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Characterization of the blood-feeding patterns of Culex quinquefasciatus in San Bernadino County, California

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CHARACTERIZATION OF THE BLOOD-FEEDING PATTERNS OF *CULEX QUINQUEFASCIATUS* IN SAN BERNARDINO COUNTY, CALIFORNIA

by

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Characterization of the Blood-Feeding Patterns of *Culex quinquefasciatus* in San Bernardino County, California

Abstract

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Culex quinquefasciatus has been identified as one of the most prominent vectors of West Nile virus (WNV) in Southern California. WNV is a zoonotic disease that is endemic in North America and is known to primarily cause flu-like symptoms in humans, and in rare cases, life-threatening conditions. The goal of this study was to identify which animal species are most frequently fed upon by these mosquitoes in this region. To examine the relationship between blood-feeding patterns and West Nile virus activity in San Bernardino County, the feeding patterns of *Cx. quinquefasciatus* are determined in a variety of habitat types, which was the primary focus of this study. Furthermore, potential shifts in seasonal blood-feeding patterns of this population of *Cx. quinquefasciatus* towards increased mammalian feeding was examined. The WNV activity in the county during 2011 was also analyzed. Over 740 *Cx. quinquefasciatus* samples were collected by West Valley Mosquito and Vector Control District in San Bernardino County during 2011 from 34 different sites. DNA from the bloodmeals was extracted and purified, and a 658-base pair region of DNA located in the mitochondrial gene *cytochrome c-oxidase I* (*COI*) was amplified. This was followed by DNA sequencing of the PCR product, and identification of the individual sequences using the Bar Code of Life Data Systems. A total of 683 bloodmeals were successfully identified. These bloodmeals belong to 29 vertebrate species across four different habitats. It was found that species richness was not significantly different

between habitats, even though the sample sizes for each habitat varied. Across habitats, the highest percentage of avian bloodmeals were taken from House Sparrows and House Finches. Bloodmeals were identified from five mammalian species which included Humans. A seasonal shift towards increased mammalian bloodmeal prevalence was observed in urban habitats. It was found that WNV activity during 2011 in San Bernardino County was relatively low when compared to the following six years.

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Chapter 1: Literature Review

Zoonotic Diseases and Vectors

Nearly two-thirds of human infectious diseases and emerging infectious diseases originate from animal origins, which also account for nearly 60% of all modern pandemic threats (Rizzoli et al. 2015). These infectious diseases that are transmitted between animals and humans are termed zoonotic diseases. Throughout human history, zoonotic pathogens have globally caused significant mortality, morbidity, and loss to wildlife, livestock, and human hosts (Gebreyes et al. 2014). Devastating diseases such as HIV, malaria, and influenza are considered zoonoses (Belay et al. 2017; Kemunto et al. 2018). These diseases can be transmitted through indirect or direct close contact between animals and humans, either through the air, through a bite, or through contact with infected bodily fluids (Kemunto et al. 2018).

Many zoonotic infections are transmitted through a vector, such as a mosquito, tick, or flea. According to World Health Organization (WHO 2018), vector-borne zoonotic diseases are human diseases caused by parasites, bacteria, or viruses that are transmitted by vectors from an infected animal host to a human. These diseases account for a significant amount of global devastation and are responsible for more than 700,000 deaths a year (Omodior et al. 2018; WHO 2018). Many of the most calamitous zoonoses are caused by viruses. Zoonotic amplification refers to the ability of a virus to amplify inside of an animal, and subsequently be transmitted to other animals, through a vector. Often, vector-borne pathogens are maintained in nature in cycles involving vectors and susceptible reservoir hosts that can naturally produce high levels of viremia in their blood (Weaver 2005).

For viral infections, disease outbreaks can occur when the virus overflows into domesticated animal and human populations, typically occurring after intense zoonotic amplification during warmer weather (Kilpatrick et al. 2008).

The most devastating global disease vector is the mosquito. Mosquitoes (Diptera: Culicidae) are responsible for more human death than any other organism due to their ability to transmit a wide range of dangerous vector-borne pathogens (Gebreyes et al. 2014; Molaei et al. 2010; Omodior et al. 2018). These diseases include, but are not limited to: Dengue, Zika, Chikungunya, Malaria and West Nile (Omodior et al. 2018). The global devastation of mosquito-borne illnesses highlights the urgent need for improved prevention and control strategies, and these approaches must begin with better understanding of the factors that drive transmission and amplification of these diseases.

Transmission of Mosquito-borne Viruses

To best protect the health of animal and human populations that are at risk of infection, it is critical to understand the natural dynamics of vector-borne diseases through the observation of the species of mosquitoes able to transmit and amplify the diseases within local populations. Transmission intensity is one of the most important factors used to observe transmission and amplification of these diseases among animal populations, because it can be used to determine the likelihood of a potential vector-borne illness outbreak. It is determined by both contact rate between hosts and vectors, and host competence, which is the ability of a host species to maintain and transmit the virus to a competent vector (Rizzoli et al. 2015). Additionally, vector competence, the proportion of vectors transmitting virus per total infected, is another extremely important factor involved in vector-host disease transmission (Goddard 2002; Weaver 2005). Vector competence is the ability of a vector to become infected with a pathogen, and then

successfully transmit the virus under controlled laboratory conditions (Reisen 2012). The pathogen must be able to develop and survive inside of the vector and must penetrate the salivary glands of the arthropod before being injected into a new host (Colpitts et al. 2012). Vector competence is also affected by biotic factors such as mosquito abundance and longevity of the mosquito (Goddard 2002). In addition to vector capacity, understanding the dynamics of host preferences and blood-feeding patterns is essential to assessing mosquitoes' overall vector capacity within a given population (Kilpatrick et al. 2006; Molaei et al. 2007; Simpson et al. 2012). Furthermore, the transmission of vector-borne diseases within an animal or human population is directly related to the feeding behavior and feeding preferences of the vectors themselves (Ciota 2017; Molaei et al. 2007). For a specific disease, the most competent vectors will not be efficient vectors if they do not frequently feed on competent hosts, and conversely, less competent vectors can drive epidemics if they frequently feed on competent hosts and are abundant (Ciota et al. 2017).

West Nile Virus

In the United States, one of the most prominent vector-borne illnesses is West Nile virus (WNV) (*Flaviviridae*: *Flavivirus*) (CDC 2010). West Nile virus is a viral pathogen that is part of a group of mosquito-borne viruses that are also found in the United States, including St. Louis encephalitis virus and western equine encephalomyelitis virus (Kwan et al. 2010). West Nile virus has been detected in 43 different mosquito species and it is typically maintained in an enzootic cycle that primarily consists of infected passerine birds and mosquitoes (Apperson et al. 2004; Kramer et al. 2008). However, typically during late summer and early fall, WNV may also infect humans and other mammals (Fonseca et al. 2004; Kilpatrick et al. 2006). These shifts towards increased mammalian feeding patterns have been previously demonstrated in other

studies, that correlated with the migration and dispersal of common avian species (Kilpatrick et al. 2006; Thiemann et al. 2011). According to the Center for Disease Control and Prevention (CDC 2018b), WNV has been found in every state in the continental United States. By 2017, WNV had caused a reported 48,183 cases of human disease, and 2,163 deaths in the United States (CDC 2018c). WNV eventually became considered an endemic disease, due to repeated seasonal outbreaks of disease in humans and equines (Andreadis et al. 2012; Fechter-Leggett et al. 2012; Worwa et al. 2018). Humans and equines are epidemiologically termed incompetent and 'dead-end hosts' because of their susceptibility to become infected, but inability amplify the virus in high enough quantities to infect other biting mosquitoes (Fonseca et al. 2004; Rizzoli et al. 2015).

Host competence is a critical factor that affects the transmission of WNV by mosquitoes. The competence of the potential host that the mosquito could feed on is strongly reliant on dose of the virus, and environmental temperature. Dose refers to the ability of the host, specifically avian hosts, to produce high amounts of viremia in their bloodstream after being bitten by an infected mosquito. Furthermore, increased temperatures typically increase viral replication rates in mosquitoes which can accelerate dissemination of the disease to susceptible hosts (Ciota 2017). Human WNV epidemics require bridge vectors, which are arthropods that "bridge" the gap between humans and animals and can lead to the spread of pathogenic infections among humans that are typically only transmitted among specific animal species (CDC 2018a; Molaei et al. 2010). For WNV, mosquitoes are the "bridge vector" because humans and other mammals have the "dead-end" host status, meaning they are able to become infected with the virus, but are unable to replicate enough virus in their bloodstream to infect other biting mosquitoes (Fonseca et al. 2004). WNV can infect hundreds of vertebrate species in the field, however relatively few

taxa are able to develop enough viremias to infect blood-feeding mosquitoes (Kramer et al. 2008). Host taxa that have been found to be very competent include: Corvidae which include American Crows (*Corvus brachyrhynchus*), Western Scrub-Jays (*Aphelocoma californica*), and Common Ravens (*Corvus corax*); House Sparrows (*Passer domesticus*); American Kestrels (*Falco sparverius*); House Finches (*Haemorhous mexicanus*); American Robins (*Turdus migratorius*) (Nemeth et. al 2009; Reisen et al. 2006; Wheeler et al. 2009). The most efficient WNV vectors, which are primarily *Culex* spp., are mosquitoes known to feed on both competent hosts and humans (Colpitts et al. 2012).

Culex **Mosquitoes and West Nile Virus in California**

WNV was first detected in California from a *Culex tarsalis* mosquito pool when an infected virus was isolated in 2003 near El Centro, Imperial County, and has since been isolated from several other mosquito species (McAbee et al. 2008; Molaei et al. 2010). The distribution of WNV in California comprises diverse biomes, including cool and wet habitats found in wetland and coastal regions, hot and dry desert habitats, urban dwellings, and mixed agricultural areas throughout the state, especially as temperatures rise throughout the year (Reisen et al. 2004; Reisen et al. 2012). The primary vectors of WNV responsible for recent WNV epidemics in California are from the *Culex* family of mosquitoes including *Cx. tarsalis, Culex pipiens,* and *Culex quinquefasciatus* (Kilpatrick et al. 2006; Kwan et al. 2010; McAbee et al. 2008; Molaei et al. 2010). These mosquitoes are capable of maintenance transmission among populations of birds between outbreaks and are known to drive the horizontal transmission of WNV during human outbreaks (Ciota 2017; Reisen 2012). Enzootic transmission cycles are distinct based on the primary vector and host species composition in different regions of California (Hamer et al. 2011). In rural areas, it has been found that *Cx. tarsalis* is the primary vector of WNV, and in

urban and suburban areas, *Cx. pipiens* and *Cx. quinquefasciatus* are the principal vectors (Fonseca et al. 2004; Kothera et al. 2013).

In California, like in other parts of North America, the *Culex pipiens* complex consists of two primary species: *Culex quinquefasciatus, Cx. pipiens,* and their hybrids (Kothera et al. 2013). *Culex quinquefasciatus* is typically found in tropical and sub-tropical regions, and is found throughout southern North America, South of 36° latitude (Joyce et al. 2018). This species hybridizes extensively with *Cx. pipiens* across a broad latitudinal zone, which is starkly exemplified in California and has created an extremely complex genetic background (Fonseca et al. 2004; Kilpatrick et al. 2006; Kothera, Godsey, Mutebi 2010). *Culex. pipiens* is considered the principal vector of WNV in Northern California. This is because WNV outbreaks typically occur during their peak abundance period, they have been consistently demonstrated to be competent laboratory vectors of WNV, and field populations of these mosquitoes have been repeatedly found to be infected with the virus (Fonseca et al. 2004). Furthermore, mosquitoes in this genus are considered to be the most important epidemic vectors of WNV based on seasonal overlap with bird and human infection (Vaidyanathan and Scott 2006). *Culex pipiens* has two distinct subspecies found globally: form *pipiens* and form *molestus* (Ciota, Chin, Kramer 2013). It is believed that these two different forms have unique host feeding patterns and vectoral capacities.

Vector competence has been demonstrated in previous studies to vary between species of *Culex* mosquitoes. Factors affecting this may be explained partially by genetic factors, shifts in feeding behavior, variability in viremias developed in avian reservoir hosts, or seasonal variation in vector susceptibility (Vaidyanathan and Scott 2006). It has been proposed that the mixed mammal and avian feeding habits of North American *Culex* mosquitoes results from mixed

ancestry of temperate populations of these species in the *Cx. pipiens* complex. (Kothera et al. 2013). Host selection and bloodmeal acquisition patterns are critical to evaluate because they help determine the frequency with which host species appear within bloodmeals (Faraji et al. 2014). This is especially critical for studying vector-borne diseases because data on avian reservoirs and amplification hosts demonstrates varied competence and transmission efficiency among these bird species (Reisen 2012).

Other Factors Influencing WNV Transmission

Although blood-feeding patterns of vectors is a critical part of mosquito-borne WNV transmission and is the primary focus of this study, other ecological factors must be considered because they may mechanistically alter the relationships between the hosts, vector, and pathogen. These include land use over space and time and climate (Ciota 2017; Hamer et al. 2011; Hartley et al. 2012). Different species of mosquitoes have shown tendencies to prefer different habitat types, and mosquito habitat preference plays an important role in determining which populations of animals are most susceptible to WNV infection, based on their habitat (Thiemann et al. 2012b). Additionally, differential host and mosquito abundance are important to evaluate in different habitats to determine which populations of hosts are most at risk of transmitting WNV, and in which specific habitats. In addition to urban-rural differences, other studies have reported inverse associations between WNV in *Culex* mosquitoes and the percent of wetland cover, and positive associations between high avian diversity and low WNV incidence in humans (Hamer et al. 2011). This study highlights the importance of studying WNV in cities, because urban environments are characterized by less wetland area and lower avian diversity, which is associated with higher rates of WNV in *Culex* spp.

Climate and temperature have also been shown to significantly impact viral transmission, feeding behavior and host availability. In general, environmental temperature increases the viral replication rates in mosquitoes, and has been directly correlated with increased susceptibility and transmission rates; however, this is dependent on the mosquito species and virus (Ciota 2017; Kilpatrick et al. 2008). Increased temperature has also been directly linked to increased numbers of vectors able to transmit a pathogen, but the mechanism behind this is still being investigated (Kilpatrick et al. 2008). Precipitation patterns can indirectly influence the blood meal acquisition of mosquitoes by impacting their gonotrophic cycles, which is average number of days it takes mosquitoes to lay eggs after feeding on a bloodmeal (Ciota 2017; Mala et al. 2014). Previous studies have observed seasonal shifts of host preference in *Culex* mosquitoes, with blood-feeding patterns shifting feeding on almost exclusively on avian species in the early months of the summer, to increased feeding on mammalian species during late summer and early fall (Thiemann et al. 2011). These shifts are critical to evaluate because they can mark potential WNV outbreaks among certain populations in specific regions. It is believed that this potential shift in host feeding preference may have resulted from changes in the availability of preferred avian hosts within a specific region due to seasonal environmental changes. (Kilpatrick et al. 2006).

Blood-Feeding Patterns of *Culex* Mosquitoes

Understanding blood-feeding patterns and related factors can reveal where and when particular vertebrate hosts are at risk of infection, as well as which competent hosts may be able to maintain and amplify transmission of the WNV (McPhatter et al 2017; Thiemann et al. 2012b). *Culex* mosquitoes are known to be opportunistic feeders, primarily feeding upon a variety of bird species, and in some cases have also been found to fed on mammals, reptiles, and

amphibians (Molaei et al. 2007; Thiemann et al. 2012b; Zinser, Ramberg, Willott 2004). Common hosts for *Culex* mosquitoes are frequently highly competent species including American Robins (*Turdus migratorius*), House Sparrows (*Passer domesticus*), and American Crows (*Corvus brachyrhrnchos*), however variation in vertebrate species' competencies varies spatially and temporally (Apperson et al. 2004; Ciota et al 2017; Simpson et al. 2012). Another study evaluating host feeding preference in WNV endemic areas demonstrated that *Culex* mosquitoes preferentially feed on specific avian species independent of host density (Kilpatrick et al.2006). Inverse associations have also been demonstrated between non-passerine bird species richness and human WNV cases, meaning that when there is a broader diversity of bird species in an area, especially birds that are unable to amplify WNV virus, then the number of human WNV cases reportedly decreased (Hamer et al. 2011). This is also significant because it suggests that there is a potential "dilution effect" occurring in bird communities with higher diversity (Allen et al. 2008; Reisen, Lothrop, Thiemann 2013). In a study conducted by Allen et al., it was found that WNV infection in mosquitoes and WNV incidence in humans increased with lower bird diversity and increased vertebrate reservoir competence of the bird community which could indicate that high host diversity may reduce mosquito infection prevalence (Allen et al. 2008).

The frequency of utilizing mammals as hosts could be dependent on both vector genetics and host availability (Ciota 2017; Kilpatrick et al. 2006). For example, genetically distinct populations of a single species of vector sometimes show varying host feeding patterns (Ciota 2017). The transmission and devastation of vector-borne diseases such as WNV within a population is directly related to the feeding behavior and feeding preferences of the vectors themselves (Andreadis et al. 2012). Therefore, understanding the host preferences and blood-

feeding patterns of mosquitoes and how they change over space and time is essential to assessing their vector capacity within a given population (Apperson et al. 2004).

Chapter 2: Introduction

Mosquitoes are one of the biggest natural threats to human health in the world due to their ability to transmit a wide range of dangerous vector-borne pathogens to humans (Caraballo 2014; Omodior et al. 2018). In the United States, the mosquito genus *Culex* is primarily responsible for carrying the Flaviviruses: St. Louis encephalitis virus (SLEV), and West Nile virus (WNV) (*Flaviviridae*, *Flavivirus*), both of which are now considered endemic diseases due to repeated seasonal outbreaks of disease in humans and equines (Kwan et al. 2010). WNV has been detected in multiple *Culex* species, including the most common species in California: *Culex tarsalis*, *Culex pipiens*, and *Culex quinquefasciatus* (Andreadis et al 2012; Fechter-Leggett et al. 2012; Molaei et al. 201 0). The virus is primarily maintained in a natural transmission cycle between infected mosquitoes and competent avian species; however, WNV epidemic outbreaks have been found to occur when the virus is introduced into domesticated animal and human populations (Apperson et al. 2004; Molaei et al. 2010). *Culex* mosquitoes are known to be opportunistic feeders who tend to feed on a variety of avian species, mammalian species, and occasionally on some reptile and amphibian species, making them ideal vectors for WNV (Molaei et al. 2007; Thiemann et al. 2012b; Zinser et al. 2004).

In southern California, the most prominent WNV vectors are *Cx. tarsalis* and *Cx. quinquefasciatus*. These mosquitoes have repeatedly demonstrated their ability to drive the horizontal transmission of WNV during human outbreaks and maintain transmission among populations of birds between outbreaks (Ciota 2017; Reisen et al. 2013). Additionally, both mosquito species are locally abundant and have demonstrated seasonally high WNV field infection rates (Braack et al. 2018; Ciota 2017). Seasonal shifts in feeding patterns have been previously detected in *Culex* populations, with feeding patterns shifting from feeding almost

exclusively on avian species in the early months of the summer, to increased feeding on mammalian species during late summer and early fall (Ciota 2017; Molaei et al. 2007; Tempelis et al. 1965; Tempelis 1975; Thiemann et al. 2011). Seasonal shifts can also indicate potential WNV outbreaks among certain populations of humans and animals in specific regions (Kilpatrick et al. 2006). Other studies have demonstrated that habitat influences the blood-feeding patterns of *Culex* mosquitoes. A study from rural northern California observed low WNV infection rates at sites where *Culex* mosquitoes fed primarily on domestic cattle and incompetent galliform birds, and higher rates where the females fed on American Crows, Yellow-billed Magpies (*Pica nuttalli)*, and American Robins (Campbell et al. 2013). This study also found that WNV transmission was most efficient in urban environments, where there was relatively low avian species diversity and more efficient WNV transmission among competent passerine species. A study conducted in Coachella Valley, California, during 1998-2002 observed a potential dilution effect that limited virus amplification to levels that it would lead to outbreak of WNV in human and equine populations in rural environments (Reisen, Lothrop, Thiemann 2013).

Culex quinquefasciatus is the focus of the current study due to its local abundance, its involvement in previous WNV outbreaks, and its close proximity to diverse populations of both avian and mammalian populations. This is a collaborative study with the West Valley Mosquito and Vector Control District and aims to evaluate the host-feeding preferences of *Cx. quinquefasciatus* in four different habitat types, in San Bernardino County, to determine which populations of hosts are most at-risk of WNV transmission based on their environment. This information can be then used to determine which populations of birds and other animals found in the same geographical location are most at risk for WNV infection. Furthermore, this study

highlights the natural dynamics of vector borne disease transmission within San Bernardino County, which is extremely important because it is a heavily human-populated region and hosts a variety of diverse habitats composed of a rich variation of avian and mammalian species. The information derived from this study should assist local vector control districts in southern California in creating better surveillance methods and more efficient vector control intervention strategies by adding to the knowledge of *Cx. quinquefasciatus* feeding patterns.

The ultimate goal of this project is to further characterize the feeding patterns of *Cx. quinquefasciatus* in Southern California based on habitat type, avian host availability, and WNV activity. The first objective is to evaluate the differential host-feeding preferences based on habitat type. Because *Cx. quinquefasciatus* have generally been found in urban and suburban environments, and therefore near human populations, it is critical to understand the blood-feeding patterns of these mosquitoes in these habitats. Also, many of these samples were collected in rural environments, so the investigation into the feeding patterns of mosquitoes in these habitats will be important as well. The second objective is to observe potential shifts in seasonal blood-feeding patterns of this population of *Cx. quinquefasciatus*, which could differ depending on host species diversity during different months of the year and could affect the transmission of WNV to mammals. The last objective is to observe potential relationships between blood-feeding patterns and known WNV prevalence in Southern California.

Chapter 3: Methodology

Mosquito Collection

Approximately 960 individual *Culex quinquefasciatus* samples were collected in San Bernardino County as part of a WNV surveillance program conducted by the West Valley Mosquito and Vector Control District (WVMVCD). These mosquitoes were collected between January 18 and December 14, 2011 and were chosen because of the high frequency of the collections executed during this period as mosquitoes were collected weekly or biweekly by the WVMVCD. The collection methods employed by the district included CDC-style CO_2-baited encephalitis vector survey (EVS) traps, gravid traps, and resting boxes (Cummings 1992; Newhouse et al. 1966). The WVMVCD primarily used a novel mini resting box design that was developed at the district, and periodically used gravid traps and EVS traps to capture blood-fed mosquitoes (Cheng, M.L.et al. 2013). The mosquitoes in this study were collected from 32 different collection sites concentrated near the cities of Ontario and Chino in San Bernardino County (Figure 1). The collection sites were categorized as urban, suburban agriculture, rural, and rural-dairy based on characteristics such as anthropological land use, population density, and vegetation (McPhatter et al. 2017). Following collection, the mosquitoes were identified to species, anaesthetized using triethylamine, placed in individual Eppendorf tubes, and stored at -80°C.

DNA Extraction and Purification

Culex quinquefasciatus individuals were first inspected to determine the degree of bloodmeal digestion using the Sella scale. This scale classifies the blood-fed mosquito by the degree of digestion of the bloodmeal. The bloodmeal digestion is ranked 1-7, with 1 indicating a completely empty abdomen, and 7 indicating an abdomen full of eggs with no visible blood

(Detinova et al. 1962; Santos et al. 2019). The mosquitoes ranked 2-6 that presented dark or black blood in their abdomens were selected for DNA extraction. The mosquitoes were first smashed using a pestle in individual tubes, then genomic DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit© (Qiagen Inc., Valencia, CA), following standard protocols outlined by the manufacturer, and eluted in 20 µl of AE Buffer. The extracted DNA was stored in Eppendorf tubes at -20°C.

Nested Polymerase Chain Reaction

A nested PCR developed by Thiemann *et al.* (2012a) was used to determine the source of the bloodmeal for each individual mosquito sample. For the first PCR, the four primers used (Table 1a) were designed to flank the mitochondrial cytochrome-c oxidase gene (*COI*) to amplify a 1900 bp segment. The forward and reverse primers utilized were AvTrpF1, AvSerR1, MaTrpF1, and MaSerR1. To amplify products from all known and unknown DNA sources, a mixture of these 20 µM primers was used. For each 25 µl PCR reaction, 2 µl of DNA template, 0.75 µl of each primer, 15 µl of nuclease-free water, and 5 µl of 5x FIREPol® Master Mix (Solis BioDyne, Mountain View, CA) was used. The 5x FIREPol® Master Mix is a premixed solution containing FIREPol® DNA polymerase, 5X Reaction Buffer B (0.4 M Tris-HCL, 0.1 M $(NH_4)_2SO_4$, 0.1% w/v Tween-20), 12.5 mM $MgCl_2$, and 1 mM dNTPs (200 µM dATP, 200 µM dCTP, 200 µM dGTP, and 200 µM dTTP). The thermocycling parameters were as follows: 94°C for 5 min; 25 cycles of 94°C for 0:30 s, 61°C for 0:20 s, 72°C for 2:30 min; and 72°C for 5:00 min. For the second part of the nested PCR, primers and protocols from previously published papers were utilized to amplify the 658bp barcoding region of *COI*, using the first PCR product as DNA template (Cooper et al. 2007; Ivanova, Dewaard, Hebert 2006; Thiemann et al. 2012). The forward primers VF1, VF1d, and VF1i were mixed at a ratio of 1 VF1: 1 VF1d: 2 VF1i.

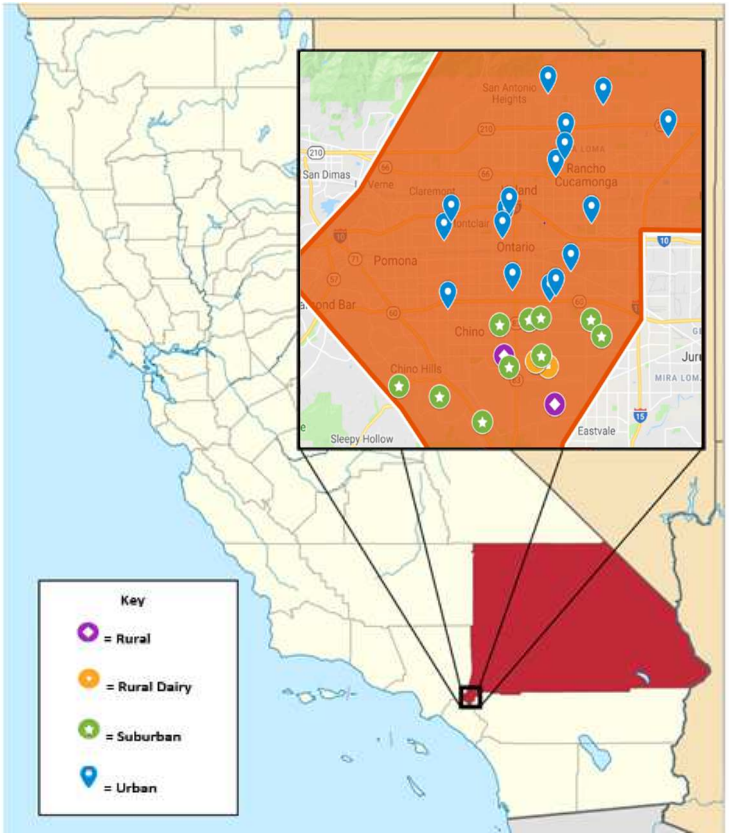

Figure 1: Geographic distribution of collection sites in San Bernardino County, CA during 2011. Shown on the map are two rural sites, three rural-dairy sites, ten suburban sites, and seventeen urban sites.

Table 1a: Primer information for PCR 1. The primers utilized were used to amplify a 1,900-bp segment of the *COI* mitochondrial gene. Degenerative code notation: Y= C/T, R= A/G, W= A/T, B= C/G/T. From Thiemann et al. 2012a.

Primer Name	**Primer Sequence**
AvTrpF1	5'-GGCCTTCAAAGCCTTAAAYAAGAGTT-3'
AvSerR1	5'-RRGGWWCGAYTCCTTCCTTTCTT-3'
MaTrpF1	5'-AGACCRAGRGCCTTCAAAGCYCT-3'
MaSerR1	5'-BRGGRGGTTCGATTCCTTCCTT-3'

Table 1b: Primer information for PCR 2. The primers utilized were used to amplify a 658-bp region of the *COI* mitochondrial gene. Degenerative Code Notation: Y= C/T, R= A/G, W= A/T. (I = Inosine; wobble base pair; not degenerative code) From Thiemann et al. 2012a.

Primer Name	**Primer Sequence**
VF1	5'-TTCTCAACCAACCACAAAGACATTGG-3'
VF1d	5′-TTCTCAACCAACCACAARGAYATYGG-3′
VF1i	5′- TTCTCAACCAACCAIAAIGAIATIGG-3′
VR1	5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′
VR1d	5′-TAGACTTCTGGGTGGCCRAARAAYCA-3′
VR1i	5′-TAGACTTCTGGGTGICCIAAIAAICA-3′

The target amplicon was amplified using Hotstar Taq® Plus DNA Polymerase (Qiagen Inc., Valencia, CA). Each reaction contained 1 µL PCR product from the previous reaction, 19.9 µL nuclease-free water, 0.5 µl dNTPs (50 µM of each dATP, dTTP, dCTP, and dGTP), 2.5 µl 10X buffer (Tris-Cl, KCl, $(NH_4)_2SO_4$, 15 mM $MgCl_2$), 0.5 µl of each primer mix (20 µM), and 0.1 µl of Hotstar Taq® DNA Polymerase (0.5 U). The cycling parameters were as follows: 95°C for 6:00 min; 30 cycles of 95°C for 0:30 s, 45°C for 0:15 s, 72°C for 0:30 s; and 72°C for 7:00 min. The PCR products were run on 1.5% agarose gels stained with GelGreen dye (Biotium, Fremont, CA). PCR products that did not produce bands were subject to two more trials of the nested PCR procedure.

Amplicon Purification

PCR products that exhibited a strong band during visualization of the DNA fragments were then treated with ExoSAP-IT® (Thermofisher, Santa Clara, CA) in accordance with the manufacturer's instructions to remove unwanted primers and dNTPs (Thiemann et al 2012a). The primer VF1d was used in the sequencing reaction. The purified products were sent for Sanger DNA Sequencing to Quintara Biosciences (South San Francisco, CA).

DNA Sequence Analysis

The sequences were identified to species using the Identify Specimen feature of the Barcode of Life Data Systems (BOLD; www.boldsystems.org). BOLD is an integrated bioinformatics database aiding the acquisition, storage, analysis, and publication of DNA barcode records, and is freely available to all researchers (Ratnasingham and Herbert 2007).

Bird Relative Abundance

The database eBird (https://ebird.org/explore), a network of bird observations reported primarily by citizens and compiled by the Cornell Lab of Ornithology, was utilized to determine

which bird species were present and the frequency of bird sightings in inland southern California during January-December 2011. This database reports the weekly frequency of avian species during a set time period to monitor avian prevalence. The reported frequency of a particular species represents the proportion of citizen-submitted checklists reporting specific species in a given area (Lagoze 2014; Thiemann et al. 2012b).

Detection of WNV in Non-Bloodfed Mosquitoes

Mosquitoes collected concurrently with those from this study were tested for WNV as a part of the WVMVCD surveillance program. Non-bloodfed mosquitoes were pooled based on species, collection site, and date of collection. The WVMVCD tested 1169 pools for the WNV using Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) at their on-site laboratory (Su 2017). All the pools were tested for the WNV envelope gene, which is a common gene target for WNV analysis (Herman 2015).

Statistical Analysis

Species richness was compared between the habitats using R-model for Rarefaction (https://rdrr.io/rforge/vegan/man/rarefy). This type of analysis accounts for the different sample sizes collected from each habitat. The Shannon's diversity index (H) was used as a measure of species abundance and richness to quantify host diversity within each habitat (Shannon 1948). Hutcheson t-tests were utilized to compare the diversity measurements between habitats (Hutcheson 1970). Species richness and Shannon diversity index (Shannon 1948) were also used as measures to determine if the feeding patterns of the mosquitoes directly affect WNV in urban environments. This was conducted by calculating the species richness and host diversity in urban collection sites where WNV-positive were collected, and in urban collection sites where WNV-positive pools were not collected. Comparisons between the two types of urban collection

sites were analyzed using Hutcheson t-tests (Hutcheson 1970). The feeding patterns of *Cx. quinquefasciatus* on selected host species in different habitat types were analyzed between habitats using chi-square (χ^2) testing as done in previous feeding pattern studies (Molaei et al. 2010; Thiemann et. al 2012b). Potential seasonal shifts between avian and mammalian species were assessed using contingency table chi-square (χ^2).

Chapter 4: Results

During 2011, blood-fed *Cx. quinquefasciatus* were collected from 32 (17 urban, 10 suburban, 2 rural, and 3 rural-dairy) locations in and around the cities of Ontario, Rancho Cucamonga, and Chino, of San Bernardino County, by the West Valley Mosquito and Vector Control District. In total, DNA was extracted from 739 of these bloodmeals, and 683 (92.4%) of the bloodmeals were successfully identified to host species.

Mosquito Collection

The collections in this study were made using gravid traps (GRVD), resting boxes (RB), and CDC-style encephalitis vector survey (EVS) traps (Cheng, M.L.et al. 2013; Molaei et al. 2007; Thiemann et al. 2012b). GRVD traps and RB traps were the most frequently reported trap types of the collected blood-fed mosquitoes, regardless of the habitat. Combined, gravid traps and resting box collections accounted for 94.7% of collections (27.9% and 66.9%, respectively), while EVS traps accounted for 5.3% of collections (Table 2).

Bloodmeal Analyses of *Culex quinquefasciatus*

Throughout the course of this study, 955 blood-fed mosquito samples were visually assessed for the presence of a bloodmeal. 216 (22.6%) of these samples were unusable because they were barely engorged, meaning only a partial meal was taken by the mosquito, or the abdomen was full of eggs and had no visible blood. In total, DNA was extracted from 739 samples, and 683 (92.4%) of these samples had the host bloodmeal source successfully identified. From urban habitats, 197 (90.0%) bloodmeals were positively identified. From suburban habitats, 117 (92.1%) bloodmeals were successfully identified. In rural and rural-dairy habitats, 178 (94.2%) and 191 (94.6%) positive bloodmeals were identified, respectively.

Table 2: Number of blood-fed *Cx. quinquefasciatus* individuals collected in each trap type, separated by habitat type. GRVD= Gravid Trap; RB= Resting Boxes; EVS= CDC-style encephalitis vector survey traps.

	Collection Method			
	GRVD Trap	RB Trap	EVS Trap	**Total**
Urban	119	74	4	**197**
Suburban	40	56	21	**117**
Rural	-	174	4	**178**
Rural-Dairy	44	147	-	**191**
Total	**203**	**451**	**29**	**683**

Overall, *Cx. quinquefasciatus* fed on 28 different avian (n=23) and mammalian (n=5) species across the four different habitat types.

Habitat Analysis

There were 29 different host species identified from *Cx. quinquefasciatus* bloodmeals from all habitats combined, and host species richness by habitat ranged from 10-17 different host species in any one habitat. Species richness represented in identified bloodmeals was not found to be significantly different between the habitats ($p>0.05$) when analyzed using the rarefaction model in R, which accounted for the differences in sample size between the habitats when rarefied to a sample size of 100 (Figure 2). Furthermore, Shannon diversity indices were calculated for each habitat and used to compare diversity between each habitat (Figure 2). The calculated Shannon diversity indices were as follows: Urban H= 1.764; Suburban H= 1.458; Rural H= 1.542; Rural-Dairy H= 1.247. Significant differences in host species diversity between habitats were found between urban and rural-dairy habitats ($t=4.312$, $df=386$, $P=0.00002$) and rural and rural-dairy habitats ($t=2.349$, $df=355$, $P=0.019$), but not between any other habitat comparisons. While there was no difference in host species richness, and only two instances of differences in host species diversity between habitats, the patterns of blood-feeding did vary by habitat as summarized here.

Urban. Of the 197 bloodmeals identified from *Cx. quinquefasciatus* bloodmeals in urban habitats, 176 (89. 3%) of the total bloodmeals were taken from avian hosts, and 21 (10.7%) were taken from mammalian hosts. Bloodmeals were identified from 15 different avian species and 2 different mammalian species (Table 3). The greatest number of bloodmeals were identified from House Sparrows (n= 77, 43.8% of avian bloodmeals; 39.1% of total bloodmeals) and House Finches (n= 62, 35.2% of avian bloodmeals; 31.5% of total bloodmeals). Bloodmeals from

House Sparrows were collected from 17 urban sites, while bloodmeals from House Finches were collected from 14 urban sites. Of the remaining avian host species, none accounted for more than 7.0% of total bloodmeals identified from urban habitats. These include Northern Mockingbirds (n= 11, 6.3 % of avian bloodmeals; 5.6% of total bloodmeals) and Domesticated Chickens (n= 7, 4.0% of avian bloodmeals; 3.6% of total bloodmeals). Avian species that were fed upon in urban habitats that were not identified in any other habitat include: American Crows, California Scrub Jays, Great Egrets, one Black-crowned Night-Heron, one Cockatiel, one Cooper's Hawk, and one White-crowned Sparrow. Mammalian bloodmeals were identified from 7 collection sites. The two mammalian species that were fed upon at urban sites are Domestic Dogs (n=13, 61.9% of mammalian bloodmeals; 6.6% of total bloodmeals) and Humans (n=8, 38.1 % of mammalian bloodmeals; 4.1% of total bloodmeals). Human bloodmeals were identified from 4 of the 17 urban collection sites.

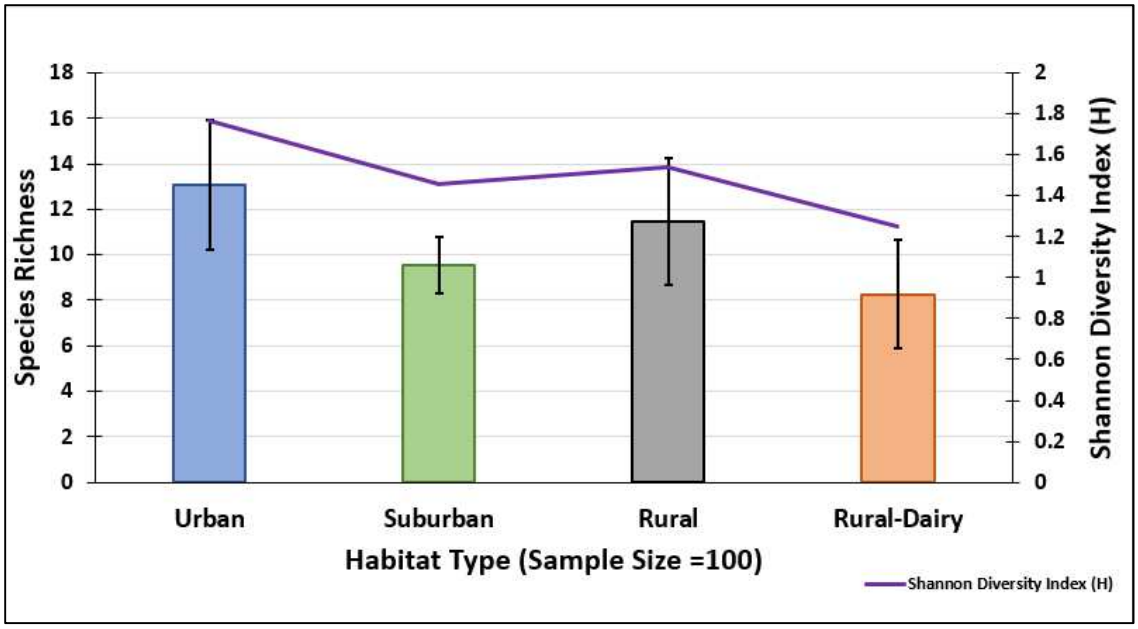

Figure 2: Rarefied host species richness by habitat accounting for sample size, using the Rarefaction Model in R. Purple line represents the calculated Shannon Diversity Index (H) for each habitat. Error bars represent the 95% confidence intervals. Analysis done using the vegan: Community Ecology Package (https://rdrr.io/rforge/vegan/man/rarefy).

Suburban. In suburban habitats, 117 bloodmeals were identified from *Cx. quinquefasciatus* mosquitoes. 100 (85.4%) of the total bloodmeals were taken from avian hosts, while 17 (14.6%) were taken from mammalian hosts. In these habitats, bloodmeals were identified from six different avian species and 4 different mammalian species (Table 4). Like urban habitats, House Sparrows (n= 54, 54.0% of avian bloodmeals; 46.2% of total bloodmeals) and House Finches (n= 36, 36.0% of avian bloodmeals; 30.6% of total bloodmeals) were the most frequently fed upon avian species at suburban collection sites. House Sparrows were fed upon at all 10 suburban collection sites and House Finches were fed upon at 8 of these collection sites. Other avian species that were fed upon multiple times at suburban sites include Domesticated Chickens (n= 4, 4.0% of avian bloodmeals; 3.4% of total bloodmeals) and Northern Mockingbirds (n= 4, 4.0% of avian bloodmeals; 3.4% of total bloodmeals). A sample was identified from a Double-crested Cormorant at one suburban site. This species was not identified as a host from any other habitat. Mammalian bloodmeals were identified from 4 different host species at 4 suburban collection sites. Domestic Dogs (n= 11, 64.7% of mammalian bloodmeals; 9.4% of total bloodmeals) accounted for the majority of the bloodmeals identified. The other mammalian species fed upon were Humans (n= 3, 17.6% of mammalian bloodmeals; 9.4% of total bloodmeals), Domesticated Cows (n= 2, 11.8% of mammalian bloodmeals; 1.7% of total bloodmeals), and one Domesticated Horse (5.9% of mammalian bloodmeals; 0.9% of total bloodmeals). The mosquitoes that fed on Domesticated Cows could have traveled from a dairy farm to a near-by suburban site, following bloodmeal acquisition. The three Human bloodmeals came from two suburban collection sites.

Rural. 178 bloodmeals were identified from *Cx. quinquefasciatus* samples from 2 rural collection sites, with 169 (94.9%) of the total bloodmeals deriving from avian hosts, and 9

(5.1%) of the total bloodmeals deriving from mammalian hosts. A total of 13 avian species and 2 mammalian species that were fed upon (Table 5). Both avian and mammalian bloodmeals were identified from both rural collection sites. The most frequently fed upon avian species was Domesticated Chickens (n= 94, 55.6% of avian bloodmeals; 52.8% of total bloodmeals). Other avian species that were commonly fed upon include House Sparrows (n= 34, 20.1% of avian bloodmeals; 19.1% of total bloodmeals) and House Finches (n= 23, 13.5% of avian bloodmeals; 12.9% of total bloodmeals). There were also individual bloodmeals identified that were taken from avian hosts that were not identified in any other habitats. These species include a Song Sparrow, a Greater Roadrunner, and a Zebra Finch. The mammalian species that were identified are Domesticated Cows (n= 8, 88.9% of mammalian bloodmeals; 4.5% of total bloodmeals) and a Coyote (11.1% of mammalian bloodmeals; 0.56% of total bloodmeals).

Rural dairy. Lastly, 191 bloodmeals were identified from *Cx. quinquefasciatus* collected from the three rural-dairy collection sites. Seventy-eight (40.8%) of the total bloodmeals were taken from avian hosts and 113 (59.2%) were taken from mammalian hosts. A total of 10 avian species and 1 mammalian species were fed upon in these habitats (Table 6). Avian and mammalian bloodmeals were identified from all 3 rural-dairy collection sites studied. The most frequently fed upon avian species in this habitat were House Sparrows (n=36, 46.2% of avian bloodmeals; 18.8% of total bloodmeals) and Domesticated Chickens (n= 27, 34.6% of avian bloodmeals; 14.2% of total bloodmeals). Other avian species that were identified in multiple bloodmeals include House Finches (n= 5, 6.4% of avian bloodmeals; 2.6% of total bloodmeals), Rock Pigeons (n= 3, 3.7% of avian bloodmeals; 1.7% of total bloodmeals), and Northern Mockingbirds (n= 2, 2.6% of avian bloodmeals; 1.0% of total bloodmeals). The avian species that were exclusively fed upon in rural-dairy habitats are Eurasian Collared-Doves and Western

Kingbirds. The only mammalian species that was fed upon in rural-dairy habitats is Domesticated Cows (n= 113, 100% mammalian bloodmeals; 59.2% of total bloodmeals).

Comparative habitat blood-feeding analysis. Across habitats, the percentage of feedings by *Cx. quinquefasciatus* was significantly different among many of the host species, even when accounting for sampling size difference between habitats (Figure 3). From any single site, the proportions of total bloodmeals was significantly different among all habitats for House Sparrows (χ^2=29.12, df=3, P<0.0001), Domesticated Chickens (χ^2=146.60, df=3, P<0.0001), and House Finches (χ^2=60.45, df=3, P<0.0001). In rural, rural-dairy, and suburban habitats, the proportion of total bloodmeals was significantly different for Domesticated Cows (χ^2=214.93, df=2, P<0.0001). It was also found that the proportion of total bloodmeals was significantly different for Humans (χ^2=8.00 df=1, P=0.005) between urban and suburban collection sites, which were the only habitat types that Human bloodmeals were identified from.

Table 3: Urban habitats. Number and percentage of the avian- and mammalian-derived bloodmeals identified from *Culex quinquefasciatus* (N= 197) in urban habitats in San Bernardino County, 2011. GRVD= Gravid Trap; RB= Resting Boxes; EVS= CDC-style encephalitis vector survey traps.

	Number/Collection Method					
Host	GRVD	RB	EVS	Species Total	% of Avian	% of Total
Avian						
House Sparrow, *Passer domesticus*	44	32	1	77	43.8	39.1
House Finch, *Haemorhous mexicanus*	45	16	1	62	35.2	31.5
Northern Mockingbird, *Mimus polyglottos*	7	4		11	6.3	5.6
Chicken, *Gallus gallus domesticus*	1	5	1	7	4	3.6
Mourning Dove, *Zenaida macroura*	3	1		4	2.3	2
American Crow, *Corvus brachyrhynchos*	1	2		3	1.6	1.5
California Scrub Jay, *Aphelocoma californica*	2			2	1.1	1
European Starling, *Sturnus vulgaris*		2		2	1.1	1
Great Egret, *Ardea alba*	1	1		2	1.1	1
American Kestrel, *Falco sparverius*			1	1	0.6	0.5
Black-crowned Night-Heron, *Nycticorax nycticorax*		1		1	0.6	0.5
Cockatiel, *Nymphicus hollandicus*		1		1	0.6	0.5
Cooper's Hawk, *Accipiter cooperii*	1			1	0.6	0.5
House Wren, *Troglodytes aedon*	1			1	0.6	0.5
White-crowned Sparrow, *Zonotrichia leucophrys*	1			1	0.6	0.5
					% of Mammalian	
Mammalian						
Domestic Dog, *Canis lupus*	6	7		13	61.9	6.6
Human, *Homo sapiens*	6	2		8	38.1	4.1

Table 4: Suburban habitats. Number and percentage of the avian- and mammalian-derived bloodmeals identified from *Culex quinquefasciatus* (N= 117) in suburban habitats in San Bernardino County, 2011. GRVD= Gravid Trap; RB= Resting Boxes; EVS= CDC-style encephalitis vector survey traps.

	Number/Collection Method					
Host	GRVD	RB	EVS	Species Total	% of Avian	% of Total
Avian						
House Sparrow, *Passer domesticus*	8	34	12	54	54	46.2
House Finch, *Haemorhous mexicanus*	19	15	2	36	36	30.6
Chicken, *Gallus gallus domesticus*	1	2	1	4	4	3.4
Northern Mockingbird, *Mimus polyglottos*	2	1	1	4	4	3.4
Double-crested Cormorant, *Phalacrocorax auritus*		1		1	1	0.9
House Wren, *Troglodytes aedon*	1			1	1	0.9
					% of Mammalian	
Mammalian						
Domestic Dog, *Canis lupus*	7	2	2	11	64.7	9.4
Human, *Homo sapiens*	2	1		3	17.6	2.6
Domesticated Cow, *Bos taurus*			2	2	11.8	1.7
Domesticated Horse, *Equus ferus caballus*			1	1	5.9	0.9

Table 5: Rural habitats. Number and percentage of the avian- and mammalian-derived bloodmeals identified from *Culex quinquefasciatus* (N= 178) in rural habitats in San Bernardino County, 2011. EVS= CDC-style encephalitis vector survey traps; RB= Resting Boxes.

	Number/Collection Method				
Host	EVS	RB	Species Total	% of Avian	% of Total
Avian					
Chicken, *Gallus gallus domesticus*	2	92	94	55.6	52.8
House Sparrow, *Passer domesticus*	2	32	34	20.1	19.1
House Finch, Haemorhous mexicanus		23	23	13.5	12.9
European Starling, *Sturnus vulgaris*		5	5	3	2.8
Rock Pigeon, *Columba livia*		3	3	1.8	1.7
Barn Owl, *Tyto alba*		2	2	1.2	1.1
Northern Mockingbird, *Mimus polyglottos*		2	2	1.2	1.1
American Kestrel, *Falco sparverius*		1	1	0.6	0.56
Greater Roadrunner, *Geococcyx californiancus*		1	1	0.6	0.56
Mourning Dove, *Zenaida macroura*		1	1	0.6	0.56
Red-tailed Hawk, *Buteo jamaicensis*		1	1	0.6	0.56
Song Sparrow, *Melospiza melodia*		1	1	0.6	0.56
Zebra Finch, *Taeniopygia guttata*		1	1	0.6	0.56
				% of Mammalian	
Mammalian					
Domesticated Cow, *Bos taurus*		8	8	88.9	4.5
Coyote, *Canis latrans*		1	1	11.1	0.56

Table 6: Rural-dairy habitats. Number and percentage of the avian- and mammalian-derived bloodmeals identified from *Culex quinquefasciatus* (N= 191) in rural-dairy farm habitats in San Bernardino County, 2011. GRVD= Gravid Trap; RB= Resting Boxes.

	Number/Collection Method				
Host	GRVD	RB	Species Total	% of Avian	% of Total
Avian					
House Sparrow, *Passer domesticus*	6	30	36	46.2	18.8
Chicken, *Gallus gallus domesticus*	7	20	27	34.6	14.2
House Finch, *Haemorhous mexicanus*	1	4	5	6.4	2.6
Rock Pigeon, *Columba livia*		3	3	3.7	1.7
Northern Mockingbird, *Mimus polyglottos*		2	2	2.6	1
American Kestrel, *Falco sparverius*		1	1	1.3	0.5
Eurasian Collared-Dove, *Streptopelia decaocto*		1	1	1.3	0.5
European Starling, *Sturnus vulgaris*	1		1	1.3	0.5
Red-tailed Hawk, *Buteo jamaicensis*		1	1	1.3	0.5
Western Kingbird, *Tyrannus verticalis*		1	1	1.3	0.5
				% of Mammalian	
Mammalian					
Domesticated Cow, *Bos taurus*	29	84	113	100	59.2

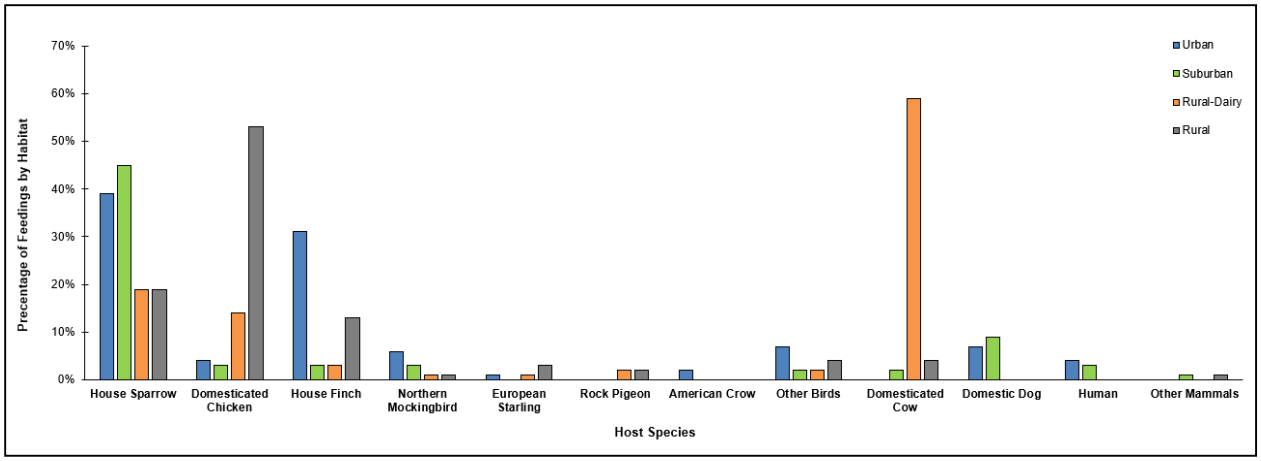

Figure 3: Proportions of vertebrate hosts of *Culex quinquefasciatus* in urban (N=197 feedings), suburban (N=117 feedings), rural-dairy (N=191 feedings), and rural habitats (N=178 feedings) in San Bernardino County, 2011.

Seasonal Bloodmeal Analysis

The seasonal blood-feeding analysis between habitats for *Cx. quinquefasciatus* during 2011 is shown in Figure 3. Statistical analysis was conducted using contingency table chi-square (χ^2) tests to observe shifts in mammalian bloodmeal prevalence from pre-September (January-August) to post-September (September-December). There was no significant change found in mammalian bloodmeal prevalence from pre-September to post-September in suburban, rural, and rural-dairy habitats (S: χ^2= 8.548, df=1, P= 0.003; R: χ^2= 1.931, df=1, P=0.165; RD: χ^2= 2.258, df=1, P=0.133) (Figure 4b, 4c, 4d). However, in urban habitats, there was a statistically significant increase in mammalian bloodmeals identified from post-September collections (χ^2= 8.548, df=1, P= 0.003) (Figure 4a).

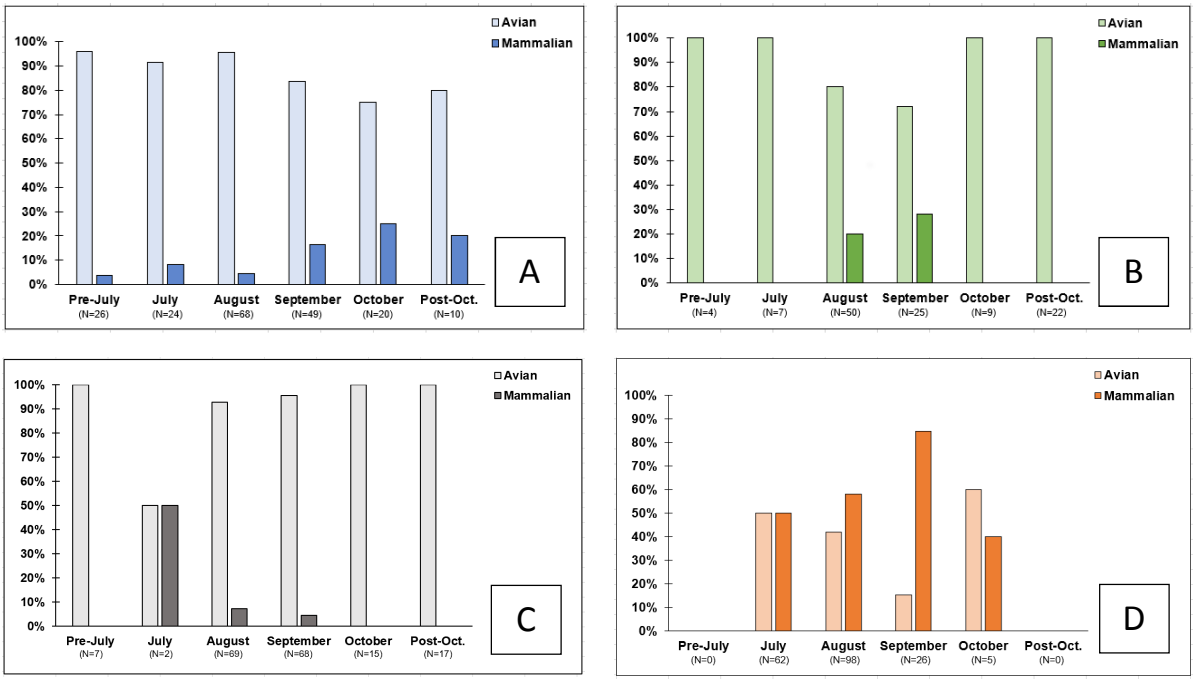

Figure 4: Percentage of identified *Cx. quinquefasciatus* bloodmeals obtained from avian and mammalian hosts collected in San Bernardino County, California, during 2011. "N" represents the number of total bloodmeals collected during each month. Lighter colored shades represent avian-derived bloodmeals and darker colored shades represent mammalian-derived bloodmeals. (A)Urban Habitats; (B) Suburban Habitats; (C) Rural Habitats; (D) Rural-Dairy Habitats.

Bird Population Estimates

The weekly frequency of occurrence of some common wild bird species found in San Bernardino County from January to December 2011 are shown in Figure 5. These data provide a general, qualitative look at the occurrence of common avian species across San Bernardino County but does not provide avian abundance at specific collection sites within each habitat studied. Certain birds such as the House Finch, American Kestrel, Mourning Dove, House Sparrow, and Red-tailed Hawk were frequently sighted throughout the year. According to the reported sightings, other bird species such as the American Crow, California Scrub-Jay, European Starling, Rock Pigeon, and White-crowned Sparrow demonstrated significant drops in frequency of occurrence during varying time periods throughout the year. This could have occurred because of the migratory patterns of the birds, or simply because these specific species were not in view of bird watchers during the time of reporting their sightings because according to the reported bird frequencies, most of these species are year-round inhabitants of this region (ebird.org).

West Nile Virus Activity

During 2011, 1169 pools of *Cx. quinquefasciatus* were tested for WNV RNA by the WVMVCD, revealing 114 pools were positive (Table 7). Ninety-four positive WNV pools were detected from 13 different urban collection sites. Eighteen positive pools were detected from 7 different suburban collection sites. Two positive pools were collected from 1 rural collection site. Host species diversity and host species richness were both analyzed for urban collection sites since these sites had the most WNV-positive pools collected from them. Host species richness for urban collection sites with positive-WNV pools is 9, while the host species richness for urban collection sites with negative pools is 12. The Shannon diversity indices were

calculated as 1.493 for urban collection sites with positive pools, and 1.723 for urban collection sites with negative pools. It was found that there was no significant difference in host species diversity between urban sites with WNV-positive pools and urban sites with no WNV-positive pools (t= 1.232, df= 116, P=0.221).

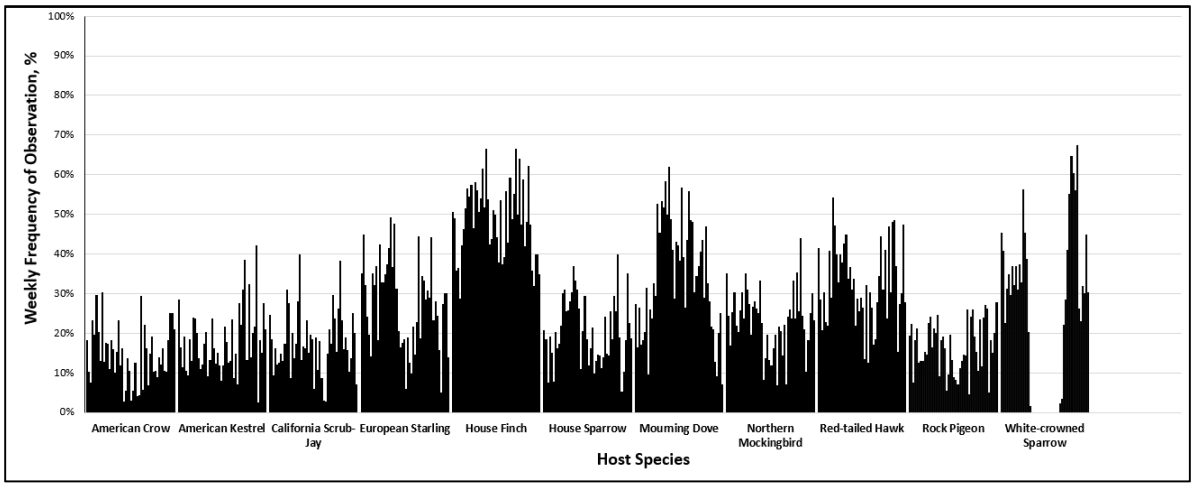

Figure 5: Weekly frequency estimates of avian species based on bird population analysis in San Bernardino County, 2011 (eBird).

Table 7: Number of *Cx. quinquefasciatus* pools that tested positive for WNV in San Bernardino County during 2011, separated by the collection month and habitat type.

Collection Month	No. Positive Pools- Urban	No. Positive Pools- Suburban	No. Positive Pools- Rural	**Total**
July	2			**2**
August	35	8	1	**44**
September	31	8		**39**
October	25	2	1	**28**
November	1			**1**
Total	**94**	**18**	**2**	**114**

Chapter 5: Discussion

This molecular and epidemiological study provides an insightful overview of the blood-feeding patterns of *Culex quinquefasciatus* in San Bernardino County. It contributes insight into the interactions among the mosquito vector, avian reservoirs, and "dead end" hosts such as domestic animals and humans. Throughout this study, the potential role of *Cx. quinquefasciatus* was examined in the enzootic and epidemic transmission of WNV, and its host-feeding patterns of a variety of vertebrate species were determined through the molecular sequencing of the mitochondrial cytochrome-c oxidase I gene (*COI*) of vertebrate species. It was found that *Cx. quinquefasciatus* fed on a broad range of avian and mammalian species and demonstrated temporal and spatial differences in feeding patterns, as previously demonstrated (Molaei et al. 2007; Molaei et al. 2010; Zinser et al. 2004). Differential host feeding patterns were examined between habitats—urban, suburban, rural, and rural-dairy collection sites— and throughout seasons within San Bernardino County, CA. These results were compared to bird population estimates and WNV prevalence in the county during 2011.

Mosquito Collection

Different types of collection methods were utilized by the WVMVCD to capture as many blood-fed mosquitoes as possible in the varying habitats. Each trap type is specifically designed to capture mosquitoes at different stages of their development and host-seeking by using an attractant to lure the mosquito to the trap. It is important to understand the differences between traps, because sampling method bias may be occurring based on the trap type used in the study (Thiemann and Reisen 2012). CDC-style encephalitis vector survey traps, which accounted for 5.3% of the collections, used CO_2 as an attractant for host-seeking mosquitoes. Typically, these mosquitoes have either taken a partial bloodmeal, which may be bias towards defensive hosts, or

have not taken a bloodmeal (Thiemann and Reisen 2012). Gravid traps, which accounted for many more of the mosquito collections in this study, are designed to attract gravid female mosquitoes who are looking for a water source to lay their eggs and have been shown in previous studies to successfully attract *Cx quinquefasciatus* (Kline et al. 2006; Reisen et al. 2004; Thornton et al. 2016). These traps have also demonstrated the ability to attract blood-fed mosquitoes that have not yet completely digested their bloodmeals, which was also the case in this study because they accounted for 27.9% of total collections. Resting boxes were the most prolific collection method utilized, accounting for 66.9% of all blood-fed *Cx. quinquefasciatus* collections. These resting boxes are typically placed on the ground in shady, open areas, and attract mosquitoes that are looking for a dark, cool place to rest and develop their eggs after taking a bloodmeal from a host (Crans 1989).

Molecular Bloodmeal Analysis of *Culex quinquefasciatus*

DNA was extracted from 739 of 955 *Cx. quinquefasciatus* blood-fed samples examined. The samples that were not subject to extraction did not present a visible bloodmeal, were too desiccated for use, or were completely gravid with no remaining trace of blood. 92.4% of the extracted bloodmeals were successfully identified through nested-PCR and DNA sequencing. The failed identification of 56 samples could be attributed to low levels of undigested blood within the engorged mosquitoes, or human error while performing the PCR techniques (Santos et al..2019). However, the latter is unlikely because each negative sample was subjected two additional rounds of PCR testing to account for such errors. In total, 28 different avian and mammalian species were identified as hosts of *Cx. quinquefasciatus* in San Bernardino County during 2011. These results are consistent with findings from other studies in different regions of

the United States that also demonstrated that *Cx. quinquefasciat*us feeds on a wide range of vertebrate species (Apperson et al. 2004; Edman 1974; Molaei et al. 2007; Molaei et al. 2010).

Habitat Host Species Analysis

Within the study region, the host species richness of each habitat ranged between 10-17 species. Each habitat was categorized based on characteristics such as population density, vegetation, and anthropological land use. These characteristics may have affected the number of host species present at each collection site and the geographic distribution of individual mosquitoes within these habitats (Meyer-Stieger et al. 2016; McPhatter et al. 2017). However, host species richness was found to be not significantly different between the habitats following rarefaction (Figure 2), so the differences observed between the habitats could have been due to sample number, not habitat type. Host species diversity was only found to be significantly different between urban and rural-dairy habitats and between rural and rural-dairy habitats (Figure 2). Within each habitat, avian bloodmeals were the most common and demonstrated diverse ranges of avian hosts being fed on, compared to mammalian hosts. In urban and rural habitats, 15 and 13 different avian species were fed upon, respectively. This shows that despite differences in habitat types, *Cx. quinquefasciatus* will feed on a broad spectrum of avian species.

Avian Bloodmeal Analysis

Across habitats, the highest percentage of avian bloodmeals were taken from the Passeriform species: House Sparrows (Urban: 39.1% of total bloodmeals; Suburban: 46.2% of total bloodmeals, Rural: 19.1% of total bloodmeals; Rural-Dairy: 18.8% of total bloodmeals) and House Finches (Urban: 31.5% of total bloodmeals; Suburban: 30.6% of bloodmeals; Rural: 12.9% of total bloodmeals; Rural-Dairy: 2.6% of bloodmeals). These Passeriform species were previously determined to be common hosts of *Cx. quinquefasciatus* in other studies and have

demonstrated their ability to be competent hosts of WNV (Edman 1974; Garcia-Rejon et al. 2010; Molaei et al. 2010; Reisen et al. 2005). Another bird species that was fed upon in every habitat were Domestic Chickens (Urban: 3.6% of total bloodmeals; Suburban: 3.4% of bloodmeals; Rural: 52.8% of total bloodmeals; Rural-Dairy: 14.2% of total bloodmeals). The proportion of total bloodmeals for these three species was statistically significant between habitats potentially indicating differential feeding on hosts present in each type of environment. Nevertheless, the abundance of these bird species was not measured at each habitat type, so concrete preferential feeding patterns on these hosts by the mosquitoes could be concretely ruled as significant. There were unique bloodmeals identified from 13 different avian host species that were not found in any other habitat. This may have been observed because of the broad diversity and differential abundance of hosts present in each habitat and collection site. A few bloodmeals were identified in urban and suburban collection sites from bird species commonly found in wetland habitats, including Great Egrets, a Double-crested Cormorant, and a Black-crowned Night Heron. The most likely reason these bloodmeals were identified from these sites is because after feeding in a wetland or riparian area, the mosquito may have been able to fly a short distance to an urban or suburban habitat. It has been found that on average, *Cx. quinquefasciatus* can travel less than 100 meters following bloodmeal acquisition (Greenberg et al. 2013).

Furthermore, there are avian species that were frequently identified as hosts in other *Cx. quinquefasciatus* studies that were either not identified in any bloodmeals or were rarely identified. In a study conducted in another region of Southern California, Mourning Doves and American Robins were identified as frequently fed upon hosts (Molaei et al 2010). A different *Cx. quinquefasciatus* study conducted in Harris County, Texas also noted that Mourning Doves

and American Robins were fed upon in higher proportions than many other species (Molaei et al. 2007). However, American Robins were not identified as hosts in this study. Additionally, bloodmeals identified from Mourning Doves were only found in 2 habitats (U: 2.0% of total bloodmeals; R: 0.56% of total bloodmeals), even though they were frequently sited throughout the region in 2011 (Figure 5). This could indicate that Mourning Doves were under-utilized hosts, especially since they were spotted by vector control technicians at some of the collection sites. However, more research must be done on the relationship between Mourning Doves and *Cx. quinquefasciatus* in this region to draw any substantial conclusions.

Mammalian Bloodmeal Analysis

In total, bloodmeals were identified from 5 mammalian hosts across all habitats. Bloodmeals identified from Domesticated Cows were found in 3 habitats (S: 1.7% of total bloodmeals, R: 4.5% of total bloodmeals; R-D: 59.2% of total bloodmeals), bloodmeals from Domestic Dogs were identified in 2 habitats (U: 6.6% of total bloodmeals; S: 9.4% of total bloodmeals), one sample from a Coyote was identified from a rural collection site, and one sample from a Domesticated Horse was identified from a suburban collection site. In previous studies conducted, Domestic Dogs were a frequently fed upon host of *Cx. quinquefasciatus* (Garcia-Rejon et al. 2010; Molaei et al. 2007). It was concluded that this was a credible finding because Domestic Dogs were common in the region. This could also be the case for this current study, since many of the collection sites were in residential areas of San Bernardino County. However, the prevalence of dogs within this region must be further examined. Domesticated Cows were by far the most frequently fed upon mammalian species because they are very prevalent at the dairy farms and in rural regions of San Bernardino County. The feedings on Domesticated Cows were found to be statistically significant between habitats. There are many

parts of the county that are open farmland that were not technically considered rural-dairy collection sites in this study, which could also contribute to the large number of identified feedings on this host throughout the county. Human bloodmeals accounted for a small number of total bloodmeals throughout the study region and were only fed upon in two habitats (U: 4.1% of total bloodmeals; S: 2.6% of total bloodmeals), even though there is a large population of humans in the county. This is consistent with findings in previous papers as well where human bloodmeals accounted for very few bloodmeals identified (Molaei et al. 2007; Reisen et al. 1990). However, it was found that the proportions of feedings on humans between urban and suburban habitats was significantly different.

Seasonal Bloodmeal Analysis

In addition to *Cx. quinquefasciatus* bloodmeal analysis by habitat, seasonal bloodmeal analysis was also investigated in this study to analyze any significant shift in mammalian feeding throughout the season. Seasonal shifts in the host-feeding patterns of *Cx. quinquefasciatus* have been reported in previous studies, with blood-feeding shifting from primarily on avian species in early summer to higher proportions of mammalian host feeding in mid-late summer (Molaei et al 2007; Tempelis 1975). This shift is important to analyze because it can mark potential WNV outbreaks among certain populations of animals in specific environments (Kilpatrick et al. 2006). These studies demonstrated significant decreases in avian-derived bloodmeals from June until October. A previous study conducted in California concerning the species *Cx. tarsalis*, also observe a significant shift towards blood-feeding on mammals during September, which corresponded to the seasonal migration of common birds in that region (Thiemann et al. 2011). Out of the four habitats studied, the only significant difference between mammalian bloodmeals identified from collections made during pre-September to post-September occurred in urban

habitats (χ^2= 8.548, df=1, P= 0.003) (Figure 4). This is important because urban habitats typically have denser human populations than other habitats, which was demonstrated by the number of human bloodmeals identified in urban habitats compared to the other three habitats.

West Nile Virus Activity

As part of their WNV surveillance program in 2011, the WVMVCD tested pools of up to 50 mosquitoes using RT-qPCR. Of the 1169 pools they tested, 114 were positive for WNV. These positive pools came from 21 different collection sites in 3 of the 4 habitats studied (U: 94 positive pools; S: 18 positive pools; R: 2 positive pools), indicating that WNV was present in the region, but was not particularly widespread. Additionally, host species diversity was found to not be significantly different between urban collection sites with WNV-positive pools and urban collection sites with no WNV-positive pools.

West Nile Virus activity in the local wild bird population was monitored by the WVMVCD by testing brain samples from dead birds using RT-qPCR for evidence of infection (Su 2017). In 2011, 52 dead birds were collected in response to reports from citizens of San Bernardino County and various animal control agencies by phone calls directly to the vector control district, or to the California Department of Public Health Dead Bird Program Hot Line or website agency (McCaughey et al. 2003). It was found that 18 out of 52 dead birds collected by the district tested positive for WNV. There were WNV-positive samples identified from 16 American Crows, 1 House Sparrow, and 1 House Finch. These results are in line with the high frequencies of reported sightings of these species in the area, and with previous studies, where avian species in the families of Corvids and Passeriforms were suggested to be amplifying hosts of WNV that were important for infecting *Cx. quinquefasciatus* to facilitate virus transmission to humans (Nemeth et al. 2009; Reisen et al. 2006; Wheeler et al. 2009).

The reported incidence of clinical West Nile virus infection was 4 cases in San Bernardino County during 2011 (Westnile.ca.gov 2019). Additionally, 2011 was a particularly low year for WNV prevalence in California. The total number of reported WNV cases was 158, while in the following years (2012-2017), reported WNV cases ranged from 379-801. In San Bernardino County, from 2012-2017, the reported number of WNV cases ranged from 8-57. The years exhibiting greater numbers of cases could have possibly seen this increase because the outbreaks of WNV occurred in areas or into populations where there was little or no background immunity to the virus (Johnston and Conly 2000). Furthermore, the variation in the number of WNV cases could be due to ecological factors such as increased temperature, higher mosquito abundance, and higher prevalence of competent host species in areas near human populations. Even though most cases of WNV infection are asymptomatic, it could be expected that in a population of over 2.1 million people, more clinical cases of WNV disease would be reported (Molaei et al. 2007). This trend may be occurring because the mosquitoes prefer to not feed on humans; however, a more likely reason is that during warmer months, people tend to stay indoors more often and are less likely to be exposed to mosquitoes during peak activity (Molaei et al. 2007; Molaei et al. 2010).

Conclusion

The results of this study confirm findings from previous studies show *Cx. quinquefasciatus* to be an opportunistic feeder, feeding on a variety of avian and mammalian species. Across all habitats observed—urban, suburban, rural, and rural-dairy collection sites—and throughout the year, the mosquito primarily fed on WNV-competent hosts such as House Sparrows and House Finches, but also were found to feed on many other avian hosts and on mammalian species such as Domesticated Cows, Domestic Dogs, and Humans. The prevalence

of WNV discovered in non-bloodfed mosquito pools collected from these sites further demonstrates that *Cx. quinquefasciatus* is one of the main vectors of WNV in Southern California. Because of species' association with human hosts and avian hosts, it is concluded that the species has continued to be involved in the enzootic transmission of the virus among birds, and incidentally to mammalian hosts. These mosquitoes must continue to be properly surveilled and controlled to protect the communities of animals and humans residing in the area.

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