

Culex pipiens Bloodmeal Analysis from Suffolk Virginia

Presentation by Jay Kiser

jkiser@suffolkva.us

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

Culex pipiens Complex

Culex pipiens

Northern house mosquito

Primary WNV Vector



Culex quinquefasciatus

Southern house mosquito

Primary WNV Vector



Hybrid Zone of *Culex pipiens* and *quinquefasciatus*



Northern limit of
Cx. quinquefasciatus

Latitude 39° N.

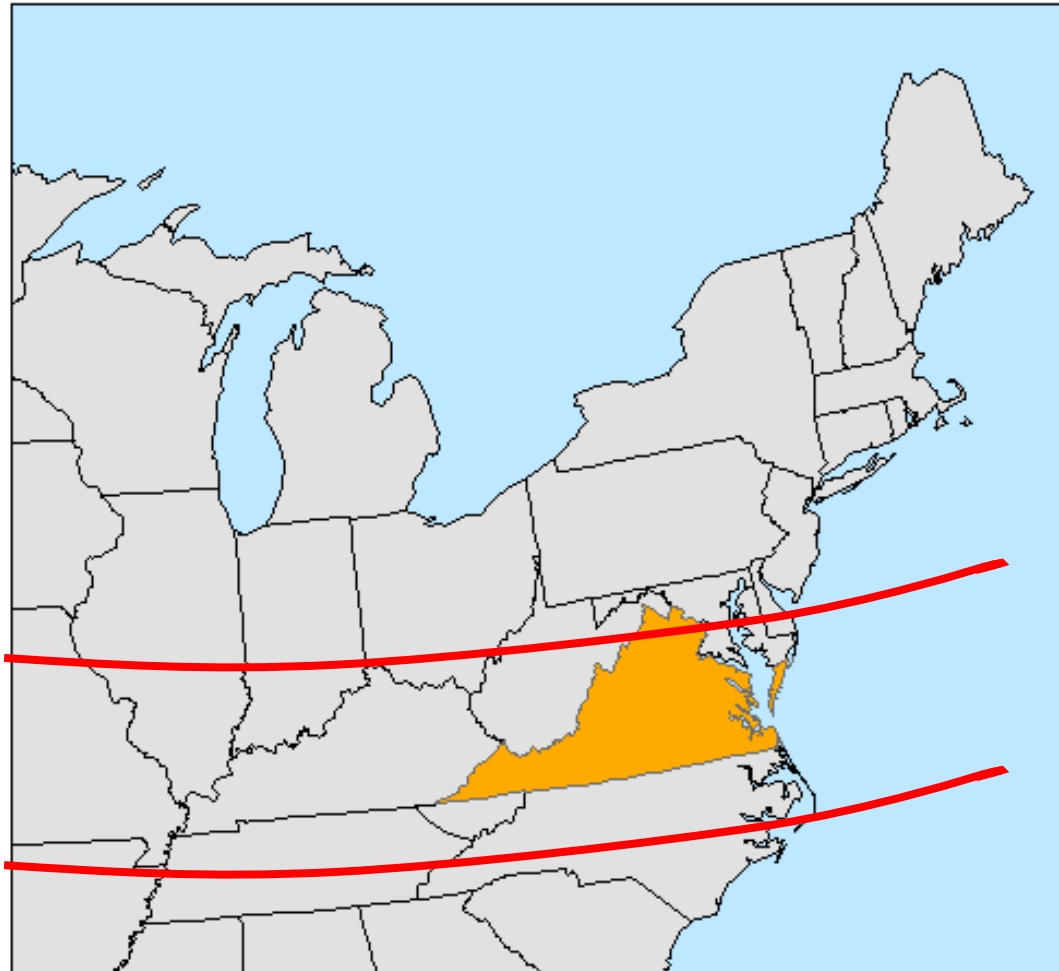


Latitude 36° N.



Southern limit
of *Cx. pipiens*

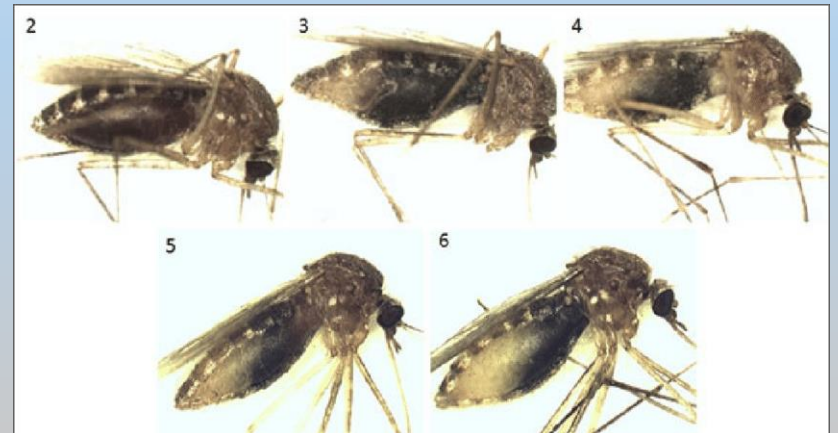
Hybrid Zone of *Culex pipiens* and *quinquefasciatus*



Bloodfed *Culex pipiens* Collections

2019-2020

- Spring, summer, and fall
- Trap types
 - Mostly gravid traps
 - Some BG and CDC
- 365 collected
 - 281 (77%) w/usable results
 - 84 (23%) not usable
 - Not enough blood
 - Blood was degraded
 - No vertebrate DNA markers found



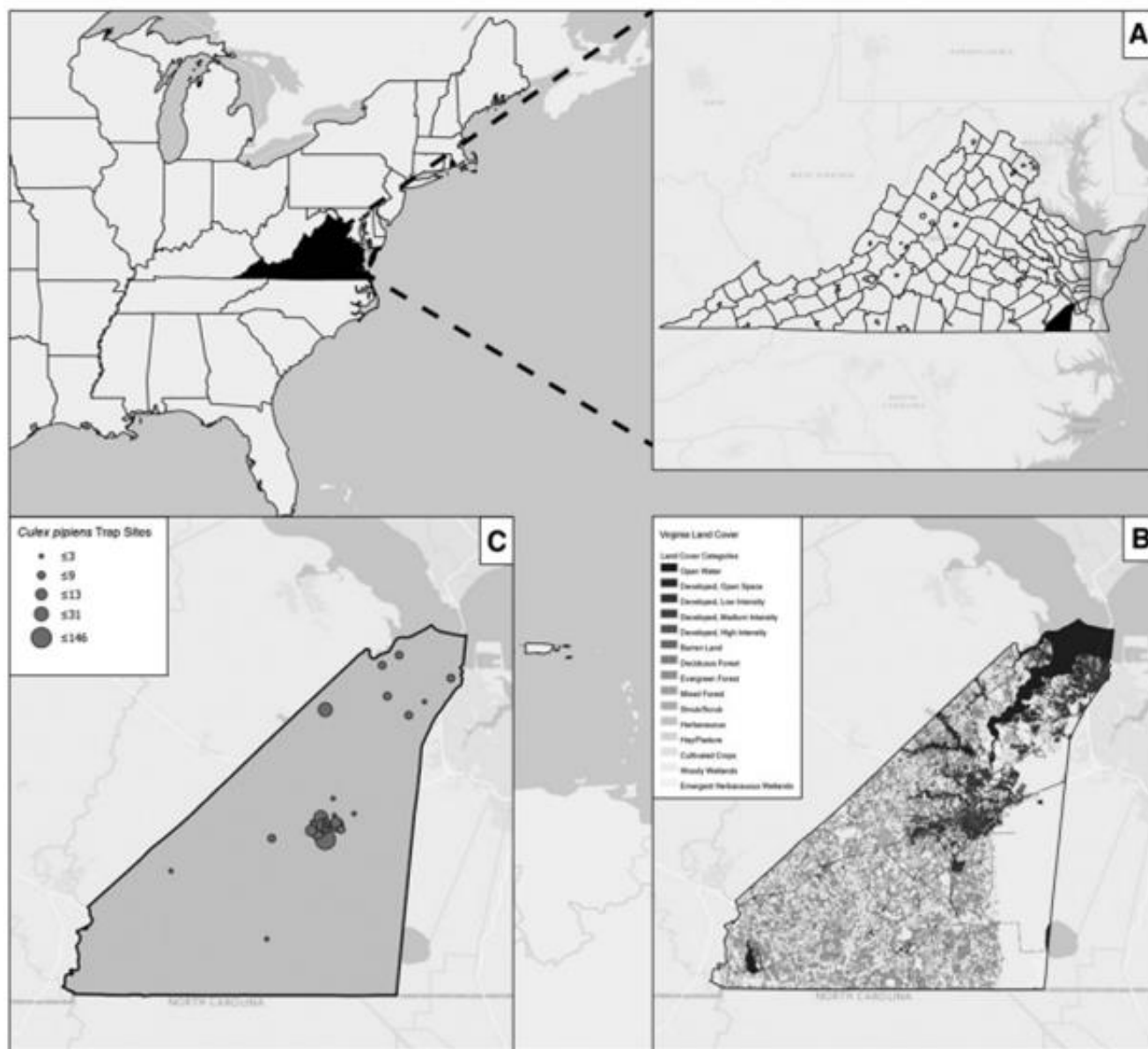


FIG. 1. Mosquito trapping sites in Suffolk, Virginia, 2019–2020. **(A)** Geographic location of Suffolk in the corner of southeastern Virginia. **(B)** Land cover of the region. **(C)** Mosquito trap sites with total number of engorged *Culex pipiens* collected at each site.

Preserving Samples



- Place mosquitoes into centrifuge tubes individually
 - Label tube



- Store in freezer
 - -18 F° short term
 - -80 F° long term

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

Bloodmeal Analysis Results

281 Cx pipiens specimens

- 255 specimens w/bird blood (only)
 - 19 species total
 - 141 American Robins
 - 57 Northern Cardinals
 - 14 Carolina wrens
- 13 specimens w/mammal blood (only)
 - 3 species total
 - 6 cats
 - 6 Virginia Opossums
 - 1 dog
- 12 Combo of blood
 - bird and mammal
- 1 Reptile, brown snake

Bloodmeal Analysis Results

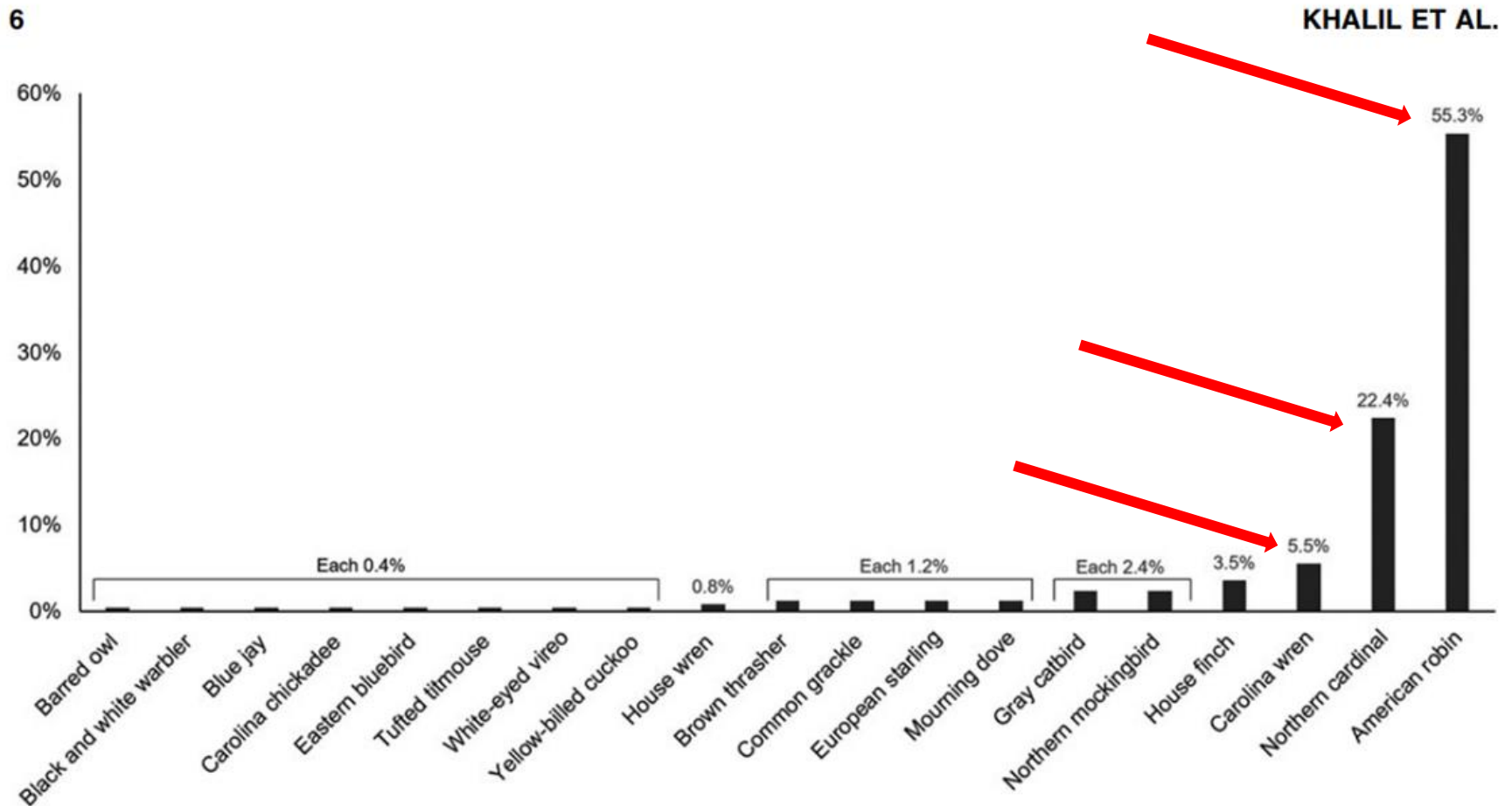
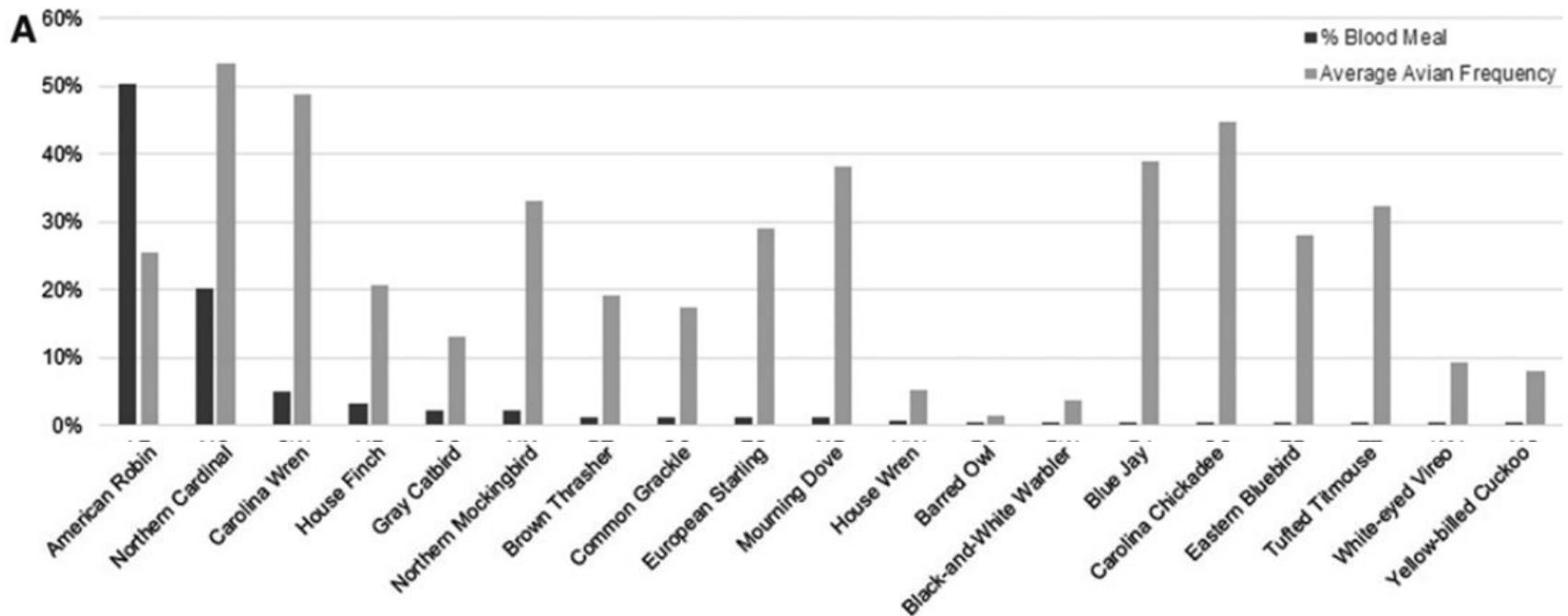


FIG. 2. Percentage of *Culex pipiens* avian-derived bloodmeals Suffolk, Virginia, 2019–2020.

Bloodmeal % vs Avian Frequency

HOST ASSOCIATIONS OF *CULEX PIPPIENS* IN VIRGINIA

7



Seasonal Differences on Bird and Mammal Feeding

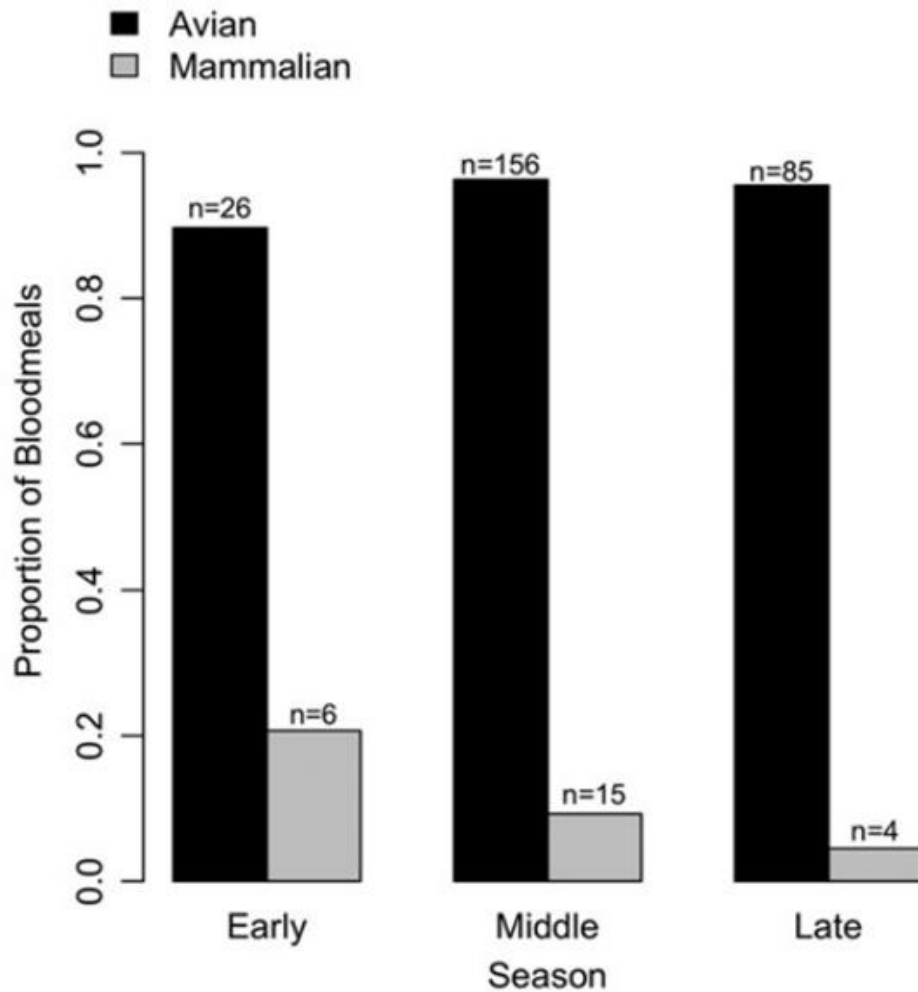
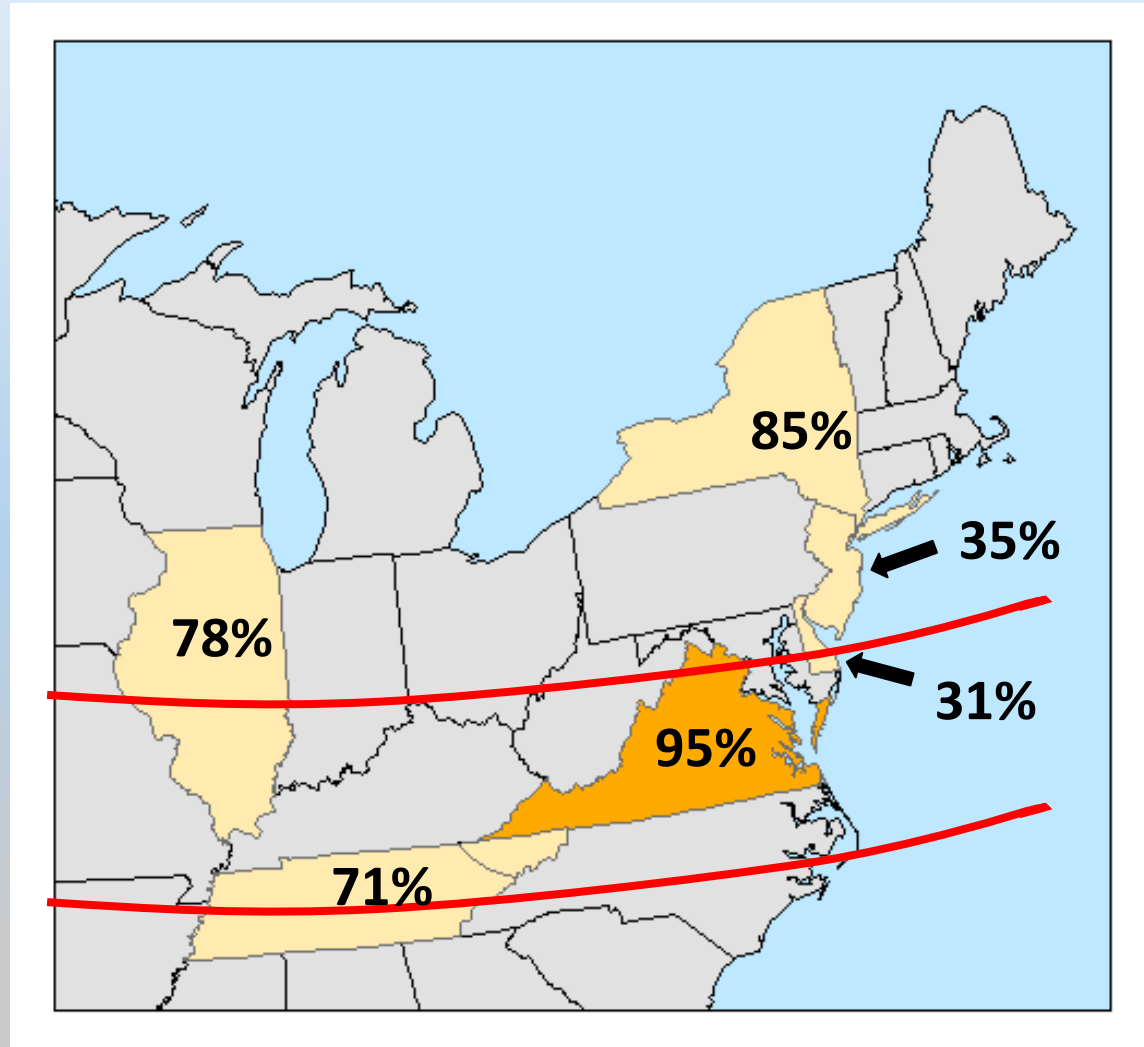


FIG. 4. Proportion of bloodmeals obtained from avian and mammalian hosts each season in Suffolk, Virginia, 2019–2020. Early season defined as May and June; Middle defined as July and August; and Late defined as September, October, and November.

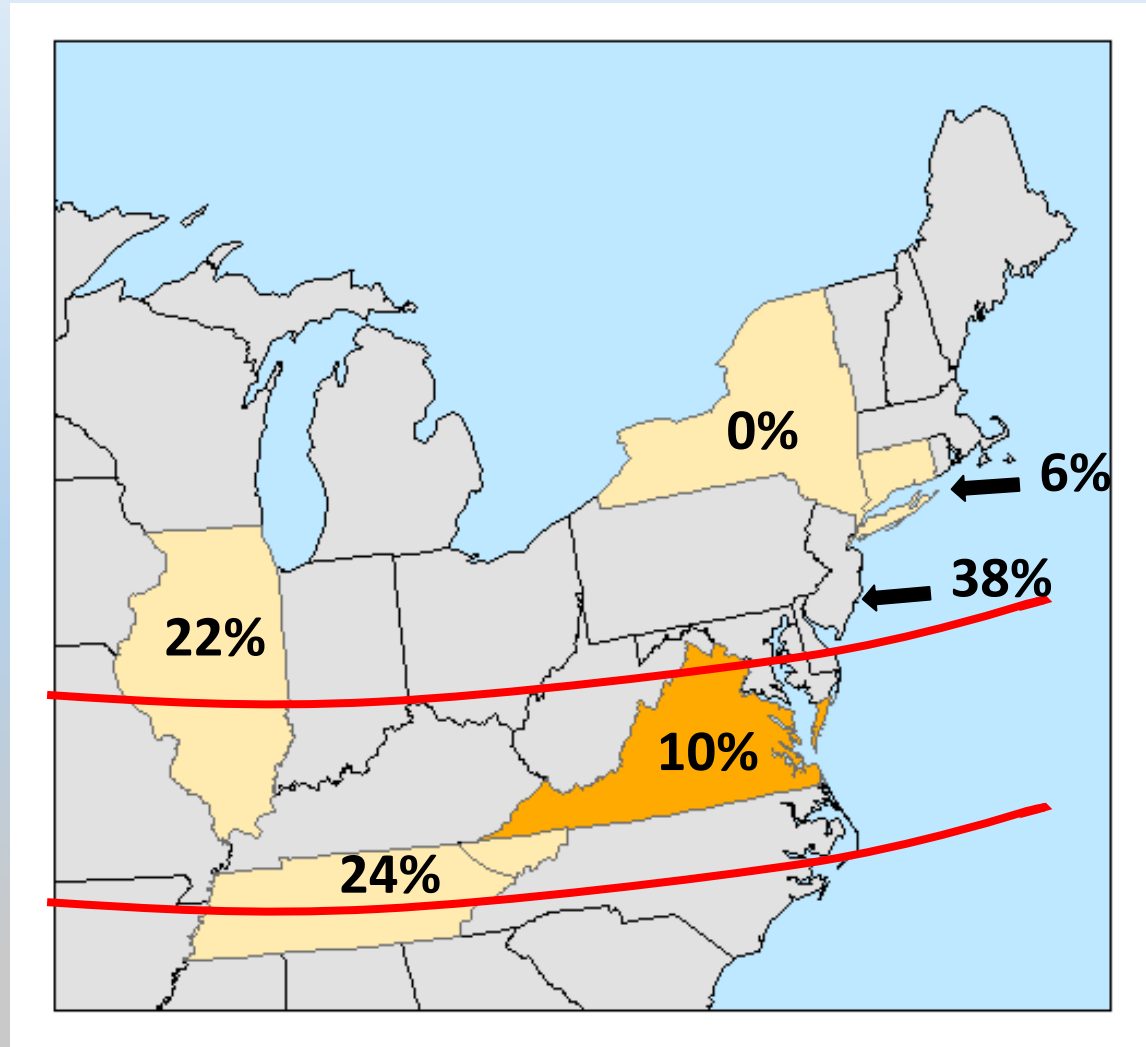
- Spring, Summer, and Fall
- No significant difference but
 - Birds slight increase
 - Mammals slight decrease

Culex pipiens feeding on birds from studies in nearby states



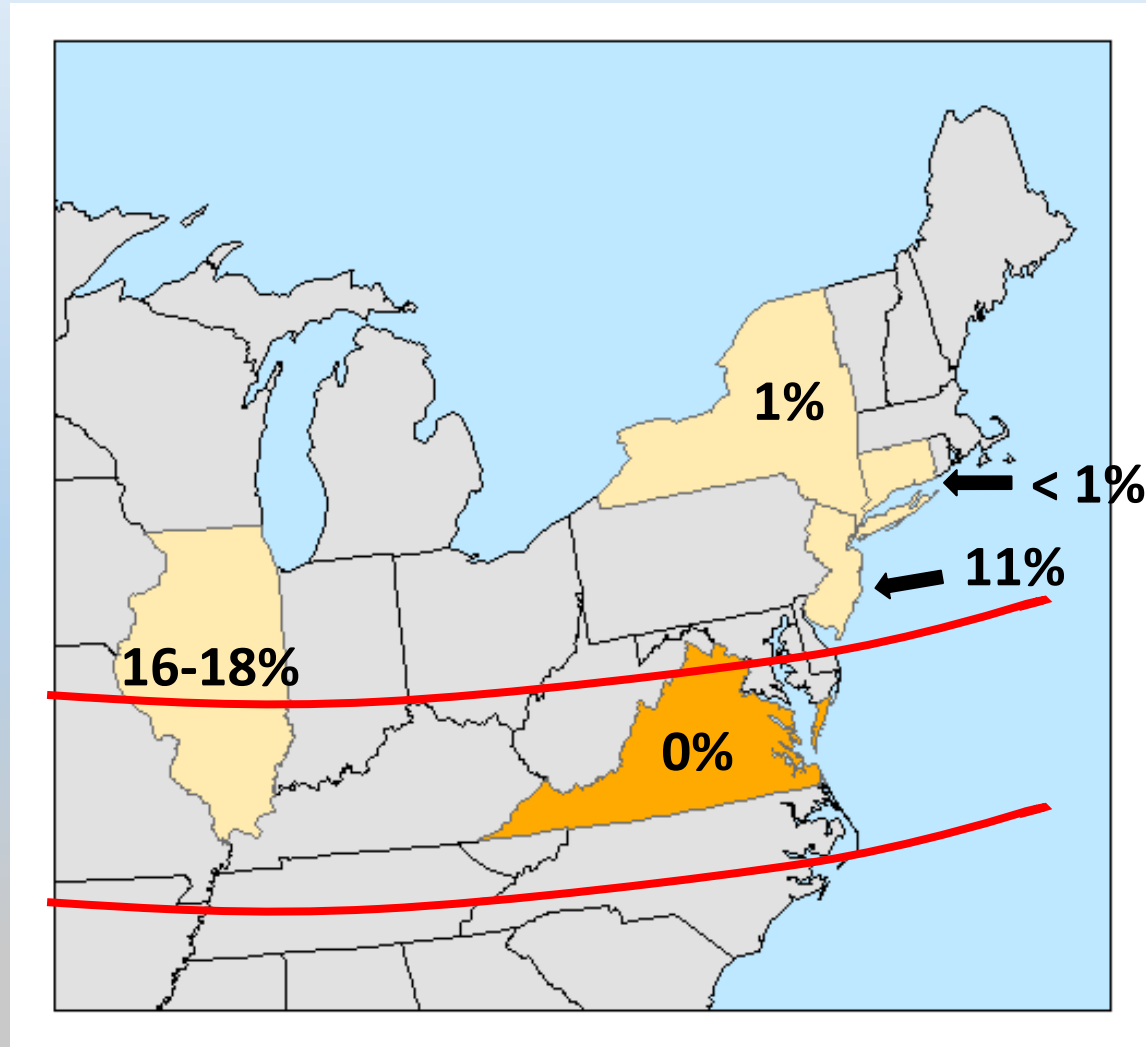
Numbers are referenced in the Khalil et al 2021

Culex pipiens feeding on mammals from studies in nearby states



Numbers are referenced in the Khalil et al 2021

Culex pipiens feeding on humans from studies in nearby states



Numbers are referenced in the Khalil et al 2021

Culex pipiens Complex

Culex pipiens



Culex quinquefasciatus



Culex pipiens f. *molestus*



Culex pipiens f. molestus

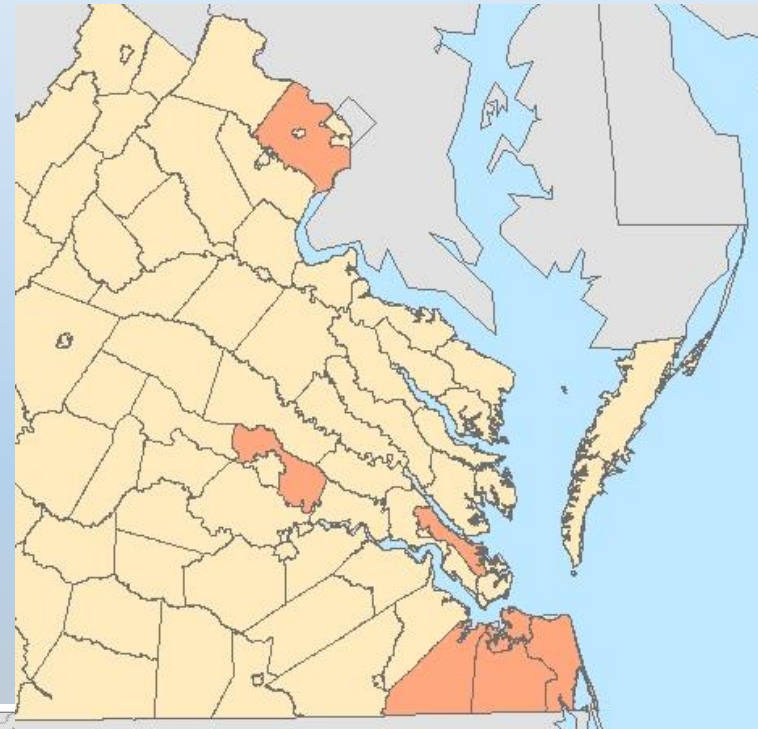


- First described in Africa
 - Varying origin stories
 - Populations found in some big cities
 - Around the world and US
- Subterranean habitat
- Feeds more on mammals and humans than *Cx. pipiens pipiens*
- Autogenous
 - Able to lay first egg raft without a bloodmeal
- Stenogamous
 - able to mate in a restrictive space. No nuptial flight required

What species do we have in Hampton Roads? The state of Virginia?

2021

- VA jurisdictions were asked to participate
 - Collect blood fed *Culex pipiens*
- 406 blood fed *Cx pipiens*
 - from 167 traps locations
 - from 8 VA jurisdictions
- Fairfax also collected 74 blood fed *Cx. pipiens* from 2019



What species do we have in Hampton Roads? The state of Virginia?

2022



- Test last year's samples
 - Bloodmeal analysis
 - Vector borne disease testing
 - DNA analysis
 - *Cx. pipiens* vs *Cx quinquefasciatus*
 - Any other members of the complex present

From Results

- Better understand our mosquitoes
- More accurate pesticide resistance times



Thank You!

2019-2020 Project

- Connecticut Agricultural Experiment Station
 - Goudarz Molaei
 - Noelle Khalil
 - Eliza Little
 - Karen Yuan
 - Michael Misencik
 - Philip Armstrong
- Suffolk Virginia
 - Karen Akaratovic
 - Charles Abadam
 - Kaitlyn Price
 - Cassidy McKelvie

2021 Collections

- Chesapeake
- Fairfax
- Henrico
- Norfolk
- Portsmouth
- Suffolk
- Virginia Beach
- York

Questions?



Jay Kiser

Jkiser@suffolkva.us