Journal of the American Mosquito Control Association, 40(2):92–101, 2024

ENHANCING EASTERN EQUINE ENCEPHALITIS VIRUS SURVEILLANCE IN NEW JERSEY: OPTIMIZED COLLECTION OF *CULISETA MELANURA*

ANNA C. FAGRE,[1,2] RAYMOND A. SOTO,[1,2] REED MAGLEBY,[2,3] MERVIN KEITH Q. CUADERA,[3] ALEXANDRIA SUN,[4] KIM CERVANTES,[3,*] SCOTT C. CRANS,[4] NICHOLAS A. PANELLA,[1] JOAN L. KENNEY,[1] AUTUMN ANGELUS,[4] KRISTEN L. BURKHALTER,[1] DANA WOELL,[3] KALANTHE HORIUCHI,[1] BRAD J. BIGGERSTAFF,[1] J. ERIN STAPLES,[1] ROXANNE CONNELLY,[1] STACEY W. MARTIN[1] AND NICHOLAS KOMAR[1]

ABSTRACT. Eastern equine encephalitis virus (EEEV) causes the most clinically severe neuroinvasive arboviral disease in the United States. The virus is endemic in eastern and Gulf Coast states and the Great Lakes region, causing cases annually. To detect EEEV circulation in its enzootic cycle before the virus infects humans and other mammals, mosquito control agencies in New Jersey have conducted mosquito surveillance using a series of permanent wooden resting box sites since 1975. We conducted 2 field studies, 1 evaluating resting traps and 1 evaluating efficacy of CO_2 lures, to optimize collection of *Culiseta melanura*, the primary enzootic vector of EEEV. Resulting mosquito samples were subjected to molecular analysis to determine EEEV infection rates. Corrugated plastic boxes trapped more bloodfed *Cs. melanura* than other resting trap types (resting boxes, Centers for Disease Control and Prevention [CDC] resting traps, or fiber pots) and were similar to resting boxes in total number of female *Cs. melanura* caught. Further, non-baited CDC light traps were more successful in trapping host-seeking *Cs. melanura* than those baited with dry ice, a CO_2 lure. The EEEV RNA was identified in *Cs. melanura*, *Aedes vexans*, *Anopheles quadrimaculatus*, and *Uranotaenia sapphirina*. Our findings indicate that corrugated plastic boxes and non–CO_2 baited traps could improve detection of *Cs. melanura*. Mosquito control agencies are encouraged to periodically assess their surveillance strategy for EEEV.

KEY WORDS Eastern equine encephalitis virus, mosquito surveillance, New Jersey, trap comparison

INTRODUCTION

Eastern equine encephalitis virus (EEEV) causes the most clinically severe domestic arboviral encephalitis, with a case fatality rate of approximately 30% for those with neurological disease (Feemster 1938, Goldfield and Sussman 1968, Przelomski et al. 1988, Lindsey et al. 2018). Most EEEV infections in humans are asymptomatic with less than 5% of seropositive individuals developing clinical disease (Goldfield and Sussman 1968). Annually, an average of 11 cases are reported in the United States, with most cases occurring in Northeastern or Gulf Coast states (Lindsey et al. 2018, Brown et al. 2021). However, 38 human cases were reported from 10 states, resulting in 19 deaths (a case fatality rate of 50%) during 2019 (Brown et al. 2021, Vahey et al. 2021).

In the northeastern USA, EEEV circulates in an enzootic cycle between passeriform birds and *Culiseta melanura* (Coquillett) in freshwater swamps. Other mosquito species serve as bridge vectors, which acquire infection from an avian reservoir and, after adequate incubation, transmit the virus to humans and other mammalian dead-end hosts (Crans et al. 1994, Molaei et al. 2015). The EEEV is endemic in New Jersey, with the state's first outbreak of 32 human cases occurring over an 8 wk period in 1959 (Goldfield and Sussman 1968). Since then, sporadic EEEV cases and outbreaks in susceptible vertebrate hosts (primarily humans and horses) have been observed in New Jersey. In the coastal regions of New Jersey, enzootic circulation is driven by *Cs. melanura* in Atlantic white-cedar (*Chamaecyparis thyoides* [L.]) swamps. Inland regions have the highest numbers of *Cs. melanura* in red maple (*Acer rubrum* [L.]) swamps (Holden et al. 1954, Crans et al. 1994). Historically, monitoring of *Cs. melanura* populations early in the season has provided valuable information about enzootic circulation of EEEV, allowing mosquito control agencies to implement enhanced surveillance and vector control strategies.

Less well-established, however, are the bridge vector species driving spillover transmission from the enzootic cycle in swamps into residential or agricultural areas. These species tend to exhibit more catholic feeding behavior, allowing for efficient transmission between avian amplification hosts and incidental hosts (i.e., horses and humans). While the relative importance of bridge vector species varies geographically and seasonally, several species from genera *Aedes*, *Culex*, and *Anopheles* have been implicated throughout the northeastern USA, in addition to *Coquillettidia perturbans* (Walker) (Armstrong and Andreadis 2010). In New Jersey, *Ae. sollicitans* (Walker) and *Cq. perturbans* have been cited as the species with greatest bridge vector transmission potential, based on field-collected mosquitoes screened during epizootic and epidemic periods (Crans et al. 1986).

[1] Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, 3156 Rampart Rd, Fort Collins, CO 80521.

[2] Epidemic Intelligence Service, Centers for Disease Control and Prevention, 1600 Clifton Rd, Atlanta, GA 30333.

[3] New Jersey Department of Health, 140 E Front St, Trenton, NJ 08608-1203.

[4] New Jersey Department of Environmental Protection, 501 E State St, Trenton, NJ 08625.

* Author to whom correspondence should be addressed: Kim Cervantes, New Jersey Department of Health, 140 E Front St, Trenton, NJ 08608-1203 (kim.cervantes@doh.nj.gov).

To monitor EEEV activity, vector control programs use a variety of mosquito resting trap types, which are used as diurnal resting places by mosquitoes (primarily those in genera *Culiseta* and *Anopheles*) (Burbutis and Jobbins 1958, Edman et al. 1968, Crans 1995, Howard et al. 2011). Resting trap types include traditional wooden resting boxes, fiber pots, corrugated plastic boxes (FlexCon), and Centers for Disease Control and Prevention (CDC) resting traps (BioQuip Products). The traditional wooden resting boxes used to trap *Cs. melanura* by many mosquito surveillance programs are 1 cubic foot, open on 1 end, and painted flat black on the outside with a red interior (Crans 1995). Fiber pots are readily available, inexpensive, lightweight, and effective at trapping *Cs. melanura* (Komar et al. 1995). Corrugated plastic boxes, structurally similar to traditional wooden resting boxes but 18″ deep (compared to the 12″ deep resting box), offer advantages in being both lightweight and collapsible for transport. The CDC resting traps resemble fiber pots but contain a battery-powered fan that prevents mosquitoes from escaping after they enter the trap.

To detect EEEV circulation in its enzootic cycle prior to viral infections occurring in humans and other mammals, New Jersey has conducted a surveillance program using a series of sites in the southern half of the state since 1975. Each site contains either 25 or 50 wooden resting boxes visited by county vector control staff on a weekly basis from the beginning of June through mid-November. Mosquitoes are aspirated from boxes and female *Cs. melanura* are pooled and tested for EEEV. Positive test results inform surveillance and control measures in surrounding communities.

In addition to monitoring resting mosquito populations, New Jersey conducts supplemental *Cs. melanura* and bridge vector surveillance using several types of host-seeking mosquito traps. One of these is the CDC light trap, often baited with dry ice to emulate CO_2 emitted from a mammalian host. Because *Cs. melanura* is an ornithophilic species and birds emit lower quantities of CO_2 than mammals, use of dry ice may hinder collection of *Cs. melanura* (Hachiya et al. 2007).

Here, we implement 2 trap-type comparison studies and present data to aid in optimization of EEEV surveillance methods. We assessed the performance of alternative resting trap types in addition to the wooden resting boxes used in the state-run surveillance program, as well as CDC light traps baited with and without dry ice. We also conducted molecular analysis on mosquitoes collected during site characterization and trap comparison studies to estimate EEEV infection rates.

MATERIALS AND METHODS

Mosquito trapping was conducted at an existing resting box surveillance site in Camden County, NJ, over a 9-day period (September 8 to 16, 2021) corresponding to CDC w 36–37. Mosquitoes were trapped during 2 trap-type comparison studies (September 13 to 16, 2021) and during preliminary characterization of the site (September 8 to 9, 2021). The site is ~100 feet (~30.5 m) in elevation and composed of coniferous and deciduous woodlands, including Atlantic white cedar swamp.

Site characterization

During initial site characterization (September 8 to 9, 2021), mosquitoes were collected using a combination of resting boxes (10 trap nights), a CO_2-baited CDC light trap (1 trap night), and a CDC resting trap (1 trap night).

Resting trap comparison

For the resting trap-type comparison (September 13 to 16, 2021), we compared 4 trap types, including 1) traditional black wooden resting boxes (12″(30 cm) H 12″(30 cm) W x 12″(30 cm) deep), 2) CDC resting traps from BioQuip Products (Panella et al. 2011), 3) black corrugated plastic boxes from Flexcon (12″(30 cm) H x 12″(30 cm) W x 18″(45 cm) deep) and 4) fiber pots painted black on the inside (Komar et al. 1995) (Fig. 1). Over 4 consecutive nights, we implemented a four-treatment, four-period crossover design (Hedayat and Stufken 2003), placing 12 traps of each type (48 total traps per night) in a grid approximately 5 m apart using a predetermined random sequence (Fig. 1).

CDC light trap with and without CO_2 comparison

To determine if using dry ice as a CO_2 source impacts trapping efficacy of *Cs. melanura* using CDC light traps (John W Hock, Gainesville, FL; Model 512), a 2-treatment, 2-period crossover design was employed (Hedayat and Stufken 2003). On the first night, 4 CO_2-baited CDC light traps and 4 non-baited CDC light traps (8 traps in total) were placed in a row approximately 50 m apart, using a predetermined random sequence. A two-day washout period (nights 2 and 3) was implemented to prevent residual carryover (i.e., "trap-out") effects from the first night. On the fourth night, the opposite trap type was used at each location within the sampling site (8 traps in total) to control for position-specific effects. On each night, traps were operated from dusk to dawn.

Mosquito collection and processing

For both field studies, mosquitoes were collected from traps each morning within a four-hour period, transported on dry ice, and stored at –80°C. Mosquitoes collected from wooden boxes, corrugated plastic boxes, and fiber pots were anesthetized with triethylamine (TEA) and aspirated using a handheld aspirator. Mosquitoes were morphologically identified using the criteria of Darsie and Ward (2005) and sorted by species, sex, and bloodfed status on a chill table. Unfed *Cs. melanura*, in addition to all other mosquito species (regardless of engorgement status), were processed at New Jersey Public Health and Environmental Laboratories (NJPHEL). Bloodfed *Cs. melanura* were sent to Division of Vector-Borne Diseases (DVBD), Centers for Disease Control and Prevention, in Fort Collins, Colorado, for further molecular analyses.

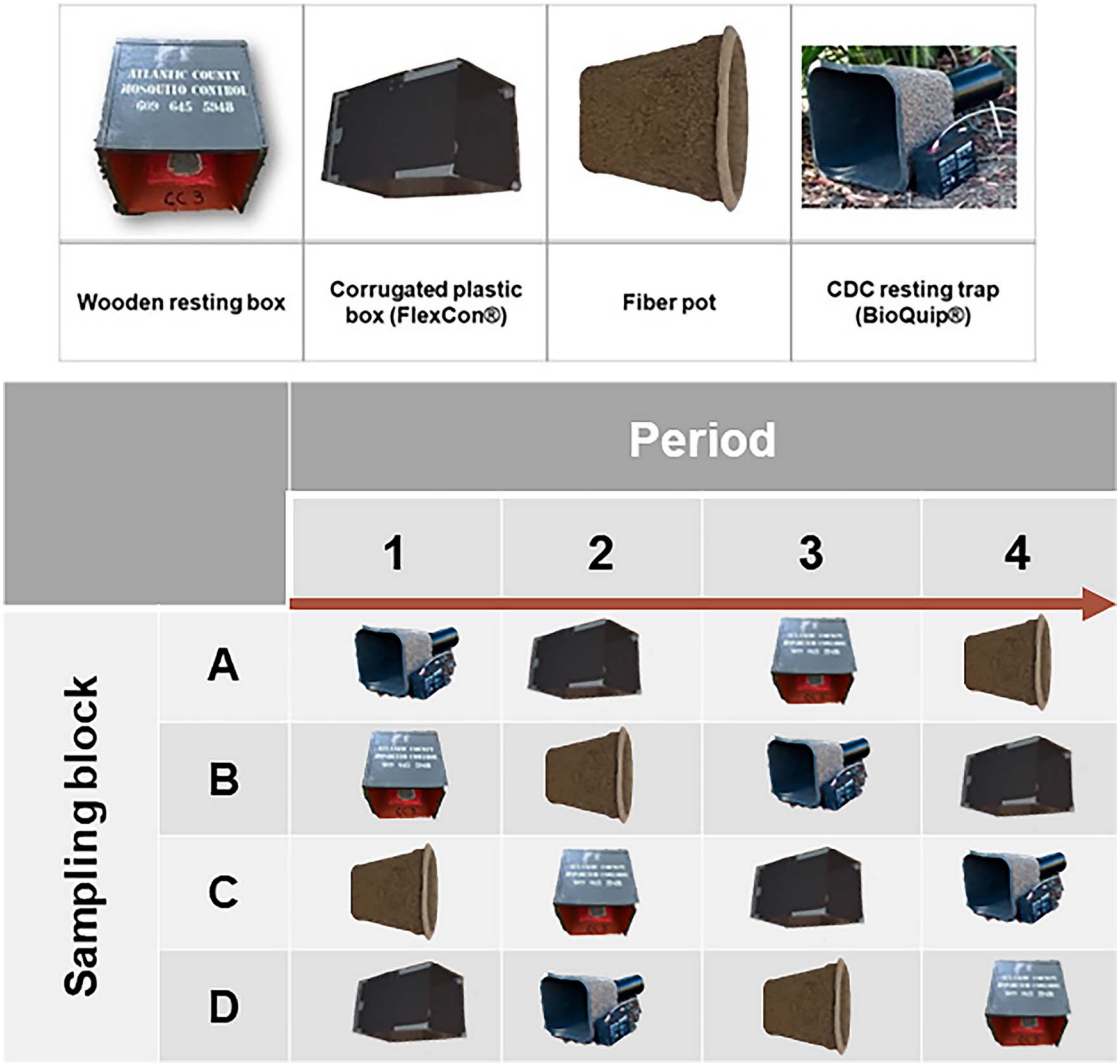

Fig. 1. Image of 4 resting trap types used in the resting trap comparison study with an example of the four-treatment, four-period crossover design. Note: images not to scale.

Statistical analysis

For the resting trap study, trap efficacy was assessed by comparing total number of female *Cs. melanura* (both bloodfed and unfed) and bloodfed-only *Cs. melanura*, referred to as "all" and "bloodfed," respectively. For the CO_2-baited trap study, total number of (host-seeking) female mosquitoes were counted and sorted by genus for analysis. Poisson regression was used to compare trap types using these counts, and we evaluated period and trap location as potential adjustment factors. Pairwise comparisons were calculated as mean ratios (MR) (95% confidence intervals [CI]), computed by exponentiating estimates of model parameters. Model selection was based on likelihood ratio chi-squared tests, with significance level 0.05. Firth's adjustment maximum likelihood (Firth 1993) was used to estimate model parameters, and final estimates (95% equal-tailed, percentile CI) were computed by bootstrap (Davison and Hinkley 1997) with 1,000 replicates. Poisson models were tested for overdispersion (Dean 1992), and Tukey's method was used for multiple comparisons adjustment as required. Analysis was conducted using R version 4.0.3 (The R Project for Statistical Computing [r-project.org]). Infection rate estimates were calculated by species, reported as maximum likelihood estimate using Firth's correction with 95% skew-corrected score CI (PooledInfRate R Package, version 1.5, CDC DVBD [see www.cdc.gov/westnile/resourcepages/mosqsurvsoft.html]).

Molecular testing of mosquitoes for EEEV RNA

All mosquitoes collected during site characterization activities and trap comparison studies were

subjected to molecular testing. Abdomens of bloodfed *Cs. melanura* were homogenized individually and RNA extracted using QIAamp Viral RNA MiniKit (QIAGEN, Valencia, CA) at CDC DVBD. Extracted nucleic acid was subjected to real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) targeting EEEV, and those testing positive were subjected to confirmatory qRT-PCR (Lambert et al. 2003). To test for viral dissemination, the legs of abdomen-positive mosquitoes were tested using the same extraction process and qRT-PCR assay.

All other mosquito species, in addition to unfed *Cs. melanura*, were tested for EEEV RNA at NJPHEL. Briefly, mosquitoes were pooled by trap night, trap type, and species before homogenization by adding 500µl of TE buffer and bead beating with a copper bb in a Qiagen TissueLyser at a frequency of 300 1/sec for 5 min followed by centrifugation. RNA was extracted using QIAamp DNA Blood BioRobot MDx kit (Qiagen, Catalogue #965152) and target detection performed using a quadruplex real-time RT-PCR assay developed in NJPHEL. Primers and probes were based off the CDC singleplex assay for EEEV (Lambert et al. 2003) and performed in a multiplex along with primer/probes for Jamestown Canyon virus, West Nile virus, and St. Louis encephalitis virus.

RESULTS

Mosquito identifications and sorting

A total of 2,175 mosquitoes were collected during site characterization (n = 279) and the trap comparison studies (n = 1896). Overall, 2,020 were identified to the species level, with 20 species represented. The remaining 155 specimens were identified to the level of genus (*Aedes, Anopheles, Culex*) or species complex (*Cx. pipiens/restuans/salinarius*). *Culiseta melanura* comprised 20.14% (n = 434/2,155) of these mosquitoes, and 103 were engorged.

Analysis of resting trap-type comparison: Model fitting for the resting trap-type comparison indicated that neither period ($P = 1.0$) nor trap location ($P = 0.36$) contributed significantly to model fit, and the test for overdispersion was not significant ($P = 0.94$), indicating the Poisson model sufficiently characterized variation. Of the 4 resting trap types under evaluation, corrugated plastic boxes caught the highest number of *Cs. melanura* mosquitoes (mean per trap night: 3.15; 95% CI: 2.63–3.74) (Table 1). Corrugated plastic boxes and resting boxes were equally effective at trapping female *Cs. melanura* (MR: 0.87; 95% CI: 0.65–1.16; see Supplemental Table 1.1), and both were more effective at trapping *Cs. melanura* than fiber pots or resting traps (Fig. 2). Corrugated plastic boxes trapped the highest mean number of bloodfed *Cs. melanura* compared to all other trap types (mean per trap night: 0.95; 95% CI: 0.68–1.30) (Table 2), including the wooden resting boxes (mean per trap night: 0.58; 95% CI: 0.38–0.86) currently in use by NJDEP (Fig. 3).

Table 1. Mean number of *Culiseta melanura* female mosquitoes trapped per trap night over all locations and periods (CP = black corrugated plastic boxes; RB = traditional wooden resting boxes; FP = fiber pots; RT = CDC resting traps).

Trap type	Mean number of female *Culiseta melanura* (95% CI)
CP	3.15 (2.63–3.74)
RB	2.79 (2.31–3.34)
FP	1.19 (0.88–1.57)
RT	0.52 (0.33–0.79)

Analysis of CDC light traps baited with and without CO_2

For the CO_2-baited trap comparison, model analysis indicated that neither period ($P = 1.0$) nor trap location ($P = 0.45$) significantly contributed to the fit, and the test for overdispersion was not significant ($P = 0.79$). Of the 498 mosquitoes collected during the CO_2 analysis, CDC light traps baited with CO_2 caught more mosquitoes, regardless of species (n = 307, 62%) than CDC light traps without CO_2 (n = 191, 38%). However, the mean number of *Cs. melanura* collected from non-baited CDC light traps per trap night was 2.19 (95% CI: 1.09–4.40) times higher than the mean number of *Cs. melanura* collected from CO_2-baited CDC light traps ($P = 0.03$). Furthermore, the non-baited CDC light traps were more favorable for *Cs. melanura*, which comprised 12.6% (24/191) of mosquitoes collected without CO_2 bait compared to 3.3% (10/307) when CO_2 was present ($P < 0.01$) (Fig. 4).

Viral testing: The 331 unengorged *Cs. melanura* collected during site characterization activities and trap-type comparison studies were split into 133 pools, 2 of which tested positive for EEEV, resulting in an estimated infection rate (IR) of 6.1 (95% CI: 1.1–19.6) per 1,000 mosquitoes. The abdomens and legs of 2 of the 103 engorged *Cs. melanura* tested positive for EEEV RNA (IR: 19.42 (95% CI: 5.34–68.05) per 1,000 mosquitoes).

Other mosquito species from which EEEV RNA was amplified included *Ae. vexans* (Meigen) (IR: 15.3 (95% CI: 0.9–75.7) per 1,000 mosquitoes), *An. quadrimaculatus* Say (IR: 21.4 (95% CI: 5.7–56.4) per 1,000 mosquitoes) and *Uranotaenia sapphirina* (Osten Sacken) (IR: 107.2 (95% CI: 6.6–409.3) per 1,000 mosquitoes) (Table 3).

DISCUSSION

In evaluating mosquito traps for EEEV surveillance in New Jersey, we determined that corrugated plastic boxes are a viable alternative to wooden resting boxes for the collection of resting *Cs. melanura*. We also showed that refraining from use of CO_2 as a bait in CDC light traps resulted in collection of a higher

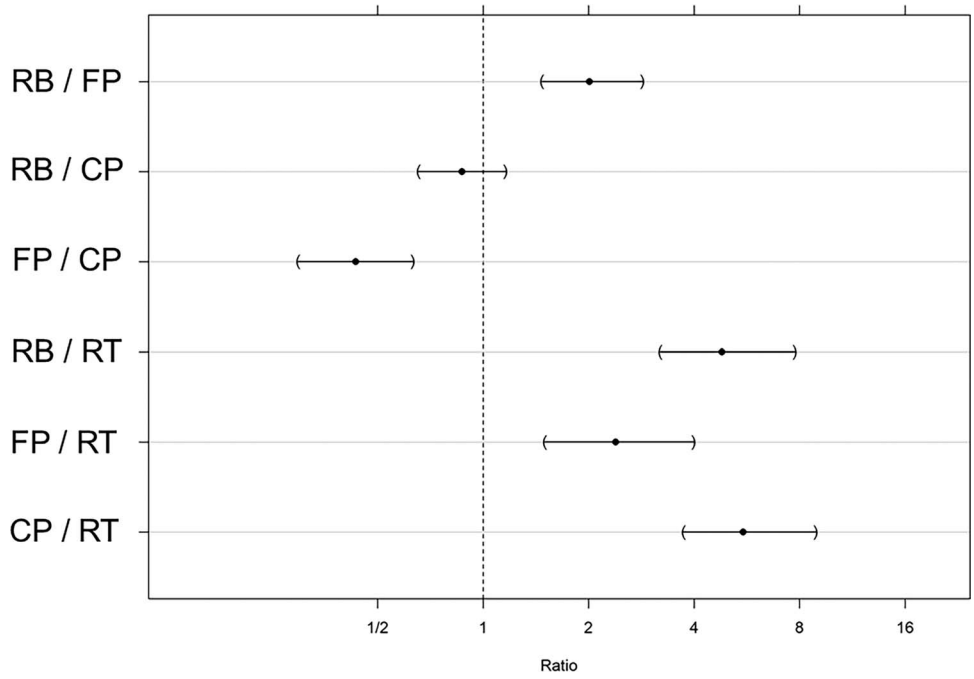

Fig. 2. Ratios of estimated mean numbers (95% CIs) of *Culiseta melanura* females trapped per trap night for each pair of trap types, adjusted for trap location and trapping period and multiple comparisons. A dashed line marks equality of the means. For specific ratio measurements, see Supplemental Table 1.1 (CP = black corrugated plastic boxes; FP, fiber pots; RB, traditional wooden resting boxes; RT, CDC resting traps).

number of host-seeking *Cs. melanura* and reduced collection of non-target species.

In our resting trap comparison conducted shortly after the peak of EEEV activity in New Jersey, corrugated plastic boxes outperformed the historically used wooden resting boxes in trapping bloodfed *Cs. melanura*. The black interior of the corrugated plastic boxes may have provided visual cues to attract mosquitoes, as previous studies have demonstrated the color preference of *Aedes* mosquitoes to be black, followed by red (Brett 1938; Brown 1954; Browne and Bennett 1981). Further, the greater depth of the corrugated plastic boxes (18″ (45 cm) deep compared to the 12″ (30 cm) depth of wooden resting boxes) might have resulted in a more substantial microclimatic gradient, providing a more favorable ambient temperature to resting *Cs. melanura*. However, without direct measurements of temperature and RH within each trap, differentiation of these effects from other abiotic factors (e.g., light) remains difficult. An important advantage of the corrugated plastic boxes is their maneuverability in the field—the material is more lightweight than wooden resting boxes, and the boxes easily collapse down when not in use. Fiber pots and CDC resting traps were not as effective as wooden resting boxes or corrugated plastic boxes in trapping *Cs. melanura*. Interestingly, battery-powered CDC resting trap demonstrated the lowest success rate in trapping *Cs. melanura*. This contrasts with our previous findings demonstrating that fiber pots caught as many *Cs. melanura* as wooden resting boxes when operated during July–October (Komar et al. 1995), and that addition of a battery-powered fan to CDC resting traps increased efficacy in trapping *Culex* spp. vectors of West Nile virus in Colorado (Panella et al. 2011). Geographic and seasonal variations in humidity and temperature might influence the relative efficiency of different trap types for different mosquito genera because temperature has been shown to impact mosquito phenology and behavior (Mushegian et al. 2021). Therefore, trap-type comparisons should be performed at different locations and at different times of the transmission season to determine optimal trap types for *Cs. melanura*.

Table 2. Mean number of bloodfed *Culiseta melanura* female mosquitoes trapped per trap night over all locations and periods (CP = black corrugated plastic boxes; RB = traditional wooden resting boxes; FP = fiber pots; RT = CDC resting traps).

Trap type	Mean number of bloodfed female *Culiseta melanura* (95% CI)
CP	0.95 (0.68–1.30)
RB	0.58 (0.38–0.86)
FP	0.57 (0.37–0.85)
RT	0.07 (0.02–0.21)

We also characterized whether presence of dry ice as a CO_2 source influenced the relative efficacy of CDC light traps in collection of *Cs. melanura* because different volatiles emulating those emitted by their preferred host could impact trap efficacy and species diversity

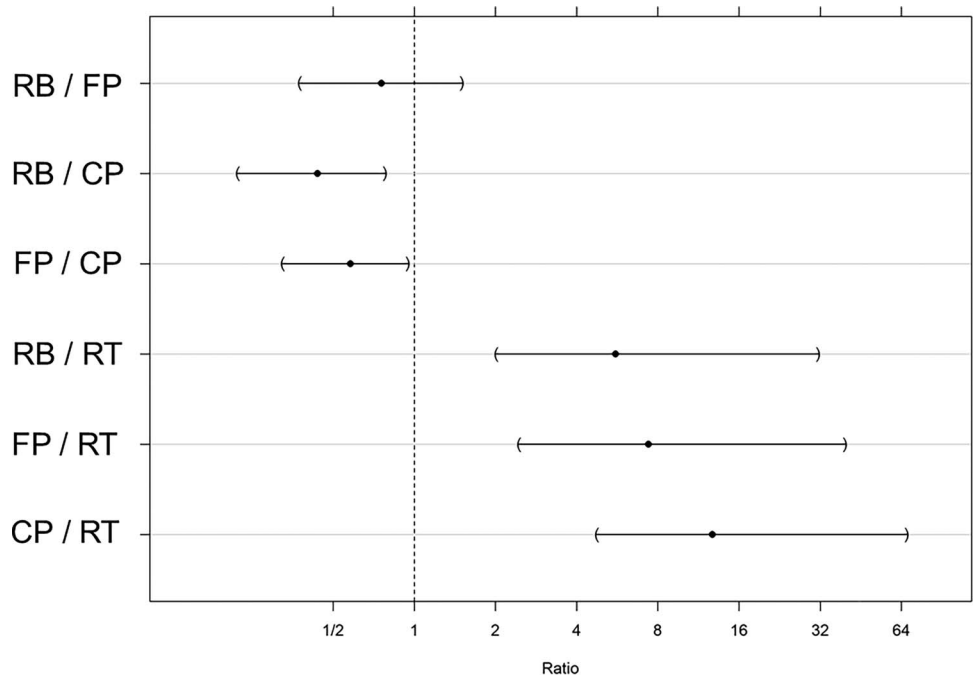

Fig. 3. Ratios of estimated mean numbers (95% CIs) of bloodfed *Culiseta melanura* female mosquitoes trapped per trap night for each pair of trap types, adjusted for trap location and trapping period and multiple comparisons. A dashed line marks equality of the means. For specific ratio measurements, see Supplemental Table 1.2 (CP = black corrugated plastic boxes; FP, fiber pots; RB, traditional wooden resting boxes; RT, CDC resting traps).

(Kline and Mann 1998, Bernier et al. 2008, Verhulst et al. 2010, Busula et al. 2017, Díez-Fernández et al. 2020, Giordano et al. 2021). Congruent with previous studies, our study demonstrated that non-CO_2 baited CDC light traps caught more *Cs. melanura* per trap night than those baited with dry ice as a CO_2 source (Hachiya et al. 2007). This may be due to this mosquito species' ornithophilic feeding preferences, as birds emit smaller quantities of CO_2 than mammals (Cooperband and Cardé 2006). Further, *Cs. melanura* comprised a greater proportion of overall mosquitoes in non-CO_2 baited traps, resulting in reduced trapping of 'non-target' species. Thus, programs or agencies targeting primarily *Cs. melanura* (*e.g.,* by EEEV vector surveillance programs) might consider foregoing use of CO_2 as a means of reducing non-target species and expediting the sorting process. Not using dry ice in EEEV surveillance programs also has implications for cost, effort, and convenience. Dry ice is costly, sublimates more drastically under extreme ambient conditions, and can present hazards to individuals handling it in the field. However, the lack of CO_2 might decrease the yield of potential bridging vectors so trap types used should be based on the specific goal of the surveillance program (e.g., identification of enzootic or bridging vectors for EEEV).

In preparing for and conducting the trap-type comparison study, we had the opportunity to further investigate the potential contribution of *Cs. melanura* and other mosquito species to EEEV amplification in southern New Jersey. The EEEV was detected not only in the enzootic vector, *Cs. melanura*, but in 3 other species of mosquitoes (*Ae. vexans*, *An. quadrimaculatus*, and *Ur. sapphirina*). *Aedes vexans* is a suspected bridge vector of EEEV transmission, having been the first species from which EEEV was isolated in Connecticut (Wallis et al. 1960). While *Ae. vexans* has ecological and life history characteristics that make it an optimal bridge vector (e.g., aggressively anthropophilic feeding behavior and large broods following heavy rains), laboratory competence studies indicate *Ae. vexans* might not play a significant role in EEEV transmission (Nasci and Mitchell 1996, Vaidyanathan et al. 1997, Armstrong and Andreadis 2010). *Anopheles quadrimaculatus* is known to feed on many mammal species, including white-tailed deer (Molaei et al. 2009), a species where evidence of EEEV infection has been described (Tate et al. 2005, Schmitt et al. 2007, Mutebi et al. 2011). Further, *An. quadrimaculatus* is competent for EEEV in the laboratory and has been suggested as a potential epidemic vector for EEEV (Vaidyanathan et al. 1997, Moncayo et al. 2000). Given this species' local abundance and shared habitat with *Cs. melanura*, further monitoring of this species to characterize its potential as a bridge vector of EEEV in New Jersey is warranted.

Lastly, we detected EEEV RNA in 1 pool of *Ur. sapphirina*, a species known to prefer feeding on annelids (e.g., worms and leeches), but which has also been shown to feed on amphibians, reptiles, birds, and humans (Molaei et al. 2008, Reeves et al. 2018,

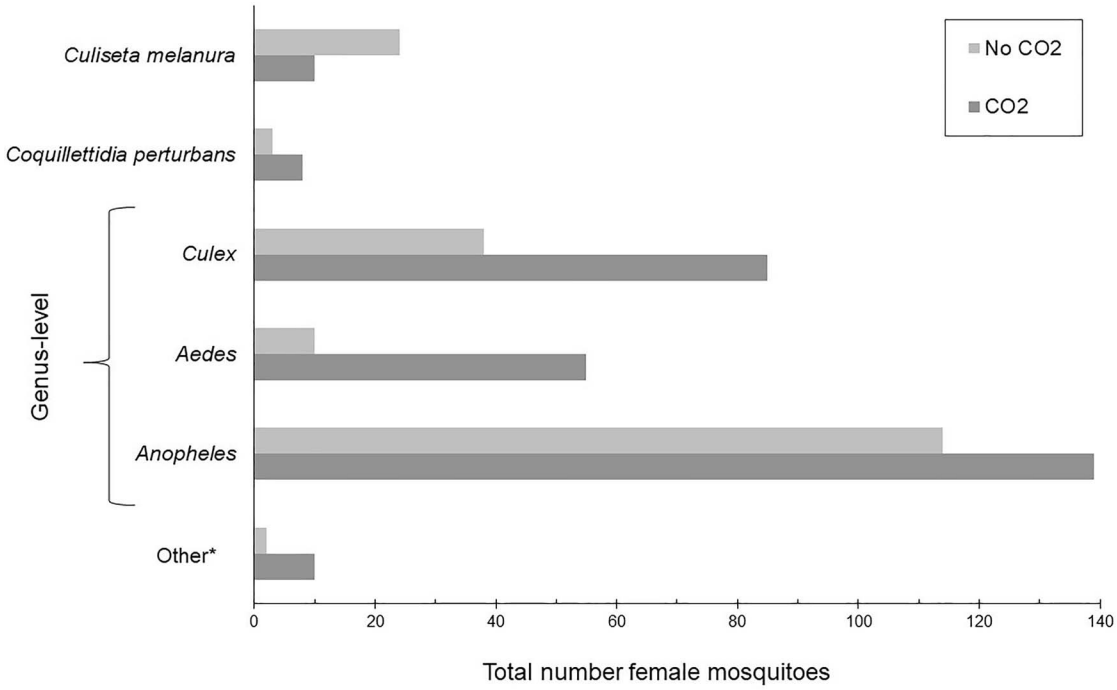

Fig. 4. Number of female mosquitoes, by genus, trapped using CDC light traps with (n = 307) and without (n = 191) the use of dry ice as a CO_2 source (8 trap nights/treatment). *Culiseta melanura* and *Coquillettidia perturbans* were analyzed at the species level. * "Other" category includes *Psorophora* spp. and *Uranotaenia* spp.

Khalil et al. 2023). Its predilection to feed on ectotherms has resulted in its incrimination as a potential overwintering host of EEEV (White et al. 2011, Bingham et al. 2012, Graham et al. 2012). Other reports detecting EEEV RNA in *Ur. sapphirina* in the southern USA postulate that in addition to the well-characterized enzootic cycle between *Cs. melanura* and passeriform birds, an additional transmission cycle might exist

Table 3. EEEV testing data for pools of mosquito species other than *Cs. melanura* collected during site characterization and trap-type comparison studies conducted in Camden County (September 8–16, 2021). For each species, the number of mosquito pools and total number of mosquitoes are provided, as is the estimated prevalence of each virus with infection rates represented as bias-corrected maximum-likelihood estimate (MLE) with 95% skewness-corrected score interval (per 1,000 individuals). Mosquitoes identified to genus level only (n = 13) were not included in this table, and species in Pipiens group (*Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius*) (n = 142) were analyzed together. Note that we do not report estimates for samples of (an arbitrary value of) 5 or fewer individuals, for which inferences are expected to be unreliable.

Mosquito species	Number of pools	Number of mosquitoes	Number of positive pools	EEEV infection rate (95% CI)
Aedes albopictus	1	1	0	—
Ae. atlanticus	1	3	0	—
Ae. canadensis	7	18	0	0.0 (0.0–175.9)
Ae. sticticus	1	5	0	—
Ae. triseriatus	1	1	0	—
Ae. vexans	13	67	1	15.3 (0.9–75.7)
Anopheles crucians s.l.	123	562	0	0.0 (0.0–6.8)
An. punctipennis	56	71	0	0.0 (0.0–51.3)
An. quadrimaculatus s.l.	77	131	3	21.4 (5.7–56.4)
Coquillettidia perturbans	9	16	0	0.0 (0.0–193.6)
Culex erraticus	139	604	0	0.0 (0.0–6.3)
Cx. pipiens, Cx. restuans, Cx. salinarius	55	142	0	0.0 (0.0–31.8)
Cx. territans	42	88	0	0.0 (0.0–41.8)
Psorophora ciliata	1	1	0	—
Ps. columbiae	2	2	0	—
Ps. ferox	4	7	0	0.0 (0.0–354.3)
Uranotaenia sapphirina	7	9	1	107.2 (6.6–409.3)
Total	539	1,728	7	

between *Ur. sapphirina* and ectotherms (Cupp et al. 2003, Graham et al. 2012).

The trap comparison studies were subjected to limitations inherent to the opportunistic nature of this field survey and its short duration. Intensive trapping in specific locations during a 1-week period limits extrapolation of our results to the rest of the year. However, we conducted both trap-type comparison studies during epidemiologic wk 37, a period during which *Cs. melanura* populations have remained active (according to historical data available through Rutgers University, https://vectorbio.rutgers.edu/reports/vector/). Additionally, the limited trapping period and optimizing the trapping for *Cs melanura* might have restricted the identification of potential bridging vectors. Further, these studies took place at 1 study site in Camden County, and caution should be exercised in translating these results to other settings—particularly those with differing ecology. Lastly, small mosquito sample sizes resulted in sometimes relatively wide, and imprecise, confidence intervals. To further corroborate the results of the trap-type comparison studies before implementation in a routine surveillance program, additional trap-type comparisons are needed (preferably at different sites throughout New Jersey or other states in the northeastern USA).

The EEEV infection causes severe neurologic illness in humans and other dead-end hosts, and detection of viral activity early in the enzootic cycle allows for prompt implementation of the vector control measures required to disrupt epizootic transmission into human or domestic animal populations. Evaluation of vector monitoring programs is an important step in assessing overall arboviral surveillance and can aid in obtaining a more granular understanding of local EEEV ecology. Each jurisdiction with EEEV circulation is encouraged to periodically reassess their vector control program to optimize their early detection and prevention of EEEV transmission.

SUPPLEMENTAL MATERIAL

Data tables describing estimated mean ratios of *Culiseta melanura* trapped during the resting trap-type comparison analysis.

Supplemental Table 1.1. Ratios (95% CIs) of estimated mean numbers of all *Culiseta melanura* female mosquitoes trapped per trap night for each pair of trap types, adjusted for trap location, trapping period, multiple comparisons (CP, black corrugated plastic boxes; FP, fiber pots; RB, traditional wooden resting boxes; RT, CDC resting traps).

Comparison of trap types	Ratio of mean number of female *Culiseta melanura* (95% CI)
RB / FP	2.01 (1.47–2.84)
RB / CP	0.87 (0.65–1.16)
FP / CP	0.43 (0.30–0.63)
RB / RT	4.80 (3.20–7.75)
FP / RT	2.39 (1.50–3.98)
CP / RT	5.53 (3.73–8.88)

Supplemental Table 1.2. Ratios (95% CIs) of estimated mean numbers of bloodfed *Culiseta melanura* female mosquitoes trapped per trap night for each pair of trap types, adjusted for trap location, trapping period, and multiple comparisons (CP, black corrugated plastic boxes; FP, fiber pots; RB, traditional wooden resting boxes; RT, CDC resting traps).

Comparison of trap types	Ratio of mean number of bloodfed female *Culiseta melanura* (95% CI)
RB / FP	0.76 (0.38–1.50)
RB / CP	0.44 (0.22–0.78)
FP / CP	0.58 (0.33–0.95)
RB / RT	5.58 (2.01–31.44)
FP / RT	7.39 (2.45–39.57)
CP / RT	12.75 (4.76–67.02)

ACKNOWLEDGMENTS

Thank you to the staff at the New Jersey Department of Environmental Protection, New Jersey Department of Health, and the following mosquito control agencies in New Jersey for their assistance in collecting, sorting, and identifying mosquitoes: Atlantic County Office of Mosquito Control, Bergen County Division of Mosquito Control, Camden County Mosquito Commission, Cape May County Department of Mosquito Control, Gloucester County Mosquito Control Division, Hudson County Regional Health Commission, Hunterdon County Mosquito and Vector Control, Mercer County Mosquito Control, Middlesex County Mosquito Extermination Commission, Monmouth County Mosquito Control, Ocean County Mosquito Commission, Passaic County Mosquito Control, Salem County Mosquito Control, and Sussex County Office of Mosquito Control. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention. Nicholas Komar and Nicholas Panella are named in a patent for the Centers for Disease Control Resting Mosquito Trap.

REFERENCES CITED

Armstrong PM, Andreadis TG. 2010. Eastern equine encephalitis virus in mosquitoes and their role as bridge vectors. *Emerg Infect Dis* 16:1869–1874.

Bernier UR, Allan SA, Quinn BP, Kline DL, Barnard DR, Clark GG. 2008. Volatile compounds from the integument of White Leghorn Chickens (*Gallus gallus domesticus* L.): candidate attractants of ornithophilic mosquito species. *J Sep Sci* 31:1092–1099.

Bingham AM, Graham SP, Burkett-Cadena ND, White GS, Hassan HK, Unnasch TR. 2012. Detection of eastern equine encephalomyelitis virus RNA in North American snakes. *Am J Trop Med Hyg* 87:1140–1144.

Brett GA. 1938. On the relative attractiveness to *Aëdes aegypti* of certain coloured cloths. *Trans Royal Soc Trop Med Hyg* 32:113–124.

Brown AWA. 1954. Studies on the responses of the female *Aedes* mosquito. Part VI.–The attractiveness of coloured cloths to Canadian species. *Bull Entomol Res* 45:67–78.

Brown SC, Cormier J, Tuan J, Lier AJ, McGuone D, Armstrong PM, Kaddouh F, Parikh S, Landry ML, Gobeske KT. 2021. Four human cases of Eastern equine encephalitis in Connecticut, USA, during a larger regional outbreak, 2019. *Emerg Infect Dis* 27:2042–2051.

Browne SM, Bennett GF. 1981. Response of mosquitoes (Diptera: Culicidae) to visual stimuli. *J Med Entomol* 18:505–521.

Burbutis PP, Jobbins D. 1958. Studies on the use of a diurnal resting box for the collection of *Culiseta melanura* (Coquillett). *Bull Brooklyn Entomol Soc* 53:53–58.

Busula AO, Takken W, de Boer JG, Mukabana WR, Verhulst NO. 2017. Variation in host preferences of malaria mosquitoes is mediated by skin bacterial volatiles. *Med Vet Entomol* 31:320–326.

Cooperband MF, Cardé RT. 2006. Comparison of plume structures of carbon dioxide emitted from different mosquito traps. *Med Vet Entomol* 20:1–10.

Crans WJ. 1995. *Resting boxes as mosquito surveillance tools*. In: Proceedings of the 82nd Annual Meeting of the New Jersey Mosquito Control Association. 1995 March 7–10; NJ

Crans WJ, Caccamise DF, McNelly JR. 1994. Eastern equine encephalomyelitis virus in relation to the avian community of a coastal cedar swamp. *J Med Entomol* 31:711–728.

Crans WJ, McNelly J, Schulze TL, Main A. 1986. Isolation of eastern equine encephalitis virus from *Aedes sollicitans* during an epizootic in southern New Jersey. *J Am Mosq Control Assoc* 2:68–72.

Cupp EW, Klingler K, Hassan HK, Viguers LM, Unnasch TR. 2003. Transmission of eastern equine encephalomyelitis virus in central Alabama. *Am J Trop Med Hyg* 68:495–500.

Darsie RF, Ward RA. 2005. *Identification and geographical distribution of the mosquitoes of North America, north of Mexico*. Gainesville, FL: University Press of Florida.

Davison A, Hinkley D. 1997. *Bootstrap methods and their application*. Cambridge, United Kingdom: Cambridge University Press.

Dean CB. 1992. Testing for overdispersion in Poisson and binomial regression models. *J Am Statistical Assoc* 87:451–457.

Díez-Fernández A, Martínez-de la Puente J, Gangoso L, Ferraguti M, Soriguer R, Figuerola J. 2020. House sparrow uropygial gland secretions do not attract ornithophilic nor mammophilic mosquitoes. *Med Vet Entomol* 34:225–228.

Edman JD, Evans FD, Williams JA. 1968. Development of a diurnal resting box to collect *Culiseta melanura* (Coq.). *Am J Trop Med Hyg* 17:451–456.

Feemster RF. 1938. Outbreak of encephalitis in man due to the eastern virus of equine encephalomyelitis. *Am J Pub Health Nations Health* 28:1403–1410.

Firth D. 1993. Bias reduction of maximum likelihood estimates. *Biometrika* 80:27–38.

Giordano BV, Allen BT, Wishard R, Xue R-D, Campbell LP. 2021. Light trap collections of mosquitoes (Diptera: Culicidae) using dry ice and octenol attractants in adjacent mosquito control programs. *Florida Entomologist* 103:499–504.

Goldfield M, Sussman O. 1968. The 1959 outbreak of eastern encephalitis in New Jersey. I. Introduction and description of outbreak. *Am J Epidemiol* 87:1–10.

Graham SP, Hassan HK, Chapman T, White G, Guyer C, Unnasch TR. 2012. Serosurveillance of eastern equine encephalitis virus in amphibians and reptiles from Alabama, USA. *Am J Trop Med Hyg* 86:540–544.

Hachiya M, Osborne M, Stinson C, Werner BG. 2007. Human eastern equine encephalitis in Massachusetts: predictive indicators from mosquitoes collected at 10 long-term trap sites, 1979–2004. *Am J Trop Med Hyg* 76:285–292.

Hedayat AS, Stufken J. 2003. Optimal and efficient crossover designs under different assumptions about the carryover effects. *J Biopharm Stat* 13:519–528.

Holden P, Miller BJ, Jobbins DM. 1954. Isolations of Eastern equine encephalomyelitis virus from mosquitoes (*Culiseta melanura*) collected in New Jersey, 1953. *Proc Soc Exp Biol Med* 87:457–459.

Howard JJ, Oliver J, Kramer LD. 2011. Assessing the use of diurnal resting shelters by *Culiseta melanura* (Diptera: Culicidae). *J Med Entomol* 48:909–913.

Khalil N, Shepard JJ, Foss K, Molaei G. 2023. Evidence of *Uranotaenia sapphirina* (Diptera: Culicidae) feeding on annelid worms in the Northeastern United States. *J Med Entomol* 60:425–431.

Kline DL, Mann MO. 1998. Evaluation of butanone, carbon dioxide, and 1-octen-3-OL as attractants for mosquitoes associated with north central Florida bay and cypress swamps. *J Am Mosq Control* Assoc 14:289–297.

Komar N, Pollack RJ, Spielman A. 1995. A nestable fiber pot for sampling resting mosquitoes. *J Am Mosq Control Assoc* 11:463–467.

Lambert AJ, Martin DA, Lanciotti RS. 2003. Detection of North American eastern and western equine encephalitis viruses by nucleic acid amplification assays. *J Clin Microbiol* 41:379–385.

Lindsey NP, Staples JE, Fischer M. 2018. Eastern equine encephalitis virus in the United States, 2003–2016. *Am J Trop Med Hyg* 98:1472–1477.

Molaei G, Andreadis TG, Armstrong PM, Diuk-Wasser M. 2008. Host-feeding patterns of potential mosquito vectors in Connecticut, U.S.A.: Molecular analysis of bloodmeals from 23 species of *Aedes*, *Anopheles*, *Culex*, *Coquillettidia*, *Psorophora*, and *Uranotaenia*. *J Med Entomol* 45:1143–1151.

Molaei G, Armstrong PM, Abadam CF, Akaratovic KI, Kiser JP, Andreadis TG. 2015. Vector-host interactions of *Culiseta melanura* in a focus of eastern equine encephalitis virus activity in southeastern Virginia. *PLoS One* 10:e0136743.

Molaei G, Farajollahi A, Armstrong PM, Oliver J, Howard JJ, Andreadis TG. 2009. Identification of bloodmeals in *Anopheles quadrimaculatus* and *Anopheles punctipennis* from eastern equine encephalitis virus foci in northeastern U.S.A. *Med Vet Entomol* 23:350–356.

Moncayo AC, Edman JD, Turell MJ. 2000. Effect of eastern equine encephalomyelitis virus on the survival of *Aedes albopictus*, *Anopheles quadrimaculatus*, and *Coquillettidia perturbans* (Diptera: Culicidae). *J Med Entomol* 37:701–706.

Mushegian AA, Neupane N, Batz Z, Mogi M, Tuno N, Toma T, Miyagi I, Ries L, Armbruster PA. 2021. Ecological mechanism of climate-mediated selection in a rapidly evolving invasive species. *Ecol Lett* 24:698–707.

Mutebi JP, Lubelczyk C, Eisen R, Panella N, Macmillan K, Godsey M, Swope B, Young G, Smith RP, Kantar L et al. 2011. Using wild white-tailed deer to detect eastern equine encephalitis virus activity in Maine. *Vector Borne Zoonotic Dis* 11:1403–1409.

Nasci RS, Mitchell CJ. 1996. Arbovirus titer variation in field-collected mosquitoes. *J Am Mosq Control Assoc* 12:167–171.

Panella NA, Crockett RJ, Biggerstaff BJ, Komar N. 2011. The Centers for Disease Control and Prevention resting trap: a novel device for collecting resting mosquitoes. *J Am Mosq Control Assoc* 27:323–325.

Przelomski MM, O'Rourke E, Grady GF, Berardi VP, Markley HG. 1988. Eastern equine encephalitis in Massachusetts: a report of 16 cases, 1970–1984. *Neurology* 38:736–739.

Reeves LE, Holderman CJ, Blosser EM, Gillett-Kaufman JL, Kawahara AY, Kaufman PE, Burkett-Cadena ND. 2018. Identification of *Uranotaenia sapphirina* as a specialist of annelids broadens known mosquito host use patterns. *Commun Biol* 1:92.

Schmitt SM, Cooley TM, Fitzgerald SD, Bolin SR, Lim A, Schaefer SM, Kiupel M, Maes RK, Hogle SA, O'Brien DJ. 2007. An outbreak of eastern equine encephalitis virus in free-ranging white-tailed deer in Michigan. *J Wildl Dis* 43:635–644.

Tate CM, Howerth EW, Stallknecht DE, Allison AB, Fischer JR, Mead DG. 2005. Eastern equine encephalitis in a free-ranging white-tailed deer (*Odocoileus virginianus*). *J Wildl Dis* 41:241–245.

Vahey GM, Mathis S, Martin SW, Gould CV, Staples JE, Lindsey NP. 2021. West Nile virus and other domestic nationally notifiable arboviral diseases—United States, 2019. *MMWR Morb Mortal Wkly Rep* 70:1069–1074.

Vaidyanathan R, Edman JD, Cooper LA, Scott TW. 1997. Vector competence of mosquitoes (Diptera:Culicidae) from Massachusetts for a sympatric isolate of eastern equine encephalomyelitis virus. *J Med Entomol* 34:346–352.

Verhulst NO, Andriessen R, Groenhagen U, Bukovinszkine Kiss G, Schulz S, Takken W, van Loon JJ, Schraa G, Smallegange RC. 2010. Differential attraction of malaria mosquitoes to volatile blends produced by human skin bacteria. *PLoS One* 5:e15829.

Wallis RC, Taylor RM, Henderson JR. 1960. Isolation of eastern equine encephalomyelitis virus from *Aedes vexans* in Connecticut. *Proc Soc Exp Biol Med* 103:442–444.

White G, Ottendorfer C, Graham S, Unnasch TR. 2011. Competency of reptiles and amphibians for eastern equine encephalitis virus. *Am J Trop Med Hyg* 85:421–425.