# Ecology of *Anopheles gambiae* in Brazil*

Causey, O. Deane, L. M. Deane, M. P.
Laboratory of the Serviço de Malaria do Nordeste at Fortaleza, Brazil and
International Health Division of The Rockefeller Foundation.

An account of the introduction, dispersion and extermination of *Anopheles gambiae* in Brazil, and of the progress and termination of the severe malaria epidemics caused by this mosquito have been recorded in a comprehensive report of the Malaria Service of the Northeast, by Soper and Wilson (1943). Certain observations made by the personnel of the central laboratory of this service constitute the subject of the present paper. Unfortunately a number of problems in the ecology of *Anopheles gambiae* were never solved because of the necessary destruction of the experimental material. The successful prosecution of the control program during the early part of the year 1940 had made it seem possible that *Anopheles gambiae* might be eradicated from Brazil within a short time, and in view of this prospect it was deemed unwise to jeopardize the ultimate success of the campaign by permitting free breeding of this mosquito even in the laboratory and the experimental areas.

## LABORATORY STUDIES

In Africa at least two distinct varieties of *Anopheles gambiae* are recognized, one with light integument, breeding in fresh water, and another with darker integument breeding in brackish or salty water. Only the light colored variety, breeding in fresh water, has been encountered in Brazil. The numerous specimens examined here have shown remarkably little variation and correspond closely to the excellent description of the African species given by Evans (1938).

* Publicado originalmente em *The American Journal of Tropical Medicine*, v. 23, n. 1, p. 73 - 94, 1943.

## Laboratory colony of *Anopheles gambiae*

Several colonies of *Anopheles gambiae* were established in the laboratory to provide material for the study of problems in the biology of these mosquitoes, and for the testing of larvicides and insecticides. Questions raised by the field staff were frequently either answered directly by laboratory experiments, or given preliminary investigation which served to orient or elaborate the field experiments. Certain observations of these colonies may be of general interest.

The technique for breeding *Anopheles gambiae* in captivity in the tropics is simple if sufficient wild females can be obtained to supply an abundance of eggs. Gravid females captured from infested houses were kept in small screened cages 28 x 28 x 38 cm, in a screened room. Petri plates containing wet cellucotton covered with filter paper, were provided in each cage for oviposition. The eggs were usually kept on the filter paper 24 hours before being transferred to water. Upon hatching, the larvae were placed in shallow pans in an open air insectary fully exposed to the sun, and fed crushed bread crumbs, or yeast and corn starch. The pupae were transferred to small dishes of water in the screened cages and kept in the screened room which ranged in temperature from about 22.0° to 30.0°C. The adults were fed water and honey and offered pigeon or human blood at frequent intervals, usually daily. It was found from experience that egg production was more prolific when mosquitoes were grouped in large numbers in one cage, than when the same population was distributed in several cages. The success or failure of establishing a second generation stock in the laboratory may depend on this simple precaution.

## Hours at which females oviposit in the *Anopheles gambiae* colony

Anopheline mosquitoes rarely oviposit during the daylight hours, and the females in the *gambiae* colony were no exception to this rule. There are no data, however, to show at what time during the night oviposition does take place. For information on this point hourly observations and records were made of oviposition in the colony during five nights. The results indicate that *Anopheles gambiae* females in captivity may deposit eggs at any hour during the night, although five of the nine batches were deposited between 8 and 11 p.m. The recorded temperature at the time batches of eggs were found varied between 27.3° and 25.3°C while the range for the nights on which these observations were made was from 28° to 22°C.

## Number of eggs laid by individual *Anopheles gambiae* female

It is generally recognized that wild females are larger and stronger than laboratory bred specimens and that they live longer even in captivity. This greater vitality is also manifested by the greater number of eggs laid at each oviposition. Counts were made of eggs from individual females isolated in separate cages. Thirteen wild females laid an average of 192 eggs per batch while 17 laboratory bred specimens laid an average of 94 eggs. Many of the mosquitoes were dissected to determine the number of retained eggs. That retention is not a factor in the lower yield by the laboratory mosquitoes was shown by the records. Among the laboratory bred females, seven individuals were found to have retained one to ten eggs, while nine specimens showed complete oviposition. Only one of the dissected seven wild females contained eggs. This was a specimen that laid 95 eggs and retained 126 eggs.

## Percentage hatching of *Anopheles gambiae* egg

Observations on the hatching rate were made on a total of 14,587 eggs. These were obtained from wild females and from the various generations of laboratory bred mosquitoes. The eggs were

counted, and kept on wet filter paper for about 24 hours. This preliminary incubation insured almost immediate hatching on being placed in water, and a minimum loss from adherence to the sides of the vessel. The larvae were counted after 48 hours. The percentage hatched in different batches of eggs from any given generation showed far greater variation than the total difference in percentage between generations. The average hatching rate for all generations was 77.9 per cent.

## Duration of the larval and pupal stages of *Anopheles gambia*

The duration of each stage in development of *Anopheles gambiae* depends upon a number of factors such as the amount of sunshine, the temperature, the quantity and quality of food, and the amplitude of living space. Therefore when it was desired to know the probable shortest time required for *Anopheles gambiae* to pupate, favorable conditions were arranged for the incubation of the newly hatched larvae. Small bowls 10 cm in diameter, containing water and small amounts of yeast and starch, were prepared 15 to 18 hours in advance of use to provide satisfactory bacterial growth for the feeding larvae. Transfer to new media was made each day. Four larvae were considered ample population for these bowls. They were fully exposed to the sun throughout the day, and were kept in a closed room during the night. Under these circumstances one larva pupated in four days and 22 hours after hatching and the remainder pupated within the next three hours.

That this incubation period is not unusually short is indicated by experiments conducted in the field. In a region where the soil is sandy and rich in organic matter vertical boles were made in the ground 50 cm. in diameter and about 40 cm. in depth. The inflow of underground water caused the sides of the holes to cave in thereby increasing the diameter but decreasing the depth of water irregularly from 0 to 20 cm. This condition of variable depth is particularly favorable for *Anopheles gambiae* larvae which spend much time feeding on the bottom. The holes were immediately covered with screen cages and each seeded with 200 eggs which had been oviposited on the

previous day. Hatching probably began at once, but routine observations were commenced the following day when first stage larvae were seen. In one case three adult *Anopheles gambiae* were found in the cage on the sixth day. No food material was added to the water before or during the experiment.

Numerous observations indicate that when eggs, larvae or pupae are kept in the shade during development, duration of the respective stage is greatly prolonged, irrespective of food or amplitude of living space.

The effect of crowding on the duration of the larval stage was unwittingly demonstrated in many of the earlier experiments, before favorable conditions were recognized. A prolonged larval period, irregular pupation, and smaller yield of both pupae and adults were manifestations of unfavorable conditions. In a series of experiments involving more than 13,000 eggs, which were incubated in batches of 300 and 500 in small basins 28cm in diameter, the duration of the period between oviposition and pupation varied between seven and 27 days. Two thirds of the pupations occurred between the twelfth and eighteenth days. Further observations on the effect of crowding were made in an experiment using three pans of equal size, 30 cm. in diameter. These were planted with 50, 200 and 500 eggs, and kept in the sun throughout the day, and in one of the laboratory rooms during the night. Crushed bread crumbs were added on alternate days. The first pupae appeared in all pans on the seventh day after immersion of the eggs, but while pupation was complete by the tenth day in the pan with the fewest larvae, pupation continued until the twentieth and twenty- first days in the more crowded pans. The percentages of larvae pupating in the three pans were 82, 46 and 37.6 respectively and the percentages of adults emerging were 70, 41.5 and 29.8 respectively.

Detailed observations on the duration of each larval and pupal stage of *Anopheles gambiae* were made on a group of 125 newly hatched larvae distributed equally among five small enamel pans.

Records were made at hourly intervals of all changes, whether molting or deaths, until the last adult emerged at 21 days and 7 hours. When molting occurred the new stage was transferred to a new bowl, or to a screened cage in the case of adults. The bowls were kept in the open air insectary during the day where they were exposed to the sun, and in a closed room at night. Bread crumbs were added daily, but the water was not changed in the bowls during the experiment. This fact and the use of relatively small bowls for the initial population of twenty-five larvae, probably contributed to a slowing up of the early development. In any event the results can be assumed to represent the behavior of larvae and pupae only under the conditions of the experiment. Its significance is found in the continuous picture which the experiment furnishes of the complete sequence of events under one set of conditions. The results are given in table 1 in which are listed only hours when significant changes took place. The first molting into second stage larvae occurred at 45 hours. The first third stage larvae appeared at 94 hours, and the first fourth stage larvae at 141 hours. Pupation began at 197 hours and the first adult emerged 25 hours after pupation. It is interesting that the second adult emerged 19 hours after pupation which is the shortest pupal stage in our records. In all, 69 of the original larvae produced adults, which gives an emergence rate of 55 per cent.

## Longevity of *Anopheles gambiae* mosquitoes

The longevity of adult mosquitoes is of particular interest in determining how long control measures should be maintained after the last larval focus is observed. Many factors may operate to alter the life span in nature, among them weather conditions, natural enemies and availability of food. The records of one laboratory experiment furnish data on the longevity of a group of laboratory bred *Anopheles gambiae* mosquitoes under more or less optimum conditions of food and protection. The pupae emerged in a screened cage 28 x 28 x 38 cm. supplied with water and honey. There were 118 females and 108 males. The first blood meal was offered 4 days after completion of pupation

and the first eggs were deposited 4 days later. Human blood was offered daily and eggs were produced until the twenty-third day. Population counts were not made before the fifteenth day when 73.7 per cent females and 85.1 per cent males were survivors. Thereafter dead mosquitoes were removed and survivors recorded daily until the thirty-ninth day when all specimens were dead. There was a gradual and rather regular decrease of both males and females throughout the period of observation.

Table 1 – Development of *Anopheles gambiae* in the laboratory. Duration of larval and pupal stages

| Hours after hatching | Stages | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st larval | | 2nd larval | | 3rd larval | | 4th larval | | Pupal | | Ecdysis | | Adult |
| | Live | Dead | Live | Dead | Live | Dead | Live | Dead | Live | Dead | | | Total |
| 1 | 125 | 0 | | | | | | | | | | | |
| 6 | 124 | 1 | | | | | | | | | | | |
| 45 | 106 | 17 | 2 | | | | | | | | | | |
| 47 | 96 | 18 | 11 | | | | | | | | | | |
| 77 | 2 | 22 | 99 | 2 | | | | | | | | | |
| 93 | 0 | 22 | 100 | 3 | | | | | | | | | |
| 94 | 0 | 22 | 71 | 5 | 27 | | | | | | | | |
| 122 | 0 | 22 | 2 | 8 | 93 | | | | | | | | |
| 141 | 0 | 22 | 0 | 8 | 72 | 3 | 20 | | | | | | |
| 197 | 0 | 22 | 0 | 8 | 2 | 8 | 84 | | 1 | | | | |
| 212 | 0 | 22 | 0 | 8 | 0 | 8 | 86 | | 1 | | | | |
| 222 | 0 | 22 | 0 | 8 | 0 | 8 | 60 | | 26 | | | 1 | 1 |
| 231 | 0 | 22 | 0 | 8 | 0 | 8 | 52 | | 32 | 2 | | 1 | 1 |
| 232 | 0 | 22 | 0 | 8 | 0 | 8 | 52 | | 29 | 2 | 3 | 1 | 4 |
| 332 | 0 | 22 | 0 | 8 | 0 | 8 | 12 | 2 | 1 | 12 | 33 | 27 | 60 |
| 463 | 0 | 22 | 0 | 8 | 0 | 8 | 0 | 2 | 1 | 16 | 35 | 33 | 68 |
| 511 | 0 | 22 | 0 | 8 | 0 | 8 | 0 | 2 | 0 | 16 | 35 | 34 | 69 |
| Percentage Mortality | | 17.6 | | 7.8 | | 8.4 | | 2.3 | | 18.8 | | | |
| Per cent adults emerged | | | | | | | | | | | | | 55.0 |

## FIELD STUDIES

In order that *Anopheles gambiae* might be studied under natural conditions while the control and eradication program was in progress two small districts, Cumbe and Corrego dos Rodrigues, were designated as control areas, left untreated and placed under the supervision of the laboratory personnel. Cumbe was especially suitable for the study of mosquito feeding and breeding habits because of its natural isolation, the presence of varied types of breeding places throughout the year, abundant *Anopheles gambiae*, and a high incidence of malaria in the human population.

### The presence of man as a factor in attracting *Anopheles gambiae*

The fact that *Anopheles gambiae* is domestic in its habits has been observed by all who have studied this mosquito. One of the greatest manifestations of its domesticity is the number of both male and female specimens found in human dwellings day and night. In view of the close association of *Anopheles gambiae* with man it was of interest to discover whether the attracting factor is the ready supply of human blood in the house or the protection afforded by the house itself. Numerous observations have shown that even at the height of the mosquito population *gambiae* is rarely present in vacant houses. During routine examinations conducted at intervals of four or five days throughout February, March and April in Cumbe, records were made of the number of persons sleeping in each room and of the number, sex, and condition of *gambiae* captured in each after being searched ten minutes.

The results on seven houses which were occupied and vacated at intervals demonstrate the influence of human occupants. When captures were made in a day following occupancy the previous night, a total of 1420 *Anopheles gambiae* was found at sixty-eight of the seventy visits, or an average of 20 *gambiae*, mostly engorged

females, per house per visit. When these houses were unoccupied the night before the captures were made, a total of only 14 mosquitoes was obtained in eight of the sixty visits, or an average of about 0.2 *gambiae* per house per visit. The one house which was never slept in during the period of observation, was negative for *gambiae* fifteen of the seventeen times examined.

The importance of the human factor is further shown by a study of individual rooms. It should be pointed out that the rooms in these houses are not distinctly separated, as the walls rarely extend to the ceiling or roof. In spite of this lack of complete separation, counts made of the number of *Anopheles gambiae* captured from sleeping rooms and from rooms not so used, showed that the former offered far greater attraction. During February, March and April 1523 visits were made to sleeping rooms and a total of 6792 specimens of *Anopheles gambiae* were captured, or an average of 4.5 per visit. During the same period 1506 visits to other rooms in these houses yielded a total of 1853 mosquitoes or an average of 1.2 per visit. In Table 2 are given the number of visits, total captures, and the average number of *gambiae* captured per visit from rooms not used as sleeping quarters and from rooms used by one, two, three, four, and five or more people. The results show that while rooms in which no persons slept, gave an average of 1.2 *gambiae* per visit, rooms with one person gave an average of 4.2 *gambiae* per visit, rooms in which two people slept gave an average of 3.6 specimens per visit, and rooms in which three, four, and five or more persons slept gave averages of 5.4, 5.9 and 5.4 *Anopheles gambiae* per visit. The figures strongly indicate that while there is a marked difference between the number of *Anopheles gambiae* captured in rooms used as sleeping quarters and the number captured in other rooms, there is no marked relationship between the number of mosquitoes and the number of persons sleeping in the rooms.

Table 2 – *Anopheles gambiae* captured in individual rooms with relation to the number of persons sleeping in them during the night previous

| Number of persons sleeping in rooms previous night | Number of rooms visited | Total visits | Total *gambiae* captured | Average *gambiae* captured per visit |
|---|---|---|---|---|
| 0 | 99 | 1,506 | 1,853 | 1.2 |
| 1 | 47 | 366 | 1,545 | 4.2 |
| 2 | 50 | 602 | 2,170 | 3.6 |
| 3 | 26 | 297 | 1,614 | 5.4 |
| 4 | 7 | 146 | 858 | 5.9 |
| 5+ | 8 | 112 | 605 | 5.4 |
| 1 or more | 138 | 1,523 | 6,792 | 4.5 |

## The influence of light on the occurrence of Anopheles gambiae in houses

There was, however, an obvious relationship between the number of *Anopheles gambiae* and the light intensity of any given room. The intensity of light was measured by taking two readings, one from the lightest spot looking toward the darkest, and vice versa, with an Electro Bewi photometer. These two readings were averaged. In order to simplify the records, rooms that had an average light intensity of nine or more were labelled as light 1; those with readings of 8.5 to seven were called light two; readings of 6.5 to five were called light three; readings of 4.5 to two**,** light four; and those with readings less than two, light five.

In analysing the light factor all rooms of similar light intensity were grouped and the average number of *Anopheles gambiae* per visit determined. The results of these observations are given in Table 3, and show a tenfold difference between the number of *Anopheles gambiae* captured in the darkest and lightest rooms. In this table no differentiation is made between sleeping rooms and other rooms of the house.

Table 3 – *Anopheles gambiae* captured in individual rooms with relation to light intensity of each

| Light intensity * | Number of rooms visited | Total visits | Total *gambiae* captured | Average *gambiae* captured per visit |
|---|---|---|---|---|
| 1 | 8 | 112 | 48 | 0.4 |
| 2 | 55 | 962 | 1,170 | 1.2 |
| 3 | 81 | 1,423 | 2,576 | 1.8 |
| 4 | 49 | 874 | 3,201 | 3.7 |
| 5 | 47 | 837 | 3,566 | 4.3 |

***** Measured with Electro Bewi photometer: 9 + = light 1, 8.5 – 7 = light 2, 6.5 – 5 = light 3, 4.5 – 2 = light 4, – 2 = light 5.

Table 4 – Average number of *Anopheles gambiae* captured per visit from rooms of given light intensity and given number of night occupants

| Light intensity* | Number of persons sleeping in rooms | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5+ |
| 1 | 0.1 | 0 | 0 | 0.5 | 0 | 0 |
| 2 | 0.6 | 3.3 | 0.8 | 1.2 | 1.3 | 3.1 |
| 3 | 0.7 | 3.6 | 2.9 | 4.9 | 4.5 | 6.6 |
| 4 | 1.3 | 8.0 | 4.0 | 10.9 | 7.6 | 3.7 |
| 5 | 3.0 | 4.0 | 6.1 | 7.0 | 9.8 | 5.3 |

*Measured with Electro Bewi photometer: 9 + = light 1, 8.5 – 7 = light 2, 6.5 – 5 = light 3, 4.5-2 = light 4, -2 = light5.

The average number of *Anopheles gambiae* captured per visit in rooms of each light intensity, in the absence of human occupants or in the presence of a stated number of persons during the night previous to capture, is summarized in Table 4. It should be noted that in these observations the rooms of light one were too few in number to supply adequate data. In other rooms with less light there appears to be a correlation between the average number of *gambiae* captured per visit, and the darkness of the room, irrespective of the absence of persons, or the number of persons sleeping in the room, when less than five people were present. When there were five or more people in a room, there were other factors, perhaps greater activity of the occupants, which counteracted the light factor.

These results demonstrate the influence of light on the number of *gambiae* found in the various rooms of a house. On inspection of the results in rooms of light five the average number of *Anopheles gambiae* captured appears to be correlated with the number of night occupants, but the irregular results found at other light intensities tend to counteract this apparent correlation, and to invalidate the general impression that the greatest number of *gambiae* are found in rooms in which the largest number of persons sleep.

## Length of time *Anopheles gambiae* remains in houses

In an effort to determine the importance of shelter and host in attracting *Anopheles gambiae* a series of experiments was conducted in a dry sandy region far removed from all potential breeding places. In the late afternoon 200 mosquitoes from the laboratory colony were liberated in each house in which resided a family of four, a laboratory assistant or a mule, during the period of observation. It was not possible to draw conclusions as to host preference from these experiments because the mosquitoes began leaving the house immediately upon being released and less than two per cent could be found in the dwelling 12 hours later, regardless of the type of host inside. Their departure was observed during both day and night, and

perhaps like that of the mosquitoes described by de Meillon (1930) in Africa, was due to the fact that the houses were too hot and dry. That this migration was not caused by disturbance of the adults in being transported and released was demonstrated by a similar migration of adults allowed to emerge from pupae inside the house.

Experiments conducted in Cumbe during the rainy season when *Anopheles gambiae* was abundant also demonstrated a rapid turnover in the mosquito population inside houses. A method of staining mosquitoes for subsequent identification was first worked out in the laboratory. Mosquitoes were sprayed with a 2 per cent aqueous solution of methylene blue or a similar solution of acid fuchsin applied with a de Vilbiss spray gun. The dyes showed no lethal effect, there being no difference in death rate between sprayed and unsprayed mosquitoes over a period of many days. The stain on the treated mosquitoes was easily detected by placing them separately on blotting paper and moistening each with a drop of alcohol, which dissolved the stain and colored the paper beneath.

Four houses known to harbor large numbers of *Anopheles gambiae* were selected for these experiments. The houses were carefully searched by four persons simultaneously and all specimens of both *Anopheles gambiae* and *Nyssorhynchus* counted and identified *in situ* on the walls and roof. Then the specimens were again sought out and sprayed with one of the dye solutions. On April 22, one hundred twenty-five *Anopheles gambiae* were sprayed with methylene blue in house 51; five hundred twenty-six with acid fuchsin in house 56; fifty-six *gambia*e and eight *Nyssorhynchus* with methylene blue in house 24; and ninety *gambiae* and ten Nyssorhynchus with acid fuchsin in house 23. From April 23 to April 26 inclusive daily captures were made from these houses and all other houses in the immediate vicinity. Four men especially trained in capturing mosquitoes spent from 40 to 60 minutes each, or a total of 160 to 240 minutes per house. The captured mosquitoes were taken to the laboratory and tested for the presence of

stain. Although the dispersion of the mosquitoes from these houses was less than in the experiments conducted in a dry sandy region far removed from water, it is significant that of 125 specimens stained in house 51 only 24 were collected the following day, and in addition 54 unstained *gambiae* were found. This would seem to indicate a large daily turnover in the mosquito population. On the succeeding three days, 7, 6 and one blue stained specimens of *Anopheles gambiae* and 50, 53, and 29 unstained specimens were collected. None with red stain was captured from this house.

From house 56 in which 526 specimens of *Anopheles gambiae* had been stained red, 103 were discovered the following day together with 345 unstained specimens. On the succeeding three days 20, three and two stained specimens were recovered as well as 241, 131 and 163 unstained *gambiae*. None containing blue stain was captured. These two houses, 51 and 56 are 350 meters apart and about 550 meters removed from houses 23 and 24.

From house 23 in which 90 *gambiae* had been stained red, 11 red and 51 unstained specimens were captured on the following day. On each of the two succeeding days one red and one blue *gambiae* were captured, and 29 and 21 unstained specimens. No stained specimens and only 12 unstained specimens were collected on the fourth day.

From house 24 in which 56 *Anopheles gambiae* had been stained blue, three red and 16 unstained specimens of *gambiae* were captured on the following day. On the second day one blue and ten unstained specimens of *Anopheles gambiae* were captured. During the remaining two days of observation only four *gambiae,* all unstained, were found. It is of interest to note that in the course of these experiments there was an interchange of red and blue stained mosquitoes only in houses 23 and 24 which were relatively close together, about 100 meters apart.

In the last experiments conducted in houses which were more than a kilometer removed from those described above no counts were made of the mosquitoes sprayed. From house 38 in which the mosquitoes were stained blue, one blue stained specimen was captured on the third day, and 5, 8 and eight unstained specimens during the three days of observation. From house 40 in which the mosquitoes were stained red three daily captures yielded two, 1, and 2 respectively red stained *gambiae* and 6, 9 and 12 unstained specimens. Other houses in the vicinity were also searched and an occasional stained mosquito captured in dwellings within an area of about 200 meters. Since large numbers of stained mosquitoes evidently departed from the original houses one would expect to find many of them in these neighboring dwellings but in this study remarkably few were captured. It is possible that after leaving the house some were blown long distances and it is probable that many were killed before finding shelter.

## Wind as a factor in dispersion of *Anopheles gambiae*

Although it was found that *Anopheles gambiae* migrated less from houses in Cumbe during the rainy season than they did from houses situated in a hot dry region ten kilometers away, it was shown that more than four-fifths of the mosquito population in any given house changed within 24 hours after observations were begun. In this locality where extremely strong prevailing winds blow during the day and night, especially throughout the dry season, many of the migrating mosquitoes are probably borne long distances by the wind. Some of the young mosquitoes on their way to houses for the first time doubtlessly experience the same fate. The occasional lulls in the wind which favor mosquito activity, followed by periods of increased wind velocity, make ideal conditions for the dispersion of mosquitoes.

In the spread of *Anopheles gambiae*, water and land traffic in which mosquitoes are carried, and natural migration, have been important factors, but it is perhaps more than just a coincidence that the dispersion has been in the direction of the prevailing winds. The

strong trade winds blowing north and west along the coast from Natal, and landward from the sea south and west up the Jaguaribe valley, both coincide with the spread of *Anopheles gambiae* in northern Brazil.

## Feeding places of *Anopheles gambiae*

Throughout the study of *Anopheles gambiae* at the central laboratory and in the experimental areas, specimens of this mosquito were observed feeding only inside houses. In order to test the regularity of this habit both human and animal bait were planted just outside houses that had been shown to be heavily infested with *Anopheles gambiae*. In the experiments conducted both by day and by night, no specimen of *Anopheles gambiae* was captured on either man or horse outside the house. Specimens of *Nyssorhynchus*, however, were collected on animal bait, each time the traps were set up at night.

It seemed possible that the close proximity of the bait to the house might be responsible for the negative results, in that the latter held greater attraction for *gambiae*. Therefore in other experiments these traps were set up near breeding places known to be producing large numbers of *Anopheles gambiae*, and which were from 40 to 60 meters distant from the hearest house. In a series of ten representative captures on animal and human bait conducted during April, 483 culicine mosquitoes were captured during the day and night, while 166 *Anopheles albitarsis* and twelve other *Nyssorhynchus* damaged beyond recognition were captured at night. *Anopheles gambiae* was not found on the bait at any time. These experiments and those reported in the previous paragraph confirm observations made throughout the investigation, namely that *Anopheles gambiae* feeds only inside houses. During February, March and April 11, 420 adults were captured in houses in Cumbe in 736 hours and 44 minutes. Of these 9609 were females, 6967 of which were engorged. In the same months no *Anopheles gambiae* was found during 129 hours and 10 minutes spent in making outdoor captures on animal bait.

## Feeding habits of *Anopheles gambiae*

The close association of *Anopheles gambiae* with man strongly suggests that man is the preferred host. The fact that unoccupied houses hold little attraction for these mosquitoes further substantiates this theory. Although it has been found that when confined in cages *Anopheles gambiae* will feed on horses, goats, cows, pigs, chickens and pigeons there are only rare instances on record to show that *gambiae* feeds on any of these animals in nature.

Since *Anopheles gambiae* is rarely found very far removed from man the presence of several specimens of this mosquito in an unoccupied house visited in April was so unusual as to warrant an investigation. It was discovered that a herd of goats had been sleeping there. In order to ascertain whether the goats attracted the mosquitoes, these animals were left in the house for further observation. Two goats were also confined in each of two other houses which had been occupied by human beings during March, but which were vacant during April.

Mosquito captures in these and other houses were made frequently during March and April and the number, sex and condition of the specimens recorded. The results are given in table 5 and confirm earlier observations, that only a few *gambiae* are to be found in unoccupied houses and that those found are usually males. After goats were placed in the houses, both males and females were captured but not so abundantly as when the houses were occupied by man. The fact that engorged females were found, however, indicated that they probably fed upon the goats. Unfortunately the blood meal could not be identified in the laboratory as sera for precipitin tests were not at the time available.

Table 5 – *Anopheles gambiae* captured in houses when empty and when occupied by goats or by human beings

| Houses | Number of captures | Males | | Unfed females | | Engorged females | | Both sexes | |
|---|---|---|---|---|---|---|---|---|---|
| | | Total captured | Average per capture | Total captured | Average per capture | Total captured | Average per capture | Total captured | Average per capture |
| Empty | 18 | 5 | 0.3 | 0 | 0 | 0 | 0 | 5 | 0.3 |
| With goats | 13 | 7 | 0.5 | 3 | 0.2 | 9 | 0.7 | 19 | 1.5 |
| With human dwellers | 15 | 37 | 2.5 | 45 | 3.0 | 227 | 15.1 | 309 | 20.6 |

In order to learn what animals were fed upon by *Anopheles gambiae* under natural conditions, precipitin tests using the technique of Rice and Barber (1935) were made on several batches of engorged females. The antisera had been prepared in Italy and shipped to Brazil where they remained at room temperature for several months before being put on ice. Many of the vials contained serum too weak or too old to give specific tests with known bloods, while others proved satisfactory only when prepared in concentrations considerably stronger than recommended by Rice and Barber. The limited supply of sera precluded the examination of a large number of specimens. The antisera available were for the blood of man, horse, cow, sheep, pig, chicken and dog. Of the 72 *gambiae* stomachs tested, all positives were with human antisera, 60 giving strong and ten weak reactions. Although the number of specimens examined is small it is perhaps significant that all positive results were obtained with human antisera.

## Susceptibility of *Anopheles gambiae* to malaria

The best evidence for the susceptibility of *Anopheles gambiae* to malaria parasites is the severe epidemics of malaria that have followed in the wake of this mosquito as shown by the sudden increase in the incidence of the disease throughout the greatest part of

the *gambiae* infested regions in Brazil. In order to get some numerical and comparative data on the infection rate of *Anopheles gambíae*, dissections were made on all species of *Anopheles* encountered in the experimental areas. During this study 1891 specimens of *gambiae* were dissected of which 5.6 per cent were found to be infected with malaria parasites. In addition 201 specimens of a species in the *tarsimaculatus* series 1.5 per cent of which were positive, and 314 *Anopheles albitarsis*, all of which were negative, were examined. Of the 1838 glands of *gambiae* examined, 29 or 1.6 per cent were positive for sporozoites, and of 1833 stomachs, 87 or 4.7 per cent were positive. Of the 186 stomachs of the *tarsimaculatus* species examined two**,** or 1.1 per cent were positive, and of 190 glands of this species one, or 0.5 per cent was positive. The results show an unexpectedly low rate for both stomach and gland infection *of Anopheles gambiae*, although all specimens were kept until the blood meal was digested and the majority were kept for a sufficient time to permit the development of oocysts.

Table 6 – Parallel human and *Anopheles gambiae* infection rates by months

| Month | Locality | Per cent positive bloods | Per cente gametocyte carriers | Per cent infected gambiae |
|---|---|---|---|---|
| January | Cumbe | 64.7 | 20.9 | 9.1 |
| February | Cumbe | 45.4 | 17.9 | 4.9 |
| March | Cumbe | 80.7 | 14.2 | 5.1 |
| April | Cumbe | 89.7 | 15.7 | 5.8 |
| May | Cumbe | 29.5 | 9.3 | 5.1 |
| May | Corrego dos Rodrigues | 68.2 | 12.1 | 10.0 |
| June | Corrego dos Rodrigues | 46.0 | 13.6 | 0 |

It should be noted that the large number of mosquitoes captured during the frequent surveys each month undoubtedly reduced the number of mosquitoes having an opportunity for multiple feedings, and thereby altered the natural infection rate. This assumption is supported by the record of infection rates by months as shown in table 6. The mosquitoes collected in Cumbo during February, March, April and May were from a population subjected to frequent captures while those collected from Cumbe in January and from Corrego dos Rodrigues in May were from previously unmolested populations. These latter rates of 9.1 and 10.0 per cent respectively, were nearly twice as high as for the other months and probably more nearly represent the true natural infection rate in these localities. The failure to find infected mosquitoes in Corrego dos Rodrigues during June is explained partly by the few specimens examined and by the fact that extensive fumigation of houses was in progress.

## Significance of infection rate

The fact that 5.6 per cent of the dissected specimens of *Anopheles gambiae* were positive is not particularly significant in itself but becomes significant when this infection rate is compared with that of the other species of Anophelines present in the area. *Anopheles albitarsis* which is perhaps the most abundant mosquito in Cumbe, rarely enters houses except when attracted by bright lights, and was never found infected with malaria parasites. The *tarsimaculatus* species although domestic in habit was rarely found infected. It is apparent that *Anopheles gambiae* was the real vector of malaria in Cumbe.

For a better evaluation of the infection rate of *Anopheles gambiae* in Cumbe it is necessary to consider the gametocyte carriers. Swellengrebel (1935) has pointed out that in order to determine the susceptibility of a species of mosquito to malaria at least 8 per cent of the children should be gametocyte carriers. During the period in which dissections were made in Cumbe the rate for the children was approximately 20 per cent and the gametocyte rate for the entire

population ranged from 20.9 per cent to 9.3 per cent. The percentage of the population positive for gametocytes and parasites, and the infection rate of *Anopheles gambiae* are shown in table 6 for Cumbe and Corrego dos Rodrigues.

Table 7 – Infection rate of *Anopheles gambiae* from houses grouped according to frequency of finding gametocyte carriers

| Number of months gametocyte carriers were found in houses | Number of houses in each group | Inhabitants | | | Mosquitoes | | |
|---|---|---|---|---|---|---|---|
| | | Number examined | Number of carriers | Per cent Carriers | Number captured | Number positive | Per cent positive |
| 4 | 5 | 119 | 41 | 34.4 | 131 | 11 | 8.4 |
| 3 | 13 | 301 | 69 | 22.9 | 409 | 24 | 5.8 |
| 2 | 15 | 344 | 46 | 13.3 | 581 | 31 | 5.3 |
| 1 | 13 | 171 | 14 | 8.1 | 386 | 13 | 3.3 |
| 0 | 14 | 153 | 0 | 0 | 274 | 9 | 3.2 |

The influence of the gametocyte rate upon the mosquito infection rate is indicated by comparing the degree of infection of mosquitoes from houses grouped according to frequency of finding gametocyte carriers. It should be pointed out that while the mosquitoes were captured during the routine searches made several times at intervals throughout the month, the gametocyte rate was determined by blood samples taken only once each month. It is recognized that this method of sampling does not detect the true number of gametocyte carriers in any given house throughout the month, but by placing in separate groups those houses that were found positive, four, three, two, one and zero times of the four monthly examinations, those with approximately equivalent carriers could be selected. The infection rate of *Anopheles gambiae* and percentage of carriers from these houses are shown in Table 7. In arranging the houses in this manner it was found that those in which gametocytes were demonstrated at the greatest number of monthly visits also showed the highest percentage of gametocyte carriers. There also appears to be a direct relationship between the per

cent of positive mosquitoes captured and the per cent of carriers in any given group of houses. This correlation is to be expected since more than 70 per cent of the mosquitoes were engorged when captured, and due to the tendency of mosquitoes to remain quiescent for several hours following a blood meal, the occupants of the house from which they were taken were most probably the donors of the blood. Moreover, since captures were being made frequently and the hazards to mosquito life were great in migration from house to house, there was increased likelihood that the infection demonstrated in the mosquito after maturation in the laboratory, was acquired in this last blood meal. In view of this statement, the finding of 3.2 per cent positive mosquitoes in houses where no gametocyte carriers were demonstrated at any of the monthly surveys, calls for comment. It should be noted that there were known malaria patients in all but one house in Cumbe and that there probably were gametocyte carriers present at some time during the month even in those houses where none was demonstrated at the routine surveys. On the other hand the tendency of *Anopheles gambiae* to migrate might also account for the finding of some few positive mosquitoes in houses with no apparent gametocyte carriers.

## Experimental infection of *Anopheles gambiae* mosquitoes

In view of the apparently low infection rate of *Anopheles gambiae* captured in areas with a large number of gametocyte carriers, it seemed of particular interest to study experimental infections of *Anopheles gambiae*. Among a total of 151 laboratory bred mosquitoes which were fed on five persons 66 specimens were dissected seven to 13 days after the infective blood meal. Six of these or 9.1 per cent showed oocysts in the stomach. The glands were negative for sporozoites. Mosquitoes fed on three of the five persons failed to become infected. In one of these cases it was discovered that a single tablet of atebrin had been taken three days prior to the examination and mosquito blood meal. The *Plasmodium falciparum* gametocytes in the patient's blood were numerous and appeared normal nevertheless.

The other two negative experiments were on *Plasmodium vivax* carriers. The two persons on whom mosquitoes became infected were *Plasmodium falciparum* carriers. The specimens which were dissected after feeding on these cases showed a stomach infection rate respectively of 33.3 per cent (five positive among fifteen dissected) and 4.2 per cent (one positive among twenty-four dissected.)

## Breeding places of *Anopheles gambiae*

The preferential breeding places of *Anopheles gambiae* in northeast Brazil were small and shallow collections of fresh water, well exposed to sunlight, with little or no vegetation and little organic matter.

During the dry season the majority of such breeding places are to be found in the drying beds of the rivers where residual pools of various sizes form in the large expanses of dry or water-soaked sand and gravel. Here the inhabitants dig their shallow wells for drinking water, and the well pits and ditches for irrigation of the river-bed crops of potatoes and green vegetables. Here too, hoofprints left in the soft sand readily fill by seepage from the superficial underground water.

Outside the river beds *gambiae* larvae are also found in the muddy or clean water gathered in the hoof marks at the margin of ponds used as drinking places for domestic animals, and in small shallow collections of seepage and surface springs, in shallow wells near houses, and in the irrigation ditches of new or recently harvested sugar cane plantations. It may be noted that many of these preferential gambiae breeding places are the result directly or indirectly of human activity.

In the rainy season the preference for a restricted type of water collection is not so clear, and *gambiae* larvae may be found in many different kinds of deposits, even in those with much vegetation and some shade. At this season the rivers offer no facilities for breeding. Outside the rivers new breeding places are formed wherever rain water accumulates in suitable locations, as in borrow pits, in natural

depressions, in trails produced by the wheels of vehicles and in the depressions left by animal hoofs. Larvae were very rarely found in flowing streams, or in the main body of water of large reservoirs or lagoons. Their presence in such locations could usually be explained by transportation during flooding of their normal breeding places.

The distribution and character of potential breeding places for *Anopheles gambiae* were carefully studied in the Cumbe area. As many water collections as could be visited routinely by one laboratory assistant were marked, and observations made on permanence, physical features, salinity and presence or absence of *gambiae* larvae.

The sources of the permanent water collections around Cumbe consist principally in the springs and seepages near the base of the sand dunes on the south, east and northeast of the village. Broad ditches connect these seepages with numerous smaller and shallower ditches, which irrigate the sugar cane plantations and are in turn drained into the Jaguaribe river. During the greater part of the year these collections are unshaded and show little vegetation, but by the time the sugar cane attains maximum growth toward the end of the rainy season, shaded ditches have become numerous and algae and grass are found abundantly in some of them. In addition, there are about 40 large deep wells from which water for irrigation is taken by wind driven pumps, and several shallow wells or *cacimbas*. Besides these permanent collections are many temporary deposits formed only during the rainy season by accumulation of rain water in natural depressions and borrow pits. Isolated hoofprints and hoofprints around the margins of big ponds, account for innumerable temporary foci.

In Table 8 is shown the distribution of *Anopheles gambiae* larvae in Cumbe according to the type of water collection in which they were found during the period with free breeding and during the control campaign, and the evidence of eradication.

The results of the monthly examination on potential and actual breeding places indicate that shallow wells were preferred by *Anopheles gambiae*, almost two thirds of the total examinations on them showing *gambiae* larvae. Only a few wells were included in this study but the findings here are in agreement with observations made in other experimental areas where shallow wells constituted the principal breeding places. The borrow pits were second with 39.2 per cent positives. The percentage of positive ponds was apparently high (16.7) but it must be explained that with one exception, *gambiae* larvae were not found in the large body of water constituting the pond, but only in the small depressions or hoofprints which abounded at the margins. The proportion of positive ditches in March and April was much smaller than in February due in part to the greater quantity of shade furnished by the growing sugar cane, but perhaps also to the fact that during the rainy season fishes gain access to these ditches. All examinations on deep wells were negative for *gambiae* larvae.

## Influence of vegetation and shade on *Anopheles gambiae* breeding

The various types of water collections were classified according to the amount and kind of vegetation at each monthly examination and the presence or absence of *gambiae* larvae. Although *gambiae* larvae were found in a greater proportion of collections with no visible vegetation, they were often discovered in collections with algae, especially in ditches, and borrow pits, and occasionally in temporary pools with grass. During the monthly surveys in Cumbe *gambiae* larvae were never found breeding in water with lilies and were found only once in an irrigation ditch covered with *Pistia*, although many ditches with this type of vegetation were examined. During the dry month of January water collections with no vegetation were found positive for *gambiae* larvae in a larger percentage of examinations than were collections showing some or much vegetation. With the advancing wet season, and increased *gambiae* breeding places, a large proportion

of water accumulations with more or less vegetation harbored *gambiae* larvae. Nevertheless, considering the period as a whole, bodies of water without vegetation were found to furnish the preferred breeding places.

*Anopheles gambiae* larvae were rarely found in very shaded collections of water in Cumbe, although many of these were examined. A fair number of breeding places occurred however, among partially shaded water collections.

## Influence of salinity on *Anopheles gambiae* breeding places

The topography of Cumbe is such that all the water sources on the south, east and northeast are fresh throughout the year, while the deep and shallow wells in the northwest area are frequently brackish and may even be strongly salty at times. The Jaguaribe river borders Cumbe on this side and at high tide invades a portion of the area. The river water is distinctly salty during the dry season and the soil retains enough salt from these floodings to make brackish any collections of water in the locality. At the peak of the rainy season, however, in April and May, and sometimes as early as in March, dilution with rain may render the water fresh to the taste. The significance of this sequence of conditions on *Anopheles gambiae* breeding was emphasized by observations made in adjacent borrow pits in this area.

Table 8 – Water collections examined monthly for *Anopheles gambiae* larvae before (1), during (2), and after (3) the eradication campaign in Cumbe

| Water collections | 1 | | | 2 | | | 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | January to April | | | May | | | June to Deccember | | |
| | Number examined | Positive for *gambiae* | Per cent positive for *gambiae* | Number examined | Positive for *gambiae* | Per cent positive for *gambiae* | Number examined | Positive for *gambiae* | Per cent positive for *gambiae* |
| Isolated hoofprints | 90 | 7 | 7.8 | 64 | 4 | 6.2 | 30 | 0 | 0 |
| Small temporary pools | 89 | 18 | 20.2 | 17 | 0 | 0 | 82 | 0 | 0 |
| Large temporary ponds (marginal hoofprints)* | 24 | 4 | 16.7 | 7 | 0 | 0 | 9 | 0 | 0 |
| Borrow pits | 240 | 94 | 39.2 | 64 | 3 | 4.5 | 106 | 0 | 0 |
| Irrigation ditches | 2,019 | 59 | 2.9 | 457 | 1 | 0.2 | 3,361 | 0 | 0 |
| Shallow wells | 36 | 22 | 61.1 | 16 | 0 | 0 | 42 | 0 | 0 |
| Deep wells | 56 | 0 | 0 | 10 | 0 | 0 | 82 | 0 | 0 |
| Total | 2,554 | 204 | 8.0 | 635 | 8 | 1.3 | 3,712 | 0 | 0 |

* With one exception *Anopheles gambiae* larvae were never found in the pond proper but only in marginal hoofprints.

On February 7 a borrow pit that had been excavated on February 2 was examined in the northwest section of Cumbe. It measured about 2 meters in diameter, was proportionally shallow, and completely exposed to sunlight. It contained fresh water, and first and second stage *gambiae* larvae. On February 16 this breeding place was again found positive for *gambiae.* On the same day another borrow pit about one meter distant was also examined. This second collection was likewise fully exposed to the sun but was deeper and narrower and contained brackish water and no *gambiae* larvae. Apparently this borrow pit had been dug deep enough for entrance of the salty underground water. On March 3 the pit was again examined and many *gambiae* larvae were found. The water now proved to be fresh from dilution with the heavy rains that had fallen several days previously. On March 27 and on April 23 *gambiae* larvae were also found in both borrow pits, and the water from both was fresh to the taste.

The influence of salinity on development of *Anopheles gambiae* was further tested in four experimental shallow holes in Cumbe. These were made about 50 cm. in diameter at the upper surface and 50 cm. deep. Hole A was dug in land where the underground water is fresh, and the earth contains organic matter. Hole B was dug nearer the margin of the river, in a place which is invaded by salt water from the river during the high tides, and where the underground water has a salty taste. Later two other holes C and D were constructed in the region near B. All holes were immediately protected by screened cages. Sodium chloride content of the water taken from each was measured and a counted number of *gambiae* eggs from wild females was deposited on the water. In hole A, containing fresh water the eggs hatched and many of the larvae developed into adults. The eggs deposited in the other holes, which contained amounts of salt ranging from 3.1 to 7.4 per cent all failed to develop. Holes C and D were restocked with eggs on several successive days but the salt concentrations of 3.3 and 3.1 per cent respectively, inhibited all development.

Laboratory experiments on the breeding of *Anopheles gambiae* in saline solutions.

Preliminary tests were made using eggs laid by wild females placed in batches of 200 on 1, 2, 5 and 10 per cent solutions of sodium chloride, and on fresh water for control of viability. The solutions were kept in white enamel bowls with a thin layer of sand at the bottom. Many larvae hatched in the control and some also in the 1 and 2 per cent solutions, very few in the 5 per cent solution and none in the 10 per cent solution. None of the larvae in saline solutions reached the second stage, while 32 adults were reared in the fresh water control. It appeared that salt concentrations greater than 2 per cent were rapidly lethal.

A number of experiments were made in which the salt was dissolved in the following concentrations: 0.25, 0.50, 0.75, 1, 1.25, 1.50, 1.75, and 2 per cent. A thin layer of sand was placed on the bottom of large glass dishes on which the level of the contained solutions was recorded with a wax pencil, and to which daily additions of fresh water were made to maintain the original concentrations. A group of 100 eggs was placed in each dish. The larvae were fed daily with minced toasted bread. A total of three or four experiments was made at each concentration. Salt concentrations of 0.25 and 0.50 per cent seemed to have little if any detrimental effect on the hatching of eggs, or the development of larvae to the adult stage. In some instances there appeared to be an acceleration of larval growth at 0.25 per cent. At 0.75 and 1 per cent there were marked reductions in the number of adults emerging, although the percentage of larvae hatched was effected but little. No adults emerged in concentrations greater than 1 per cent while the percentage of larvae hatched fell gradually with increasing concentrations up to and including 2 per cent.

Oviposition on saline solutions by *Anopheles gambiae*

The preferential use of fresh water for oviposition by *gambiae* females was shown in a series of laboratory tests. Petri dishes

containing cotton and filter paper moistened with distilled water and concentrations of 1, 2, 5 and 10 per cent sodium chloride were placed in each cage with a group of gravid *gambiae* females from Cumbe. The number of eggs oviposited in each solution and their appearance, were recorded on the following day. In a series of twelve experiments 15,033 eggs were oviposited, about 53 per cent of which were deposited on fresh water, and another 33 per cent on the one per cent saline solution. Of these less than 0.4 per cent remained white. At the time of oviposition mosquito eggs are white in color but become black within the first hour, unless arrested in development by some injurious agent. That these white eggs which failed to turn black represent permanently damaged eggs was demonstrated by washing and resuspending them on fresh water. No larvae were ever produced from the permanently white eggs. More than 90 per cent of the 1,207 eggs oviposited on 2 per cent saline remained white, while 98 per cent of the 863 on the 5 and 10 per cent saline solutions remained white. It should be noted that the mosquitoes in each cage had free choice among five Petri dishes for oviposition but that in addition to salinity there were other factors which might influence the ultimate selection of place, such as the position of the dishes in the cages and the number of mosquitoes per cage. Variable numbers of gravid females according to availability at the moment, were used and that some of the cages were undoubtedly overcrowded, was evident when the data were summarized and analyzed. Nevertheless the results appeared clearcut in demonstrating the preference of the gravid females for oviposition on fresh or only slightly brackish water, and the lethal effect of brackish or salt water on *gambiae* eggs.

## Survival of *Anopheles gambiae* in the dry season

It might appear to the casual observer that *Anopheles gambiae*, in common with other Anophelines, would find breeding conditions untenable during the dry season in northeast Brazil. However the type of breeding places normally selected by *Anopheles gambiae*,

and the close association of this mosquito with human habitation are biological characteristics of the species which insured adequate breeding throughout the year. The shallow wells dug for drinking water, usually in close proximity to houses, were the preferred water collections for oviposition. The manner of construction of these wells is dependent upon the depth to the water table. When the water is near the surface only small holes are made, but when the level is low the top of the well is wide with an excavated walk leading down to the water. In both cases the water is fully exposed to the direct rays of the sun for several hours each day and usually free of surface vegetation. In deep wells with straight sides, *Anopheles gambiae* was never found.

The shallow wells and furrows made for irrigation purposes in the drying river beds, and the excavated holes and hoofprints, filled by seepage from the high underground water table in these same areas, were other dry season foci. During the rainy season the shallow temporary pools, ditches, and animal tracks containing fresh water without vegetation, become innumerable, and *gambiae* breeding likewise multiplied.

That these conditions were highly favorable for *Anopheles gambiae* was attested by the rapid spread of this mosquito up the Jaguaribe River valley in numbers sufficient to produce one of the most severe epidemics of malaria ever recorded.

It is apparent to those familiar with the terrain and with the biology of the mosquito that the eradication of *Anopheles gambiae* from Brazil was not due primarily to unfavorable conditions in the country but rather to an extensive and ably conducted program aimed at both the larvae and adults of the species. Moreover the fact that the major success was achieved against *gambiae* during the rainy season of a very wet year when breeding was abundant and extensive, affords excellent proof of the efficiency of the control measures.

## *Anopheles gambiae* in Cumbe

*Anopheles gambiae* was known to have been prevalent in Cumbe during the malaria epidemic of 1939 and to have continued unrestricted breeding after September 1939 when all control measures were suspended to allow the area to return to natural conditions before investigations were commenced in 1940. The character and situation of the water collections in the area made ideal conditions for the permanent maintenance of *gambiae.* Here breeding continued at an increasing rate while other areas were being rapidly cleaned, and ceased only after control measures were instituted by the control service of the Malaria Service of the Northeast, in May 1940.

In January 1940 regular inspection and capture of larvae and adults were commenced in Cumbe as a part of the laboratory study of an isolated malarious community. The population consisted of an average of 291 persons residing in 57 to 60 houses dispersed over an area of 1.6 square kilometers. Every house in the area was visited about six times a month. A rough index of *gambiae* mosquito density was arrived at by recording the average number of adults captured per round of visits each month, and the number of houses found to harbor them. A representative portion of the water collections in the area was inspected each month (Table 8). An index of the prevalence of *Anopheles gambiae* breeding was determined by the percentage of water collections examined which were found with *gambiae* larvae. The results of the year's observations on adults and larvae are summarized in Table 9.

Table 9 – *Anopheles gambiae* in Cumbe in the year 1940 before during and after the eradication campaign, with a record of rainfall

| Period | Month | Days | Visits | *Anopheles gambiae* | | | | | | Rain-fall |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Adults | | | Larvae | | | |
| | | | | Houses examined | Houses positive | Average *gambiae* per cycle | Water deposits examined | Water deposits positive | Per cent positive | |
| | | | | | | | | | | *mm* |
| Before | Jan. | 25-31 | 2 | 57 | 42 | 237 | 738 | 30 | 4.1 | 86.5 |
| | Feb. | 7-27 | 6 | 58 | 53 | 455 | 525 | 72 | 13.7 | 101.4 |
| | Mar. | | 6 | 59 | 57 | 745 | 824 | 52 | 6.3 | 233.5 |
| | Apr. | 4-27 | 6 | 59 | 57 | 703 | 467 | 50 | 10.7 | 421.2 |
| | Jan.-Apr. | 1-19 | | | | 535 | 2,554 | 204 | 8.0 | 86.5 |
| During | May | 6-8 | 1 | 59 | 35 | 275 | | | | |
| | | 9-11 | 1 | 59 | 16 | 29 | | | | |
| | | 13-15 | 1 | 59 | 15 | 44 | 635 | 8 | 1.3 | 260.4 |
| | | 16-18 | 1 | 59 | 4 | 7 | | | | |
| | | 20-22 | 1 | 59 | 2 | 2 | | | | |
| | | 23-25 | 1 | 59 | 0 | 0 | | | | |
| After | June | | 6 | 60 | 0 | 0 | 695 | 0 | 0 | 98.2 |
| | July | | 6 | 59 | 0 | 0 | 406 | 0 | 0 | 45.2 |
| | Aug. | | 6 | 60 | 0 | 0 | 558 | 0 | 0 | 3.4 |
| | Sept. | | 6 | 59 | 0 | 0 | 608 | 0 | 0 | 4.2 |
| | Oct. | | 6 | 59 | 0 | 0 | 447 | 0 | 0 | 0 |
| | Nov. | | 6 | 58 | 0 | 0 | 452 | 0 | 0 | 0 |
| | Dec. | | 6 | 58 | 0 | 0 | 546 | 0 | 0 | 15.9 |
| | June-Dec. | | | | 0 | 0 | 3,712 | 0 | 0 | |

• Rainfall not available for Cumbe. Figures from Aracatí, 10 kilometers distant.

During the four months before eradication measures were begun the average number of *gambiae* captured per cycle was 535, with an average of 703 for April, the month in which control was initiated. An average of 8 per cent of the water collections examined were found to harbor *gambiae* larvae, and in April 10.7 per cent. The figures show that *Anopheles gambiae* was reproducing at a high rate and under suitable conditions at the time control measures were begun. During the period of intensive eradication measures in May there was progressive reduction in the number of mosquitoes captured and a marked reduction in the number of water collections shown to harbor larvae. In June and the six months following, no *Anopheles gambiae* specimens, either adults or larvae, were discovered. This rapid reduction and elimination of the *gambiae* population during May becomes more significant when it is noted that it was accomplished in the period following the peak of rainfall for the year, at which time one might normally expect the maximum mosquito population. lt was a vivid demonstration of the rapid results to be obtained by the combined attack on larvae with Paris green and on adults with Pyrocide as used by the Malaria Service of the Northeast (Soper and Wilson 1943)

## SUMMARY

Several colonies of *Anopheles* gambiae were established in the laboratory. Egg production by colony bred females was considerably less than that of wild females, the former averaging 94 and the latter 192 eggs per batch. Oviposition occurred throughout the night but most frequently during the hours before midnight. No difference was observed in the hatching rate of eggs from wild and laboratory bred females. In a series of experiments in the laboratory involving more than 13,000 eggs the duration of the period between oviposition and pupation varied between seven and 27 days. When kept under favorable conditions larvae were brought through to pupation in four days and 22 hours. The shortest pupal stage observed was 19 hours.

*Anopheles gambiae* was shown to be highly domestic by the fact of its presence in occupied houses and rooms used as sleeping quarters, and by its relative absence from unoccupied houses and rooms not slept in by man. A direct correlation was demonstrated between the number of *Anopheles gambiae* discovered and the darkness of any given room.

Migration from houses situated in a moist region was less than from houses in a hot dry region. In the former, however, more than four-fifths of the mosquito population in any given house changed within 24 hours after observations were begun.

Experiments using both man and animal bait inside and outside of houses revealed that *Anopheles gambiae* feeds only inside houses and that man constitutes the preferred host.

Dissections on the three species of *Anopheles* prevalent in the experimental areas showed *Anopheles gambiae* to be the principal vector of malaria with an infection rate of 5.6 per cent as compared with rates of 1.5 and 0 for a species of the *tarsimaculatus* series and *Anopheles albitarsis* respectively. The gametocyte rate of the human population varied between 9.3 per cent and 20.9 per cent, during the period when dissections were made.

Sixty-six laboratory bred *Anopheles gambiae* dissected after feeding on five human gametocyte carriers showed an infection rate of 9.1 per cent. Mosquitoes fed on three of the carriers failed to show oocysts while the stomach infection rate of specimens fed on the other two cases were 33.3 and 4.2 per cent.

Preferred breeding places of *Anopheles gambiae* were found to be small collections of fresh water, comparatively free of vegetation, fully exposed to the sun, and usually near human habitation. Such collections were available in northeast Brazil throughout the year.

Although *gambiae* larvae were never found in brackish water in nature, laboratory experiments showed that development could take place up to one per cent sodium chloride concentration.

REFERENCES

EVANS, A. M. Mosquitoes of the Ethiopian Region, Part II, Anophelini. The British Museum, London, 1938.

DE MEILLON**,** B. Entomological Studies – Observations on *Anopheles funestus* and *Anopheles gambiae* in the Transvaal. S. Afr. Inst. Med. Res., 1934, 6, 195-248.

RICE, J. B., AND BARBER, M. A. Malaria studies in Greece. Jl**.** Lab. Clin. Med., 1935, 20, 876-883.

SOPER, F. L. and WILSON, D. B. *Anopheles gambiae* in Brazil, 1930 to 1940. 1943. Semination and Eradication, 1942.

SWELLENGREBEL, N. H. Lectures, Second International Course in Malariology. League of Nations Eastern Bureau, Singapore, 1935.