PHOTOPERIOD AND TEMPERATURE INFLUENCES ON DIAPAUSE IN EGGS OF THE FLOODWATER MOSQUITO *AEDES NIGROMACULIS* (LUDLOW) (DIPTERA: CULICIDAE) [1]

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ABSTRACT. Eggs of the mosquito *Aedes nigromaculis* from central Washington (46° N. lat.) entered diapause in response to a sub-optimum temperature, the percentage of diapause increasing as the length of exposure to the sub-optimum temperature increased. A maternal effect on egg diapause was noted, especially in response to certain photoperiods and photoperiod—temperature combinations.

INTRODUCTION. Eggs of floodwater mosquitoes may hatch immediately upon completion of embryonation if flooded by water of a suitable temperature; however, those which remain exposed to the air for a few days after embryonation may or may not hatch when later flooded, depending upon the preceding environmental conditions. This non-responsive state can either be a result of diapause, or of other physical factors that are termed deconditioning (Horsfall, 1956).

Eggs which fail to hatch soon after embryonation become somewhat dried. Such eggs are less likely to hatch, a condition referred to as "deconditioned." These deconditioned eggs can be "conditioned" for hatching by exposure for a few hours to either high humidity or free water. Since this conditioning period is brief, "deconditioned" eggs are considered as differing from diapausing eggs. Reactivation from the diapause state is a longer process than conditioning, often requiring months of exposure to environmental conditions such as low temperature (Horsfall, 1956; Harwood and Horsfall, 1959; Clements, 1963). Such a dormant state immediately resulting from unfavorable environmental conditions, but rapidly reversed by return to favorable conditions, is termed quiescence.

Diapause may be characterized as follows: the environmental or genetic triggering of neuroendocrine mechanisms, resulting in a reduction in biosynthetic activities and respiration, producing a state of arrested development which prepares the individual, in advance, for approaching unfavorable conditions (Lees, 1956; Harvey, 1962). This arrest is due to physiological mechanisms rather than to suppression by immediate environmental factors (Beck *et al.*, 1963). Diapause inducing experiments measure only the results of a sequence of physiological responses which ultimately determine the response of the individual. Each individual has a different stimulus requirement for eliciting this all or none response of eclosion (deWilde, 1962).

Facultative diapause is found in multivoltine species where the arrest in development is environmentally influenced, rather than automatically induced as in the obligatory diapause of univoltine species. One environmental condition is photoperiod which, acting as a token stimulus, bears little relation to the immediate well-being of the individual but, being the only environmental influence which precisely relates to the time of year, synchronizes the life cycle with the seasons (Lees, 1955). Lower mean fall temperatures may enhance a short photoperiod causing greater numbers to enter diapause. The type of diapause found in a species, there-

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fore, largely determines its distribution (Way, 1962).

Aedes nigromaculis is found only in Mexico, central and western United States, and southern Canada. The larvae are usually found in alkaline water but may also occur in temporary pools of fresh, brackish, or foul barnyard water. The adults are strong fliers (Carpenter and LaCasse, 1955; Horsfall, 1955). Although this mosquito is the primary mosquito of flood irrigated pastures in California, during this study it was found in large numbers in only one irrigated pasture near Mesa, Franklin County, Washington. *A. dorsalis,* and *A. vexans* were also found in this pasture, both occurring in larger numbers than *A. nigromaculis* until mid-August after which *A. nigromaculis* began to increase in numbers, becoming almost the only mosquito present by late September and early October. Since all of the *A. nigromaculis* females died immediately after oviposition in the laboratory, the abundance of adults in the field during the fall suggests a relationship of temperature with egg hatching in this species. Although Carpenter and LaCasse (1955) report that the females are active during the day, becoming more aggressive during the evening, I found a great decrease in host seeking activity as dusk approached. Differences in mean California and Washington evening temperatures might serve as an explanation.

Telford (1958) indicated that *A. nigromaculis* has staggered hatching with up to a dozen or so broods per year. He also stated that it does not hatch during the first spring flooding, indicating either it has a high water temperature requirement for hatching or it is slower in coming out of diapause in the spring. High temperature was considered to result in maximum egg hatch, with properly conditioned eggs hatching very quickly upon submersion.

In a later work, Telford (1963) indicated that the hibernation of *A. nigromaculis* in central California constitutes a true facultative egg diapause of the mature embryo. He indicated that descending temperatures in the fall initiated diapause, whereas ascending temperatures terminated diapause in the spring. He stated that 60° to 70° F (15.5° to 21° C) initiated diapause in the fall but terminated it in the spring. Telford reported that seasonal photoperiod changes are insignificant as effectors of diapause in this species. He indicated that the temperature during embryonic development had little or no effect on the induction of diapause, but there was a profound effect of temperature on the mature embryo. Eggs embryonated at 60° to 80° F (15.5° to 26.5° C) and stored at 80° F hatched regardless of the temperature of the flooding medium (40° to 80° F) (4.5° to 26.5° C). Eggs embryonated at 80° F but stored at 60° F did not hatch regardless of the temperature of the flooding medium. Of the eggs embryonated at 60° F, only those stored at 60° F and flooded at this, or lower, temperatures did not hatch. Only when the storage temperature of 40° F was used did a flooding temperature of 80° F fail to cause hatch. In general, the lower the storage temperature the greater the number of eggs failing to hatch.

Telford (1963) relates the temperature relationship of this species to its seasonal appearance as follows: Eggs laid in warm summer weather hatch immediately upon flooding in the summer even though the irrigation water (well water) may be cold. Eggs exposed to the declining temperatures of fall gradually enter diapause, with the result that the temperature and other conditions of the flooding medium are more critical to eclosion. This continues until eggs do not hatch in the fall. The entire procedure is reversed in the spring.

MATERIALS AND METHODS. The experimental methods are the same as those previously described (McHaffey, 1969, McHaffey and Harwood, 1970, McHaffey, 1972); consequently, they will only briefly be repeated for this paper.

Females were field-collected by aspirator, transported to the laboratory, identified, and placed into constant tempera-

ture chambers (25° C) at a photoperiod characteristic of the date of collection, determined by adding 1 hour ("civil twilight") to the calendar day length. Incandescent bulbs provided the light source and guinea pigs were used as a blood source.

Absorbent tissue paper moistened with distilled water was the oviposition substrate. Eggs were collected every 3 days or less by washing the eggs from the tissue with distilled water into a pan for collection on a 200-mesh stainless steel screen. Eggs were then washed into a common evaporating dish, thereby being randomized before transfer to filter paper.

The eggs were deposited on pieces of air-dried alcohol-sterilized filter paper, placed on moist cheesecloth in glass petri dishes, which were then sealed with rubber petri dish sealers. The dishes were labeled without conscious selection, and placed in BOD incubators with a timed incandescent light source. The substrate in these sealed dishes remained moist for several months. The storage temperatures used were 32, 25, and 10° C, hereafter referred to as super-optimum, optimum, and sub-optimum, respectively. For the purpose of this study, 25° C was designated as optimum temperature even though an actual optimum was not determined. Eggs scheduled for changing-temperature regimes (e.g. 25–10° C) were held for 10 days at each temperature, the change from one temperature to another being abrupt rather than gradual.

After exposure to the prescribed regime the dishes were removed from the chambers and held at room temperature for 2 hours. The eggs were then washed into an appropriately marked plastic and wire mesh envelope comprised of a thin plastic sheet on one face and a 200-mesh stainless steel screen on the other face. The screen retained eggs and larvae while permitting exchange of dissolved gases and circulating water but excluding gas bubbles.

Envelopes containing eggs were lowered into an aerated, distilled water-filled container maintained at 28° C in a constant temperature water bath. The eggs were kept in this aerated, well-oxygenated water for 30 minutes; then the air stream was replaced with nitrogen. The nitrogen rapidly displaced the dissolved oxygen in the water, producing a potent hatching stimulus (Judson, 1960). The oxygen depletion rate by nitrogen displacement was from the air saturation point of 6.2 ppm oxygen to 2.1 ppm oxygen in 30 minutes (average of 9 readings). The flow rate for the nitrogen was standardized, and the air flow rate was sufficient to maintain a maximum oxygen reading while in use.

After 30 minutes in aerated water followed by 30 minutes in nitrogenated water, the contents of the egg envelopes were washed with distilled water into labeled jars. The hatch was recorded by counting the larvae removed with an eye dropper. The remaining eggs were transferred onto a new piece of alcohol-sterilized filter paper, placed again in a labeled petri dish with moist cheesecloth, sealed with rubber sealers, and returned to their previous chamber for an additional period of time. Eggs remaining at the end of any experiment, generally after the third hatching trial, were cleared with a 0.5 percent sodium hypochlorite solution (Mortenson, 1950) to determine viability based upon the appearance of the embryo. Eggs were categorized as viable if the embryo was creamy white, had eye spots, a hatching spine, and distinct abdominal segmentation. Yellowish-brown embryos were designated nonviable and were excluded from the totals. Viable eggs remaining at the end of an experiment were considered to be in diapause.

The figures represent the results from the experimental regimes after 1, 2, and 3 hatching attempts. The bar graphs representing three hatching attempts show 95 percent confidence limits for the accumulative totals after the first and third attempts only. The data were analyzed by use of a binomial confidence limits table in the publication by Mainland *et al.* (1956).

EXPERIMENT 1. The purpose of the

initial experiment was to determine if photoperiod would induce egg diapause at an "optimum" temperature of 25° C and, if so, the number of days required to induce diapause at this temperature. Sixteen hours was selected as a likely non-diapause-inducing photoperiod and 11 hours as a likely diapause-inducing photoperiod. Eggs were exposed initially for 2, 3, and 4 weeks prior to the first hatching attempt with two subsequent hatching attempts at 1-month intervals.

The eggs, which were from females captured during a 16-hour natural daylight, were kept on a 16-hour photoperiod at 25° C for 3 days after oviposition before being incorporated into the experiment.

There was no significant difference between photoperiod treatments after an initial exposure of 14 or 21 days (Fig. 1). In the 28-day initial exposure group, however, a significantly greater hatch occurred in the 16-hour photoperiod, indicating that photoperiod has some effect upon egg hatching—the longer the exposure to the short photoperiod, the greater the number of eggs entering diapause. This effect, however, is nullified by sequential flooding and drying periods at this temperature.

The previous experiment indicated that eggs from 16-hour females were affected by an 11-hour photoperiod only after 28 days of exposure. Additional experiments at 25° C, indicated that eggs of 16-hour females could not be induced to diapause by 14½, 13½, and 8-hour photoperiods.

EXPERIMENT 2. The next experiment was designed to determine if certain constant temperature-photoperiod combinations could induce egg diapause. The eggs, which were from 16-hour females, were maintained on a 16-hour/25° C regime 3 days before being incorporated into the experiment.

There was no significant difference between photoperiod treatments under both the super-optimum (32° C) and optimum (25° C) temperatures (Table 1). These results indicate that photoperiod has no effect upon egg diapause at either of these temperatures and that both temperatures are optimum for hatching of this species.

The greatest number of diapause eggs were produced under the sub-optimum temperature regimes (10° C). There was no significant difference between photoperiod treatments at 10° C until after the third hatching, when a significantly greater accumulative hatch had occurred in the long photoperiod regime. These results indicate that even though the holding temperature remains at a sub-optimum level, a significant number of eggs break diapause after 80 days of exposure to a long photoperiod, and a short photoperiod keeps a significantly greater number of the eggs in diapause.

Eggs in the previous experiments were subjected to various constant temperature and photoperiod regimes. In nature, of course, eggs are subjected to daily and seasonal temperature changes. The results of an experiment designed to determine the effect of increasing and decreasing temperature under constant photo-period regimes indicated that diapause induction in eggs from 16-hour females occurs as a result of prolonged exposure to a sub-optimum temperature, rather than photoperiod or temperature-photoperiod combinations.

The previous experiments utilized eggs oviposited by females captured during a 16-hour natural daylight and maintained in the laboratory at this photoperiod. These experiments indicated that a sub-optimum temperature has a much greater ability to induce diapause in eggs of these females than does photoperiod. The following experiments were conducted to determine if maternal influence is a factor in egg diapause.

EXPERIMENTS 3 AND 4. The next two experiments utilized eggs oviposited by females captured during a 13½-hour natural photoperiod. The females were placed in a 13½-hour/25° C chamber in the laboratory, all eggs being kept under this regime until they were 3 days old, before being incorporated into an experiment.

Under various constant temperature-photoperiod combinations, egg diapause was induced by a sub-optimum temperature (10° C) and also by photoperiod. Photoperiod also influenced the induction of egg diapause under various changing temperature—constant photoperiod regimes. (Fig, 2).

In the 16-hour photoperiod, the constant optimum temperature regime (25–25° C) produced significantly fewer diapausing eggs than the decreasing temper-

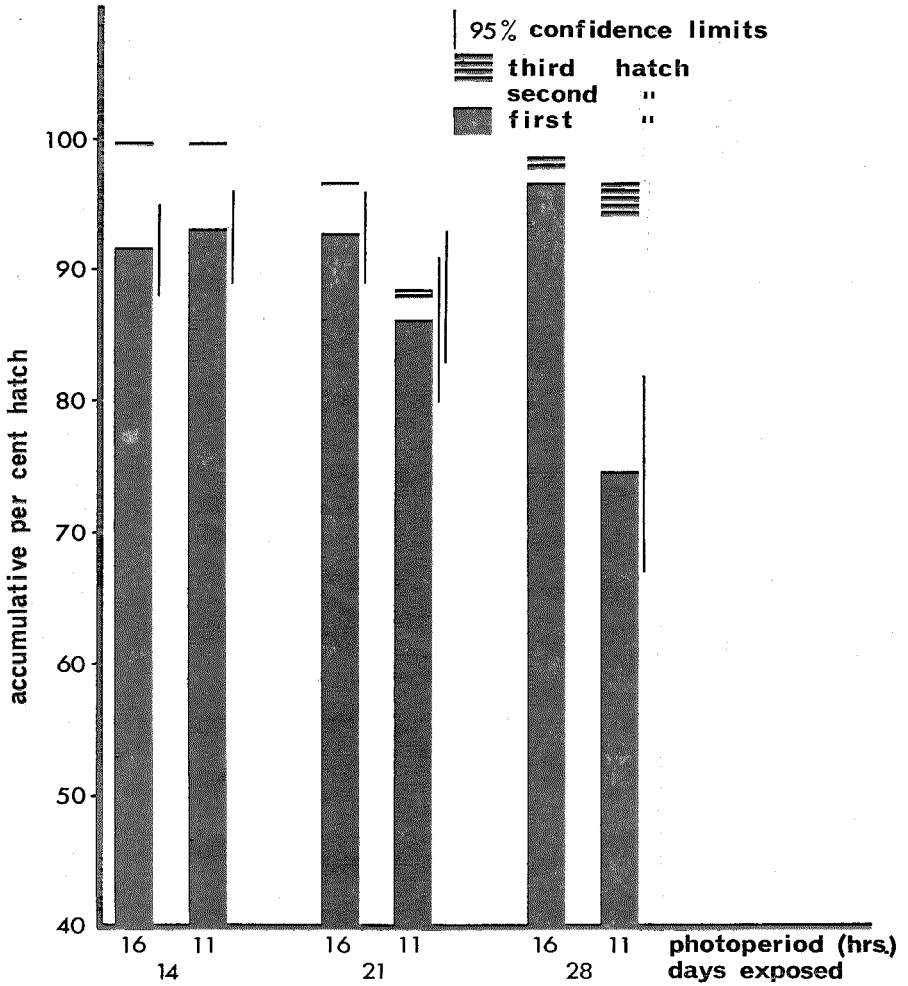

FIG. 1.—Hatching of eggs from 16-hour females in response to three initial lengths of exposure to two photoperiods at 25° C (*Aedes nigromaculis*).

TABLE 1.—Hatching of eggs from 16-hour females in response to six temperature-photoperiod treatments (*Aedes nigromaculis*)

Egg Treatment		First Hatch (+ 21 days)		Second Hatch (+ 1 month)		Third Hatch (+ 1 month)		
Temperature (° C)	Photoperiod (Hrs)	Percent Hatch	95% C. L.*	Accum. % Hatch	95% C. L.*	Accum. % Hatch	95% C. L.*	N
32	16	94.8	92–97	99.3	.....	99.7	.....	290
	11	91.2	87–94	99.2	.....	99.6	.....	261
25	16	87.0	83–91	90.6	87–94	96.4	.....	276
	11	90.8	87–94	95.7	.....	97.2	.....	283
10	16	9.0	5–15	15.4	10–22	53.2	45–61	156
	11	11.5	8–17	17.0	12–23	31.0	25–38	200

* Confidence limits.

ature regime (25–10° C) which produced significantly fewer diapausing eggs than the constant sub-optimum temperature regime (10–10° C). The increasing temperature regime (10–25° C) produced a hatch intermediate between the decreasing temperature and constant optimum temperature regimes.

These results indicate clearly that some eggs from the 13½-hour females enter diapause after only a short exposure (10 days) to a sub-optimal temperature (25–10° C regime), even in a 16-hour photoperiod, but that a large number enter diapause when the exposure time to this sub-optimum temperature is extended (10–10° C regime). The effect of an exposure to a 10-day sub-optimum temperature is mostly overcome by a subsequent 10-day exposure to an optimum temperature (10–25° C regime).

Under the 11-hour photoperiod, the hatch under the constant optimum temperature regime and the increasing temperature regime were equal, both being significantly greater than either the decreasing temperature or the constant sub-optimum temperature regimes. The constant sub-optimum temperature regime produced the greatest number of diapausing eggs.

The results clearly indicate that a sub-optimum temperature induces diapause, the degree of influence depending upon the length of exposure and the time of exposure within the regime. The effect of a sub-optimum temperature is competely reversed by subsequent exposure to an optimum temperature (10–25° C regime).

Inter-photoperiodic comparisons show that there was no photoperiod effect under the constant optimum-temperature regime. The decreasing temperature regime (25–10° C) and the constant sub-optimum temperature regime (10–10° C), however, showed a photoperiod and temperature effect on egg diapause, since significantly more diapausing eggs were produced in the short photoperiod regimes. Short photoperiod apparently must be coupled with a sub-optimum temperature to maximally influence diapause in eggs from 13½-hour females.

EXPERIMENTS 5 AND 6. The next two experiments utilized eggs laid by females captured during a 12½-hour natural photoperiod. These females and their eggs were kept on a 12½-hour/25° C regime in the laboratory until the eggs were 3 days old at which time they were incorporated into the experiment.

The results of the first experiment utilizing eggs from 12½-hour females indicated that under constant photoperiod temperature treatments, temperature has a greater influence on egg diapause than does photoperiod. The changing temperature-constant photoperiod regimes indicate that maximum diapause in these eggs is produced by a sub-optimum temperature (10° C).

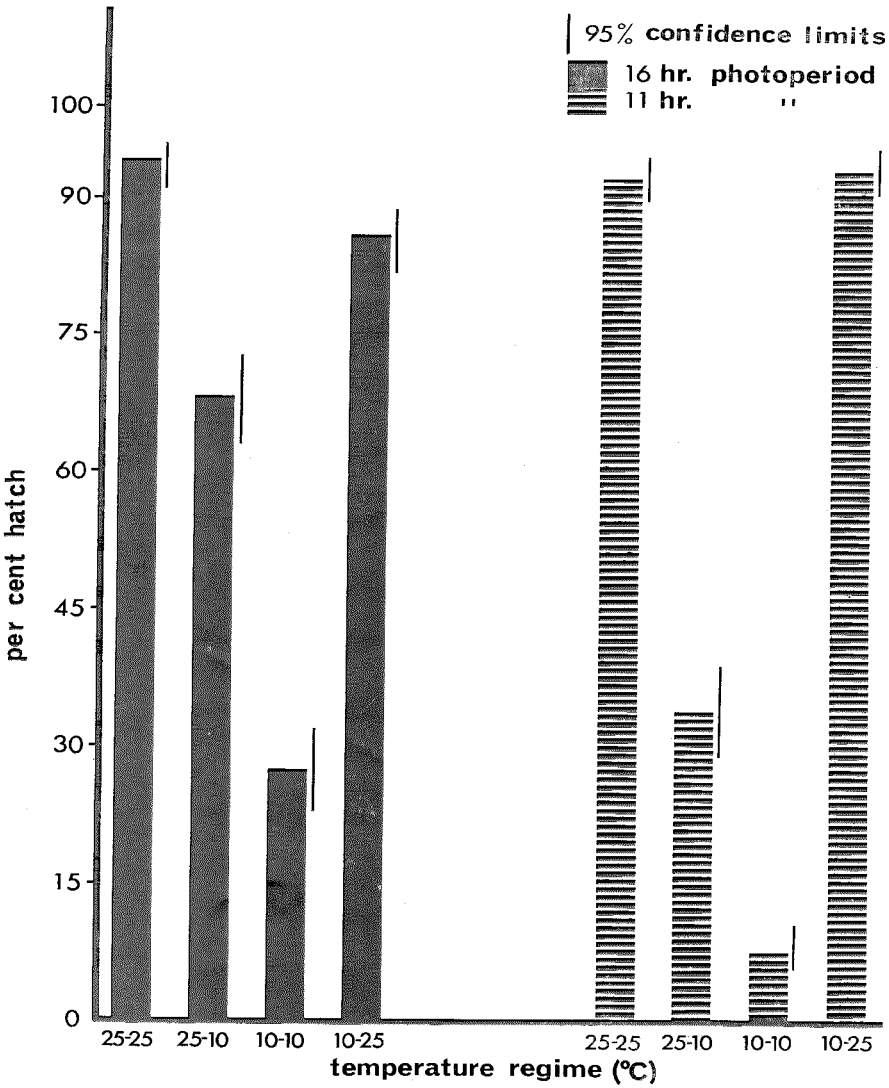

FIG. 2.—Hatching of eggs from 13½-hour females in response to four temperature regimes under two constant photoperiods (*Aedes nigromaculis*).

INTER-EXPERIMENTAL COMPARISONS. Maternal influences on egg diapause can be more clearly seen when the results from the previous constant temperature-photoperiod regimes which utilized eggs from females of three different photoperiod backgrounds, are grouped together.

At 32° C the initial hatch of eggs kept in a 16-hour photoperiod decreased as the female photoperiod decreased from 16 to 12½ hours, the differences between the 16 and 12½-hour female groups being slightly significant (Fig. 3). There was no significant difference in egg hatch between female groups in the 11-hour photoperiod.

At 10° C and a 16-hour egg photoperiod, the 16-hour female group produced significantly more diapause eggs throughout the second hatching attempt than did the 13½-hour female group. The 13½ and 12½-hour female groups were not significantly different from each other until after the third hatching attempt. In the 11-hour photoperiod, the final accumulative hatch from the 16 and 13½-hour female groups were significantly less than from the 12½-hour female group. The only eggs which in the final analysis exhibited a photoperiod effect at a constant sub-optimum temperature (10° C) were those from the 16-hour females.

The data obtained from egg groups maintained in five photoperiods at 25° C are shown in Figure 4. There was no significant difference in hatch between the female groups when eggs were placed on 16 and 14 ½-hour photoperiods. On a 13½-hour egg photoperiod, the initial hatch from 12½-hour females was significantly less than either of the other two female groups. With eggs kept on an 11-hour photoperiod, the initial percentage of hatch decreased as the length of the female photoperiod decreased. The 8-hour egg photoperiod produced a significant difference in hatch between the 13½-hour and 12½-hour female groups.

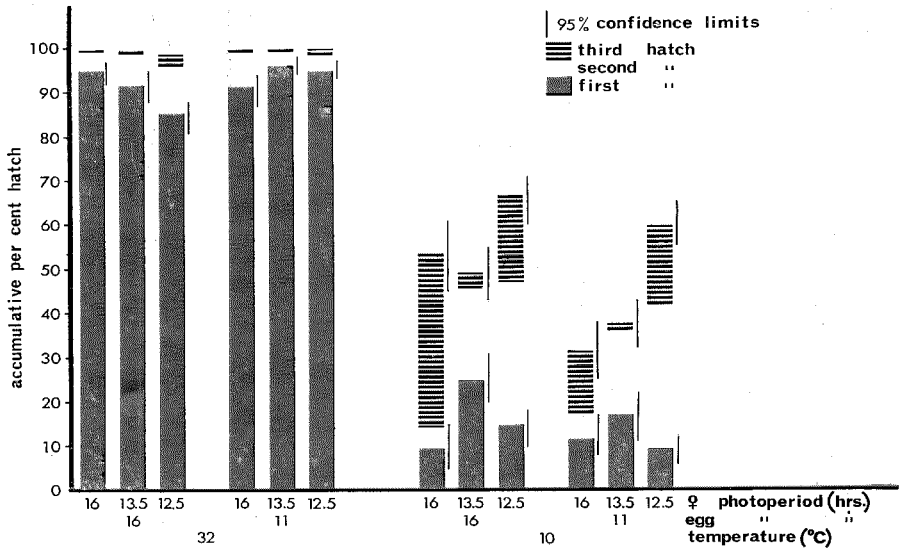

FIG. 3.—Hatching of eggs from females of three different photoperiod backgrounds in response to combinations of two photoperiods and two temperatures (*Aedes nigromaculis*).

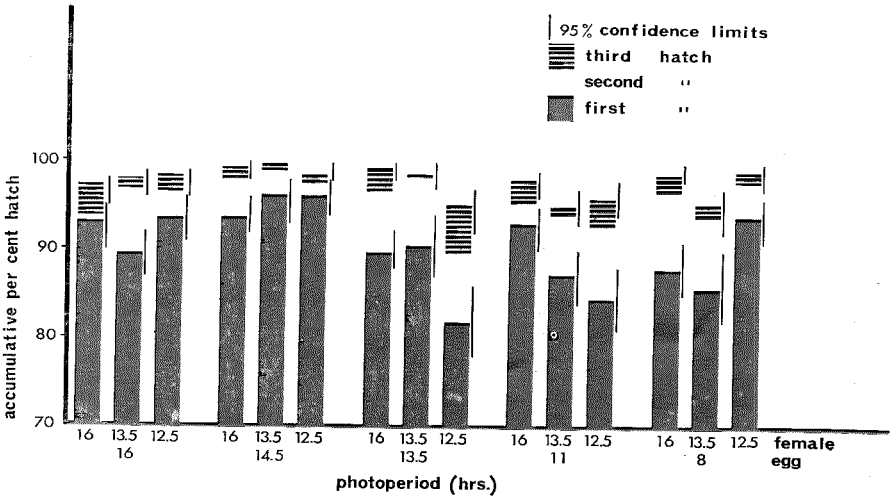

FIG. 4.—Hatching of eggs from females of three different photoperiod backgrounds in response to five photoperiods at 25° C (*Aedes nigromaculis*).

Differences in response between female groups to the same changing temperature-constant photoperiod regimes can be seen in Figure 5.

Under a 16-hour egg photoperiod, the following statements can be made: (1) the 16 hour females produced the greatest percentage of diapausing eggs under the optimum temperature regime (25–25° C); (2) under the decreasing temperature regime (25–10° C) and the increasing temperature regime (10–25° C), there was an increase percentage of hatch as the length of the female photoperiod decreased; and (3) eggs from the 13½-hour females produced the smallest percentage of diapause eggs in the constant sub-optimum temperature regime (10–10° C).

In an 11-hour photoperiod: (1) the constant temperature regime (25–25° C) and the increasing temperature regime (10–25° C) produced the greatest percentage of diapause in eggs from the 16-hour females; (2) the decreasing temperature regime (25–10° C) produced the greatest percentage of diapause eggs from the 13½-hour females; and (3) there was no maternal effect upon egg diapause at the constant sub-optimum temperature (10–10° C).

Only the 13½-hour females group produced eggs which showed a combined temperature-photoperiod effect on egg diapause. The primary influence for egg diapause in eggs from the 16 and 12½-hour females was temperature.

CONCLUSIONS. In eggs from 16-hour females, photoperiod had no effect on egg hatch at 32° C. Generally speaking, at 25° C the two photoperiods tested (16 and 11 hours) had no effect on egg diapause until after 28 days of initial exposure to the 11-hour photoperiod. Under a constant sub-optimum temperature (10° C) no photoperiod effect was shown until after the third hatching attempt when the accumulative hatch in the short photoperiod (11-hour) was less than that in the long photoperiod (16-hour). Perhaps a significant number of eggs can break diapause after 3 months of exposure to a sub-optimum temperature if the photoperiod is long, but in a short photoperiod they are unable to do so.

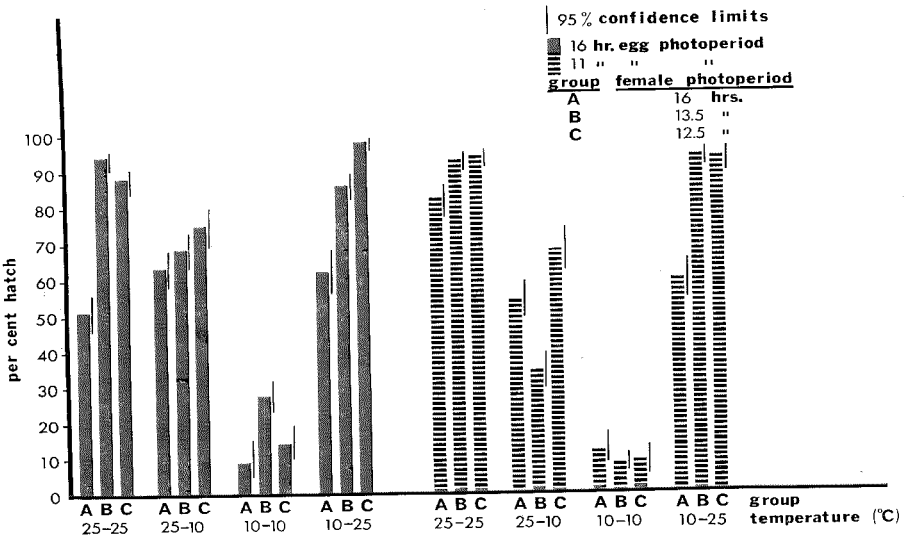

FIG. 5.—Hatching of eggs from females of three different photoperiod backgrounds in response to four temperature regimes under two constant photoperiods (*Aedes nigromaculis*).

In eggs from the 13½-hour females, photoperiod had a slight effect at 25° C as shown by a trend toward a decrease in hatch as the egg photoperiod decreased from 14½ to 8 hours. A combined photoperiod-temperature effect on the induction of egg diapause was indicated in the decreasing temperature regime (25–10° C) and the constant sub-optimum temperature regime (10–10° C).

In eggs from the 12½-hour females, there was no significant difference in hatch between temperature regimes from one egg photoperiod to another. These tests indicate that diapause in eggs from these females results only from a sub-optimum temperature and is in no way influenced by photoperiod.

A maternal influence on egg diapause was noted in most of the constant optimum-temperature and alternating-temperature regimes. Since these differences were quite variable it is difficult to make any generalizations; however, it will be noted that a photoperiod and temperature effect on egg diapause only occurred in eggs from the 13½-hour females.

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