Journal of the American Mosquito Control Association, 20(2):138–145, 2004

SELECTED MOSQUITO VECTORS OF WEST NILE VIRUS: COMPARISON OF THEIR ECOLOGICAL DYNAMICS IN FOUR WOODLAND AND MARSH HABITATS IN DELAWARE

JOHN B. GINGRICH[1] AND LAURA CASILLAS[2]

ABSTRACT. We compared human landing, CO_2-baited light trap, CO_2-baited Omni-Fay trap, and gravid trap collections of mosquitoes at 4 woodland and marsh sites with varied vegetative habitats in Delaware during summer 2001. Landing collections provided more consistent, but sometimes smaller, collection numbers than light traps. Proportions of parous mosquitoes were also higher in landing than in light trap or Omni-Fay collections about 65% of the time. Circadian feeding rhythms were observed for 3 suspected vector species selected on the basis of 1999–2000 laboratory vector competence data and their presence at local habitats. These species included *Aedes vexans, Culex salinarius,* and *Cx. pipiens pipiens. Aedes vexans* fed after dusk, primarily before 2200 h and again around dawn (0600–0700 h). Both *Cx. salinarius* and *Cx. p. pipiens* exhibited peak feeding activity after dusk and in predawn periods, as well as extended feeding periods up until midnight. Time of feeding is an important factor in evaluating vector–host associations and risks of human outbreaks.

KEY WORDS Arthropod, vector, ecology, mosquitoes, West Nile virus, Delaware

INTRODUCTION

By the end of 2001, West Nile virus (WN) in the western hemisphere had spread to 44 states, the District of Columbia, Canada, and the Cayman Islands (MMWR 2002). By December 2002, WN had been isolated from 36 field-collected mosquito species (MMWR 2002). Of these, at least 31 species are found in Delaware and the eastern shore of Maryland. This is a particularly long list of suspected vectors for a nontropical disease. Although West Nile fever exhibits low case fatality rates in healthy humans, WN is an extremely facile virus with a wide range of hosts, both vertebrate and invertebrate. Also, it causes high mortality in some avian species, especially birds in the family Corvidae, such as crows and blue jays. Given the wide geographic distribution and long flight ranges of many suspected mosquito vectors, as well as information gaps on their bionomics, focusing pest management efforts on the most important species (on the basis of early vector competence data [Sardelis et al. 2001, Turell et al. 2001]) or on the habitat types that breed potential vector species has been difficult. Consequently, spraying operations have often been broader in area and time of application than necessary. Laboratory studies on vector competence of potential vector species cited above, as well those from Goddard et al. (2002), have helped to reduce the list of potential WN vectors, but with mosquito management budgets strained by the wide range of potential species and habitats that could be sources for WN outbreaks, it is desirable to further focus our intervention efforts on species that exhibit strong temporal and spatial associations with humans and avian amplifying hosts.

The overall objective of this research was to compare the bionomics of selected suspected vector species with the use of different collection methods in order to determine better ways to assess their risk of WN transmission to humans. Specifically, we wanted to determine bionomic factors for each suspected vector species on a longitudinal basis, as follows: 1) abundance per person-night or per trap-night (a measure of vector density), 2) number of generations per year, 3) proportions of parous mosquitoes, and 4) circadian feeding rhythms. We supposed these data would vary greatly during the course of each breeding season. On the basis of these factors, we sought to identify mosquito species that are likely vectors of WN and to better project when and where to concentrate mosquito management efforts.

MATERIALS AND METHODS

Mosquito study sites for 2001 were chosen from year 2000 nuisance mosquito collections by the Delaware Department of Natural Resources and Environmental Control. The selected sites had a demonstrated presence of certain suspected vector species that had exhibited multiple isolations of virus in New York in 2000 (Bernard et al. 2001), particularly *Aedes vexans* (Meigen), *Culex pipiens pipiens* L., and *Cx. salinarius* Coq. A heavily wooded site (Kenton), a mixed meadow and woodland site (Delaware Park), a coastal salt marsh site (Port Penn), and a mixed brackish water/fresh water site (Primehook) were selected. We performed our mosquito collections at biweekly intervals from 1 June to 30 September, 2001, making 9 collections per site.

We used 4 types of collection methods—human landing, the Hock CO_2-baited light (CDC-style) trap, the Omni-Fay CO_2-baited trap, and an infusion-baited gravid trap. The baits for the gravid traps were infusions of fermented grass clippings

[1] Department of Entomology and Wildlife Ecology, University of Delaware, Newark, DE 19716.
[2] Superfund Emergency Response and Removal Branch, EPA Region 7, Kansas City, MO 66101.

or oak leaves, depending on dominant plants present at the site. Landing collections were made only by the authors,[3] who collected landing mosquitoes immediately after they alighted on the exposed legs and feet of 1 person per site for periods of 7.5 h/night (0530–0750 and 1900–2350 h). Collectors trapped landing mosquitoes for 50 min of every hour during the collection period. Omni-Fay and light traps were placed approximately 30–50 ft away from the landing collection site in opposite directions. These traps were run overnight from 1900 to 0800 h, coinciding with the landing collections. Gravid traps were placed in a shaded area within the area occupied by the other traps but were run for a 24-h period.

The wide range in types of collections that we used enabled us to compare mosquito species coming to humans versus those coming to traps. For landing collections, we also obtained data on landing periods by placing individual mosquitoes in vials and marking them according to the hour of arrival. Collections were returned to the lab alive and quick frozen at −80°C prior to further work.

All mosquitoes from landing collections were identified to species by morphological keys (Darsie and Ward 1981) and counted. For Omni-Fay and light trap collections, all mosquitoes were counted, but because collections often numbered over 1,000, 50–100 mosquitoes were randomly selected and identified to species. The proportion of each species in that sample was used to extrapolate the total number of each species present in the entire collection. The most likely WN vectors from 1999 and 2000 research reports (Bernard et al. 2001) and from vector competence studies (Turell et al. 2001) were dissected for parity evaluation. Priority species for dissection among those mosquitoes found at our study sites were, therefore, *Ae. vexans, Cx. p. pipiens,* and *Cx. salinarius.* We had also hoped for sufficient numbers of other species, particularly *Ae. albopictus, Ochlerotatus sollicitans,* and *Oc. triseriatus,* but these species were scarce at our sites. Mosquitoes of each of the 3 primary species were dissected and then examined for parity by observing ovarian tracheolar coiling (Detinova 1962) under a compound microscope. Parous mosquitoes were tallied to provide the percentage of parity.

Abundance was calculated as the number of mosquitoes for each type of collection per person (or per trap) per collection period. Circadian feeding activity for landing mosquitoes was divided into 4 periods, arranged in the order 0530–0750, 1900–2050, 2100–2250, and 2300–2350 h. The number of generations per year was estimated by graphing abundance and proportions of parous mosquitoes per season, then observing the occurrence of peaks. Parity proportions were determined as the number of parous mosquitoes of each species divided by the total number of each species in each dissected collection. Comparison of parity proportions were made between landing and light trap or landing and Omni-Fay trap collections at the same site and week in which 9 or more (usually many more) specimens had been dissected for each compared species. If either collection had insufficient numbers (<9 of a given species), no comparison was made. For landing collections, the *n* values of mosquitoes dissected and checked for parity varied according to the number trapped (9 or far more, with a few exceptions) and in good enough condition for dissection. Actual dissected numbers are shown in the figures. An adjusted chi-square test was used to test statistical differences between the collections.

[3] The authors were the only ones collecting mosquitoes and took reasonable precautions and medical actions to monitor their health throughout the study.

RESULTS

Collections: Twenty-nine species of mosquitoes were trapped at our sites during the 2001 collection season (Table 1). Species collected and abundance estimates varied according to the type of trapping method used. Of the 3 suspected vector species given detailed attention (*Ae. vexans, Cx. p. pipiens,* and *Cx. salinarius*), 2 species (*Ae. vexans* and *Cx. p. pipiens*) displayed greater numbers in light trap than landing collections and 1 species (*Cx. salinarius,* Table 2) appeared in comparable or inconsistent numbers in both types of collections. Out of a possible 9 collections from 4 sites (total possible = 36 collections), there were 5 species for which landing collections consistently displayed the greatest abundance (*Ae. cinereus, Oc. canadensis, Oc. sticticus, Oc. excrucians,* and *Cx. territans*) and 10 species for which CO_2-baited light trap collections consistently exhibited the greatest abundance (*Oc. cantator, Cx. erraticus, Coquillettidia perturbans, Cx. p. pipiens, Anopheles bradleyi, An. punctipennis, Oc. sollicitans, Oc. trivitattus, Uranotaenia sapphirina,* and *Ae. vexans*). Two species were comparably trapped or displayed inconsistent trapping trends at different sites, as shown by mean abundance in landing collections or light traps, including *Cx. salinarius* and *Oc. triseriatus.* Variability in abundance estimates was greater in Hock light trap collections than in landing collections based upon standard deviation values of the major species trapped by both methods. Additionally, Hock traps often collect species that rarely, if ever, feed on humans, such as *Cx. restuans* and *Ur. sapphirina.*

Omni-Fay traps, in general, collected far fewer mosquitoes than landing collections or CO_2-baited light traps (Table 1). However, 6 species trapped in low numbers (*Ae. albopictus, An. crucians, Cs. melanura, Oc. mitchellae, Cx. restuans,* and *Ps. columbiae*) exhibited slightly greater abundance in Omni-Fay traps compared with the landing or light trap collections. *Culex restuans* was predominantly trapped (n = 78, Table 1) in gravid traps, occa-

Table 1. Species of mosquitoes and total number of mosquitoes collected from 4 sites in Delaware by 4 different trapping methods during June–September, 2001. Landing collections were from humans, Hock traps used light and dry ice attractants, Omni-Fay traps used dry ice attractant only, and gravid traps were baited with various infusions of leaves or grass.

Species	No. mosquitoes				
		Trap			
	Landing Collection	Hock Trap	Omni-Fay	Gravid	Total
Aedes albopictus	2	0	8	3	13
Ae. cinereus	6	0	0	0	6
Ae. vexans	414	5,639	1,683	0	7,736
Anopheles bradleyi	30	192	0	0	222
An. crucians	0	0	14	0	14
An. punctipennis	59	423	46	5	533
An. quadrimaculatus	337	1,693	594	7	2,631
Coquilletidia perturbans	347	1,985	600	0	2,932
Culex erraticus	45	110	51	1	207
Cx. p. pipiens	349	958	400	109	1,816
Cx. restuans	0	9	11	78	98
Cx. salinarius	2,836	7,019	1,876	18	11,749
Cx. territans	1	0	0	6	7
Culiseta inornata	0	0	0	1	1
Cs. melanura	2	2	3	0	7
Ochlerotatus atlanticus	1	0	1	0	2
Oc. canadensis	650	494	511	0	1,655
Oc. cantator	246	1,464	698	0	2,408
Oc. excrucians	9	0	0	0	9
Oc. mitchellae	1	0	5	0	6
Oc. sollicitans	56	198	53	0	307
Oc. sticticus	15	0	8	0	23
Oc. taeniorhynchus	36	166	42	0	244
Oc. triseriatus	37	102	26	18	183
Oc. trivitattus	2	37	0	0	39
Psorophora ciliata	24	51	18	0	93
Ps. columbiae	12	3	18	0	33
Ps. ferox	39	82	51	0	172
Uranotaenia sapphirina	0	47	22	1	70
Total (n = 29)	5,556	20,674	6,739	247	33,216

sionally in Omni-Fay traps, only once in a light trap, and once in a landing collection.

Parity: Landing collections yielded higher proportions of parous mosquitoes in paired species comparisons than Hock light traps in 19 of 31 data sets. Hock light traps indicated higher parity proportions in only 9 of 31 data sets with paired comparisons within species during the same collection week. This difference was significant ($P < 0.05$). Similarly, there were higher proportions of parous mosquitoes in 23 of 34 paired comparisons of landing and Omni-Fay trap collections. The opposite was true in only 10 of 34 paired comparisons, yielding $P < 0.05$ in favor of landing collections.

Seasonal abundance and parity estimates: Because of the greater consistency of data observed in landing collections, detailed comparisons of seasonal abundance and parity were made with the use of landing collection data only.

At Primehook, the 2 predominant species were *Cx. p. pipiens* and *Cx. salinarius*. *Culex p. pipiens* exhibited only 1 peak in parity and abundance, occurring during June 18 and 25, 2001, respectively. However, a peak in parity typically indicates a previous abundance peak as mosquitoes emerge and age from a major brood. We estimate that there was an earlier peak emergence period of *Cx. p. pipiens* that was missed because of the late starting date of our collections. *Culex salinarius* exhibited 2 peak periods of abundance at Primehook—July 16 and August 27, 2001—but the proportion of parous mosquitoes suggested that there was an earlier abundance peak in late May or early June (Fig. 1).

At Delaware Park, the 2 predominant species were *Cx. p. pipiens* and *Ae. vexans*. *Culex p. pipiens* exhibited 1 peak in abundance, but parity data were insufficient to draw other conclusions. There were 3 peaks in abundance of *Ae. vexans* at Delaware Park, occurring at June 18, July 16, and August 13, 2001 (Fig. 2). However, a high proportion of parity in the 1st week of June suggested a prior peak in abundance sometime in May before the start of collections (Fig. 2). *Aedes vexans* exhibited 2 peaks in abundance (June 18 and August 13, 2001) at Kenton, but parity data were inconclusive in indicating prior abundance peaks.

Table 2. Mean abundance and standard deviations of 4 species of mosquitoes at 4 sites (Primehook, Delaware Park, Kenton, and Port Penn, DE) collected by landing collections (LC) and Hock CO_2-baited light traps (HT).

Species	Trap method	Abundance[1,2] Primehook	Delaware Park	Kenton	Port Penn
Cx. p. pipiens	LC	27.0 ± 37.6	7.2 ± 7.6	N/A	N/A
	HT	67.4 ± 143.1	18.6 ± 28.6	N/A	N/A
Cx. salinarius	LC	120.7 ± 101.7	3.7 ± 3.2	N/A	213.3 ± 242.5
	HT	109.8 ± 151.4	74.3 ± 122.4	N/A	594.0 ± 600.1
Ae. vexans	LC	9.7 ± 18.2	22.2 ± 26.0	10.0 ± 12.1	N/A
	HT	62.9 ± 105.8	265.7 ± 451.6	33.5 ± 62.6	N/A
Oc. canadensis	LC	N/A	N/A	64.0 ± 70.0	N/A
	HT	N/A	N/A	56.9 ± 70.3	N/A

[1] N/A, excluded because there were 5 or more pairs of 0s among the 9 seasonally paired collections.
[2] Abundance for LC is expressed as mosquitoes/person/night. Abundance for HT is expressed as mosquitoes/trap/night.

At Port Penn, *Cx. salinarius* was the dominant species collected. Peaks in abundance occurred at July 16 and September 10, 2001 (Fig. 3). However, the proportion of parous mosquitoes exhibited 2 peaks, occurring at June 18 and August 27, 2001. High parity observed on June 18, 2001, suggested a prior earlier emergence that was not detected because of our late starting date (Fig. 3).

Circadian rhythms: Circadian feeding rhythms and parity proportions were determined for the major suspected vector species at each site. These feeding patterns were observed for each collection date to determine whether there were seasonal shifts in feeding rhythms and whether parous mosquitoes predominated during certain feeding periods. These data are most easily observed in graphic form, although there were no parity data for time periods in which the species was absent (shown as 0s).

Ochlerotatus canadensis was the dominant species in Kenton, although the most prevalent suspected vector was *Ae. vexans*. However, biting periods of these species were different because *Ae. vexans* tended to feed later in the evening (after sunset), whereas *Oc. canadensis* feeding often occurred at dawn or just before sunset. Also, *Oc. canadensis* exhibited a much shorter seasonal abundance than *Ae. vexans*.

Aedes vexans was the dominant species at Delaware Park in 2001. In the early part of the collec-

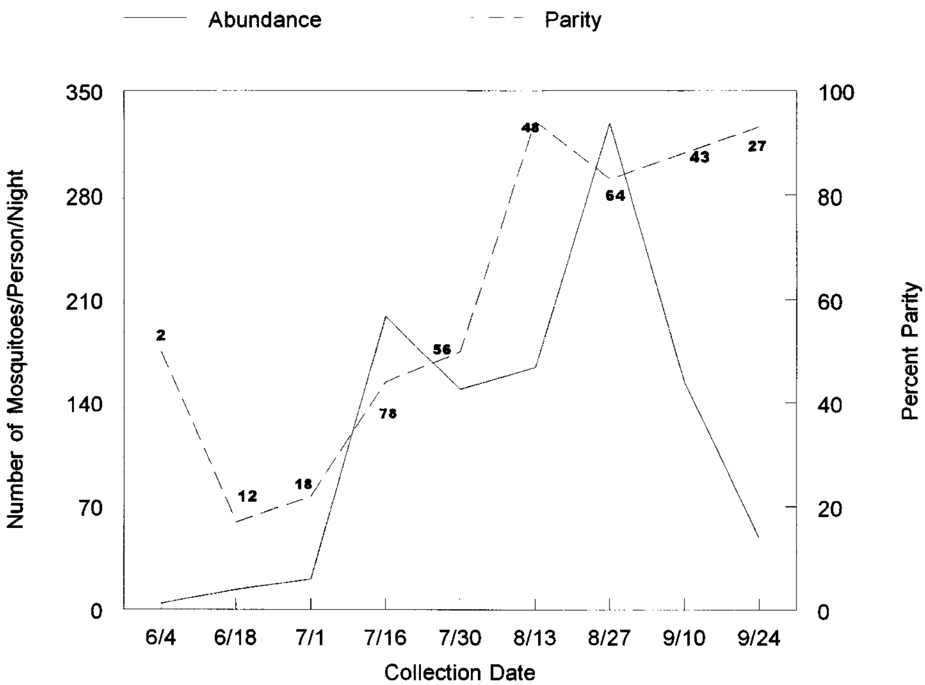

Fig. 1. Landing abundance (mosquitoes/person/night) and percent parity (number dissected shown at data point) of *Cx. salinarius* at Primehook, DE.

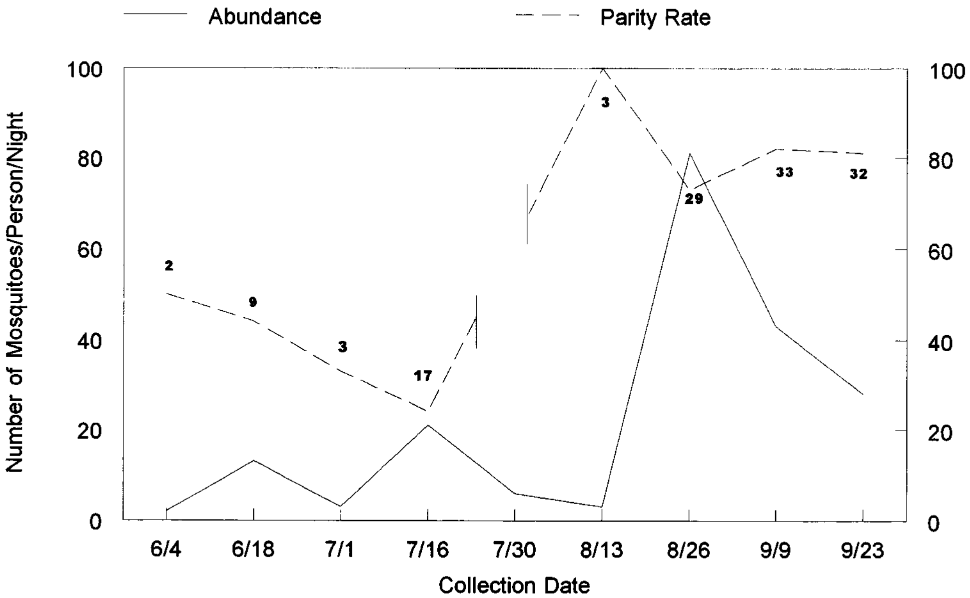

Fig. 2. Landing abundance (mosquitoes/person/night) and percent parity (number dissected shown at data point) of *Ae. vexans* at Delaware Park.

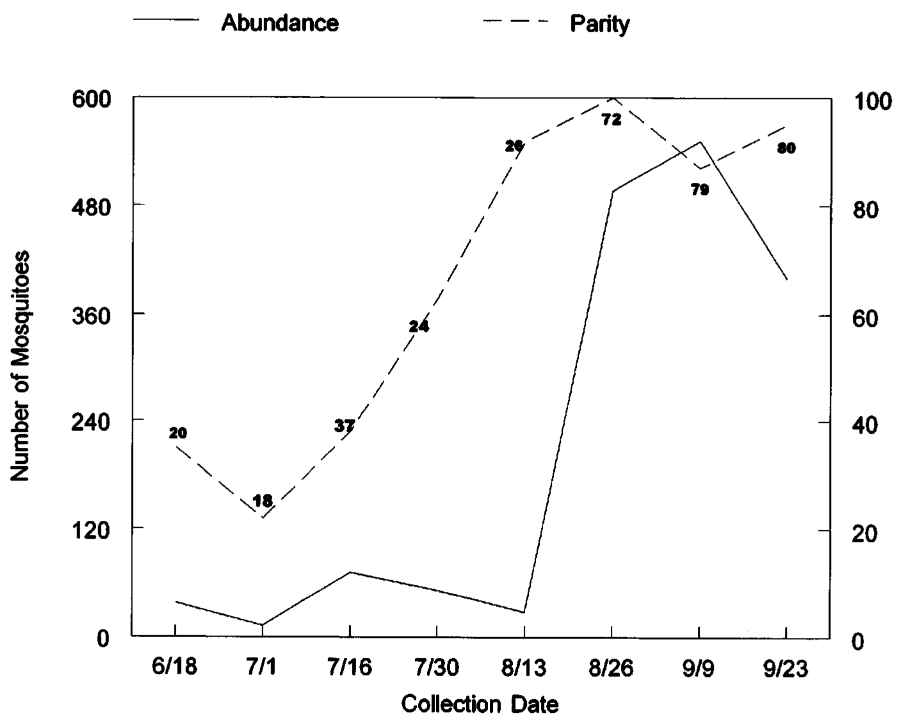

Fig. 3. Landing abundance (mosquitoes/person/night) and percent parity (number dissected shown at data point) of *Cx. salinarius* at Port Penn, DE.

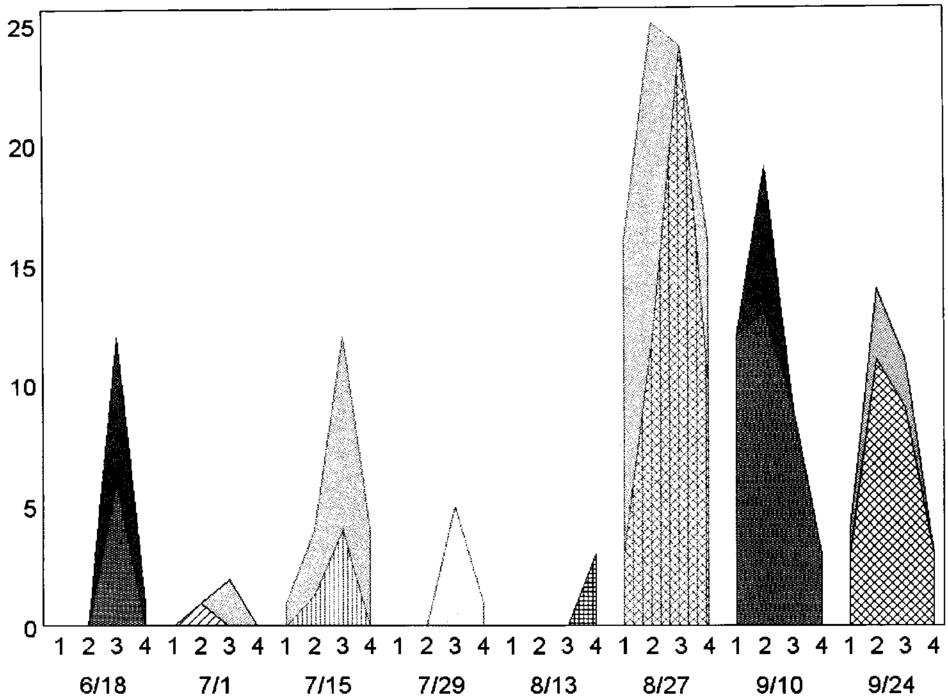

Fig. 4. Number of *Ae. vexans* landing (outer graph) versus number parous (inner graph) at Delaware Park during 8 seasonal collections (June 18 to September 24, 2001) and 4 nightly collection periods: 1, 0530–0750 h; 2, 1900–2050 h; 3, 2100–2250 h; 4, 2300–2350 h. Different hatching for inner and outer graphs is used for visual contrast only.

tion season (June 3 to July 15), *Ae. vexans* exhibited peak abundance and parity during the period from 2100 to 2250 h (Fig. 4). By late August (August 27), peak landing collection periods shifted to early morning or early evening (1900–2050 h), with peak parity generally occurring later than the peak landing period. *Culex salinarius* also generally exhibited peak landing and parity from 2100 to 2250 h up until June 18 at Primehook. However, during an extremely warm night on July 1, peak landing was delayed until after 2300 h, although peak parity occurred earlier (Fig. 5). By September 10, peak landing periods had shifted to 1900–2050 h, and by September 23, it occurred in the early morning. Peak parity during September, however, occurred later than peak abundance. *Culex salinarius* exhibited this same general pattern of activity at Port Penn (not shown).

Culex p. pipiens landing peaks at Delaware Park in 2001 generally followed the same seasonal pattern as *Cx. salinarius* at Port Penn, with peak abundance usually occurring from 2100 to 2250 h up until August 27, after which time it occurred from 1900 to 2050 h. *Culex p. pipiens* abundance at Primehook followed a circadian pattern similar to *Cx. salinarius* but shifted to earlier landing periods in the early morning or early evening (1900–2050 h) by July 30.

DISCUSSION

The type of collections that provide the best information relevant to transmission of human diseases has been a source of contention among researchers. A number of malaria studies that have used landing collections for mosquitoes have indicated that human landing collections are essential to obtaining good anopheline vector data (Parsons et al. 1974, Ulloa et al. 1997, Strickman et al. 2000). In part, this is because many anopheline species are poorly attracted by light and other types of traps. This comparison has been made infrequently for arboviral vectors, although *Cx. annulirostris,* a vector of Murray Valley encephalitis in Australia, is more frequently taken in human landing than in light trap collections (Jones et al. 1991). This is in part because *Culex* spp. typically tend to predominate as *Flavivirus* vectors, and *Culex* spp. are generally said to be attracted to light traps. However, numerous *Aedes/Ochlerotatus* and some anopheline species are suspected vectors of WN, and, until more evidence is gathered on the importance of these potential vectors, it seems wise to investigate a broad spectrum of species. Another complication with trap collections was the greater collection-to-collection variation in abundance compared with human landing collections, as was evident from the

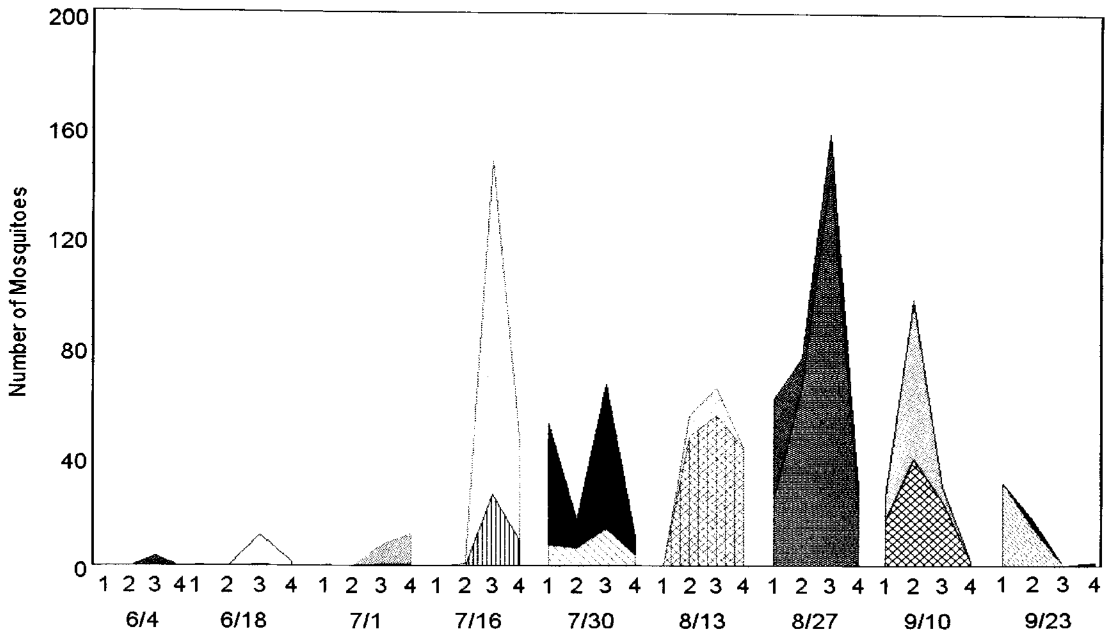

Fig. 5. Number of *Cx. salinarius* landing (outer graph) versus number parous (inner graph) at Primehook, DE, over 9 seasonal collections (June 4 to September 23, 2001) and 4 nightly collection periods: 1, 0530–0750 h; 2, 1900–2050 h; 3, 2100–225 h; 4, 2300–2350 h. Different hatching is for visual contrast only.

extremely high standard deviations obtained from our trap data. The rate of dry ice sublimation, the direction and speed of wind, the relationship of carbon dioxide plumes to fan intakes, the trap height, and faults with traps all contribute to this variation. By contrast, humans adjust to the wind by moving to protected areas and only collect human-attracted mosquitoes. In assessing the relative importance of vector species, attraction to landing on humans would seem to be a key factor in the epidemiological relevance of the data.

Data on proportions of parous mosquitoes are extremely important to virus transmission studies on vector species because parity and virus replication in mosquitoes is directly related to longevity. In our studies, it was evident that parity became markedly higher in the latter half of the collection season (by August 1 or 13) for *Ae. vexans* and *Cx. salinarius,* and proportions of parous mosquitoes remained high through September. If this pattern repeats from year to year, it would suggest that this is an important factor conducive to WN transmission in August and September. Parity data for *Cx. salinarius* were remarkably similar at the Port Penn and Primehook sites, suggesting that this pattern is not a site-specific phenomenon.

The mosquito species studied exhibited consistent circadian feeding patterns. *Aedes vexans* generally fed from 2100 to 2250 h, but shifted to 1900 to 2050 h in late August, probably related to the earlier sunset, as has been previously demonstrated by Murphey et al. (1967). The circadian feeding rhythm of *Cx. salinarius* was similar, including the shift to feeding from 1900 to 2050 h in late August. Circadian feeding patterns are important, in that they indicate opportunities for association between the mosquito and its hosts.

ACKNOWLEDGMENTS

We gratefully appreciate the use of laboratory facilities provided by Judy Hough-Goldstein and Charles Mason of the Department of Entomology/Wildlife Ecology, University of Delaware. William Meredith of the Division of Fish and Wildlife, Delaware Department of Natural Resources and Control, supported this project with funding and encouragement that were essential for its success. Special thanks are also due Thomas Moran and David Saveikis, Mosquito Control Sections, Delaware Department of Natural Resources and Control, for providing information regarding potential field sites and logistical support at critical periods.

REFERENCES CITED

Bernard KA, Maffei JG, Jones SA, Kauffman EB, Ebel GD, Dupuis AP II, Ngo KA, Nicholas DC, Young DM, Shi PY, Kulasekera VL, Eidson M, White DJ, Stone WB, NY State WNV Surveillance Team, Kramer LD. 2001. West Nile virus infection in birds and mosquitoes, New York State, 2000. *Emerg Infect Dis* 7:679–685.

Darsie RF, Ward RA. 1981. *Identification and geographical distribution of the mosquitoes of North America, north of Mexico.* Mosquito Systematics Supplement Volume 1, 313 p. Fresno, CA: American Mosquito Control Association.

Detinova TS 1962. *Age-grouping methods in Diptera of medical importance, with special reference to some vectors of malaria* Geneva, Switzerland: World Health Organization.

Goddard LB, Roth AE, Reisen WK, Scott TW. 2002. Vector competence of California mosquitoes for West Nile virus. *Emerg Infect Dis* 8:1385–1389.

Jones RE, Barker-Hudson P, Kay BH. 1991. Comparison of dry ice baited light traps with human bait collections for surveillance of mosquitoes in northern Queensland, Australia. *J Am Mosq Control Assoc* 7:387–384.

[MMWR] Morbidity and Mortality Weekly Report. 2002. Provisional surveillance summary of the West Nile virus epidemic—United States, January–November, 2002. *Morbid Mortal Wkly Rep* 51:1129–1133.

Murphey FA, Burburtis PP, Bray DF. 1967. Bionomics of *Cx. salinarius* Coquillet. II. Host acceptance and feeding by adult females of *Cx. salinarius* and other mosquito species. *Mosq News* 27:366–374.

Parsons RE, Dondero TJ, Hoo CW. 1974. Comparison of CDC miniature light traps and human biting collections from mosquito catches during malaria vector studies in peninsular Malaysia. *Mosq News* 34:211–214.

Sardelis MR, Turrell MJ, Dohm DJ, O'Guinn ML. 2001. Vector competence of selected North American *Culex* and *Coquillettidia* mosquitoes for West Nile virus. *Emerg Infect Dis* 7:1018–1022.

Strickman D, Gaffigan T, Wirtz RA, Benedict MQ, Rafferty CS, Barwick RS, Williams H. 2000. Mosquito collections following local transmission of *Plasmodium falciparum* malaria in Westmoreland County, Virginia. *J Am Mosq Control Assoc* 16:219–222.

Turell MJ, O'Guinn ML, Jones JW. 2001. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. *J Med Entomol* 38:130–134.

Ulloa A, Rodriguez MH, Rodriguez AD, Roberts DR. 1997. A comparison of two collection methods for estimating abundance and parity of *Anopheles albimanus* in breeding sites and villages of southern Mexico. *J Am Mosq Control Assoc* 13:238–244.