# PUPATION AND EMERGENCE IN *AËDES TAENIORHYNCHUS* (WIED.).



BY

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# PUPATION AND EMERGENCE IN *AËDES TAENIORHYNCHUS* (WIED.).

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Ethological investigations of the salt-marsh mosquito, *Aëdes taeniorhynchus* (Wiedemann), have disclosed a rather special importance to the time of day of emergence. Interest has therefore focused on the happenings around this critical time in the insect's life. In order to understand emergence and the behaviour of the mosquitos immediately after emergence—including such important habits as mating and migrating—it was found necessary to have further information on some aspects of the development of the aquatic stages.

Field observations and rearing experiments were undertaken for this purpose at intervals between October 1951 and January 1953. It was hoped that a few experiments would be sufficient, but the problem was soon found to be considerably more complicated than expected, and the experiments reported here are only preliminary. From them, however, a few rather puzzling facts have been obtained, and an understanding of them may only be obtained by further experiments. Excellent conditions for such experiments will be provided in the proposed Entomological Research Centre of the Florida State Board of Health. The interruption occasioned by the establishing of this centre can be advantageously used in summarising the knowledge so far obtained.

## Experimental Technique.

Since the technique varied from one experiment to the next, with some experiments being made in order to find the most suitable method, a short description of each experiment will follow.

*Experiment 1.*

About 30,000 fourth-instar larvae and a few pupae were transported in 60 half-pint ice cream cardboard cartons from the Big Pond in Fort Pierce the 75 miles to Archbold Biological Station, Lake Placid on 7 October 1951. They were placed in a room in which temperature and light could be regulated, the temperature at 28° to 30°C.* daytime and 19° to 22° night-time; " Sunrise " was simulated at 0730–0800 hr., and " Sunset " at 1945–2015 hr. As this experiment was made in order to get information on the behaviour of imagines of known age, only a few data on the larval development were recorded.

*Experiment 2.*

Larvae were obtained from a mangrove swamp on Sanibel Island (off the south-west coast of Florida), 20 November 1951. Emergence had been in progress several days. Most of the collection was of fourth-instar larvae, but there were also a few third-instar larvae and pupae. The natural water in the mangrove pools was rather dark and had a specific gravity of 1·005 at 22°.

The samples were taken to the laboratory at Lake Placid as fast as possible but many larvae died during transport. The remaining, healthy-looking larvae (all fourth-instar) were transferred to ice-cream cartons, 100 larvae to each. The surface area of a carton is 55 sq. cm., and the volume of water was kept at

* All temperatures in this paper are given in degrees Centigrade.

100 ml. by frequently adding distilled water which was kept in the room to avoid temperature shock. The experiment comprised five series:—

A. Natural water. No feeding until 0945 hr. on 24 November.
B. Natural water. Fed with dog biscuit once a day from 20 November.
C. As in B, but transferred to distilled water on 26 November.
D. At once transferred to distilled water; fed as B and C.
E. A single carton with 200 larvae in which the natural water remained unchanged and the dead larvae and exuviae were not removed. Fed as B, C, and D.

In series A there were 10 cartons, in B and C four cartons each, in D two cartons, and in E, as mentioned above, there was but one carton with 200 larvae. The cartons were placed on tables in the mosquito chamber. The temperature was kept at 27° to 28°; sunrise was simulated at 0900 hr. and sunset at 2100 hr.

*Experiment 3* (29.xi. to 2.xii.1951).

Rain-produced, fourth-instar larvae and pupae were brought to the field laboratory on Sanibel Island from a natural breeding area on the island, 29 November 1951. They were kept in jars at room temperature (18° to 21°, mean 20°) and the emergence studied.

*Experiment 4* (16 to 21.xii.1951).

A piece of sod from the Fort Pierce study area was flooded at the Archbold Station and the five emerging larvae kept in a one-gallon beaker. They were fed with standard food (pulverised dog biscuit + dry yeast, 1:1). Tungsten light 0730–2230 hr. plus ultraviolet light 1000–1030 hr.

*Experiment 5* (14–29.ii.1952).

Rain-produced (2 to 3 February) fourth-instar larvae and a few pupae collected on Sanibel Island, 14 February 1952, and brought to the field laboratory for study of emergence. Kept at room temperature (17° to 23°, mean 20°). There were five series:—

A. 1,000 larvae were separated from the general collection at 1400 hr. on 14 February. On 17 to 21 February, 620 adults emerged but there was no pupation after 20 February. The remaining larvae were fed with pulverised dog biscuit and 60 more adults emerged between 25 and 29 February.
B. 109 individuals, pupated 14 February, 1400–1800 hr.
C. 219 individuals, pupated 15 February, 0000–0900 hr.
D. 178 individuals, pupated 15 February, 0000–2200 hr.
E. 136 individuals, pupated 15 February, 1000–2200 hr.

*Experiment 6* (3 to 6.v.1952).

A batch of tide-produced larvae on Sanibel Island was brought to the field laboratory for observation. Pupation had just begun in the field. Kept under natural light conditions and at room temperature (mean 26°). Pupae were removed at hourly intervals and the resulting emergence recorded every hour.

*Experiment 7* (3 to 10.xi.1952).

An egg-rich sod from Sanibel Island was flooded at the Archbold Biological Station. Half the larvae were kept singly in small glasses with 5 ml. water and fed with varying numbers of drops of standard food, 0·5 gm. ground dog biscuit plus 0·5 gm. dry yeast suspended in 50 ml. water. Temperature, 28°; light 1200–2300 hr. The other half of the larvae were kept, 5 together, in gallon beakers as in Experiment 4.

*Experiment 8* (14 to 23.xi.1952).

Larvae were obtained as in the previous experiment and observations similarly made at the Archbold Station. Fed as in Experiment 7; temperature 28°. Seventeen series with different combinations of light, hatching time, and medium, the latter being distilled water (H), 2 per cent. NaCl solution (S), or natural water from a mangrove area (M) on Sanibel Island.

A. (AH, AS, AM) in daylight, hatched 14 November at 0000 hr.
B. (BM) ,, ,, ,, ,, ,, ,, 0800 ,,
C. (CM) ,, ,, ,, ,, ,, ,, 1600 ,,
D. (DH, DS, DM) artificial light 2300–0900 hr. hatched 14 November at 0000 hr.
E. (EH, ES, EM) ,, ,, ,, ,, ,, ,, ,, ,, ,, 0800 ,,
F. (FH, FS, FM) ,, ,, ,, ,, ,, ,, ,, ,, ,, 1600 ,,
G. (GH, GS, GM) in darkness, inspected and handled by weak dark red light. Hatched 14 November at 0000 hr.

*Experiment 9* (1 to 8.xii.1952).

An egg-laden sod from Sanibel Island was flooded with tapwater in pans at Archbold Station and the sod removed after about 36 hours. Fed with standard food as in Experiment 7; temperature 29°. Four series, with varied hatching and light times:—

A. Flooded 1 December at 0000 hr. Light 2200 hr. "midnight" 1600 hr.
B. ,, ,, ,, ,, 0800 ,, ,, ,, ,, ,, ,, ,,
C. ,, ,, ,, ,, 1600 ,, ,, ,, ,, ,, ,, ,,
D. ,, ,, ,, ,, 0000 ,, Constant darkness.

*Experiment 10* (21 to 29.i.1953).

The same type of sod as in the preceding experiments was used but most of the eggs, now 5 or 6 months old, seemed to be dead. Of six series started, only two successfully produced larvae:—

A. Flooded 21 January at 0930 hr. Larvae poured off at 1120 hr. Kept in constant light.
B. As in A but a lid was placed over the pan every day between 2000 and 1000 hr.

## Duration of Larval Stage.

*Effect of temperature.*

The duration of the larval stage is dependent on several factors. Water temperature is indisputably important and under natural breeding conditions is probably the main factor. During the summer months in Florida, when the mean temperature is about 30°, the interval from hatching to pupation is not usually more than five or six days. A frequent situation is for the first three instars to last one day each and the fourth instar 2 to 3 days, or a little less than the first three instars put together. In the colder seasons, larval development may last 2 to 3 weeks.

Recent observations made under actual field conditions on Sanibel Island in late July and early August showed that the larval period could be as short as 3 to 4 days. This occurred in water exposed to direct sunlight and not over 1 to 3 inches deep, where the temperature ranged between 27° and 38° throughout the day. These pools were in a grassy, mowed-over field; the larvae could not escape the warm water. The same rainfall had hatched eggs in many depressions on the island. In one large swale, where the water averaged 10 to 12 inches in depth, with many large areas 18 inches deep, and where there was plenty of shade, it was observed that it took 6 to 7 days for 5 per cent. and 9 days for about 10 per cent. of the larvae to reach pupation. In the shallow pools mentioned earlier there was little difference in temperature between top and bottom, but in this larger and deeper swale, water temperatures averaged 33° at the surface and 28° to 29° six to twelve inches below the surface. It was noted that the larvae spent most of their time at these cooler depths.

Unfortunately, all the experiments in which the larvae were hatched from eggs in the laboratory were made at 28° or 29°. There are therefore no experimental data on the influence of temperature on the larval development.

*Effect of other factors.*

Even at uniform temperature, however, there is a considerable variation in the duration of the larval stage. Table I gives the data for all the experiments

TABLE I.

Age of pupation for all experiments in which hatching time was known.

| Experiment number | 4 | 7A | 7B | 8 | 9 | 10 |
|---|---|---|---|---|---|---|
| Number of pupae .. .. .. | 5 | 11 | 4 | 403 | 770 | 18 |
| % pupated on:— | | | | | | |
| 5th day (96–119 hr. old) .. | 100 | | 75 | 13 | 12 | 50 |
| 6th day (120–143 hr. old) .. | | 91 | 25 | 49 | 24 | 44 |
| 7th day (144–167 hr. old) .. | | | | 34 | 12 | 6 |
| 8th day (168–191 hr. old) .. | | 9 | | 4 | 46 | |
| 9th day (192–215 hr. old) .. | | | | | 6 | |
| Mean age (in hours) at pupation .. | 98 | 133 | 121 | 141 | 158 | 138 |

Only pupation previous to 10th day is considered in this Table. Temperature (28°–29°C.) and feeding conditions uniform for all experiments.

in which the age of the larvae was known. It will be seen that in the experiments with few individuals the duration of the larval stage is shorter than in the "big" experiments, nos. 8 and 9. In Experiment 8, about 7 larvae remained, unpupated, after the ninth day while in Experiment 9 the pupation by the ninth day represented less than half the larvae. In the earlier experiments, in which the age of the larvae was unknown, there had been experienced the same phenomenon of prolonged larval stage, usually combined with very high mortality.

That the factor involved is hunger was demonstrated in Experiments 2 and 5. In Experiment 2, fourth-instar larvae were brought in from the field; 1,000 larvae were fed with dog biscuit every day and yielded 442 pupae, another batch of 1,000 larvae were not fed for the first 88 hours, by which time only 6 per cent. had pupated. In the larval group which had been fed, pupation went on, but no pupae appeared among the unfed larvae during the next four days. Pupation was resumed only 60 hours after these starved animals were fed, but the total number of pupae from this batch of 1,000 larvae was only 223. In the fifth experiment the same thing occurred. In series A, containing 1,000 fourth-instar larvae, pupation stopped after 3 to 4 days of hunger. The remaining larvae pupated only after feeding was started.

The delay in development and high mortality in Experiment 9 were probably also caused by insufficient food intake. In that case, however, it was not because there was too little food present but because too many animals were kept together in large pans with but little water. Most of the feeding takes place at the bottom, and it was observed that the feeding larvae were incessantly disturbed by those wriggling to the surface for respiration. Under crowded conditions it might be dangerous for larvae in a feeding stage if the depth of water is less than 3 to 4 cm. At the other extreme, living completely isolated seems also to delay development. In Experiment 7, half of the individuals (7B) were kept, five together, in gallon beakers (as in Experiment 4); 5 pupated on the fifth day and 3 on the following day, all of them when 115 to 124 hours old. Among the nine larvae isolated in small vials (7A) the first pupated at 139 hours and the last when 189 hours old.

The larvae probably feed mostly during the first half of each instar. In the case of fourth-instar larvae introduced into waters containing radioactive phosphorus for marking, it was observed that the increase in larval radioactivity took place mostly in the early part of the stage (Dr. Maurice W. Provost, personal communication).

In Experiment 8 an attempt was made to evaluate the rôle of water characteristics in setting the duration of the larval stage. Table II gives the results.

TABLE II.

Age at pupation in different types of water.

| Type of water | Distilled water | 2% NaCl | Mangrove water |
|---|---|---|---|
| Number of pupae | 191 | 117 | 95 |
| % pupated on:— | | | |
| 5th day (96–119 hr. old) .. | 12 | 12 | 15 |
| 6th day (120–143 hr. old) .. | 47 | 42 | 59 |
| 7th day (144–167 hr. old) .. | 34 | 44 | 25 |
| 8th day (168–192 hr. old) .. | 7 | 2 | 1 |
| Mean age (in hours) at pupation .. | 141 | 139 | 132 |

Data from Experiment 8.

The fastest development occurred in the mangrove water but the differences were too small to warrant a definite conclusion. No important error, if any, was made in pooling the results of the three series.

*Sex differences.*

From the earliest experiments it was evident that those larvae which later became male adults pupated before those which became female imagines. In all experiments it was found that the first pupae isolated in a brood became mostly males while the last ones became females. From the experiments in which the time of hatching was known and in which the pupae were isolated, it is possible to give figures for the duration of the development of the two sexes. It is seen in Table III that the larvae of females were considerably slower in developing than those of males. Before discussing these findings any further, another peculiarity of pupation must be considered.

*The 24-hour rhythm in pupation.*

It was evident from the earliest experiment that emergence of imagines was not evenly distributed over the 24 hours but had a definite daily maximum; this will be discussed below. In the second experiment the same was found to be

TABLE III.

Sexual differences in the duration of the larval stage.

| Sex | Male | Female |
|---|---|---|
| Number of individuals .. .. | 90 | 89 |
| % pupated on:— | | |
| 5th day (96–119 hr. old) .. | 23 | 6 |
| 6th day (120–143 hr. old) .. | 64 | 36 |
| 7th day (144–167 hr. old) .. | 13 | 57 |
| 8th day (168–192 hr. old) .. | | 1 |
| Mean age (in hours) at pupation .. | 129 | 145 |

Data from Experiments 4, 7, 8D, 8E, 8F, and 10.

TABLE IV.

Distribution by percentage of pupation over 24 hours in all experiments with dark and light periods.

| Experiment | 2 | 4 | 6 | 7 | 8A–F | 9A–C | 10 | Mean |
|---|---|---|---|---|---|---|---|---|
| Number of animals | 754 | 5 | 1,886 | 15 | 315 | 432 | 18 | 3,424 |
| Hour of pupation:— | | | | | | | | |
| 00–03 .. .. | 7 | 0 | 0 | 0 | 4 | 9 | 0 | 3·2 |
| 03–06 .. .. | 4 | 0 | 0 | 0 | 1 | 1 | 0 | 1·1 |
| 06–09 .. .. | 5 | 0 | 0 | 0 | 3 | 0 | 6 | 1·5 |
| 09–12 .. .. | 8 | 0 | 0 | 0 | 9 | 2 | 0 | 2·8 |
| 12–15 .. .. | **17** | 0 | 1 | **40** | **27** | 11 | 11 | 8·4 |
| 15–18 .. .. | **34** | 0 | 11 | **47** | **27** | 9 | 17 | 17·7 |
| 18–21 .. .. | **17** | **80** | **67** | 13 | **22** | **43** | **44** | 48·2 |
| 21–24 .. .. | 8 | **20** | **21** | 0 | 7 | **25** | **22** | 17·1 |

Time is given as hours (00 to 24) beyond experimental midnight (00 hour) which is the midpoint of the period of darkness, whether natural or artificial.

TABLE V.

Distribution by percentage of pupation in relation to different 24-hour datum types.

| | Alternating light and darkness | | | All darkness |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Hour made 00 below | Exp. midnight (as in Table IV) | Midnight E.S.T. | Hatching hour | Midnight E.S.T. |
| 00 – 03 .. .. | 3 | 8 | 12 | 12 |
| 03 – 06 .. .. | 1 | 7 | 16 | 3 |
| 06 – 09 .. .. | 2 | 7 | 8 | 23 |
| 09 – 12 .. .. | 3 | 15 | 16 | 17 |
| 12 – 15 .. .. | 8 | 10 | 12 | 6 |
| 15 – 18 .. .. | 18 | 11 | 10 | 7 |
| 18 – 21 .. .. | 48 | 19 | 12 | 16 |
| 21 – 24 .. .. | 17 | 23 | 14 | 16 |

Based on same data as Table IV, plus Experiments 8G and 9D (column 4).

the case with pupation, and this has been confirmed by all the experiments recorded in Table IV. Briefly stated, it is seen that 83 per cent. of all pupation took place during the evening periods (15 to 24 hours after experimental midnight) and 48 per cent. in the period just after sunset (18 to 21 hours after experimental midnight). That this period of maximum pupation is determined by the distribution of light and darkness is beyond doubt, as the following will attest.

The pupation times in Table IV are arranged according to hours (00 to 24) beyond experimental midnight, which is the midpoint of the period of darkness, whether natural or artificial. Arranged according to " civil time ", *i.e.*, Eastern Standard Time (Table V, col. 2) the daily maximum nearly disappears; if arranged according to hatching time (Table V, col. 3) the maximum disappears. Neither is a daily rhythm of pupation discernible in the case of larvae reared in darkness (Table V, col. 4). It is obvious then that the 24-hour pupation rhythm occurs in the mean only when the experiments are pooled according to the actual change of light and darkness. The maximum occurs around sunset.

With this knowledge it might be worth while to examine the hour of hatching of the eggs in relation to experimental midnight. In Experiments 8 and 9 hatching was effected at 8-hour intervals with this point in view. Unsuitable technique delayed larval development in the ninth experiment, so Table VI presents the data from Experiment 8 only. Differences attributable to hatching hour were very slight, which can only mean that the tendency to pupate around sunset has just as much accelerating as retarding effect on the larval development.

TABLE VI.

Duration of the larval stage in relation to the hour of hatching.

| Experiment 8, Series:— | A + F | B + D | C + E |
|---|---|---|---|
| Number of individuals | 187 | 55 | 73 |
| Hatching hour | 00 hr. | 08 hr. | 16 hr. |
| % pupated on:— | | | |
| 5th day ( 96–119 hr. old) .. | 16 | 7 | 10 |
| 6th day (120–143 hr. old) .. | 43 | 53 | 63 |
| 7th day (144–167 hr. old) .. | 34 | 40 | 27 |
| 8th day (168–192 hr. old) .. | 7 | | |
| Mean age (in hours) at pupation .. | 140 | 140 | 136 |

Data from Experiment 8.

## The Pupal Stage and Emergence.

*Influence of temperature.*

Of the three factors found to affect the rate of larval development, one may at once be excluded—nutrition—since the pupae do not eat.

Concerning the effect of temperature, the experiments here considered gave a somewhat better knowledge in the case of pupae than of larvae. In series B of the fifth experiment, pupal development took place at a rather constant temperature of 20·8°, while in Experiment 6 the temperature had a mean of 25·5°. In Experiments 4 and 7 to 10 the mean temperature was fairly constant at about 29°. The duration of the pupal stage in these experiments, as reflecting the temperature effect on rate of development, is summarised in Table VII. Fig. 1 shows that the reciprocal of the duration is a straight-lined function of the temperature. Taking the crudeness of the experiments into account, the approximation is surprisingly good. As the only factor at variance in these groups of experiments was the temperature, it seems justified to consider temperature as the only environmental factor of importance in establishing the duration of the pupal stage.

TABLE VII.

Duration of pupal stage in relation to temperature.

| | | | |
|---|---|---|---|
| Experiment .. .. | 5B | 6 | 4, 7–10 |
| Number of individuals .. | 109 | 1,716 | 450 |
| Temperature (°C.) .. | 20·8 | 25·5 | 29·0 |
| Pupal duration (in hr.) | | | |
| male .. .. | 61·9 | 44·7 | 36·7 |
| female .. .. | 60·0 | 45·7 | 37·5 |

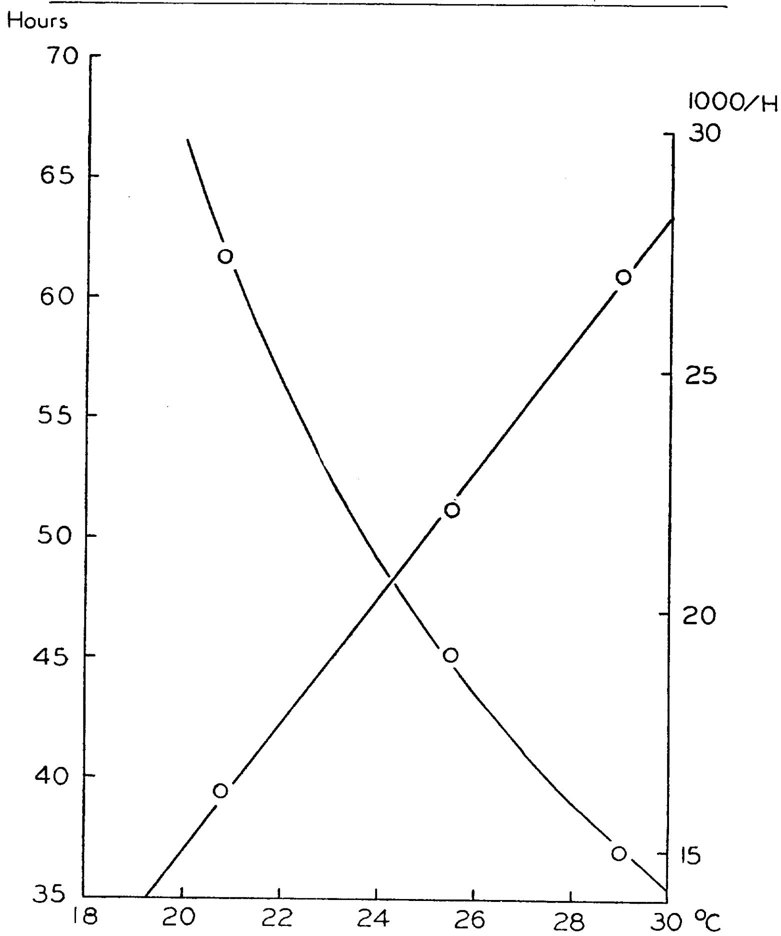


Fig. 1.—Duration of the pupal stage and the velocity of the pupal development in relation to temperature in *Aëdes taeniorhynchus*.

*The 24-hour rhythm in emergence.*

In all the experiments without any exception, and in all observed cases in nature, it has been found that the emergence is not evenly distributed over the 24-hour period but has rather a marked maximum at a certain time of the day, as was found also to be the case with pupation. There is, however, one important difference; maximum pupation was always found to be around the change from light to darkness, but maximum emergence might take place at any time of the day. The reason for this is of course easily understood. If the maximum of pupation, as the effect of the day-night rhythm, is fixed to the period of sunset and the duration of the pupal stage is influenced solely by temperature, it follows that there must be a maximum of emergence which will be at the same time every day during the emergence of the same brood but which may be at any hour of the day, depending on temperatures. " Brood " in the sense just used means a group of animals resulting from a simultaneous hatch and developing at the same temperatures. In experiments with different temperatures and in different breeding areas or seasons in the field, the resulting maximum of emergence will occur at different times of the day.

As will be seen from Table VIII all experiments with daily changes from light to darkness and with the temperature at 29° gave a maximum emergence

TABLE VIII.

Distribution by percentage of the emergence over the 24 hours, experimental midnight (00 hr.) being the midpoint of the period of darkness whether natural or artificial, or civil (E.S.T.) midnight in the case of continuous darkness.

| | Alternating light and darkness | | | All darkness |
|---|---|---|---|---|
| Experiment .. .. .. | 5B | 6 | 4, 7, 8A–F, 9A–C, 10 | 8G, 9D |
| Number of individuals | 109 | 1,716 | 564 | 133 |
| Temperature (°C.) | 20·8 | 25·5 | 29 | 29 |
| Exp. hour of emergence | | | | |
| 00 – 03 .. .. .. | 7·4 | 0·2 | 9·4 | 20·1 |
| 03 – 06 .. .. .. | 22·0 | | 22·0 | 10·8 |
| 06 – 09 .. .. .. | 49·5 | | 32·7 | 12·9 |
| 09 – 12 .. .. .. | 17·4 | | 23·1 | 8·2 |
| 12 – 15 .. .. .. | | 0·8 | 1·9 | 4·9 |
| 15 – 18 .. .. .. | | 9·1 | 7·4 | 16·4 |
| 18 – 21 .. .. .. | 2·8 | 68·7 | 0·8 | 11·0 |
| 21 – 24 .. .. .. | 0·9 | 21·7 | 2·7 | 15·7 |

in the morning, corresponding to the 37-hour duration of the pupal stage superimposed on sunset time pupation. In Experiment 5B the emergence maximum was also in the morning, corresponding to the pupal duration of 62 hours, very close to 24 hours more than at 29°. And in Experiment 6, maximum emergence occurred at the same time of day (sunset) as the maximum pupation; the duration of the pupal stage at 25·5° was 45 hours or almost two days. And finally, the last column of Table VIII shows that when the animals developed in darkness there was no daily maximum of emergence.

*Sexual difference in emergence.*

In the preceding remarks no attention has been paid to the difference between males and females in pupal duration because this difference is very small, 0 to 2 hours. But, as shown above, there is a rather marked difference in the larval duration which is carried over to the emergence. For the group of experiments given in Table III the mean age at pupation was found to be 129 hours for males and 145 for females. The mean age for the emergence of the same 179 animals was 166 hours (males) and 182 hours (females) so that the sexual difference in both larval and pupal duration is 16 hours.

As mentioned above, emergence takes place over several days with a maximum each day at the same time. The sexual differences in development will therefore cause a preponderance of males during the first emergence maxima and of females in the last. The largest homogenous material including complete brood histories available from the experiments are the pooled experiments 8A and 8F. They give a very typical picture (fig. 2). The emergence lasted five

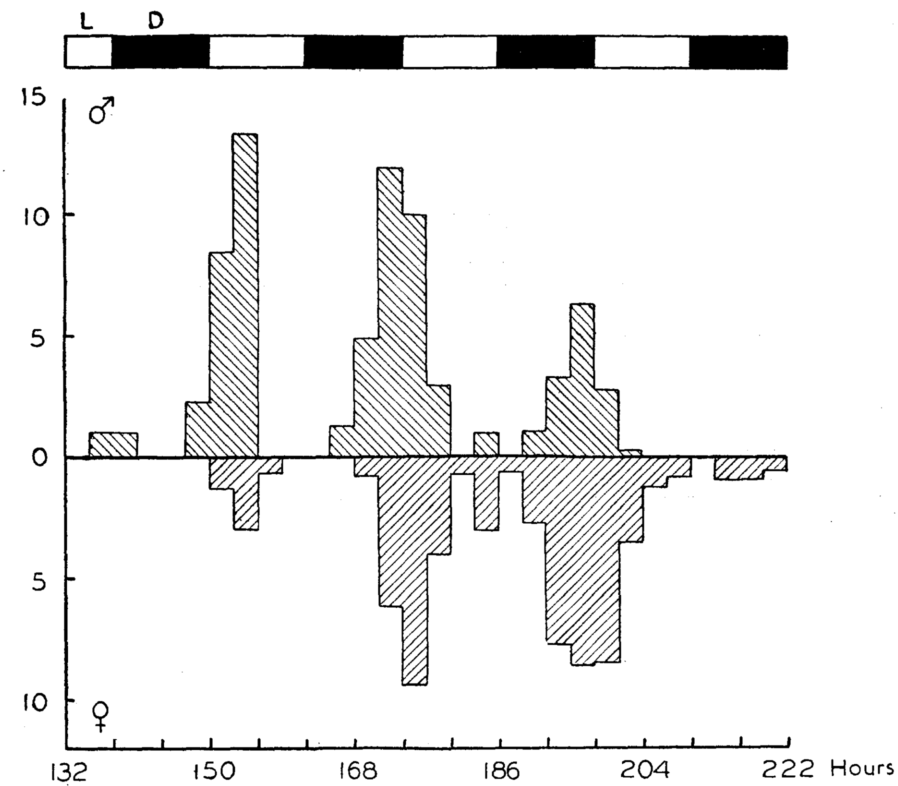


Fig. 2.—Distribution of the emergence of male and female imagines of *Aëdes taeniorhynchus* hatched simultaneously from the eggs.

days, of which the first had a small maximum consisting solely of males. On the second day there emerged the largest number of males, and a few females. The third day, nearly the same number of each sex emerged. The fourth day more females than males emerged. And finally on the last day a few females and no males emerged.

From fig. 2 it will be seen that the females within each maximum emerged a little later than the males, which is probably normal. The difference is, however, so small that it was not always discernible in material analysed by 3-hour intervals. It is most likely that it is the difference between males and females in the duration of the pupal stage, as mentioned above usually 1 to 2 hours, which causes this delay in the appearance of the females during the daily emergence maxima.

## Conclusions.

The tentative conclusions which may be drawn from these preliminary experiments are the following. The duration of larval life in *Aëdes taeniorhynchus* is determined by temperature and nutrition, pupation occurring ordinarily around the change from day to night. But it must be borne in mind that the critical moment when the process of transformation begins must precede the moment of pupation (*i.e.*, actual casting of the last larval skin) by an unknown number of hours. When the process is once started it seems to continue unremittingly to the emergence of the adult, and at a rate influenced only by temperature.

If the temperature of the water in the pools in which the larvae are living in the field is known, it should be possible to predict at what hour of the day the emergence maxima will take place. This hour is probably of some importance from the viewpoint of applied entomology, because unpublished observations suggest the possibility of a certain relationship between the hour of emergence, the hour when the migration starts, and, possibly also, the range of migration.

## Summary.


A series of field observations and rearing experiments were carried out in Florida to gain information on the duration of the larval stage and the time of day of pupation and emergence of the salt-marsh mosquito, *Aëdes taeniorhynchus* (Wied.).

The technique adopted in a series of experiments is described.

It was found that the duration of the larval stage is dependent on several factors of which water temperature is probably the main factor. Hunger was also shown to be important either through lack of food or overcrowding. Larvae which later became male adults pupated before those which became female imagines.

The period of maximum pupation is determined by the distribution of light and darkness, 83 per cent. of all pupation taking place during the evening periods, 15 to 24 hours after experimental midnight, and 48 per cent. in the period just after sunset, 18 to 21 after experimental midnight. The tendency to pupate around sunset is thought to have just as much accelerating as retarding effect on the larval development.

Temperature is considered to be the only factor of importance in determining the duration of the pupal stage which varies from 61 hours at 20·8°C. to 45 hours at 25·5° and 37 hours at 29°.

Both in nature as well as under controlled conditions in the laboratory it was found that, for a large number of larvae hatched simultaneously, the emergence of adults usually lasts three or fours days, with a maximum at the same hour on each of these days. The period of emergence is proportional to the duration of the post-embryonic development and may under unfavourable conditions last weeks.

Maximum emergence of imagines has been found to occur at various times of the day. This is as should be expected since the hour of pupation is fixed to a certain period of the day, and the duration of the pupal stage is solely dependent on the temperature.

The difference in the duration of pupation in males and females is very small but owing to the rather marked sexual difference in the duration of the larval stage, 129 hours for males against 145 for females at 29°, there is a

preponderance of males during the first emergence maxima and of females in the last.

It is concluded that if the temperature of the water in which the larvae are living in the field is known, it should be possible to predict at what hour of the day the emergence maxima will take place.

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