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Diel Oviposition Patterns of *Aedes albopictus* (Skuse) and *Aedes triseriatus* (Say) in the Laboratory and the Field

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ABSTRACT: The oviposition patterns of *Aedes albopictus* and *Aedes triseriatus* were observed in preliminary field experiments during the summer of 1995 and in the laboratory the following winter. *Aedes albopictus* exhibited a diel periodicity of oviposition in the field, ovipositing a significantly greater number of eggs during the day than during the night ($P = 0.0001$). Laboratory observations for 40 consecutive hours indicated that *Ae. albopictus* oviposited only during the hours of light, with a broad peak of oviposition activity occurring in mid-afternoon. *Aedes triseriatus*, however, oviposited during all periods of the day and night in the field. A significantly greater number of eggs were oviposited in traps open 24 hours than in traps open only during the day ($P = 0.01$), whereas there was no significant difference in the number of eggs deposited in traps open 24 hours and those open only during the night ($P = 0.14$). In the laboratory, *Ae. triseriatus* oviposited during all periods of light and dark, with a distinct peak of oviposition activity occurring during the evening crepuscular period.

Keyword Index: *Aedes albopictus, Aedes triseriatus*, oviposition, periodicity.

INTRODUCTION

The oviposition cycle of *Aedes aegypti* (L.) has been studied extensively in both the laboratory (Haddow and Gillett 1957, Haddow et al. 1961, Gillett 1962) and in the field (McClelland 1968; Chadee and Corbet 1987, 1990a, 1990b; Corbet and Chadee 1990, 1992). In contrast, the oviposition cycles of *Aedes albopictus* (Skuse) and *Aedes triseriatus* (Say) have received less attention. Gubler (1971), working with *Ae. albopictus* in India, performed some preliminary experiments in the field, and Chadee and Corbet (1989) studied the periodicity of oviposition by a strain of *Ae. albopictus* from Singapore in the laboratory. More recently, Abu Hassan et al. (1996) examined the oviposition rhythm of *Ae. albopictus* in the field in Malaysia. Oviposition patterns of *Ae. triseriatus* have been observed in the laboratory using a strain from Georgia (Hayes and Morlan 1957) and have been studied more extensively in the field in Wisconsin (Loor and DeFoliart 1970). Only Hayes and Morlan (1957) have used mosquito populations from the southeastern U.S., and comparable studies have not been conducted for southeastern U.S. populations of *Ae. albopictus* or North Carolina populations of *Ae. triseriatus*. Both *Aedes* species are sympatrically distributed in urban areas of the southeastern U.S., where they colonize container habitats (Moore et al. 1990). As urban pests and potential vectors of disease agents (Sudia et al. 1971, Watts et al. 1972, Mitchell et al. 1987, Grimstad et al. 1989, Scott et al. 1990), mosquito abatement agencies often monitor population levels of these species. Gravid mosquitoes are of particular interest since pathogen transmission and nuisance activity are dependent on the blood-feeding activity of females. Knowledge of times of peak oviposition activity can be important in the surveillance of mosquito populations, especially in relation to the use of oviposition traps. Accordingly, our study was undertaken to determine the time of day that southeastern populations of these mosquitoes oviposited in the field and to define their specific times of peak oviposition activity under laboratory conditions.

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MATERIALS AND METHODS

Field Oviposition Studies

Twelve ovitraps, consisting of No. 10 tin cans (*ca.* 4 L volume) painted glossy black inside and out with Rustoleum® and partially filled with tap water, were placed at approximately 25 m intervals around the perimeter of a residence in Raleigh, NC. A red velour paper strip (2.5 x 15 cm) was clipped to the inside of each trap as an oviposition substrate. Ovitraps were operated for either 12 or 24 hour intervals depending on which of the following conditions they were randomly assigned: open day, open night (ODON); closed day, open night (CDON); open day, closed night (ODCN). In the "open day" condition, traps were operated from 0700 hours to 1900 hours, and when in the "open night" condition, traps were operated from 1900 hours to 0700 hours. Ovitraps were closed by covering them with an opaque polypropylene, "snap-on" lid. After a 3.5 day period, ovistrips were retrieved, and the conditions of the traps were changed. There were three replicate ovitrapping periods completed from June 28 and July 10, 1995. Within each period, there were four replicates of each trap condition; and at each site, each ovitrap was operated once in each of the three conditions.

After the collection period, ovistrips were placed on moist paper towels in sealed clear plastic containers and exposed to a long day (14 hours of light) photoperiod. After *ca.* seven days, the ovistrips were placed in polypropylene tubs and flooded with distilled water. A small amount of a liver powder:baker's yeast mixture was added to each container to promote the production of bacteria and to stimulate the eggs to hatch. Larvae were reared to maturity, killed in hot water, preserved in ethanol, and subsequently identified to species and counted.

Mosquito Colony Origin and Maintenance

Aedes albopictus and *Ae. triseriatus*, collected as larvae and pupae in New Hanover County, North Carolina, in the summer of 1994, were maintained in a rearing facility at a relative humidity of *ca.* 75 percent and a temperature of ca. 26°C under a photophase: scotophase of 14h:10h. Larvae were fed a mixture of 2:1 liver powder:baker's yeast (wt.:wt.) using a standardized feeding schedule (Gerberg et al. 1994). Adults were maintained in Plexiglas® oviposition cages (30 x 30 x 30 cm), fitted with cotton surgical socking tops, and provided with a 10 percent sucrose solution *ad libitum*. To obtain eggs, females were routinely fed on citrated pig blood from a natural membrane condom fitted with a modified aquarium heater (Benzon and Apperson 1987). After field collections were made, *Ae. albopictus* and *Ae. triseriatus* were reared continuously, without separating generations, for one and six months, respectively, until colonies were established. After establishment, the colonies were named F_1. The F_3 and F_4 generations were used in laboratory experiments.

Laboratory Oviposition Studies

Females were fed to repletion on a human forearm four days prior to initiating the experiments. Preliminary experiments indicated that the peak oviposition period for *Ae. albopictus* and *Ae. triseriatus* occurred approximately four days after blood-feeding. Groups of ten 4-7 day-old females were placed in six oviposition cages immediately after blood-feeding. A 10 percent sucrose solution was supplied in each cage. In addition, ten males were aspirated into each cage to assure that females were mated. At 0600 hours on the fourth day after blood-feeding, a single filter-paper (Fisherbrand grade P8, Fisher, Pittsburgh, PA) lined 125 ml specimen cup (Fisher No. 09-800), spray-painted black and containing 30 ml of tap water, was placed in the center of each oviposition cage. Each cup was replaced with a freshly prepared oviposition cup every hour thereafter for 40 consecutive hours. Filter papers were removed and all eggs deposited during the one-hour exposure period were counted.

Insects were entrained to a 14h:10h photophase: scotophase light regimen throughout development and during our oviposition experiments. The lighting system consisted of a single bank of two 40W tube-fluorescent lights that were placed approximately 0.25 m over the cages. From 0700 to 0800 hours and from 2000 to 2100 hours, crepuscular light was provided by a single 40W incandescent bulb.

Statistical Analysis

For the field experiments, the number of eggs deposited by each species under each trap condition was subjected to a square root transformation to normalize variances and analyzed by a three-way analysis of variance (ANOVA) to determine if mosquito species, sampling period, or trap condition affected the numbers of eggs collected in the ovitraps. Significantly different means were segregated using Fisher's least-significant-difference (LSD) test (SAS 1989).

RESULTS

Aedes albopictus

In field experiments, *Ae. albopictus* oviposited primarily during the day (TABLE 1). The mean number of eggs deposited during the ODCN condition (75.5 eggs/period) by *Ae. albopictus* was significantly greater

($P = 0.0001$) than the mean number of eggs oviposited during the CDON condition (17.8 eggs/period). In addition, there was no significant difference ($P = 0.74$) between the numbers of eggs oviposited in traps operated under the ODON condition (70.4 eggs/period) and traps operated under the ODCN condition.

From the field experiments, it was determined that *Ae. albopictus* oviposits primarily during the day and that *Ae. triseriatus* generally oviposits at night, but apparently lays during the day as well. Laboratory studies were conducted to determine exactly when each species oviposits.

Aedes albopictus exhibited a diurnal periodicity in its oviposition behavior in the laboratory, confirming our observations in the field (Fig. 1). Oviposition activity was initiated with the morning crepuscular period (0700 hours), and ceased with the onset of darkness (2200 hours). There was a slight early morning peak of oviposition activity between 0800 and 1100 hours, followed by a broad peak of oviposition activity which occurred between 1200 and 2000 hours. During this latter period, 92.2 percent of all eggs were oviposited. Peak oviposition activity occurred during a three-hour period between 1300 and 1600 hours, when 45.4 percent of all eggs were laid.

Aedes triseriatus

Aedes triseriatus oviposited mainly at night, but also during the day (TABLE 1). The mean number of eggs laid by *Ae. triseriatus* during the CDON condition (21.3 eggs/period) was greater but not significantly different ($P = 0.25$) from the number of eggs deposited during the ODCN condition (7.6 eggs/period), or the ODON condition (26.2 eggs/period) ($P = 0.14$). *Aedes triseriatus* oviposited during all periods of the day and night in the laboratory; however, a distinct peak of oviposition activity was exhibited during the evening crepuscular period (2000-2100 hours) (Fig. 1). During the periods preceding 2000-2100 hours, an average of 3.0 percent of all eggs were oviposited per hour, whereas during the peak oviposition period, 34.8 percent of all eggs were oviposited.

DISCUSSION

In both field and laboratory studies, *Ae. albopictus* demonstrated a periodicity in its oviposition behavior. In the laboratory, no eggs were deposited in periods of total darkness. In the field studies, however, *Ae. albopictus* deposited some eggs when traps were open at night and closed during the day. It is highly probable that the comparatively smaller number of eggs deposited at night were oviposited during the crepuscular periods and not during times of total darkness. Since the traps were closed for periods of 12 hours between 0700 hours and 1900 hours, and the day length in North Carolina in June and July is greater than 12 hours, it is likely that eggs were deposited during the light periods immediately before 0700 hours and after 1900 hours.

Chadee and Corbet's (1989) laboratory experiments demonstrated that *Ae. albopictus* exhibited a diurnal periodicity of oviposition with a peak of activity occurring from around 1600 to 1800 hours (with sunset at 1800 hours). Most (92.5%) of the eggs were laid in their study between 1400 and 1800 hours. The field studies of Abu Hassan et al. (1996) also showed that *Ae. albopictus* exhibited a diurnal oviposition rhythm. They reported a peak in oviposition activity between 1500 and 1700 hours, accounting for 42.3 percent of the total number of eggs oviposited. Our results correspond more closely to those reported by Gubler (1971) than to Chadee and Corbet (1989) or Abu Hassan et al. (1996). As reported by Gubler (1971), we found a more widely dispersed period of oviposition activity. In our study, peak oviposition activity occurred between 1300 and 1600 hours when 45.4 percent of the total number of eggs were deposited. Gubler (1971) showed that a majority (about 76%) of eggs were laid between 1100 and 1700 hours. Both Gubler (1971) and Chadee and Corbet (1989) found that *Ae. albopictus* exhibited a late afternoon peak in oviposition activity, approximately two hours before sunset. Abu Hassan et al. (1996) also found a late afternoon peak in oviposition activity, but the relation of the activity peak to sunset is unknown because the times of sunrise and sunset were not reported. In our laboratory study, oviposition peaked about four hours prior to the crepuscular period. In addition, mosquitoes used in our experiments exhibited a slight peak of morning oviposition activity which occurred between 0900 and 1000 hours, after which activity decreased until 1200 hours. Gubler (1971) also found a peak in oviposition in the morning, whereas, Chadee and Corbet (1989) did not.

It is possible that the differences between our results and those of Chadee and Corbet (1989) and Abu Hassan et al. (1996) may be explained by differences in *Ae. albopictus* strains studied. It is unlikely that Chadee and Corbet's (1989) use of single females would explain the differences, since they obtained similar results in a colony cage containing approximately 200 females. An additional explanation of differences between our results and Chadee and Corbet's (1989) may relate to differences in thermal regimes. Their insectary temperature was 28 ± 1°C, whereas the temperature in our experiments was *ca.* 26°C.

Abu Hassan et al. (1996) reported that *Ae. albopictus*

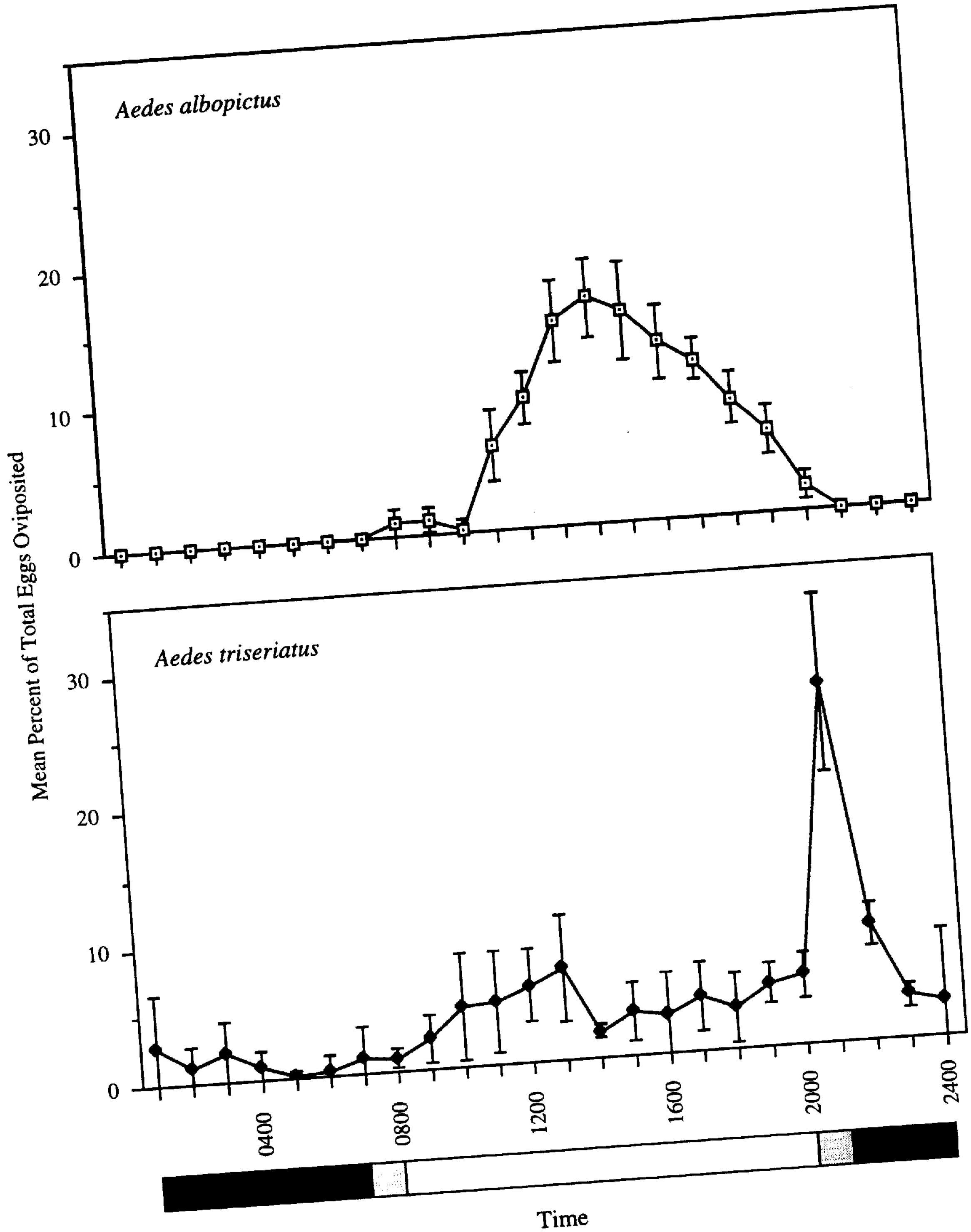

Figure 1. Mean percentage (± SE) of the total eggs oviposited by *Aedes albopictus* and *Aedes triseriatus* per hour. Light bar represents photophase, black bars represent scotophase, and gray bars represent crepuscular periods. N = 6 oviposition cages with 10 females each.

oviposited during the night hours in the field. Neither our laboratory experiments or Chadee and Corbet's (1989) experiments confirmed this behavior. It is possible that the lack of night-time oviposition by *Ae. albopictus* in the laboratory is an artifact, resulting from differences in mosquito strains or environmental conditions such as temperature or season. For *Ae. aegypti*, Corbet and Chadee (1990) confirmed in the field their laboratory observations (Chadee and Corbet 1987) which revealed a lack of oviposition during night hours.

Unlike *Ae. albopictus*, *Ae. triseriatus* oviposited during all periods of the day and night, with a peak occurring at the evening crepuscular period in the laboratory. These results confirm the field data of Loor and DeFoliart (1970), who found that in Wisconsin, *Ae. triseriatus* exhibited a peak in oviposition during the period of one hour before to one hour after sunset. They also found that oviposition occurred at low levels at other times during a 24 hour period. Hayes and Morlan (1957) found in the laboratory that oviposition occurred mainly at night. Similarly, in our field experiments, more eggs were oviposited at "night" than during the "day." However, in our field experiments, eggs were collected over 12 hour periods, and therefore precisely when the eggs were laid could not be determined. More eggs were oviposited in the CDON condition than in the ODCN condition, probably because the former condition encompassed the evening crepuscular period. Results of our laboratory experiments indicated that oviposition activity peaks during the evening crepuscular period. In addition, the number of eggs oviposited by *Ae. triseriatus* in the CDON condition was highly variable (TABLE 1) and therefore, not significantly different from either the ODCN or the ODON conditions. This variability reflects the behavioral tendency of *Ae. triseriatus* to oviposit during all periods of the day and night. The large amount of variation in egg numbers in the CDON condition probably reflects differences in local trap conditions, such as temperature and/or the amount of sunlight. Loor and DeFoliart (1970) found that at temperatures below 64°F (*ca.* 18°C) oviposition activity ceased. In addition, Corbet and Chadee (1990) showed that the location of an ovitrap, relative to the amount of sunlight the trap receives, can significantly affect the numbers of eggs deposited in the trap.

The oviposition activity of *Ae. albopictus* and *Ae. triseriatus* was similar to that of another container-breeding mosquito, *Ae. aegypti* (Chadee and Corbet 1987, Corbet and Chadee 1990), in that all three species oviposited during the day. In the wet season in Trinidad, West Indies, Corbet and Chadee (1990) demonstrated that the pattern of oviposition in the field by *Ae. aegypti* was bimodal, consisting of two well-defined peaks which occurred in the early morning and late afternoon. However, in the laboratory, a single peak of oviposition activity by *Ae. aegypti* was observed by Haddow and Gillett (1957) and Gillett (1962) just prior to the onset of darkness. The oviposition pattern of *Ae. triseriatus* resembled the oviposition pattern of *Ae. aegypti* in the laboratory, except that *Ae. triseriatus* initiated peak oviposition activity at the onset of the crepuscular period, whereas the egg-laying activity of *Ae. aegypti* peaked during the late afternoon. In addition, *Ae. triseriatus* oviposited at a low, fairly constant level during all periods of light and dark, whereas *Ae. aegypti*

TABLE 1. Mean number of eggs oviposited by *Aedes albopictus* and *Aedes triseriatus* under three different ovitrap conditions.

		Mean number of eggs (± SE) laid per sampling period[a]	
Trap Condition[b]	n	*Aedes albopictus*	*Aedes triseriatus*
ODCN	12	75.5 (9.5) a	7.6 (4.1) b
CDON	12	17.8 (5.5) b	21.3 (11.9) ab
ODON	12	70.4 (9.4) a	26.2 (7.8) a

[a]Numbers of eggs per ovitrap were subjected to square root transformation prior to statistical analysis. Mean values within each species followed by the same letter are not significantly different ($P > 0.05$) by LSD.

[b]ODCN = open during day, closed during night; CDON = closed during day, open during night; ODON = open during day and night.

laid eggs primarily during daylight. *Aedes albopictus* was similar to *Ae. aegypti* in that it only oviposited during the light period, but *Ae. albopictus* showed a much broader, single peak of oviposition in the laboratory. A slight morning peak of oviposition was observed for *Ae. albopictus*, but it only represented about 2 percent of all eggs oviposited. In comparison, approximately 40 percent of all eggs were laid during the morning peak by *Ae. aegypti* in the field (Corbet and Chadee 1990).

The field experiments reported here are preliminary, and to adequately characterize the diel oviposition patterns of both species under field conditions, additional traps and more samples encompassing crepuscular light periods are needed. However, the differences in the number of eggs per trap found between the "night" and "day" sampling periods (albeit insignificant *for Ae. triseriatus*) strongly suggest that *Ae. albopictus* predominantly oviposits diurnally, while *Ae. triseriatus* primarily exhibits nocturnal oviposition habits.

Our results indicate that both *Ae. albopictus* and *Ae. triseriatus* exhibit a diel periodicity in egg laying, with the majority of eggs for both species oviposited after 1200 hours. Consequently, in designing surveillance programs for these species, placement of oviposition traps in the field should be made in the morning before noon.

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