PHOTOPERIOD-INDUCED DIAPAUSE IN A NORTH CAROLINA STRAIN OF *AEDES SOLLICITANS*: PHOTOSENSITIVITY OF FULLY FORMED AND DEVELOPING EMBRYOS[1]

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ABSTRACT. The role of photoperiod in the induction of diapause in eggs laid by field-collected adult *Aedes sollicitans* mosquitoes was examined at different temperatures. Short-day photoperiods induced a much higher incidence of diapause at 15° than at 22°C, whether eggs were 0, 1, 2, 3, 4 or 5 days old postoviposition on exposure. A 6-week exposure to a 6:18 to 10:14 (L:D) photoperiod at 15° caused 87–100% of the eggs tested to enter diapause. The diapause incidence was significantly different for 0- to 5-day-old eggs subjected to a 10:14 photoperiod at 15° and to a combination of 10.5:13.5 photoperiod and a 21:5°C thermoperiod. Hatchability of 0- to 5-day-old eggs did not differ significantly after a 6-week exposure to other photoperiod/temperature combinations tested. However, evidence of photoperiod/temperature interaction during embryogenesis was observed following exposure to different short-day photoperiods at both 22 and 15°C.

INTRODUCTION

Depending upon the strain or geographical location of the species population, embryonic diapause in the mosquito *Aedes sollicitans* (Walker) may be induced by low temperatures (Bidlingmayer and Schoof 1956, Elmore and Fay 1958, Mallack et al. 1964, Woodard et al. 1968) or photoperiod/temperature interaction (Anderson 1970). *Aedes sollicitans* enters diapause as a fully formed embryo. However, both developing and fully formed embryos of a Connecticut strain of the species have been reported (Anderson 1970) sensitive to short-day photoperiod. In the case of developing *Ae. sollicitans* embryos, it is likely that the photosensitive period does not span the entire duration of embryogenesis. Investigations with *Ae. triseriatus* (Say) (Kappus 1964[2]) and *Ae. taeniorhynchus* (Wiedemann) (Parker 1986) have shown variation in the effect of photoperiod/temperature interaction during the period of embryogenesis. The degree of a mosquito's reaction to a short-day photoperiod may also be a factor that influences whether photoperiod/temperature interaction during the period of embryogenesis will have a noticeable effect on the subsequent rate of induction or the subsequent incidence of diapause induced.

This investigation was conducted to determine the role of photoperiod in the initiation of embryonic diapause in a North Carolina (NC) strain of *Ae. sollicitans* and the effect of photoperiod/temperature interaction on *Ae. sollicitans* at different stages of embryonic development, which begins immediately after eggs are laid and may be completed in 3 to 4 days at 27° and in 8 to 10 days at 22°C (Nayar 1985).

MATERIALS AND METHODS

Experiments were conducted with eggs laid by *Ae. sollicitans* adult females periodically collected during the summer of 1982, 1983 and 1984 in Pamlico County, North Carolina and maintained in the laboratory at 27 ± 1°C, 16:8 (L:D) photoperiod, and 70–80% RH. Experimental eggs were collected within 24 hr after they were laid. The eggs from each collection were divided into 6, or multiples of six, groups of approximately 150 eggs per group. Six groups of eggs (derived from the same collection) were exposed to each treatment: a 1-, 2-, 3-, 4-, 5- or 6-week exposure to a given photoperiod/temperature combination. One of the 6 egg groups was exposed to the treatment at daily intervals from day 0 to day 5 after the eggs were collected. In each experiment, 0- to 5-day-old eggs (postoviposition) were held in the same petri dish during the experiment (Parker 1985). In a preliminary investigation, eggs were dechorionated with sodium hypochlorite on days 0–4 postoviposition and the approximate stage of development of embryos was determined.

Eggs were subjected to a range of photoperiods at a constant 10, 15, 22 and 27 ± 1°C for 1–6 weeks in photoperiod- and temperature-controlled incubators. Temperature during the photophase usually is higher than that during the scotophase of photoperiods in nature. Thus eggs were also subjected to a 11.3:12.7 photoperiod at a thermoperiod of 24:9°C and to a 10.5:13.5 photoperiod at a thermoperiod of 21:5°C. The higher and lower temperature of

[1] Paper No. 11283 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7601. Project No. 5553.

[2] Kappus, K. D. 1964. The photoperiodic induction of diapause in eggs of *Aedes triseriatus* (Say). Ph.D. dissertation, Ohio State Univ., Columbus, OH.

each thermoperiod coincided with photophase and scotophase, respectively, and they approximated mean maximal and minimal air temperatures that may be encountered during October or November in natural sites. Photophase approximated daylengths experienced during October or November.

The effect of different photoperiod/temperature combinations on eggs 0 to 5 days old was initially examined 1 week after the exposure of the 5-day-old eggs and at weekly intervals thereafter. The effect of a combination was based on the percentage of eggs that failed to hatch immediately after the treatment was terminated. To stimulate hatching, approximately 50 eggs were pipetted into a glass tube within a 10-ml snap-cap vial that contained a 1:1000 aqueous solution (by weight) of Bacto nutrient broth (Difco Laboratories, Detroit, MI). Eggs remained submerged in the nutrient broth for 5 hr after treatments at 22 and 27°C, and up to 24 hr after those at lower temperatures. Newly hatched larvae were counted. The number of hatched larvae was divided by the sum of hatched larvae and unhatched viable eggs to determine percentage hatch. The percentage hatch from each tube was treated as a replicate in statistical analyses.

The failure of eggs to hatch was considered an expression of diapause. Unhatched eggs were checked for viability as described by Mortenson (1950).

Analysis of variance was used to determine whether photoperiod/temperature interaction and age had a significant effect on hatchability. Duncan's new multiple range test (Edwards 1966) was used to segregate significant differences between means for eggs of different age groups and for egg exposed to different photoperiods.

RESULTS

Seventy percent of 100 eggs dechorionated within 24 hr after they were laid collapsed in the sodium hypochlorite solution; no development was evident in 10% of the eggs and approximately 20% of the eggs had embryos developed to the germband stage. The labral groove, cephalic and caudal poles were distinguishable on day 1, and segmentation of the abdomen on day 2. Eye spots were evident on a few embryos on day 2 and on all embryos on day 3 postoviposition. Embryos of 4-day-old eggs appeared fully formed.

Effect of photoperiod at different constant temperatures.

27°C. No reaction to photoperiod occurred at 27°. Essentially all (>90%) of the 0- to 5-day-old eggs hatched after each 1- to 6-week exposure to either a 6:18, 8:16, 10:14, 12:12 or 14:10 photoperiod.

22°C. A marked reduction (44–85%) in hatchability was caused by photoperiods with a 6 to 10 hr photophase at 22° (Table 1). Differences between the effect of a 6-week exposure to the photoperiods varied least when eggs were 0 days old on exposure (Table 1). However, after a 6-week exposure to each photoperiod tested at 22°, hatchability of 0- to 5-day-old eggs did not differ significantly ($P > 0.05$).

15°C. A 6:18 to 10:14 photoperiod at 15° induced diapause in 87–100% of the eggs 0 to 5 days old on exposure (Table 1). Diapause was induced rapidly. Maximal hatch, observed after either week 1 or 2 of exposure to the photoperiods, ranged from 23.6–35.5% of the eggs exposed on days 0 to 2 and from 50–52% of the eggs exposed on day 5 postoviposition. However, after week 1 of exposure to a 14:10 photoperiod, 69, 94, 83 and 92% of the 0-, 1-, 2- and 5-day-old eggs hatched, respectively. Following a 6-week exposure to the 14:10 photoperiod, each 0- to 5-day-old group of eggs displayed a significantly higher percentage hatch than that observed for similar groups of eggs stored under the 6:18 to 12:12 photoperiods for a similar duration ($P < 0.05$) (Table 1).

Among photoperiods tested at 15°, only the 10:14 photoperiod caused significant ($P < 0.05$) variation in the incidence of diapause among eggs 0 to 5 days old on exposure. The photoperiod induced the lowest and highest diapause incidence when eggs were 0 and 5 days old on exposure, respectively. Among eggs 0 to 3 days old (embryonating) on exposure, the 2-day-old eggs subsequently showed the lowest hatch and it was significantly ($P < 0.05$) lower than that of eggs that were 0 and 1 day old on exposure to the photoperiod (Table 1).

10°C. Hatchability was affected by temperature alone and there was no significant difference ($P > 0.05$) between percentage hatch of the 0- to 5-day-old eggs after a 6-week exposure to treatments (Table 1). Regardless of photoperiod or the duration of a treatment, all or > 90% of the eggs exposed on days 0 to 2 postoviposition failed to hatch immediately after the treatment was terminated. However, after each duration of exposure to treatments, survivorship was essentially total (> 90%), even that of embryos of eggs subjected to treatments on day 0 postoviposition. Immediately after the termination of a 6-week exposure to a 6:18 photoperiod, 131 of the 132 embryonated eggs exposed on day 0 postoviposition failed to hatch but 98% of them (131) hatched following a 4-week conditioning period.

The 5-day-old eggs showed a 40–50% reduction in hatchability following week 1, and a 90%

Table 1. Hatchability of 0- to 5-day-old (postoviposition) *Aedes sollicitans* eggs after a 6-week exposure to different photoperiod/temperature combinations.

		Age (day) when exposed to photoperiod					
		0	1	2	3	4	5
Temp (°C)	Photoperiod L:D	*x% hatch ± SEM	*x% hatch ± SEM	*x% hatch ± SEM	*x% hatch ± SEM	*x% hatch ± SEM	*x% hatch ± SEM
22	6:18	21.4 ± 6.2a	14.9 ± 4.3a	16.6 ± 2.2a	25.7 ± 2.9a	27.2 ± 5.4a	30.1 ± 6.4a
	8:16	23.1 ± 11.1a	21.9 ± 10.1a	23.0 ± 1.4ab	22.5 ± 2.1a	36.5 ± 1.8ab	35.4 ± 1.4a
	10:14	31.3 ± 2.4a	56.2 ± 3.1b	43.9 ± 5.2b	48.3 ± 5.7b	51.9 ± 9.6bc	56.2 ± 7.7ab
	12:12	82.4 ± 2.5b	70.5 ± 7.0b	76.1 ± 11.0c	80.9 ± 3.4c	69.8 ± 6.7c	87.6 ± 7.4c
	14:10	69.3 ± 1.7b	68.5 ± 8.5b	69.5 ± 7.0c	62.3 ± 5.8d	69.1 ± 9.7c	70.1 ± 4.1bc
15	6:18	3.4 ± 2.4a	4.8 ± 1.4a	5.5 ± 3.6a	1.4 ± 0.7a	0.0 ± 0.0a	0.0 ± 0.0a
	8:16	5.0 ± 5.0a	7.0 ± 0.6a	4.4 ± 3.1a	2.9 ± 0.7a	2.9 ± 1.4a	0.0 ± 0.0a
	**10:14	13.1 ± 1.2a(a)	5.6 ± 1.6a(b)	1.4 ± 0.7a(c)	2.7 ± 1.4a(bc)	3.3 ± 1.8a(bc)	0.7 ± 0.0a(c)
	12:12	36.7 ± 8.8b	32.8 ± 7.2b	29.6 ± 6.7b	22.6 ± 1.8b	33.0 ± 8.0b	33.6 ± 5.7b
	14:10	70.1 ± 2.3c	70.1 ± 5.7c	52.1 ± 8.8c	68.3 ± 5.9c	57.0 ± 7.0c	58.7 ± 9.5c
10	6:18	0.7 ± 0.7a	1.4 ± 0.7a	3.6 ± 2.6a	0.7 ± 0.7a	0.7 ± 0.7a	2.7 ± 0.8a
	8:16	0.7 ± 0.7a	0.0 ± 0.0a	0.7 ± 0.7a	0.0 ± 0.0a	1.4 ± 0.7a	0.0 ± 0.0a
	10:14	0.0 ± 0.0a	4.1 ± 1.7a	0.0 ± 0.0a	0.7 ± 0.7a	0.8 ± 0.8a	3.2 ± 0.7a
	12:12	7.0 ± 3.8a	2.2 ± 1.3a	1.5 ± 0.7a	0.8 ± 0.8a	1.4 ± 0.7a	0.7 ± 0.7a
	14:10	5.6 ± 3.9a	3.2 ± 1.6a	4.0 ± 0.9a	2.2 ± 0.9a	2.2 ± 1.3a	6.9 ± 4.1a

* Means within a vertical column that are followed by the same letter are not significantly different at the 5% level of probability (Duncan's new multiple range test). A mean is based on a sample of 80–154 viable eggs.

** Means within parentheses that are followed by a different letter are significantly different at the 5% level of probability (Duncan's new multiple range test).

reduction following week 4 of exposure to the 6:18 to 14:10 photoperiods at 10°. Under each of the treatments, all or nearly all of the 5-day-old eggs failed to hatch following week 6 of exposure (Table 1).

Effects of photoperiod/thermoperiod interactions.

Effects of a 10.5:13.5 photoperiod and 21:5° C per 24 hr. After a 6-week exposure to the photoperiod/thermoperiod combination, the 0- to 5-day-old eggs exhibited a similar percentage hatch (Table 2) to that exhibited by eggs subjected to the 10:14 photoperiod at 15° (Table 1). The rate of diapause induction in 5-day-old eggs was also rapid (Fig. 1A), as noted for 5-day-old eggs subjected to the 10:14 photoperiod at 15° (Fig. 1B). A weaker effect of the photoperiod/thermoperiod combination on initial hatchability was the chief difference observed between effects of the treatment and effects of the 10:14 photoperiod and 15° combination, whether eggs were 0 or 5 days old on exposure (Figs. 1A, 1B).

Effects of a 11.3:12.7 photoperiod and 24.9°C per 24 hr. A 1- to 3-week exposure had no effect on the 5-day-old eggs; >90% of the eggs hatched after a 1- to 3-week exposure period (Fig. 1C). However, a marked reduction in hatchability commenced following week 3 of exposure (Fig. 1C), and it coincided with a sharp increase in percentage hatch of eggs 0–2 days old on exposure. For the 0- to 2-day-old eggs, maximal percentage hatch (75–88%) occurred after week 4 of exposure, then sharply declined as indicated (Fig. 1C) for eggs exposed to the treatment on day 0 postoviposition.

Although embryos of the 5-day-old eggs entered diapause at the fastest rate, there was no significant difference ($P > 0.05$) between the percentage hatch displayed by the 0- to 5-day-old eggs following week 6 of exposure to the treatment (Table 2). After week 6 of exposure, hatchability of eggs 0 and 5 days old was most similar to that of 0- and 5-day-old eggs subjected to the 12:12 photoperiod at 15° for a 6-week duration (Figs. 1C, 1D).

DISCUSSION

Data presented suggest that photoperiod is an important factor in stimulating the induction of embryonic diapause in the NC strain of *Ae. sollicitans* at a wide range of temperatures. Among temperatures tested, however, a constant 15°C was best for photoperiod to induce diapause, regardless of the state of embryonation of eggs on exposure. Results suggest that the critical photoperiod at 15° (that which induced 50% diapause) was between the 12:12 and 14:10 photoperiods. Based on percentage hatch of eggs that were 5 days old (embryonated) on exposure, a 6:18, 8:16 and 10:14 photoperiod at 15° were just as effective in initiating diapause as was a constant 10°C, which nullified effects of photoperiod. A constant 27°C, the highest temperature tested, also suppressed reaction to photoperiod.

Results indicate that reaction to photoperiod was not nullified by a very low temperature, 5°C, experienced only during scotophase. The 10.5:13.5 photoperiod and 21:5°C thermoperiod combination induced an identical percentage (99.3%) incidence of diapause in 5-day-old eggs to that induced in 5-day-old eggs by a 10:14 photoperiod at 15°, after a similar 6-week exposure period. The effect of these two treatments may have ecological significance, despite the fact that temperature fluctuates in nature. A 10 and a 10.5 hr daylength may be experienced by field populations of the NC strain of *Ae. sollicitans* during November. At 35°09'N lat. (Bayboro, NC), the mean daily maximum and minimum air temperature for November 1981–83 was 20 and 6.7°C, respectively (data

Table 2. Effect of two photoperiod/thermoperiod combinations on the hatchability of 0- to 5-day-old of *Aedes sollicitans* eggs after a 6-week storage period. High and low temperatures coincided with photophase and scotophase, respectively.

Age (day) when exposed	Photoperiod/thermoperiod combination			
	L:D 10.5:13.5 at 21:5°C		L:D 11.3:12.7 at 24:9°C	
	No. eggs viable	*$\bar{x}$% hatch ± SEM	No. eggs viable	*$\bar{x}$% hatch ± SEM
0	128	13.9 ± 1.2a	114	48.2 ± 5.8a
1	134	8.3 ± 3.1ab	131	54.2 ± 11.0a
2	135	4.8 ± 2.7bc	130	59.2 ± 2.7a
3	144	5.2 ± 3.1bc	132	40.7 ± 4.7a
4	126	0.7 ± 0.1c	132	53.0 ± 1.3a
5	114	0.7 ± 0.7c	143	30.7 ± 5.3a

* Means within a column followed by the same letter are not significantly different at the 5% level of probability (Duncan's new multiple range test).

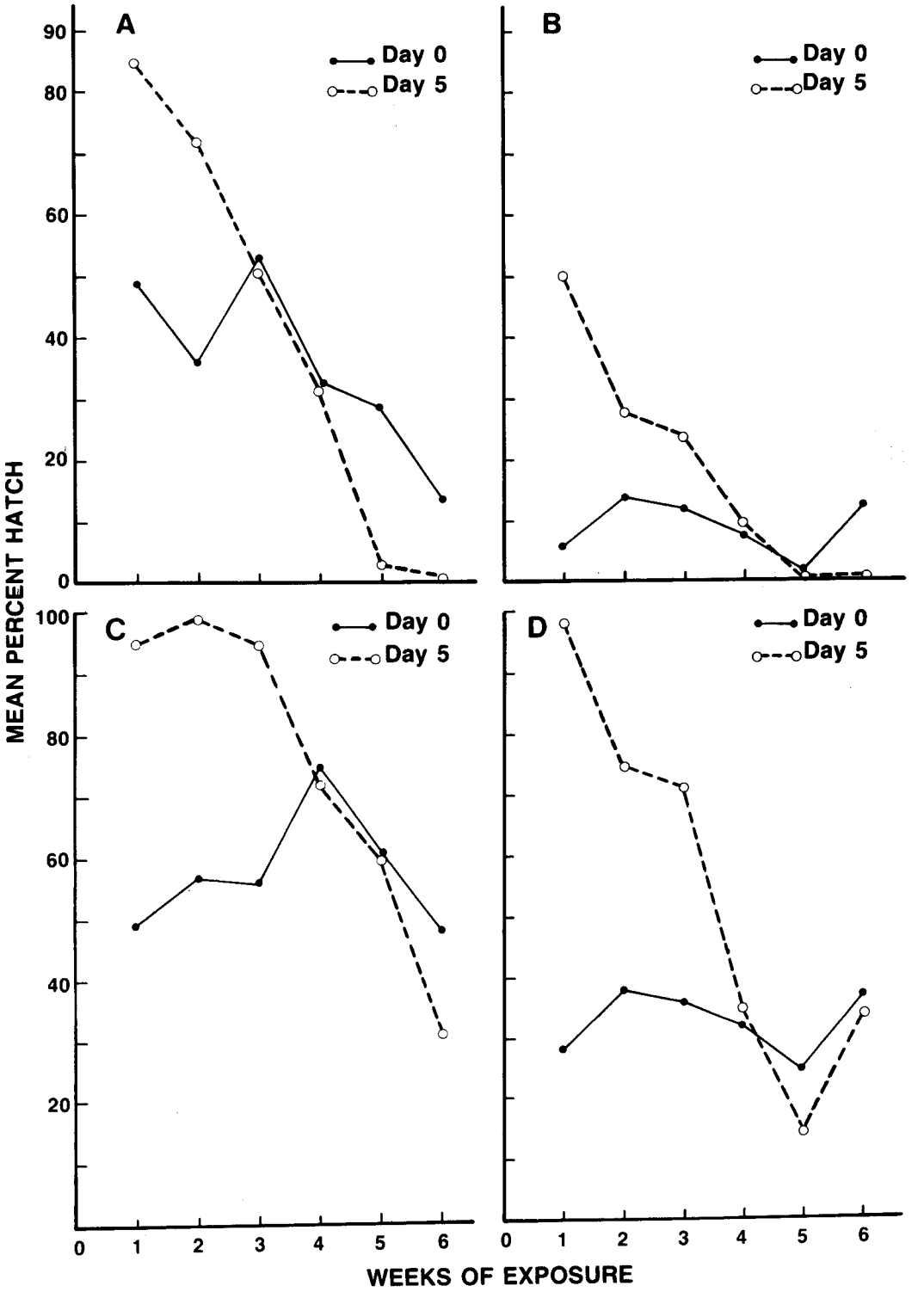

Fig. 1. Hatchability of 0- and 5-day-old *Ae. sollicitans* eggs following a 1- to 6-week exposure to: (A) a 10.5:13.5 photoperiod at 21:5° (photophase:scotophase regime), (B) a 10:14 photoperiod at 15°, (C) a 11.3:12.7 photoperiod at 24.9° (photophase:scotophase regime), and (D) a 12:12 photoperiod at 15°C. A datum point is based on a sample of: (A) 114–156, (B) 104–165, (C) 114–152, and (D) 89–143 viable eggs.

provided by the National Climatic Data Center, Ashville, NC). Generally, adult *Ae. sollicitans* are still present in the field during November (Carpenter and Chamberlain 1943, B. T. Hale and A. Prost, unpublished data).

Anderson (1970) reported that the late larval instars, pupa, adult, developing embryo and fully formed embryo of a Connecticut strain of *Ae. sollicitans* were sensitive to a 10:14 photoperiod at 23°C. Results suggest that developing embryos as well as fully formed embryos of the NC strain of *Ae. sollicitans* were also sensitive to photoperiod. Eggs 0 days old on exposure to a 6:18 photoperiod at 22° exhibited a slightly lower hatch than those 5 days old on exposure. They also exhibited a low percentage hatch after each duration of exposure to a 10:14 and 12:12 photoperiod at 15°, which was not due primarily to effects of temperature on development. However, the weaker effect of the 10:14 photoperiod on eggs 0 days old than on eggs 5 days old on exposure suggest that effects of 15° on physiological processes during early embryogenesis modified subsequent reaction to the photoperiod. Temperature is known to affect reaction to photoperiod (Danilevskii 1965, Beck 1980, Saunders 1982), and its effect during embryogenesis may vary with the state of development of the embryo (Khelevin 1958). Zero-day-old *Ae. taeniorhynchus* eggs also showed a weaker reaction to a 10:14 photoperiod at 15°C than did eggs that were 5 days old on exposure to the treatment (Parker 1986).

The precise stage of embryonic development at which photosensitivity commenced was not determined and it was not defined in Anderson's (1970) investigation. However, results suggest a relationship between the state of embryonation (at 27°) of eggs 2 days after they were laid and the onset of photosensitivity, as reported for *Ae. taeniorhynchus* (Parker 1986). Among the 0- to 3-day-old embryonating eggs, eggs 2 days old subsequently exhibited the lowest percentage hatch under both the 10:14 photoperiod at 15° and the 10.5:13.5 photoperiod at 21:5°. Kappus (1964[2]) reported that *Ae. triseriatus* embryos were sensitive to photoperiod as early as 5 days after eggs were laid; the stage of development attained by the embryos on day 5 was not reported.

Although evidence suggest that *Ae. sollicitans* may be sensitive to photoperiod during embryogenesis, there is probably a greater selection pressure on fully formed embryos than on developing embryos to react to photoperiod stimuli in nature. Because of the marked decline in the number of adults late in the season, fewer developing than fully formed embryos of the NC strain of *Ae. sollicitans* are likely to encounter temperatures found most favorable for diapause induction by photoperiod in laboratory experiments.

ACKNOWLEDGMENTS

The cooperation of the Pamlico County Health Department is gratefully acknowledged and I especially thank Mr. L. Hatton for helping me collect *Ae. sollicitans* adult females during summer 1982. I am grateful to Mrs. D. Jones for typing the manuscript and to Drs. C. Apperson, R. Axtell, J. Myers and R. M. Roe for reviewing the manuscript.

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