STUDIES IN THE ECOLOGY OF THE NARROW-MOUTHED TOAD, *MICROHYLA CAROLINENSIS CAROLINENSIS*[1]

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Microhyla has received little attention from students of the Amphibia. The most extensive material is that of Wright (1932), Bragg (1943, 1950a, 1950b), and Freiburg (1951). This study contributes to our knowledge of the subspecies *Microhyla carolinensis carolinensis* and to our general understanding of anuran behavior.

The nomenclatural history has been outlined by Hecht and Matalas (1946) who concluded that *M. olivacea* and *M. mazatlanensis* should be considered as subspecies of *M. carolinensis*. Their view is followed in this paper but further evaluation of the systematics of these populations is required.

METHODS

The detailed procedures for both the field and laboratory researches are presented in pertinent sections of this report; only the general approach and sources of material are considered here.

Field studies during an eighteen-month period gave the author an opportunity to become thoroughly familiar with the species. Knowledge gained from seven weeks of observation in Louisiana and Florida was especially valuable.

Population samples were taken at several places near New Orleans, and from pine forests near Angie, Washington Parish, Louisiana. The New Orleans area material came from four localities: levees along the Bonnet Carré Spillway (St. Charles Parish, La.); levees along the Mississippi River near the Huey P. Long Bridge (Jefferson Parish, La.); abandoned fields at Camp Plauche (near Harahan, Jefferson Parish, La.); and, cypress swamps in Tulane University's Sarpy Wildlife Refuge (near Norco, St. Charles Parish, La.).

From the examination of 579 individuals (fresh and preserved) data were gathered for study of population composition, reproductive cycles, growth, and food habits. Snout-vent length measurements were made with a vernier caliper. Secondary sex characters were noted. The abdominal cavity was opened and the gonads were examined. Stomach contents of 203 individuals were analyzed.

Breeding behavior was observed in the field and laboratory. Ma-

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[2] The work was done under the direction of Dr. Fred R. Cagle, to whom the author wishes to express his gratitude for the inspiration and criticism that made the study possible. Dr. Harley N. Gould, of Newcomb College (now emeritus), also gave generous aid in interpretation of the cytological material.

cerated fresh pituitary glands were injected intraperitoneally to stimulate breeding activity in the laboratory.

Environment

Environmental Factors

Field observations suggest that there are two environmental factors which limit, more than any others, the distribution of this species in Louisiana. These two factors, moisture and cover, are closely related. The importance of moisture to these frogs and their extreme susceptibility to drying were demonstrated in trapping studies at Camp Plauche.

Traps used in these studies were constructed from 1/16″ mesh wire screening. The area was an abandoned and partially overgrown dump, providing abundant cover in the form of piles of tar paper, plaster, tin cans, and scattered boards and timbers. There was a sparse to dense covering of low grasses with an upper stratum of dense weeds including such forms as ragweed (*Ambrosia* spp.) and goldenrod (*Solidago* spp.). In the immediate area of the traps there was a stand of low willow (*Salix nigra*) which shaded some of them. Despite the shade it was necessary to check these traps within two hours after sunrise in order to prevent death of almost all individuals from desiccation. Even on cloudy days death resulted within four or five hours if the animals were not removed from their exposed position.

Desiccation makes these frogs vulnerable to predators, especially small ants (*Iridomyrmex humilis*).

Another apparent effect of moisture was the variation in the effectiveness of hand collecting methods at various periods during the year in the Camp Plauche area. In the moist spring months *Microhyla* was abundant under surface cover such as logs, old cardboard, and other debris. As the weather became drier, specimens became more and more difficult to discover in this manner. All cover had been carefully replaced and no animals had been removed from the area. By August and Stepember several hours of intensive search failed to reveal a single specimen. Possibly there had been a migration of the population from the immediate area to contiguous places, but thorough search failed to reveal individuals. Sporadic trapping during this period produced few frogs. This indicates that at least a few individuals remained and sought out retreats at some depth in the earth. By the following spring the accessible population had returned to its original level.

Many other factors influence the distribution of the frogs. Substrate in the habitat may range from permanently or temporarily flooded swamp or marsh to dry sandy pinelands. The amount of litter, duff, and humus differs greatly. That the substrate does affect population density was indicated by studies of pineland populations. Anderson, Liner and Etheridge (1952) found that the size of *Microhyla* populations in a Louisiana pineland area fluctuated with the depth of the organic layers of the soil.

In all habitats there seems to be a distinction between simple overhead cover, as characterized by boards, paper, tin cans, logs, or stones, and more complicated covers, where various crevices, crannies, or burrows are used. In cypress swamps near New Orleans *Microhyla* is often found buried in rotten logs and stumps, usually in relatively loose, soft wood. One individual was taken in a dead stump eight feet above the ground. Stumps have also provided the greatest concentrations thus far discovered. On March 15, 1950, a low hollow stump with an inside diameter averaging thirty inches produced twenty-six juveniles in a stratum six inches thick. Crevices between leaf bases of palmetto plants (*Sabal minor*) are used by *Microhyla* in swamps and in open thickets at the edge of the marshes. These log and palmetto covers often may be completely isolated by water of considerable depth during part of the year. Piles of hay or straw, and other decaying vegetation, such as the muskrat houses in marshes, are also favored cover types. All of these covers have at least two common factors, moisture and a loose structure which permits easy penetration.

Under certain conditions a short semi-burrow is constructed by the frog. Typically these are found where there is relatively loose overhead cover and the soil is granular. Under these circumstances a pear-shaped depression is hollowed out so that only the top of the animal's head is visible. This behavior was commonly shown by individuals on open levees.

Location of suitable breeding sites is also a factor that can exercise a limiting effect upon any anuran species. The wide adaptability of the present species, as shown in detail in a following section on breeding behavior, makes it unlikely that this factor can be of major importance anywhere in Louisiana.

Habitats

The foregoing remarks upon certain of the ecological requirements provide a groundwork for discussion of the ecological distribution of the species in Louisiana. Plant associations provide the best key to this distribution. The habitats favored by *Microhyla* may be divided into forest communities and non-forest or field and marsh communities.

Microhyla occurs in four major forest types: cypress-gum swamp, bottomland hardwood, live-oak ridges, and pine-oak uplands. The bottomland hardwoods and live-oak ridges have received little study.

A cypress-gum swamp in the Sarpy Wildlife Refuge has been studied intensively. The area is covered with a second growth cypress-gum forest interspersed with pure stands of cypress (*Taxodium distichum*) and of cut grass (*Zizaniopsis miliacea*) marsh. There are numerous "borrow pits" along the roads, and several sloughs and bayous partially covered by duckweed (*Lemna* spp.), water hyacinth (*Eichhornia crassipes*) and water fern (*Azolla caroliniana*). The water level fluctuates over a three foot interval. During a fourteen-month period the forest floor was uncovered for only about four months in late spring and summer. When the water is at a depth of only six or eight inches, there are numerous stumps, logs and hummocks exposed. The most productive collecting sites are floating or partly sunken logs, stumps, and the bases of palmetto plants.

The pine-oak community is essentially a dry environment in the areas studied. Longleaf pine (*Pinus palustris*) and post oak (*Quercus stellata*) are major forest trees. Along small streams and in moist depressions there are cypress-gum associations. Frequent burning removes much of the litter and the soil often has no considerable organic layer. Despite this, there appear to be large amphibian and reptile populations.

The non-forest habitats differ markedly from the forest types. They are abandoned fields, grassy levees, short grass areas (such as pastures, lawns, and golf courses), and marshes. Pure palmetto stands seem best considered as an intermediate type as they most often occur along the boundary zone between forest and marsh.

In abandoned fields and grassy levees the cover is about the same although the vegetation is different. The levees are typically covered with low dense grass, but may be grazed and thus belong in the short grass group. Abandoned fields are characterized by a mixture of grasses, weeds, and shrubs.

In urban areas, such as lawns and golf courses, the evaluation of the cover is difficult. There are always hedgerows or gardens where some cover exists, but thorough examination of such areas is difficult and no definite conclusion as to cover preference can be made.

Marshland communities are somewhat analagous to the cypress swamps in respect to water relationships, but have very different cover facilities. Such ridges as may be present may support live-oak (*Quercus virginiana*) or willow. Dr. Cagle tells me that where muskrats are present the frogs have been found in number in the lodges. Any hummock of organic material above the surface will often have one or more individuals buried in it.

Behavior

Diel Activity Cycle

Captive animals in outdoor terraria were observed at hourly or half-hourly intervals through the late afternoon and evening. The frogs burrowed in rotten wood during the day and moved to the surface at early dusk. Most individuals watched from concealment until it became almost fully dark and then began to move about in the open. Activity was typically much reduced by 10 to 11 P.M. Usually only one or two individuals out of six to ten in the terrarium were visible after midnight, whereas all were visible at dusk.

Field observations in Audubon Park, New Orleans, substantiated this pattern. Individuals were found moving about on the short grass of the golf course on many occasions at night. The peak of abundance was usually reached at the time full darkness began.

Movement

Locomotion on land is usually described as a scramble or crawl. This describes the escape behavior when disturbed. Locomotion of undisturbed animals may be described as a walk. Individuals have been observed in terraria and in the field to walk slowly and rather deliberately with the body well off the ground. When disturbed in the open where there is no cover readily available they will hop, covering distances up to twelve inches. They do not land well when hopping and usually fall at an angle so that they roll and have to regain their balance before starting the next hop, often in a different direction. Such erratic movement could have escape value.

In the water they are buoyant and swim well. Pope's statement (1919) that they do not dive is incorrect. Calling males and pairs in amplexus usually dive immediately when disturbed and individuals of both sexes floating in flood waters in the Bonnet Carré Spillway dived readily to escape capture.

Trapping yielded data on movement. The traps used at Camp Plauche were constructed of fine wire screen stapled to wooden frames. The frame consisted of one-inch strips nailed together to form a square, ten inches on a side. Between two square frames a single heavy piece of board, one or two inches wide and eighteen inches long was nailed. Screening was then attached to this frame to form a rectangular cage. The sides and one end were closed and a funnel of the same screening was set into the open end. This funnel projected inward for about eight inches and had an opening about four inches in diameter at the inner end. Drift fences eight to ten inches in height were constructed of asphalt siding stapled to stakes at intervals of about two feet. The angle of the fences leading into the mouth of the trap was about forty-five degrees. Before the fence was put down, the ground was scraped bare and, if possible, a shallow trench three inches wide was dug. The lower edge of the fence was bent and laid flat to the ground in the trench to form an apron on the inner side of the V, earth and debris were scraped over it and packed down to discourage animals from burrowing beneath the fence. Since it was hoped to study breeding movements, as well as normal activity, with these traps, they were placed around a small temporary pond. Five traps were used at the start of the study. All traps faced outward and were connected with an unbroken line of fence so that the pond was entirely fenced off. The line of the traps and the fence formed a clover-leaf around the pond with the traps at the angles between the leaflets.

Towards the end of the trapping period, which lasted from April 19 to May 22, two outgoing traps were added. They did not operate efficiently since there was no apron on the pond side of the fence and escape beneath it was probably not difficult.

All individuals found alive in the traps were tattooed with a number on the under surface of the thigh, sexed, measured, and released on the inner side of the fence. Since one of the returns had a faded and illegible number, it was feared that the markings were not holding and that many of the supposedly new individuals caught in the traps might be recaptures. For this reason six frogs were tattooed and kept in rotten wood in a moist terrarium for a period of two months and their markings were periodically examined. Although there was considerable fading, all but one of the numbers were clearly legible. The one illegible mark had faded, but there was no possibility of overlooking the fact that the individual had been marked. Therefore it is assumed that all unmarked individuals taken in the traps were being caught for the first time.

Trapping results show that considerable movement must have occurred (fig. 1). The first ten days of trap operations preceded the opening of the breeding season. In this period the average nightly catch was 2.7 individuals (1.0 males, 0.7 females, 0.7 juveniles). After the start of the breeding season there were twenty-three days of trap operation with an average nightly catch of 3.1 individuals (2.0 males, 0.45 females, and 0.5 juveniles). Nine trapped specimens were found dead and dried. These were included in the average nightly catch but could not be sexed or aged and thus cause a slight discrepancy between the two sets of figures. There were only five recoveries of marked individuals. All of these were less than six days after release and most were taken by lifting logs or other cover inside the fence. One was taken six days after its release inside the fence in an outgoing trap.

It appears that a large population was present and that there was considerable individual movement, both before and during the breeding season. An increase in total movement during the breeding season seems to be indicated by the trapping data. This increase occurs entirely among the males, and there is an apparent decrease in movement among females and juveniles.

The relative difference in movement of the sexes is strikingly demonstrated by examining the three large population samples which were based on movement and comparing them with samples taken by hand collecting. The sex ratios of all three samples are strongly in favor of males. Of 69 adults trapped at Camp Plauche, 52 were males and 17 were females (fig. 1). A sample taken from a pipeline ditch (Anderson, Liner, and Etheridge, 1952) during the breeding season consists of 147 males and 80 females. A February sample from an aggregation caused by rising flood waters has less disparity between the sexes (94 males and 55 females). The sex ratio was close to 1:1 in samples taken by hand collecting. Of 57 individuals taken in March, 29 were males and 28 females. Twenty-two of these came from the cypress swamps of the Sarpy Wildlife Refuge and 35 from the levee near the Huey P. Long Bridge. The swamp sample was made up entirely of immature individuals and consisted of 11 males and 11 females. The levee sample had 18 males (7 adults and 11 juveniles) and 17 females (13 adults and 4 juveniles).

These contrasting data in regard to sex ratios present a problem

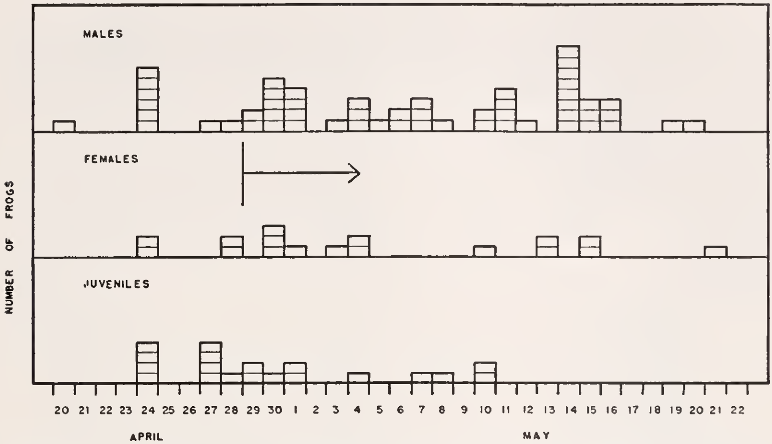

Figure 1. Summary of trapping data at Camp Plauche. Breeding activity (indicated by arrow) continued from April 29th throughout the study period. Each rectangle indicates one individual. Rainfall occurred on January 23-24, 27-30, and May 13-15.

in judgment. Either the movement is equal and the population truly predominantely male or else the sexes are about equal in numbers and the males are much more active. The case for greater activity on the part of the males seems the better one.

It is apparent this movement is related to the amount of moisture and the intensity of breeding drives. Temperature obviously also controls the activity. Samples taken in cold weather show a much greater percentage of empty stomachs than do those taken in warmer weather, indicating that periods of cold (50°F, or less) may prevent feeding over considerable intervals. In the New Orleans area field observations show that inactivity due to cold is sporadic and that the animals are generally active throughout the year.

Food Habits

Dickerson (1906) stated that *Microhyla* are generally eaters of ants. Stomachs of 203 specimens were examined. These represent a year-round sampling from all habitats, though cypress-gum, short-grass and pineland habitats are best represented. Most food items are relatively undamaged in the stomach and can be identified readily. Food organisms were identified to order or family. A count was made of the individuals of each form present (Table 1). When the organisms were broken, heads were counted as whole individuals. Food items were sorted into piles and the percent of the total volume estimated for each form (Table 2). Stomachs are described as "full," "partly full," and "almost empty" (Tables 2, 3). Despite their smaller number the 40 "full" stomachs are probably the most representative indication of food habits. Organisms preyed upon by *Microhyla* range from 0.4 to 6.3 mm in length, averaging about 3 mm.

TABLE 1.
NUMBER OF INDIVIDUALS OF FOOD ORGANISMS IN FULL STOMACHS

Food Organisms	Mean	Maximum
Mollusca (snails)	1.5	2
Crustacea (Isopoda)	—	1
Arachnida (spiders)	2.0	4
Arachnida (mites)	1.2	2
Collembola	13.3	21
Isoptera	30.6	78
Coleoptera		
Carabidae	1.5	2
Curculionidae	1.6	4
Elateridae	1.2	2
Erotylidae	—	2
Scydmaenidae	2.0	2
Staphylinidae	2.9	8
Unidentified	1.4	3
Total Coleoptera	3.5	9
Lepidoptera	—	1
Hymenoptera		
Formicidae	65.6	248
Unidentified	1.0	1
Unidentified insects	2.5	7

Ants, termites, and small beetles are the principal items. Some of the beetles (scydmaenids, clavigerids, and staphylinids) are commonly associated with ants. Many of the organisms taken are secretive, suggesting that the frogs may feed while under cover as well as during the period of surface activity.

Food remains leave the stomach by late afternoon. When the material enters the large intestine, it is formed into fecal pellets 3 to 5 mm long and 2 to 4 mm in diameter.

Predation

No instance of natural predation has been observed in the field. Wright (1932) reported that *Microhyla* had been eaten by a garter snake (*Thamnophis sirtalis*). Anderson (1942) reported an instance of predation by the copperhead. Blair's (1936) report of *Microhyla* dwelling in tarantula burrows indicates that they may live unscathed in close association with formidable predators.

The secretion of *Microhyla carolinensis* has been thought to give it some protection from predators. If a bit of the mucous secretion touches one's eye, a violent burning sensation may persist for more than an hour. This secretion was also found to be irritating to the mucous membranes of the mouth and throat.

The functions of this skin secretion in protection of the animal from attack by ants was observed in the field on April 5, 1951. The following is an excerpt from field notes of that date.

> "Two individuals were found under a post which was swarming with small brown ants. When a frog was placed in the aroused colony numerous ants immediately

> attacked it. The frog sat very quietly and much material was secreted by the skin glands. The ants rapidly became entangled in this thick layer and their attention was diverted into attempts to escape rather than continued attack upon the frog. After a short while the frog ducked under a few lumps of dirt. When uncovered it was found free of ants. The frog was replaced among the ants and the procedure repeated. The next time the frog sought cover it could be seen that the mucous and the entangled ants were brushed off when the animal passed any object. (Captain Joseph Jones, U.S.A.F. identified the ants as *Iridomyrmex pruinosus.*)."

Field notes also show that this secretion may be toxic to other amphibians.

> "Thirty plus *Microhyla* in small bottle with four *Hyla cinerea* and one *H. squirella.* Upon reaching the laboratory *H. cinerea* and the *H. squirella* appeared dead. When washed in several rinses of water one of the *H. cinerea* recovered somewhat. It was placed in an aquarium and by noon the next day was stronger and had moved a little, but it weakened rapidly when handled. The tongue was partly extruded through the side of the

TABLE 2.
AVERAGE PERCENT BY VOLUME OF STOMACH CONTENTS

Food Organisms	Full Stomachs (Percent)	Partly Full Stomachs (Percent)
Mollusca (snails)	15.5	54.0
Crustacea (Isopoda)	22.5	—
Chilopoda	22.5	—
Arachnida (spiders)	9.3	9.1
Arachnida (mites)	1.3	6.3
Collembola	15.0	14.0
Isoptera	67.8	73.9
Hemiptera	3.7	11.4
Dermaptera	8.0	9.0
Coleoptera		
Carabidae	1.5	—
Clavigeridae	1.0	—
Curculionidae	15.5	19.8
Elateridae	10.0	18.2
Erotylidae	—	20.2
Scydmaenidae	3.5	5.0
Unidentified	7.5	17.0
Total Coleoptera	15.8	21.6
Lepidoptera	10.0	30.0
Hymenoptera		
Formicidae	69.0	58.2
Unidentified	5.5	—
Unidentified insects	17.6	19.2
Plant fragments	9.7	18.1
Sand	97.6	80.8
Unidentified material	41.7	38.2

mouth and the mouth contained much slime and some blood. The next day the animal was found dead. Several of the *Microhyla* showed ill effects, but recovered shortly."

Some predation under abnormal conditions was found. *Microhyla* in the traps, handicapped by dehydration, were attacked and killed by small ants. Numerous ants would usually first be trapped in the frog's secretion and killed. On one occasion the headless body of a frog was found in a trap. It had apparently been killed and partially eaten by some small mammal.

Voice

In addition to the breeding call, which will be discussed in detail later, two other sounds are produced. Both are apparently protest notes. The first and most common one is a faint clicking produced when the individual is handled. The second, a chirp or squawk

TABLE 3.
FREQUENCIES OF OCCURRENCE OF FOOD ORGANISMS

Food Organisms	All Stomachs (203)	Full Stomachs (40)	Partly Full Stomachs (50)	Almost Empty * Stomachs (65)
Mollusca (snails)	4	2	2	—
Crustacea (Isopoda)	3	2	—	1
Chilopoda	2	2	—	—
Arachnida (spiders)	16	8	4	2
Arachnida (mites)	27	4	13	10
Collembola	7	3	3	1
Isoptera	12	6	3	1
Hemiptera	14	7	5	2
Dermaptera	2	1	1	—
Coleoptera				
Carabidae	2	2	—	—
Clavigeridae	1	1	—	—
Curculionidae	12	6	6	—
Elateridae	8	5	2	1
Erotylidae	1	—	1	—
Scydmaenidae	5	4	1	—
Staphylinidae	18	12	5	1
Unidentified	35	16	11	8
Total Coleoptera	58	23	20	10
Lepidoptera	2	1	1	—
Hymenoptera				
Formicidae	114	29	43	36
Unidentified	4	2	1	1
Unidentified insects	19	7	4	8
Shed skin	10	—	—	—
Plant fragments	36	9	13	10
Sand	26	5	9	8
Unidentified	25	6	13	6

* Other stomachs: Empty—32, Not noted—10.

sometimes given when the frog is roughly handled, apparently also replaces the clicking noise when the animal is in water. These sounds are made by both sexes.

Reproduction

Gonadal Cycle

The reproductive behavior of most North American anurans has been assumed to be strongly cyclic. This conception is based on the sharply delineated breeding seasons of many frogs and toads inhabiting the northeastern portions of the continent. Bragg (1950a) on the basis of his field observations in Oklahoma, has strongly challenged the application of this view to several species. The question of cyclic versus non-cyclic patterns indicates a need for study of the gonadal cycles of many forms. The study of Glass and Rugh (1944) of the normal cycle of spermatogenesis of *Rana pipiens* in Vermont and the study of Fischer and Richards (1950) on the ovarian cycle of *Acris crepitans* in Oklahoma are the best sources of comparative information.

Sections of 44 testes and 31 ovaries were studied. Some ovaries and testes for cytological preparations were taken from fresh specimens and fixed in Bouin's, while others were taken from specimens which had been fixed in formaldehyde, washed in a deformalizing solution and placed in 50 percent alcohol, according to standard museum practice. There did not seem to be any great difference in the results obtained by these two methods. The gonads were embedded in paraffin and sectioned at eight microns. Mayer's haematoxylin or Galigher's haematoxylin was used, the latter usually proving more satisfactory.

Male Reproductive Cycle.—The appearance of the seminiferous tubules of the anuran testis has certain dissimilarities from that of the mammalian testis. The various stages of spermatogenesis are not evenly dispersed around the wall of the tubule, but are found in clusters which project out into the lumen and are distinct from other clusters. Rugh (1951) has suggested that each of these larger clusters may come from a single spermatogonium. There is no zonation inward from the tubule wall, but a linear arrangement of clusters along the wall. Sertoli nuclei are scattered close to the wall and do not occur in clusters. Spermatogonia occur singly or in small clusters.

The first step in interpretation of testes slides is identification of the several stages of spermatogenesis. Rugh (1951) describes the stages in some detail as found in *Rana pipiens*. His descriptions of spermatozoa, spermatids and secondary spermatocytes are readily applicable to the present species, but difficulty was encountered in identifying primary spermatocytes on the basis of his illustrations and descriptions. Rugh stated that nuclei of primary spermatocytes are irregularly spherical, very large, and never numerous. These characters readily fit nuclei which appear to belong to sertoli cells, but leave unidentified another type which is a major component of the seminiferous tubule wall. Because of this difficulty it is believed that a description of the stages as interpreted in this study is necessary.

Spermatozoa are easily identified. The heads are small, darkly stained, and bullet-like in shape. They may occur in clusters in the sertoli cells or scattered in the lumen of the tubules. All cells which have elongated nuclei are classed as spermatozoa.

Spermatids are small in size and usually consist of dense, darkly stained, circular nuclei, each surrounded by a ring of clear cytoplasm. These cells occur in clusters which extend into the lumen from the tubule wall and each cluster contains a large number of individual cells (mean 41.9). They usually differ from the secondary spermatocytes only in size and in the larger number of cells in a cluster.

Secondary spermatocytes occur in clusters containing a small number (mean 20.9) of cells larger than those found in spermatid clusters. Each cell consists of a medium-sized, dense, darkly stained nucleus with an outer ring of clear cytoplasm.

Primary spermatocytes occur in clusters which contain still fewer individuals (mean 13.5) and do not project very far into the lumen. The nuclei are large and granular, but are not dense or darkly staining.

Spermatogonia are much like the primary spermatocytes in appearance, but are smaller and often occur singly rather than in clusters. Since the change to primary spermatocytes is gradual, no exact criterion for separation is possible.

Sertoli cells have very large, pale granular nuclei. These may be irregular or regular and rounded in outline. They always occur singly, close to the wall of the tubule, and are never found in clusters. There are only a few (two to eight) visible in each tubule. They are often associated with clumps of spermatozoa which are obviously in sertoli cells.

Breeding activity of *Microhyla* in Louisiana occurs from the latter part of April until the middle of September. Testes of mature frogs taken throughout the year show all the maturation stages of spermatogenesis, but the relative numbers and the relative amount of space allocated to each stage varies greatly (fig. 2).

Testes of mature individuals taken in October are characterized by the presence of small numbers of spermatozoa scattered about in the lumen of the tubules. A few spermatozoa also are in sertoli cells, but nowhere do they occur in dense masses. Primary spermatocytes are the major component of the tubule wall.

Some November individuals show a condition similar to that found in October. In others spermatozoa have begun to increase in number, appearing in concentrations in sertoli cells. There is a marked decrease in the number of primary spermatocytes. These trends continue and by January the massed spermatozoa in the sertoli cells are so numerous as to cause a reduction in the size of the lumen. The primary spermatocytes have lost their place as the major component of the tubule wall and are found only in small scattered clusters.

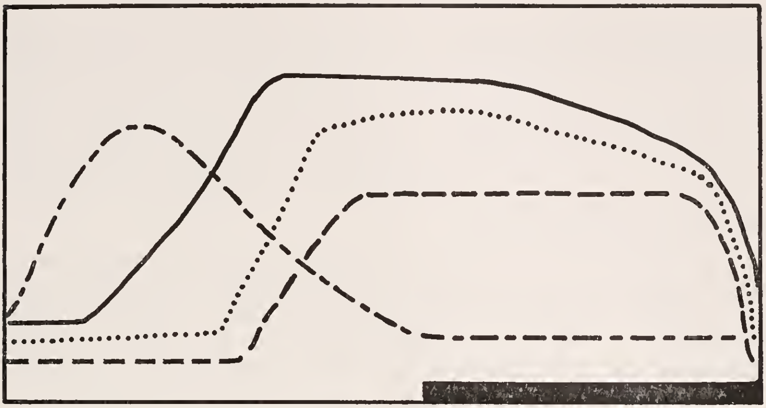

Figure 2. Spermatogenesis in *Microhyla c. carolinensis.* Solid line =spermatozoa; dashed line=spermatids; long dash-short dash line=primary spermatocytes; dotted line=secondary spermatocytes; wide solid black line at base of figure=breeding season.

In February the massing of the spermatozoa in sertoli cells reaches its peak. Massed spermatozoa nearly fill the lumen of the tubule and often extend outward to the tubule wall. Primary and secondary spermatocytes and spermatids occur in small clusters. The accumulation of the spermatozoa in the sertoli cells seems to be a slow, gradual process in the months after the close of the breeding season. During this period large numbers of secondary spermatocytes and spermatids do not occur.

The massed condition of the spermatozoa persists through the early part of the breeding season. With the initiation of breeding, however, there is a gradual thinning out of the spermatozoa in the sertoli cells and some appearance of massing in the lumen of the tubule.

For two months preceding the breeding season and during the first two months of the latter period a peak in general activity is reached. This is marked by the massing of the spermatozoa and by a great increase in the numbers of secondary spermatocytes and spermatids (fig. 2). This increase begins in March and the number of secondary spermatocytes and spermatids remains high through May and June.

In June there is a slow reduction in the number of secondary spermatocytes and spermatids, paralleled by a thinning out of the spermatozoa. There appears to be a very slight increase in the number of primary spermatocytes at this time. These trends continue through July and August.

In summary, the male gonadal cycle may be divided into two major phases. The first is the slow build up of spermatozoa following the cessation of breeding activity. This phase culminates in February with the massed spermatozoa filling the tubules. The second phase is initiated in March with the appearance of numerous secondary spermatocytes and spermatids. During the early part of the breeding season there is a height of spermatogenic activity characterized by the draining of mature spermatozoa from the supplies in the sertoli cells and a partial replacement of these from the developing secondary spermatocytes and spermatids. With the end of the breeding season the supply of the latter stages is no longer maintained and the spermatozoa are thus drained to a very low level, preparatory to the start of the first phase once again.

Secondary Sexual Characters and the Male Cycle.—Sexual dimorphism in *Microhyla* occurs in size, coloration, presence of vocal sacs, and presence of dermal modifications in the male. The size relationship will be discussed at another point in this paper, but the modification of skin pigmentation and structure show some cyclic fluctuations and will be considered here.

The black throat does not show marked cyclic changes and is characteristic of more than 85 percent of the males 22 mm or more in snout-vent length throughout the year. The throat of the male is usually abruptly darker than the abdomen and pectoral region. Some difficulty may be encountered in applying this character to strongly pigmented individuals. There is a slight intensification of this dark throat color during the breeding period.

Two secondary sex characteristics may be observed only in the breeding season. One, the presence of chin tubercles, was first described by Mittleman (1950). Tubercles may occur in a single or double row along the ventral surface of the lower jaw and may range from one to 28 in number. They are conical and project approximately 0.07 mm, as measured with an ocular micrometer scale. In the June sample of 129 males displaying a black throat, 114 showed tubercles. The second characteristic is the wrinkling or roughness of the skin of the whole throat area of the male. Wrinkling may be caused by the development of the vocal sac. There may also be small rough patches and occasionally transverse bands of tiny tubercles. There is great variation in these throat characteristics.

Female Reproductive Cycle.—The present study utilizes data derived from the study of gonad sections and the measurement of gross features of the female gonads. Examination of the gross changes in the ovary proved especially informative. This organ consists of a double-walled sack, normally divided into three to five lobes. The oogonia and the developing oocytes lie between the inner and outer walls of the sack. As the oocytes grow they bulge into the lumen of the ovary and are closely covered by the very thin inner membrane which forms most of the follicular wall. During their growth the oocytes pass through three distinct stages. Analysis of the occurrence of these stages furnishes a basis for determining the reproductive cycle.

The first of the oocyte stages is characterized by a clear, transparent cytoplasm having a whitish, granular-appearing nucleus. These cells have a diameter of from 0.08 to 0.45 mm, falling between 0.11

and 0.34 mm in most cases. These are present in all ovaries throughout the year. This clear stage is the only one present in all frogs less than 19 mm in length and in some up to a length of 22 mm.

Oocytes belonging to the second and succeeding stage of development become opaque and milky-white. They range in size from 0.23 to 0.77 mm and are found throughout the year in ovaries of most frogs over 20 mm in snout-vent length.

Oocytes belonging to the third and final stage are pigmented. These range in diameter from 0.69 to 1.49 mm. The smaller ones are pigmented throughout and are a dark brown color. The larger ones show a fully developed differentiation of the animal and vegetal poles, the former being a very dark brown, the latter white. The pigmented oocytes are present in individuals of more than 23 mm in length from January until breeding takes place. They are absent in all ovaries from late September until January.

In addition to developing oocytes, the walls and lumen of the ovary may contain degenerating pigmented oocytes. Some of these may be quite large and retain their shape while others are indicated only by small amorphous concentrations of dark pigment in the ovarian wall. These are believed to be oocytes which failed to ovulate or were caught in the ovarian tissues after ovulation. Presence of these oocytes suggests previous breeding, though they do not occur in all females which have had previous breeding seasons.

The ovaries of 142 frogs were examined. The ovarian wall was broken and the fragments studied under a dissecting microscope. The presence of various stages of oocyte development was noted and a sample of each class measured with an ocular micrometer. Since a random sample of each of the various stages could not be taken, ten or more oocytes of each class were measured representing the extremes and the apparent general trend. As the ovaries enlarge with the growth of the pigmented oocytes, many of the cells become angular. It is necessary therefore to search for oocytes which are still nearly spherical in making measurements.

The various oocyte types as outlined above also show certain cytological differences. The nuclei of the small oocytes, belonging to the clear variety, are regular in outline and have a few dark-staining, rounded nucleoli near the edge. The cytoplasm of these small oocytes may sometimes show large, radially oriented vacuoles. These vacuoles are reduced in size with the growth of the oocytes and eventually disappear entirely. They may contain small, dark-staining bodies and it seems possible that these have some relation to the yolk nuclei reported in *Bufo* by King (1908) and in *Acris* by Fisher and Richards (1950). The nuclei of the white oocytes show the greatest cyclic change. There is increasing irregularity of the nuclear margin, an increase in the number of nucleoli and a movement of the latter toward the center of the nucleus as the breeding season approaches. These trends are continued as the oocytes become pigmented and a highly irregular germinal vesicle, or nucleus, results. This has a dense scattering of small nucleoli at its center. The pigment in these maturing oocytes is laid down mostly around the outer rim of the cytoplasm.

The oviduct of the female and the fat bodies in both sexes display variation in correlation with that in the gonads. The oviduct is a white tube, wavy in its anterior part and much convoluted in the posterior glandular portion. It fluctuates greatly in size, being relatively slim throughout the post-breeding period in the fall and winter and enlarging greatly as the breeding season approaches. The fat bodies exhibit a reversed cycle. This occurs to some extent in both sexes but is clearly marked only in the female. The fat bodies are enlarged during the fall and winter and are reduced in size and finally almost disappear with the maximum development of the ovaries at the beginning of the breeding season. It is possible that they store materials used in the developing oocytes.

The results of the oocyte measurements give a clear picture of the female reproductive cycle. Females which have recently deposited their eggs have, with the exception of a few pigmented eggs that were not ovulated, only clear and white oocytes in the ovary. The ovary appears as a white, angular structure, molded about the posterodorsal surface of the digestive tract and the posterior end of the body cavity. In section the ovaries also show oocytes of two classes. The larger ones have a palely staining cytoplasm which seems to indicate some yolk accumulation. The margins of the nuclei are irregular. The nucleoli are near the edge of the nucleus in some cells, or scattered inward to the centers in others. The smaller oocytes have a less variable, more darkly stained nucleus. The nuclear margins are regular or slightly scalloped and the nucleoli are near the surface. The smallest oocytes often have numerous large vacuoles arranged radially around the outer edge of the cytoplasm. The most noticeable features of these sections is the presence of collapsed follicular sacs.

There were no mature individuals available from the months of September and October, but the November and December samples furnish information which makes an evaluation of the period possible. Apparently there is little growth in oocyte size during the months of early fall and winter. The most notable feature is the disappearance of the collapsed follicles. There is considerable variation in the size of the oocytes. The larger ones have irregularly margined nuclei, and the nucleoli are scattered throughout the nucleus. Some small oocytes show vacuolated cytoplasm and others do not.

In January the first pigmented oocytes appear. From this time on they increase rapidly in number. Growth of pigmented oocytes follows a smooth curve with the exception of the March sample which was taken in 1951 (the other samples were taken in 1949-50). The apparent explanation for this discrepancy is that the 1949-50 winter and spring were exceptionally mild, while the 1950-51 winter was cold. If this effect is actually due to climatic conditions, it indicates considerable plasticity in the female reproductive cycle. This tardy development was apparently compensated for rapidly, for the breeding season began two weeks earlier in 1951 than it did in 1950.

During the period of development of pigmented oocytes there appears to be a decrease in the size of the white oocytes. This may be explained on the assumption that the larger white oocytes are becoming pigmented, and fall in another class, leaving only smaller cells behind and thus reducing the average size.

As a result of the great growth of the pigmented follicles the ovaries increase rapidly in size, almost surrounding the digestive tract. The white and clear oocytes are crowded into the corners between the maturing pigmented oocytes. All classes of oocytes are now angular in shape due to pressure from others nearby. With this ovarian enlargement there occurs the great enlargement of the oviducts, especially the glandular portions.

Discussion.—The gonadal cycle of male *Microhyla* contrasts sharply with that found for *Rana pipiens* in Vermont by Glass and Rugh (1944). The differences are understandable when the breeding seasons and activity periods of the two animals are considered. *Rana pipiens* is, in Vermont, a form with a restricted period of activity and a long hibernation; physiological processes are at their height during the warm months of the year and are slow during the long periods of hibernation. In the spring there is a short, definite, breeding period, preceding the seasonal optimum of environmental conditions, which gives the tadpoles a favorable period for growth and transformation and gives the adult a chance to prepare for the coming year. In *Microhyla* in southern Louisiana there is no period of hibernation, and there is no need for a rapid build up of reserves or an early preparation for the coming breeding season of the next year. There is also no problem of time for larval development.

Another interesting problem arising in relation to reproductive cycles is the matter of the timing of individual activity during an extended breeding season. Why one female deposits her eggs in April and another does so in September is not known. There seemed to be little variation in the degree of preparation for breeding among the females of the June sample. If the time of individual breeding is fixed by inheritance, interesting evolutionary implications arise, such as reproductive isolation resulting in fragmentation of sympatric populations.

The female reproductive cycle described here agrees with that given by Fisher and Richards (1950) for *Acris* in some respects, and differs considerably in others. Fisher and Richards do not emphasize the external morphology of the oocytes and thus comparison is difficult. They report a range of stages found in each ovary and this is interpreted as an indication of differential rates of development. In *Microhyla* it seems apparent that this variation cannot be attributed to differential rates but rather indicates that the ovary contains, at any given time, oocytes destined for use in at least two and possibly three breeding seasons. This situation apparently differs sharply from the ovarian cycle of *Rana pipiens,* for Rugh (1951) reports that after ovulation "the ovary has been freed of mature eggs and contains only oogonia with no pigment and little if any yolk." Although oogonia may be found in most sections of *Microhyla* ovaries, they are never common, while white and clear oocytes are always abundant. The pattern of nuclear change with oocyte growth seems to be identical with that reported by Fisher and Richards (1950) and Rugh (1951). However, Fisher and Richards (1950) seem to imply that such a phenomenon as the movement of the nucleoli toward the center of the nucleus occurs in all oocytes in a given ovary at once. This is not true of *Microhyla* for the nuclear condition is correlated with the gross morphology and small, clear-type oocytes always have few nucleoli and these are always near the nuclear border.

Reproductive Behavior

Field observations of the reproductive behavior of *Microhyla* are difficult because of the shyness of calling males and the dense vegetation in most breeding sites. However, techniques for field study of certain aspects have been worked out, and with practice it becomes easier to discover the males in their calling positions. Two sets of field observations, one at Century, Florida on July 15, 1950 and the other at New Orleans on July 28, 1950 provided much valuable material.

In addition to field observations, specimens injected with whole pituitary gland after the method standardized by Rugh (1935) were studied in the laboratory. Glands used were from ranid frogs of three species. Average doses in terms of glands from mature individuals of these donor species are as follows: *Rana pipiens,* two; *Rana clamitans,* one-half; *Rana catesbeiana,* one-third. Injections were made with a 1.5 ml syringe and #22 needle. Care was taken not to inject more than 0.05 ml of water into any individual. Individuals were placed in a large container with shallow water and some material upon which they could climb about. When amplexus began they were transferred to small aquaria for observation.

Information on other questions of reproductive behavior was obtained by examination of ovaries and oviducts.

It has been demonstrated above that a definite gonadal cycle exists. This cycle is considered as the primary factor in control of reproductive behavior. It may be assumed to be under hormonal control but has shown variations apparently attributable to climatic conditions.

Environmental Factors.—Weather conditions have been shown to influence the degree of general activity as well as breeding activity. Most authors are agreed that rain stimulates breeding behavior. Thus Wright (1932) states: "This subterranean species normally breeds and mates at night. Heavy, warm rains of one to four inches may precipitate mating in daylight and rarely one captures a croaking male at mid-day." Barbour (1941) reported a large chorus developing after a cloudburst. Bragg (1950a) on the other hand, stated that breeding of *M. c. carolinensis* in Oklahoma is not much influenced by rainfall. In this respect it should be noted that most authors, in describing time of breeding of this and other frogs, do not distinguish between occurrence of calling individuals and actual mating. There is no evidence that the two are necessarily synchronous. The notes of the writer show that choruses, like movement, are often initiated by rain. The first breeding of the 1950 season did not take place until after two days of rain. Until that time there had been no calling or other breeding activity. On the second night just after the rain had ceased large choruses were found at several localities and pairs were found in amplexus. Shorter rains as little as four days previously had had no effect and it seems obvious that this prolonged and very heavy rainfall was needed to initiate breeding.

Light, with its apparent inhibiting effect upon movement, also influences the breeding behavior. Daylight choruses are common at the height of the breeding season even in very dry weather, but no evidence of diurnal amplexus or egg deposition has been noticed.

Great adaptability is shown in regard to breeding sites. Males have been found calling, and tadpoles seen, in wheel ruts, ditches, small permanent ponds and margins of cypress lakes. These can be classified as being deep water or shallow water sites. Deep water sites are those with water one to six feet deep. Breeding activity takes place in such areas only when a dense mat of floating vegetation such as water hyacinth (*Eichhornia crassipes*) or alligator weed (*Alternanthera philoxeroides*) is present. Shallow water sites are less than one foot in depth and some have a maximum depth of only one or two inches. These sites may be temporary or permanent waters. Wright (1932) recorded a great mortality of eggs due to drying of temporary sites in the Okefinokee swamp.

Vegetation in shallow water may be dense or non-existent, but calling males have been seen using sticks, lumps of earth, crevices in the earth or in logs, loose bark, sparse short grasses and large clumps of tall grasses, alligator weed and water hyacinth as cover. This reaction is apparently based upon two factors: the shyness of the animal which keeps it partially under cover, and the need of some sort of support for maintenance of proper calling position.

The bottom of breeding ponds ranges from mud to decomposing organic matter to hard clay.

Observations made near New Orleans on July 28, 1950 suggest that there may be some sort of location sense in regard to traditional breeding sites. A location in Jefferson Parish was used heavily all through the spring. It consisted of a weedy pond, about 2.5 feet in maximum depth and 60 feet in diameter, with a wide belt of alligator weed around the shore. *Acris* called from the short and sparse weed nearer the center of the pond and *Microhyla* from the dense, tall weed along the edge. At some time between June 1st and July 20th an abandoned field stretching behind this pond was cleared with a bulldozer and the pond filled in. On July 28 after a rainy day *Microhyla* were found calling from puddles and crannies between lumps of earth. There was no living vegetation but cover was furnished in some pools by root stubs or bits of floating board. There was no apparent difference between any two parts of the field of approximately five acres. Despite this apparent complete similarity of possible breeding places over the whole area, the large chorus was found only in the immediate area of the former pond.

As there is a daily cycle of normal activity, there is also a cycle of breeding activity. It has been stated that during periods of intense breeding activity many individuals may remain in the breeding area and call throughout the day. In these and in all other cases there is a movement of individuals to the breeding sites at dusk. Observations at a small artificial pool in Audubon Park, New Orleans, show that the peak of this movement is just at full darkness. The males precede the females in moving to the breeding sites, the peak of male movement preceding that of the females by ten to twenty minutes.

Voice.—The call is usually described as a sheep-like bleat. Burt (1932) noted that in Louisiana and Mississippi he heard two calls, one "goat-like" and the other a "soft murmur." The writer has never been able to hear anything resembling this latter call either in field or laboratory. Some variations in the normal call take place when amplexus begins. The normal call has a duration of about 1.4 seconds at 25°C (water temperature).

The calling posture is constant as reported by various authors (Wright 1932, Barbour 1941). The body is usually in a vertical or near vertical position, immersed in water up to the axilla and supported against some object by the forelimbs.

While calling, the male does not move about, but retains his position without change for long periods of time. Of six individuals marked by placing tagged sticks near them at 8:45 P.M. (Century, Florida July 16, 1950), all retained positions at 9:00 P.M., four retained position at 9:30 P.M. and at 10:00 P.M. Since eggs were found in the regions occupied by the two that left their positions, it is likely that they had mated successfully. Again, near New Orleans on July

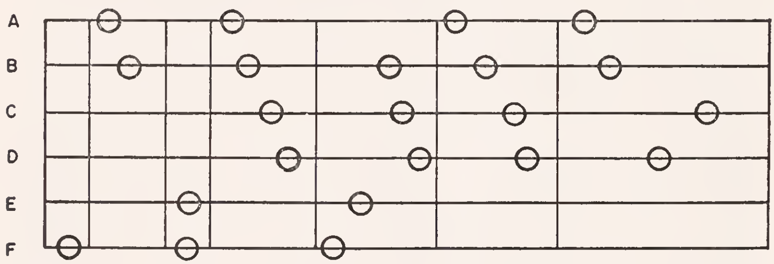

Figure 3. Calling patterns, July 28, 1950. Each letter and corresponding line represents a single individual. Vertical lines indicate pauses between series of calls.

TABLE 4.
CALLING PATTERNS IN A SMALL CHORUS

38 series of calls (total studied)	
34 —	2 or more individuals
20 —	3 or more individuals
14 —	4 or more individuals
5 —	5 or more individuals
3 —	6 individuals

Calling frequencies

Individuals	A	B	C	D	E	F
Total Calls	29	25	16	23	11	10
Started series	9	3	0	8	3	6
Called alone	0	0	0	0	3	1

Frequency of calling sequences

Combinations of two individuals	Combinations of three individuals	Combinations of four individuals
(30 possible comb.)	(240 possible comb.)	(420 possible comb.)
AB — 20	ABC — 9	ABCD — 3
AD — 4	ABD — 2	ABDC — 1
BC — 15	ADB — 2	ABDF — 1
BD — 2	ADE — 1	BDFE — 1
CD — 4	BCD — 4	BCDE — 1
DA — 9	BDC — 1	CDEG — 1
DB — 3	BDF — 1	DABC — 4
DC — 1	DAB — 6	DABD — 1
DE — 2	DFE — 1	EABC — 1
DF — 2	CDE — 1	EDAD — 1
ED — 1	EAB — 1	FDAB — 1
FA — 6	EDA — 1	FEDA — 1
FD — 1	FAB — 2	FADE — 1
FE — 3	FAD — 1	FABC — 2
	FDA — 1	
	FED — 1	

28th six individuals under observation maintained their positions with very little change for two and one-half hours. One remained in position and called for three hours before beginning to move about.

The calling of a group around a small pool was studied in both the Florida and New Orleans areas. A predominant pattern was perceived in the Florida chorus. A technique applied at a New Orleans site permitted the observer to record and analyze this pattern. A number of parallel lines were drawn, each representing an individual and designated by a letter. Each time an individual called a dot was placed on the line representing that individual. Since the calls of the individuals in a small aggregation are in bursts with intervals of silence between, each series is written as one would write a bar of music. Figure 3 gives an example of calling records obtained in this way and Table 4 presents the analysis of the data obtained for 38 series of calls around one pool. There were six males calling around this pool which was about three feet in diameter.

They could all be observed easily during this period.

When the data are summarized relationships between calling individuals are shown. Of the 38 series of calls, 34 have two or more individuals represented (Table 4). With 6 individuals calling there were 30 possible combinations of two. Of these possible sequences 15 occurred a total of 75 times. On the basis of equal chance and calling activity for each individual it would be expected that each of 30 combinations would have occurred slightly less than three times as an average. Actually one sequence (AB) occurred 20 times, another (BC) occurred 15 times, and a third (DA) occurred nine times. If those series which had three individuals calling are considered there are 120 possibilities, but only 16 occurred a total of 35 times. The chances that any sequence should occur more than once is thus small, yet the pattern ABC occurred nine times, the pattern DAB occurred six times, and the pattern BCD occurred four times. Considering series of four individuals there are 240 possible combinations; 14 series occurred a total of 20 times, and DABC occurred four times, ABCD three times, and FABC twice. There is thus a definite pattern, revolving around four individuals: A, B, C, and D. Examining the status of the remaining two individuals, E and F, it is seen in Table 4 that they are the only ones which called alone, that is, without being part of a series. It is further noted that they called less than the other individuals, but usually either called alone or began the series. Turning back to the original four it is seen that A and B called the most, but that B was not aggressive, rarely initiating a series of calls, while A and D were both aggressive in this sense. In contrast, C never started a series. Carrying these relationships further it is found that B followed A in 20 of the 25 series in which it took part, and that C followed B in 15 out of 16 series in which it took part.

The significance of these relations is not understood. A, apparently the dominant member of the group, remained in position and kept calling the longest, but did not succeed in attracting a female. Two other members of the group (exactly which ones could not be determined at the time) did go into amplexus some time after calling observations were made.

There seems to be no restriction upon calling densities. Calling individuals may be as close as one inch apart (three or four individuals at times) or as far as fifteen or twenty feet.

In the Jefferson Parish area studies on calling pattern there were numerous small complexes similar to the one studied. An attempt was made to apply the same method to groups as was applied to individuals and thus determine if there were relations between them also. The results did not indicate any significant patterns.

Sex Recognition.—The phenomena of sex recognition are obscure. On several occasions it has been possible to detect a faint vibration when males were handled, similar to that found in *Bufo*, but a function in sex recognition is not indicated by observations of mating behavior.

There are very few errors in recognition and only in one instance (in the laboratory) has a male been found clasping or attempting to clasp another male. Both individuals in this one case had received heavy doses of pituitary gland. The males have never been observed to clasp inanimate objects as male *Bufo* do.

Initiation of amplexus has been observed in water in laboratory aquaria. The female floats in a position characteristic of the species, with hind legs widely spread. The male, appearing to move about at random, will, when behind her, swim into clasping position and work up her back until the normal amplexus can be achieved. This procedure is not believed to illustrate the normal courtship behavior.

The following excerpt is from field notes taken at the Jefferson Parish area, July 28th, 1950. These notes were taken at the small pool described above where the studies on calling pattern had just been made. The observer sat about two feet from the pool. To his left was individual *A* in calling position behind a floating board. Directly across the pool (about 20 inches wide at this point) was individual *B*. Directly in front of the observer on the near bank were three individuals called in the notes the "trio". They were about one inch apart in a triangular formation. They were partly hidden by some sticks and other debris and behind them were at least one, possibly two other frogs which were hidden completely. The notes read:

> "During 9:00 to 9:30 interval the moon becomes brighter as the sky clears. The inter-group intervals seem to get longer and the intra-group calling density may be less. Individual seen moving across middle of pool, perhaps middle one of trio which has gone—it dives to avoid light and a small individual simultaneously appears on land near third of trio—it moves past in front of and almost touching the latter, which calls with increased frequency. An individual appears again in the middle of the pool briefly and the new individual near the trio vanishes.
>
> "Three grunts seem to come from near B's position and an individual seen scrambling up the bank from that area—B does not react. Another individual seen about one inch from B. Two more grunts heard, but position not definite, one has a croaklike quality—location established for these sounds—a pair in amplexus on board near A.
>
> "Nine minutes later a large individual comes down bank near the trio. The latter (two and possibly three are still present) begin to call rapidly. "A" speeds up calling in response. All three of trio members in original position, the newcomer moves between them and disappears. Three minutes later a pair in amplexus appears in that area."

These notes illustrate the difficulty of observation. They also illustrate several points. One is that calling males respond to the approach of another individual, presumably a female, by increasing the calling rate, but do not move from position to attempt amplexus, although the individual may pass very close. There appears to be two possibilities : (1) the wandering, non-calling individuals are females, and some action on the part of the female other than appearance or nearness and movement must serve as a release mechanism for amplexus; (2) these individuals are actually males and their sex is recognized by other males without amplexus or other contact. Observations indicate that most of the wandering individuals in a chorus are females.

The grunts and croaks noted at the approximate time of occurrence of amplexus have also been observed in the laboratory when terraria were set up in such a way that the males could call normally and the females move to them.

Noble and Aronson (1942) found that female *Rana pipiens* were not receptive to amplexus until ovulation had taken place. In *Microhyla* ovulation may sometimes begin either just before or after amplexus has started. Dissection of a female taken in amplexus and preserved forty-five minutes later revealed that ovulation had just begun. Approximately twenty percent of the eggs had reached the oviduct. Examination of thirteen other females taken in amplexus showed ovulation still in process in six individuals and complete in seven. Two unmated females found moving about in the same chorus had nearly completed ovulation. Observations in both field and laboratory indicate that egg deposition took place in from one and one-half to two hours after amplexus began. Thus ovulation is rapid and the females become receptive to males at or before its commencement. This pattern of rapid ovulation and early receptiveness might be of value to the species in arid climates, permitting the animals to take advantage of sudden rains for breeding purposes.

Amplexus is semi-pectoral or axillary depending on size relationships. It may be inguinal in cases where the female is not receptive, or in cases where a male grasps a male. In amplexus, as observed in pituitary injected individuals in the laboratory, the male rests on top of the female, clasping her strongly in a semi-pectoral embrace. The arms of the male pass just behind those of the female. The thumbs dig into the pectoral area just posterior and ventral to the insertion of the female's forearms. The palms are turned out and forward. There is considerable size disparity and the snout of the male just reaches the line between the female's eyes. The back of the male is just above the water surface, the thighs are flexed, with the shanks pointing dorso-laterally and the feet laterally and anteriorly. The body of the female is at an angle of about forty-five degrees to the surface of the water. Her thighs are partially extended, the shanks point postero-laterally, the feet laterally and slightly anterior.

Egg Deposition.—At the beginning of amplexus the male and female cooperate in swimming movements. As the time for egg deposition nears, the male ceases swimming actions and cocks his hind legs tightly in against his body.

Egg deposition can be anticipated by noting certain features of the behavior of the clasping pair. About twenty minutes before the time of deposition the female becomes restless and moves about. The interval between initiation of this behavior and egg deposition was the same in the field on July 28 as in the laboratory. In the field the female traveled about one foot overland to a deeper pool and back again carrying the male on her back. At about this time there is a change in the swimming movements of the female. She now swims with very short, jerky, simultaneous movements of the hind limbs.

At the start of egg deposition the female may raise the hind limbs and rear part of the body to the surface of the water and bring the extended hind limbs forward until they are at right angles to the midline on the same plane as the water surface. This produces a swaybacked position. The feet are then moved in short forward strokes, moving the animal backward one to two inches. The strokes then shorten further and the legs move ventrally. (This preparatory stage was omitted in those individuals seen in the field, but was exhibited by two of three pairs in the laboratory.) The female then extends the legs tensely in a ventro-lateral position with the toes bent downward, bringing the cloaca upward above the water surface. As she does this the male slides forward so that his cloacal opening is about one-eight inch anterior to that of the female. He brings his feet together so that they curve around her buttocks on either side of the cloaca with the ankle joint at about the cloaca level. In this position the eggs appear to boil from the cloaca of the female. About thirty eggs are laid in each such ejaculation.

No information has been obtained on the duration of the clasp after egg deposition is complete. In the laboratory the clasping position apparently may be retained indefinitely if the pair are kept in the water. If they have an opportunity to leave the water, it may be broken relatively soon and exit from the water may possibly stimulate the male to release his grip.

Discussion.—Bragg (1950b) has asserted that two major breeding patterns are discernible in North American anurans. These he designated as mesic and xeric. The mesic pattern, as he has defined it, is basically cyclic, little influenced by rainfall, and with physiological control vested in hormonal and other internal factors. In comparison, he defined the xeric pattern as non-cyclic, with the breeding independent of internal control to a great extent and initiated by rainfall. He recognized also, a third, or intermediate pattern. *Microhyla c. carolinensis* was considered by him to be a mesic form and *Microhyla c. olivacea* a xeric form. His basis for this separation was the belief that the breeding of *M. c. carolinensis* was more restricted as to season and was little influenced by rainfall. Unfortunately he had not had full opportunity to observe this form and make comparison. The cyclic gonadal pattern of *carolinensis* is certainly influenced to some degree by rainfall, particularly as to initiation of the breeding season. There is some breeding activity independent of rainfall but this activity is much reduced. The gonadal cycle appears to be adaptable to either a mesic or xeric behavior pattern and an existing pattern might be merely an effect of the local climate.

One factor in the breeding behavior which Bragg postulated for forms showing xeric behavior patterns is supported by material gathered here. This is the possibility that ovulation may not actually begin until after amplexus has started and the fact that it is quite rapid.

The social aspects of the reproductive behavior are so little studied that there can be little evaluation of them at this time. The significance to be given to the difference and similarities between the actions of egg deposition in *Microhyla* and in *Rana pipiens* as described by Noble and Aronson (1942) is also uncertain.

Reproductive Potential

The sample taken from the ditch at Angie in June was used for study of the reproductive potential of the species. In utilizing these data it is assumed that the sample is representative of the breeding females of the whole population as regards to both composition and the condition of unspent females. This means that it is also assumed that while males exhibit more movement than females, mature females of all size groups, in the equivalent physiological condition, move equally. (Although the breeding season had been underway for some time, no spent females were found in the sample.)

A total ovarian count was made upon each of 66 females with mature oocytes. The compliment of eggs per female ranged from 152 to 1,089. The mean number of eggs was 510 and the total was 33,657. An analyses by year classes shows that the three-year-olds bear the burden of the reproduction (Table 5).

The fact that so few two-year old individuals are recorded is difficult to explain. In view of the indications noted below that some of these may not breed until their third year, it seems possible also that many may not breed until late in the season. Periodic samples at the breeding ponds should prove useful in answering this question.

Growth and Development

The development of the embryo and the tadpole is not reported in this paper, although they have received some study. Deckert (1914) and Ryder (1891) have published short notes. Wright (1932) reported considerable material, and Orton (1946) discussed the growth and color pattern of the tadpole.

Both embryonic development and growth of the tadpole through metamorphosis are rapid, as would be expected in a species that often breeds in temporary rain pools.

Snout-vent measurements were made on 633 frogs. Preserved, freshly killed, and live individuals were used. The percentage of error in measurement of each type was calculated. This percentage was small in all methods (preserved, 1.2; freshly-killed 3.8; alive, 2.1). Measurements of a test series of individuals measured in all three conditions were used to determine if corrective factors were necessary for fresh and live material. The corrective factor of 0.989 indicated

TABLE 5.
REPRODUCTIVE POTENTIAL: ANALYSIS BY YEAR CLASSES

Age (yrs.)	Size (mm)	No. of Specimens	Mean No. of Eggs	Total Eggs	Percent of Total Production
2	23.0 — 24.4	6	377.8	2,267	6.7
3	24.5 — 26.9	44	466.3	20,516	60.9
4	27.0 — —	16	680.8	10,894	32.4

for fresh material as contrasted with preserved material is small enough so that in this study it may be ignored. It was found necessary to apply a corrective factor of 0.914 to measurements of live individuals.

The samples were found to be affected by the collecting situations studied. One was forced movement, caused by rising flood waters in the Bonnet Carré Spillway. The second was a combination of general feeding and breeding movement bringing the animals into traps at the Camp Plauche area and the third was apparently primarily breeding movement which caused the frogs to become trapped in a pipeline ditch near Angie, Louisiana.

Snout-vent frequencies were plotted for each month (fig. 4). A class interval of 0.7 mm seemed best suited for both large and small samples.

Study of these frequency distributions show that no sample is fully representative of the population. Year classes can be delineated only approximately and an accurate growth rate cannot be calculated. Only a rough outline of growth can be given.

Individuals transform at about 10.0 mm from May until October. Since some may thus have four months start on others of the same year class in their growth, the difficulties of delimiting the year classes are not surprising. The effect of this seems to be diminished somewhat in older individuals. A tentative separation into year groups for the months of May and June shows a progressively smaller range per group as size increases. It appears that most individuals are 13.0 to 19.0 mm in their first spring following transformation, 21.0 to 24.0 mm the second spring, 24.5 to 26.9 mm the third spring, and from 27 to 35 mm in succeeding years. This arrangement is substantiated by attainment of sexual maturity in the 21 to 24 mm size class. These estimates are found to coincide roughly with Wright's (1932) estimates of the early growth of the species in the Okefinokee. He found the transformation size to range from 8.5 to 12.0 mm with a mean of 10.8 mm. He then gave the year classes for the following spring as 15.0 to 20.0 mm for the first year group. However, he thinks they are 20 to 25 or 27 mm in the second spring and 27 to 36 mm in the third. The break at 26 to 27 mm in the curves given here is slight, but it occurs often enough to be significant. When the June samples are plotted with an 0.5 mm interval this break is greatly emphasized. Wright's classification would indicate that growth of maximum sized individuals is 8 mm from transformation until the first spring, 7 mm in the second year, and 9 mm in the third. This maintenance of an extremely rapid growth seems improbable in the light of the general understanding of the growth of reptiles and amphibians.

The size groups are demonstrated best if we combine the samples from October through February. Four classes indicate a maximum life span of five or more years (fig. 4).

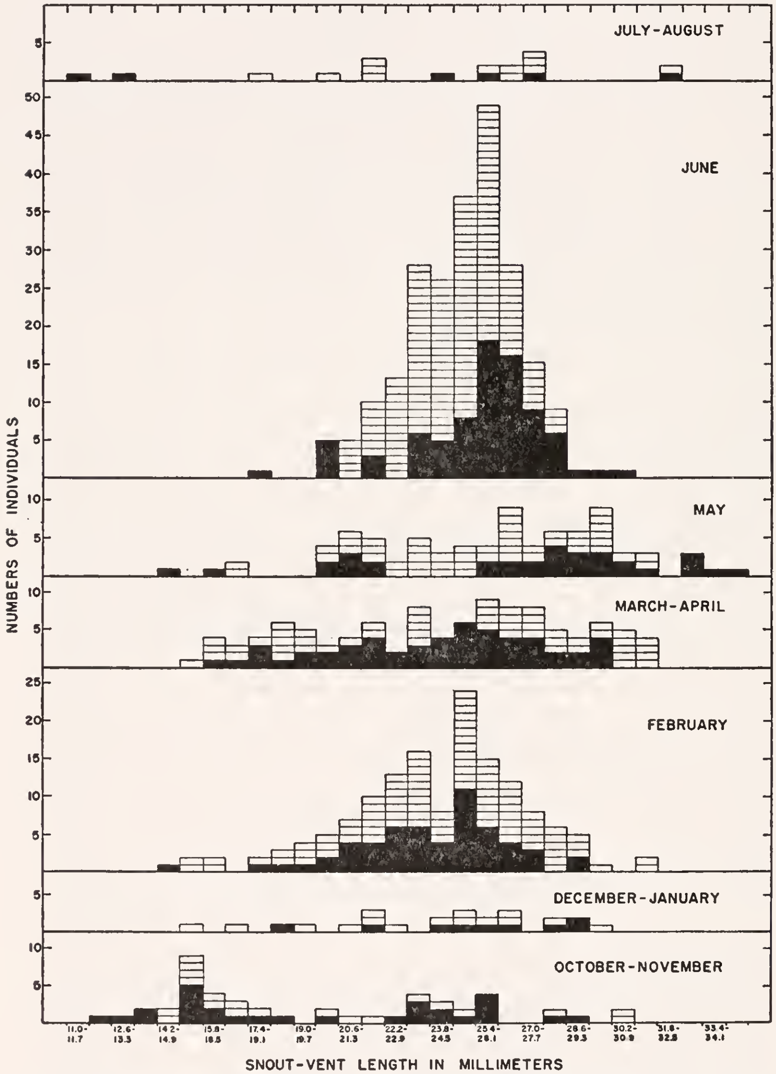

Figure 4. Size frequencies in population samples, sexes combined. Open rectangles = males; solid rectangles = females.

Individuals attaining sexual maturity by their second spring range in size from 21 to 24 mm. Several criteria are used to indicate the attainment of maturity. The criteria used for males are (1) the appearance of secondary sex characters, (2) the presence of mature spermatozoa, as indicated by smears of testes of both fresh and preserved individuals, and (3) the development of the gonads as indicated by the study of histological sections. The secondary sex characters of the female are largely negative and the examination of the ovary is the only criterion.

A distinction must be made between the determination of the smallest sexually mature size class at the breeding season and the actual time at which the individuals take up the adult gonadal cycle. A discussion of the relative importance of the two dates is academic, for both are important.

Analysis of the June sample shows that the secondary sex characters first develop in males of 21.0 to 22.9 mm snout-vent length. The blackening of the throat seems to occur first and the tubercles to appear at a slightly larger size (from 21.5 to 23.9 mm). Mature spermatozoa were found in smears of testes of individuals 21.0 mm or larger, but were sometimes absent in individuals of 23.0 to 23.4 mm.

Smears of testes taken throughout the year show that mature and active spermatozoa often are present before there is any external indication of sex. Spermatozoa have been found in smears of testes of individuals as small as 18.4 mm. In histological sections a very few spermatozoa were found in the testes of an individual measuring only 15.5 mm in snout-vent length. This individual was taken in October, showing that spermatozoa may be present during the year in which the individual transformed, a full year and a half before the first breeding activity is expected. Further study of sections of testes of immature individuals allows an estimation of the time at which the condition of the gonad approximates and begins to follow the seasonal cycle of adult individuals. The immature testis is characterized by very small tubules, each containing only a few cells. The arrangement of the cells in the tubules is very loose. They are not in clumps as found in mature testes. These characteristics are retained through the first winter and then are gradually lost so that by the middle or end of the second summer the mature condition is approximated and the annual cycle begins.

Study of June females demonstrates that pigmented eggs first occur at 23.0 mm but are lacking in some individuals up to 24.5 mm. In all monthly samples, comparison of the minimum size in which white oocytes are found and the maximum size in which only clear ones are found shows a considerable overlap. These facts indicate that some females may not deposit eggs until their third year.

There are insufficient specimens in the critical early fall period to allow an estimation of the time at which the young females develop the adult cyclic pattern.

Sexual dimorphism in size has been remarked upon by Wright

TABLE 6.
SEXUAL DIMORPHISM IN SIZE

Size Groups (mm)	Males No.	Males Percent of Total males	Females No.	Females Percent of Total females
22.2 — 23.7	62	16.6	27	10.4
23.8 — 25.3	75	20.1	44	16.9
25.4 — 26.9	85	22.8	64	24.6
27.0 — 28.5	38	10.2	32	12.3
28.6 — 30.1	19	5.1	19	7.3
30.2 — 31.7	15	4.0	4	1.5
31.8 — —	1	.3	6	2.3

(1932). This is manifest in the larger maximum size of females and by the relative percentages of male and female individuals in the larger size groups. The maximum size for males is 32.2 mm and the maximum for females 34.3 mm. A relatively larger percentage of females in the samples fall in the larger size groups (Table 6). This dimorphism is slight.

Shedding was observed in six females used in breeding experiments. Shed skins are found occasionally in the stomachs and may be identified by the outlines of toes or fingers (Table 3).

POPULATIONS

Some information about populations can be derived from the larger samples. The trapping data from Camp Plauche also gives a little information about the density and composition of a population.

In the discussion of movement, reference was made to the sex ratios found in various population samples. The relatively large percentage of males was considered to be due, in part at least, to greater movement by that sex. In support of this it was pointed out that samples collected by hand showed a less unbalanced relation.

The sex ratios are more nearly even in the smaller size classes. This could be attributed to an actual difference in the ratios in juvenile and adult populations or explained on the assumption that the relatively greater movement of the males does not express itself fully in immature individuals. It is impossible to tell whether either one, or both, of these factors contribute to the observed result.

The continuous catch of unmarked individuals at Camp Plauche indicates a large population, but since most recoveries were not made in the traps it is not possible to attempt a population estimate.

Interesting variations in size composition are seen in the different samples (fig. 4). A large percentage of immature individuals occurred only in the October-November and March-April samples. Both of these were hand collected samples. The proportion of immature individuals is considerably higher in the October-November sample than in any other. This is to be expected at the end of the breeding season but an additional significance is also suggested. This sample was collected in the cypress swamps of the Sarpy Wildlife Refuge and corroborates field observations which indicate a vastly different size composition in these swamp populations as compared with those from the abandoned fields and levees. Further comparative samples of both breeding and non-breeding populations must be taken before this situation can be interpreted. Some observations in the field suggest an ecological segregation of different size groups in the swamps.

The most abnormal of the samples from the point of view of composition is the June sample. This is found to be almost entirely composed of mature individuals and indicates a great difference in the movement of adult and juvenile individuals during the breeding season, rather than a true picture of the population.

Conclusions

1. *Microhyla carolinensis carolinensis* is highly adaptable in regard to habitat requirement. Moisture seems to be the most critical habitat requirement.

2. There is considerable nocturnal movement by all individuals. The males wander more than the females and juveniles.

3. A wide range of small organisms is taken as food. The major food items are ants, beetles, and termites.

4. There is little evidence of predation.

5. The reproductive behavior is cyclic. The gonadal cycle is, however, very different from that of *Rana pipiens* in the northeast. This difference seems to be correlated with seasonal activity as controlled by temperature.

6. Very little modification of the gonadal cycle, if any, would be required to permit breeding activity along the pattern which Bragg (1950a) designated as non-cyclic. The determining factor is probably environmental.

7. The breeding behavior is complex and is in need of further study. The phenomena of sex recognition found in other North American anurans were not observed in this subspecies.

8. The calling patterns of small groups indicate some sort of complex social relationship between calling individuals.

9. Population composition varies with the habitat. Further studies in the population dynamics of this subspecies are needed.

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