VECTOR-BORNE AND ZOONOTIC DISEASES
Volume XX, Number XX, 2021

DOI: 10.1089/vbz.2021.0069

Host Associations of *Culex pipiens*: A Two-Year Analysis of Bloodmeal Sources and Implications for Arboviral Transmission in Southeastern Virginia

Noelle Khalil,[1,2] Eliza A.H. Little,[2,3] Karen I. Akaratovic,[4] Jay P. Kiser,[4] Charles F. Abadam,[4] Karen J. Yuan,[5] Michael J. Misencik,[1,2] Philip M. Armstrong,[1,2,5] and Goudarz Molaei[1,2,5]

Abstract

Understanding vector-host interactions is crucial for evaluating the role of mosquito species in enzootic cycling and epidemic/epizootic transmission of arboviruses, as well as assessing vertebrate host contributions to maintenance and amplification in different virus foci. To investigate blood-feeding pattern of *Culex pipiens*, engorged mosquitoes were collected on a weekly basis at 50 sites throughout Suffolk, Virginia, using Centers for Disease Control and Prevention miniature light traps, BG-Sentinel traps, and modified Reiter gravid traps. Vertebrate hosts of mosquitoes were identified by amplifying and sequencing portions of the mitochondrial *cytochrome b* gene. Of 281 *Cx. pipiens* bloodmeals successfully identified to species, 255 (90.7%) contained solely avian blood, 13 (4.6%) mammalian, 1 (0.4%) reptilian, and 12 (4.3%) both avian and mammalian blood. Nineteen avian species were identified as hosts for *Cx. pipiens* with American robin ($n = 141$, 55.3% of avian hosts) and northern cardinal ($n = 57$, 22.4%) as the most common hosts. More American robin feedings took place in areas of higher development. Three mammalian species were also identified as hosts for *Cx. pipiens* with Virginia opossum and domestic cat as the most common hosts in this class (each $n = 6$, 46.2% of mammalian hosts). There was no significant seasonal difference in the proportion of bloodmeals obtained from avian hosts, but there was a decrease in the proportion of bloodmeals from mammalian hosts from spring to fall. One engorged specimen of *Cx. pipiens* with Virginia opossum-derived bloodmeal tested positive for West Nile virus (WNV), and another with black-and-white warbler-derived bloodmeal tested positive for eastern equine encephalitis virus. Our findings, in conjunction with the results of vector competence studies and virus isolation from field-collected mosquitoes, lend additional support that *Cx. pipiens* serves as the principal enzootic vector and potential epizootic/epidemic vector of WNV in southeastern Virginia.

Keywords: *Culex pipiens*, bloodmeal analysis, vector, West Nile virus, Virginia

Introduction

Members of the *Culex pipiens* complex play a significant role in transmitting arboviral pathogens that infect humans in North America, Europe, and elsewhere (Fonesca et al. 2004, Farajollahi et al. 2011, Brugman et al. 2018). The principal involvement of this species complex in the transmission and maintenance of West Nile virus (WNV) has been established after the introduction of this virus to the Western hemisphere in 1999 (Molaei et al. 2006, Colpitts et al. 2012). West Nile virus (WNV) has since become the leading mosquito-borne disease in the United States and was responsible for more than 21,000 human disease cases and nearly 1200 deaths in the country from 2009 to 2018 (McDonald et al. 2021). The virus is maintained in an enzootic cycle involving avian hosts and bird-biting mosquitoes (Rossi et al. 2010). Although various mosquito species can perpetuate this cycle in nature, *Cx. pipiens* is considered an

[1]Department of Environmental Sciences, The Connecticut Agricultural Experiment Station, New Haven, Connecticut, USA.
[2]Center for Vector Biology & Zoonotic Diseases, The Connecticut Agricultural Experiment Station, New Haven, Connecticut, USA.
[3]Department of Entomology, The Connecticut Agricultural Experiment Station, New Haven, Connecticut, USA.
[4]Suffolk Mosquito Control, Department of Public Works, Suffolk, Virginia, USA.
[5]Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, Connecticut, USA.

important primary vector due to its abundance in the region, inclination for blood feeding on birds (Apperson et al. 2004, Molaei et al. 2006), vector competence (Turell et al. 2005), and high prevalence of WNV isolated from field-collected mosquitoes (Bernard et al. 2000, Andreadis 2012). Moreover, previous reports indicate that depending on location, a fraction of bloodmeals from *Cx. pipiens* is mammalian derived, suggesting its ability to partake in epidemic and epizootic transmission of WNV to humans and equines (Apperson et al. 2004, Molaei et al. 2006, Hamer et al. 2009).

In the United States, members of the *Cx. pipiens* complex include *Cx. pipiens* form pipiens, *Cx. pipiens* f. molestus, *Culex quinquefasciatus*, and hybrids of the species existing where their geographic ranges overlap (Farajollahi et al. 2011, Andreadis 2012, Molaei et al. 2012). Behavioral and physiological differences exist among members of the complex, most notably in propensity for avian or mammalian blood feedings (Farajollahi et al. 2011, Molaei et al. 2012, Fritz et al. 2015). Hybridization could influence host selection of *Cx. pipiens* across different regions among other important factors such as host abundance and availability (Huang et al. 2009, Molaei et al. 2012, Fritz et al. 2015). Evidence points to hybridizations of *Cx. pipiens* f. molestus and *Cx. pipiens* f. pipiens, as well as *Cx. pipiens* f. pipiens and *Cx. quinquefasciatus* between 36°N and 39°N latitudes (Barr 1957), yet limited studies have investigated the host-feeding patterns of *Cx. pipiens* in mid-Atlantic states within this zone. A study of *Cx. pipiens* in Washington DC and Maryland at the northern limit of the hybrid zone indicates a shift in feeding preference from avian to mammalian hosts as the season progresses (Kilpatrick et al. 2006b). The underlying reasons for the shift are not well understood; however, introgression between the avian-feeding *Cx. pipiens* f. pipiens and the mammalian-feeding *Cx. pipiens* f. molestus was believed to influence the probability that these mosquitoes feed on humans (Kilpatrick et al. 2007).

Despite the potential role of *Cx. pipiens* as an epidemic/epizootic vector of WNV in the mid-Atlantic region, studies on blood-feeding behavior have yet to be conducted in Virginia. This area, particularly southeastern Virginia, features an abundance of wetland habitats and stagnant water pools, including reservoirs and marshes ideal for mosquito breeding. In addition, the availability of numerous wildlife and bird sanctuaries in the region provides ample hosts for blood-feeding mosquitoes (Loftin et al. 2006). Spatio-temporal analyses of host-feeding patterns in a given locality are crucial for evaluating vectorial capacity in mosquitoes and identifying vertebrate hosts involved in amplification and maintenance of arboviruses in nature. Such analyses are key to better guide local mosquito control measures and help prevent human epidemics of arboviral pathogens.

In an attempt to extend the knowledge of *Cx. pipiens* as an enzootic and epidemic vector, this study examines the host-feeding pattern of *Cx. pipiens* by means of bloodmeal analysis of mosquitoes collected from Suffolk, Virginia. This study was conducted in an area with the potential of hybridization between members of the complex, but because we did not perform population genetic studies, we will use the term *Cx. pipiens* only throughout the article. Vertebrate hosts of mosquitoes were identified to species level by amplifying and sequencing portions of the mitochondrial *cytochrome b* gene. The main objectives of this study were to (1) quantify the degree of interaction of *Cx. pipiens* with vertebrate hosts and whether interactions can be explained solely by host frequency and abundance, (2) assess the likelihood of temporal shifts in blood feeding by season and spatial patterns in relationship to urban development, (3) determine contribution of vertebrate hosts to amplification and maintenance of arboviruses in nature, and (4) evaluate the potential for *Cx. pipiens* to serve as a bridge vector and the implications for epidemic transmission to humans in the region.

Materials and Methods

Ethics statement

Permissions were obtained for field sites on privately owned parcels, and all other sites were located on properties owned or operated by the City of Suffolk. No special permissions were required as some of the co-authors work for the City of Suffolk Public Works Department and were authorized to collect mosquitoes on these sites. The field activities associated with this study did not involve endangered or protected species.

Study sites

The city of Suffolk (36°44′ 29″ N 76° 36′ 36″ W) is located in southeastern Virginia positioned between the upland and lowland regions of the coastal plain province (Fig. 1). The city is part of the greater Hampton Roads area and is situated ∼15 km south of the Chesapeake Bay and 50 km west of the Atlantic Ocean. Many wetland habitats are featured in the area, including marshes, reservoirs, and freshwater swamps. The three watersheds in the city are the Chowan River watershed, the James River watershed, and the Great Dismal Swamp watershed. It is the largest city in the state by land area at ∼1036 km^2. The majority of the land is zoned as agriculture (59%), followed by mixed urban, suburban, and commercial (26%), and conservation (15%). The conservation district comprises two national wildlife refuges. In southeastern Suffolk, the Great Dismal Swamp National Wildlife Refuge encompasses 148 km^2, roughly one-third of the refuge's total area. The Nansemond National Wildlife Refuge is located in the northern part of the city along the Nansemond River and encompasses roughly 1.66 km^2 of tidal marsh and grassland habitats. Among Virginia's independent cities, Suffolk has the second lowest human population density with roughly 85,000 residents.

Mosquito collection

Mosquitoes were collected on a weekly basis from May to November 2019 and 2020 at 68 sites throughout Suffolk, Virginia (50 sites set regularly and 18 intermittently). Three trap types were used: Centers for Disease Control and Prevention (CDC) miniature light traps (BioQuip Products, Rancho Dominguez, CA) baited with CO_2, BG-Sentinel traps (Biogents, Regensburg, Germany) baited with both a chemical lure and CO_2, and modified Reiter gravid traps baited with a fermented mixture of chicken manure, alfalfa, yeast, and water. CDC traps were set most frequently at 32 traps per

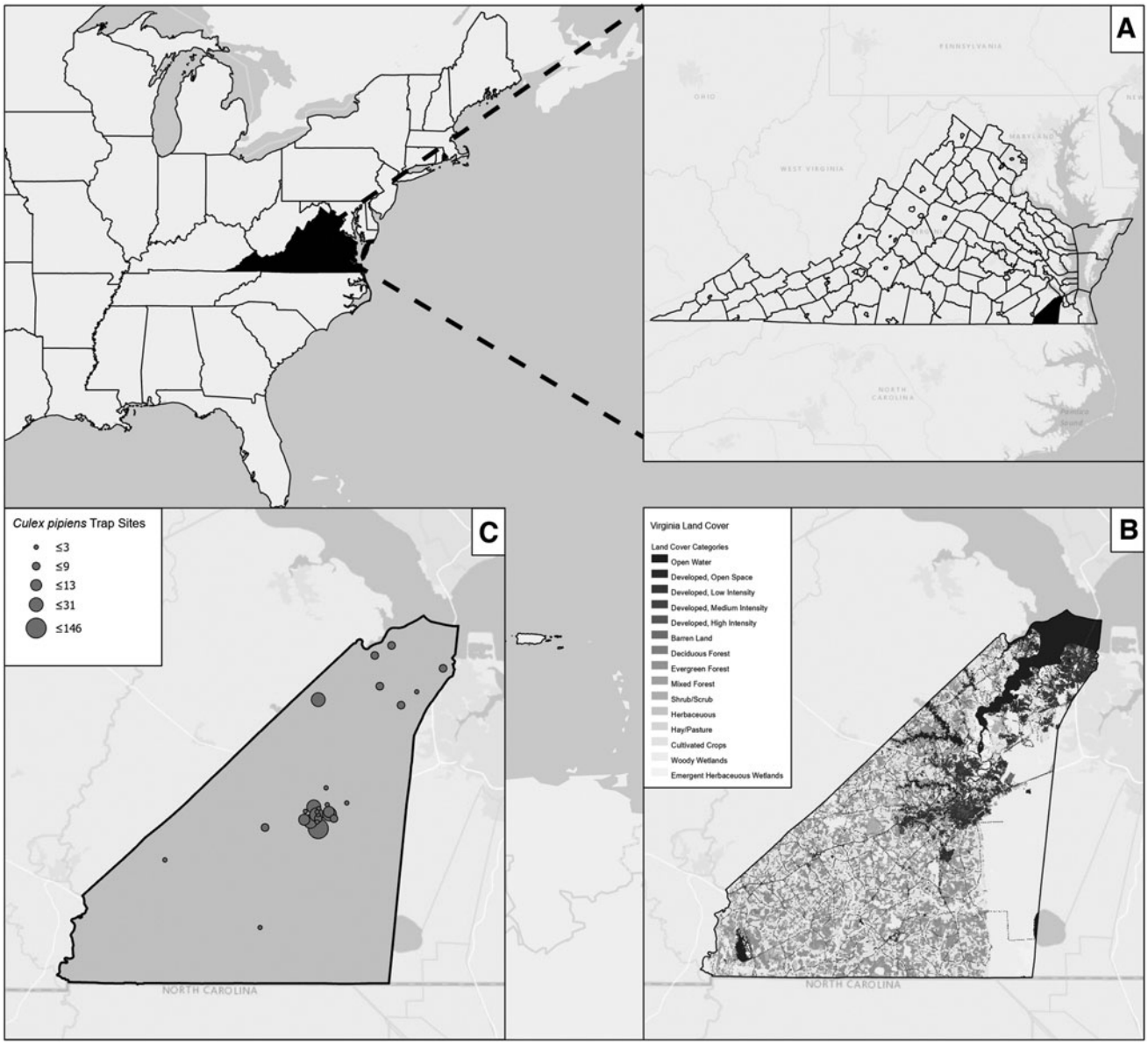

FIG. 1. Mosquito trapping sites in Suffolk, Virginia, 2019–2020. **(A)** Geographic location of Suffolk in the corner of southeastern Virginia. **(B)** Land cover of the region. **(C)** Mosquito trap sites with total number of engorged *Culex pipiens* collected at each site.

week, whereas BG and gravid traps were set at 19 and 11 traps per week, respectively. In some cases, multiple trap types were set at the same site due to varying efficiencies in collecting different mosquito species. Reiter gravid traps were the most effective for collecting *Culex* species, capturing over 86% of the *Cx. pipiens/Culex restuans* throughout the city. Most traps were concentrated around developed areas with residential or commercial land surrounded by swamp and woodland habitats. Traps were set between the hours of 12:00 PM and 3:00 PM and picked up the following morning between 6:30 AM and 9:30 AM. Upon collection, traps containing live mosquitoes were transported to the City of Suffolk Mosquito Control (CSMC) laboratory. Mosquito specimens were then placed in a −18°C freezer for immobilization and were morphologically identified to the species level using a dissecting microscope and a regional identification guide (Harrison et al. 2016). Blood-fed *Cx. pipiens* specimens were individually separated into labeled 2.0-mL microcentrifuge tubes and stored at −18°C.

DNA isolation of blood-fed mosquitoes

Mosquito abdomens were dissected on clean microscope slides using a sterile razor blade with the aid of a dissecting microscope. Dissected abdomens were placed in individual 1.5-mL microcentrifuge tube for bloodmeal analysis and corresponding heads/thoraxes were reserved in separate 2.0-mL tubes for virus testing. DNA was extracted by using either DNAzol BD (Molecular Research Center, Cincinnati, OH) or the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA). Both methods were conducted using the manufacturer's recommendations with minor modifications as previously documented (Molaei et al.

2006, 2015, Little et al. 2021). The DNAzol BD protocol consisted of homogenizing the abdomen in a 1.5-mL tube containing 400 μL of DNAzol BD using a disposable micropestle (USA Scientific, Ocala, FL). This was followed by the addition of 15 μL of proteinase K to each tube, a 10-minute incubation at 70°C, and centrifugation at 18,000 × *g* for 10 min. Following the transfer of the supernatant to a separate 1.5-mL tube, DNA was precipitated by adding 100% ethanol and 3 μL of Poly Acryl Carrier (Molecular Research Center) before incubating on ice for 10 min. DNA pellets were washed twice with 75% ethanol, air dried, and reconstituted in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). For DNeasy Blood & Tissue Kit, abdomens were homogenized in 180 μL buffer ATL using a micropestle, and 20 μL of proteinase K was added to each tube. DNA extraction was continued according to the manufacturer's recommendation, and DNA was eluted with 42 μL of elution buffer. Tubes containing isolated DNA were stored at −20°C for bloodmeal analysis.

Bloodmeal analysis

PCR assays were performed using isolated genomic DNA as templates to identify vertebrate sources. Portions of mitochondrial *cytochrome b* gene were amplified using both avian- and mammalian-specific primer pairs, as previously documented (Molaei and Andreadis 2006, Molaei et al. 2006). Avian primers were 5′–GACTGTGACAAAATCCC NTTCCA–3′ (forward) and 5′–GGTCTTCATCTYHGGY TTACAAGA–3′ (reverse) with an amplicon size of 508. Mammalian primers were 5′–CGAAGCTTGATATGAA AAACCATCGTTG–3′ (forward) and 5′–TGTAGTTRTC WGGGTCHCCTA–3′ (reverse) with the amplicon size of 772 (Ngo and Kramer 2003, Molaei et al. 2006, 2007). A Taq PCR Core Kit (Qiagen) was used for all reactions consistent with the manufacturer's instructions. A total volume of 40 μL was prepared for the assays, including 3 μL DNA, 3.2 μL of each primer (0.1–0.5 μM), 4 μL 10 × Qiagen PCR Buffer (containing 15 mM MgCl2), 0.8 μL dNTP mix (10 mM each), 0.2 μL Taq DNA polymerase (1.25 U/reaction), and 25.6 μL diH_2O. PCR reactions were performed using Veriti Dx Thermal Cycler (Applied Biosystems, Foster City, CA), and cycling conditions were as described in earlier studies (Molaei et al. 2006, 2007). Samples with either no amplification products or inconclusive sequencing results were further analyzed in subsequent PCR assays using universal vertebrate primer pairs: 5′–TGTAAAACGACGGCCAGTTCTCAACCAACCACAA RGAYATYG–3′ (forward) and 5′–CAGGAAACAGCTATG ACTAGACTTCTGGGTGGCCRAARAAYCA–3′ (reverse), with an amplicon size of 658 bp (Ivanova et al. 2007). PCR amplicons were purified using QIAquick PCR purification kit (Qiagen) and sequenced using a 3730xl DNA Analyzer (Applied Biosystems) at the Keck Sequencing Facility, Yale University (New Haven, CT). For species identification, sequences were annotated using ChromasPro version 2.1.8 (Technelysium Pty Ltd., Tewantin, Australia) and submitted to GenBank (NCBI) for comparison with publicly available sequences.

Avian frequency analysis

Estimated frequencies of birds throughout Suffolk and adjacent counties were obtained through eBird, a database managed by the Cornell Laboratory of Ornithology (eBird 2021). Available worldwide through its website, the eBird project aims to track abundance and distribution of bird species by documenting checklist data provided by professional and recreational birdwatchers. Frequency data for a particular species are generated by compiling reports of the species from checklists of a specified date and location. The timeframe in which frequency data were obtained for this study was May through November 2019 and 2020. Chi-squared tests were used to assess whether the observed biting frequency of *Cx. pipiens* on certain bird species differed from the expected biting frequency as determined from eBird data.

Spatiotemporal analyses

Temporal shifts in avian and mammalian blood feeding by season were analyzed using logistic regression. The data were divided into three seasons for 2019 and 2020: early (May and June); middle (July and August); and late (September, October, and November).

Spatial patterns in blood feeding were investigated with respect to the impact of urban landscapes. Data were obtained from the 2016 National Land Cover Database (NLCD) and were further classified into four classes characterizing water, developed, undeveloped, and agricultural land cover (Little et al. 2021). The proportion of developed land within a 1000-meter radius of each trap location was calculated, and logistic regression was used to measure the influence of urban landscapes on *Cx. pipiens* blood feeding.

All statistical analyses were completed using R Statistical Software version 3.6.2 (R, 2019), and all maps were created in ArcGIS version 10.8 (Esri, Redlands, CA).

Virus testing

Head and thorax of each blood-fed *Cx. pipiens* were homogenized for 4 min at 25 cycles per minute in a 2-mL tube containing a single copper BB, 0.5 mL phosphate-buffered saline, 30% heat-inactivated rabbit serum, 0.5% gelatin, and 1 × antibiotic/antimycotic using a MM 300 Mixer Mill (Retsch, Newtown, PA) as previously described (Andreadis et al. 2004, Armstrong et al. 2012). Homogenates were centrifuged at 4500 × *g* for 7 min at 4°C, followed by inoculation of 100 μL of each mosquito sample into a 25 cm^2 flask containing Vero cells. The flasks were placed on a rocker for 10 min at room temperature to allow for virus adsorption, and essential media containing 5% fetal bovine serum, 2% sodium bicarbonate, 1% antibiotic-antimycotic, and 1% L-glutamine (Gibco, ThermoFisher Scientific, Waltham, MA) were added. The cells were incubated for 1 week at 37°C and checked daily for cytopathic effect (CPE) starting on day 3 postinoculation. Viral RNA was extracted from CPE-positive cell cultures and corresponding mosquitoes using QIAamp Viral RNA Mini Kit (Qiagen, Germantown, MD) and screened for WNV and eastern equine encephalitis virus (EEEV) in real-time RT-PCR assays using the TaqMan RNA-to-Ct 1-Step Kit (Applied Biosystems) (Lanciotti et al. 2000). The primer-probe set WNV10533fwd, WNV10625rev, and WNV10560-FAM were used in amplification of WNV (Tang et al. 2006), and both primer-probe sets EEE1858, EEE1926c, and EEE1881probe and EEE411F, EEE527R, and EEE463probe in amplification of EEEV (Armstrong et al. 2012).

Nonblood-fed mosquitoes were grouped into pools of ≤50 individuals by trap location, species, and collection date, and stored in microcentrifuge tubes at −18°C until virus testing. Specimens were screened for evidence of infection with WNV and EEEV at the CSMC laboratory from 2010 to 2020. Two types of in-house antigen assays were used: VecTest (Medical Analysis Systems, Camarillo, CA) and VecTOR Test (VecTOR Test Systems, Thousand Oaks, CA). Mosquito pools were screened using a combination of vector tests only, vector tests with RT-PCR confirmation, or RT-PCR only by the Virginia Division at Consolidated Services (DCLS) and the Pennsylvania Department of Health (PADOH).

Results

Mosquito species diversity and abundance

From 2019 to 2020, adult mosquito surveillance throughout the City of Suffolk, Virginia, took place at 68 trap sites with a total of 4124 trap nights: 1599 CDC, 1283 BG-Sentinel, and 1242 Reiter gravid trap nights. A total of 464,084 female mosquitoes were collected belonging to 9 genera and 41 species. Blood-fed *Cx. pipiens* were found at 28 of the 68 trap sites. These 28 trap sites consisted of 2392 trap nights using 946 BG-Sentinel traps, 238 CDC miniature light traps, and 1208 modified Reiter gravid traps. At these sites, a total of 195,558 female mosquitoes were collected belonging to 9 genera and 32 species (Table 1). Of these, the most frequently collected species were *Aedes albopictus* ($n=59{,}073$, 30.2%), followed by *Culiseta melanura* ($n=54{,}431$, 27.8%) and *Cx. pipiens* ($n=39{,}859$, 20.4%). The remaining species comprised 21.6% ($n=42{,}195$) of total mosquitoes collected (Table 1).

Bloodmeal analysis

A total of 365 blood-fed *Cx. pipiens* collected in 2019 and 2020 were subjected to bloodmeal analysis, and vertebrate hosts of 281 (77.0%) mosquitoes were successfully identified to species (Table 2). Of these 281 specimens, 255 (90.7%) contained solely avian, 13 (4.6%) mammalian, 1 (0.4%) reptilian, and 12 (4.3%) both avian and mammalian blood.

Table 1. Number and Percentage of Adult Female Mosquitoes (by Species) Collected from 28 Sites in Suffolk, Virginia, 2019–2020

Mosquito species	n	%
Aedes albopictus	59,073	30.2
Culiseta melanura	54,431	27.8
Culex pipiens	39,859	20.4
Culex restuans	12,511	6.4
Culex salinarius	9663	4.9
Aedes canadensis	5463	2.8
Psorophora ferox	4316	2.2
Culex erraticus	2206	1.1
Anopheles quadrimaculatus	2014	1.0
Aedes vexans	1193	0.6
Aedes atlanticus	747	0.4
Other	4082	2.1
Total	195,558	

Nineteen avian species were identified as hosts for *Cx. pipiens* (Table 2 and Fig. 2). These species were members of four orders with the majority classified as Passeriformes ($n=250$, 98.0% of avian and 89.0% of total). The remaining avian bloodmeals were from Columbiformes ($n=3$, 1.2% and 1.1%), Cuculiformes ($n=1$, 0.4% and 0.4%), and Strigiformes ($n=1$, 0.4% and 0.4%). The most common avian species that served as hosts for *Cx. pipiens* was American

Table 2. Number and Percentage of Avian-, Mammalian-, and Reptilian-Derived Bloodmeals from *Culex pipiens* Collected in Suffolk, Virginia, 2019–2020

Vertebrate hosts Common name (species name)	*Frequency of bloodmeals* n *(%)*
Avian	
American robin (*Turdus migratorius*)	141 (50.18)
Northern cardinal (*Cardinalis cardinalis*)	57 (20.28)
Carolina wren (*Thryothorus ludovicianus*)	14 (4.98)
House finch (*Carpodacus mexicanus*)	9 (3.20)
Gray catbird (*Dumetella carolinensis*)	6 (2.14)
Northern mockingbird (*Mimus polyglottos*)	6 (2.14)
Brown thrasher (*Toxostoma rufum*)	3 (1.07)
Common grackle (*Quiscalus quiscula*)	3 (1.07)
European starling (*Sturnus vulgaris*)	3 (1.07)
Mourning dove (*Zenaida macroura*)	3 (1.07)
House wren (*Troglodytes aedon*)	2 (0.71)
Barred owl (*Strix varia*)	1 (0.36)
Black-and-white warbler (*Mniotilta varia*)	1 (0.36)
Blue jay (*Cyanocitta cristata*)	1 (0.36)
Carolina chickadee (*Poecile carolinensis*)	1 (0.36)
Eastern bluebird (*Sialia sialis*)	1 (0.36)
Tufted titmouse (*Baeolophus bicolor*)	1 (0.36)
White-eyed vireo (*Vireo griseus*)	1 (0.36)
Yellow-billed cuckoo (*Coccyzus americanus*)	1 (0.36)
Mammalian	
Domestic cat (*Felis catus*)	6 (2.14)
Virginia opossum (*Didelphis virginiana*)	6 (2.14)
Dog (*Canis lupus familiaris*)	1 (0.36)
Reptilian	
Brown snake (*Storeria dekayi*)	1 (0.36)
Mixed	
American robin and Virginia opossum (*T. migratorius* and *D. virginiana*)	6 (2.14)
Northern cardinal and Virginia opossum (*C. cardinalis* and *D. virginiana*)	2 (0.71)
American robin and Domestic cat (*T. migratorius* and *F. catus*)	1 (0.36)
Northern cardinal and Domestic cat (*C. cardinalis* and *F. catus*)	1 (0.36)
Mourning dove and Virginia opossum (*Z. macroura* and *D. virginiana*)	1 (0.36)
Northern mockingbird and Virginia opossum (*M. polyglottos* and *D. virginiana*)	1 (0.36)
Total	281 (100)

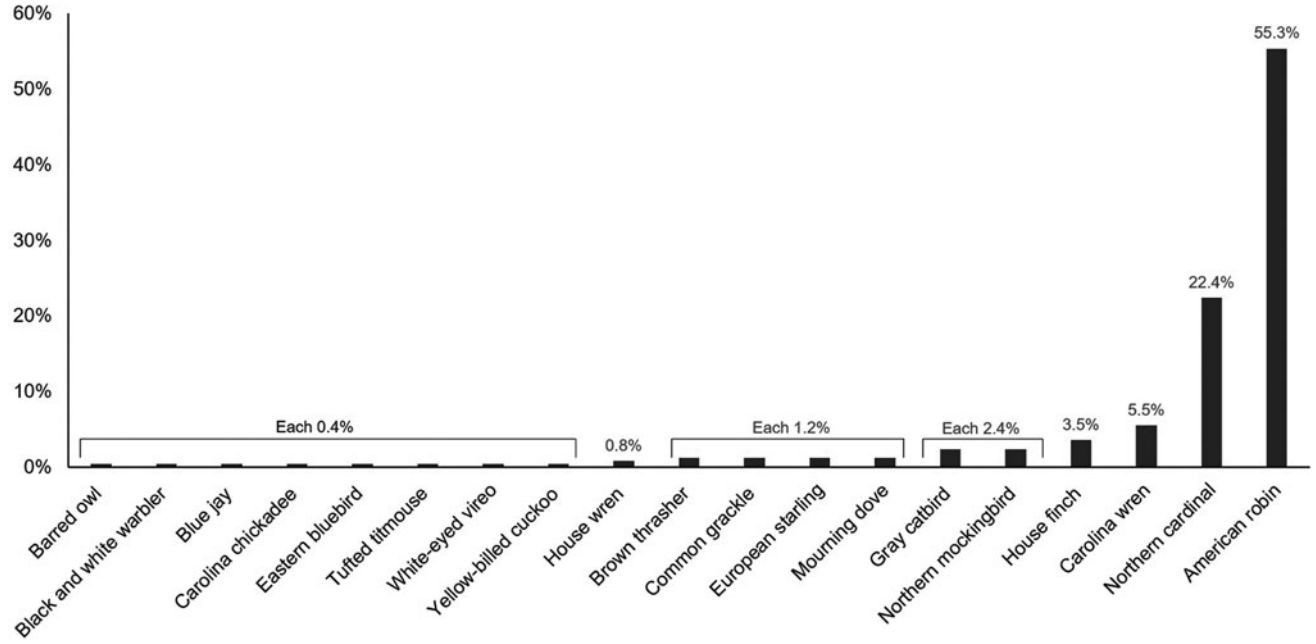

FIG. 2. Percentage of *Culex pipiens* avian-derived bloodmeals Suffolk, Virginia, 2019–2020.

robin, *Turdus migratorius* ($n = 141$, 55.3% of avian and 50.2% of total), followed by northern cardinal, *Cardinalis cardinalis* ($n = 57$, 22.4% and 20.3%); Carolina wren, *Thryothorus ludovicianus* ($n = 14$, 5.5% and 5.0%); house finch, *Carpodacus mexicanus* ($n = 9$, 3.5% and 3.2%); gray catbird, *Dumetella carolinensis* ($n = 6$, 2.4% and 2.1%); and northern mockingbird, *Mimus polyglottos* ($n = 6$, 2.4% and 2.1%). The remaining 13 bird species comprised 8.6% of avian feedings and 7.8% of total feedings.

Of the three mammalian species identified as hosts for *Cx. pipiens*, Virginia opossum, *Didelphis virginiana* ($n = 6$, 46.2% of mammalian and 2.1% of total) was the most frequent, comprising 83.3% ($n = 10$) of mixed bloodmeals, followed by domestic cat, *Felis catus* ($n = 6$, 46.2% and 2.1%), constituting the remaining 16.7% ($n = 2$) of mixed bloodmeals. Dog, *Canis lupus familiaris* ($n = 1$, 7.7% and 0.4%), was identified as the third mammalian host.

Avian abundance and expected frequency of host-specific bloodmeals

Monthly frequencies from eBird for each avian species and corresponding percentages of bloodmeals are depicted in Fig. 3. Northern cardinal and Carolina wren have been reported as the most observed bird species from May through November in the region. The percentage of bloodmeals for most of the species, including Carolina wren, blue jay, Carolina chickadee, and others, was lower than anticipated, considering their estimated frequencies. To assess any difference between the observed biting frequency of select bird species and the expected biting frequency as determined from eBird data, chi-squared analysis was performed. American robins and northern cardinals were the only species assessed in this analysis as they represented the majority of bloodmeals, 55.3% and 22.4% of avian bloodmeals, respectively. The observed biting frequency of American robins was higher than expected, given the avian frequency data obtained from eBird (chi-squared = 90.08, df = 1, $p < 0.0001$), while the observed biting of northern cardinals was lower than expected, given the bird frequency data (chi-squared = 115.9, df = 1, $p < 0.001$).

Seasonal shifts and spatial patterns of blood feedings

Logistic regression was used to assess temporal shifts in blood feeding by season. While there was no significant difference in the proportion of bloodmeals taken from avian hosts by season, there was a notable decrease in the proportion of bloodmeals taken from mammalian hosts over the seasons, with the highest proportion in early season and the lowest proportion in late season (Fig. 4 and Table 3).

Logistic regression analysis was also used to assess spatial differences in blood feeding by the degree of urban development around each trap location. No relationship was observed between mammal biting and percent development within 1000 meters of trap sites (odds ratio [OR] = 0.996, 95% confidence interval [CI] = [0.980–1.015], p value = 0.661). There was also no relationship detected between avian feeding and percent development within the 1000 meters of trap sites (OR = 1.003, 95% CI = [0.993–1.013], p value = 0.559). However, in regard to feeding on American robins, it was observed that more feedings on this bird species took place in areas of higher development. For each unit increase in the percent development within 1000 meters of traps, there is a 4% increase in robin biting (OR = 1.04, 95% CI = [1.027–1.055], p value <0.001).

Arbovirus infection

Screening of head and thorax of individual blood-fed *Cx. pipiens* by cell culture and PCR resulted in two viral isolates from two separate specimens. One specimen tested positive for WNV, which was collected on August 6, 2019, from a suburban neighborhood east of Lake Kilby (36° 43′ 30.504″ N, 76° 36′ 13.248″ W), and the bloodmeal source

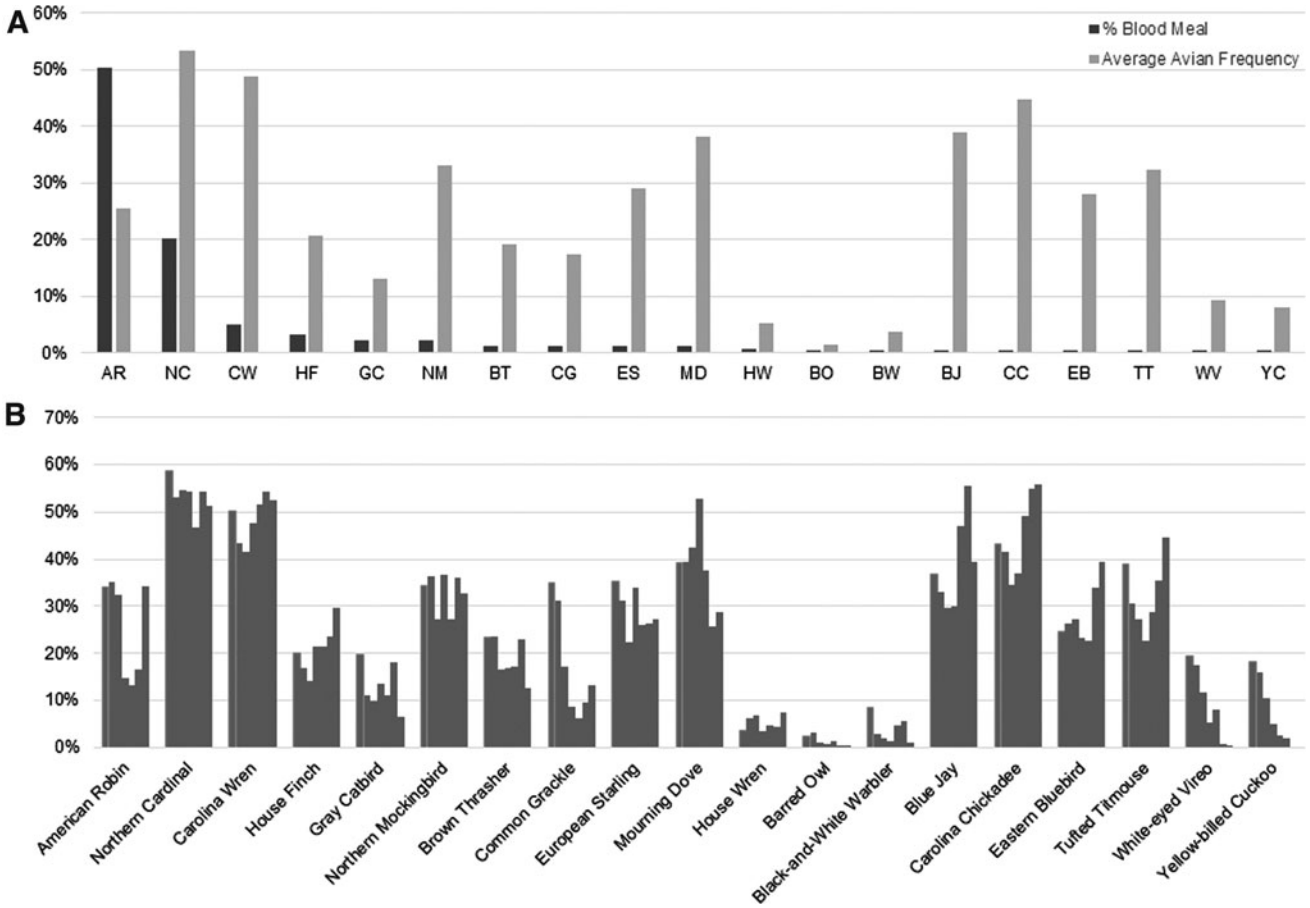

FIG. 3. Frequency of avian species and avian-derived bloodmeals of *Culex pipiens* in Suffolk, Virginia, 2019–2020. **(A)** Percentage of avian-derived bloodmeals in *Cx. pipiens* compared with the average avian frequencies in the City of Suffolk, Virginia, and surrounding cities/counties (City of Chesapeake, Isle of Wight County, City of Portsmouth, and Southampton County) May through November 2019–2020. **(B)** Monthly frequencies of avian species based on point count data in the City of Suffolk, Virginia, and surrounding cities/counties (City of Chesapeake, Isle of Wight County, City of Portsmouth, and Southampton County) May through November 2019–2020.

was a Virginia opossum. The second mosquito was determined to be infected with EEEV. This specimen was collected on October 1, 2019, from a location adjacent to the northwest border of the Great Dismal Swamp (36° 44′ 34.386″ N, 76° 32′ 49.2972″ W) and had fed on a black-and-white warbler.

Between 2010 and 2020, a total of 45,417 mosquito pools were screened for WNV and EEEV, including 25,499 pools for WNV and 19,918 pools for EEEV (Table 4). Of the 3323 *Cx. pipiens/restuans* pools, 166 (5.0%) tested positive for WNV (minimum infection rate [MIR] = 1.677), and no pool was tested positive for EEEV. Of the 16,807 *Cs. melanura* pools screened for WNV, 58 (0.3%) tested positive (MIR = 0.085), and of the 18,653 pools screened for EEEV, 611 (3.3%) tested positive (MIR = 0.803).

Discussion

This study provides insight into the role of *Cx. pipiens* as a vector of WNV and potentially other arboviruses by determining its host associations in Suffolk, Virginia. Our results show this mosquito species predominantly feeds on passerine birds. We also document a small proportion of bloodmeals from mammalian hosts and one reptilian host. The identification of *Cx. pipiens* feeding patterns and the ensuing potential for viral transmission is crucial for better understanding of its vectorial capacity, particularly in such an area with a range of wetland habitats, wildlife reserves, and developed urban areas with an abundance of vertebrate hosts.

Disparities in blood-feeding pattern of *Cx. pipiens* exist depending on location. Our analysis of *Cx. pipiens* in Suffolk, Virginia, reveals that 90.7% of specimens fed on avian hosts. This finding is in agreement with other studies that have reported primarily ornithophilic feeding behavior of this mosquito species with little propensity to feed on mammals (Molaei et al. 2006, Montgomery et al. 2011). However, some studies have reported a lower percentage of avian-derived bloodmeals with varying degrees of interaction with mammalian hosts. For instance, the percentage of bird feedings comprises 34.7% in New Jersey, 71.4% in Tennessee, 84.6% in New York (Apperson et al. 2004), 31.0% in Delaware (Gingrich and Williams 2005), and 77.6% in Illinois (Hamer et al. 2008).

American robin served as the most frequent (50.2%) host for *Cx. pipiens* in this study. Our finding is consistent with results of previous investigations in which 28.0% of

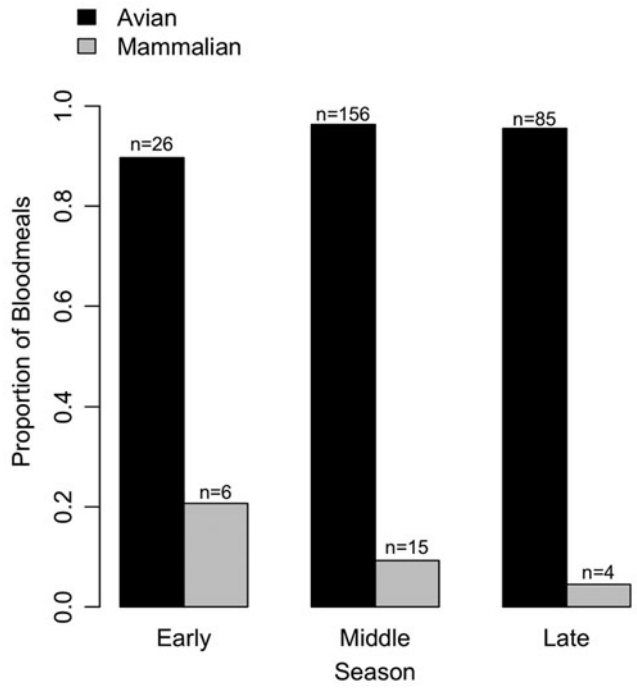

FIG. 4. Proportion of bloodmeals obtained from avian and mammalian hosts each season in Suffolk, Virginia, 2019–2020. Early season defined as May and June; Middle defined as July and August; and Late defined as September, October, and November.

bloodmeals in Illinois (Hamer et al. 2008), 37.7% in Connecticut (Molaei et al. 2006), and 45.1% in Tennessee (Savage and Kothera 2012) were from this bird species. The majority of *Cx. pipiens* with American robin-derived bloodmeals in our study (81.6%) was collected from a single site in an urban area. More American robin feedings were also observed in areas of higher urban development in our study. In laboratory investigations, American robins have been identified as moderately competent reservoir hosts of WNV (Komar et al. 2003, VanDalen et al. 2013). By integrating abundance, bloodmeal frequency, and reservoir competence values of American robins in Maryland and Washington D.C., Kilpatrick et al. (2006a) concluded that the species was likely responsible for infecting roughly 59.3% of *Culex* mosquitoes with WNV. In light of these findings, our bloodmeal analysis results suggest that American robins serve as major virus amplification hosts in Suffolk, Virginia.

Northern cardinal was identified as the second most frequent host (20.3%) for *Cx. pipiens* in our analysis, in agreement with previous studies reporting this passeriformes species as a frequent host (Apperson et al. 2004, Savage et al. 2007, Savage and Kothera 2012). Although the role of northern cardinals in the transmission cycle of WNV is not entirely understood, previous antibody analyses indicate that northern cardinals have relatively high seroprevalence, suggesting that these birds are frequently exposed to WNV and may act as competent reservoir hosts for this virus (Komar et al. 2005, Gibbs et al. 2006, Marshall et al. 2006, Gingrich et al. 2010).

Carolina wren was also identified as a common source of bloodmeals for *Cx. pipiens* in our study. An investigation of viremia profiles and mortality in songbirds revealed a WNV antibody prevalence of 17.5% for Carolina wrens in nature and a mortality rate of nearly 30% after experimental infection with WNV (Kilpatrick et al. 2013). The spatial overlap of Carolina wrens with questing mosquitoes and antibody prevalence as hosts indicates that this species might be involved in the transmission of WNV.

Except for American robins, the percentage of bloodmeals obtained from most avian species differed from what would be expected based on the average avian frequency in the region. One possible explanation for disproportionate feeding on avian hosts is the spatial overlap of host habitat and host-seeking mosquitoes. Previous studies have demonstrated that the abundance of foraging *Cx. pipiens* increases at higher levels in the canopy where some avian hosts commonly nest and roost (Anderson et al. 2004, 2006, Drummond et al. 2006, Savage et al. 2008, Janousek et al. 2014). It is worth noting that a previous bloodmeal analysis of *Cs. melanura* collected at the same sites also revealed the greatest percentage of feedings from American robins and northern cardinals (Molaei et al. 2015), suggesting that these two bird species are abundant and most accessible at some or all sites. The capability of some avian species to exhibit defensive behavior against attacking mosquitoes could also be a potential factor for fewer blood feedings on species with high estimated frequency in the region (Edman et al. 1974, Anderson and Brust 1997, Darbro and Harrington 2007).

A small fraction of *Cx. pipiens* (4.6%) obtained bloodmeals from mammalian hosts, suggesting the occasional involvement of this species in epizootic/epidemic transmission of WNV in the region. Varying proportions of feedings on mammals have been documented in Tennessee and New York (Apperson et al. 2004), Connecticut (Molaei et al. 2006), Illinois (Hamer et al. 2008), and California (Montgomery et al. 2011). The three mammalian hosts in this study included cat, domestic dog, and Virginia opossum. We did

TABLE 3. LOGISTIC REGRESSION MODEL RESULTS OF THE EFFECT OF SEASON ON *CULEX PIPIENS* AVIAN AND MAMMALIAN BLOOD FEEDING IN SUFFOLK, VIRGINIA

	Avian			*Mammalian*		
	OR	*95% CI*	p *Value*	*OR*	*95% CI*	p *Value*
Intercept	8.667	(3.052, 36.347)	<0.001	0.261	(0.096, 0.601)	0.003
Middle season	3.000	(0.604, 12.139)	0.137	0.391	(0.142, 1.188)	0.078
Late season	2.452	(0.459, 11.821)	0.259	0.180	(0.043, 0.683)	0.012

CI, confidence interval; OR, odds ratio.

Table 4. Number of Mosquito Pools Tested for West Nile Virus and Eastern Equine Encephalitis Virus, Number of Pools That Tested Positive for West Nile Virus and Eastern Equine Encephalitis Virus, and Their Associated Minimum Infection Rate Values from 2010 to 2020

Mosquito species	*No. of pools tested for WNV*	*WNV+ pools*	*WNV MIR*	*No. of pools tested for EEEV*	*EEEV+ pools*	*EEEV+ MIR*
Cs. melanura[a]	16,807	58	0.085	18,653	611	0.803
Cx. pipiens/restuans[a]	3323	166	1.677	142	0	0
Ae. albopictus[a]	2314	0	0	356	0	0
Cx. salinarius[a]	1213	0	0	58	0	0
Cx. erraticus[a]	1032	0	0	3	1	8.772
Ae. vexans[a]	434	0	0	130	0	0
Coquillettidia perturbans[a]	297	0	0	297	0	0
Uranotaenia sapphirina[b]	0	0	0	271	3	1.245
Aedes triseriatus[a]	70	0	0	4	0	0
An. quadrimaculatus[b]	4	0	0	4	0	0
Aedes japonicus[c]	4	0	0	0	0	0
Culex territans[c]	1	0	0	0	0	0
Total	25,499	224	—	19,918	615	—

[a]A combination of VecTest, VecTOR Test, and RT-PCR was used to test these mosquitoes.
[b]Only RT-PCR was used to test these mosquitoes.
[c]Only VecTOR Test was used to test these mosquitoes.
EEEV, eastern equine encephalitis virus; MIR, minimum infection rate; WNV, West Nile virus.

not identify feedings from humans, despite the fact that the majority of our collection sites was located in developed areas. It is possible that because the mammalian hosts identified in this study spend considerable time outdoors, particularly during peak mosquito activity, they are more accessible to host-seeking mosquitoes than humans, who may avoid exposure to mosquito bites (Molaei et al. 2007). This low frequency of human-derived bloodmeals may explain the scarcity of WNV disease cases in humans in Suffolk, Virginia. However, other studies have reported varying degrees of feeding of *Cx. pipiens* on humans. Human-derived feedings represented 17.9% and 16.4% of total feedings in two studies conducted in Illinois (Hamer et al. 2008, 2009), 10.8% in New Jersey (Apperson et al. 2004), but merely 0.5% in Connecticut (Molaei et al. 2006), and 1.1% in New York (Patrican et al. 2007).

Mixed blood feedings from both avian and mammalian hosts represented 4.3% of total bloodmeals in this study. An analysis of *Cx. pipiens* in Connecticut also revealed mixed blood feedings in 3.9% of the specimens (Molaei et al. 2006). In earlier investigations of *Cx. quinquefaciatus* host feeding, it was reported that 2.2% of this mosquito species obtained mixed bloodmeals in Orange, Riverside, and San Bernardino Counties in southern California (Molaei et al. 2010), and 8.3% in Harris County, Texas (Molaei et al. 2007), exemplifying this member of the complex as a bridge vector. The presence of both avian and mammalian blood in an engorged specimen further suggests that *Cx. pipiens* promotes transmission of arboviruses such as WNV to mammalian hosts as a bridge vector, in addition to its prominent role in enzootic transmission.

In our study, a single bloodmeal was obtained from a reptilian host, the brown snake. This reptile was also reported as a host for *Cx. pipiens* in New York (Patrican et al. 2007). The contribution of reptiles to the WNV transmission cycle has been previously investigated (Klenk and Komar 2003, Klenk et al. 2004, Steinman et al. 2006, Dahlin et al. 2016), although whether they serve as amplifying or dilution hosts remains uncertain. Snakes have also been implicated as overwintering hosts for EEEV and can thus contribute to the seasonal enzootic amplification of the virus (White et al. 2011). We identified a single specimen of *Cx. pipiens* positive for EEEV in our study.

There was no temporal change in the proportion of avian-derived bloodmeals observed in this study. However, there was a decrease in the proportion of bloodmeals obtained from mammalian hosts over the seasons (Fig. 4 and Table 3). Previous investigations have demonstrated a shift in feeding of *Cx. pipiens* from American robins to other avian hosts or to mammalian species, an occurrence attributed to the decline in robin abundance as the season progressed (Kilpatrick et al. 2006b, Molaei et al. 2006, Hamer et al. 2009), or to physiological changes in mosquitoes (Chaves et al. 2010). However, our analysis shows that American robin remained the primary host in late summer and early fall, consistent with bloodmeal analyses of *Cx. pipiens* in Shelby County, Tennessee (Savage et al. 2007), and some counties in New York (Patrican et al. 2007).

Variations in bloodmeal composition across regions are attributed, in part, to the genetic makeup of *Cx. pipiens* populations, in addition to such factors as host availability and abundance. Hybridization between the avian-feeding *Cx. pipiens* f. pipiens and mammalian-feeding members of the complex (*Cx. quinquefasciatus* and *Cx. pipiens* f. molestus) is hypothesized to result in increased tendency to feed on mammalian hosts (Fonesca et al. 2004, Kilpatrick et al. 2007, Huang et al. 2009, Fritz et al. 2015). A study conducted in the mid-Atlantic, United States, revealed a greater proportion of mammalian feedings by *Cx. pipiens* (Kilpatrick et al. 2006b), and subsequent genetic analysis of the same population showed a higher proportion of ancestry from *Cx. pipiens* f. molestus, suggesting the influence of genetics on feeding patterns (Kilpatrick et al. 2007). Although this study site is located in an established hybridization zone between 36°N and 39°N latitudes (Barr 1957), the population structure of the *Cx. pipiens* mosquitoes has not been investigated in this region.

One engorged *Cx. pipiens* specimen tested positive for WNV in our study. The source of bloodmeal was a Virginia opossum. Detection of antibodies against WNV has been documented in opossums (Dietrich et al. 2002, Bentler et al. 2007), and the virus has been successfully isolated from this marsupial species (Bosco-Lauth et al. 2014). In a more recent study, WNV was determined to be responsible for the death of a Virginia opossum in Quebec, Canada (Lamglait and Lair 2019). In addition, a single engorged specimen of *Cx. pipiens* tested positive for EEEV in our analysis, and the source of bloodmeal was identified a black-and-white warbler. Little is known about the role of black-and-white warblers in maintenance and amplification of EEEV.

Screening of un-engorged specimens of *Cx. pipiens/restuans* for evidence of arbovirus infection revealed 5.0% (MIR = 1.677) of pools tested positive for WNV. *Culiseta melanura* was the only other mosquito species that tested positive for WNV at only 0.3% (MIR = 0.085), highlighting the role of *Cx. pipiens* as the principal vector of WNV in southeastern Virginia. No EEEV-positive pools were detected for *Cx. pipiens/restuans*, while 611 pools of *Cs. melanura* tested positive for EEEV (MIR = 0.803). These findings suggest that although *Cx. pipiens* has the potential to be involved in EEEV transmission, other mosquito species may play bigger roles as vectors in transmission of this arbovirus.

Conclusions

We find that *Cx. pipiens* mosquitoes in Suffolk, Virginia, feed primarily on passeriformes birds, including American robins and northern cardinals, capable of supporting WNV amplification. *Culex pipiens* also acquires bloodmeals from mammalian hosts, although at a much less frequency. Our findings, in concert with vector competence and WNV isolation from field-collected mosquitoes, lend support for *Cx. pipiens* to serve as the principal enzootic vector and potential epizootic/epidemic vector of WNV in southeastern Virginia.

Acknowledgments

We would like to thank the Connecticut Agricultural Experiment Station seasonal research assistants, Mallery Breban, Sarah Bonello, and Fiona Quigley for technical assistance. We would also like to extend appreciation to Cassidy McKelvie and Kaitlyn Price, interns at the City of Suffolk Mosquito Control, for their help in mosquito collection.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

Funding for this research project was, in part, provided by Laboratory Capacity for Infectious Disease Cooperative Agreement Number U50/CCU6806-01-1 from the U.S. Centers for Disease Control and Prevention. This publication was also funded, in part, by the City of Suffolk, Virginia, Public Works Department. The findings and conclusions in this article are those of authors and do not necessarily represent the official positions of the funding agencies.

References

Anderson RA, Brust RA. Interrupted blood feeding by *Culex* (Diptera: Culicidae) in relation to individual host tolerance to mosquito attack. J Med Entomol 1997; 34:95–101.

Anderson JF, Andreadis TG, Main AJ, Kline DL. Prevalence of West Nile virus in tree canopy–inhabiting *Culex pipiens* and associated mosquitoes. Am J Trop Med Hyg 2004; 71:112–119.

Anderson JF, Andreadis TG, Main AJ, Ferrandino FJ, et al. West Nile Virus from female and male mosquitoes (Diptera: Culicidae) in subterranean, ground, and canopy habitats in Connecticut. J Med Entomol 2006; 43:1010–1019.

Andreadis TG. The contribution of *Culex pipiens* complex mosquitoes to transmission and persistence of West Nile Virus in North America. J Am Mosq Control Assoc 2012; 28: 137–151.

Andreadis TG, Anderson JF, Vossbrinck CR, Main AJ. Epidemiology of West Nile virus in Connecticut, USA: A five-year analysis of mosquito data 1999–2003. Vector Borne Zoonotic Dis 2004; 4:360–378.

Apperson CS, Hassan HK, Harrison BA, Savage HM, 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.

Armstrong, PM, Prince N, Andreadis TG. Development of a multi-target TaqMan assay to detect Eastern Equine Encephalitis Virus variants in mosquitoes. Vector Borne Zoonotic Dis 2012; 12:872–876.

Barr AR. The distribution of *Culex p. pipiens* and *Cx. p. quinquefasciatus* in North America. Am J Trop Med Hyg 1957; 6:153–165.

Bentler KT, Hall JS, Root JJ, Klenk K, et al. Serologic evidence of West Nile virus exposure in North American mesopredators. Am J Trop Med Hyg 2007; 76:173–179.

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

Bosco-Lauth A, Harmon JR, Lash RR, Weiss S, et al. West Nile Virus isolated from a Virginia opossum (*Didelphis virginiana*) in Northwestern Missouri, USA, 2012. J Wildl Dis 2014; 50:976–978.

Brugman VA, Hernández-Triana LM, Medlock JM, Fooks AR, et al. The role of *Culex pipiens* L. (Diptera: Culicidae) in virus transmission in Europe. Int J Environ Res Public Health 2018; 15:389.

Chaves LF, Harrington LC, Keogh CL, Nguyen AM, et al. Blood feeding patterns of mosquitoes: Random or structured? Front Zool 2010; 7:3.

Colpitts TM, Conway MJ, Montgomery RR, Fikrig E. West Nile virus: Biology, transmission, and human infection. Clin Microbiol Rev 2012; 25:635–648.

Dahlin CR, Hughes DF, Meshaka WE Jr, Coleman C, et al. Wild snakes harbor West Nile virus. One Health 2016; 2:136–138.

Darbro JM, Harrington LC. Avian defensive behavior and blood-feeding success of the West Nile vector mosquito, *Culex pipiens*. Behav Ecol 2007; 18:750–757.

Dietrich G, Montenieri JA, Panella NA, Langevin S, et al. Serologic evidence of West Nile virus infection in free-ranging mammals, Slidell, Louisiana, 2002. Vector Borne Zoonotic Dis 2005; 5:288–292.

Drummond CL, Drobnack J, Backenson PB, Ebel GD, et al. Impact of trap elevation on estimates of abundance, parity rates, and body size of *Culex pipiens* and *Culex restuans* (Diptera: Culicidae). J Med Entomol 2006; 43:177–184.

eBird. 2021. eBird: An online database of bird distribution and abundance [web application]. eBird, Cornell Lab of Ornithology, Ithaca, New York. Available at www.ebird.org. (Accessed March 2, 2021).

Edman JD, Webber LA, Schmid AA. Effect of host defenses on the feeding pattern of *Culex nigripalpus* when offered a choice of blood sources. J Parasitol 1974; 60:874–883.

Farajollahi A, Fonseca DM, Kramer LD, Kilpatrick AM. ''Bird biting'' mosquitoes and human disease: A review of the role of *Culex pipiens* complex mosquitoes in epidemiology. Infect Genet Evol 2011; 11:1577–1585.

Fonesca DM, Keyghobadi N, Malcolm CA, Mehmet C, et al. Emerging vectors in the *Culex Pipiens* complex. Science 2004; 303:1535–1538.

Fritz ML, Walker ED, Miller JR, Severson DW, et al. Divergent host preferences of above- and below-ground *Culex pipiens* mosquitoes and their hybrid offspring. Med Vet Entomol 2015; 29:115–123.

Gibbs SE, Allison AB, Yabsley MJ, Mead DG, et al. West Nile virus antibodies in avian species of Georgia, USA: 2000–2004. Vector Borne Zoonotic Dis 2006; 6:57–72.

Gingrich JB, O'Connor L, Meredith WH, Pesek JD, et al. Epidemiology of West Nile Virus: A silent epiornitic in Northern Delaware in 2007 without associated human cases. J Am Mosq Control Assoc 2010; 26:274–286.

Gingrich JB, Williams GM. Host-feeding patterns of suspected West Nile virus mosquito vectors in Delaware, 2001–2002. J Am Mosq Control Assoc 2005; 21:194–200.

Hamer GL, Kitron UD, Brawn JD, Loss SR, et al. *Culex pipiens* (Diptera: Culicidae): A bridge vector of West Nile virus to humans. J Med Entomol 2008; 45:125–128.

Hamer GL, Kitron UD, Goldberg TL, Brawn JD, et al. Host selection by *Culex pipiens* mosquitoes and West Nile virus amplification. Am J Trop Med Hyg 2009; 80:268–78.

Harrison BA, Byrd BD, Sither CB, Whitt PB. *The Mosquitoes of the Mid-Atlantic Region: An Identification Guide*. Cullowhee, NC: Mosquito and Vector-borne Infectious Diseases Laboratory Publication, Western Carolina Univ, 2016.

Huang S, Hamer GL, Molaei G, Walker ED, et al. Genetic variation associated with mammalian feeding in *Culex pipiens* from a West Nile virus epidemic region in Chicago, Illinois. Vector Borne Zoonotic Dis 2009; 9:637–642.

Ivanova NV, Zemlak TS, Hanner RH, Herbert PD. Universal primer cocktails for fish DNA barcoding. Mol Ecol Notes 2007; 7:544–548.

Janousek WM, Marra PP, Kilpatrick AM. Avian roosting behavior influences vector-host interactions for West Nile virus hosts. Parasit Vectors 2014; 7:399.

Kilpatrick AM, Daszak P, Jones MJ, Marra PP, et al. Host heterogeneity dominates West Nile virus transmission. Proc Biol Sci 2006a; 273:2327–2333.

Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, et al. Genetic influences on mosquito feeding behavior and the emergence of zoonotic pathogens. Am J Trop Med Hyg 2007; 77:667–671.

Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, et al. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. PLoS Biol 2006b; 4: e0040082.

Kilpatrick AM, Peters RJ, Dupuis AP II, Jones MJ, et al. Predicted and observed mortality from vector-borne disease in small songbirds. Biol Conserv 2013; 165:79–85.

Klenk K, Komar N. Poor replication of West Nile virus (New York 1999 strain) in three reptilian and one amphibian species. Am J Trop Med Hyg 2003; 69:260–262.

Klenk K, Snow J, Morgan K, Bowen R, et al. Alligators as West Nile virus amplifiers. Emerg Infect Dis 2004; 10:2150–2155.

Komar N, Langevin S, Hinten S, Nemeth N, et al. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerg Infect Dis 2003; 9:311–322.

Komar N, Panella NA, Langevin SA, Brault AC, et al. Avian hosts for West Nile Virus in St. Tammany Parish, Louisiana, 2002. Am J Trop Med Hyg 2005; 73:1031–1037.

Lamglait B, Lair S. Fatal West Nile Virus infection in a Virginia opossum (*Didelphis virginiana*) with pulmonary lepidic-predominant adenocarcinoma. J Wildl Dis 2019; 55: 990–994.

Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, 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; 38: 4066–4071.

Little EAH, Harriott OT, Akaratovic KI, Kiser JP, et al. Host interactions of *Aedes albopictus*, an invasive vector of arboviruses, in Virginia, USA. PLoS Negl Tropl Dis 2021; 15: e0009173.

Loftin KC, Diallo AA, Herbert MW, Priyarshadan PG, et al. Five-year surveillance of West Nile and eastern equine encephalitis viruses in Southeastern Virginia. J Environ Health 2006; 68:33–40.

Marshall JS, Zuwerink DA, Restifo RA, Grubb TC. West Nile Virus in the permanent-resident bird community of a fragmented Ohio landscape. Ornithol Monogr 2006; 60:79–85.

McDonald E, Mathis S, Martin S, Staples E, et al. Surveillance for West Nile Virus Disease—United States, 2009–2018. MMWR 2021; 70:1–15.

Molaei G, Andreadis TG. Identification of avian- and mammalian-derived blood meals in *Aedes vexans* and *Culiseta melanura* (Diptera: Culicidae) and its implication for West Nile virus transmission in Connecticut, USA. J Med Entomol 2006; 43:1088–1093.

Molaei G, Andreadis TG, Armstrong PM, Anderson JF, et al. Host feeding patterns of *Culex* mosquitoes and West Nile virus transmission, Northeastern United States. Emerg Infect Dis 2006; 12:468–474.

Molaei G, Andreadis TG, Armstrong PM, Bueno R Jr, et al. Host feeding pattern of *Culex quinquefasciatus* Say (Diptera: Culicidae) and its role in transmission of West Nile virus in Harris County, Texas. Am J Trop Med Hyg 2007; 77:73–81.

Molaei G, Armstrong PM, Abadam CF, Akaratovic KI, et al. Vector-host interactions of *Culiseta melanura* in a focus of Eastern Equine Encephalitis Virus activity in Southeastern Virginia. PLoS One 2015; 10:e0136743.

Molaei G, Cummings RF, Su T, Armstrong PM, et al. Vector-host interactions governing epidemiology of West Nile virus in southern California. Am J Trop Med Hyg 2010; 83:1269–1282.

Molaei G, Huang S, Andreadis TG. Vector-host interactions of *Culex pipiens* complex in northeastern and southwestern USA. J Am Mosq Control Assoc 2012; 28:127–136.

Montgomery MJ, Thiemann T, Macedo P, Brown DA, et al. Blood-Feeding Patterns of the *Culex pipiens* Complex in Sacramento and Yolo Counties, California. J Med Entomol 2011; 48:398–404.

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

Patrican LA, Hackett LE, Briggs JE, McGowan JW, et al. Hostfeeding patterns of *Culex* mosquitoes in relation to trap habitat. Emerg Infect Dis 2007; 13:1921–1923.

Rossi SL, Ross TM, Evans JD. West Nile virus. Clin Lab Med 2010; 30:47–65.

Savage HM, Aggarwal D, Apperson CS, Katholi CR, et al. Host choice and West Nile virus infection rates in blood-fed mosquitoes, including members of the *Culex pipiens* complex, from Memphis and Shelby County, Tennessee, 2002–2003. Vector Borne Zoonotic Dis 2007; 7:365–386.

Savage HM, Anderson M, Gordon E, Mcmillen L, et al. Host-seeking heights, host-seeking activity patterns, and West Nile Virus infection rates for members of the *Culex pipiens* complex at different habitat types within the hybrid zone, Shelby County, TN, 2002 (Diptera: Culicidae). J Med Entomol 2008; 45:276–288.

Savage HM, Kothera L. The *Culex pipiens* complex in the Mississippi river basin: Identification, distribution, and bloodmeal hosts. J Am Mosq Control Assoc 2012; 28:93–99.

Steinman A, Banet-Noach C, Simanov L, Grinfeld N, et al. Experimental infection of common garter snakes (Thamnophis sirtalis) with West Nile virus. Vector Borne Zoonotic Dis 2006; 6:361–368.

Tang Y, Hapip CA, Liu B, Fang CT. Highly sensitive TaqMan RT-PCR assay for detection and quantification of both lineages of West Nile virus RNA. J Clin Virol 2006; 36:177–182.

Turell MJ, Dohm DJ, Sardelis MR, O'guinn ML, et al. An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile Virus. J Med Entomol 2005; 42:57–62.

VanDalen KK, Hall JS, Clark L, McLean RG, et al. West Nile virus infection in American Robins: New insights on dose response. PLoS One 2013; 8:e68537.

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

Address correspondence to:
Goudarz Molaei
Department of Environmental Sciences
The Connecticut Agricultural Experiment Station
123 Huntington Street
PO Box 1106
New Haven, CT 06504
USA

E-mail: goudarz.molaei@ct.gov