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THE DEVELOPMENT AND LONGEVITY OF HAEMAGOGUS MOSQUITOES UNDER LABORATORY CONDITIONS[1]

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The importance of the mosquitoes of the genus *Haemagogus* in the epidemiology of sylvatic yellow fever led us to undertake laboratory experiments with the mechanism of virus transmission with the Villavicencio species. The results of the virus studies have been published in a series of papers by Bates and Roca (1945a, 1945b, 1946a, 1946b, 1946c), which include some notes on the vector mosquitoes. We became particularly interested in the effect of environmental temperature on the establishment and development of the virus in the mosquitoes, and it seemed that temperature conditions favorable for the virus were also favorable for the mosquitoes. The object of the present paper is to summarize these experiments from the entomological point of view.

Mosquito studies were concerned entirely with the Villavicencio population of *Haemagogus* which, in earlier papers, was erroneously called *Haemagogus capricornii*. The population has recently been named *Haemagogus spegazzinii falco* by Kumm, Osorno and Boshell (1946). The three name types (*capricornii*, *spegazzinii* and *falco*) are remarkably similar mosquitoes, distinguished chiefly by slight morphological characters of the male genitalia. The Villavicencio population seems to be morphologically homogeneous, corresponding to the *falco* type. It has been possible to establish laboratory colonies of certain *Haemagogus* species (Osorno, 1944; Hovanitz, 1946), but all attempts with *falco* have been unsuccessful, due to the failure of the adults to mate under cage conditions. Consequently, our studies have been concerned either with adults caught in the forest or with first genera-

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tion material from such adults. Methods of capture, and the results of field studies, have been published previously (Bates, 1944; 1945); laboratory methods of handling infected mosquitoes have been described in some detail by Bates and Roca (1945a) and are the same as those used in the experiments with adults described in the present paper.

SPEED OF LARVAL DEVELOPMENT

Our interest in larval development was primarily practical—to find the most satisfactory method of obtaining adults for experimental purposes. Most of our studies of culture media involved different types of infusion. It is difficult to standardize such infusions so that one experiment can be compared with another, or work at one laboratory repeated in another laboratory. As an example, the results of one set of parallel experiments are summarized in Table I. This shows the

TABLE I

EXPERIMENT WITH GROWTH OF HAEMAGOGUS LARVAE IN VARIOUS CULTURE MEDIA
(100 first stage larvae in 200 cc. of medium, kept at room temperature, mean 25° C.)

Medium	Pupation (Day)					Surviving Larvae on 13th Day	Total Survival through 13th Day
	9	10	11	12	13		
Pure infusion of *Inga* leaves	0	0	0	0	0	36	36
10 per cent *Inga* infusion + breadcrumbs	0	0	0	5	3	43	51
10 per cent *Inga* infusion + dog biscuit	0	0	4	8	10	11	33
10 per cent *Inga* infusion + brewer's yeast	3	10	10	4	6	5	38
Hay infusion (no extra food)	5	16	14	1	13	44	93
Tap water + bread-crumbs	0	0	0	0	0	96	96
Tap water + dog biscuit	0	0	0	0	0	73	73
Tap water + brewer's yeast	1	24	13	12	29	17	96

very considerable difference in rate of growth (as well as in survival) in different media. The infusion of *Inga* leaves (*Inga* is a common leguminous tree of the local forests) was long used as a standard medium in the laboratory; yet as can be seen from this table, certain lots of infusion, at any rate, were very unsatisfactory indeed. Results with brewer's yeast were very consistent, and this was later adopted as a standard medium both for raising larvae and for stimulus of egg hatching. The favorable effect of yeast on the growth of mosquito larvae is well known (e. g., experiments of Trager, 1937), and it is used in many types of routine culture media. We used the dried product put up in tablet form by vitamin manufacturers. While this yeast in tap water seemed to be an entirely adequate food for *Haemagogus capri-*

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cornii, it was not sufficient for *Aëdes serratus* or *Psorophora ferox*, though these did well with yeast added to a strong hay infusion.

We made various experiments to determine the effect of temperature on larval growth rate. The results from one set of parallel experiments are summarized in Table II. In this case the larvae were kept individually in shell vials containing tap water to which brewer's yeast was added as food. The amount of yeast was not standardized: rather we attempted to maintain a balance so that some yeast was always present, yet not enough to cloud the water. The yeast, of course, grew more rapidly at high than at low temperatures, so that less was needed at high temperatures. If the yeast grew sufficiently to cloud the water, the larvae were apt to die. The method of feeding each larva separately is subjective, but the results were much more consistent than the results of experiments with known and definite amounts of yeast. In this later type of experiment, a yeast concentration suitable for larvae kept at, say, 20°, would be lethal for larvae kept at 30°. Attempts to grow larvae at a constant temperature of 35° failed, but we do not

TABLE II

GROWTH OF HAEMAGOGUS LARVAE AT VARIOUS TEMPERATURES

(Parallel experiments with 44 larvae in each lot in individual tubes with tap water and brewer's yeast)

Temperature	Average Length of Stages in Days					Total Time Egg to Adult
	I	II	III	IV	Pupal	
20° C.	5.1	2.9	3.1	8.0	6.9	26.0
25° C.	3.4	1.8	2.2	5.7	4.9	18.0
Room temperature (mean 25.5° C.)	2.9	1.3	2.0	4.7	4.1	15.0
30° C.	2.4	1.2	1.6	4.3	3.0	12.5

know whether the failure was the result of a direct effect of the temperature on the larvae, or was caused indirectly by effect on the culture medium.

In the experiments summarized in Table II, the 20° and 30° temperatures were maintained in incubators with fair accuracy, subject only to the hazards of our local power source. The 25° temperature was obtained in a cellar, which during the period of the experiment showed a mean temperature of 24.6°, with an absolute maximum of 25.5° and minimum of 23.0°; the diurnal fluctuation was normally less than 1°. "Room temperature" during the experiment showed a mean of 25.5°, an average maximum of 27.9°, an average minimum of 23.0, an absolute maximum of 30.5° and an absolute minimum of 21.5°. There seems to be a slight acceleration of development under the variable "room temperature" conditions; otherwise the time-temperature curve for *Haemagogus spegazzinii* is similar to that obtained with other species of mosquitoes (e. g., by Headlee, 1942; Hurlbut, 1943; Huffaker, 1944), though development at any given temperature is

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slower with *Haemagogus spegazzinii* than with *Aëdes aegypti* or *Anopheles quadrimaculatus*.

The difference in relative length of the four larval stages in mosquitoes is interesting. Possibly the relative length of any given stage is a specific characteristic, since various authors, working with different species, have arrived at different figures (Bates, 1941). In the case of *Haemagogus spegazzinii*, an average of the figures quoted in Table II gives the following proportions for each stage: I, 26 per cent of total larval developmental period; II, 14 per cent; III, 17 per cent; IV, 43 per cent. The distribution under the various temperature conditions is closely similar. The fourth stage seems always to be the longest in mosquito larvae; the possible significance of this has been commented on by Huffaker (1944).

It has often been observed that with mosquitoes, as with other insects, males develop faster than females; but exact figures on the amount of the difference have not come to our attention. The developmental period in the temperature experiments is analyzed by sex in

TABLE III

LENGTH OF DEVELOPMENTAL PERIOD BY SEX IN *Haemagogus spegazzinii*

Temperature	Developmental Period in Days		
	Males	Females	Difference
20° C.	25.6	27.1	1.5
25° C.	17.7	18.2	0.5
Room temperature	14.8	15.4	0.6
30° C.	12.3	12.6	0.3

Table III. To check whether the slowing of female development might be a characteristic of some particular period, such as fourth stage larva or pupa, the data for the 20° group were analyzed in detail. The difference in speed is apparently spread over most of the period of development: the average length of the first stage was the same in both sexes, but the second stage was 0.2 days shorter in males, the third stage also 0.2 days shorter, the fourth stage 0.7 days shorter, and the pupal stage 0.4 days shorter.

SIZE OF ADULTS

It has long been known that the lower the temperature of the larval environment, the larger the adults (within a given species, of course). This is based primarily on the observation that spring mosquitoes in the temperate zone are larger than summer specimens of the same species. The subject became of some importance in Europe with the discovery that size was a differentiating character between two anopheline populations in the Netherlands, one a malaria vector and the other not. The history of this has been reviewed by Swellengrebel and de Buck (1938). Nevertheless there seem to be no published data

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on the relation between larval environmental temperature and adult size. To obtain such data, we saved the adults from the temperature experiment summarized in Table II. The right wing and right metathoracic leg of each mosquito were mounted dry on a slide and measured with an ocular micrometer. Various measurements were taken, but the most satisfactory, as a general index of size, seemed to be the wing length measured from the cleft of the alula to the apex—the alula rather than the base of the wing being taken to avoid error because of differences in the point at which the wing was broken. The data from these measurements are summarized in Table IV.

When we realized how much larger and how much hardier the mosquitoes raised at 20° were than those raised at higher temperatures, we adopted 20° as a routine temperature for raising adults for a series of attempts at colonizing the species. Unfortunately, we did not make any clearly comparable tests of adult longevity of mosquitoes raised at

TABLE IV

RELATION OF LARVAL TEMPERATURE TO ADULT SIZE
(Length of wing, from cleft of alula to apex in mm.)

Temperature	Males		Females	
	No. Specimens	Length and Standard Deviation	No. Specimens	Length and Standard Deviation
20° C.	17	2.69 ± .09	16	3.21 ± .06
25° C.	14	2.56 ± .07	21	3.01 ± .08
Room temperature	21	2.61 ± .07	13	3.01 ± .05
30° C.	18	2.43 ± .08	12	2.84 ± .05

various temperatures, but we had a clear impression that the 20° adults survived adverse conditions much better than those raised at room temperature or at 30°: they were more active in cages, bit more readily (even though unfertilized) and withstood periodic exposures to high temperatures such as would result from leaving the cages for half an hour or more daily in bright sunshine. We did not succeed, however, in inducing sexual activity in such adults.

ADULT LONGEVITY UNDER LABORATORY CONDITIONS

Mosquitoes of the genus *Haemagogus* were early suspected as vectors of sylvatic yellow fever, but attempts to obtain virus transmission in the laboratory failed because of difficulty in keeping the mosquitoes alive through the extrinsic incubation period of the virus (Kumm and Frobisher, 1932; Antunes and Whitman, 1937). In these early experiments conventional methods of maintaining mosquitoes were used: the mosquitoes were kept in cages, "protected from the wind and covered with a layer of wet absorbent cotton in order to increase the humidity" (Antunes and Whitman). We used similar methods and obtained similar results: most mosquitoes died in five or six days, and only an

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occasional specimen would live through the presumed incubation period of the virus. We found that longevity could be increased if the mosquitoes were kept at low temperatures (20°), but this was of little help since the extrinsic incubation period of the virus would also be greatly prolonged. We made a great many experiments, attacking the problem from various points of view, such as frequency of blood meals, type of food other than blood, type of cage, humidity and temperature; and we eventually worked out quite satisfactory techniques for our purposes. For the most part, these experiments were not controlled with sufficient care to be of value for the analysis of problems of mosquito physiology, and there is no point in summarizing them in detail. They do, however, illustrate the difficulty of laboratory experimentation with problems like longevity, and they demonstrate the specific nature of the physiological adaptations that govern longevity under laboratory conditions, since techniques that were successful with *Haemagogus spegazzinii* were unsuccessful with other diurnal forest mosquitoes and vice versa.

The literature on factors governing insect longevity is enormous, and a very considerable number of studies have been made of the survival of various mosquito species under laboratory conditions. The subject is of obvious practical importance, since the efficiency of a given species as a vector of disease depends, in part, on its relative longevity. Because the study of longevity in nature is very difficult, laboratory study offers a promising line of attack. Outstanding studies of this type, with general reviews of the literature, are those by Russell and Rao (1942) and Sinton and Shute (1938).

The method that we eventually adopted for the maintenance of infected haemagogus has been described in detail in a previous article (Bates and Roca, 1945a). Essentially it consisted in keeping the mosquitoes in individual flat-bottomed glass vials (25x50 cm. in size) with a layer of moist cotton in the bottom covered with a disc of filter paper (to prevent the mosquitoes' getting entangled in the cotton) and plugged with a cup of aluminum (or monel) wire screening containing a small wad of cotton soaked in sugar solution for food. The vials were kept in racks in an environment with air in constant movement: we put fans in all incubators, and when mosquitoes were kept in open rooms, we found it advisable to place them near an electric fan. It seemed, in other words, important that the mosquitoes have a source of moisture always available (the wet cotton in the bottom of the vials) but that they be maintained in a relatively dry atmosphere (usually about 70 per cent relative humidity) with the air in constant movement. Satisfactory survival could also be obtained in a cage with large numbers of mosquitoes if the bottom was covered with moist cotton, and the cage kept near a fan.

We made a total of 290 infection experiments with yellow fever virus in mosquitoes, principally *Haemagogus spegazzinii*. Most of these experiments are of no use for longevity studies, however, since specimens were removed and killed at varying intervals to test for virus development. In some cases the "control" mosquitoes showed that the lot had received no virus, and such lots were often kept specifically for longevity tests. Also, as a general rule, no mosquitoes were

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removed from an infected lot during the first 10 days; thus comparison can be made of percentage of survival in the 10-day period under various environmental conditions.

It is interesting that the method of individual vials was satisfactory only with diurnal forest mosquitoes of the canopy zone, such as *Haemagogus* and *Sabethoides* (Bates, 1944); mosquitoes of the forest floor zone, such as *Aëdes serratus* and *Psorophora ferox* required quite different treatment. Thus one lot of 26 *Aëdes serratus* kept under standard haemagogus conditions in vials at a constant temperature of 30° had a mean life of 4.9 days and a maximum survival of 7 days; the mean life *Haemagogus spegazzinii* under identical conditions was 13.8 days (based on 512 specimens in 14 experiments), with maximum survival about 50 days. In 42 different lots of *spegazzinii* kept under these conditions, survival after 10 days was almost always over 50 per cent, with an average survival for this period of 63 per cent.

On the other hand, *Psorophora ferox* and *Aëdes serratus* showed excellent survival in small cages in a subterranean room where the

TABLE V

SURVIVAL OF MOSQUITOES IN SMALL CAGES IN SUBTERRANEAN ROOM
(Temperature fairly constant at 25°)

Species	No. Experiments	No. Mosquitoes	Survival in Days	
			Mean	Average Maximum
Haemagogus spegazzinii.....	8	295	7.6	13.3
Aedes serratus.....	5	138	26.7	51.8
Psorophora ferox.....	5	86	20.8	36.8
Psorophora cingulata.....	4	135	13.4	25.8

temperature was quite constant at 25°, and the humidity nearly saturated. Data from a series of comparable experiments under these conditions with three diurnal forest mosquitoes and one crepuscular species (*Psorophora cingulata*) are summarized in Table V. Under these conditions, one would judge *Aëdes serratus* to be a long-lived species and *Haemagogus spegazzinii* a short-lived one—precisely the opposite of the results obtained under the conditions described in the preceding paragraph.

The experiments mentioned above were all made with mosquitoes caught as adults and brought to the laboratory. They were thus of unknown age at the start of the experiment. Longevity experiments with such mosquitoes, however, gave quite consistent results; in the case of *Haemagogus spegazzinii*, transmission experiments were made with wild caught mosquitoes throughout the year, with no difference in average survival from one time of year to another. There was, however, a very great difference in survival between wild caught haemagogus and specimens raised in the laboratory at 30°, with yeast as larval food. Four hundred and four such specimens were used in infection exper-

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iments; only 28 per cent survived the first 10 days in vials at 30°, in contrast with an average survival of 63 per cent of wild caught specimens (based on 1,423 specimens) over the same period. Comparable experiments were not made with adults bred from larvae kept at lower temperatures, but as remarked above, we have the impression that such adults were much hardier. At any rate, it is clear that in the case of laboratory-bred mosquitoes, the larval culture conditions may have a controlling influence on adult longevity.

It has often been demonstrated that virus infection has no obvious adverse effect on the vector mosquito. To check this a series of 27 consecutive infection experiments in which haemagogus were maintained in vials at 30° were selected and divided into three categories: first those in which the source animal was subsequently found not to have circulated virus at the time of the experiment, so that the mosquitoes were uninfected; second, those in which the source animal was circulating only a small amount of virus, so that few mosquitoes were infected; and third, those in which the source animal was circulating a

TABLE VI

AVERAGE SURVIVAL IN DAYS OF *Haemagogus spegazzinii* IN INDIVIDUAL TUBES UNDER VARIOUS TEMPERATURE CONDITIONS

Temperature	No. Experiments	No. Mosquitoes	Average Survival
20° C. constant	3	66	19.4
25° C. constant	3	128	18.3
26.6° C. (20 Hr. 25° C, 4 Hr. 35° C.)	3	154	14.5
30° C. constant	14	512	13.8
35° C. constant	3	132	4.8

large amount of virus, so that about 90 per cent of the mosquitoes were found to be infected. After 10 days, 60 per cent of 334 non-infected mosquitoes, 74 per cent of 283 partially infected mosquitoes, and 60 per cent of 784 heavily infected mosquitoes were still alive.

EFFECT OF TEMPERATURE ON HAEMAGOGUS ADULTS

The effect of temperature on the development of virus in haemagogus mosquitoes has been discussed in a previous paper (Bates and Roca, 1946b). Transmission experiments were carried out routinely at constant temperature of 30°; but since such temperature conditions would be unknown in nature, we made a few transmission experiments at various other constant and alternating temperatures. We found that virus development in mosquitoes kept for 20 hours at 25° and four hours at 35° daily was almost as rapid as that in mosquitoes kept at 30° constantly. In one set of parallel experiments, for instance, the minimum incubation period for the virus was 28 days at 25°, 23 days at 25°–30°, 12 days at 25°–35°, and 10 days at 30°. We failed to get transmissions at constant temperatures of 20° or 35°. It thus appeared that the four-hour daily exposure to 35° had a very considerable accel-

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erating effect on virus development not explainable by the mean temperature, which calculated on an hourly basis would be 26.6°. We thought it would be interesting to attempt to measure the effect of these temperature conditions on the mosquito.

The average survival of haemagogus mosquitoes in individual vials under various temperature conditions is summarized in Table VI. The figures are based on few experiments and relatively few specimens; but the results with various individual experiments are quite consistent, and the survival figures are probably significant. It is interesting that survival is shortened by the four-hour daily exposure to 35° below what would be expected from the hourly mean (26.6°).

As a check on the influence of temperature on a physiological process in the mosquito, we kept detailed records on oviposition in several experiments. The results are summarized in Table VII. These mosquitoes all engorged on a monkey at the start of the experiment (the

TABLE VII

OVIPOSITION OF HAEMAGOGUS UNDER VARIOUS TEMPERATURE CONDITIONS
(88 mosquitoes in each lot)

	TEMPERATURE			
	25° C.	25° C.-35° C.	30° C.	35° C.
Per cent alive at 10 days.....	53	47	43	2
Average day of first eggs....	7.2	6.2	6.2	6.5
Per cent laying eggs.....	61	57	39	5
Total number eggs in 10 days	1144	1121	742	57
Number eggs per mosquito laying.....	21	22	22	11

infectious meal) and subsequently received no food except sugar solution. It is interesting that the number of mosquitoes laying eggs and the number of eggs laid are practically the same at 25° constant temperature, and at the 25°–35° alternating temperature. Egg development, however, is speeded up by the daily exposure to 35°, being the same (6.2 days) as in mosquitoes kept at a constant temperature of 30°. Egg development in the mosquito seems to act like virus development. The number of mosquitoes laying eggs is appreciably reduced at the constant temperature of 30°, more so than would be explicable by the increased mortality. The number of eggs per mosquito laying, however, remains the same: in other words, a certain proportion of individuals are inhibited from oviposition by the constant temperature of 30°. A constant temperature of 35° seems to be very unfavorable for *Haemagogus spegazzinii* from every point of view.

DISCUSSION

Haemagogus spegazzinii is a diurnal forest mosquito with a peak of activity toward mid-day; it is found most abundantly in the forest canopy or in open sunny clearings or around forest margins. Its bright

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metallic coloration may be an adaptation to this relatively dry and warm environment. The larvae have been found in tree-holes, most commonly in holes with very narrow apertures; they are scarce in relation to the number of adult mosquitoes, and it seems likely that the chief breeding place is some type of tree-hole or container habitat that has been overlooked in forest studies. Conditions favorable for haemagogus survival in the laboratory are very different from conditions favorable for the survival of the mosquitoes of the forest floor zone (*Psorophora ferox* and *Aëdes serratus*), and it is difficult to avoid the conclusion that these laboratory differences reflect physiological adaptations to the different natural environments. A constant high relative humidity seems to be definitely unfavorable for haemagogus and these mosquitoes are very short lived when kept in a subterranean room in small cages with added moisture—conditions that seem to be ideal for the mosquitoes of the forest floor zone (Table V). The mean life in cages in such a room was 7.6 days; whereas at the same temperature (25°) in individual vials with screen stoppers, in an incubator provided with an electric fan, the mean life was 18.3 days (Table VI). It is well known that many mosquitoes avoid extremely high humidities (Thomson, 1938), but in general the higher the humidity, the longer the survival (Hundertmark, 1938; Leeson, 1939). It would be interesting to investigate the mechanism of the unfavorable effect of the cellar environment on haemagogus.

The correspondence between conditions favorable for haemagogus mosquitoes and conditions favorable for yellow fever virus is interesting. A constant temperature of 30° was very favorable for virus development, but relatively unfavorable for the mosquitoes; twenty hours daily at 25° and four hours at 35°, however, proved to be almost equally favorable for the virus, and much better for the mosquitoes than the constant temperature. It is very likely that the mosquito would be subject to an alternating temperature of this order of magnitude in nature, since 25° is close to the mean forest temperature at the floor zone, where conditions are quite constant day and night, and 35° is a very likely body temperature for daily periods of activity in a sun-loving mosquito like haemagogus.

It is interesting to speculate as to the possible nature of the "optimum" temperature for these various processes. With regard to larval development, the "optimum" is generally considered to be the temperature at which the greatest speed of development coincides with a minimum mortality (e. g., Mosna, 1937) or perhaps the highest temperature at which clearly adverse effects are not apparent. Yet "optimum" carries the connotation of "most favorable for the species," which surely involves many factors other than mere speed of growth. In haemagogus, for instance, the size (and apparently the hardiness) of the adult is in part a function of the larval temperature, and this would have to be taken into account in attempting to determine what larval temperature would be most favorable for the species. In the case of adults, survival under protected conditions is longer the lower the temperature, but in nature this longer survival might be counterbalanced by the slowing up of physiological processes such as egg development, and the consequent greater hazard of accident to the

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mosquito before these processes were carried through. In this case, the highest temperature at which adverse effects are not demonstrable might well be the "optimum" for the species. That such adverse effects appear is shown by the decreased oviposition, for instance, at 30° and 35° in the present studies. The fact that egg development proceeds at the same rate at the 25°–35° alternation of temperature as at the constant temperature of 30°, and that oviposition and survival are much greater at the alternating temperature, might well be taken as indicating that some such conditions as these were "most favorable for the species." They are certainly most favorable for the virus!

SUMMARY

Experiments were carried out testing the effect of food and larval temperature on the development of *Haemagogus spegazzinii*. Brewer's yeast was found to be an excellent food for this species, though inadequate for *Aëdes serratus* or *Psorophora ferox*. Larval development required 26 days at 20°, 18 days at 25° and 12.5 days at 30° C. Adults developing from larvae kept at lower temperatures were larger (and apparently hardier) than those from larvae kept at higher temperatures. Data are given on the difference in size. Of the total period of larval development, 26 per cent was spent in the first stage, 14 per cent in the second, 17 per cent in the third, and 43 per cent in the fourth, the proportions being the same at the various temperatures tested. The males developed faster than the females, the increase in developmental speed being demonstrable from the second larval stage on.

Survival of *Haemagogus spegazzinii* was very unsatisfactory with mosquitoes kept in cages provided with moisture and maintained in a room at 25° with a high relative humidity. Survival of mosquitoes of the forest floor zone (*Aëdes serratus*, *Psorophora ferox*, *P. cingulata*) was excellent under these conditions. *Haemagogus* seemed to require an adequate supply of available mositure, but a relatively dry atmosphere; and best results were obtained with mosquitoes in individual screen-stoppered vials with constant air movement provided by an electric fan. Survival of *Aëdes serratus* and simiilar mosquitoes was very poor under these conditions.

Survival of haemagogus adults bred from larvae kept at 30° was very poor, as compared with survival of wild caught adults. Infection with yellow fever virus had no effect on adult longevity.

In general, the lower the temperature, the longer the survival of adults within the range tested (20°–35°). Unfavorable effects measurable in decreased oviposition were apparent at 30° and 35°. Speed of egg development (and virus development) was almost the same at an alternating temperature of 20 hours daily at 25° and 4 hours at 35° as at a constant temperature of 30°; the proportion of specimens ovipositing and the average length of life were both greater at the alternating temperature. The difference between behavior at 25° and at the 25°–35° alternation cannot be accounted for by the increase in mean temperature, since this, calculated on an hourly basis, was only 26.6°.

The relation between the laboratory results and the habits of the mosquito in nature is discussed, together with some comment on the significance of the concept of "optimum temperature."

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