be only a few days, while in the bullfrog and green frog, there may be a month or more between the onset of the males' sexual activity and ovulation of the females. When ovulation does occur, the abdominal pressure resulting from the large amount of jelly now surrounding the eggs is a major factor in causing the cessation of the females' warning croak. Under such conditions the sexually excited males retain their clasp upon the females. The clasp and abdominal pressure constitute a multiple stimulus giving rise to the egg-laying reflexes; oviposition in turn causes release. A hormonal mechanism may also be a factor causing cessation of the croak and oviposition.

SUMMARY AND CONCLUSIONS

1.—The voices of the male and female *Rana pipiens* are described. The significance of each croak is discussed and the variation with the sexual cycle is noted.

2.—A detailed description of oviposition is made on the basis of forty-one cases. Certain phases of this behavior which have been studied quantitatively are: (a) duration of the oviposition; (b) ejaculatory movements of the male; (c) release of the female by the male at the conclusion of oviposition.

3.—A series of experiments on the normal mating pattern of *Rana pipiens* suggests the following conclusions:

a. The male shows no ability to discriminate between a male, female or pair at a distance.

b. Sex recognition in *Rana pipiens* occurs only after the male has attempted to clasp the sex object and depends upon the girth and warning voice of the female. Small girth plus the warning croak cause the clasping male to release. Large girth and absence of the warning croak (silence) cause the male to maintain his clasp.

c. Oviposition reflexes of the female never occur normally without the initial stimulus of a clasping male. It is possible to elicit these reflexes experimentally by the use of mechanical devices which simulate the male's clasp.

d. Ejaculatory pumps of the male follow the egg-laying movements of the female. The ventral surface of the male's body, particularly the pectoral region and ventral surface of the forelimbs, is presumably sensitive to the female's oviposition movements.

e. Pseudo-oviposition (egg-laying reflexes) can be induced in gravid, non-ovulated females

which have been given intraperitoneal injections of physiological saline solution. Pseudo-oviposition includes all elements of normal egg-laying (with the exception of egg extrusion) although some phases are quantitatively reduced.

f. A fundamental clasp pattern is common to both male and female and is subject to seasonal modifications in the male.

4.—Since the increased girth and loss

of the warning croak are the major factors responsible for the male's maintenance of amplexus, and since these two factors are associated with ovarian maturation and ovulation, it is concluded that the female *Rana pipiens* exhibits estrous (receptive) behavior analogous to that of mammals.

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PLATE I

Fig. 1. Normal amplexus. Note flexion of male's hind legs.

Fig. 2. Beginning of oviposition; male in downstroke. Note position of female's hind legs and toes.

Fig. 3. Oviposition nearing completion. Male in upstroke. Note how egg mass is deposited between female's legs.

Fig. 4. Oviposition in deep water. The pair is just below the surface clinging to blades of grass; male in downstroke. The male's head is concealed by a twig. Note the position of the female's hind legs.

Fig. 5. End of oviposition. The male, about to release, is showing the pre-release movements.

Fig. 6. Female *Rana clamitans* clasping another female of the same species.

