VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 4, Number 4, 2004

Research Paper

Epidemiology of West Nile Virus in Connecticut: A Five-Year Analysis of Mosquito Data 1999–2003

THEODORE G. ANDREADIS,[1] JOHN F. ANDERSON,[1] CHARLES R. VOSSBRINCK,[1] and ANDREW J. MAIN[1,2]

ABSTRACT

Two hundred and ten isolations of West Nile virus (WNV) were obtained from 17 mosquito species in six genera in statewide surveillance conducted in Connecticut from June through October, 1999–2003. *Culex pipiens* (86), *Culex salinarius* (32), *Culex restuans* (26), *Culiseta melanura* (32), and *Aedes vexans* (12) were implicated as the most likely vectors of WNV in the region based on virus isolation data. *Culex pipiens* was abundant from July through September and is likely involved in early season enzootic transmission and late season epizootic amplification of the virus in wild bird populations. Epidemic transmission of WNV to humans in urban locales is probable. The abundance of *Cx. restuans* in June and July and isolations of WNV in early July suggest that this species may play an important role as an enzootic vector involved in early amplification of WNV virus among wild birds. Its involvement as a bridge vector to humans is unlikely. *Culex salinarius* was the most frequently captured *Culex* species and was abundant in August and September when virus activity was at its height. Frequent isolations of WNV from this species in September when the majority of human cases were reported in union with its abundance at this time of the year, demonstrated vector competence, and broad feeding habits, make *Cx. salinarius* a likely bridge vector to humans, horses and other mammals. Multiple isolations WNV from *Cs. melanura* collected in more rural locales in late August and September, provide supportive evidence to suggest that this predominant avian feeder may play a significant role in epizootic amplification of the virus among wild bird populations in these environs. *Aedes vexans* was the only species of *Aedes* or *Ochlerotatus* from which multiple isolations of WNV were made in more than one year and was among the most frequently trapped and abundant species throughout the season. Since *Ae. vexans* predominately feeds on mammals it is unlikely to play a significant role in epizootic amplification of WNV, however, because of its abundance and aggressive mammalian and human biting behavior it must receive strong consideration as a bridge vector to humans and horses. The occasional virus isolations obtained from *Aedes cinereus* (4), *Uranotaenia sapphirina* (3), *Ochlerotatus canadensis* (2), *Ochlerotatus trivittatus* (2), *Ochlerotatus sollicitans* (2), *Ochlerotatus sticticus* (2), *Psorophora ferox* (2), *Anopheles punctipennis*, *Anopheles walkeri*, *Ochlerotatus cantator*, *Ochlerotatus taeniorhynchus*, and *Ochlerotatus triseriatus* in conjunction with their inefficient vector competency and host feeding preferences indicate that these species likely play a very minor role in either the enzootic maintenance or epizootic transmission of WNV in this region. The principal foci of WNV activity in Connecticut were identified as densely populated (>3,000 people/mi^2) residential communities in coastal Fairfield and New Haven Counties, and in the case of 2002, similar locales in proximity of the city of Hartford in central Hartford County. In almost all instances we observed a correlation both temporally and spatially between the isolation of WNV from field-collected mosquitoes and subsequent human cases in these locales. In most years the incidence of human cases closely paralleled the number of virus isolations made from mosquitoes with both peaks falling in early September. We conclude that the isolation of WNV from field-collected mosquitoes is a sensitive indicator of virus activity that is associated with the risk of human infection that habitually extends from early August through the end of October in Connecticut. Key Words: West Nile virus—Epidemiology—Mosquito—Vectors—*Culex pipiens*—*Culex restuans*—*Culex salinarius*—*Culiseta melanura*—*Aedes vexans*. Vector-Borne Zoonotic Dis. 4, 360–378.

[1]Connecticut Agricultural Experiment Station, New Haven, Connecticut.
[2]American University in Cairo, Cairo, Egypt.

INTRODUCTION

MOSQUITO SURVEILLANCE for West Nile virus (WNV) has been conducted in Connecticut since the virus was first detected in North America during the summer of 1999 (Anderson et al. 1999, Lanciotti et al. 1999). The objectives of this program have been to (1) identify potential mosquito vectors; (2) quantify the seasonal abundance and spatial distribution patterns of mosquitoes in suburban/urban and rural foci; and (3) assess the role of various species in enzootic maintenance and epizootic amplification in wild bird populations, and epidemic transmission to humans.

Initial surveillance studies conducted in the

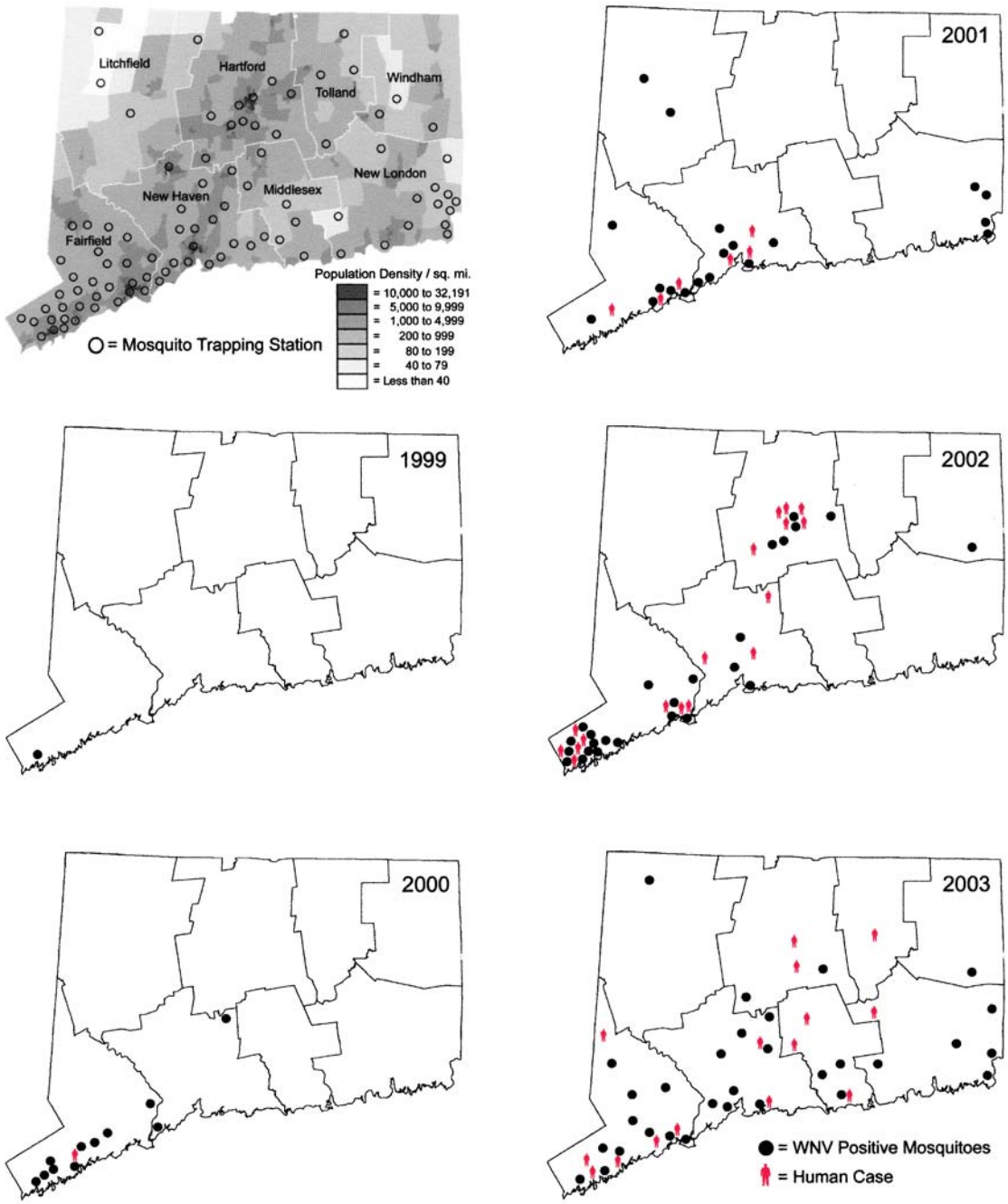

FIG. 1. Geographic distribution of West Nile virus isolations from mosquitoes and human cases in relation to human population density and mosquito trapping stations in Connecticut 1999–2003.

northeastern United States in the year following the outbreak (Andreadis et al. 2001b, Bernard et al. 2001, Kulasekera et al. 2001, Marfin et al. 2001, White et al. 2001) largely incriminated *Culex* species as the most important vectors of WNV in the region. National surveillance data reported to the federal Centers for Disease Control and Prevention via the ArboNET surveillance network (CDC 2002) supported these initial findings with five species, *Culex pipiens*, *Culex restuans*, *Culex salinarius*, *Culex quinquefasciatus*, and *Culex tarsalis* accounting for 55% of the WNV-positive pools obtained from mosquitoes collected in 28 states in 2002. Since 1999, a total of 43 WNV-infected mosquito species have been reported (CDC 2003).

Despite these observations, there have been no in depth studies that have examined the relationship between mosquito populations and the incidence of WNV both within and outside recognized WNV foci. These objectives have been specifically addressed in Connecticut through the establishment and maintenance of a statewide network of fixed mosquito trapping sites that have been continually monitored from June through October for the 5 years 1999–2003. The results of these investigations are reported herein and examined in relation to the epizootiology and epidemiology of WNV in the region.

MATERIALS AND METHODS

Mosquito trapping and identification

Mosquito trapping was conducted from June through October, 1999–2003, at 91 locations statewide (Fig. 1). Approximately one-third of the sites were located in densely populated residential locales along the urban/suburban corridor that extended from lower Fairfield and New Haven Counties, up through the Connecticut River Valley and into lower Hartford County. Trap sites included parks, greenways, golf courses, undeveloped wood lots, sewage treatment plants, dumping stations, and temporary wetlands associated with waterways. Trapping locations in the other five counties (Litchfield, Middlesex, New London, Tolland, and Windham) were established in more sparsely populated rural settings that included permanent fresh-water swamps (red maple/white cedar) and bogs, coastal salt marshes, horse stables, and swamp-forest border environs. Trapping frequency was variable but was minimally made once every ten days at each trap site over the course of the entire season. The mean number of trap nights per site was 18 in 1999, 21 in 2000, 23 in 2001, and 31 in 2002 and 2003.

Two trap types were used in 2000 through 2003: (1) a CO2 (dry ice)–baited CDC miniature light trap with an aluminum dome, and (2) a CDC gravid mosquito trap (Reiter 1983). A sod grass-infusion was utilized in the gravid traps in 2000 and 2001 (Lampman and Novak 1996), while commercial rabbit chow (Purina Mills LLC, St. Louis, MO) was used in 2002 and 2003. Trapping was done exclusively with CDC light traps in 1999. Typically, traps were placed in the field during the late afternoon and retrieved the following morning. Adult mosquitoes were transported alive to the laboratory where they were promptly identified on chill tables with a stereomicroscope using descriptions and keys of Darsie and Ward (1981) and Means (1979, 1987). Mosquitoes were pooled by species, collecting site, trap type, and date. The number of mosquitoes per pool ranged from 1 to 50. Mosquitoes were stored at −80°C until tested for virus.

Virus isolation and identification

Each frozen mosquito pool was disrupted in 1–1.5 mL of phosphate buffer saline (PBS) containing 0.5% gelatin, 30% rabbit serum, antibiotic, and antimycotic. This was accomplished either by trituration with alundum in a mortar and pestle, or by shaking in a 2-mL centrifuge vial containing a copper BB pellet. Mosquitoes in the centrifuge vials were shaken at 25 cycles per second for 4 min in a Retsch Laboratory Vibration Mill MM 300 (Irvine, CA). All samples were centrifuged at 4°C for 10 min at 520*g*. A 100-μL aliquot of the supernatant was inoculated onto a monolayer of Vero cells growing in a 25-cm^2 flask at 37°C in 5% CO_2. Cells were examined for cytopathologic effect at 3–7 days following inoculation.

WNV was identified from Vero cell positive cultures either by reverse transcription–polymerase chain reaction (RT-PCR) (1999–2001), or

by TaqMan RT-PCR (2002 and 2003) (Lanciotti et al. 2000). For the RT-PCR procedure, Vero cell cultures showing lytic activity were pelleted and processed using a Qiagen Rneasy mini protocol. The Rneasy column was eluted twice with 40 μL of RNAse free cell culture water. Two micro-liters of the column eluate was reverse transcriptase amplified using the Perkin-Elmer GeneAmp EZ rTh RNA PCR kit. Three different sets of primers representing five primer sites unique to WNV were used for redundancy: (1) WN-233F (GACTGAAGAGGGCAATGTTGAGC) and WN-1189R (GCAATAACTGCGGACYTCTGC); (2) WN-200F (TCAATATGCTAAAACGCGG) and WN-540R (TTAGAGAGGGTAACTGCTCC); (3) WN-451F (GTGCTATCAATCGGCGGAGCTC) and 540R. Gene amplification was done on an MJ Research PTC-200 DNA Engine. The protocol was as follows: 60°C for 30 min, 94°C for 2 min followed by 40 cycles of 94°C for 45 sec, 50°C for 30 sec, and 60°C for 1 min 30 sec. PCR product was run in a 1.5% agarose gel stained with ethidium bromide and electrophoresed at 20 V/CM for approximately $^1/_2$ h. Band size was checked against the AmpliSize size markers from BioRad.

In the TaqMan assay, RNA was extracted from a 70-μL sample of each Vero cell positive culture using a QIAamp viral RNA kit (QIAGEN, Valencia, CA). Negative and positive control samples were included in each test. The negative control was double-processed tissue culture water (Sigma, St. Louis, MO). The positive control was *Cx. pipiens* isolate 8770. Primers were WNENV-forward 1160 (TCAGCGATCTCTCCACCAAAG) and WNENV-reverse 1229 (GGGTCAGCACGTTTGTCATTG). WNENV-probe 1186 (TGCCCGACCATGGGAGAAGCTC) was used with 5′ end labeled with the FAM reporter dye and the 3′ end labeled with the TAMRA quencher dye. A 25-μL reaction volume using the TaqMan RT-PCR Ready-Mix Kit (PE Applied Biosystems) was prepared with 2.5 μL of RNA, 0.25 μL of each primer, 0.15 μL of probe, 12.5 μL of 2 $\times$ buffer, 0.5 μL of RT-PCR enzyme, and 8.85 μL of water. Real time assays were done in a Smart Cycler (Cepheid, Sunnyvale, CA). Samples were subjected to one cycle of 50°C for 20 min, 95°C for 10 min, and then 50 cycles of 95°C for 15 sec and 60°C for 60 sec. Specimens with a cycle threshold value of <37 were considered to be infected with virus.

Minimum field infection rates (MIR) for estimating WNV infection rates in pooled mosquito samples were calculated using the methodology of Biggerstaff (2003). Data were analyzed by ANOVA and regression analysis (Jandel Corp. 1995).

Human population and climatological data analyses

Human population data for Connecticut were obtained from the U.S. Census 2000 (U.S. Census Bureau 2002) and the Connecticut State Department Office of Policy and Management (CT OPM 2003). The relationship between human population density and mosquito abundance was ascertained for selected species by plotting the mean number of mosquitoes collected per trap night in each of the 5 years 1999–2003 versus human population density in the respective township where the mosquito collections were made. These relationships were analyzed by linear regression (Jandel Corp. 1995). Information on the illness onset dates for all confirmed human cases of WNV were obtained from the Connecticut State Department of Public Health.

Temperature and rainfall data were obtained from the NOAA Climatological Data for New England (NCDC 2003). Deviations from the norm were calculated for June through September of each year from data reported at 22 official stations located in the central and coastal divisions of the State that included 87 of 91 trapping locations.

RESULTS

Mosquito collection and virus isolation data

The mosquito collection data for 1999–2003 are summarized in Table 1. A total of 717,283 female mosquitoes representing 38 species in nine genera were collected from the field, identified and processed for virus isolation (52,499 pools). The most commonly trapped species were *Ochlerotatus canadensis* (n = 106,964, 15.5% of total), *Coquillettidia perturbans* (106,557,

Table 1. Mosquito Species Trapped and Tested for West Nile Virus in Connecticut, 1999–2003

Genus	Species	No. mosquitos collected		No. pools tested
		Gravid trap	*Light trap*	
Aedes	*cinereus*	1,596	52,656	3,921
	vexans	505	75,310	4,600
Anopheles	*barberi*	41	12	45
	crucians	—	55	16
	punctipennis	106	11,516	2,693
	walkeri	118	1,580	779
	quadrimaculatus	69	4,581	535
Coquillettidia	*perturbans*	2,283	104,274	4,164
Culex	*erraticus*	—	4	1
	pipiens	7,795	14,519	3,096
	restuans	5,744	18,991	3,098
	salinarius	523	34,701	2,843
	territans	118	332	287
Culiseta	*inornata*	—	1	1
	melanura	546	35,088	3,221
	minnesotae	21	99	27
	morsitans	84	1,136	429
Ochlerotatus	*abserratus*	18	8,446	582
	atropalpus	—	15	14
	aurifer	214	10,147	627
	canadensis	1,183	105,781	4,588
	cantator	119	15,451	1,493
	communis	—	357	39
	diantaeus	—	1	1
	excrucians	21	3,751	655
	fitchii	1	6	3
	grossbecki	—	1	1
	japonicus	1,697	2,588	1,769
	provocans	—	111	10
	sollicitans	89	11,247	711
	sticticus	396	40,625	1,802
	stimulans	35	3,907	543
	taeniorhynchus	42	23,045	863
	triseriatus	1,708	13,092	2,711
	trivittatus	724	72,132	3,306
Orthopodomyia	*signifera*	—	17	17
Psorophora	*ferox*	67	15,058	1,299
Uranotaenia	*sapphirina*	155	10,632	1,609
Total		26,018	691,265	52,499

15.4%), *Aedes vexans* (75,815, 11.0%), *Ochlerotatus trivittatus* (72,856, 10.5%), and *Aedes cinereus* (54,252, 7.8%), which collectively accounted for more than one-half (58.1%) of all specimens. Other notably abundant species included *Ochlerotatus sticticus* (41,021, 5.9%), *Culiseta melanura* (35,634, 5.2%), and *Ochlerotatus taeniorhynchus* (23,087, 3.3%). *Anopheles punctipennis* (11,622, 1.7%) was the most frequently captured anophelene.

Five species of *Culex* were collected. *Culex salinarius* (35,224, 5.1%), *Cx. restuans* (24,735, 3.6%), and *Cx. pipiens* (22,314, 3.2%), were the most numerous. *Culex territans* (450, 0.1%) was infrequently found, and *Culex erraticus* was trapped on one occasion. *Culex pipiens* and *Cx. restuans* were commonly collected in gravid traps (36.6% and 23.8% of their respective totals, excluding 1999), while almost all (98.5%) of the *Cx. salinarius* females were collected in CO_2-baited light traps. Among the *Aedes* and *Ochlerotatus*, only two container-breeding species, *Ochlerotatus japonicus* and *Ochlerotatus triseriatus* were collected with any degree of

regularity in the gravid traps when compared to their collection from light traps (39.6% and 11.5% of their respective totals).

The virus isolation data are summarized in Table 2. A total of 210 isolations of WNV were obtained from 17 mosquito species in six genera. Eighty-four percent of all virus isolations were obtained from four species: *Cx. pipiens* ($n = 86$, 41.0%), *Cx. salinarius* ($n = 32$, 15.2%), *Cs. melanura* ($n = 32$, 15.2%), and *Cx. restuans* ($n = 26$, 12.4%). The overall minimum field infection rates (MIRs) (calculated from all trap collections 2000–2004, excluding 1999 collection data when no gravid traps were used) for *Cx. pipiens* were 3.98 per thousand among females collected in the gravid traps, and 4.00 per thousand among females collected in the light traps. The overall MIRs for *Cx. restuans* were similarly equivalent with 1.22 per thousand in the gravid trap collections and 1.03 per thousand in the light trap collections. The only other mosquito species from which multiple isolations of WNV were obtained in more than 1 year was *Ae. vexans* ($n = 12$, 5.7%).

Yearly MIRs for the five mosquito species from which multiple WNV isolations were obtained in more than 1 year were further calculated at the county level (Table 3). These were determined from season long trap collections of each respective species only in those counties where WNV isolations were made. The highest rates were obtained for *Cx. pipiens* (range = 1.2 to 30.7, overall mean = 5.7) followed by *Cx. salinarius* (range = 0.5–16.6, mean = 3.5), *Cs. melanura* (range = 0.7–11.6, mean = 2.8), and *Cx. restuans* (range = 0.4–4.8, mean = 2.0). Median MIR values among these four species were not significantly different by ANOVA ($p < 0.01$). There was no statistically significant association between MIRs and the number of human cases of WNV infection at the county level for any of the species individually or collectively (linear regression analysis).

Spatial geographic distribution of virus activity

The geographic distribution of the WNV isolations from mosquitoes in relation to human population density and locally acquired human cases of WNV in the state is shown in Figure 1. The number of trap sites from which infected mosquitoes were recovered progressively increased from one location in 1999, to 11 in 2000, 19 in 2001, 25 in 2002, and 31 in 2003. With the exception of 2003, this increase in the number of locales where WNV positive mosquito pools were detected, was associated with a corresponding increase in the number of lo-

TABLE 2. ISOLATIONS OF WEST NILE VIRUS OBTAINED FROM MOSQUITOES COLLECTED FROM CDC LIGHT (L) AND GRAVID (G) TRAPS IN CONNECTICUT, 1999–2003

	1999	*2000*		*2001*		*2002*		*2003*		
Mosquito species	*L*	*G*	*L*	*G*	*L*	*G*	*L*	*G*	*L*	*Total*
Cx. pipiens	1	1	4	3	12	26	28	1	10	86
Cx. salinarius	—	—	2	—	14	1	2	1	12	32
Cx. restuans	—	1	3	1	1	3	7	2	8	26
Cs. melanura	—	—	3	2	4	—	—	—	23	32
Ae. vexans	1	—	—	—	3	—	4	—	4	12
Ae. cinereus	—	—	—	—	1	—	—	—	3	4
Ur. sapphirina	—	—	—	—	1	—	1	—	1	3
Oc. canadensis	—	—	—	—	1	—	—	—	1	2
Oc. trivittatus	—	—	—	—	—	—	1	—	1	2
Oc. sollicitans	—	—	—	—	2	—	—	—	—	2
Oc. sticticus	—	—	—	—	—	—	—	—	2	2
Oc. cantator	—	—	—	—	1	—	—	—	—	1
Oc. taeniorhynchus	—	—	—	—	1	—	—	—	—	1
Oc. triseriatus	—	—	—	—	1	—	—	—	—	1
Ps. ferox	—	—	—	—	—	—	—	—	2	2
An. punctipennis	—	—	—	—	1	—	—	—	—	1
An. walkeri	—	—	—	—	—	—	—	—	1	1
Totals	2	14		49		73		72		210

Table 3. County-Based Minimum Field Infection Rates (per Thousand Mosquitoes) of West Nile Virus in Connecticut Mosquitoes, 1999–2003

Year and county	No. human cases	Mosquito species				
		Cx. pipiens	Cx. restuans	Cx. salinarius	Cs. melanura	Ae. vexans
1999						
Fairfield	0	1.2	—[a]	—	—	0.5
2000						
Fairfield	1	1.4	1.7	—	0.8	—
New Haven	0	1.4	—	0.5	—	—
2001						
Fairfield	3	5.2	0.4	2.6	0.7	0.2
Litchfield	0	—	—	—	11.6	—
New Haven	3	1.5	0.8	1.6	3.2	—
New London	0	—	—	—	0.4	0.7
2002						
Fairfield	8	7.8	2.3	1.6	—	0.8
Hartford	6	7.1	4.8	—	—	0.3
New Haven	3	3.8	—	—	—	—
Windham	0	4.8	—	—	—	—
2003						
Fairfield	6	2.9	1.4	0.5	0.7	0.1
Hartford	2	2.4	3.0	—	3.5	—
Middlesex	3	30.7	—	—	4.7	—
New Haven	2	4.3	1.5	1.0	1.2	0.4
New London	1	—	—	—	1.8	—
Windham	0	—	—	16.6	—	—
Median[b]		3.8[a]	1.6[a]	1.6[a]	1.5[a]	0.4[b]
Mean ± SE		5.7 ± 2.2	2.0 ± 0.5	3.5 ± 2.2	2.8 ± 1.1	0.4 ± 0.1

[a]No WNV isolations made from that species.
[b]Median values followed by a common letter are not significantly different ($p < 0.01$) by Kruskal-Wallis one-way ANOVA on ranks (Jandel Corp. 1995).

cally acquired human cases ($n = 0$, 1, 6, and 17, respectively). With a few exceptions in 2001, the large majority of virus isolations made from 1999 to 2002 were obtained from mosquitoes collected in densely populated (>3,00 people/mi^2) residential locales along the urban and suburban corridor extending through coastal Fairfield and New Haven Counties in the southwestern corner of the state. This was the same general area where the preponderance of human cases was reported each year.

An expansion of virus activity into central Hartford County, with five human cases in the vicinity of the city of Hartford (population density of >5,000 people/mi^2) was observed in 2002. This was coincident with the isolation of WNV from five species of mosquitoes (*Cx. pipiens* = 9, *Cx. restuans* = 2, *Ae. vexans*, *Oc. trivittatus*, and *Ur. sapphirina* 1 each) collected from five sites that were in close proximity. The year 2002 was also notable by the presence of two additional foci of concentrated virus activity in coastal Fairfield County where human population density was similarly high. These included the (1) Greenwich/Stamford area (population density = 2,890 people/mi^2) in the extreme southwestern most panhandle corner of the state where five human cases were reported and 35 isolations of WNV were made from four species (*Cx. pipiens* = 27, Cx. restuans = 3, *Cx. salinarius* = 3, and *Ae. vexans* = 2) collected from eleven different trapping sites in the region; and the (2) Bridgeport/Stratford area (population density = 5,800 people/mi^2) where three human cases were reported and 12 isolations of WNV were made from two species (*Cx. pipiens* = 11, *Cx. restuans* = 1) from three local trap sites. It is noteworthy that, in 2002, 74% of the 73 virus isolations made from mosquitoes were obtained from *Cx. pipiens* (Table 2).

A major expansion of virus activity throughout the southern two thirds of the state was seen in 2003 with either human cases or virus

isolations from mosquitoes detected in all eight counties. Several human cases ($n = 6$) and multiple WNV isolations from mosquitoes ($n = 33$ from 11 sites) were again found in the densely populated regions of coastal Fairfield and New Haven Counties. However, for the first time high levels of WNV activity were detected in mosquitoes ($n = 39$ isolations from 20 sites) with accompanying human cases ($n = 9$) in more sparsely populated (<1,000 people/mi^2) regions of the state. This was coincident with the isolation of WNV from 12 species of mosquitoes, nearly one-third (32%) of which were obtained from *Cs. melanura*. Unlike prior years, there were no apparent foci of infection and comparatively fewer WNV isolations were made from *Cx. pipiens* (15% of total in 2003 vs. 50% in 1999, 35% in 2000, 31% in 2001, and 74% in 2002) (Table 2).

Climatological data

An analysis of climatological data (Fig. 2) showed that 1999, 2001, 2002, and 2003 were consistently warmer than normal from June through September, with an overall monthly average temperature deviation from the norm of 1.2°C in 1999, 0.7°C in 2001, 1.3°C in 2002, and 0.8°C in 2003. The summer of 2000, by contrast was cooler with an average monthly temperature deviation of −0.6°C. Summer rainfall amounts were typical in 2001 and 2002, but above average rainfall was consistently recorded in all four months of 2000 and in three of four months in 2003. Rainfall amounts in 1999 were below average in June and July but considerably above average (16.2 cm from the norm) in September.

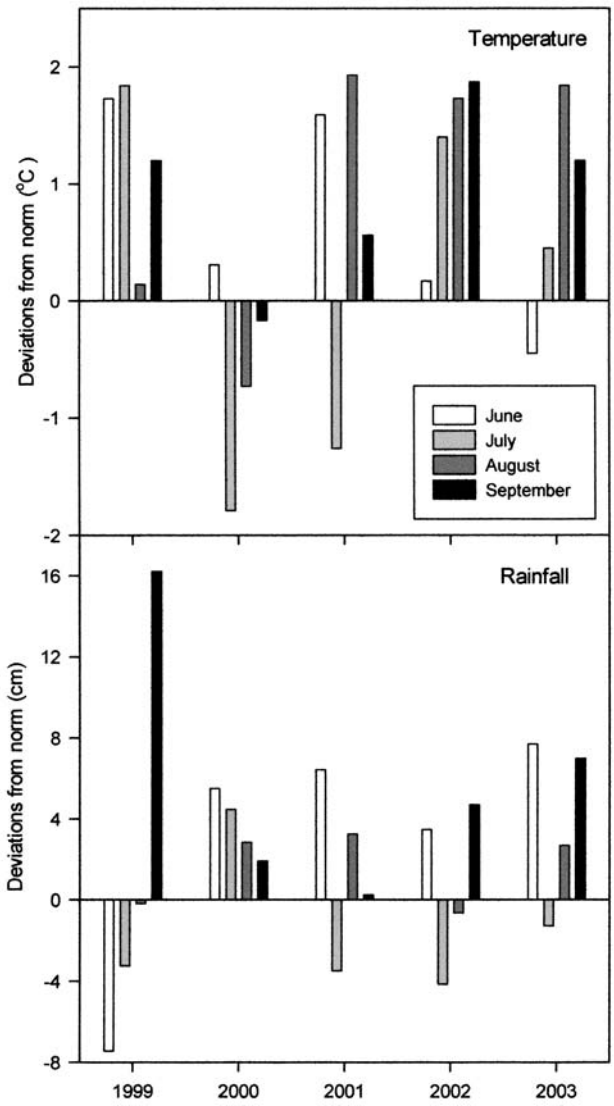

FIG. 2. Mean monthly temperature and rainfall deviations from the norm, June through September in Connecticut, 1999–2003.

Temporal distribution of virus activity

A weekly summary of the number of WNV isolations made from mosquitoes from 1999–2003 and the illness onset dates of confirmed human cases in each year are shown in Figure 3. The earliest isolations of WNV from mosquitoes were made in early July, and although they were comparatively few in number (2.4% of total isolations), they were generally made 5–6 weeks prior to the illness onset dates of the first human cases. The one exception was in 2002, when the difference was only 2 weeks. In 2000, 2001, and 2003, these early virus isolations from mosquitoes were followed by a 2–3-week period in which no further virus activity was detected in mosquitoes. A steady continuous increase in the number of virus isolations was regularly initiated in early August and typically lasted 5–6 weeks, with the greatest number of isolations being made during the first and second weeks of September. Sharp increases were observed in 2001 and 2003, while those in 2000 and 2002 were more gradual. In most years, peak virus activity in mosquitoes as determined by the number of isolations was followed by a noticeably more acute decline

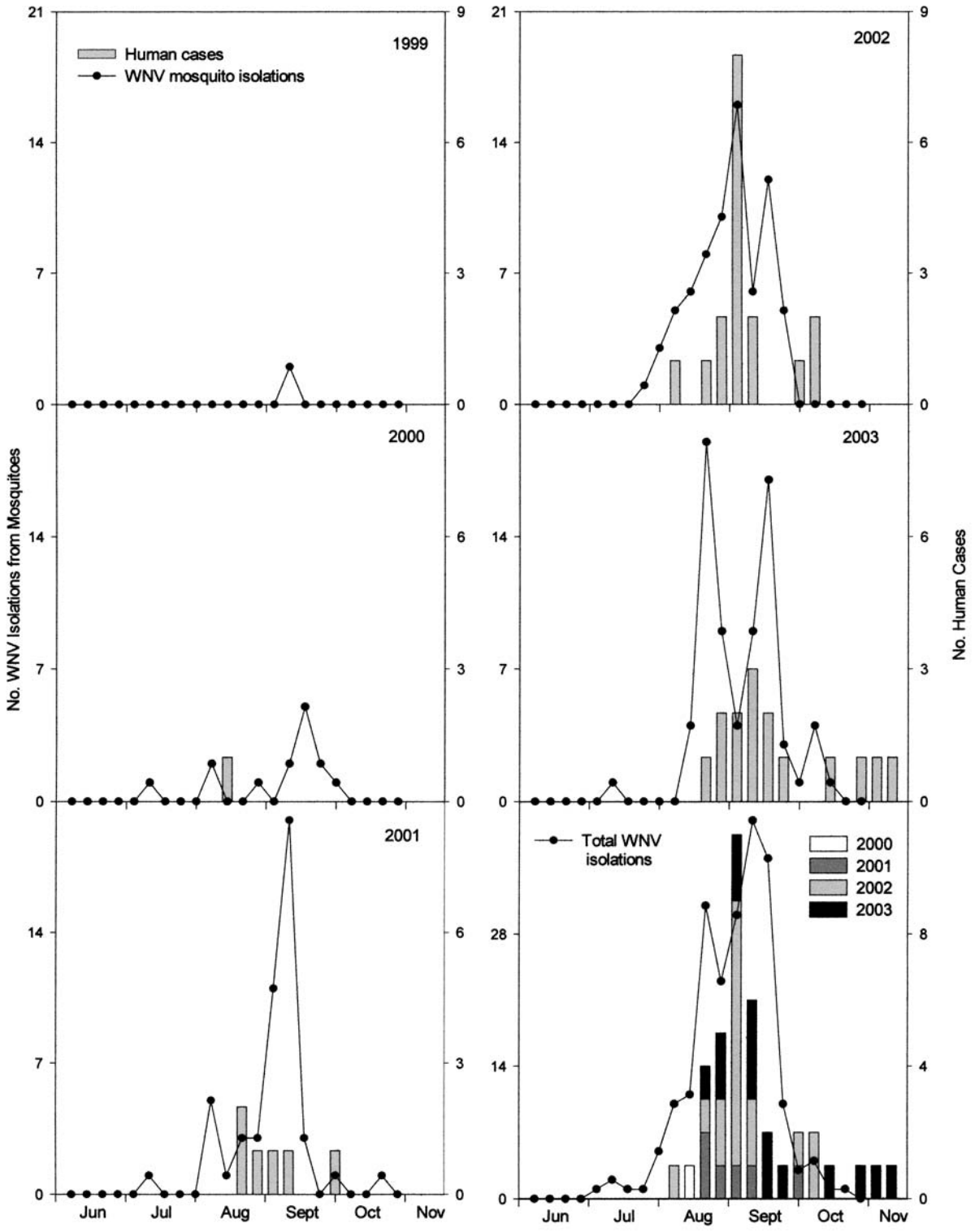

FIG. 3. Weekly incidence of West Nile virus isolations from mosquitoes and date of onset of locally acquired human cases in Connecticut, 1999–2003.

that continued through the latter half of September and early October. On the whole, the incidence and magnitude of reported human cases of WNV closely paralleled the number of virus isolations made from mosquitoes with both peaks generally falling during the same time period, early September. It is notable that, in 2002 and 2003, several human cases were re-

ported to have illness onset dates that were 2–4 weeks after the last virus isolations were obtained from mosquitoes.

Species-specific abundance and virus isolation data

A summary of the total weekly collection and virus isolation data from 1999–2003 for those mosquito species from which multiple WNV isolations were obtained in more than 1 year (*Cx. pipiens*, *Cx. restuans*, *Cx. salinarius*, *Cs. melanura*, and *Ae. vexans*) are shown in Figure 4, along with the virus isolation data for the remaining 12 species. *Culex pipiens* collections increased steadily from June through July and peaked during the first week of August. They remained abundant through August and September but exhibited a gradual decline through October. A few early isolations of WNV were obtained from *Cx. pipiens* in mid and late July (4.6% of total), but the majority of virus isolations were made in August (44.2%) and September (48.8%), when populations were on the decline, and only two (2.3%) were made in October.

Culex restuans, by contrast, was more abundant during the early summer months of June and July, and peak collections were recorded in early July. Trap collections decreased steadily through July and August and few adults were trapped in September. Some of the earliest virus isolations were obtained from collections of *Cx. restuans* that were made in early July (7.7% of total), but most were obtained in August (50%) and early to mid-September (42.3%), when populations were quite low.

The collection trends observed with *Cx. salinarius* populations were similar to those seen with *Cx. pipiens*. A steady gradual increase was observed from June through mid-August, followed by a comparable decline through September and October. The virus isolation data for *Cx. salinarius* differed from *Cx. pipiens* in that very few isolations were made before mid-August (3.1% of total), and most (71.8%) were made in September.

Culiseta melanura was consistently collected throughout the season, and there were three discernable peaks of adult abundance that were apparent over the 5-year period: mid-June, late July to early August, and mid-September. Despite the early summer abundance of this species, the incidence of WNV isolation was decidedly shifted to the latter months. The first isolations were not made until mid-August and they continued to be made into mid-October, several weeks after the last isolations were recorded for any other species, some of which (e.g. *Cx. pipiens*, *Cx. salinarius*) were comparable in abundance at that time.

Aedes vexans was the most commonly trapped species from which multiple isolations of WNV were obtained in more than 1 year. Three peaks of progressively decreasing adult abundance were evident: late June, mid-August, and early September. The earliest isolations of WNV were not recorded until mid-August, and most (91.7%) were made from mosquitoes collected during the first 3 weeks of September, which was the shortest period of virus activity recorded for any of the five aforementioned species.

A summary of the virus isolation data for the remaining twelve species from which only an occasional isolation was made is also shown in Figure 4. With the exception of the one isolation that was obtained from *Oc. canadensis* collected on July 5, 2001, all other isolations from these species were made over a 6-week period extending from mid-August through mid-September.

The relationships between human population density and the abundance of *Cx. pipiens*, *Cx. restuans*, *Cx. salinarius*, *Cs. melanura*, and *Ae. vexans* based on mean collections from trap sites in 73 municipalities over the 5-year period 1999–2003 are individually shown in Figure 5. Regression analysis revealed a statistically significant positive linear correlation ($r = 0.78$, $p < 0.001$) between human population density and the mean number of adult female *Cx. pipiens* collected per trap night in the respective locales. No significant associations of this type were revealed with any of the other aforementioned species. WNV-positive *Cx. pipiens* were recovered from a variety of locales where the human population density ranged from a low of 170 people/mi^2 to high of 8,721 people/mi^2. However, an overwhelmingly high percentage (89.5%) of the positive trap sites ($n = 17$ of 19) were located in municipalities ($n = 31$ of 73)

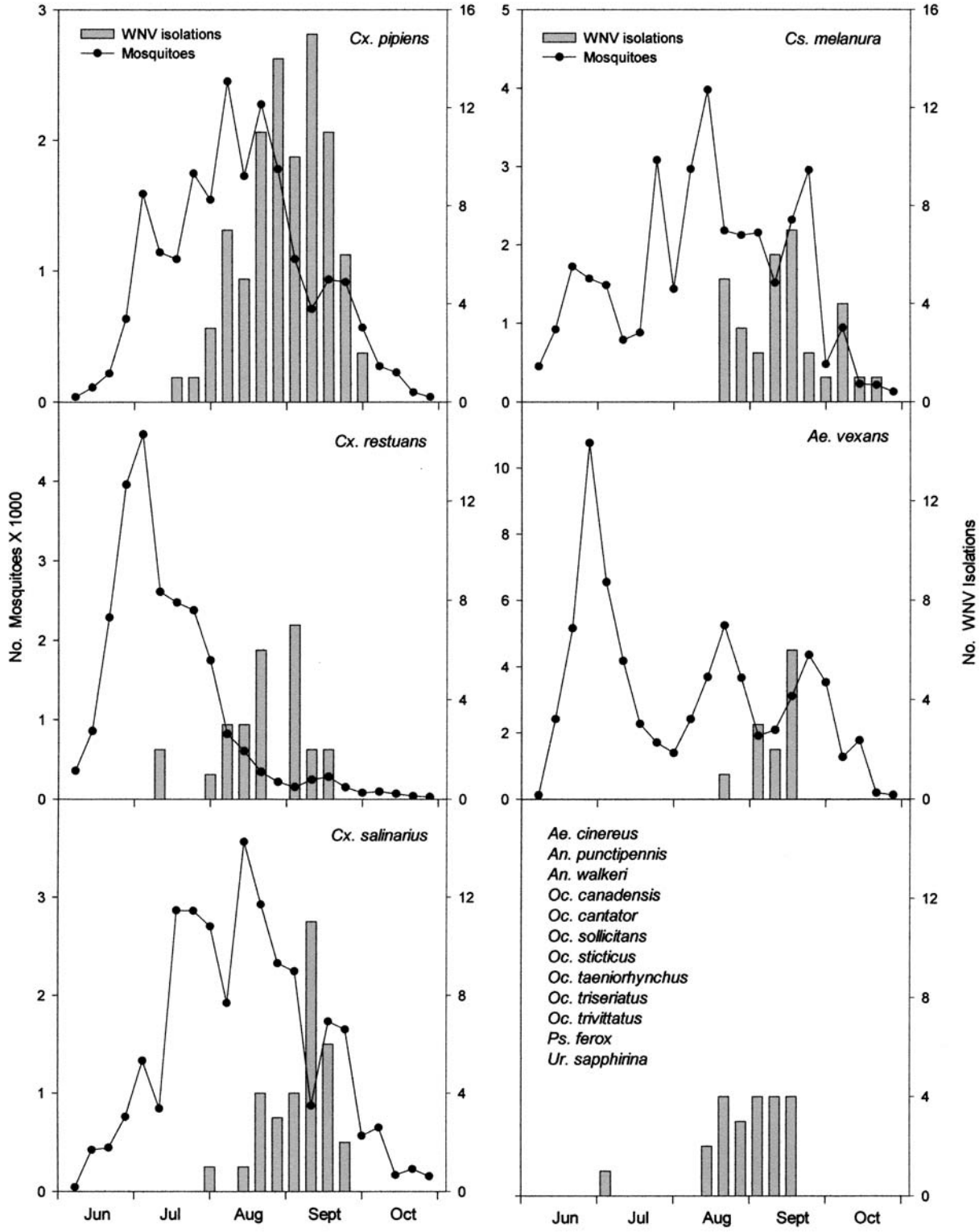

FIG. 4. Total weekly abundance and West Nile virus isolations obtained from *Cx. pipiens*, *Cx. restuans*, *Cx. salinarius*, *Cs. melanura*, *Ae. vexans* and weekly incidence of West Nile virus isolations obtained from the additional 12 mosquito species for 1999–2003.

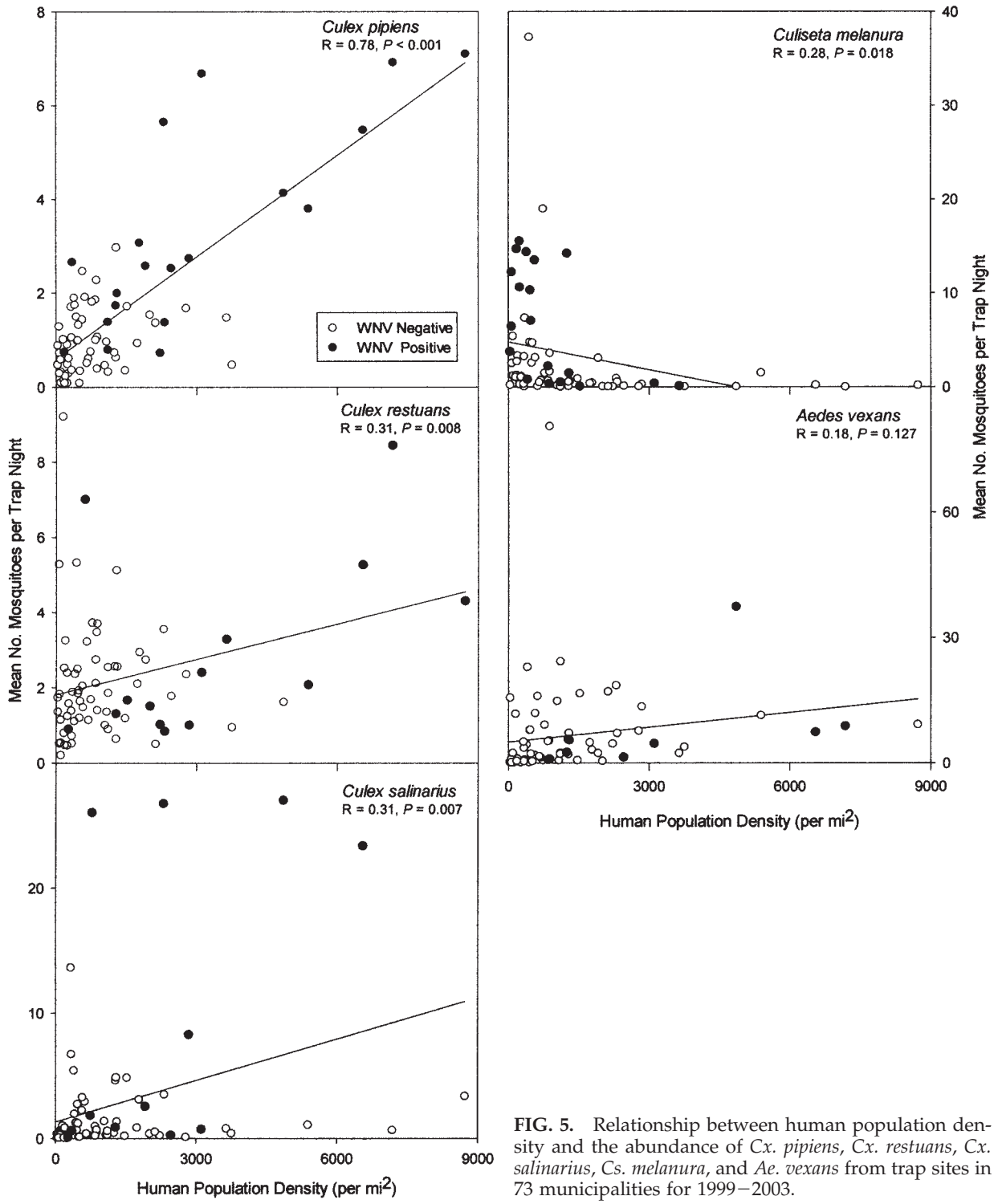

FIG. 5. Relationship between human population density and the abundance of *Cx. pipiens*, *Cx. restuans*, *Cx. salinarius*, *Cs. melanura*, and *Ae. vexans* from trap sites in 73 municipalities for 1999–2003.

where the human population density exceeded 1,000 people/mi^2. Comparable values for *Ae. vexans* were 87.5% ($n = 7$ of 8), *Cx. restuans* 85.7% ($n = 12$ of 14), *Cx. salinarius* 63.6% ($n = 7$ of 11), and *Cs. melanura* 31.6% ($n = 6$ of 19).

DISCUSSION

The mosquito collection and virus isolation data obtained in our investigations conducted over the period, 1999 through 2003, clearly im-

plicate five species—*Cx. pipiens*, *Cx. restuans*, *Cx. salinarius*, *Cs. melanura*, and to lesser degree *Ae. vexans*—as the most likely vectors of WNV in this region. The preponderance of isolations from *Cx. pipiens* (40% of the total and more than 2.5 times more than any other species) further suggests that this species is the most important. These conclusions are consistent with early mosquito surveillance studies conducted in the northeastern United States (Nasci et al. 2001b, Andreadis et al. 2001b, Bernard et al. 2001, Kulasekera et al. 2001, Marfin et al. 2001, White et al. 2001) and are supported by laboratory vector competence studies (Turell et al. 2000, 2001, 2004, Sardelis et al. 2001, Goddard 2002) that have shown *Cx. pipiens*, *Cx. salinarius*, *Cx. restuans*, and *Ae. vexans* to be moderately efficient vectors. Laboratory transmission of WNV has yet to be demonstrated with *Cs. melanura*, but this species does develop disseminated infection after feeding on viremic chickens (Turrell et al. 2004).

Our repetitive yearly sampling in the same geographic locales further helped to reveal the principal foci of WNV activity in the state. These were identified as densely populated (>3,000 people/mi^2) residential communities in coastal Fairfield and New Haven Counties, and in the case of 2002, similar locales in and around the city of Hartford in central Hartford County (population density >5,000 people/mi^2). In almost all instances, we observed a correlation both temporally and spatially between the isolation of WNV from field-collected mosquitoes and reported human cases in these local communities. Furthermore, in most years the incidence of human cases closely paralleled the number of virus isolations made from mosquitoes, with both peaks falling during early September. We conclude that the isolation of WNV from field-collected mosquitoes is a sensitive indicator of virus activity that is associated with the risk of human infection that habitually extends from early August through the end of October in Connecticut.

The dissimilar patterns of virus activity observed in 2002 and 2003 may be explained in part by local climatological events that appeared to impact resident mosquito populations. An analysis of rainfall and temperature data from June through September showed 2002 to be the driest (monthly mean = 0.8 cm above norm) and warmest (monthly mean = 1.3 C° above norm) recorded over the 5-year period 1999–2003, while the summer of 2003 was notably the wettest with a monthly mean rainfall amount of 4.03 cm above normal (Fig. 2).

Virus activity during the summer of 2002, as determined by the number of virus isolations from mosquitoes and subsequent human cases, was highly concentrated in three densely populated urban centers where the population density exceeded 10,000 people/mi^2. Virus activity in 2003, by contrast, was more widely distributed, and although several human cases and numerous virus isolations were similarly obtained from mosquitoes collected from the same general regions of coastal Fairfield County, no highly concentrated foci of virus activity were apparent in the urban centers as in 2002.

These diverse patterns of virus activity were associated with measurable differences in the abundance of three presumed vectors—*Cx. pipiens*, *Cx. salinarius*, and *Cs. melanura*—in our trap collections and subsequent prevalence and distribution of WNV in these and other mosquito species in each of the 2 years. In 2002, significantly greater numbers of *Cx. pipiens* were collected throughout the season (mean number mosquitoes/trap night $\pm$ SE = 3.2 $\pm$ 0.5 in 2002 vs. 1.3 $\pm$ 0.2 in 2003, $p < 0.001$, Kruskal-Wallis one-way ANOVA on ranks). This was markedly reflected in the 12-fold greater numbers of adult females that were collected in the gravid traps in 2002 (5,438 vs. 444 in 2003) rather than in the light trap collections, which were virtually equivalent (3,156 in 2002 vs. 2,887 in 2003). Virus isolations were obtained from six different mosquito species, and nearly three quarters (74%) of these were made from *Cx. pipiens*. This contrasted sharply with the mosquito collection and virus isolation data for 2003, where significantly greater numbers of *Cx. salinarius* (6.3 $\pm$ 1.0 mean number mosquitoes/trap night in 2003 vs. 1.6 $\pm$ 0.2 in 2002, $p < 0.001$) and *Cs. melanura* (5.9 $\pm$ 0.7 mean number mosquitoes/trap night in 2003 vs. 1.1 $\pm$ 0.1 in 2002, $p < 0.001$) were collected, and WNV isolations were obtained from twice as many mosquito species ($n = 12$). Furthermore, only

15% of the virus isolations made in 2003 were from *Cx. pipiens*, while 32% were obtained from *Cs. melanura* and 18% from *Cx. salinarius*. Although more in-depth studies that directly measure mosquito abundance are warranted, our observations in 2002 and 2003 suggest that hot dry summers may foster conditions (e.g., high organic content and reduced flushing action of run-off in catch basin habitats) (Munstermann and Craig 1976), that facilitate increased populations of *Cx. pipiens* and therein lead to more highly concentrated foci of virus activity, especially in urban centers where our analyses (Fig. 5) demonstrate this species predominates. Conversely, we might also anticipate less focal and more widespread virus activity and associated human risk of infection during excessively wet summers that produce an abundance of other presumed vector species such as *Cx. salinarius* and *Cs. melanura.*

The ornithophilic feeding preference of populations of *Cx. pipiens* from northern latitudes is well established (Crans 1964, Means 1968, Spielman 1971, Tempelis 1975, Magnarelli 1977, Apperson et al. 2002) and clearly support a major role for this mosquito in transmission of WNV to birds in this region of the United States. Our early season isolations of WNV in July when trap collections were increasing, and multitude of isolations in late August and September further lead us to conclude that *Cx. pipiens* is likely involved in both early season enzootic transmission as well as late season epizootic amplification of the virus in wild bird populations. The former conclusion for early season enzootic involvement is congruent with the detection of WNV in hibernating *Cx. pipiens* (Nasci et al. 2001a) and the demonstration of vertical transmission of the virus by this species in the laboratory (Dohm et al. 2002). The latter conclusion for late season amplification in wild bird populations is supported by a concurrent study conducted at a local WNV focal center in coastal Fairfield County, Connecticut, where Anderson et al. (2004) reported finding significantly greater numbers of WNV-infected *Cx. pipiens* from traps placed in the tree canopy when compared to similar traps placed near the ground.

If WNV does over winter locally in hibernating mosquitoes as is generally thought (Nasci et al. 2001a), it remains unclear why we were unable to detect the virus in June, after host-seeking females leave their hibernacula (Spielman 1971). Either the prevalence of viral infection in the emerging population was exceedingly low and below a collection threshold that we could not attain with our trapping efforts, or alternatively the level of virus in individual mosquitoes was below the detection limits of our Vero cell cultures. According to Lanciotti et al. (2000) the TaqMan assay has a 10-fold greater sensitivity than do Vero cell cultures (0.1 PFU vs. 1PFU) for detecting WNV in mosquito pools. This is supported by the recent study of Nasci et al. (2002) who reported the detection of 36% more WNV positive pools with field-collected mosquitoes using the TaqMan procedure than with the Vero cell plaque assay, leading them to conclude that use of the former assay would likely provide the highest probability of detecting early season transmission when mosquito infection rates are low. In either event, the repeated detection of WNV from field-collected mosquitoes in the same geographic locales is supportive evidence for local yearly reemergence in these regions.

The role that *Cx. pipiens* may play in epidemic transmission of WNV virus to humans in the northeastern United States continues to be problematic. The majority of the reports in the literature clearly indicate that local populations from Connecticut (Magnarelli 1977), Massachusetts (Spielman 1971), New Jersey (Crans 1964), and New York (Means 1968, Tempelis 1975, Apperson et al. 2002) predominately feed on birds and are reluctant to feed on humans. However, Apperson et al. (2004) identified mammalian-derived blood meals in 38% of blooded *Cx. pipiens* ($n = 109$), 10.8% of which were human-derived, collected from natural and man-made resting sites in suburban areas of New Jersey. The involvement of *Cx. pipiens* as an epidemic as well as epizootic vector appeared probable. This interpretation is consistent with our observations in 2002 where 78% of the viral isolations obtained from mosquitoes collected in the three urban focal centers where 11 human cases were detected, were made from *Cx. pipiens*. Conversely, Apperson et al. (2004) additionally identified 84.6% avian and no mammalian derived blood meals in

blooded *Cx. pipiens* ($n = 19$) collected from more rural hardwood forests locales in Westchester County (north of New York City), New York, thus implying differences in the host feeding preferences of resident populations. Differences in host feeding preferences have previously been reported in farm and woodland populations of *Cx. pipiens* in New York by Means (1968), who observed *Cx. pipiens* inhabiting commercial bird farms to routinely engorge on ducks and pheasants but to hardly ever bite humans, while populations in sylvan environments would attack humans readily. Spielman (1964, 1971, 2001) have reported brief episodes of human biting by heterozygote forms of urban *Cx. pipiens* in Boston during periods of interbreeding between anautogenous (diapausing, eurygamous) males and autogenous (non-diapausing, stenogamous) females in September and December. Fonseca et al. (2004), in an examination of highly polymorphic DNA microsatellite loci, have further demonstrated the existence of "hybrid" bird and human biting populations of *Cx. pipiens* in the northeastern United States, which differ genetically and behaviorally from the Palearctic aboveground, anautogenous (bird-biting) *Cx. pipiens pipiens* form, and the underground, autogenous (human-biting), *Cx. pipens molestus* form. Although supportive evidence on the host feeding preferences of these two Paleartic forms is lacking, they hypothesize that the Nearctic "hybrids" may serve as the bridge vectors that contribute to the severity and range of the WNV epidemic in North America. Clearly, further investigations on the population biology, and spatial and temporal host feeding preferences of this mosquito complex in urban and rural habitats are warranted.

The abundance of *Cx. restuans* in our trap collections in June and July and isolations of WNV in early July (Fig. 4) support an earlier supposition (Andreadis et al. 2001b) that this mosquito may play an important role as an enzootic vector involved in early amplification of WNV among wild birds in the northeastern United States. In addition to being the most abundant *Culex* species at this time of the year, our data show that it is widely distributed throughout the region (Table 1) and that it occurs in both urban and rural environs (Fig. 5). This conclusion is fully consistent with its well-documented ornithophilic feeding preferences (Means 1968, Tempelis 1975, Magnarelli 1977, Irby and Apperson 1988, Apperson et al. 2002, 2004). However, there are a number of reports in the literature (Barr 1958, Hayes 1961, Murphey et al. 1967, Means 1968, 1987) that indicate that, although *Cx. restuans* prefers feeding on birds, females bite humans and a human-derived blood meal has been identified from one of 29 blooded females collected in suburban New Jersey (Apperson et al. 2004). These findings taken in concert with the multiple isolations of WNV obtained from this species in August and September, do not preclude its involvement as a bridge vector to humans. However, because of its low abundance at this time of the year, transmission to humans is likely to be a comparatively rare occurrence.

Culex salinarius was the most frequently captured *Culex* species in our investigations and was locally abundant in August and September when virus activity was at its height. In contrast to *Cx. pipiens* and *Cx. restuans*, *Cx. salinarius* is a well-recognized generalist that feeds indiscriminately on both birds and mammals and is reported to readily bite humans (Crans 1964, Murphey et al. 1967, Suyemoto et al. 1973, Edman 1974, Cupp and Stokes 1976, Means 1987, Irby and Apperson 1988). Studies by Apperson et al. (2002, 2004) with local populations from New Jersey and New York reaffirmed the wide-ranging feeding habits reported in these prior investigations; with identification of mammal to bird feeding ratios of 4:1 in blooded females collected from a WNV focus in Queens, New York in 2000, and 3:1 in blooded females collected from WNV endemic peri-urban areas in New Jersey in 2001 with 8.6% of the mammalian blood meals being identified as human derived. Our frequent isolations of WNV from this species in September when the majority of human cases were reported in union with its abundance at this time of the year, demonstrated vector competence (Sardelis et al. 2001), and broad feeding habits, make *Cx. salinarius* a likely bridge vector to humans, horses and other mammals as suggested previously

by others (Andreadis et al. 2001b, Kulasekera et al. 2001, Sardelis et al. 2001, Apperson et al. 2004).

Our repetitive isolations of live WNV from *Cs. melanura* collected in more rural locales in late August and September of 2000, 2001, and 2002 provide supportive evidence to suggest that this predominant avian feeder may play a significant role in epizootic amplification of the virus among wild bird populations, especially passerines, its preferred host (Crans 1964, Means 1968, Edman et al. 1972, Muul et al. 1975, Nasci and Edman 1981, Magnarelli 1977, Irby et al. 1988, Apperson et al. 2004) in these sylvan environments. Although the vector competence of Cs. *melanura* for WNV has not been fully evaluated, females are susceptible to infection and develop disseminated infections after feeding on viremic chickens in the laboratory (Turrell et al. 2004), and according to Turrell et al. (2001), nearly all individuals that develop disseminated infection are as a rule, capable of transmitting the virus. *Cs. melanura* was typically abundant in June and July. However, the lack of virus isolations until late August, almost certainly rule out early involvement in enzootic transmission of WNV for this species in most years. On the other hand, the late season isolations of WNV obtained from this species in October of 2003, several weeks after the last isolations were detected for any other species are noteworthy as they were coincident with the identification of four human cases with illness onset dates extending from mid October to mid November of that same year. Furthermore, while the evidence for human biting by *Cs. melanura* is scarce, Apperson et al. (2004) have identified human derived blood meals in two of 68 blooded females collected from New Jersey, thus establishing human feeding by this species in the northeast region. Therefore, despite its decided feeding preference for birds, we cannot entirely dismiss the possible involvement of *Cs. melanura* in occasional transmission of WNV to humans.

Aedes vexans was the only species of *Aedes* or *Ochlerotatus* from which multiple isolations of WNV were made in more than 1 year. It was among the most frequently trapped species in our investigations and it was abundant throughout the entire season, June through September. Despite its abundance however, only 12 isolations of WNV were obtained from 4,600 pools, the most of any of the 38 species that were tested over the 5-year period. With one exception, all of the isolations were made from females collected during the first three weeks of September when the bulk of human cases were reported. The role that *Ae. vexans* may play in transmission of WNV to humans and horses in this region is unclear. *Aedes vexans* is a moderately competent laboratory vector (Turell et al. 2001, 2004) and is an aggressive human biter. It is most active in the early evening but will also bite during the day, and according to Means (1979) readily attacks humans in houses, especially on warm, humid nights during late summer. With a few exceptions (Cupp and Stokes 1973, Hassan et al. 2003), most studies have shown that *Ae. vexans* predominately feeds on large and small mammals including horses, and rarely on birds (Edman 1971, Suyemoto et al. 1973, Magnarelli 1977, Ritchie and Rowley 1981, Nasci 1984, Irby and Apperson 1988, Apperson et al. 2002, 2004, Ngo and Kramer 2003). Therefore, if we accept the premise that wild birds are the principal reservoir hosts for WNV, then it is logical to deduce that its lack of preference for birds would severely limit the opportunity for *Ae. vexans* to acquire the virus and thus reduce its vectoral capacity. Therefore, we conclude that *Ae. vexans* is unlikely to play a significant role in epizootic amplification of WNV, but because of its abundance and aggressive mammalian and human biting behavior must receive strong consideration as a bridge vector to humans and horses.

The occasional virus isolations obtained from the remaining twelve species, *Ae. cinereus*, *An. punctipennis*, *An. walkeri*, *Oc. canadensis*, *Oc. cantator*, *Oc. sollicitans*, *Oc. sticticus*, *Oc. taeniorhynchus*, *Oc. triseriatus*, *Oc. trivittatus*, *Ps. ferox*, and *Ur. sapphirina* are consistent with the detection of a few WNV RNA positive pools from *An. punctipennis*, *Oc. cantator*, *Oc. triseriatus*, *Ps. ferox*, in New York in 2000 (Bernard et al. 2001, Kulasekera et al. 2001, White et al. 2001) and *Oc. taeniorhynchus* from Florida in 2001 (Hribar et al. 2003). Our isolations from

An. walkeri, *Oc. canadensis*, *Oc. sollicitans*, *Oc. sticticus*, *Oc. trivittatus*, and *Ur. sapphirina* are new documented records for WNV in the United States. With the exception of *Ur. sapphirina*, which is reported to feed on reptiles and amphibians (Irby and Apperson 1988), all of the other aforementioned species are predominantly mammalian (including human) feeders. However, occasional avian derived blood meals have been identified from *An. punctipennis*, *Oc. triseriatus* (Irby and Apperson 1988, Apperson et al. 2004), *Oc. sollicitans* (Crans 1964, Cupp and Stokes 1976), *Oc. taeniorhynchus* (Edman 1971), *Oc. trivittatus* (Nasci 1984), *Ps. ferox* (Edman 1971, Magnarelli 1977, Irby and Apperson 1988), and *Ur. sapphirina* (Cupp and Stokes 1976), which could account for the infrequent but occasional virus isolation. Vector competency for WNV has been demonstrated for *Oc. canadensis*, *Oc. sollicitans*, *Oc. cantator*, *Oc. triseriatus*, and *Oc. taeniorhynchus*, but according to Turell et al. (2000, 2001, 2004), these species are relatively inefficient vectors. *Psorophora ferox* is unable to transmit the virus even when infected (Turell et al. 2004), and the vector competence of *Ae. cinereus*, *An. punctipennis*, *An. walkeri*, *Oc. sticticus*, *Oc. trivittatus*, and *Ur. sapphirina* have not been evaluated. Our findings in concert with the inefficient vector competency and reported host feeding preferences indicate to us that these twelve mosquitoes likely play a very minor role in either the enzootic maintenance or epizootic transmission of WNV in this region.

Despite the wide distribution and moderate abundance of the newly recognized introduced species from Asia, *Oc. japonicus* in Connecticut (Andreadis et al. 2001a), we obtained no isolations of WNV from any of the 1,769 pools of this species that were put into cell culture. This was somewhat surprising, since WNV RNA positive pools has been previously detected in a few females collected in New York (Bernard et al. 2001, White et al. 2001) and *Oc. japonicus* is among the most highly efficient laboratory vector species evaluated to date (Sardelis and Turell 2001, Turell et al. 2001). On the other hand, our findings are fully consistent with the identification of white-tailed deer (*Odocoileus virginianus*) derived blood meals in 100% (n = 54) of *Oc. japonicus* collected recently from New York (Apperson et al. 2004) and thus support our conclusion that this species plays little or no role in transmission of WNV in this region.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of our support staff—John Shepard, Michael Thomas, Jodie Ortega, Amanda Rahmann, Bonnie Hamid, Nicholann Halladay, Michael Vasil, and Terrill Goodman—and thank the following individuals for their assistance in collecting, identifying and processing mosquitoes for virus testing in one or more seasons: Daniel Altneu, Melanie Baron, Dawn Berube, Kathleen Blessing, Jason Cabral, Edward Calandrella, John Capotosto, Eric Carlson, John Duarte, Grecekia Elliot, Ronald Ferrucci Jr., Shannon Finan, Deborah Floch, Kristen Frank, Hannah Ginese, Sarah Hubbard, Kelli Jones, Charles Lagoy, Michael MacAloon, Jason Martin, Charles McGee, Susan McKnight, Mathew Meeneghan, Laura Mickowski, Michael Misencik, Ryan Monroe, Lori Napolitano, Lisa Nigro, Anja Pennell, Kevin Porter, Kelly Shanley, Michael Spada, and Kyle Startz. We also express our gratitude to Dr. Randall Nelson, Connecticut Department of Public Health for providing access to the human case histories, and Paul Capotosto and Roger Wolf, Connecticut Department of Environmental Protection, the Stamford Health Department, Ledgelight Health District, and the US Navy for assistance in trapping. Funding for this research was provided by Laboratory Capacity for Infectious Diseases Cooperative Agreement Number U50/CCU116806-01-1 from the Centers for Disease Control and Prevention, United States Department of Agriculture Specific Cooperative Agreement Number 58-6615-1-218, and Hatch Grant CONH00768.

REFERENCES

Anderson, JF, Andreadis, TG, Vossbrinck, CR, et al. Isolation of West Nile virus from mosquitoes, crows, and a Cooper's hawk in Connecticut. Science 1999; 286:2331–2333.

Anderson, JF, Andreadis, TG, Main, AJ, et al. Prevalence of West Nile virus in tree canopy-inhabiting *Culex pip-*

iens and associated mosquitoes. Am J Trop Med Hyg 2004; 71:112–119.

Andreadis, TG, Anderson, JF, Munstermann, LE, et al. Discovery, distribution and abundance of the newly introduced mosquito *Ochlerotatus japonicus* (Diptera: Culicidae) in Connecticut, USA. J Med Entomol 2001a; 38:774–779.

Andreadis, TG, Anderson, JF, Vossbrinck, CR. Mosquito surveillance for West Nile virus in Connecticut, 2000: Isolation from *Culex pipiens*, *Cx. restuans*, *Cx. salinarius*, and *Culiseta melanura*. Emerg Inf Dis 2001b; 7:670–674.

Apperson, CS, Harrison, BA, Unnasch, TR, et al. Host-feeding habits of *Culex* and other mosquitoes (Diptera: Culicidae) in the borough of Queens in New York City, with characters and techniques for the identification of *Culex* mosquitoes. J Med Entomol 2002; 39:777–785.

Apperson, CS, Hassan, HK, Harrison, BA, et al. Host feeding patterns of established and potential mosquito vectors of West Nile virus in the eastern United States. Vector-Borne Zoonotic Dis 2004; 4:71–82.

Barr, AR. The mosquitoes of Minnesota. Univ Minn Agr Exp Sta Tech Bull 228.

Bernard, KA, Maffei, JG, Jones, SA, et al. West Nile virus infection in birds and mosquitoes, New York state, 2000. Emerg Infect Dis 2001; 7:679–685.

Biggerstaff, BL. *PolledInfRate: A Microsoft® Excel Add-In to Compute Prevalence Estimates from Pooled Samples.* Fort Collins, CO: Centers for Disease Control and Prevention, 2003.

Centers for Disease Control and Prevention, Provisional surveillance summary of the West Nile virus epidemic–United States, January–November 2002. MMWR 2002; 51:1129–1133.

Centers for Disease Control and Prevention. West Nile virus: entomology [On-line]. Available: www.cdc.gov/ncidod/dvbid/westnile/mosquitoSpecies.htm, 2003.

Connecticut Office of Policy and Management. Census 2000 population counts for Connecticut municipalities and counties [On-line]. Available: www.opm.state.ct.us/pdpd3/data/estimate.htm, 2000.

Crans, WJ. Continued host preference studies with New Jersey mosquitoes, 1963. Presented at the 51st Annual Meeting of the NJ Mosquito Extermination Association 1964.

Cupp, EW, Stokes, GM. Identification of bloodmeals from mosquitoes collected in light traps and dog-baited traps. Mosq News 1973; 13:39–41.

Cupp, EW, Stokes, GM. Feeding patterns of *Culex salinarius* Coquillett in Jefferson Parish, Louisiana. Mosq News 1976; 6:332–335.

Darsie, RF Jr, Ward, RA. Identification and geographic distribution of mosquitoes of North America, north of Mexico. Mosq Syst 1981; 1(Suppl):1–313.

Dohm, DJ, Sardelis, MR, Turell, MJ. Experimental vertical transmission of West Nile virus by *Culex pipiens* (Diptera: Culicidae). J Med Entomol 2002; 39:640–644.

Edman, JD. Host-feeding patterns of Florida mosquitoes I. *Aedes*, *Anopheles*, *Coquillettidia*, *Mansonia* and *Psorphora*. J Med Entomol 1971; 687–695.

Edman, JD. Host-feeding patterns of Florida mosquitoes III. *Culex* (*Culex*) and *Culex* (*Neoculex*). J Med Entomol 1974; 11:95–104.

Edman, JD, Webber, LA, Kale II, HW. Host-feeding patterns of Florida mosquitoes II. *Culiseta.* J Med Entomol 1972; 9: 429–434.

Fonseca, D.M., Keyghobadi, N, Malcolm, CA, et al. Emerging vectors in the *Culex pipiens* complex. Science 2004; 303:1535–1538.

Goddard, LB, Roth, AM, Reisen, WK, et al. Vector competence of California mosquitoes for West Nile virus. Emerg Infect Dis 2002; 8:1385–1391.

Hassan, HK, Cupp, EW, Hill, GF, et al. Avian host preference by vectors of eastern equine encephalomyelitis virus. Am J Trop Med Hyg 2003; 69:641–647.

Hribar, LJ, Vlach, JJ, Demay, DJ, et al. Mosquitoes infected with West nile virus in the Florida Keys, Monroe county, Florida, USA. J Med Entomol 2003; 40:361–363.

Hayes, RO. Host preferences of *Culiseta melanura* and allied mosquitoes. Mosq News 1961; 21:179–187.

Irby, WS, Apperson, CS. Hosts of mosquitoes in the coastal plain of North Carolina. J Med Entomol 1988; 25:85–92.

Jandel Corp. *SigmaStat 2.0 for Windows, Version 2.0.* San Rafael, CA: Jandel Corp., 1995.

Kulsekera, VL, Kramer, L, Nasci, RS, et al. West Nile virus infection in mosquitoes, birds, horses and humans, Staten Island, New York, 2000. Emerg Infect Dis 2001; 7:722–725.

Lampman, Rl, Novak, RJ. Oviposition preferences of *Culex pipiens* and *Culex restuans* for infusion-baited traps. J Am Mosq Control Assoc 1996; 12:23–32.

Lanciotti, RS, Roehrig, JT, Deubel, V, et al. Origin of the West Nile virus responsible for an outbreak of encephalitis in the Northeastern United States. Science 1999, 286:2333–2337. Emerg Infect Dis 2001; 7:626–630.

Lanciotti, RS, Kerst, AJ, Nasci, RS, et al. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan Reverse Transcriptase-PCR Assay. J Clin Microbiol 2000; 8:4066–4071.

Magnarelli, LA. Host feeding patterns of Connecticut mosquitoes. Am J Trop Med Hyg 1977; 26:547–52.

Marfin, AA, Peterson, LR, Eidson, M, et al. Widespread West Nile virus activity, eastern United States, 2000. Emerg Inf Dis 2001; 7:722–725.

Means, RG. Host preferences of mosquitoes (Diptera: Culicidae) in Suffolk County, New York. Ann Entomol Soc Am 1968; 61:116–120.

Means, RG. Mosquitoes of New York. Part I. The genus *Aedes* Meigen with identification keys to genera of Culicidae. NY State Mus Bull 1979; 430a:1–221.

Means, RG. Mosquitoes of New York. Part II. Genera of Culicidae other than *Aedes* occurring in New York. NY State Mus Bull 1987; 430b:1–180.

Munstermann, LE. Craig, GB Jr. *Culex* mosquito populations in the catch basins of northern St. Joseph County, Indiana. Proc Indiana Acad Sci 1977; 86:246–252.

Muul, I, Johnson, BK, Harrison, BA. Ecological studies of *Culiseta melanura* (Diptera: Culicidae) in relation to eastern and western equine encephalomyelitis viruses

on the eastern shore of Maryland. J Med Ent 1975; 11:739–748.

Murphey, FJ, Burbutis PP, Bray DF. Bionomics of *Culex salinarius* Coquillett. II. Host acceptance and feeding by adult females of *C. salinarius* and other mosquito species. Mosq News 1967; 7:366–74.

Nasci, RL. Variations in the blood-feeding patterns of *Aedes vexans* and *Aedes trivittatus* (Diptera: Culicidae). J Med Entomol 1984; 21:95–99.

Nasci, RL, Edman, JD. Blood-feeding patterns of *Culiseta melanura* (Diptera: Culicidae) and associated sylvan mosquitoes in southeastern Massachusetts eastern equine encephalitis enzootic foci. J Med Entomol 1981; 18:493–500.

Nasci, RL, Savage, HM, White, DJ, et al. West Nile virus in overwintering *Culex* mosquitoes, New York City, 2000. Emerg Infect Dis 2001a; 7:742–747.

Nasci, RL, White, DJ, Sirling, H, et al. West Nile virus isolates from mosquitoes in New York and New Jersey, 1999. Emerg Infect Dis 2001b; 7:626–630.

Nasci, RS, Gottfried, KL, Burkhalter, KL, et al. Comparison of Vero cell plaque assay, TaqMan® reverse transcriptase polymerase chain reaction RNA assay, and Vectest™ antigen assay for detection of West Nile virus in field-collected mosquitoes. J Am Mosq Control Assoc 2002; 18:294–300.

National Climatic Data Center. Climatological data: New England [On-line]. Available: www5.ncdc.noaa.gov/pdfs/cd/newengland, 2003.

Ngo, KA, Kramer, LD. Identification of mosquito bloodmeals using polymerase chain reaction (PCR) with order-specific primers. J Med Entomol 2003; 40:215–222.

Reiter, P. A portable, battery-powered trap for collecting gravid Culex mosquitoes. Mosq News 1983; 43:496–498.

Ritchie SA, Rowley, WA. Blood-feeding patters of Iowa mosquitoes. Mosq News 1981; 41:271–275.

Sardelis, MR, Turell, MJ. *Ochlerotatus j. japonicus* in Frederick County, Maryland: discovery distribution, and vector competence for West Nile virus. J Am Mosq Control Assoc 2001; 17:137–141.

Sardelis, MR, Turell, MJ, Dohm, DJ, et al. Vector competence of selected North American *Culex* and *Coquillettidia* mosquitoes for West Nile virus. Emerg Infect Dis 2001; 7:1018–1022.

Spielman, A. Studies on autogeny in natural populations of *Culex pipiens* in nature. I. Reproductive isolation between autogenous and anautogenous populations. Am J Hyg 1964; 80:175–183.

Spielman, A. Studies on autogeny in natural populations of *Culex pipiens.* II. Seasonal abundance of autogenous and anautogenous populations. J Med Entomol 1971; 8:555–561.

Spielman, A. Structure and seasonality of Nearctic *Culex pipiens* populations. NY Acad Sci 2001; 951:220–234.

Suyemoto, W, Schiefer, BA, Elderidge, BF. Precipitin tests of blood-fed mosquitoes collected during the VEE surveillance survey in the southern United States in 1971. Mosq News 1973; 33:392–395.

Tempelis, CH. Host-feeding patterns of mosquitoes, with a review of advances in analysis of blood meals by serology. J Med Entomol 1975; 11:635–653.

Turell, M, Dohm, DJ, Sardelis, MR, et al. An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. J Med Entomol 2004 (in press).

Turell, M, O'Guinn, M, Dohm, DJ, et al. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. J Med Entomol 2001; 38:130–134.

Turell, M, O' Guinn, M, Oliver, J. Potential for New York mosquitoes to transmit West Nile virus. Am J Trop Med Hyg 2000; 62:413–414.

United States Census Bureau. U.S. Census 2000: Connecticut: 2000 [On-line]. Available: www.census.gov/prod/2002/pubs/c2kprofoo-ct.pdf, 2002.

White, DJ, Kramer, LD, Backenson, PB, et al. Mosquito surveillance and polymerase chain reaction detection of West Nile virus, New York state. Emerg Infect Dis 2001; 1:643–649.

Address reprint requests to:
Dr. Theodore G. Andreadis
Connecticut Agricultural Experiment Station
123 Huntington St.
P.O. Box 1106
New Haven, CT 06504

E-mail: theodore.andreadis@po.state.ct.us