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SEASONAL DISTRIBUTION, BLOOD-FEEDING HABITS, AND VIRUSES OF MOSQUITOES IN AN OPEN-FACED QUARRY IN CONNECTICUT, 2010 AND 2011

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ABSTRACT. Seasonal abundance of mosquitoes, their viruses, and blood–feeding habits were determined at an open-faced quarry in North Branford, CT, in 2010 and 2011. This unique habitat had not previously been sampled for mosquitoes and mosquito-borne viruses. Thirty species of mosquitoes were identified from 41,719 specimens collected. *Coquillettidia perturbans*, *Aedes trivittatus*, and *Ae. vexans* were the most abundant species and represented 34.5%, 17.7%, and 14.8% of the totals, respectively. Jamestown Canyon virus was isolated from 6 species of mosquitoes collected from mid-June through July: *Cq. perturbans* (3 pools), *Ae. cantator* (3), *Ae. trivittatus* (2), *Ae. aurifer* (1), *Ae. excrucians* (1), and *Culex pipiens* (1). West Nile virus was cultured from 8 pools of *Cx. pipiens* and from 1 pool of *Culiseta melanura* collected from mid-August through late September. Cache Valley virus was isolated from 4 species of mosquitoes in 3 genera from about mid-August through late September 2011: *Cq. perturbans* (5 pools), *Ae. trivittatus* (2), *Anopheles punctipennis* (1), and *An. quadrimaculatus* (1). Nine different mammalian hosts were identified as sources of blood for 13 species of mosquitoes. White-tailed deer, *Odocoileus virginianus*, were the most common mammalian hosts (90.8%), followed by raccoon, *Procyon lotor* (3.1%), coyote, *Canis latrans* (2.4%), and human, *Homo sapiens* (1.2%). Exclusive mammalian blood-feeding mosquitoes included: *Ae. canadensis*, *Ae. cantator*, *Ae. excrucians*, *Ae. japonicus*, *Ae. vexans*, *An. punctipennis*, and *Cx. salinarius*. Fourteen species of birds, mostly Passeriformes, were identified as sources of blood from 6 mosquito species. Five species that fed on mammals (*Ae. thibaulti*, *Ae. trivittatus*, *Ae. cinereus*, *Cq. perturbans*, and *Cx. pipiens*) also fed on birds.

KEY WORDS Blood meal identification, Cache Valley virus, Connecticut, Jamestown Valley virus, mosquito distribution, West Nile virus

INTRODUCTION

The geographical and seasonal distribution of mosquitoes throughout Connecticut has been reported periodically from 1904 through 2005 (Britton and Viereck 1904, Matheson 1945, Wallis 1960, Andreadis et al. 2005), and studies at specific locations have also reported abundance of mosquitoes (Wallis 1953; Magnarelli 1978; Andreadis 1986, 1988; Morrison and Andreadis 1992; Andreadis et al. 2001). However, none of these studies reported collections of mosquitoes and tested them for arboviruses from the open-faced quarry on Totoket Mountain, a trap-rock massif, one of Connecticut's more unique ecological settings located in North Branford, CT. We hereby report our findings of mosquitoes, arboviruses, and the identification of blood meals in 2010 and 2011.

MATERIALS AND METHODS

Tilcon open-faced quarry

The Tilcon Connecticut, Inc., open-faced quarry on Totoket Mountain in North Branford, CT, is often called the longest open-face rock quarry in the world, with a main face of ±3 mi. This quarry supplies crushed stone aggregate and hot mixed asphalt. The quarry is located immediately northeast of the junction of Route 80 and Route 22 and runs north more or less parallel to Route 22 and immediately west of Lake Gaillard in New Haven County (Fig. 1). The uniqueness of this property prompted us to request permission to sample this land for mosquitoes and viruses. A license agreement was signed on July 12, 2010, allowing The Connecticut Agricultural Experiment Station to place mosquito traps on the North Branford Quarry, owned by Tilcon Connecticut, Inc. An employee of the company always accompanied 1 of the authors (JFA) during the setting and collection of mosquito traps.

Mosquito collections

Mosquitoes were collected from July 15, 2010, through October 26, 2010, and between June 8, 2011, and October 18, 2011. Collections were usually made at weekly intervals with Centers for Disease Control and Prevention miniature light traps baited with dry ice. Traps were placed at 26 locations during the 2 years of sampling around the perimeter of the quarry

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Fig. 1. Aerial view of the North Branford, CT, Tilcon Connecticut, Inc., open-faced rock quarry showing locations of collection sites.

near permanent bodies of water, temporary pools, forest areas, and near storage areas for equipment tires (Fig. 1). All traps were hung from a branch of a tree, usually at a height of 2 to 4 ft above the ground. Mosquito Magnet Experimental traps (MMX Traps) were placed at 3 locations in 2011. Two of the MMX traps were placed about 15 ft above the ground, and 1 was placed 4 ft above the ground. Traps were placed in the field during the afternoon and retrieved the following morning. Containers with mosquitoes from each trap were placed in a cooler with ice packs to keep mosquitoes alive and brought to the laboratory. Mosquitoes were knocked-down with cold temperatures or anesthetized with carbon dioxide. Mosquitoes were frozen at –80°C.

Mosquito identification

Mosquitoes were thawed, identified to species on a cold plate under a microscope, and placed into groups of 25 or less according to species, date of collection, and location (Andreadis et al. 2005). Mosquitoes were kept on ice until they were tested for viruses. Mosquitoes containing blood were identified, refrozen, and tested later for blood-meal identification.

Virus isolation and identification

Mosquitoes were tested for arboviruses in Vero cells by methods described previously (Anderson et al. 2004). Pooled mosquito specimens were triturated in a 2.0-ml centrifuge tube containing a copper BB pellet and 0.5–1.0 ml of phosphate-buffered saline with 0.5% gelatin, 30% rabbit serum, and 1% 100× antibiotic and antimycotic (10,000 units/ml of sodium penicillin G, 10,000 µg/ml of streptomycin sulfate, and 25 µg/ml of amphotericin B; Invitrogen, Carlsbad, CA) in 0.85% saline. A Vibration Mill MM 300 (Retsch Laboratory, Irvine, CA), set at 25 cycles/sec and placed inside a biosafety hood, was used to grind the mosquitoes for 4 min. After centrifugation at 4°C for 7 min at 5200 × *g*, a 100-µl inoculum from each sample was placed onto a 24-h-old monolayer of Vero cells growing in a 25-cm^2 flask at 37°C in an atmosphere of 5% CO_2. Inoculum was added to each flask after growth medium had been decanted. The flask was rocked for 5 min, new growth medium was added, and the flask was returned to the CO_2 incubator. Cells were examined daily for cytopathogenic effect 3–7 days following inoculation.

West Nile virus (WNV) was identified by a TaqMan reverse transcriptase–polymerase chain reaction (RT-PCR) assay. The QIAamp Viral RNA Mini Kit protocol (Qiagen, Valencia, CA) was used to extract ribonucleic acid (RNA) from a 70-µl sample of infectious Vero cell-growth medium. The RT-PCR protocol was used to identify isolates of WNV (Lanciotti et al. 2000). Specific procedures are detailed in Anderson et al. (2015).

Jamestown Canyon virus (JCV) and Cache Valley virus (CVV) were identified using the Titan One-Tube RT-PCR system (Roche Diagnostics, Indianapolis, IN). The RNA was extracted from viral isolates using the Viral RNA Kit (Qiagen). Primers BUNS+new: 5-TGACCAGTAGTGTACTCCAC-3_ and BUNS-new: 5_-CAAGCAGTAGTGTGCTCCAC-3_, which targeted the conserved terminal ends of the S-segment of the *Orthobunyavirus* genus, were used (Dunn et al. 1994; Armstrong and Andreadis 2006, 2007). The amplification product of each unknown *Orthobunyavirus* virus was digested with 2 restriction enzymes in separate reactions: EcoRV cut JCV at 364 base pairs (bps) and 627 bps, and SwaI severed CVV at 411 bps and 539 bps. Each restriction enzyme master mix consisted of 13.55 µl of water, 2.0 µl of 10× reaction buffer, 0.2 µl of 100× BSA, and 0.25 µl of the specific restriction enzyme, to which was added 4 µl of the amplified reagent, and

then the mixture was incubated overnight at 37°C. Each digestion product was separated on a 2% agarose gel and stained with ethidium bromide.

Field infection rates

The field infection rates for each species of mosquito infected with a specific virus for each week in 2011 was determined per 1,000 specimens using the bias-corrected maximum likelihood estimation method (Biggerstaff 2006).

Blood meal identification

Deoxyribonucleic acid (DNA) was extracted from blood of individual engorged females using a DNeasy Blood and Tissue Kit (Qiagen). The abdomen of each engorged specimen was added to a 1.5-ml microcentrifuge tube, to which 180 µl of animal tissue lysis (ATL) buffer and 20 µl of proteinase K were added. Tissues were lyzed overnight at 56°C following the protocol for the Purification of Total DNA from Animal Blood or Cells (Qiagen). Extracted DNA was used as a template in the PCR assay. Primers were based on vertebrate mitochondrial *cytochrome b* sequences using established protocols (Molaei and Andreadis 2006; Molaei et al. 2006a, 2006b, 2007). Polymerase chain reaction amplicons were purified using the QIAquick PCR Purification Kit (Qiagen). Sequencing of both DNA strands was performed using the sequencer 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA) at the DNA Analysis Facility on Science Hill, Yale University, New Haven, CT. Sequences were annotated using ChromasPro version 1.22 (Technelysium Pty Ltd., Tewantin, Queensland, Australia) and identified from the GenBank DNA sequence database by using a BLAST search (BLASTN) of the National Center for Biotechnology Information. To avoid contamination, laboratory staff frequently replaced gloves and restrained from speaking, coughing, sneezing, and any other activities to further prevent accidental introduction of human DNA to DNA samples from engorged mosquitoes and/or PCR reactions. Furthermore, DNA samples from the engorged mosquitoes were isolated in a separate laboratory space, and PCR reactions were prepared in a Class II, Type A2 Biosafety Cabinet (LABCONCO, Kansas City, MO), equipped with high-efficiency particulate air (HEPA) filtration and built-in ultraviolet lamp. In addition to positive controls, negative (no DNA) reactions accompanied each group of samples amplified at any given time, and adequate care was taken while opening and closing laboratory tubes and vials.

RESULTS

Seasonal distribution

Thirty species were identified from the 41,719 specimens from 26 sites in 2010 and 2011 (Table 1).

Table 1. Total female mosquito specimens collected by species at the open-faced quarry, North Branford, CT, 2010, 2011.

Species	2010	2011	Total
Aedes abserratus	0	11	11
Ae. aurifer	0	35	35
Ae. canadensis	1	683	684
Ae. cantator	93	1,479	1,572
Ae. cinereus	10	1,289	1,299
Ae. excrucians	0	53	53
Ae. japonicus	33	475	509
Ae. sollicitans	2	9	11
Ae. sticticus	0	256	256
Ae. taeniorhynchus	1	3	4
Ae. thibaulti	0	1,408	1,408
Ae. triseriatus	5	46	51
Ae. trivittatus	29	7,359	7,388
Ae. vexans	785	5,375	6,162
Anopheles crucians	0	5	5
An. punctipennis	51	410	461
An. quadrimaculatus	55	50	105
An. walkeri	98	131	229
Coquillettidia perturbans	3,576	10,799	14,375
Culex pipiens	31	1,833	1,864
Cx. restuans	12	331	343
Cx. salinarius	87	1,522	1,609
Cx. territans	0	23	23
Culiseta melanura	16	857	873
Cs. minnesotae	0	2	2
Orthopodomyia signifera	0	4	4
Psorophora ciliata	0	4	4
Ps. columbiae	0	6	6
Ps. ferox	4	1,767	1,771
Uranotaenia sapphirina	27	578	605
Total	4,916	36,803	41,719

Coquillettidia perturbans (Walker), *Aedes trivittatus* (Coq.), and *Ae. vexans* (Meigen) were the most abundant species, and they represented 34.5%, 17.7%, and 14.8% of the totals, respectively. Seasonal distributions of the 16 relatively abundant species in 2011 are shown in Figures 2 and 3.

Ten species had bimodal distributions, though some may have been trimodal. Peaks of abundance often were not equal. *Aedes canadensis* (Theobald) and *Ae. sticticus* (Meigen) were most abundant in June and early July, with smaller peaks in September (Fig. 2). *Aedes japonicus* (Theobald) reached a peak of activity from late June through mid-July and became more numerous in late August through mid-September. *Aedes trivittatus* was relatively abundant from late June through mid-July and from late August through much of September. *Aedes vexans* was abundant from late June to mid-July and from late August through late September.

Anopheles punctipennis (Say) reached peak abundance in late June through mid-July, with a smaller surge in late August through early September (Fig. 3). *Culex pipiens* L. reached peak abundance in mid-July and mid- to late August. *Culiseta melanura* (Coq.) was relatively abundant in July but was more abundant in September.

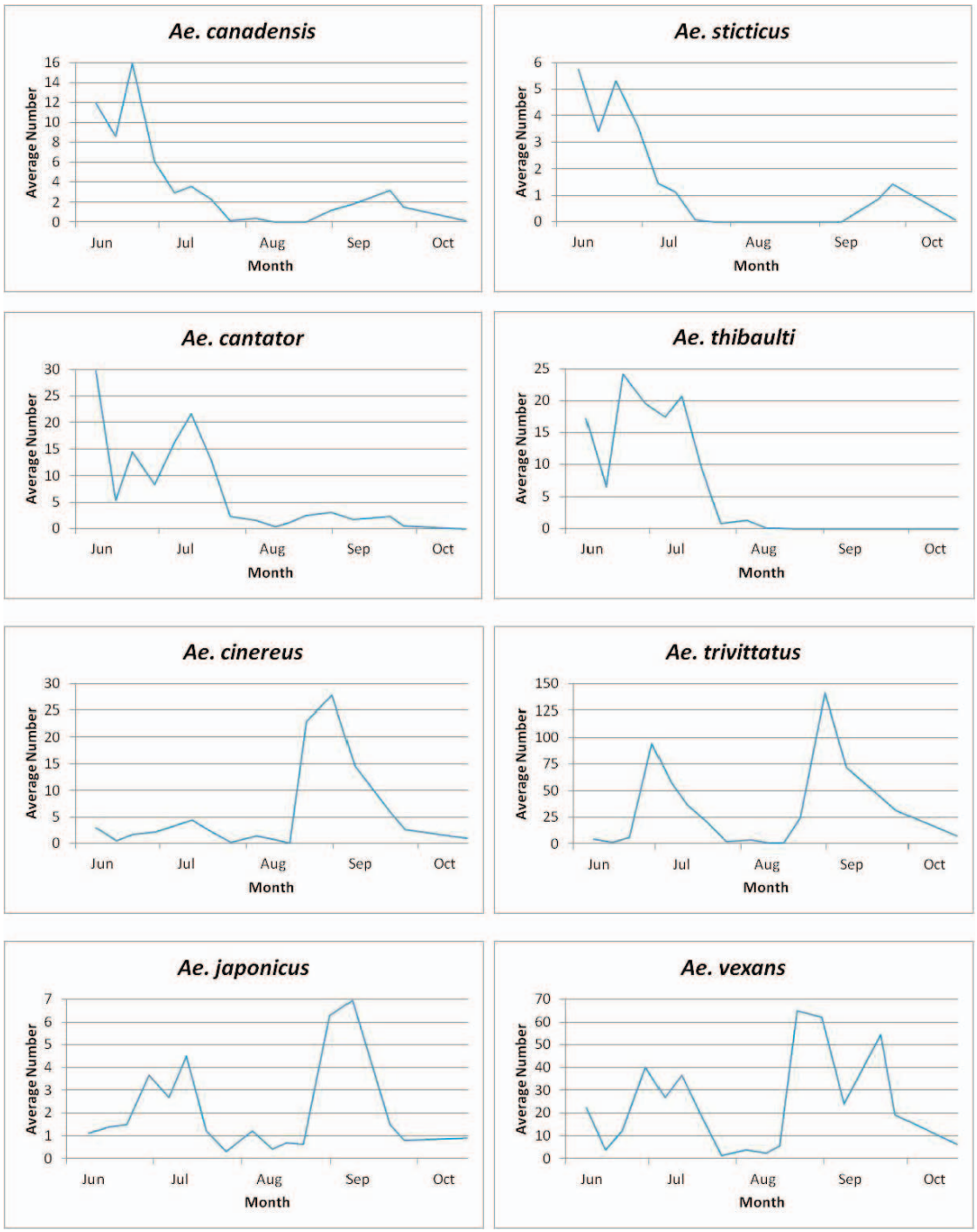

Fig. 2. Average number of specimens per collection night for 8 species of *Aedes* at the Tilcon Connecticut open-faced quarry property, North Branford, CT, June 8–October 18, 2011.

Psorophora ferox (von Humboldt) had a modest peak of activity in mid-July but appeared in much greater numbers in late August through late September. *Uranotaenia sapphirina* (Osten Sacken) became relatively abundant in mid-July through early August, with a second flush of activity occurring from late August through the 3rd wk of September.

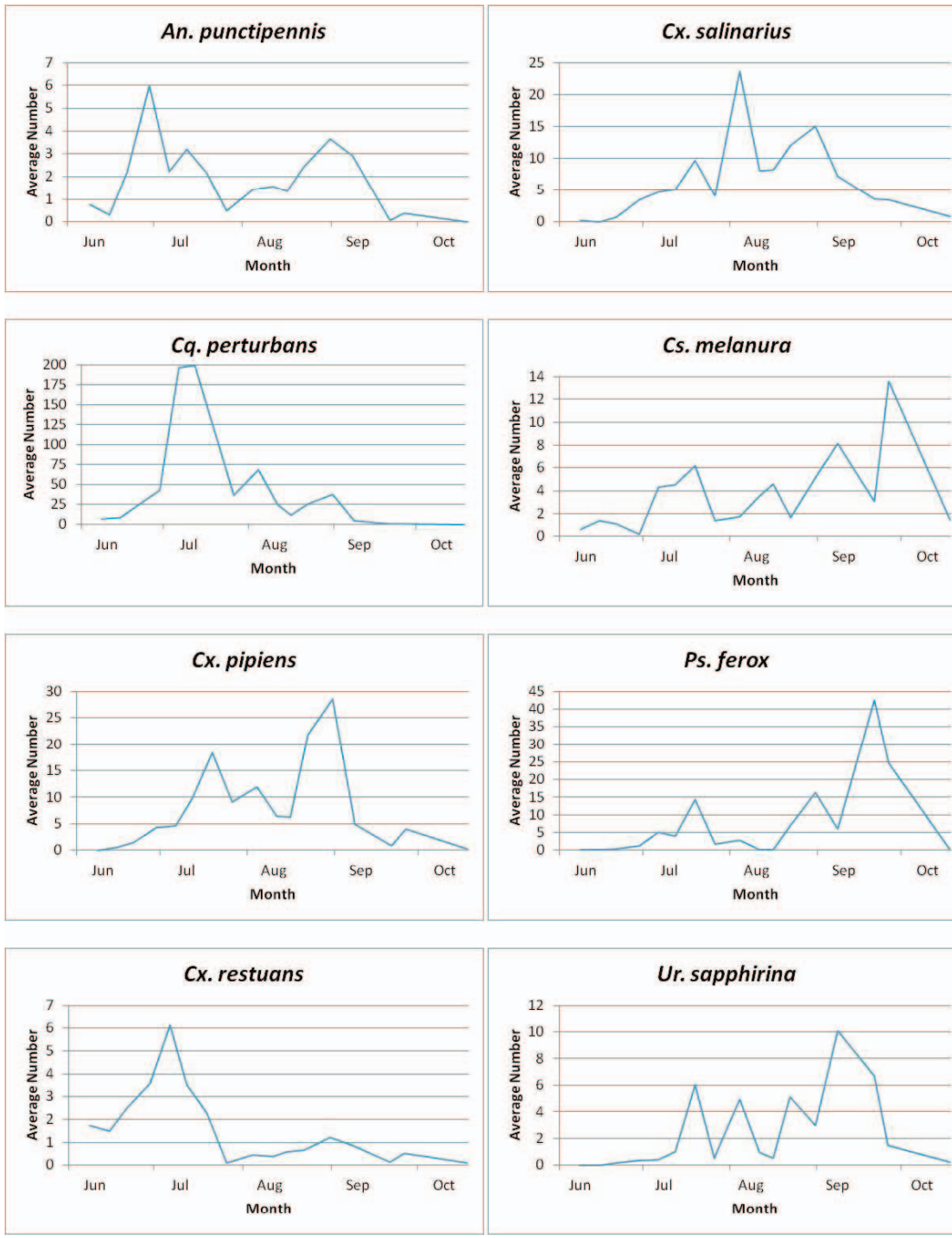

Fig. 3. Average number of specimens per collection night for 8 species from 5 genera at the Tilcon Connecticut open-faced quarry property, North Branford, CT, June 8–October 18, 2011.

Other species usually had a single flush of activity, though relative abundance could extend for 6 wk. *Aedes cinereus* Meigen was most abundant in late August into early September (Fig. 2). *Aedes cantator* (Coq.) and *Ae. thibaulti* (Dyar and Knab) were most abundant from early June through mid-July. *Coquillettidia perturbans* was relatively numerous from late June through August, with peak abundance in early

Table 2. Viruses isolated from mosquitoes at the open-faced quarry, North Branford, CT, 2011.

Mosquito species	No. mosquitoes (pools)	No. isolations and maximum likelihood estimation (MLE; 95% confidence interval [CI])			Total
		West Nile	Jamestown Canyon	Cache Valley	
Anopheles punctipennis	409 (114)	0	0	1 (2.43; 0.14–11.66)	1
An. quadrimaculatus	50 (39)	0	0	1 (19.89; 1.15–91.97)	1
Culiseta melanura	857 (130)	1 (1.16; 0.07–5.60)	0	0	1
Coquillettidia perturbans	10,800 (533)	0	3 (0.28; 0.07–0.75)	5 (0.47; 0.17–1.03)	8
Culex pipiens	1,833 (175)	8 (4.53; 2.13–8.59)	1 (0.54; 0.03–2.62)	0	9
Aedes aurifer	35 (18)	0	1 (27.87; 1.66–126.08)	0	1
Ae. cantator	1,450 (167)	0	3 (2.07; 0.55–5.56)	0	3
Ae. excrucians	53 (20)	0	1 (19.49; 1.13–94.41)	0	1
Ae. trivittatus	7,360 (404)	0	2 (0.27; 0.05–0.89)	2 (0.27; 0.05–0.89)	4
Total isolations		9	11	9	29

July (Fig. 3). *Culex salinarius* Coq. was relatively abundant from late June through late September and most numerous in August. *Culex restuans* Theobald was most abundant from early June through mid-July, with peak abundance noted in early July.

Virus isolations

Three species of viruses were isolated from 9 species of mosquitoes from June 21 through September 26, 2011 (Table 2). No viruses were isolated in 2010. West Nile virus was cultured from 8 pools of *Cx. pipiens* collected from August 16 through September 8 and from 1 pool of *Cs. melanura* collected on September 26. Seven isolates from *Cx. pipiens* and the 1 from *Cs. melanura* were from specimens collected in a tree canopy–positioned MMX trap. Weekly field infection rates (maximum likelihood estimates, at 95% confidence level per 1,000 specimens) from August 16 through September 26 for all mosquito species ranged from 1.74 (0.10–8.48) on August 16 to 0.37 (0.02–1.180) on August 22. The field infection rates for *Cx. pipiens* and *Cs. melanura* were 4.53 and 1.16, respectively (Table 2).

Jamestown Canyon virus was isolated from 6 species of mosquitoes from June 21 through July 26 (Table 2). Three isolations were obtained from each pool of *Cq. perturbans* and *Ae. cantator*, and 2 were obtained from *Ae. trivittatus*. Isolations were also obtained from *Cx. pipiens*, *Ae. aurifer* (Coq.), and *Ae. excrucians* (Walker). The weekly field infection rates (maximum likelihood estimates, at 95% confidence level per 1,000 specimens) ranged from 2.13 (0.56–5.72) on June 21 to 0.40 (0.07–1.32) on July 12. Field infection rates for *Cq. perturbans*, *Ae. cantator*, and *Ae. trivittatus* were 0.28, 2.07, and 0.27, respectively (Table 2).

Cache Valley virus was obtained from 4 species of mosquitoes on August 11, and August 31 through September 21 (Table 2). Five and 2 isolations were obtained from *Cq. perturbans* and *Ae. trivittatus*, respectively. Single isolations were obtained from *An. punctipennis* and *An. quadrimaculatus* Say. Weekly field infection rates (maximum likelihood estimates at 95% confidence level per 1,000 specimens) ranged from 2.76 (0.50–8.91) on August 11 to 0.42 (0.02–2.05) on September 21. Field infection rates for *Cq. perturbans* and *Ae. trivittatus* were 0.47 and 0.27, respectively (Table 2).

Identification of blood meals

Six mosquito species fed on birds, and 13 species fed on mammals (Tables 3 and 4). Twenty-nine mosquitoes fed on 14 species of birds. Blood samples from gray catbirds (*Dumetella carolinensis* (L.)), American robins (*Turdus migratorius* (L.)), and red-winged blackbirds, (*Agelaius phoeniceu* (L.)), were each recorded from 4 mosquito specimens. Fourteen specimens of *Cs. melanura* and 7 specimens of *Cx. pipiens* fed on birds.

In total, 415 mosquitoes fed on small and large mammals, including white-tailed deer, *Odocoileus virginianus* (Zimmermann) (91%); raccoons, *Procyon lotor* (L.) (3%); and coyotes, *Canis latrans* Say (2,.%) (Table 4). Five specimens (1.2%) fed on humans *Homo sapiens* (L.). Deer were the predominate hosts for 12 of the 13 mosquito species. *Culex pipiens* was recorded feeding on 1 deer and 1 coyote. Ninety-five percent of *Ae. trivittatus* fed on white-tailed deer; the remaining specimens of this species fed on 3 other species of mammals and 2 species of birds, and 2 specimens fed on eastern-box turtles, *Terrapene carolina carolina* (L.). *Aedes cinereus* fed on white-tailed deer and on rodents. Of the *Ae. thibaulti* specimens, 78%, 20%, and 2% fed on deer, raccoons, and coyotes, respectively. Five species that fed on mammals also fed on birds (*Ae. thibaulti*, *Ae. trivittatus*, *Ae. cinereus*, *Cq. perturbans*, and *Cx. pipiens*).

DISCUSSION

Forty-nine species of mosquitoes have been recorded from Connecticut, and their life histories have been summarized from publications that began in 1904 (Andreadis et al. 2005). We report the collection of 30 species in 2010 and 2011 from the

Table 3. Number of avian blood meals identified from 6 species of mosquitoes collected at the open-faced quarry, North Branford, CT, 2010, 2011.

Bird	Mosquito species						Total
	Aedes thibaulti	*Ae. trivittatus*	*Ae. cinereus*	*Coquillettidia perturbans*	*Culex pipiens*	*Culiseta melanura*	
American goldfinch (*Spinus tristis* (L.))						1	1
American robin					1	3	4
Barn swallow (*Hirundo rustica* (L.))					1	1	2
Eastern screech owl (*Megascops asio* (L.))					1		1
Grasshopper sparrow (*Ammodramus savannarum* (Gmelin))						3	3
Gray catbird		2			1	1	4
Indigo bunting (*Passerina cyanea* (L.))					1		1
Mourning dove (*Zenaida macroura* (L.))				1		1	2
Northern cardinal (*Cardinalis cardinalis* (L.))					1	1	2
Orchard oriole (*Icterus spurius* (L.))						1	1
Red-eyed vireo (*Vireo olivaceus* (L.))						1	1
Red-winged blackbird			1	2		1	4
Wild turkey (*Meleagris gallopavo* (L.))	1	1					2
Yellow-breasted chat (*Icteria vireus* (L.))					1		1
Total	1	3	1	3	7	14	29

open-faced rock quarry on Totoket Mountain in North Branford, CT, where mosquitoes had not previously been collected and identified. Additionally, we report the isolation of 3 viruses and the identification of blood meals from 444 specimens.

Seasonality of mosquitoes at the open-faced mine was in general similar to published accounts of mosquitoes collected elsewhere in Connecticut (Andreadis et al. 2005). Notable differences were as follows. *Aedes cinereus* may have been relatively more abundant in August than reported previously. The relative abundance of *Ae. cantator* was unexpected. Although collected throughout Connecticut, this species is typically found near the upland portions of the salt marsh along Long Island Sound (Andreadis et al. 2005). Our mosquito collection site was 10 km inland. Perhaps vernal pools in the immediate vicinity of the mine were conducive for egg laying and larval development. *Aedes sticticus*, which previously has been reported to be univoltine, was bivoltine at this site, with females relatively abundant in June and again appearing in September after being absent in collections made in August.

Three species of virus were isolated from mosquitoes collected in 2011. West Nile virus has been present in Connecticut since 1999 (Anderson et al. 1999) and has caused disease in 120 humans, with 3 deaths in the state through 2014 (www.ct.gov/dph/WNV). This virus was isolated from mosquitoes collected from August 16 through September 26 and most frequently from *Cx. pipiens*, the most important vector in northeastern USA (Andreadis et al. 2004). Eight of the 9 isolations of WNV were from *Cx. pipiens*, and 6 of these plus 1 from *Cs. melanura* were from mosquitoes collected in the tree canopy. Previous studies have reported the prevalence of virus in mosquitoes inhabiting the tree canopy to be higher compared to collections obtained near ground level (Anderson et al. 2004, Andreadis and Armstrong 2007). Our relatively numerous isolations of WNV from *Cx. pipiens* beginning in August confirm the findings of Andreadis et al. (2004) that *Cx. pipiens* is likely involved in late season epizootic amplification in birds. The isolation of WNV from *Cs. melanura* on September 26 suggests that this bird-feeding mosquito also amplifies this virus in birds late in the season.

Jamestown Canyon virus was isolated from 6 species of mosquitoes belonging to the genera *Aedes*, *Culex*, and *Coquillettidia*, collected from June through July, 2011. This virus may cause a mild febrile illness in humans, and 1 documented case has been reported in Connecticut (Nelson et al. 2002). This virus is prevalent throughout Connecticut and has been reported to be maintained in 22 species of mosquitoes, particularly *Aedes* mosquitoes (Andreadis et al. 2008). *Aedes cantator* and *Cq. perturbans*, from which we obtained multiple isolations, were identified as important vectors (Andreadis et al. 2008). Our isolation of JCV from *Cx. pipiens* is unique. Two major lineages of this virus have been reported in Connecticut (Armstrong and Andreadis 2007).

Cache Valley virus was isolated from 4 species of mosquitoes in 3 genera from about mid-August through late September, 2011. Andreadis et al. (2014) reported isolating this virus, which occurs epizootically periodically and not consistently from year to year, from 16 species in Connecticut and incriminated *An. punctipennis* to be the most likely consistent vector. One of our isolations was from *An. punctipennis*. Our isolation of CVV in August and September is consistent with the findings of Andreadis et al. (2014), who reported virus infection in

Table 4. Number of mammalian blood meals identified from 13 species of mosquitoes collected at the open-faced quarry, North Branford, CT, 2010, 2011.

Mosquito species	Total	White-tailed deer	Raccoon	Coyote	Human	Cattle (*Bos taurus* (L.))	Eastern chipmunk (*Tamias striatus* (L.))	White-footed mouse (*Peromyscus leucopus* Rafinesque)	Eastern cottontail (*Sylvilagus floridanus* (Allen))	Meadow vole (*Microtus pennsylvanicus* (Ord))
Aedes canadensis	15	11	3	1						
Ae. cantator	10	10								
Ae. cinereus	16	10					3	2		1
Ae. excrucians	3	2	1							
Ae. japonicus	4	4								
Ae. thibaulti	41	32	8	1						
Ae. trivittatus	138	131	1	3	3					
Ae. vexans	49	47			1	1				
Anopheles punctipennis	2	2								
Coquillettidia perturbans	105	102		2		1				
Culex pipiens	2	1		1						
Cx. salinarius	21	17		2		1			1	
Psorophora ferox	10	9			1					
Total	416	378	13	10	5	3	3	2	1	1

mosquitoes occurring from mid-August through September. Armstrong et al. (2015) reported a new lineage of CVV in Connecticut in 2010. This virus has caused disease in humans (Sexton et al. 1997) and congenital defects in lambs (Crandell et al. 1989).

Blood-feeding patterns of mosquitoes at the open-faced quarry were consistent with reports of the feeding habits of mosquitoes collected elsewhere in Connecticut (Magnarelli 1977; Molaei et al. 2006a, 2008, 2016) and in other locations (Apperson et al. 2002, 2004; Savage et al. 2007). Three species of *Aedes*, *Cq. perturbans*, *Cx. pipiens*, and *Cs. melanura* fed on birds. The exclusive feeding of *Cs. melanura* on birds is supportive of the previous report by Molaei and Andreadis (2006), and the isolation of WNV is consistent with other studies suggesting this species is an epizootic vector among birds (Andreadis et al. 2004). The preponderance of feeding on birds by *Cx. pipiens* and the isolation of WNV from 8 pools are supportive of the importance of this species in the enzootic and epizootic transmission of WNV (Andreadis et al. 2004). The feeding of 3 species of *Aedes* and *Cq. perturbans* on both birds and mammals has been documented previously, and the suggestion has been made that such species could be bridge vectors for WNV and eastern equine encephalomyelitis (Molaei et al. 2008, 2016).

Thirteen mosquito species, 8 species of *Aedes*, 2 species of *Culex*, *Ps. ferox*, *An. punctipennis*, and *Cq. perturbans*, fed on mammals, and all, with the exception of *Cx. pipiens*, fed predominately on white-tailed deer. The isolation of JCV and CVV from 6 and 3 of these species, respectively, is supportive of other studies indicating that white-tailed deer likely are enzootic amplifiers of these viruses (Grimstad 1988, Blackmore and Grimstad 1998).

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