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Blood-Feeding Behavior of Culex tarsalis at Medicine Lake National Wildlife Refuge, Montana

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Abstract

It is known that the ornithophillic mosquito, *Culex tarsalis* is the primary vector of West Nile Virus (WNV) in Montana. An analysis of this mosquito's blood feeding behavior allows researchers to identify preferred hosts and to determine the rate at which this species parasitizes humans and other tangential hosts. Additionally, an understanding of blood feeding behavior allows an assessment of WNV risk based on the regional availability of preferred hosts and the hosts' competency in viral amplification. In this study, blood engorged *Cx. tarsalis* were captured at Medicine Lake National Wildlife Refuge over three one-week periods spaced through early, middle, and late summer. Vertebrate DNA was isolated from mosquito blood meals and a fragment of the COI gene was PCR amplified to determine host species. Additionally, avian surveys were conducted at mosquito collection sites so that each bird's relative abundance could be compared to its incidence in blood meals. The two most common host species were the Mourning Dove comprising 59% of blood meals and the American Robin comprising 33%. These species were ranked 4th and 14th, respectively in observed abundance out of the 47 species counted in this study. These results suggest that *Cx. tarsalis* at Medicine Lake NWR exhibits a feeding preference for these two species.

Introduction

West Nile Virus (WNV) is an arbovirus that is spread through an enzootic cycle between mosquito vectors and bird hosts (Bell et al., 2006). The analysis of blood-feeding behavior of competent vector mosquitoes allows the determination of host preferences and the prediction of zoonotic transmission pathways (Molaei et al., 2008; Simpson et al., 2011). Additionally, the surveillance of vector blood-feeding behavior reveals parasitism of human and other mammalian hosts which allows the prediction of tangential infections (Kilpatrick et al., 2006; Simpson et al., 2011).

The ornithophilic mosquitoes of the *Culex* genus, including *Cx. tarsalis* in Montana, are the primary mosquito vectors of West Nile Virus (Brault, 2009; Hale, 2007). Through blood feeding, a virulent mosquito can infect a competent host which can in turn amplify the virus and infect subsequent blood feeding mosquitoes (Kent et al., 2009). While most mammals and birds are susceptible to WNV infection, very few mammals are capable of producing sufficient levels of viremia to infect blood feeding mosquitoes and even among birds, only certain species are virulent competent hosts (Savage et al., 2007). Though *Culex* mosquitoes feed primarily on birds, they will also opportunistically feed on mammals, creating a risk of WNV infection for humans and livestock (Apperson et al., 2002). Studies on *Cx. tarsalis* in Colorado and California and *Cx. pipiens* in Illinois, Tennessee, and Connecticut document shifts in *Culex* feeding behavior between early and late summer (Diuk-Wasser et al., 2010; Hamer et al., 2009; Kilpatrick et al., 2005 and 2006; Molaei et al., 2008). Tempelis et al. (1965; 1967) determined that *Cx. tarsalis* in both California and Colorado fed increasingly on mammalian species in late summer, though bird species were still the most common

hosts. A more recent study in Colorado by Kent et al. (2009) also showed an increase in mammalian-derived *Cx. tarsalis* blood meals in August with Columbiformes (doves) comprising the most utilized blood source.

Studies on *Cx. pipiens* in the Midwest, South, and Eastern United States identified the American Robin, *Turdus migratorius* as the most common host species (Apperson et al., 2002; Diuk-Wasser et al., 2010; Kilpatrick et al. 2006; Molaei et al., 2008). In urban and suburban areas of Maryland and Washington D.C., Kilpatrick et al. (2006) demonstrated a seasonal shift in *Cx. pipiens'* host preference from robins to humans in late summer. This study suggested that the seasonal dispersal of robins caused *Cx. pipiens* to pursue mammalian hosts, a behavior shift that could trigger WNV epidemics, assuming an infected mosquito population. Other studies on *Cx. pipiens* in Illinois and Tennessee, however, showed no such definitive bird to mammal host shifts (Hamer et al., 2009; Savage et al., 2007). Both Hamer et al. (2009) and Savage et al. (2007) show that *Cx. pipiens*' use of mammal hosts remains constant throughout the summer, but that decreases in the use of robins do occur. In Illinois, Hamer et al. (2009) notes that the percentage of robin-derived blood meals declined as summers progressed. Savage et al. (2007) in Tennessee did not find this end-of-summer shift, most likely because robins are year-round residents of Tennessee. Instead, the study found periodic changes in blood feeding behavior that suggested robins were most heavily used during periods of nesting. Collectively, all of these studies show significant regional variation in the blood feeding behavior of *Culex* mosquitoes. While *Cx. pipiens* appears to exhibit a preference for American Robin hosts, it is not known whether populations of *Cx. tarsalis* would show similar partiality. Additionally, the studies on *Cx. pipiens* behavior were largely

conducted in urban and suburban habitats, so the feeding habits of more rural *Culex* populations are mostly unknown (Hamer et al., 2009; Kent et al., 2009).

Though regional host preferences for doves and robins were found in *Culex* populations, studies suggest that these preferences are not based entirely on host abundance. In the area of Colorado studied by Kent et al. (2009), the House Sparrow (*Passer domesticus*) was the most abundant avian species, but appeared in an average of only 4.73% of *Cx. tarsalis* blood meals surveyed throughout the summer. Similarly, Hamer et al. (2009) found House Sparrows, Common Grackles (*Quiscalus quiscula*), and European Starlings (*Sturnus vulgaris*) to be under-utilized as hosts according to their regional abundances. Certain species (avian and mammal) are more tolerant of *Culex* blood feeding than others due to defensive behaviors or morphologies (Darbro and Harrington, 2007; Edman et al., 1974; Molaei et al., 2008). Edman et al. (1974) recorded the defensive behaviors of a wide variety of birds and mammals experimentally exposed to *Cx. nigripalpus*. Defensive behaviors were characterized as any movement (e.g. pecking or ruffling of feathers) made to deter, kill, or avoid mosquitoes. The study showed that generally larger hosts attracted more mosquitoes, but the most tolerant hosts (those exhibiting the least defensive behavior) were fed upon most heavily by *Cx. nigripalpus*. Of the birds tested, passerines were generally found to be the least mosquito tolerant, while the Black-crowned Night Heron (*Nycticorax nycticorax*) and the Great Horned Owl (*Bubo virginianus*) exhibited the fewest defensive behaviors and were the most heavily fed upon. Several other studies also found Black-crowned Night Herons and congeneric Yellow-crowned Night Herons (*Nycticorax violacea*) to be very tolerant of parasitism by *Culex* mosquitoes (Maxwell and Kale, 1977; Stamm, 1958; Webber and

Edman, 1972). Darbro and Harrington (2007) exposed both House Sparrows and juvenile chickens (*Gallus gallus*) to *Cx. pipiens* and recorded more successful parasitism on the sparrows. Additionally, the rate of feeding success on sparrows by *Cx. pipiens* was significantly higher than that for *Cx. nigripalpus* (82% compared to 24%) as recorded by Edman et al. (1974). The significance of these data is not clearly understood. While *Cx. pipiens* is apparently capable of effectively parasitizing House Sparrows, studies of the natural blood feeding behavior of *Culex* mosquitoes show consistent under-utilization of sparrows relative to their abundance (Hamer et al., 2009; Kent et al., 2009; Kilpatrick et al., 2006).

The hypothesis that *Culex* mosquitoes preferentially select more vulnerable hosts is supported by studies that show or imply blood-parasitism of less mobile aggregated nesting and fledgling birds. In addition to the aforementioned studies by Hamer et al. (2009) and Savage et al. (2007) suggesting *Cx. pipiens*' use of nesting robins, Griffing et al. (2007) used video surveillance to observe that nesting robins attracted significant numbers of host-seeking mosquitoes. Studies of mosquito parasitism on nesting hosts are primarily performed in order to determine whether nesting aggregates are a source of WNV amplification (Loss et al., 2009 a.). Diuk-Wasser et al. (2010) found that large, communally roosting groups of robins were heavily parasitized by *Cx. tarsalis*, thereby allowing rapid amplification of WNV. Similarly, communally roosting American Crows (*Corvus brachyrhynchos*) have been subject to significant WNV mortality in California and Illinois, but it is not known whether the virus is amplified through elevated mosquito parasitism, or oral transmission from the preening behavior common to crows (Reisen et al., 2006; Ward et al., 2006). Thiemann et al. (2011) found that *Cx. tarsalis* at a farmstead

in Northern California primarily parasitized nesting Black-crowned Night Herons. Reisen et al. (2008) recorded WNV infection among juvenile herons at the same location, but the infection rate was not high enough to consider heron nesting colonies to be WNV amplification foci. Black-crowned Night Herons produce sufficient levels of viremia to transmit WNV, so their competency as a reservoir or amplifying host remains debated (Komar et al., 2003; Reisen et al., 2008). Another instance of nest parasitism by *Cx. tarsalis* is found in large nesting colonies of American White Pelicans (*Pelecanus erythrorhynchos*) in Montana and North Dakota (Rocke et al., 2005). Johnson et al., (2010) detected WNV in both juvenile pelicans and *Cx. tarsalis* at Medicine Lake National Wildlife Refuge (the site for this study) and through DNA sequencing of blood meals, determined that all of the mosquitoes analyzed had fed on pelicans. The high rate of WNV infection among the juvenile pelicans strongly suggests that these birds are a preferred host species. The study also tested for an association between WNV infected pelican colonies and tangential human infection rates, concluding that counties hosting infected colonies had a significantly higher (5X) risk of human infection than those without infected colonies (Johnson et al., 2010). Whether *Cx. tarsalis* exhibit a genuine preference for juvenile American White Pelicans or if they feed on the birds only according to their abundance is unknown. Additionally, an infected colony's capability of amplifying WNV, or the possibility of subsequent infections by host-alternating *Cx. tarsalis* is not understood (Johnson et al., 2010).

Because a reliance on virulent competent hosts (especially passerines) is likely to be required to sustain WNV epizootics, regional abundances of incompetent hosts is hypothesized to decrease WNV amplification and corresponding human infection risk

(Brault, 2009; Ezenwa et al., 2005; Ostfeld and Keesing, 2000). Studies in Louisiana of WNV infection rates in *Culex* mosquitoes and local bird diversity surveys by Ezenwa et al. (2005) showed that non-passerine abundance was negatively correlated with mosquito infection. This study suggests a "dilution effect" in which the availability of non-passerine hosts provides *Culex* with an alternative food source that prevents the viral amplification that would occur if passerines were the primary available hosts (Ezenwa et al., 2005; Hamer et al., 2006). The evidence that *Culex* do not select hosts based only on abundance implies that the presence of a preferred host species incapable of WNV amplification could mitigate local WNV infection risk. Alternatively stated, the presence of non-passerine bird species susceptible to mosquito parasitism could have a zooprohylactic effect on WNV (Edman et al., 1974; Ezenwa et al., 2005; Hamer et al., 2009).

Loss et al. (2009 b.) evaluated both avian diversity and WNV prevalence in Chicago, Illinois to test for the negative correlation proposed by the dilution model. The results did not show a negative correlation, though the study's measure of avian diversity did not include gulls, waterfowl, and raptors, all of which are potential hosts and have varying degrees of virulent competency (Komar et al., 2003). The investigation did, however, make the important assertion that the dilution model assumes nonselective utilization of competent and incompetent hosts by vector mosquitoes. The role of any vertebrate host in the maintenance, amplification, or dampening of WNV's enzootic cycle is entirely dependent on that species' exploitation by blood-feeding mosquitoes (Molaei et al., 2008; Hamer et al., 2011). A more accurate assessment of the dilution effect or simply tangential WNV infection risk would ideally establish any preferences in

blood-feeding behavior of vectors and then address these preferences in conjunction with overall avian diversity and host competence.

The present study attempts to examine the blood feeding behavior and preferences of *Cx. tarsalis* at Medicine Lake National Wildlife Refuge (Sheridan County, Montana) through the DNA analysis of mosquito blood meals and an evaluation of avian abundance and diversity. It is hypothesized that the most utilized hosts will be those that are both vulnerable (e.g. nesting or communally roosting species) and locally abundant.

Materials and Methods

Study Location

The study was conducted at Medicine Lake National Wildlife Refuge in Sheridan County, Montana. The refuge complex occupies 12829 hectares at approximately 590 m in elevation and holds the 3326-hectare Medicine Lake. Mosquito collections and avian surveys were conducted at three sites on the refuge: the refuge headquarters, the hedgerow near the researcher's camping area, and Bridgerman Point, the site of the American White Pelican nesting colony. Sites were chosen based on their proximity to water and the presence of tree canopy. The trapping locations at the headquarters and the camping area held predominantly Vetch (*Vicia Spp.*) and Russian Olive (*Elaeagnus angustifolia*) while Bridgerman Point's canopy consisted primarily of American Elm (*Ulmus Americana*) and Chokecherry (*Prunus virginiana*).

Mosquito Collections

Collections were conducted over three separate weeks throughout the summer: 7/10/2012 - 7/13/2012, 7/24/2012 – 7/27/2012, and 8/6/2012 – 8/10/2012. At each site, mosquitoes were collected using 9 by 9-inch wood fiber pots (Western Pulp Products, Corvallis, OR) spray-painted black on the inside following Komar et al. (1995). Pots were placed in groups of two, facing opposite directions, under thick shrub or tree canopy cover. Where possible, the pots were placed between branches or stalks at the bases of shrubs or trees, or otherwise recessed into the surrounding undergrowth. Pairs of pots were all at least 3 m apart. The number of pots per location depended on the amount of suitably dense foliage. Eighteen pots were used at the headquarters site, six at the camping area, and twenty at Bridgerman Point. Resting mosquitoes were aspirated from the pots using a Craftsman Heavy Duty Hand-Held Aspirator (BioQuip Products, Rancho Domingo, CA) and immediately stored at -20°C. Pots were aspirated in the morning, mid-day and evening. Of the captured mosquitoes, only female *Culex tarsalis* were retained for blood-meal analysis.

DNA Extraction

Captured *Culex tarsalis* were examined under a dissecting microscope for blood meals. Blood engorged abdomens were removed with a sterile razor blade and placed

individually into 1.5 ml microcentrifuge tubes. The abdomens were then homogenized with a flame-sterilized glass rod in 0.5 ml of DNA-zol BD solution (Molecular Research Center, Cincinnati, OH). DNA was isolated from the homogenate according to the DNA-zol BD Manufacturer Protocol (2008) with the following exceptions: All volumes were halved, 4 µl of Polyacryl Carrier (Molecular Research Center, Cincinnati, OH) were added during the lysis step, and 3 µl of HEPES were added to neutralize the NaOH after the DNA solubilization step. The extraction samples were stored at -20°C.

Blood-meal Analysis

Blood meal sources were determined by polymerase chain reaction (PCR) amplification of a 648 base pair fragment of the vertebrate mitochondrial cytochrome c oxidase 1 (COI) gene. The COI fragment was amplified using a 50 pmol forward primer cocktail of VF1 t1, VFid t1, and VFli t1 and a 50 pmol cocktail of reverse primers VR1 t1, VRid t1, VFli t1, both mixed at a ratio of 1:1:2 respectively, according to Ivanova et al. (2007). Each DNA sample was amplified in a 50 µl reaction volume containing 6 µl of the DNA template, 1 µl each of the forward and reverse primer cocktails, 25 µl of GoTaq Colorless Master Mix (Promega, Madison, WI), and 17 µl of nuclease free water. Amplification cycles were 1 min at 94°C; 5 cycles at 94°C for 30 sec, 50°C for 40 sec, 72°C for 1 min; 35 cycles at 94°C for 30 sec, 54°C for 40 sec, 72°C for 1 min; and 72°C for 10 min according to Ivanova et al. (2007).

Amplification products were visualized on a 1.2% agarose gel containing 1.5 µl ethidium bromide with a 100 base pair ladder and blue/orange 6x loading dye (Promega, Madison, WI). After visualization, the PCR products were cleaned by adding 10 µl of

ExoSAP-IT (USB Corporation, Cleveland, OH) to each reaction, then thermocycling for 1 cycle at 37°C for 15 min and 80°C for 15 min. After cleaning, samples were sequenced by Macrogen in Gayang-Dong Seoul, Korea. The sequences were then compared with GenBank's nucleotide sequence database using the Basic Local Alignment Search Tool (BLAST).

Avian Surveys

Birds were counted and identified at each trap location in 15 minute point surveys in the morning and evening concurrent with mosquito trapping. Species were identified by sight and sound in plots centered on each site's fiber pots and encompassing a 25 m radius around each pot. Dead birds and birds flying higher than 10 m overhead were not counted in the surveys. The relative abundance of each species was calculated, and then compared to its prevalence in blood meals. A pooled Spearman Rank analysis was used to test for a relationship between the relative abundance of each bird observation in the field and its relative abundance in blood meals. Additionally, the effects of location (each of the three survey sites) and time (each of the three survey weeks) were tested for their effects on total bird abundances using single factor Analysis of Variance (ANOVA).

Results

The primary aim of this investigation was to evaluate *Culex tarsalis'* use of avian hosts at Medicine Lake NWR. A total of 115 *Cx. tarsalis* were captured from the study

site. Nineteen of these were from the camp site, 30 from Bridgerman Point, and 66 from the refuge headquarters. After the 115 DNA samples were extracted and sequenced, 31 produced incomplete sequences from which a host species could not be determined. Of the remaining 84 samples, 66 returned avian sequences from 6 different species, the Mourning Dove, the American Robin, the Orchard Oriole (*Icterus spurious*), the House Wren (*Troglodytes aedon*), the Canada Goose (*Branta canadensis*), and the Chestnut-collared Longspur (*Calcarius ornatus*). The non-avian blood meals were from a cow (*Bos taurus*), n=1 and sheep (*Ovis aries*), n=13. Table 1 shows the number of blood meals from each of the six birds and the relative abundance of each in both the blood meals and in observation.

Table 1. Summary of Blood Meals

Species	Total Blood Meals	Blood Fed Relative Abundance	Observed Relative Abundance
Mourning Dove (*Zenaida macroura*)	39	0.591	0.0851
American Robin (*Turdus migratorius*)	22	0.333	0.0107
Orchard Oriole (*Icterus spurius*)	2	0.0303	0.001254
House Wren (*Troglodytes aedon*)	1	0.01515	0.00237
Canada Goose (*Branta canadensis*)	1	0.01515	0.000557
Chestnut-collared Longspur (*Calcarius ornatus*)	1	0.01515	0

In order to determine if *Cx. tarsalis* exhibited any preference among hosts, the densities and relative abundances of birds at the trapping locations were measured. The avian surveys catalogued 47 species. Table 2 summarizes all avian observations in the study. The Chestnut-collared Longspur was the only species to appear in a blood meal,

but not in observations. While not enumerated, cattle were frequently seen on the refuge, but sheep were not.

Table 2. Avian Observations and Relative Abundances

Species	Total =	Observed Relative Abundance =
American White Pelican (*Pelecanus erythrorhynchos*)	2137	0.2977
Yellow-headed Blackbird (*Xanthocephalus xanthocephalus*)	1160	0.1616
Brown-headed Cowbird (*Molothrus ater*)	880	0.1226
Mourning Dove (*Zenaida macroura*)	611	0.0851
Common Grackle (*Quiscalus Quiscula*)	419	0.0584
Bank Swallow (*Riparia riparia*)	352	0.0490
Western Kingbird (*Tyrannus verticalus*)	242	0.0337
Eastern Kingbird (*Tyrannus tyrannus*)	218	0.0304
Franklin's Gull (*Leucophaeus pipixcan*)	212	0.0295
Ring-billed Gull (*Larus delawarensis*)	143	0.0199
Barn Swallow (*Hirundo rustica*)	128	0.0178
Double-crested Cormorant (*Phalacrocorax auritus*)	101	0.0141
Clay-colored Sparrow (*Spizella padilla*)	82	0.0114
American Robin (*Turdus migratorius*)	77	0.0107
California Gull (*Larus californicus*)	51	0.0071
Yellow Warbler (*Dendroica petechia*)	47	0.0066
Chipping Sparrow (*Spizella passerina*)	39	0.0054
Grasshopper Sparrow (*Ammodraus savannarum*)	37	0.0052
Common Yellowthroat (*Geothlypis tricha*)	33	0.0046
Song Sparrow (*Melospiza melodia*)	28	0.0039
Cedar Waxwing (*Bombycilla cedrorum*)	23	0.0032
Red-winged Blackbird (*Agelaius phoeniceus*)	19	0.0027
Gray Catbird (*Dumetella carolinensis*)	17	0.0024
House Wren (*Troglodytes aedon*)	17	0.0024
Ring-necked Pheasant (*Phasianus colchicus*)	15	0.0021
Spotted Sandpiper (*Actitis macularius*)	11	0.0015
American Avocet (*Recurvirostra americana*)	11	0.0015
American Goldfinch (*Spinus tristis*)	10	0.0014
Orchard Oriole (*Icterus spurius*)	9	0.0013
Killdeer (*Charadrius vociferus*)	8	0.0011

Western Meadowlark (*Sturnella neglecta*)	6	0.0008
Warbing Vireo (*Vireo gilvus*)	6	0.0008
Short-eared Owl (*Asio flammeus*)	4	0.0006
Savannah Sparrow (*Passerculus sandwichensis*)	4	0.0006
Canada Goose (*Branta canadensis*)	4	0.0006
Brown Thrasher (*Toxostoma rufum*)	3	0.0004
LeConte's Sparrow (*Ammodramus leconteii*)	3	0.0004
Willet (*Tringa semipalmata*)	2	0.0003
Black-crowned Night Heron (*Nycticorax nycticorax*)	2	0.0003
European Starling (*Sturnus vulgaris*)	2	0.0003
Mallard (*Anas platyrhynchos*)	2	0.0003
Horned Lark (*Eremophila alpestris*)	1	0.0001
Northern Harrier (*Circus cyaneus*)	1	0.0001
Northern Flicker (*Colaptes auratus*)	1	0.0001
Chestnut-collared Longspur (*Calcarius ornatus*)	0	0

The Spearman Rank Correlation Coefficient (ρ) was 0.02 with a p-value of 0.865, indicating no significant correlation between relative abundance in the field versus blood meals. A second Spearman Rank analysis was performed comparing the relative abundance observed in the field and relative blood-fed abundances of only the six species found in blood meals. These data sets showed a stronger, but still statistically insignificant relationship with $\rho=0.82$ and a p-value of 0.5.

The ANOVA results testing for a seasonal effect showed no significant difference between the observed abundances during the three separate weeks of sampling. The analysis of the three locations, however, showed that Bridgerman Point exhibited significant variance ($F=15.66$, $F_{crit}=5.79$, $p=0.007$) from the other two locations. This suggests that sampling location had a significant effect on the observed abundances of birds in this study. From the avian observations, the two primary species that contributed to this difference are the American White Pelican and the Yellow-headed Blackbird, the

two most abundant species in this study. All of the pelicans except two (2135 out of 2137) were observed in the large breeding colony at Bridgerman Point. All 1160 of the Yellow-headed Blackbirds were observed in large flocks at this location.

Discussion

The results of this study suggest that *Cx. tarsalis* at Medicine Lake NWR exhibit a feeding preference for Mourning Doves. This observation is consistent with the findings of Kent et al. (2009) and Hess and Hayes (1970) in Colorado, and Tempelis et al. (1967) in California. The reasons for *Cx. tarsalis*' partiality towards doves are not fully understood, but Kent et al. (2009) propose that the unique brooding behavior of these birds makes them particularly susceptible to mosquito parasitism. Mourning Doves are multiple brooders, capable of rearing several clutches throughout a summer and typically build simple, sparse nests in low canopy cover (Westmoreland et al., 1986). During avian surveys in the present study, Mourning Dove nests containing either juvenile doves or eggs were observed at all three of the trapping locations. Though it is not known that *Cx. tarsalis* feeds preferentially on juvenile doves, the minimal feather coverage and sedentary nature of fledglings would likely educe increased parasitism from mosquitoes.

In experimental infections of birds with WNV, Komar et al. (2003) characterized adult Mourning Doves as relatively poor reservoir hosts, finding them to produce significantly lower viremias than all passerine species tested. Kent et al. (2009), however, suggest that doves can be a major source of WNV amplification because their extensive

use by *Cx. tarsalis* would likely result in higher rates of infection than would occur in populations of less preferred birds with higher viral competency. Though no studies have specifically addressed the WNV reservoir competency of juvenile doves, Mahmood et al. (2004) determined that fledgling Mourning Doves were more competent reservoirs of St. Louis encephalitis, a related flavivirus, than were adults.

The second most utilized host in this study was the American Robin, which comprised one third of all avian blood meals. In avian surveys, robins were ranked 14th in abundance with only 77 individuals observed throughout the study. The low relative abundance of this species paired with its high prevalence in blood meals implies that it is a preferred host of *Cx. tarsalis*. In Colorado, Kent et al. (2009) also found robins to be the second most prevalent *Cx. tarsalis* blood meal source and found that during several samplings (late June through mid July) robins were overused according to their abundance at a rate higher than were doves. This period appears to correspond with the nesting season of robins which could indicate that *Cx. tarsalis* target robin broods as Hamer et al. (2009) determined for *Cx. pipiens*. In the present study the largest sampling of robin blood meals (14) was taken in August, so nesting vulnerability was not likely a factor in *Cx. tarsalis*' preference. Though not abundant in surveys, the robins were typically observed in large groups of six or more in dense foliage. This communal behavior may have contributed to the robin's susceptibility, but larger groups of other passerines, especially Yellow-headed Blackbirds (*Xanthocephalus xanthocephalus*) were more frequently observed and were not found in blood meals. As a passerine, the American Robin is a very competent WNV reservoir and its exploitation by *Cx. tarsalis* would likely make it a significant contributor to WNV amplification and maintenance

(Kent et al., 2009; Komar et al., 2003). The overuse of robins by *Cx. tarsalis* at Medicine Lake (22 blood meals per 77 observations) is higher than that of Mourning Doves (39 blood meals per 611 observations), suggesting that *Cx. tarsalis* may actually prefer robins to doves, but is limited in blood feeding by their relative scarcity.

Of the remaining five avian blood meals, two were from the Orchard Oriole. This result is noteworthy in that other icterids, the Yellow-headed Blackbird, the Brown-headed Cowbird (*Molothrus ater*), and the Common Grackle (*Quiscalus quiscula*) were the second, third, and fifth most abundant species observed respectively, while the Orchard Oriole ranked 29th in abundance. The factors contributing to *Cx. tarsalis*' preference for the Orchard Oriole, but not the other much more prevalent icterids is unknown.

The Chestnut-collared Longspur was the only species to be identified in a blood meal, but not in the avian surveys. Though this species was not seen in the thick vegetation at the survey points, it was commonly observed in open fields throughout the refuge and therefore, is not considered an abnormal result.

Few conclusions can be drawn from the non-avian blood meals collected in this study. The single cow-derived blood meal was obtained in late summer, but the overall increase in blood meals with the summer's progression makes it insignificant as evidence of a shift in blood feeding behavior toward mammals as described by Hamer et al. (2009), Kent et al. (2009), and Savage et al. (2007). Due to the lack of observation of sheep on or near Medicine Lake NWR, the validity of the sheep-derived blood meals is

questionable. Additionally, the sequence matches on GenBank showed relatively low query coverage, possibly indicating incorrect matches or contamination.

Analysis of variance between the three trapping locations in this study revealed that Bridgerman Point hosted significantly higher numbers of observed birds than the other two sites. This variation could be obviously attributed to the large breeding colony of American White Pelicans and numerous flocks of Yellow-headed Blackbirds on the point. It was at this location that WNV symptomatic American White Pelicans, California Gulls (*Larus californicus*), Eastern Kingbirds (*Tyrannus tyrannus*), and Double-crested Cormorants (*Phalacrocorax auritus*) were observed. None of these species was found in *Cx. tarsalis* blood meals. Particularly odd was the absence of pelican blood meals because juvenile pelicans were abundant and seemingly vulnerable in large, sedentary groups. Additionally, large numbers (>50) of these young pelicans exhibited symptoms of WNV that would strongly suggest heavy parasitism by *Cx. tarsalis*. Previous analysis of mosquitoes at Bridgerman Point by Johnson et al. (2010) found that all of the 14 analyzed *Cx. tarsalis* had fed on pelicans. In the present study, the absence of pelican blood meals could indicate an avoidance by *Cx. tarsalis* which could also imply an alternate mode of WNV transmission among pelicans. However, fewer than half of the blood meals obtained from Bridgerman Point returned readable sequences, so further analysis and a larger sample size are required to draw more definitive conclusions.

In summary, this study shows that *Cx. tarsalis* at Medicine Lake NWR targets some hosts at a rate disproportionate to their relative abundance. The Mourning Dove and the American Robin appear to be preferred hosts. Both of these species (especially the robin) are capable of maintaining and amplifying WNV and could significantly

contribute to epizootic infections. This investigation did not document *Cx. tarsalis'* use of American White Pelicans and did not observe significant parasitism of tangential mammalian hosts. A larger sample size and broader seasonal sampling are needed to more accurately determine trends in the blood feeding behavior of *Cx. tarsalis* at this location.

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