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UMI
A STUDY OF THE SEASONAL DISTRIBUTION

OF ANOPHELES IN HOUSTON, TEXAS

Thesis

Presented to the Graduate Committee of The
William Marsh Rice Institute,
in partial fulfillment of the require-
ments for the degree of Doctor of
Philosophy

By

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A Study of the Seasonal Distribution of Anopheles
in Houston, Texas

by

E. C. Matthes

Introduction

The problem of malaria in the South, and in other parts of the United States as well, has demanded a considerable amount of research. The greater part of this work has been concerned with the control of the disease by the eradication of the anopheline mosquitoes, and much of such work recently has dealt with the ecological aspects of the problem. Such factors as temperature, humidity, larval food, plant associates, hydrogen-ion concentration of the water of larval breeding places, and many others have been considered. The effects of these various factors on the life and habits of the mosquitoes usually differ for the various species and often differ for a single species in different parts of its range. Since the life of an animal is never entirely governed by a single factor, but by a complex combination of interrelated factors, each area studied offers to some extent a condition peculiar to the location.

In the following pages an attempt will be made, after the plotting of fluctuations in the seasonal abundance of the one common Anopheles of the region, Anopheles quadrimacu-
latus Say, to evaluate the various ecological factors involved in producing these fluctuations. It will be shown that the influence of some of these factors is of very local nature; that is, certain factors may operate in quite different manners and may be of decidedly different importance in a locality where a bayou is the main source of emerging mosquitoes, on the one hand, and in a nearby locality where flooded rice fields are the main source.

This survey covered the period of time from March 1932 to January 1934. The area studied included the southern part of the city of Houston and immediate vicinity outside the city limits, and also a rice farming district 15 miles to the west of the city. The species of Anopheles encountered and studied in these areas were Anopheles quadrimaculatus, Anopheles crucians Wiedmann, Anopheles punctipennis Say, and Anopheles pseudopunctipennis Theobald. But, whereas 4 species of Anopheles were found to occur in Houston, only A. quadrimaculatus was found in sufficient numbers to make possible any comprehensive study of the factors influencing fluctuations.

Breeding Places

Howard, Dyar, and Knab (1917), in regard to A. quadrimaculatus, state that "The larvae occur in natural collections of water of a more or less permanent nature. They are more addicted to permanent stagnant water, such as the edges of sluggish rivers and marshes containing algae, less to springs
and running water, and do not occur in temporary ground pools filled by rains."
Bradley (1924) in his studies of Anopheles in the vicinity of Mound, Louisiana, states:
"It is a well known fact that during warm weather and given suitable protection, Anopheles will breed in this locality in almost any situation where water accumulates and remains for a week or ten days at a time. We have found them here in water in tin cans, rain barrels, watering troughs, open wells, cisterns, and pools and puddles of all kinds, but, while these places may produce goodly numbers of Anopheles, they are insignificant when compared to the numbers produced by the natural breeding places of these insects, which are very abundant in this locality and which may be classified as lakes, bayous, and sloughs."

In Houston, Anopheles breeding places are somewhat similar to those of Mound, Louisiana, as described by Bradley. Although A. quadrimaculatus is usually found in rather clean water, supporting considerable algal or other plant growth, it is often found breeding in abundance in association with Theobaldia incornata Williston in small stagnant pools containing very foul water. These observations show that the breeding of this species is not limited greatly by the condition of the breeding place, at least not to the extent to which many species of mosquitoes are limited by the conditions of their breeding areas.

In studying the breeding places of Anopheles in the southern part of the city of Houston, a single important area
was discovered, a spring-fed bayou (Bray's Bayou). The character of this stream is rather variable. At some places there are wide pools that may have a depth of 5 or 6 feet. At other places there are narrow rapids usually varying from 2 to 3 feet in depth. But on a whole the stream is shallow, varying from about 1 to 2 feet in depth, and having an approximate average width of about 15 feet. The bottom is composed mostly of mud and clay, but at many places the stream passes over rock or gravel beds. In the shallow areas, except above the rock and gravel beds, Chara growth is often very extensive and forms at such places a rather dense entanglement extending from the mud bottom to the surface of the water. During the height of mosquito breeding a single dip into the water above these beds, with a one-liter beaker, yields as many as 50 larvae.

During dry warm periods of the year the rather steep banks of the stream become grassy. At the water's edge the overhanging grass and the vegetation growing in the water, including the Chara, protect the developing mosquito larvae from top-feeding minnows and other fishes. Furthermore, along almost the entire course of the stream, reeds extend out into the water and afford some protection for larvae, especially where these plants serve as anchorage places for drifting algal material.

Photographs of the bayou are shown on page 5.

In the general locality of this bayou Anopheles larvae
Bray's Bayou, a breeding area of Anopheles larvae
are also found in forest and meadow puddles, but as a rule such places are of little importance except during periods of prolonged rainfall, at which time the pools persist for a sufficient length of time to permit successful mosquito breeding. It is probable that, even during these periods, the mosquitoes emerging from such breeding places never equal more than 25 per cent. of the total population that would be expected to emerge from the bayou at the same period or season providing they are amply protected from the fish.

In a second area where several thousand acres of rice was being grown, adult anopheles were found to be extremely abundant. These fields were kept flooded with 12 to 18 inches of water from May to about the first of September. Besides the rice, these fields also supported a considerable amount of various water grasses; and Chara was not uncommon. Water seepage from these farms alone, where adequate drainage did not exist, was sufficient to provide rather extensive breeding grounds for mosquitoes. Larvae in the fields, and in the puddles and ditches of seepage water outside the fields, were never as concentrated as they were above the Chara beds of the bayou, but on account of the area covered by the rice fields, larvae were much more abundant there than in the bayou.

Collecting Places

The system used in arriving at the abundance of adult mosquitoes was that of counting their numbers in a definite
area. This count was made at about noon on Friday of each week. These weekly data on the number of mosquitoes found in daytime resting-places give an idea of their relative abundance at various times or seasons of the year. During the entire period of the survey, data were kept on the number of mosquitoes found under a small concrete bridge located near the city limits of the southern part of Houston. This bridge spans a small gully and is within 300 yards of Bray’s Bayou, which has been referred to above as being the most important breeding place of Anopheles mosquitoes in the area studied. The bridge is located in a timbered section; consequently, the emergence of adult mosquitoes from the afore-mentioned forest pools contributes to the mosquito population found under the bridge. A small zoological garden is located within 800 yards of this collecting place and probably serves as a good source of food supply for adult female mosquitoes.

Barber and Hayne (1924) state that "Nocturnal dispersion of Anopheles quadrimaculatus from a resting place is nearly complete by the end of six days." Davis (1926) working with Brazilian anophelines states in his summary: "It was found in a series of experiments that only 3.1 per cent. of the anopheline mosquitoes resting in certain houses on given days in warm weather were present in the same houses on the days immediately following." Such observations, and others, indicate that the individual population of a resting place changes rather rapidly as a result of crepuscular and nocturnal acti-
vity. Realizing this condition as being important in making seasonal adult abundance studies, the writer studied a number of different resting places during the collecting periods in order to get some idea of the general abundance of mosquitoes and to use this information as a check against the data of the single collecting place being used. It was generally observed that the variations in mosquito abundance at one collecting place corresponds rather closely with the abundance of mosquitoes found elsewhere in the area. When mosquitoes are numerous under the bridge they are also numerous under other bridges, in barns, in natural cavities along the banks of the bayou, etc., and when there is a decrease of mosquitoes at one place there is usually a corresponding decrease at other places in the locality. A specific check was made, which supports the accuracy of the above observations, by counting the mosquitoes found under another bridge, spanning Bray’s Bayou, but located about one mile from the one already described. This count represented the total Anopheles population under the bridge.

Abundance of *A. quadrimaculatus* was also studied by means of weekly counts of the number of mosquitoes in marked areas on the sides, in the corners, and on the ceiling of a barn located in the rice-farming district. In all there were 30 marked areas in the barn, and they represent a total approximate space of 2,000 square inches. This barn is located within 100 yards of a rice field, and near a small permanent pond.
About 10 farm animals (horses, hogs, cows, etc.) served as a source of food for the mosquitoes. Usually about 90 per cent. of the females had partaken of blood. Observations were made at this barn over a period of 12 weeks, from July to October 1938.

Seasonal Abundance of Species

*Anopheles crucians* was found throughout the year, but was more abundant during the winter and spring months. According to rather meager collection data, its abundance was found to be about 5 to 6 per cent. of that of *A. quadrimaculatus* during the summer months; but during February, March, and the first part of April, it was actually more abundant than *quadrimaculatus* and much more abundant than either of the other two species of Anopheles found in this area. According to Matheson (1929), "This species ranges from southern New York along the coastal region to Florida, the Southern States, Mexico and Cuba."

Although rather extensive mosquito collecting was carried on during the summers of both 1932 and 1933, no specimens of *Anopheles punctipennis* were caught. This species made its appearance in October each year, reached an abundance peak during the winter months, and began to disappear during the spring months. Some idea of the relative abundance of the three species of Anopheles during early spring may be obtained by examining the data of a representative day, March 23, 1934. On that day collections made beneath numerous bridges in the
vicinity of Houston yielded 130 A. crucians, 5 A. punctipennis, and 2 A. quadrimaculatus. Larvae of these three species were found in about the same proportions as were the adults. Dyar (1928) gives the distribution of A. punctipennis as "Southern Canada, United States, Mexican tableland, Venezuela." There is some doubt about the correct identification of the Venezuela specimen. Matheson and Shannon (1922), discussing the abundance of this species in New York, state, "This is undoubt- edly the dominant anopheline in the Ithaca region." Boyd and Weathersbee (1929), studying anophelines in coastal North Carolina from November 1, 1927 to May 1, 1928, state, "In this locality three species of anophelines, A. quadrimaculatus, A. punctipennis and A. crucians are found. A. quadrimaculatus are found. A. quadrimaculatus and A. punctipennis are far more numerous than A. crucians, which is infrequent at all times." These data would indicate that A. punctipennis has an eastern distribution in the United States and that Houston is probably located somewhere near the southern limits of its range. The statement by Boyd and Weathersbee in conjunction with Houston data would also indicate a more southern range for crucians than North Carolina.

Anopheles pseudopunctipennis is much less abundant than the other three species of Anopheles. In all only 5 female specimens were collected, and these data and other records indicate that the species occurs in the Houston area from November to March. This species ranges from South America
through Mexico into Southwestern United States (Matheson 1929). It is a common species in the Rio Grande Valley of Texas. Houston seems to be located a bit too far to the north for the species to occur abundantly in this location, and it is difficult to explain why it should have been found only in the winter months.

The range of *Anopheles quadrimaculatus* is from Southern Canada through the United States into Mexico and from the Rocky Mountains east to the Atlantic Sea Board. Houston is located within its active breeding range, but the abundance of this species begins to decrease in the southern part of the State and in the northern part of Mexico. As has already been stated, this is the important species of *Anopheles* occurring in Houston, and aside from the data given above, this paper is concerned entirely with the seasonal distribution of this species.

In Graph I, curve B represents the weekly count of *A. quadrimaculatus* found under the bridge first described above, referred to in the graph as Bridge 1. Adults made their appearance during April in both the years 1932 and 1933. But, the time of emergence of adults is probably rather variable, depending on various meteorological conditions, especially temperature. The increase in the abundance of mosquitoes during spring and the first part of summer, as a rule, is rather slow, but again is variable, depending on meteorological conditions. During the latter part of summer a very
rapid increase in the number of mosquitoes usually occurs, and the peak of abundance is reached during September.

Boyd (1927) in studying the seasonal distribution of *A. quadrimaculatus* in Georgia makes the following statement:

"The charts show that after the initial appearance of adults in April or May which occurs in rather low numbers in most years, a marked rise in numbers takes place in June or July, which is maintained with considerable irregularity until October, when, before the onset of frost, a rapid decline takes place." He further states that he believes that the irregularities in his incidence curve afford a clue to the number of broods, or generations, of *A. quadrimaculatus* occurring each year. No such indication of regular generations are found in Houston. The major irregularities of the curve seem to be best explained by the effect of rainfall and temperature, as will be shown in detail below.

**Effects of Rainfall**

In the southern part of the city of Houston rainy periods, instead of increasing the number of important breeding places, render inefficient the chief breeding source (Bray's Bayou) by causing the water to rise above the vegetation growing in it and at its edges. Under such conditions the large numbers of top feeding minnows (*Gambusia affinis* Baird and Girard) and other fishes rid the stream in a definite and rapid manner of the mosquito larvae; accordingly, there occurs a subsequent, and a more or less immediate, drop in adult abundance. In
the forest and meadow puddles left standing after heavy and continued rainfall, Anopheles larvae may be found, but usually only in limited numbers.

To illustrate how rainfall may affect mosquito abundance in an opposite manner, Bradley (1932), referring particularly to lakes near Mound, Louisiana, says:

"The summer rainfall in this region is not usually sufficient to keep the surface-water areas from showing a steady decrease as the season advances. This gradual decrease in water surface tends to keep the margins of the water areas more or less free from vegetation, which encroaches on the shore line in times of stationary or rising water, and to remove from the surface of the water large quantities of flotage which are blown to the margins by the wind. This is not re-floated, except in case of exceptionally heavy rains, until the high waters of the ensuing winter. (Fig. 1) The lowering of the water level also gradually leaves the tree and brush covered marginal areas, particularly those of the lakes, dry; and in the lakes the central parts of which are devoid of aquatic vegetation the production of Anopheles ceases."

An attempt has been made to show graphically the effect of rainfall on mosquito abundance. Refer to rainfall data and mosquito abundance curves in Graph I. The information regarding the amount of rainfall was secured from the United States Weather Bureau at Houston, and the graph shows the total weekly amount of rain in inches. A critical study of the graph shows that similar amounts of rainfall do not always
show the same effect on the mosquito abundance. Intense rainfall over a short period of time tends to flush the bayou while the same amount of rain over a longer period of time may have no noticeable effect on causing the water of the bayou to rise above the Chara beds. Also the effect of rainfall as represented in the graph is at times modified by seepage water from the rice farms that flows into the bayou. But it may be noted that most of the major decreases in mosquito incidence are preceded by heavy rainfall.

A study of the daily amount of rainfall will show the approximate amount of rain required definitely to change the water level of the bayou. After a period of five or more days of dry weather, a rainfall of one inch, falling within a period of 12 hours, is enough to cause a rise in the bayou sufficient to flush the mosquito larvae from their hiding places above the Chara beds. It was observed in one particular instance that an inch of rainfall falling on damp ground and distributed over a period of three days, but falling in heavy showers, also caused a definite rise in the bayou. But insufficient data and the complication of modifying factors make further statements regarding the effect of rainfall on the water level of the bayou impossible.

During the first week of August 1932 a heavy rain caused a rise in the bayou, whereupon the adult population began to decrease immediately. During the following two weeks, more rain and a Gulf storm further contributed to the decrease in
the number of adults. The bayou receded to normal water level during the middle and latter part of August; but this recession does not seem to be the sole reason for a sharp increase in adult incidence noted at this time. This increase is also partially explained by heavy breeding taking place in the forest pools left standing after the heavy rains during the first part of the month.

During the height of adult abundance in September most of these forest pools had dried; consequently, almost the sole source of emerging anopheline mosquitoes was the bayou, which was teeming with larvae in all stages of development. Rains during the latter part of the month caused approximately a ten-inch rise in the bayou, and at the same time there came a definite drop in temperature. As a result of these conditions, a very radical decrease in adult incidence was observed.

An increased temperature and a return to normal conditions of the breeding area appear to account for the abundance peak attained during the middle of October; but rainfall or change of water level of the bayou does not explain the decrease during the last part of October. Here, temperature probably played the important part.

At the beginning of the breeding season in 1933, conditions were ideal for the developing mosquito larvae. The bayou was low and Chara beds were very extensive. This is probably the reason for a more rapid increase in the numbers of adults in April 1933 as compared with the same period in 1932. The decrease of adult population during the first part
of May 1933 again seems to be the indirect result of rain, that is, the flushing of larvae from the Chara beds and their destruction by Gambusia.

During June and the greater part of July there was some rain, but not a large amount at any one time. But the bayou remained relatively high during this period (at least a few inches above the Chara beds). This condition is probably explained by water seepage from rice farms along the bayou about 20 miles to the west of Houston. During the latter part of July there was heavy rainfall and the bayou was very high, but it receded rapidly and contributed to the definite increase of mosquitoes during the first part of August. The small drop in the curve during the middle of August is also explained by rain. During the last of this month and the first of September there was considerable rain. This rain water, along with water drained from rice farms into the bayou, caused the water level in the bayou to rise well above the Chara beds. This probably is the cause of the decrease in mosquitoes noted at this time. In the same period of time in 1932, it will be noticed that there was a small decrease in mosquitoes during the second week of September, and the cause here also was probably partially due to drainage of water from rice farms into the bayou.

During the peak of abundance in September 1933, the bayou had large beds of Chara and these beds served as protection to a large number of Anopheles larvae. Heavy rains explain the drop in the curve during the latter part of September and
the first part of October. The peak during the middle of October was favored by extensive Chara beds in the bayou. Although there was some rain during the last part of October, temperature probably also played a part in the decrease of mosquitoes at this time.

Curve C as shown in Graph I represents the number of mosquitoes counted under the other bridge that has been described, and is referred to in the graph as Bridge 2. In this curve a unit represents 10 mosquitoes as compared to 1 mosquito per unit in curve B. Although the two bridges serving as collecting places are separated by a distance of about one mile, the main source of emerging adult mosquitoes is the same bayou. Therefore, other factors being equal, both curves B and C should be very much alike. The graph shows this to be true.

Observations on the abundance of mosquitoes on the rice farm were begun during the last part of July 1933 and extended to about the middle of October of the same year. Even at the beginning of observations, mosquitoes were very abundant. It is unfortunate, therefore, that the rice farms were not discovered earlier in the summer, so that the study of the mosquito abundance in this locality would have been more nearly complete.

Curve D (Graph I) represents the count of mosquitoes in the barn on the rice farm. It will be observed that there was a rather constant and rapid increase in abundance during the month of August 1933. The peak during the last part of the
month represents a count of 4,166 mosquitoes, found in a space estimated at about 2,000 square inches. In this curve a unit represents 10 mosquitoes as compared to 1 mosquito per unit in curve B. The radical drop in this curve during the first part of September is explained by the destruction of mosquito breeding places by the drainage of the rice fields in preparation for harvesting. Obviously rainfall did not affect mosquito production on the rice farm as it did in the bayou. There was no flushing of the fields, or at least not to the extent it destroyed the shelter afforded by various plants to the mosquito larvae and thus allowed Gambusia to eat them. During the latter part of August 1933 when rains caused a decrease in mosquito abundance in the neighborhood of Bray's Bayou, due to a rise in the water of the stream, the mosquito population of the rice farm continued to increase with remarkable steadiness.

Temperature and Humidity

At various places in the above discussion reference has been made to temperature as being a governing factor in mosquito abundance. Boyd (1929) states, "The temperature of the water appears to exert an important influence in determining the distribution of breeding of quadrivulatus. Breeding does not become widespread until available places have mean temperatures of 21° C. (70° F.) or higher, and declines as the temperature goes below this limit in the fall. In addition to the warmth of the water, a minimal diurnal range of varia-
tion appears to be a requirement." Mayne (1926) regarding the effect of temperature and humidity on *A. quadrimaculatus* under laboratory conditions makes the following statements:

"It has been found by the writer that eggs may be laid at 55° to 62° F., but not laid at 40° to 54° F.; that hatching takes place at temperatures of 66° to 70° F., but not at 58° to 59° F. There is no doubt that oviposition and hatching take place within well defined limits during the inactive season."

The writer has some evidence that seems to indicate that the eggs of *A. quadrimaculatus* will hatch at a lower temperature than that given by Mayne. In a series of two experiments a number of fed female *A. quadrimaculatus*, field collected specimens, were placed in a refrigerator and kept at 11° to 14° C. These mosquitoes were kept above water in cotton plugged vials, and the temperature as observed was that of the water. In the first experiment all of 12 selected fed mosquitoes laid eggs in from 60 to 330 hours, and in every case these eggs hatched after a period varying from 178 to 216 hours, averaging about 208 hours. In the second experiment all of 38 mosquitoes laid eggs and the average period of incubation was 198 hours. At the opposite extreme of temperature eggs were observed to hatch at 35° to 37° C. within 24 to 30 hours, but at 37° C. many of the eggs died.

The temperature curve (curve A) as given in Graph I represents weekly averages of two-hour readings. The read-
ings were made from the recording sheets of a standard thermo-
graph. While it is true that the temperature of the water of
the breeding places undoubtedly is the important factor rather
than air temperature, I believe that air temperature consti-
tutes a safe guide to temperature effects, at least in the
bayou, since such readings of water temperature as were taken
showed a fluctuation of only two or three degrees Centigrade
from that of the air during the breeding season. These read-
ings were taken of surface water in the parts of the stream
actually serving as breeding grounds. In deeper parts of the
bayou and in stagnant shallows exposed to the sun the tempera-
ture of the water would undoubtedly be considerably lower or
higher respectively than the prevailing shade temperature.

It is observed that when the temperature curve drops
below 20° C., regardless of the condition of the bayou, the
number of adults decreases rapidly, and that at about 22° to
23° C. the adult incidence definitely increases providing
suitable breeding places are available.

The breeding season of the mosquitoes on the rice farm
is limited by the length of time that irrigation takes place,
and this period is so situated, extending from May to about
the first of September, that the effect of low temperature
cannot be studied. There may possibly be some effect from
high temperature during early summer, when the rice is still
small, and does not furnish enough shade to keep the shallow
water from becoming hot, but rice farm observations were
began too late for the writer to notice this effect if it did exist. During the latter part of the summer the shade furnished by the rice is sufficient to keep the water cool, and only along the bar pits and other unshaded places in the fields does the sunshine heat the water to a high temperature.

The independent effect of temperature is usually inadequate to explain the seasonal prevalence of an insect. The modifying influences of other factors, especially humidity, must be considered. Freeborn (1932) showed by laboratory experimentation the interrelationship between temperature and humidity when considering the longevity of *Anopheles maculipennis* Meigen. Mayne (1930) has conducted similar experiments with three Indian Anopheles. This work and a large amount of other research indicate that saturation deficiency of the atmosphere is an important factor in the life of an insect, and that the importance of this factor varies with different species of insects in accordance with their ability to resist desiccation.

To measure the drying power of the atmosphere a white ball atrometer was used. This instrument was placed in the areas from which adult mosquito collections were made, that is, under the bridge (Bridge 1) and in the barn on the rice farm. Freeborn (1932) noted that, "When the drying power of the air reached 70 c. c. per day, the mosquito population dwindled and recovered only when the amount of water evaporated fell below 70 c.c." Such an evaporation rate was never
reached in Houston. The maximum average for any week during the survey was 34 c.c. per day.

In Graph II data are given to show the extreme fluctuation of atrometer readings both under a bridge (Bridge 1) and in the barn on the rice farm. These data are shown in condensed form. In the case of the bridge the data cover only the time of the most active breeding season of the Anopheles in 1932, the period of May to December. Atrometer data for the rice farm are shown for the period of July to October 1933. In either locality, if humidity (as indicated by the evaporation of water from the white ball atrometer) plays any part in the seasonal distribution of the mosquito in question, the graph, at least, does not show it. Under natural conditions existing in Houston, the humidity seems so high, or of such an optimum nature, that it is impossible to demonstrate any effect the humidity may have on the seasonal distribution of A. quadrimaculatus. Furthermore, any effect produced by a combination of humidity and temperature would likewise be impossible to demonstrate without laboratory experimentation.

Temperature and humidity requirements in the lives of many insects are met by the animals themselves in seeking out sheltered, moist retreats. The writer feels that this is characteristic of A. quadrimaculatus, especially in regard to the females found in the winter time. These females seem to prefer rather moist resting places, and in Houston show a
preference for storm sewers. In addition to being moist, these sewers have a temperature, during cold weather, 4° or 5° C. higher than that of the outside.

Hibernation

Literature pertaining to the hibernation of mosquitoes is rather limited. This statement is especially true of species occurring in temperate and tropical regions of the world. In Europe probably more work has been done on this phase of the insect's life than in any other part of the world, and most of this work pertains to A. maculipennis. This species has been divided into several races or varieties on the basis of structure, habits and general physiology. It is found that in areas where both races may occur together in the winter, one race may go into complete hibernation while the other may continue to feed, breed, and transmit malaria. A very adequate summary of the races of A. maculipennis is given by Hackett, Martini and Missirol (1932).

Barber, Komp, and Hayne (1924) state that "In the southern portions of Georgia, Alabama, and Louisiana larvae of Anopheles may be found in large numbers in winter. Development of ova, larvae, and pupae continues in winter as in summer, but more slowly." In Houston, observations show that A. quadrimaculatus also survives the winter in both adult and immature forms. Breeding may be suspended during cold spells but is continued during the warmer periods of winter, as is shown by the presence of larvae in all stages
of development. That such larvae do not represent quiescent or hibernating immature forms, and also that winter egg-laying occurs is proved by the presence of larvae, in various stages of development, in such newly established breeding habitats as must require the winter deposition of ova.

Females that may be considered to be in a partial stage of hibernation occur during the winter months. At this season mosquitoes seem to have a wider range of dispersion than during the summer since they are found abundantly in sewers on the Rice Institute campus, and in smaller numbers in many other places, where they are not observed at all during the height of their abundance in the summer. They are also usually of a darker color and seldom contain fresh blood. These mosquitoes probably live for a longer period of time in the winter than in the summer as a result of lower temperature, and the darker color may possibly be explained by a difference in metabolic activity. That these mosquitoes are not in a true state of hibernation is shown by their egg-laying when subjected to the higher temperatures of the laboratory.

Freeborn (1932) observed in California that A. maculipennis in the winter is decidedly darker than the summer mosquito of the same species. The above observations may very likely represent seasonal forms applicable to the seasonal forms of Lepidoptera as described and discussed by Uvarov (1931) in his treatise of the subject of seasonal variations in
form and coloration of insects.

Of such places as were found harboring *A. quadrimaculatus* in the winter, storm sewers seem the most important. It is estimated that as many as several thousand females could be found at a storm sewer outlet at almost any time during the winter, and that the mosquitoes seem to prefer the rather darkened part of these tunnels at distances not exceeding 100 yards from the opening. As has already been stated, these sewers always contain at least a slight amount of running or standing water, and during cold weather have temperature readings exceeding by 4 or 5 degrees Centigrade the temperature outside.

Just preceding the cold period in December of 1932, a considerable amount of mosquito breeding had taken place, and immediately afterwards fourth-instar *Anopheles* larvae were found in pools that had partially frozen over. Larvae collected at this time lived for a period of at least 10 days at a temperature of 4° C. Even in the presence of an abundance of food, such larvae showed no noticeable growth, but when subjected to a temperature ranging between 20° to 30° C. completed their metamorphosis. These observations show the probable importance of the larvae of *A. quadrimaculatus* in helping to carry the species through relatively severe winter weather.

Cold weather in Houston is usually of short duration, characterized by what is known as a "norther", and lasting usually not more than 2 or 3 days. During these cold spells
the activity of the larvae is slowed down; their growth and activity is resumed, however, with the advent of higher temperatures.

Hydrogen-Ion Concentration

Hydrogen-ion concentration of water in which Anophelles mosquitoes were breeding in Houston, and on the rice farm, as determined by the calorimetric method, showed a range of a pH of 6.7 found in some forest pools to a pH of 8.1 found above the Chara beds in the bayou. This range of variation produced no noticeable effect on the development of the mosquito larvae. Senior-White (1925) in a rather extensive study of the hydrogen-ion concentration of the water serving as breeding places for mosquitoes in Ceylon showed that the range of tolerance for Anopheles was rather wide. MacGregor (1929) states, "---under natural conditions, it indicates the favorable or unfavorable associations of chemical and biological factors in the breeding places, upon which the successful or unsuccessful development of the larvae depend." No study was made of such interrelated factors but it was noticed that the pH range observed seemed to have no adverse effect upon larval food supply. Desmids, diatoms, and filamentous algae were out abundant in the breeding areas through the year. Microscopic animal life was also usually abundant.

Number of Generations

As has already been stated, no definite broods or genera-
tions of *A. quadrimalatus* are noticeable. It does not seem very probable that definite broods would be observed in Houston, since the mosquitoes breed throughout the winter, and since there are apparently no meteorological or other physical factors that definitely mark a period of breeding or cessation of breeding. The fluctuations in the abundance of mosquitoes found under the two bridges have already been explained by the influence of rainfall and temperature. In addition, it is observed (Graph I) that mosquitoes on the rice farm increased in abundance over a period of six weeks, a period of time of sufficient length to give some indication of brood cycles if they had been present. Further, it will be noted that at the height of mosquito abundance on the rice farm there had been a great decrease in mosquito incidence under the bridges. If these variations in abundance were in any way dependent on a succession of broods, then it would be expected that the cycles would coincide for the two localities, since they are separated by a distance of only 15 miles.

**Natural Enemies**

A review of literature concerning observations on the natural enemies of mosquito larvae indicates that, although a large number of different animals feed on these immature insects, the top-feeding minnows are in most places the only forms of true economic importance. *Gambusia affinis* is the important minnow found in Texas. Its extremely inquisitive
nature of investigating any slight movement in the water, its voracious feeding habits, and its adaptability to various types of environment give this fish a place of prime importance in mosquito control work. These minnows are widely distributed in the vicinity of Houston, occurring in almost all of the natural waters of this area.

It is due to the feeding mainly of this fish that the bayou is cleared of mosquito larvae after heavy rains. In the rice fields this fish is also common, but is ineffective for mosquito control because of the abundance of plant life that protects the mosquito larvae from being fed on by the fish.

_Zygonectes notatus_ Rafinesque, a top-feeding minnow not widely distributed but found in Bray's Bayou, feeds readily on mosquito larvae under laboratory conditions. A single specimen ate 14 large larvae (_Theobaldia inornata_) in 3 hours. This minnow shows considerable interest in the wiggling mosquito larvae but lacks the great inquisitiveness of _Gambusia affinis_ and has less voracious feeding habits. Although adaptable to the environment of artificial receptacles this fish requires a more highly aerated water than does _Gambusia affinis._

Other fishes collected from the bayou, including _Boleosoma camurum_ Forbes, _Pomoxis annularis_ Rafinesque, and _Opeo-poecidus emiliae_ Hay, show a slight interest in mosquito larvae. Sun Perch and Kentucky Bass (_Micropterus pseudoplites_ Hubbs), also collected in Bray's Bayou, were found to eat large num-
bers of larvae in the laboratory, but these fish probably do more harm than good in mosquito control by feeding on Gambusia affinis.

Aside from fishes it was observed under laboratory conditions that the young of Siren, collected from permanent and rather stagnant pools, eat considerable numbers of mosquitoes. In one experiment 5 large mosquito larvae were eaten in a period of 18 hours by a small Siren. In another experiment 9 larvae were eaten in 1½ hours and the same Siren in the following 2 hours ate 6 more large-sized mosquito larvae.

Water tigers or larvae of Dytiscidae also eat mosquito larvae. In one experiment 12 larvae were placed in a beaker of water along with 8 larval Dytiscidae. Four of the mosquitoes were eaten during 12 hours and after a period of 24 hours 2 mosquito larvae still remained. This would indicate that water tigers are not efficient destroyers of the wigglers. This inefficiency is probably due to their inability to capture the wigglers, even in very close quarters. But, once a wiggler is caught, the water tiger lives up to its name and reputation. The wigglers are often killed by 3 or 4 thrusts of the mandibles of the water tiger and then discarded. At other times the soft inner parts of the larvae are eaten. In another observation, which displayed the greedy habits of the Dytiscid larvae, three of them were found to be fighting over a single mosquito larva. These water tigers have a habit of thoroughly twisting, folding, and biting their prey.
Adult Dytiscidae although they feed on mosquito larvae under laboratory conditions seem even less efficient in wiggler destruction than do the larval forms. Adult specimens of Gyrinidae, Hydrophilidae, and Nepidae eat but few mosquito larvae. It would seem that on the whole, therefore, that the animal enemies of mosquito larvae aside from fishes, at least as observed in Houston, are not important in acting as a check on their distribution and growth. It was often observed that pools of water supporting large numbers of these predators also supported many mosquito larvae.

Although the subject has not been investigated, important plant enemies of mosquito larvae are not observed to occur in the Houston area. Various species of Chara have often been reported and proved to be injurious to mosquito larvae; reference is made especially to the work of Matheson and Himman (1928), (1929), and (1931). So far as has been observed in Houston, the species of Chara growing in the bayou and on the rice farms do not have an adverse effect on the development of the Anopheles larvae.

Summary and Conclusions

In Houston 4 species of Anopheles mosquitoes are found to occur, A. quadririmaculatus, A. crucians, A. punctipennis, and A. pseudopunctipennis. But, whereas 4 species do occur, probably only A. quadririmaculatus occurs in sufficient abundance to be of economic importance. The active breeding season of this species is from April to October. The other
3 species occur principally or exclusively during the colder periods of the year.

Although larvae of *A. quadrivittatus* are found to occur in a wide variety of breeding places, in the southern part of the city of Houston, a bayou is by far the most important place. In a rice farming district near Houston, larvae are abundant and widespread in the fields, and an enormous adult population is observed.

In the region of the bayou, rainfall is responsible for most of the fluctuations in abundance of the Anopheles. Rainy periods instead of increasing the number of important breeding places, render inefficient the chief breeding source, a bayou, by causing the water to rise above the vegetation growing in it, and thus leaving the mosquito larvae unprotected from top-feeding minnows. Such destruction of larvae is almost immediately followed by a rapid decrease in adult abundance. Rains do not produce the flushing effect in the rice fields, and do not affect in any way, so far as could be observed, the abundance of adults.

It is observed that when the temperature curve drops below 20° C., regardless of the condition of breeding areas, the number of adults decreases rapidly, and that at about 22° to 23° C. the adult incidence definitely increases providing suitable breeding places are available.

Humidity conditions in Houston, as studied by atmometer readings play no part, or at least not any important part, in the seasonal distribution of *A. quadrivittatus*. 
This species breeds throughout the year, but in the winter, breeding activities are much reduced. A form of partial hibernation probably occurs in adult females during the winter months. Definite broods or generations of *A. quadrimaculatus* do not occur in Houston.

In this area, *Gambusia affinis* is the only natural enemy of mosquito larvae that can be considered of true economic importance.

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NOTE TO USERS

Oversize maps and charts are microfilmed in sections in the following manner:

LEFT TO RIGHT, TOP TO BOTTOM, WITH SMALL OVERLAPS

This reproduction is the best copy available.
Graph I. Showing weekly variations in temperature in a barn on a rice farm.
Ons in temperature; and adult Anopheles abundance unrice farm. Amount of rainfall represented by shaded ar
Phlebotomus abundance under two bridges, and in areas shaded by shaded areas.