Nocturnal Activity of Mosquitoes (Diptera: Culicidae) in a West Nile Virus Focus in Connecticut

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ABSTRACT Six species of mosquitoes (Diptera: Culicidae) were collected in sufficient numbers for analysis in segregating traps set at 2-h intervals by using CO_2 and light as attractants in a West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) focus in Stratford, CT. The Kolmogorov–Smirnov one-sided test for two samples was used to analyze the data. Mosquito activity began shortly before sunset and continued until shortly after sunrise the next morning. All species had geometric means that were significantly higher during the 2-h period shortly after sunset compared with the 2-h collection before sunset. Species, known to be naturally infected with WNV, were often attracted to these traps in about equal numbers at 2-h intervals during an 8- to 10-h period commencing shortly after sunset. Differences of geometric means were not significant among the four or five 2-h collection periods commencing at sunset for *Aedes vexans* (Meigen), *Culex salinarius* Coquillett, and *Aedes cinereus* Meigen. *Aedes cantator* (Coquillett) had a significantly higher geometric mean for the 2-h period commencing at sunset, and *Coquillettidia perturbans* (Walker) was captured in significantly greater numbers during the 2-h period starting at sunset compared with periods commencing 6 h after sunset. *Culex pipiens* L. tended to have an activity pattern that was primarily nocturnal. Time of night, not meteorological conditions, was the most important factor in determining the nightly variation in the number of trapped mosquitoes. Parity rates of *Cx. pipiens* collected during specific periods of the night were not significant. In total, 39 isolations of WNV were made from seven species collected primarily during periods of total darkness. Humans are at risk of being bitten by infected mosquitoes throughout the night.

KEY WORDS West Nile virus, mosquito biting cycles, *Culex pipiens*, *Culex salinarius*, *Aedes vexans*

Flight and feeding behaviors of mosquitoes (Diptera: Culicidae) are governed in large part by periods of light and dark and are often entrained endogenously (Clements 1999). Field studies in Africa and South America >50 yr ago showed the importance of documenting biting cycles of specific species of mosquitoes in relation to transmission of human disease-causing pathogens (Kumm and Novis 1938, Bates 1944, Gillett 1951, Lumsden 1952). Humans and sometimes animals were used as bait in these experiments. In the United States, traps using light and CO_2 have been used more often to collect mosquitoes seeking blood (Service 1993), although other types of traps and biting or landing counts also have been used (Thompson and Dicke 1965, Bidlingmayer 1985, Reisen and Pfuntner 1987).

In northeastern United States, multiple isolations of West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) have been made from collections of *Culex pipiens* L., *Culex salinarius* Coquillett, *Culex restuans* Theobald, *Culiseta melanura* (Coquillett), and *Aedes vexans* (Meigen), suggesting that these species may be important in the enzootic or epidemic transmission (Andreadis et al. 2004). We now report the nocturnal activity periods of species of mosquitoes, which were relatively common in an intense enzootic focus of WNV in Connecticut. The Centers for Disease Control and Prevention (CDC) miniature light trap supplemented with dry ice is the most common type of trap in current use in northeastern United States (Andreadis et al. 2004). Therefore, mosquito collections were made at 2-h intervals in a WNV focus by using a light and CO_2 commercially available segregating trap.

Materials and Methods

Mosquito Collection. Trapping was conducted from 18 May to 1 November 2004, 25 May to 17 October 2005, and 15 June to 10 October 2006 in a wooded area on Water Pollution Control Authority land in Stratford, CT. Trapping was conducted on 25, 19, and 17 nights in 2004, 2005, and 2006, respectively. Specifics of this area have been reported previously (Anderson et al. 2004). Trapping was carried out at approximately

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weekly intervals, although no trapping was done from 20 September to 16 October 2005.

The Collection Bottle Rotator trap (model 1512, John W. Hock Company, Gainesville, FL) supplemented with dry ice was used in 2004 and 2005 to segregate mosquito collections into sequential 2-h (120-min) time periods. Carbon dioxide was dispensed at a rate of 19.1–31.7 liters/h from the dry ice container throughout the trapping period. In 2006, compressed CO_2 was dispensed over the top of the trap at a rate of 30 liters/h. Six traps were set up on each collection date. Traps were placed at three different locations ≈69 m apart. At each location, one trap was positioned ≈1.5 m above ground and another trap was placed ≈7 m above ground in the tree canopy. Mosquitoes were collected in a labeled 500-ml plastic jar during each collecting interval, they were anesthetized with CO_2 in the field, and then they were transferred to labeled glass vials sealed with a rubber stopper and gas and waterproof tape. Vials were kept on dry ice until they were transferred to a −80°C freezer in the laboratory.

Onset and cessation of biting is often correlated with times of sunset and sunrise (Lumsden 1952, Service 1993), but at the latitude where we conducted our studies (41° 10′ 41″ N), it is difficult to adjust times to both sunset and sunrise (Service 1993). Collections were each 120 min and started at 6 p.m. EDT in 2004 and 2005. For analyses of data, collection periods were established to account for changes in sunset. Period 1 included 120–90 min of daylight before sunset. Collection period 2 varied from 89 to 1 min of daylight before sunset. Collecting periods 3–5 were always made in total darkness. Minutes of daylight before sunrise for collection periods 6, 7, and 8 ranged from 41 to 0, 120 to 0, and 120 to 50, respectively.

In 2006, collection period 1 started 2 h before sunset, and collection period 2 started at sunset. Sunset began at 8:27 p.m. EDT on 15 June on the first collection date and at 6:21 p.m. EDT on 10 October on the last collection date. The last two collection periods, 6 and 7, had varying periods of daylight or none at all.

Mosquito Identification. Mosquitoes were thawed on a cold platform and were identified to species (Darsie and Ward 1981, Andreadis et al. 2005). Mosquitoes without apparent blood in their midguts were placed into groups of 50 or less by species, date, trap height, location, and time of catch.

Virus Testing and Identification. Mosquitoes were tested for virus in Vero cells by procedures described previously (Anderson et al. 2004). Identification of WNV was made by the polymerase chain reaction assay described by Lanciotti et al. (2000).

Parity Studies. Parity of *Cx. pipiens* was determined in 2006 in 160 specimens collected during time periods 2–5 (Kardos and Bellamy 1961).

Weather Data. Temperature, dew point, rainfall, and wind data were taken at 1-h intervals at the Sikorsky Airport, which was located within 1 mile of our sampling site. Hourly relative humidity was calculated from the temperature and dew point (Lawrence 2005).

Table 1. Total specimens collected by species and year, Stratford, 2004–2006

Species	Yr		
	2004	2005	2006
Cx. pipiens	2,259	791	1,039
Cx. salinarius	2,926	485	1,037
Ae. vexans	4,352	989	2,224
Ae. cinereus	2,203	376	
Cq. perturbans	1,329	2,632	
Ae. cantator	1,255	782	

Statistical Analysis. To assess differences among collecting intervals, the cumulative probability distributions for each 2-h collection period were compared using the Kolmogorov–Smirnov one-sided test for two samples (K-S test) (Conover 1980). Comparison tests were performed for each time period pair for each mosquito species. The cumulative probability distributions were then compared by year and height for each species. Numbers of specific species of mosquitoes collected during each collecting period were $\log_e$ transformed with zero count data being replaced with a 1 for presentation of data as geometric means. Analyses were based upon the K-S test (Systat 11, Systat Software, Inc., Point Richmond, CA), with $P < 0.05$.

Spearmen rank-correlation analysis ($P < 0.05$; Systat 11) was used to compare relationships among numbers of collected specimens of specific species during specific periods and meteorological variables. Pearson chi-square test (Systat 11) was used to compare the parity status of female *Cx. pipiens* collected during different time intervals in 2006.

Results

Six species of mosquitoes were analyzed by the K-S test in 2004 and 2005. Numbers collected by year are shown in Table 1. *Ae. vexans* was the most abundant species captured in the rotator trap. Significantly greater numbers of *Ae. vexans* were collected during periods 2–6 than at other times (Fig. 1). Ground-positioned traps caught significantly more *Ae. vexans* than traps placed in the canopy in both years (Table 2). The frequency of collecting *Cx. pipiens* was significantly higher during time periods 3–6 compared with time periods 1, 2, 7, and 8 (Fig. 1). Larger numbers of *Cx. pipiens* were captured in canopy-placed compared with ground-positioned traps, but differences were not significantly different (Table 2). *Cx. salinarius* was least abundant during period 1 and tended to be most abundant during periods 2–6. Collections were significantly higher by the K-S test for traps placed near the ground than in those placed in the canopy in 2004, but in 2005, differences at the two heights were not significant (Table 2).

Aedes cinereus Meigen and *Coquillettia perturbans* (Walker) were captured during periods 2–6 in significantly greater numbers than during periods 1, 7, and 8 (Fig. 2). *Aedes cantator* (Coquillett) also were captured in greater numbers during periods 2–6, but significantly greater numbers were obtained in period 2

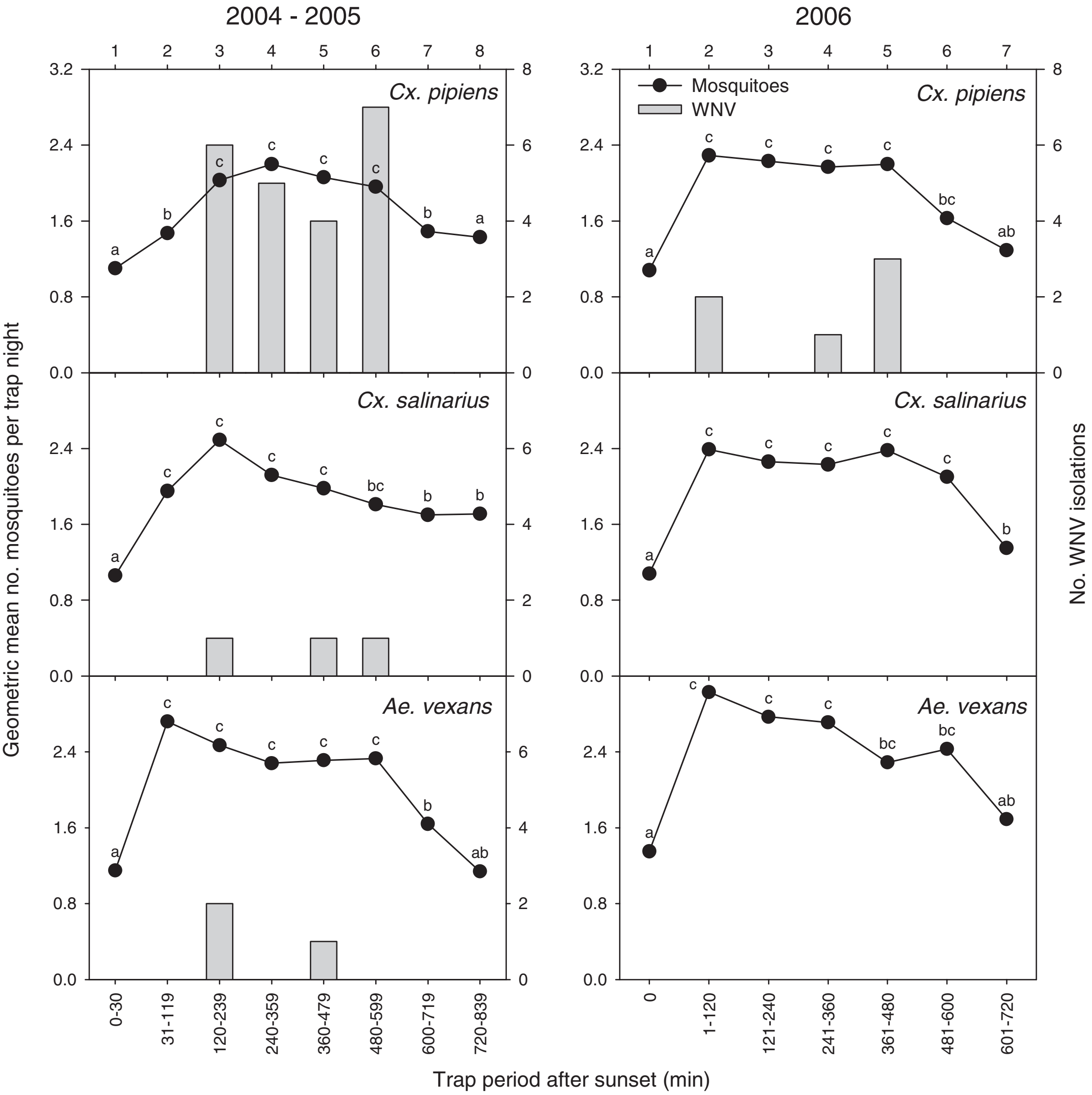

Fig. 1. Geometric means of *Cx. pipiens*, *Cx. salinarius*, and *Ae. vexans* caught at 2-h (120-min) intervals per trap per night at ground and canopy levels and total number of isolations of WNV relative to sunset, 2004–2005 and 2006. Each 120-min collection period is identified by number at the top of the figure. Collection period 1 in 2004–2005 included 120–90 min of daylight, and collection period 2 included 89–1 min of daylight. Collection period 1 in 2006 was set for 120 min of daylight immediately before sunset. Geometric means for each 120-min collection interval for each species for 2004–2005 and for 2006 with the same lowercase letter are not significantly different ($P < 0.05$; K-S test).

than in the other collecting periods. All three species were captured in significantly greater numbers at ground level than at canopy levels, except for *Cq. perturbans* captured in 2004 when differences were not significant at the two elevations (Table 2).

Ae. vexans, Cx. pipiens, and *Cx. salinarius* were captured in sufficient numbers for statistical analysis in 2006, when compressed CO_2 was used and trapping commenced 2 h before sunrise on each collecting night. All three species were collected in significantly greater numbers during periods 2–6 than during period 1 (Fig. 1). Numbers of *Cx. salinarius* captured during period 7 were significantly less than periods 2–6, but, although numbers collected during period 7 were also less for *Ae. vexans* and *Cx. pipiens*, geometric means were not significantly different from periods 5 and 6 for *Ae. vexans* and period 6 for *Cx. pipiens.* Significantly greater numbers of *Ae. vexans* and *Cx. salinarius* were collected at ground level compared with traps in the canopy, and significantly greater numbers of *Cx. pipiens* were collected in the canopy than at ground level (Table 2).

West Nile virus was isolated from mosquitoes in all 3 yr (Figs. 1 and 2). In 2004, isolations were made from 9 August to 13 September from *Cx. pipiens* ($n = 8$), *Cx. salinarius* ($n = 2$), *Ae. vexans* ($n = 3$), and *Ae. cinereus*

Table 2. Geometric means of 2-h collections per trap per night at canopy and ground levels, 2004, 2005, and 2006

Species	Ht	Yr		
		2004	2005	2006
Ae. vexans	Canopy	1.78a	1.28a	1.59a
	Ground	3.30b	1.97b	3.13b
Cx. pipiens	Canopy	2.03a	1.55a	2.15a
	Ground	1.76a	1.52a	1.48b
Cx. salinarius	Canopy	1.97a	1.28a	1.53a
	Ground	2.72b	1.45a	2.33b
Ae. cinereus	Canopy	1.43a	1.09a	
	Ground	1.92b	1.49b	
Cq. perturbans	Canopy	1.32a	1.30a	
	Ground	1.59a	2.68b	
Ae. cantator	Canopy	1.51a	1.34a	
	Ground	1.80b	1.83b	

Counts followed by the same letter within a column for a specific species and year were not significantly different ($P < 0.05$; K-S test).

($n = 1$). The virus was isolated from *Cx. pipiens* ($n = 14$) and *Cx. salinarius* ($n = 1$) from 1 August to 19 September 2005. *Cx. pipiens* yielded six isolates in 2006 from 23 August to 6 September. All isolates in 2004 and 2005 were from mosquitoes trapped in periods 3–6; in 2006, isolates were made from *Cx. pipiens* collected during periods 2, 4, and 5).

In addition, WNV was isolated from three species collected in insufficient numbers to be tested by the K-S test. In 2004, virus was isolated from *Cx. restuans* ($n = 2$) collected in time periods 3 and 4. WNV was isolated from one pool of *Psorophora ferox* (von Humboldt) collected during time period 5. In 2005, WNV was isolated from *Aedes sollicitans* (Walker) during period 6.

Parity studies were performed on 160 *Cx. pipiens* collected in 2006 within time periods 2–5. Percentage of parous females were 40, 40, 23, and 22 for time periods 2, 3, 4, and 5, respectively. There were no significant differences among time periods ($\chi^2 = 5.72$, df = 3, $P = 0.13$).

Weather data during the dates and times of mosquito collections in 2004, 2005, and 2006 are shown in Table 3. Temperatures and wind speed decreased and relative humidity increased after sunset, when significantly larger numbers of mosquitoes were collected. Precipitation tended to be low during most collection times. To examine the relationships among the numbers of collected mosquitoes, the meteorological variables (precipitation, wind speed, relative humidity, and temperature), and the time of night, a Spearman rank-correlation analysis was applied (Systat 11). During the 3 yr of this study, only temperature and time of night were significantly correlated with mosquito-catch for the three most abundant species (*Ae. vexans, Cx. salinarius,* and *Cx. pipiens*). The temperature effect was mainly seasonal, because the majority of insects were caught between mid-July and mid-August, which is the warmest time of the year. When temperatures over the course of the night were expressed as deviations from the nightly average temperature, there was no significant correlation between this diurnal temperature variation and mosquito-catch.

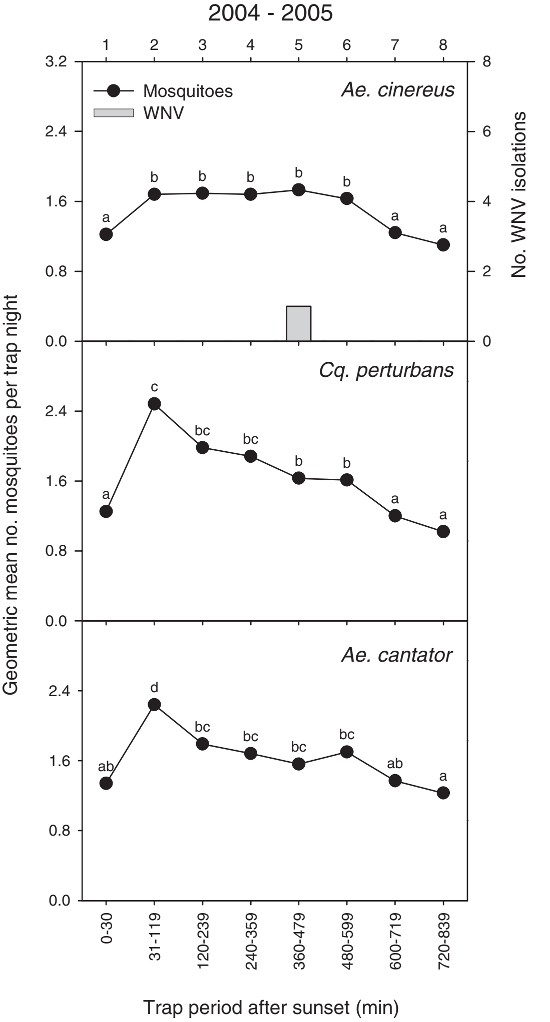

Fig. 2. Geometric means of *Ae. cinereus*, *Cq. perturbans*, and *Ae. cantator* caught at 2-h (120-min) intervals per trap per night at ground and canopy levels and total number of isolations of WNV relative to sunset, 2004–2005. Each 120-min collection period is identified by number at the top of the figure. Collection period 1 in 2004–2005 included 120–90 min of daylight; collection period 2 included 89–1 min of daylight. Geometric means for each 120-min collection interval for each species with the same lowercase letter are not significantly different ($P < 0.05$; K-S test).

Thus, time of night was the most important factor in determining the nightly variation in the number of trapped mosquitoes.

Discussion

Some species of mosquitoes are active throughout the night, whereas others have distinct feeding peaks (Gillett 1971). Our collection of mosquitoes at 2-h intervals for a total of 14 h adjusted to sunset throughout much of the mosquito season over a 2-yr (*Ae. cinereus*, *Cq. perturbans*, and *Ae. cantator*) or 3-yr (*Ae.

Table 3. Weather conditions recorded (mean ± SD) at Sikorsky Airport, Stratford, CT, during each 2-h collection period, 2004–2006

Yr	Collection period	Minutes trap operated		Temp (°C)	RH (%)	Wind speed (km/h)	Rainfall (cm/h)
		Before sunset	After sunset				
2004	1	120–90	0–30	22.4 ± 3.3	70.6 ± 14.7	15.7 ± 5.6	0.009 ± 0.018
	2	89–1	31–119	20.5 ± 3.9	73.2 ± 16.0	13.4 ± 5.0	0.005 ± 0.018
	3	0	120	19.5 ± 4.0	77.6 ± 14.1	11.3 ± 6.0	0.010 ± 0.043
	4	0	120	18.6 ± 4.2	81.9 ± 11.7	10.6 ± 6.8	0.015 ± 0.081
	5	0	120	18.1 ± 4.1	83.2 ± 12.2	10.0 ± 7.4	0.036 ± 0.155
	6	0	120	17.5 ± 4.1	85.2 ± 12.3	10.3 ± 8.4	0.025 ± 0.084
	7	0	120	17.7 ± 4.5	83.9 ± 10.2	12.1 ± 7.4	0.025 ± 0.081
	8	0	120	16.5 ± 5.5	79.3 ± 11.9	10.3 ± 6.1	0.025 ± 0.107
2005	1	120–90	0–30	23.4 ± 4.6	72.2 ± 18.9	12.9 ± 4.7	0.000 ± 0.000
	2	89–1	31–119	22.0 ± 4.8	73.2 ± 15.1	10.6 ± 4.2	0.0005 ± 0.003
	3	0	120	21.3 ± 4.2	78.4 ± 13.3	9.2 ± 5.3	0.0007 ± 0.003
	4	0	120	20.2 ± 4.4	84.1 ± 10.5	6.4 ± 4.3	0.013 ± 0.051
	5	0	120	19.8 ± 4.7	86.3 ± 8.7	6.1 ± 4.7	0.013 ± 0.051
	6	0	120	19.1 ± 4.7	87.7 ± 7.3	7.4 ± 5.3	0.051 ± 0.178
	7	0	120	19.6 ± 4.6	85.2 ± 8.2	9.3 ± 5.0	0.028 ± 0.127
	8	0	120	19.3 ± 4.1	82.1 ± 7.0	9.7 ± 8.0	0.015 ± 0.087
2006	1	120	0	23.7 ± 3.0	55.5 ± 22.5	11.7 ± 5.0	0.061 ± 0.244
	2	0	120	21.6 ± 2.5	68.5 ± 16.9	9.5 ± 6.9	0.005 ± 0.018
	3	0	120	20.2 ± 3.1	74.9 ± 11.9	9.8 ± 5.5	0.033 ± 0.135
	4	0	120	19.3 ± 3.3	77.4 ± 9.4	10.5 ± 6.0	0.000 ± 0.003
	5	0	120	18.4 ± 3.6	79.2 ± 8.2	8.8 ± 6.1	0.000 ± 0.000
	6	0	120	17.7 ± 3.5	81.0 ± 7.6	7.4 ± 5.6	0.000 ± 0.003
	7	0	120	18.2 ± 4.0	78.2 ± 10.3	7.4 ± 5.8	0.003 ± 0.008

vexans, Cx. pipiens, and *Cx. salinarius*) period shows that these six species are active throughout much of the night. Mosquito activity began around sunset and continued until about sunrise. Our collections were made and analyzed when mosquitoes were relatively abundant and when they were relatively scarce during two or three mosquito seasons. The collection of mosquitoes at 2-hr intervals throughout most of the mosquito season resulted in many collections without specimens. This type of collection is similar to the truncated log-normal distribution described previously (Southwood 1978). Thus, the log-transformed data set is not described by the normal distribution due to the large number of zero counts, and the variance calculated from such a data set would underestimate the true variance. Thus, the data were not simply log-transformed and analyzed by ordinary parametric statistics (Conover 1980, Helsel 1990). Instead, the K-S test for nonparametric statistics was used.

There have been numerous reports of bursts of mosquito activity occurring shortly after sunset (Murphey and Darsie 1962, Thompson and Dicke 1965, Knight and Henderson 1967, Carroll and Bourg 1977, Taylor et al. 1979, Lewis and Bennett 1980, Slaff and Crans 1981, Allan et al. 1982, Trueman and McIver 1984, Edman and Spielman 1988). The activity patterns for *Ae. vexans, Ae. cantator, Ae. cinereus, Cx. salinarius,* and *Cq. perturbans* seem to be primarily nocturnal-crepuscular (Edman and Spielman 1988). All species had geometric means that were significantly higher during collection period 2 compared with collection period 1. Geometric means of *Ae. vexans, Cq. perturbans,* and *Ae. cantator* in 2004 and 2005 (Figs. 1 and 2) and *Ae. vexans, Cx. pipiens,* and *Cx. salinarius* in 2006 (Fig. 1) were higher in period 2 than in the following three or four 2-h time periods, but the differences, with the exception of *Ae. cantator* and *Cq. perturbans* (Fig. 2), were not significant. *Ae. cantator* had a significantly higher geometric mean for period 2 than the other collection periods, and the mean for collection period 2 of *Cq. perturbans* was higher than all other times and significantly higher compared with periods 1 and 5–8.

Females of *Cx. pipiens* are responsive to hosts only during the night (Bowen et al. 1988). Flight activity was reported to be greatest after midnight in Ohio, although relatively large numbers were collected from 10 p.m. through 7 a.m. (Mitchell 1982). *Cx. pipiens* in our studies tended to have an activity pattern that was primarily nocturnal. In 2004 and 2005, the geometric mean for *Cx. pipiens* collected during period 2 was significantly less than the means for periods 3–6, and in 2006, significantly more specimens were collected for the four 2-h collection periods commencing at sunset than in periods 1 and 7.

Thirty-nine isolations of WNV were made from seven species (*Ae. vexans, Ae. cinereus, Ae. sollicitans, Cx. pipiens, Cx. salinarius, Cx. restuans,* and *Ps. ferox*). All isolations were made from specimens collected after sunset and usually during periods of total darkness. These were the periods when the largest numbers of specimens of each species were collected. Our isolations of WNV from mosquitoes captured during periods 3–6 during 2004 and 2005 and during periods 2, 4, 5, and 6 in 2006 suggest that infected females seeking hosts are relatively active throughout the night.

Among species from which WNV was isolated, four (*Cx. pipiens, Cx. restuans, Cx. salinarius,* and *Ae. vexans*) seem to be the important vectors of this virus to humans in northeastern United States (Andreadis et al. 2004). The preponderance of isolations from *Cx. pipiens* and its relative abundance (Anderson et al. 2006) suggest that this species is likely the most important, but it, like *Cx. restuans*, feeds predominately on birds and infrequently on humans (Magnarelli 1977; Apperson et al. 2002, 2004, Molaei et al. 2006). *Cx. salinarius*, also an abundant species, is more catholic in its feeding, obtaining blood from both birds and mammals, including humans, and it is considered a primary bridge vector of passing the virus from birds to humans (Andreadis et al. 2004). *Ae. vexans* feeds predominately on mammals, aggressively feeds on humans, and, although relatively fewer isolations have been made from this species compared with the species of *Culex*, it is relatively abundant and must also be considered as a possible bridge vector to humans (Molaei and Andreadis 2006). All of the above-mentioned species have been documented to be competent vectors (Turell et al. 2005). The fact that we trapped infected mosquitoes during all periods of darkness suggests that humans are at risk of being bitten by infected mosquitoes at almost any time during the night by any one of the above-mentioned species, although Kilpatrick et al. (2005) suggested *Cx. pipiens* as the most important species.

In 2004 and 2005, trapping commenced at 6 p.m. EDT with collection times converted to the time of sunset. In 2006, traps were set to begin operating 2 h before sunset. Data were similar for the three species collected in sufficient numbers in 2006 (*Ae. vexans, Cx. pipiens,* and *Cx. salinarius*) to the data collected for these three species collected in 2004 and 2005 (Fig. 1). All three species were collected in significantly greater numbers in the period beginning during sunset compared with the period before sunset. Because biting cycles of predominately crepuscular- and nocturnal-feeding species must be adjusted to time of sunset, we think it preferable to adjust the starting time of trapping to the time of sunset rather than starting trapping at a specific time throughout the mosquito season and then adjusting collections to time of sunset.

Parity rates of *Cx. pipiens* attracted to traps during collection periods 2–5 in 2006 ranged from 22 to 42%, but differences were not significant. These findings are similar to studies with other species (Bidlingmayer 1974, Service 1993). Thus, there seems to be no large differences among biting times of parous and nulliparous *Cx. pipiens.*

The collection of greater numbers of *Aedes* species and *Cx. salinarius* at 2-h intervals per night in ground positioned traps and greater numbers of *Cx. pipiens* in canopy-placed traps is commensurate with previous reports on the vertical distribution of mosquitoes (Main et al. 1966, Anderson et al. 2004, Darbro and Harrington 2006, Drummond et al. 2006).

Ultralow volume applications of insecticides with ground spraying equipment to control mosquitoes are often used in northeastern United States to reduce the risk of humans acquiring infection with WNV. Our data suggest that greatest application efficacy will be obtained by spraying from sunset to about sunrise.

Temperature and relative humidity after sunset tend to decrease and increase, respectively. Wind speed tends to decrease during the night. Mosquitoes were most active after sunset during collection periods 2–6. Our statistical analyses comparing relationships of the numbers of collected mosquitoes to specific time periods, precipitation, wind speed, relative humidity, and temperature showed that time of night was the most important factor in determining the nightly variation in the number of trapped *Ae. vexans, Cx. salinarius,* and *Cx. pipiens.* Thus, we focused on time of night instead of meteorological variables.

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