Spatio-Temporal Effects of Adult Mosquito Control on Vector Abundance and West Nile Virus Transmission

By

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Abstract

West Nile virus (WNV) can cause a potentially fatal neuroinvasive mosquito-borne disease. The virus is maintained in an enzootic cycle between birds and *Culex* mosquitoes but can spillover to cause infections in horses and humans. Approximately 80% of human infections are asymptomatic, but 20% develop a febrile illness and <1% result in a neuroinvasive disease which can result in long-term physical and mental disabilities. During periods of high risk for human infections, insecticides are used to rapidly reduce the abundance of infectious mosquitoes in proximity to humans, thereby reducing zoonotic transmission risk. However, the degree to which mosquito populations are reduced following insecticide applications is highly variable and difficult to measure in operational settings. New vector control strategies, like ivermectin (IVM), a drug that increases mosquito mortality when ingested, are under investigation to improve the specificity of control strategies. This dissertation assesses the spatio-temporal impacts of aerial applications of insecticides and bird-delivered ivermectin on *Culex* mosquito populations and WNV transmission dynamics.

In Chapter 1, we developed generalized additive models to estimate the duration and magnitude of reduction in abundance of *Culex (Cx.) tarsalis* and *Cx. pipiens*, the primary WNV vectors in California, following application of aerial adulticides in Sacramento and Yolo counties from 2006-2017. Aerial applications are utilized during periods of epidemic risk to rapidly reduce the abundance of infectious mosquitoes in proximity to humans. The efficacy of these applications for reducing *Culex* mosquito abundance is difficult to assess for single events due to stochastic variation in trapping success as well as natural variation in mosquito abundance due to season, land use, and weather. Peridomestic *Cx. pipiens* populations were reduced to a greater extent compared with *Cx. tarsalis* after both single and repeated spray events, likely due to the species' focal distribution within urbanized areas. In contrast, impacts of aerial sprays on *Cx. tarsalis* populations are likely diluted by the species' large dispersal ability and the broad distributions of productive larval habitat present in the study area.

In Chapter 2, we conducted a randomized field trial to investigate the impacts of a novel WNV control strategy, IVM-treated backyard chickens, on mosquito population and WNV transmission

dynamics. We placed eight flocks (4 treated and 4 untreated) of six chickens each in backyards across Davis, California for the WNV season (Jun-Sep 2019). We detected a reduction in WNV seroconversions in treated flocks, paired with increased mortality in wild *Cx. tarsalis* feeding on treated chickens and a reduction in parity in female mosquitoes near treated flocks, suggest that there was a reduction in WNV transmission around treated vs. untreated flocks resulting from ivermectin-administration. We did not detect a difference in abundance or WNV infection rates in mosquitoes.

In Chapter 3, we developed a spatially implicit dynamical model of WNV transmission in the presence of IVM-treated birdfeeders to assess the feasibility and efficacy of treating backyard birds with IVM to reduce local WNV transmission. Using field-collected data on birdfeeder usage and nocturnal roosting habits of common backyard birds to parameterize the model, we estimated that reductions up to 83.9% in infectious mosquito-days and 61.3% in infections in competent birds could be obtained under ideal conditions. Both the probability of IVM-induced mosquito mortality and the number of treated lots strongly affect the magnitude of reduction while the spatial distribution of treated lots within a neighborhood did not. Increasing the total number of treated birds in a neighborhood, irrespective of WNV competency, reduced WNV transmission intensity, indicating that IVM deployment should target a wide variety of common backyard bird species.

Taken together, the results of this dissertation provide new insights into the efficacy of existing and novel methods of vector control for reducing mosquito abundance and WNV transmission. An improved understanding of these processes will inform future vector control strategies, providing more efficient and targeted approaches to prevent arboviral transmission.

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Introduction

West Nile virus (WNV) is a potentially fatal, neuroinvasive mosquito-borne virus belonging to the Japanese encephalitis serogroup of flaviviruses (family *Flaviviridae*) [1]. Other viruses in this serogroup that commonly cause human disease include Japanese encephalitis virus, St. Louis encephalitis virus, and Usutu virus. Originally isolated in Uganda in 1937 [2], WNV caused sporadic cases and epidemics in Africa, Eurasia, and the Middle East over the next several decades [3,4]. In 1999, the virus appeared for the first time in the western hemisphere when it invaded Queens, New York, causing large-scale mortality in birds and 59 identified human cases with 7 deaths [5]. Subsequently, the virus underwent a rapid range expansion, spreading across the contiguous United States, Canada, Mexico, Central and South America, and the Caribbean over the next five years [6–9]. WNV is now the most widely distributed encephalitic flavivirus with evidence of transmission on all continents except Antarctica [4] and is the leading cause of mosquito-borne disease in the United States over the last twenty years [10]. In 2003, WNV invaded southern California and rapidly spread to all counties and has become endemic [11–14]. Since its invasion (1999-2019), 51,801 cases and 2,390 fatalities [15] have been reported across the United States with the total number of infections estimated to have exceeded 7 million [16]. While average annual incidence is highest in the northern plains states east of the Rocky Mountains, California reports more disease than any other state (15% of total reported U.S. cases) [17,18].

Approximately 80% of human infections are asymptomatic with the majority of symptomatic cases experiencing a febrile illness characterized by acute onset of fever, headache, fatigue, malaise, muscle pain, and weakness [19]. Less than 1% of all infections result in West Nile neurologic disease with manifestations including meningitis, encephalitis, or acute flaccid paralysis. This severe form of the disease has a 10% case fatality rate of approximately 10% and often results in long-term physical and mental sequelae [20]. Risk of severe disease increases with age and is associated with other comorbidities such as cardiovascular disease, chronic renal disease, hepatitis C viral infection, and immunosuppression

[21]. WNV disease has a high economic cost with a median cost of $142,321 per patient with the majority (68.3%) of charges borne by government programs [22].

WNV is maintained in an enzootic cycle between birds and mosquitoes, predominantly in the genus *Culex*, and can spillover into mammals, like horses and humans, both of which are dead-end hosts as they do not achieve high enough viremias to infect biting mosquitoes [23–26]. At least 300 bird species and more than 30 mammal species have been found to be infected with WNV, but a much smaller number of species likely play an important role in maintenance and amplification [25,27,28]. The most competent bird species, those obtaining high enough viremias to infect feeding mosquitoes, belong to the orders Passeriformes (songbirds) and Charadriiformes (shorebirds) [25,29]. Corvids, especially crows and jays, exhibit high fatality rates [29,30]. However, many bird species do not develop symptoms, despite reaching high viremias, and acquire lifelong immunity [31]. The risk of spillover increases as the number of infectious birds and competent bridge vector mosquitoes in proximity to humans increases.

The identity of amplifying and bridge vector mosquito species varies by location and sometimes season, modifying the local risk of transmission. Mosquitoes that preferentially feed on birds, like *Culex* (*Cx.*) *pipiens* and *Cx. quinquefasciatus*, tend to be efficient maintenance and amplification vectors while more opportunistic feeders generally serve as bridge vectors, infecting humans and horses [24], but species can act in multiple roles. The feeding habits of some species, like *Cx. nigripalpus* and *Cx. tarsalis* [32,33], are known to shift from primarily ornithophilic to more general throughout a season such that they would shift from amplification to bridge vectors, resulting in increased risk for spillover transmission in late summer [24]. In western North America, the primary enzootic and epizootic vectors are *Cx. tarsalis* and *Cx. pipiens* complex mosquitoes [11,24,34,35]. Female *Cx. tarsalis* breed in recently created and relatively clean rural water sources like recently flooded wetlands and rice fields [36–38], but also exploit urban locations like abandoned swimming pools [39]. Upon emergence, *Cx. tarsalis* adults may fly into urban areas seeking blood meals [40]. In contrast, *Cx. pipiens* typically breed in eutrophic artificial water sources like peridomestic containers, dairy lagoons, and storm drain systems [36,41–43], and are often most abundant in urban and suburban areas. When a female mosquito ingests an infected

blood meal, the virus must escape the midgut, disseminate throughout the body and infect the salivary glands before the mosquito can transmit the virus during subsequent blood meals [44]. The extrinsic incubation period, time from ingestion to infectiousness, is temperature dependent and shortens as temperature increases [45], so risk increases during the summer months.

Licensed, effective WNV vaccines exist for horses, but not for humans, [3] so options to prevent transmission to humans involve minimizing human-mosquito interaction through personal protective measures (i.e., wearing long sleeves, avoiding being outside at dawn and dusk when mosquitoes are active, and using insect repellent) and vector control [46,47]. In California, in response to the threat posed by mosquitoes and to protect public health, the Mosquito Abatement Act of 1915 codified the mechanism for establishing local vector control districts throughout the state [48]. These districts, along with others elsewhere in the United States, utilize integrated pest management strategies to target mosquitoes throughout their lifecycle to reduce the chance of arboviral transmission to humans. Effective control for mosquito-borne diseases begins with surveillance of mosquito populations and disease activity in both vectors and hosts [47,49]. Surveillance of adult mosquitoes commonly involves trapping host-seeking females in CO_2-baited traps and gravid females in gravid traps. Pools of collected mosquitoes are tested for viral RNA [50], indicating the presence of infected mosquitoes. Monitoring for the presence of WNV in wild birds also provides an early warning indication of the presence of the virus in local bird-mosquito cycles and detection of antibodies in sentinel flocks of chickens often indicate high risk of WNV transmission to humans [51]. Regular, systematic trapping and testing over many years allows for the identification of deviations from expected abundance trends or presence of a mosquito-borne pathogen, signaling the need to implement vector control measures. Typical WNV control strategies involve public education and source reduction, biological control with mosquito fish, spot treatments of adult and larval insecticides, or truck-mounted adulticide sprays [52,53]. Larval control measures are generally used in a proactive manner while adult-targeted measures are used reactively to rapidly respond to high-risk situations [47]. Larval control is typically more accepted by the general public. Previous studies support the effectiveness of larviciding catch basins, a common larval *Culex* habitat, to reduce the abundance of

larvae [54,55], but environmental conditions and suboptimal catch basin design can significantly reduce the efficacy [56,57] and larviciding alone is insufficient to effectively control mosquito populations and curb WNV transmission [58]. In periods of epidemic risk, emergency large-scale aerial application of ultra-low volume adulticide insecticides are employed to eliminate infected female mosquitoes in proximity to humans and break the chain of transmission [49,59]. Previous studies have found that aerial spraying reduces mosquito abundance and infection prevalence for arboviruses like WNV [60–62], St. Louis encephalitis virus [63], and western equine encephalomyelitis virus [64] and has been linked to a reduction in human WNV cases in a treated versus an untreated area [65]. However, estimates of the impact of single treatment events on abundance vary widely.

While the "gold standard" for evaluating the efficacy of control strategies is randomized controlled trials, these are not always practical or ethical to employ for the evaluation of aerial spraying; vector control and public health officials do not wish to withhold control measures from any areas with a high level of zoonotic risk. Additionally, the results from such experimental studies do not necessarily correspond to results obtained under operational field conditions. The Before/After Control/Impact (BACI) framework, originally developed in environmental science literature [66–69], uses observational data to capture changes in the natural history of a location due to some impact, like vector control [70,71]. Under the BACI framework, an impact location and at least one separate control location, sampled at various time points before and after the impact, are compared using an ANOVA to distinguish natural variability from the result of the impact [67–69]. An extension of BACI evaluation that is commonly applied to evaluate vector control, called "Mulla's Formula", utilizes mosquito surveillance data to estimate the impact of single treatment events. This method compares pre- to post-treatment numbers of collected mosquitoes inside the treatment zone and in an adjacent untreated area [72]. However, the assumptions of this method are often violated due to the underlying connectivity of highly vagile mosquito populations and stochastic variation in night-to-night trapping success unrelated to control activities, causing wide variation in estimates across space and time, with some studies even indicating increases in abundance following treatment [73–75]. Mortality of caged sentinel mosquitoes, used

estimate the impact of sprays on mosquito populations at different distances from the application equipment and in different vegetation habitats, are often paired with changes in local trap counts to estimate the effect, but these may not represent the exposure of wild mosquitoes to the insecticides. Additionally, the duration of reduction following insecticide treatment has not been studied beyond qualitative comparisons that a reduction lasted longer than or at least as long as a certain number of hours or days following spraying. No unified method exists for estimating the magnitude and duration of expected reduction in abundance achieved through aerial spraying under field conditions.

Overall, adulticide applications have limited precision to target mosquitoes involved in enzootic and zoonotic transmission without disseminating pesticides over large areas, thereby increasing the possibility of non-target effects despite precise tuning of applications to times of peak activity of target mosquitoes [52,76–79]. Widespread insecticide applications also face increasing levels of insecticide resistance in mosquito populations which render control measures ineffectual [80–82] as well as intense skepticism by some members of the public who fear the health impacts of large-scale application of pesticides, prompting the development of alternative products and strategies.

Several novel mosquito control strategies in other systems revolve around releasing modified mosquitoes to either suppress local populations or modify the vector to become refractory to pathogen transmission. Techniques like sterile insect technique (SIT), incompatible insect technique (IIT), and release of insects carrying a dominant lethal (RIDL), aim to achieve population suppression through repeated releases of large numbers of male mosquitoes that cannot produce viable offspring when mating with wild female mosquitoes [83]. With many generations of releases, the local vector population should be substantially reduced, thereby reducing disease transmission. Under SIT, males are sterilized with radiation or chemicals prior to release. In IIT, reared populations are infected with *Wolbachia pipientis* (*Wolbachia* hereafter), an endosymbiotic bacterium, and when infected males mate with females infected with a different strain of *Wolbachia* or none at all, no offspring survive due to cytoplasmic incompatibility. Accidental releases of females would undermine the success of each of these techniques so highly tune sex-sorting procedures are required. Overall, combined SIT and IIT techniques hold

promise to reduce disease transmission and encouraging results have already been reported in some locations [84,85]. The most successful RIDL strategy developed by the company Oxitec uses transgenic *Aedes* (*Ae.*) *aegypti* males that, when mated to wild females, produce offspring that die prior to emergence as an adult [86]. Field releases of these transgenic males in the Cayman Islands and Brazil resulted in 80-95% reduction in populations in small areas [87,88]. For sustained suppression, continual releases of modified mosquitoes need to occur due to immigration of wildtype mosquitoes from surrounding areas. Population replacement techniques involve releases of both male and female mosquitoes carrying a heritable trait that reduces or blocks their ability to transmit pathogens such that when these modified mosquitoes mate with wild mosquitoes, the trait spreads through the population, transforming it and thereby reducing disease transmission [83]. One leading method involves releasing both male and female *Ae. aegypti* infected with *Wolbachia*. *Wolbachia* infection in *Ae. aegypti* has been shown to limit transmission of dengue, Zika, and chikungunya viruses [89–91] and has been shown to stably infect populations following introduction (94-98% average infection 2-years post-release) because infected females have a reproductive advantage due to cytoplasmic incompatibility [92]. Another population modification strategy uses gene drive systems like CRISPR-Cas9 to introduce genes mediating disease-resistance [93,94] or sterility [95–97] into populations in a self-perpetuating manner. Development of similar population suppression or modification techniques for *Culex* have lagged with only recent studies illustrating successful manipulation of *Cx. quinquefasciatus* with CRISPR [98,99] and overall, these strategies are expensive and time consuming to develop and deploy [100], and potentially ineffective in highly dispersive *Culex* populations. Additionally, transient *Wolbachia* infections have been reported to increase WNV infection rates in mosquitoes [101] while no enhancement in dissemination or transmission rates have been found for stable *Wolbachia* infections [102].

Ivermectin (IVM), a widely used antiparasitic drug in human and veterinary medicine [103,104], is a promising alternative control strategy for WNV. IVM has previously been used in mass-drug administrations to treat onchocerciasis and lymphatic filariasis, providing hope for their eradication in the face of resistance and standard-of-care treatments with high toxicity [105,106]. It has also been shown to

be highly effective at killing *Anopheles* mosquitoes that transmit malaria parasites, resulting in reductions in malarial incidence following mass drug administrations [107,108]. Investigation of the delivery of IVM to *Culex* mosquitoes through wild birds for control of WNV transmission has been initiated recently [109]. Bird-delivered IVM provides the potential for targeted control of mosquitoes involved in maintenance and zoonotic transmission of WNV by increasing the mortality of those bird-feeding mosquitoes; mosquitoes that ingest IVM experience increased mortality [109,110] so few will likely survive long enough to take another bloodmeal at which disease transmission could occur, thus preventing future mosquito bites and blocking subsequent transmission events. IVM binds the glutamate-gated chlorine channels on invertebrate nerve and muscle cells, leading to hyperpolarization of the neurons and resulting in paralysis and death of the invertebrate [111–113]; this is a different mode of action than current pesticide products thus circumventing the current insecticide resistance issue in mosquito populations.

Lab and field studies have found that a variety of birds, including common bloodmeal hosts for *Cx. tarsalis* (doves and sparrows), readily ingest IVM-treated feed at concentrations of 200 mg IVM/kg with no adverse effect and *Cx. tarsalis* experiences high lethality upon ingestion of blood from IVM-treated doves [109]. A Phase I trial of lethality of IVM-treated blood meals and stability, palatability, and safety of IVM-treated birdseed performed jointly by Colorado State University and TDA Research, Inc. has been completed with promising results. A pilot study provided support for a reduction in the number of infected *Cx. tarsalis* within 50 m of IVM-treated feeder as compared to areas within 50 m of untreated control feeders, but the difference was inconclusive based on data from a single season [109]. During mist-netting around treated feeders at the end of the season, 87% of birds (9/10 common grackles and 4/5 house sparrows) were found to have detectable levels of IVM in their blood. Sufficient serum was obtained from one grackle to assess mosquito mortality through artificial bloodfeeding and was found to have strong mosquitocidal activity (100% mortality in 2 days) compared to that of control serum. Future work is needed to characterize bird interactions with birdfeeders and nocturnal roosting habits in relation

to feeders during the WNV season in order to assess the spatial extent and feasibility of using IVM-treated birds to control local WNV transmission.

This dissertation addresses gaps in the current knowledge of the spatial and temporal impacts of adult mosquito control on population dynamics of *Culex* mosquitoes and WNV transmission risk. It outlines an improved method of analysis of the effect of control measures through the use of observational data and investigates a new tool for WNV control. In Chapter 1, I developed a novel assessment of aerial spraying using a generalized additive modeling framework that took advantage of the large observational datasets of surveillance and control data from vector control districts to capture the baseline expected dynamics of mosquito populations and quantify the spatio-temporal effects of aerial sprays on populations of WNV vector mosquitoes under field conditions. For Chapter 2, I led a randomized field trial in Davis, California to assess the impact of IVM-treated chickens on mosquito populations and WNV transmission and concluded that IVM had the potential for local WNV control. In Chapter 3, I assessed the potential efficacy of deploying IVM-treated birdfeeders in neighborhoods to reduce local transmission of WNV using a spatially implicit compartmental model of WNV infection dynamics, informed by field-collected data on birdfeeder usage and nocturnal roosting habits of common backyard birds in Fort Collins, Colorado. Results from this chapter, namely the number and spatial arrangement of treated feeders and the bird species to target in a neighborhood for optimal control, can be used in the design of future field trials. Taken together, the results of this dissertation elucidate the effects of existing and novel control strategies on the dynamics of WNV and its mosquito vectors. These findings can be used by vector control and public health professionals to design, implement, and evaluate future effective vector control strategies.

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Chapter 1: Spatio-temporal impacts of aerial adulticide applications on populations of West Nile virus vector mosquitoes

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Abstract

Aerial applications of insecticides that target adult mosquitoes are widely used to reduce transmission of West Nile virus to humans during periods of epidemic risk. However, estimates of the reduction in abundance following these treatments typically focus on single events, rely on pre-defined, untreated control sites, and can vary widely due to stochastic variation in population dynamics and trapping success unrelated to the treatment. To overcome these limitations, we developed generalized additive models fitted to mosquito surveillance data from CO_2-baited traps in Sacramento and Yolo counties, California from 2006-2017. The models accounted for the expected spatial and temporal trends in the abundance of adult female *Culex tarsalis* and *Culex pipiens* in the absence of aerial spraying. Estimates for the magnitude of deviation from baseline abundance following aerial spray events were obtained from the models. One-week post-treatment with full spatial coverage of the trapping area by pyrethroid or pyrethrin products, *Cx. pipiens* abundance was reduced by a mean of 52.4% (95% CI: -65.6, -36.5%) while the use of at least one organophosphate pesticide resulted in a 76.2% (95% CI: -82.8, -67.9%) reduction. For *Cx. tarsalis* one-week post-treatment with full coverage resulted in a 30.7% (95% CI: -54.5, 2.5%) reduction; pesticide class was not a significant factor contributing to reduction. In comparison, repetition of spraying over three to four consecutive weeks resulted in similar estimates for *Cx. pipiens* and a somewhat smaller magnitude for *Cx. tarsalis*. aerial adulticides are effective for rapid short-term reduction of the abundance of the primary West Nile virus vectors, *Cx. tarsalis* and *Cx.*

pipiens. A larger magnitude of reduction is estimated in *Cx. pipiens*, possibly due to the species' focal distribution. Effects of aerial sprays on *Cx. tarsalis* populations are likely modulated by the species' large dispersal ability, population sizes, and vast productive larval habitat present in the study area. Our modeling approach provides a new way to estimate effects of public-health pesticides on vector populations using routinely collected observational data and accounting for spatio-temporal trends and contextual factors like weather and habitat. It does not require pre-selected control sites and expands upon past studies that have focused on effects of individual aerial treatment events.

Introduction

West Nile virus (WNV; genus *Flavivirus*, family *Flaviviridae*) causes a potentially fatal, neuroinvasive mosquito-borne disease [1]. It is maintained in an enzootic cycle between birds and mosquitoes [2,3], predominantly in the genus *Culex* [4], and can spillover to infect horses and humans, both of which are dead-end hosts vulnerable to disease [5]. *Culex* (*Cx.) tarsalis* and *Cx. pipiens* complex mosquitoes are the primary enzootic and epizootic vectors in California [6,7]. While 80% of human infections are asymptomatic, clinical manifestations can include acute febrile illness, encephalitis, flaccid paralysis, and death [8]. Often the severe form results in long-term physical and mental disabilities [9]. WNV invaded California in 2003 and has become endemic [7]. An average of 238 neuroinvasive cases occur statewide annually with approximately one-third occurring in the Central Valley, where the landscape is dominated by large-scale agriculture punctuated by cities and small towns [10].

As no human vaccine exists, prevention of human diseases relies primarily on personal protective measures (i.e. wearing long sleeves, using insect repellent, and avoiding the dawn/dust periods when mosquitoes bite) and vector control by local vector control districts or health departments [11,12]. In periods of epidemic risk when large numbers of WNV-infected *Culex* mosquitoes are detected near human population centers, large-scale aerial applications of insecticides are utilize to rapidly reduce the abundance of adult mosquitoes and disrupt virus transmission cycles, thereby reducing zoonotic transmission risk [13].

Three main classes of pesticide products have been licensed for use in aerial spray applications in California; pyrethrins, pyrethroids, and organophosphates [12,14,15]. Pyrethrins are naturally derived insecticides from chrysanthemum flowers (*Chrysanthemum cineriaefolium*) that inactivate sodium channels in the insect nervous system, resulting in paralysis and death [15]. Pyrethroids are synthetically derived pyrethrins with a similar mode of action and longer half-life. Organophosphates inhibit acetylcholinesterase, affecting neurotransmission and causing uncontrolled nerve activation and death in insects [14].

A standard method for evaluating the efficacy of an aerial spray event compares pre- to post-treatment mosquito trap counts inside the treatment zone versus changes for the same period in an adjacent unsprayed control area [16]. This method, first proposed by Mulla et al. [17], has been adapted to and widely used in evaluating the efficacy of aerial spraying for reducing the abundance of female mosquitoes and has been extended to assess changes in other indicators of risk, namely infection prevalence in mosquitoes, human cases, and reported dead birds with WNV infection [16]. However, reported estimates vary widely, with some studies even indicating occasional increases in trap counts following spray events [18–21].

Despite its wide use, the assumptions behind Mulla's formula are often violated, resulting in confounded estimates. First, treatment and control sites are often not independent due to the spatial connectivity of populations with mosquito dispersal and immigration [22]. With the connectivity and potential drift of pesticides via wind, there is the potential that insecticide sprays have wider population impacts than just the targeted spray zone [20]. Second, the difference in pre- to post- trap count ratios in and between areas are not solely due to control measures, but rather are impacted by weather, seasonality in mosquito populations, differential presence of larval breeding sources or simply stochastic variation in trapping success [18,20,21]. Overall, Mulla's formula neglects the spatio-temporal structure of mosquito populations and external factors impacting the random volatility of mosquito trapping success.

To overcome the limitations of assessing the efficacy of aerial sprays on the individual spray event basis, we paired long-term surveillance and vector control records (12 years) from Sacramento-

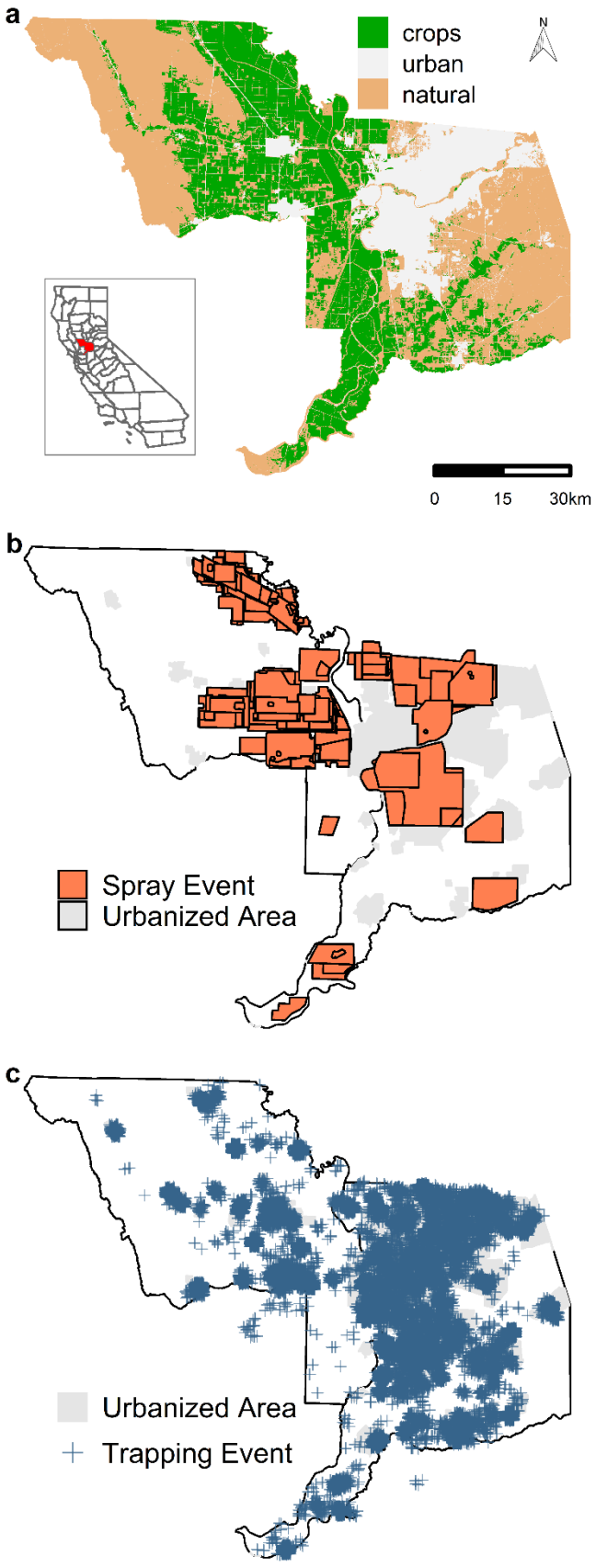

Figure 1-1: Land cover, Aerial sprays, and mosquito collections (2006-2017) in Sacramento & Yolo counties, California. (a) *Distribution of cultivated crops (primarily rice), urban, and other natural land cover types across the study area. Land cover categories were derived from the 2011 National Land Cover database [34]. Inset highlights the location of these counties in the state of California. Location of (**b**) zones targeted for aerial treatment applications and (**c**) CO_2-baited mosquito traps during 2006-2017 in Sacramento and Yolo counties. Each polygon (**b**) and point (**c**) represents a single spray or trapping event, respectively. A random shift of ≤ 1 km applied to trap locations for visualization of repeated trapping at the same location across time.*

Yolo Mosquito and Vector Control District (SYMVCD) in California to capture baseline spatio-temporal mosquito population dynamics and estimate the magnitude and duration of the impacts of aerial sprays on the abundance of *Cx. pipiens* and *Cx. tarsalis*, the predominant WNV vectors in California. We chose a generalized additive modeling (GAM) framework to capture the nonlinear population dynamics and associations inherent to mosquito collections.

Methods

Study Area

The study area encompasses Sacramento and Yolo counties, California (Figure 1-1) which have a combined area of approximately 5,126 km^2 and a population of approximately 1.73 million people in 2016 [23]. Sacramento County

is 34.12% urban and 65.88% rural with the majority of urban areas consisting of the concentrated Sacramento urban center and surrounding suburbs. In comparison, Yolo County is 4.61% urban and 95.39% rural with smaller, more dispersed urban areas [24]. These counties, located in the northern part of California's Central Valley, are characterized by a Mediterranean climate with hot, dry summers (Jul mean temperature: 25.8°C, May-Sep mean total rainfall: 3.18 cm) and mild, rainy winters (Jan mean temperature: 9.6°C, Oct-Apr mean total rainfall: 47.30 cm) [25] and extensive irrigated agriculture, especially rice and row crops such as tomatoes. Sacramento-Yolo Mosquito and Vector Control District (SYMVCD), established in 1946 to protect the public from nuisance mosquito biting and mosquito-borne diseases, manages mosquito populations in Sacramento and Yolo counties [26].

Aerial Treatments and Mosquito Collections

SYMVCD provided the spatial polygons (Figure 1-1b) and associated data detailing the date, area targeted for spraying, number of consecutive nights of spraying in the same location, and pesticide product used for all aerial sprays during the study period (1,021 unique nights of spraying during 930 spray events).

Mosquito collection records for CDC CO_2-baited EVS traps [27] from SYMVCD for the years 2006-2017 (Figure 1-1c) were obtained with permission through the CalSurv Gateway [28], an online database hosting data from California vector control agencies. Any records that indicated trap malfunctions or which unfeasibly ran longer than one night were excluded. Each record (n = 24,344) contained latitude, longitude, date, number of traps employed, and total female *Cx. tarsalis* and *Cx. pipiens* captured. Distribution of traps and spray events by year (Figure A1-1) and season (Figure A1-2) are presented in Appendix 1.

Any records that indicated trap malfunctions or which were operated for more than one night were excluded. For each species separately, we removed the collection reports corresponding to those greater than two standard deviations above the mean in each week to remove the influence of outliers (i.e., large singular deviations from broader abundance trends) on smooth functions subsequently estimated by the models.

All geographic data were projected from geographic to planar coordinates (Albers conic equal-area, EPSG 3310, NAD83) using the rgdal package in R statistical software (version 3.3.2; [29,30]) for all data processing and analysis.

Covariate Development

To isolate the effects of aerial insecticide treatments within out final statistical model, we first developed a set of spatio-temporal and environmental covariates to explain the long- and short-term trends in *Cx. tarsalis* and *Cx. pipiens* abundance. Inclusion of these covariates established a counterfactual basis in the models for the expectation in abundance in the absence of control, leaving the additional terms characterizing aerial insecticide sprays to explain any deviations attributable to the treatments.

For each remaining trap collection (n = 23,707 for *Cx. pipiens*; n = 23,678 for *Cx. tarsalis*), we derived a set of temperature variables to capture the effect of weather on trap collections. The mean temperature during the host-seeking period (dusk to dawn) and 30-year monthly average temperature were determined for each collection using 4-km resolution data provided by the PRISM Climate Group [31]. We calculated the deviation in temperature from the monthly average during the host-seeking period to capture activity rates on the night of trapping (i.e., warmer/colder than 'normal' resulting in higher/lower mosquito activity and resulting trap counts). As mosquito developmental rates are highly impacted by temperature [32,33], we also calculated the average temperature during the two-week period immediately preceding the trap collection to capture short-term effects of weather on mosquito abundance. Rainfall was not considered because amounts were negligible in the study area during the season when aerial insecticide applications occurred.

To characterize the larval habitat present around a trap location and to incorporate the sharp changes in land use across the study area, we used the 30x30m gridded land cover data from the 2011 National Land Cover database [34]. We used the classification of all pixels within a 5km radius area surrounding each trap to determine the proportion of three non-overlapping land use categories: urban, cultivated crops, and natural (Figure 1-1a). Land use categories were chosen to represent larval habitat

and bionomics of the *Cx. pipiens* and *Cx. tarsalis* [35]. The radius was chosen based on known dispersal distances for the species [36,37]. The 'urban' category encompasses all levels of developed land (i.e. structures, roads, and constructed materials). The 'crops' category represents annual irrigated crops which are predominantly rice in the study area. The remaining classifications were combined to create the 'natural' category.

We quantified the spatio-temporal intersections between spray zone polygons and trap locations to quantify the degree to which antecedent spray events impacted mosquito collections. Spatial coverage of each trap was quantified as the average proportion of the area from which a trap collects mosquitoes ('collection area') that was covered by aerial treatment zones during the four weeks preceding the collection. Using a conservative estimate on *Culex* flight distance [36–39] and to account for insecticide drift during application, we used a 5km radius collection area for both species. The temporal sequence of overlapping sprays during the four weeks preceding a collection (modeled as a factor for each unique sequence) was used to capture any lagged effects and impacts of repeated spray events. For each week preceding a collection, the total proportion of the collection area overlapping with the treatment zone was assessed. When multiple treatment zones overlapped with a single collection area in a week, we assumed an additive effect, summing the proportions of overlap from each unique spray up to a maximum of 1.0 that represented complete coverage. We used the average spatial coverage of targeted spray zones during all weeks with at least one overlapping spray event to quantify the spatial impact of sprays for each specified temporal sequence of spraying. Traps > 5km from all treatment zones had a spatial coverage of zero and corresponded to the reference level of the temporal sequence factor. These traps were included to capture baseline spatio-temporal mosquito dynamics in the absence of aerial treatments. Therefore, the impact of aerial spray events on collections was quantified with a two-fold approach, namely with the factor corresponding to the sequence of weeks when spraying overlapped during the preceding four weeks and the average proportion of the collection area that overlapped under that sequence.

According to guidelines from the California Department of Public Health, periods of high risk for arbovirus transmission are characterized in part by abnormally high mosquito abundance [12]. In order to

capture this dramatic deviation from 'normal' abundance that our smoothed modeling framework could not capture, but that precipitated aerial spray events, we also applied a prospective assessment to identify spray events closely following each collection. To account for the time required to respond to a high-risk period, we assessed the presence of overlapping treatment zones with a collection in the following four weeks on the weekly scale, similar to the above retrospective assessment of sprays.

To capture potential differences between broad classes of pesticides used (organophosphate vs. pyrethrin and pyrethroids combined), we included a binary indicator variable for whether at least one spray event associated with a particular trap collection used an organophosphate. Sample sizes were too small to further investigate differences between pyrethrins and pyrethroids or between individual insecticide products.

We considered time in a variety of ways to capture trends in mosquito abundance in two parts: typical annual seasonality and coarser spatial-temporal trend over the twelve-year study period. Using the trap collection dates, 'week' (number of weeks from the start of the study period; range 1-626) and 'day' (range 1-365) variables were created to capture a continuous yearly effect and seasonality, respectively. We interacted the 'day' variable with each category of land use (i.e. 'urban', 'crops', and 'natural') to capture the seasonal trend in these different habitats. Each individual seasonality curve was weighted by the proportion of land use in that category within 5 km of the trap collection to produce a single unified seasonal trend that reflected the specific habitat composition for that collection.

Statistical Analysis

We developed generalized additive models (GAMs) to relate nightly trap counts of female mosquitoes, either *Cx. tarsalis* or *Cx. pipiens*, to aerial adulticide applications, adjusted for variation in trap counts due to spatio-temporal mosquito dynamics. We chose GAMs because of the flexible parameterization of smooth functions of covariates to explain spatial and temporal trends [40,41]. Covariates considered to explain baseline mosquito dynamics were day or week of the year, year, location, land use, two-week average temperature, and nightly deviations from average temperature during trapping, the presence of a spray event in the following one to four weeks (high risk period), and

pesticide class used in the aerial spray. Either a smooth function or a factor were used in fitting the covariates with the choice between these forms, along with spline and basis dimension if a smooth function was chosen, based on model fit and biological relevance. Cyclic cubic regression splines were used to prevent discontinuity between the ends of the smooth representing the seasonal patterns (aka between Dec 31 and Jan 1). Thin plate regression splines were chosen for most covariates because they are isotropic and have been shown to be the optimal smoother of any given basis dimension [42]. A cubic regression spline was used in the spatio-temporal surface due to its superior performance over thin plate regression splines for the large amount of observations [43].

We fit negative binomial GAMs using the gam function in R (version 3.3.2; package mgcv) [29,44] with restricted estimation maximum likelihood (REML) as the smoothing parameter estimation method. We choses a negative binomial function to account for the over-dispersed nature of trap count data. We used backward selection guided by AIC [45] to reach our final model. In each model, we included an on offset term for the number of traps operated per trapping event. All covariates were included in the initial model and choices of interactions between covariates entered into the initial model were guided by biological relevance. We used concurvity, a measure of collinearity for smooth functions (range 0-1; 43), and visually examined deviance residuals for consistency in space and time to assess the final model fit.

Using the other covariates to establish the expected abundance of each species in the absence of aerial spraying, we estimated the mean change in predicted abundance across the range of spray regimes observed in the data, using the Bayesian posterior covariance matrix for the parameters that accounted for smoothing parameter uncertainty [43]. We simulated 10,000 random draws from the posterior distribution of the fitted model, a multivariate normal distribution with mean equal to the estimated model coefficients and covariance matrix of the parameters, to predict the abundance of each species across the spatial and temporal sequences of sprays observed in the data. For each draw, we then calculated the mean change in abundance from the baseline no-spray scenario at each point on the spatio-temporal surface, along with

the corresponding 95% confidence interval. Estimates of efficacy from the model were compared with those derived from Mulla's formula [16,17].

An R script outlining the workflow of parameter development, model fitting, and estimating change in abundance across the spatio-temporal surface presented in Appendix 2.

Results

Data overview and model selection

The relative abundance (number of females per trap-night) of *Cx. pipiens* and *Cx. tarsalis* varied spatially during the peak WNV season from late Jun to early Oct when aerial sprays occurred (Figure 1-2). Typically, higher abundance of *Cx. pipiens* was observed in urban areas whereas higher abundance of *Cx. tarsalis* was typically in non-urbanized areas near irrigated agriculture.

The final model for each species included an offset for the number of traps run per collection event; and smooth functions of space by time (on the weekly timescale across the twelve years), day of the year by each land use category ('urban', 'crops', and 'natural'), two-week average temperature, nightly deviations in average temperature during trapping, and spatio-temporal impacts of aerial spraying. Our choice of cut-off for removing outliers during model fitting did not significantly change our results.

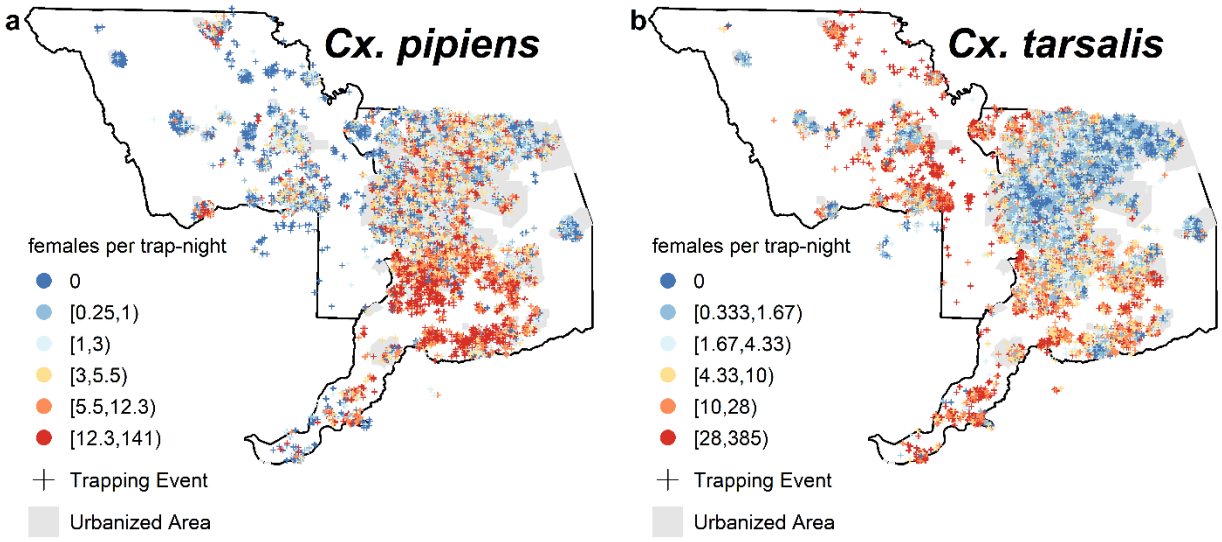

Figure 1-2: Collections of (a) Cx. pipiens and (b) Cx. tarsalis during peak WNV season. *Plus signs (+) indicate the location and number of female mosquitoes per trap-night for each collection during the period when aerial spraying occurred (late-Jun to early-Oct). Colors represent abundance quintiles by species for non-zero collections. A random shift of ≤ 1 km was applied to trap locations to aid visualization of repeated collections at the same locations during the study timeframe.*

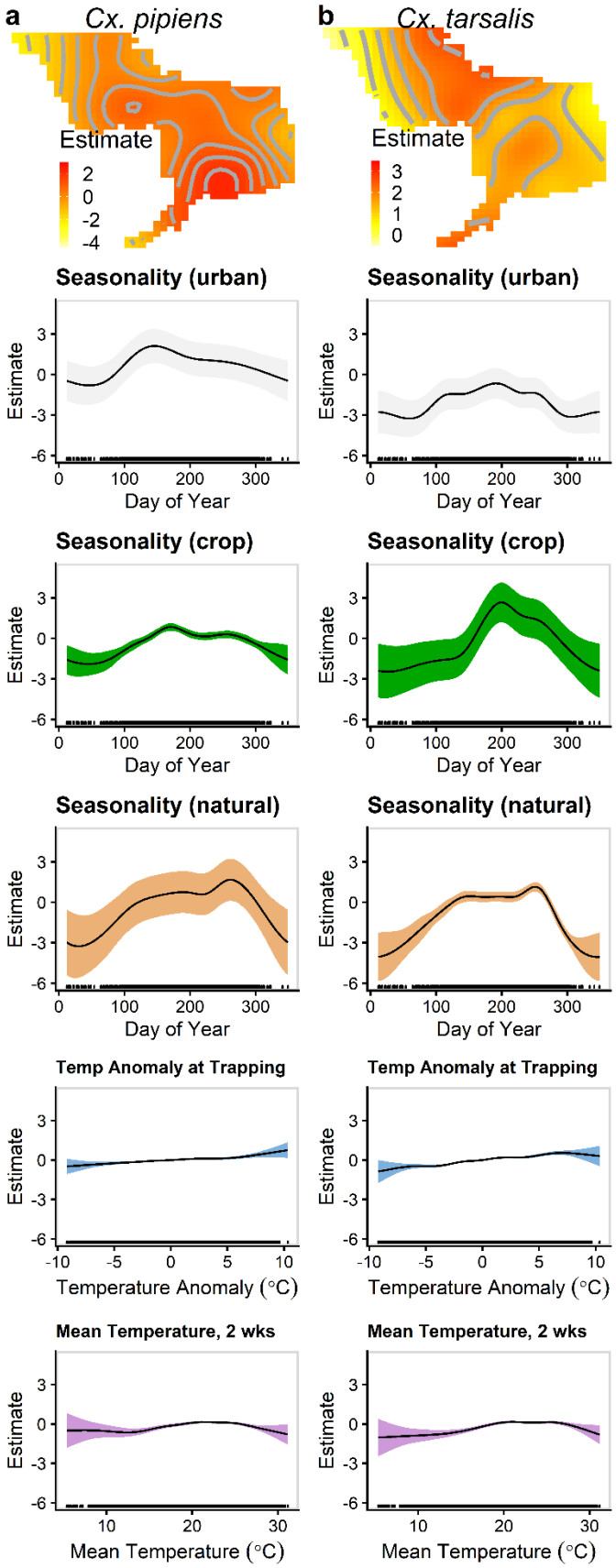

Figure 1-3: Smooth covariate functions explaining nightly abundance of (a) Cx. pipiens and (b) Cx. tarsalis. *Smooth functions from final GAMs are shown for the spatio-temporal surface (top), seasonality in a fully urban area, seasonality in a fully crop area, seasonality in a fully natural area, deviation (°C) from the 30-year monthly average temperature on the night of trapping, and the average temperature (°C) during the two-weeks prior to trapping (bottom). The shaded region represents 95% confidence interval for one-dimensional functions. A representative slice of the three-dimensional spatio-temporal surface is presented for 2011 at the midpoint of the typical WNV season (week of Aug 1). Spatio-temporal surfaces are plotted on individual axes for each species to resolve the spatial scale.*

Removing the top 0%, 4.5%, or 10% of data in each week for each species resulted in minor shifts in confidence interval widths and magnitude of some estimates, but no change to inference. The resulting smooth functions for each species are illustrated in Figure 1-3 (see Figures A1-3 & A1-4 for spatio-temporal surfaces for all years for each species). Construction of smooth functions used in the final models is outlined in Table A1-1. All smooth functions were highly significant ($P <$ 0.0001). A random intercept for site location was included to account for repeated collections at the same location, fitted using coefficients penalized by a ridge penalty [46]. We also retained, based on reductions in AIC,

parameters for the presence of sprays in the 1 & 4 and 1, 2, & 3 weeks following the trap collection for *Cx. tarsalis* and *Cx. pipiens*, respectively (Table A1-2). Based on reduction in AIC, only the model for *Cx. pipiens* retained the term indicating the presence of at least one spray event with an organophosphate pesticide during the previous four weeks, as compared to all sprays using combinations of pyrethrin or pyrethroid products (-48.9% change in abundance for ≥ 1 organophosphate, $P < 0.001$).

The largest magnitude of variability in the baseline abundance for both species was due primarily to the seasonality covariates, followed by the spatio-temporal surface. The temperature covariates contributed the smallest magnitude to establishing abundance, but all smoothed functions were highly significant ($P < 0.0001$). In all smooth functions, positive estimates correspond to increases in the population, negative estimates correspond to decreases in the population, and 0 indicates no modulation in abundance at that covariate value.

The distribution of the final model residuals was right-skewed for both species indicating the model underestimated extreme trap counts. However, no spatial or temporal pattern remained in the deviance residuals (Figures A1-5 & A1-6). Both models had low estimated concurvity values [43] for the aerial spraying smooth function with the rest of the model parameters (*Cx. pipiens*: 0.145; *Cx. tarsalis*: 0.148). This indicates that the smooth estimates for the impact of aerial spraying were not confounded by other parameters. In addition, the relatively high deviance explained value for both models (*Cx. pipiens*: 44.0%; *Cx. tarsalis*: 62.3%) indicates good model fit despite for the complex dynamics inherent in mosquito populations.

Effects of aerial insecticide treatments

A smooth surface of the spatio-temporal impacts on *Cx. pipiens* abundance is presented for treatments with only pyrethrin or pyrethroid products (Figure 1-4a) or with at least one organophosphate product (Figure 1-4b). For *Cx. tarsalis*, the difference in impact by broad pesticide class was not retained in the final model, so a single smooth is presented (Figure 1-4c). Overall, the models estimated a lower magnitude of change in *Cx. tarsalis* abundance as compared to *Cx. pipiens*. For example, following aerial spraying with full spatial coverage (i.e., 100% coverage of the area within 5 km of the trap), we estimated

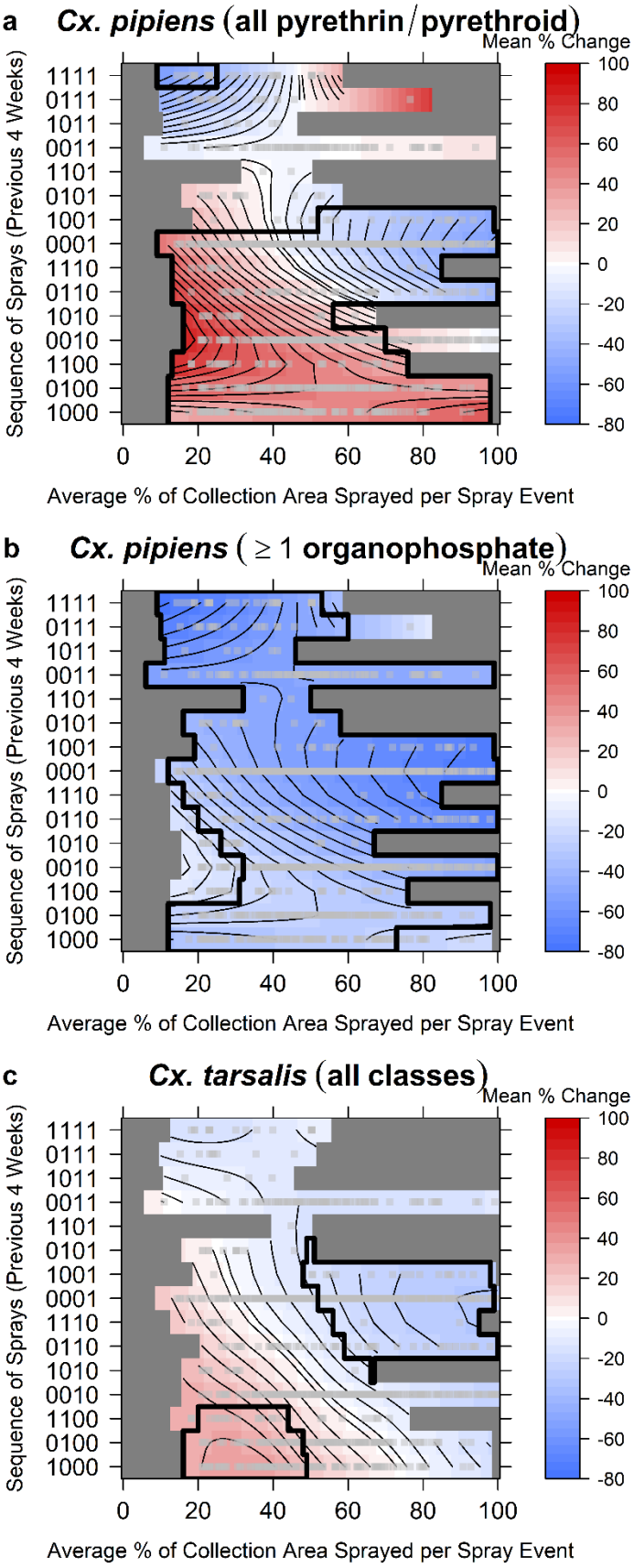

Figure 1-4: Mean changes in abundance following aerial spraying, as compared to no-spray baseline. *Estimates are shown for* ***(a, b)*** *Cx. pipiens and* ***(c)*** *Cx. tarsalis abundance changes with respect to antecedent sequence and average spatial coverage of aerial treatments. For Cx. pipiens, estimates are shown for* ***(a)*** *sprays that used only pyrethrin or pyrethroid products and for* ***(b)*** *sprays that utilized an organophosphate product at least once. For Cx. tarsalis, estimates are with any product class. Horizontal axes represent the average proportion of the 5km buffer surrounding a trap covered by a spray event and vertical axes represent the temporal sequence of aerial sprays during the four weeks preceding the trapping event. Presence (1) or absence (0) of sprays in the 1, 2, 3, and 4 weeks (R to L) prior to trap are indicated by the 4-digit sequence. The sequences of sprays are ordered from the fewest number and temporally most distant spray events (bottom) to the largest number and temporally closest spray events (top). Estimates are truncated to the range observed with the available data (grey squares indicate points present in data). Areas enclosed in a black border represent the portion of the spatio-temporal surface with significant estimates (P < 0.05)..*

a mean one-week *Cx. pipiens* abundance change of -52.4% (95% CI: -65.6, -36.5%) if all spray events had used pyrethroid or pyrethrin products. If at least one organophosphate product had been used, we estimated a -76.2% (95% CI: -82.8, -67.9%) mean change in *Cx. pipiens* abundance. In contrast, *Cx. tarsalis* populations with full spatial coverage by aerial sprays showed an estimated -30.7%

(95% CI: -54.5, 2.5%) mean change one-week post-spraying regardless of the product class.

For both species, larger reductions in abundances were estimated in areas with higher spatial coverage of aerial sprays (large proportion of spatial overlap) than those on the fringes (low proportion of spatial overlap). Sprays occurring closer in time to collections were generally estimated to result in larger reductions in abundance compared to those longer ago. At longer time lags (i.e., two to four weeks post-spraying), higher than expected abundance for both species was estimated, with the increase only occurring in *Cx. pipiens* populations following sprays with pyrethrins and pyrethroids.

The majority of temporal spray sequences lacked data across the full range of spatial overlap (0-100%); we did not estimate the change in abundance for these areas. Data were sparser or lacking for higher spatial coverage during multiple weeks of spraying. For regions of the spatio-temporal surface with data support, the reduction one-week post-spraying with full spatial coverage was the largest reduction predicted for *Cx. tarsalis*. In contrast, *Cx. pipiens* populations on the fringes of spray events for the preceding four weeks results in a similar reduction as for populations with full spatial coverage by aerial sprays one-week ago (all pyrethrin/pyrethroids: -54.3% (95% CI: -81.0, -6.2%) change; at least one organophosphate: -77.2% (95% CI: -90.4, -53.9%) change).

Comparison to conventional estimates

As a comparison for our model results, we applied the conventional approach of Mulla's formula [16,17] to the combined trapping and control records from 2006-2017 to estimate the efficacy of sprays. Considering trapping one-week before and after a spray event and using a 5km buffer around the targeted spray zone as the adjacent comparison area, we were able to calculate the effect for 36 spray events (3.87%) for *Cx. pipiens* and *Cx. tarsalis*; the majority of spray events lacked traps in all of the required spatial and temporal locations for the calculation. Most of the estimates for the change in abundance indicated a reduction in trap counts, but estimates varied widely, ranging from complete population elimination (100% decrease) up to 11,000% increases following a spray event (Figure A1-7). Most estimates for *Cx. pipiens* indicated varying degrees of reduction while those for *Cx. tarsalis* spanned reductions to increases.

Discussion

This study found that aerial insecticide treatments achieve strong short-term reductions in both *Cx. tarsalis* and *Cx. pipiens* populations. Previous studies have assessed the short-term impact of aerial spraying on mosquito abundance and highlighted the volatility of estimates across space and time. In order to overcome the limitations of using single events to estimate the efficacy of aerial spraying on reducing the abundance of WNV vector mosquitoes, we used a large dataset of surveillance and control records together with GAM models. This modeling framework allowed us to establish baseline mosquito adult abundance and identify deviations from expected nightly abundance attributed to aerial spraying (i.e. counterfactual basis) as well as the spatial and temporal impacts of aerial applications. Our results indicate that aerial sprays do achieve population reduction for both *Cx. pipiens* and *Cx. tarsalis* with heterogeneity in the magnitude and pattern of reduction between species and pesticide product.

The differences in the magnitude and pattern of estimated response between species can be attributed partially to the different bionomics of the individual mosquito species. In the study area, *Cx. pipiens* are predominantly peridomestic with larval habitats limited primarily to backyard sources and stormwater systems in urbanized areas [35,47]. Thus, areas targeted by aerial insecticide treatments would span a large fraction of any particular population, leaving few adults to repopulate the treated area from proximal unsprayed locations. In contrast, *Cx. tarsalis* breed in agricultural areas and may disperse into surrounding agricultural and urban areas [35,48,49]. This species also has a larger typical dispersal distance and achieves higher population densities than *Cx. pipiens* [35–37]. aerial insecticide treatments typically target only a small fraction of the total available habitat for *Cx. tarsalis*, often near urbanized areas, and any effect of aerial sprays could be moderated by immigration of adult *Cx. tarsalis* from surrounding unsprayed locations, potentially from distant locations [38,50]. Therefore, the best suppression for *Cx. tarsalis* populations would be achieved in isolated areas, as has been reported previously [22]. Additionally, repetition of sprays on the weekly scale is less effective at controlling *Cx. tarsalis* than *Cx. pipiens* because of the rapid immigration and emergence of new adults from large areas of productive larval habitat.

While our estimated reduction in abundance of *Culex* mosquitoes show some similarity to previous published estimates of aerial spray events from Sacramento and Yolo counties (Table 1-1), our methodology also accounted for contextual factors, resulting in more robust estimates of the average effect of spraying. Previous estimates exhibited spatial heterogeneity [19]. Utilizing covariates to capture the spatial structure, temperature deviations, and varying distribution of larval habitats removed the confounding impact of these factors on our model results. Similarly, previous estimates have varied, in part because Mulla's formula cannot fully capture spatio-temporal nuances of mosquito population dynamics. For example, Lothrop et al. [20] observed 73% increases in *Cx. tarsalis* abundances post-spraying despite observing mortality in caged sentinel mosquitoes and large reductions during previous spray events. The authors attributed the estimated increase to the dynamics of *Cx. tarsalis* populations at the time of the study, particularly the large emergence of *Cx. tarsalis* following the annual flooding of the nearby wetlands that was not captured by the Mulla's formula framework and the fact that the sprays were not impacting mosquitoes in the productive larval habitats. Previous estimates also use differing time interval lengths to estimate mosquito abundance before and after spray events. The heterogeneity in these time intervals combined with the heterogeneity of resulting estimates (Table 1-1) highlights the need to use consistent time intervals to improve generalizability of estimates between studies. As standardization of the time interval largely depends on the operational capacity of vector control districts for trapping and responding to epidemic conditions, no single recommendation may be feasible across all studies. However, we recommend that mosquito control agencies should keep the timeframe consistent across their evaluations to increase comparability of intra-agency control efforts. A similar range of estimates for change in *Culex* abundance following adulticide treatments have been reported outside California. Most are in broad agreement with our findings, although none used Mulla's formula. In Chicago, Illinois, a reduction of 54% in *Cx. pipiens* trap counts within the spray zone vs. the baseline pre-spray abundance was reported in contrast with a 153% increase outside the spray zone following two, single-night aerial spray events with a pyrethroid seven days apart [51]. An average 65.3% reduction in

Table 1-1 Estimated change in *Culex* mosquito populations following aerial spray events in California using Mulla's formula.

Location City, county	Year	Nights Sprayed[#]	Product Class	Comparison Length[†]	Species	% Change[‡]	Reference
Davis, Yolo	2006	2	pyrethrin	2 days before, 2 days after	*Cx. pipiens*	- 58.0	[19]
					Cx. tarsalis	- 25.6	
Woodland, Yolo	2006	2	pyrethrin	2 days before, 2 days after	*Cx. pipiens*	- 77.7	[19]
					Cx. tarsalis	- 46.8	
Sacramento, Sacramento	2005	3	pyrethrin	7 days before, 7 days after	*Cx. pipiens*	- 75.0	[21]
					Cx. tarsalis	- 48.7	
Sacramento, Sacramento	2006	3	pyrethrin	3 days before, 3 days after	*Cx. pipiens*	- 39.3	[85]
					Cx. tarsalis	- 57.3	
Coachella valley, Riverside	2005 (Mar)	3 alt^	pyrethroid	5 days before, 1 day after	*Cx. tarsalis*	- 93.0	[20]
	2005 (Jun)	3 alt^	pyrethroid	5 days before, 1 day after	*Cx. tarsalis*	- 77.0	[20]
	2005 (Sep)	3 alt^	pyrethroid	5 days before, 1 day after	*Cx. tarsalis*	73.0	[20]

[#] Number of consecutive nights sprayed in spray event.
[†] Length of time before and after an aerial spray event used when comparing of trap counts.
[‡] Percent change in *Culex* abundance following an aerial spray event as calculated using Mulla's formula [16,17]
^ Aerial spraying occurred on 3 alternate nights.

Cx. pipiens/*restuans* populations was observed within 24 hours of truck-mounted applications of a pyrethroid [52]. In contrast, no significant changes in *Cx. pipiens* abundance were observed following single-night truck-mounted applications of a pyrethroid in three communities near Boston, Massachusetts [53]. Up to 75% reduction in the two-day counts of female *Cx. quinquefasciatus* was reported during a month-long period with truck-mounted pyrethroid sprayed five days a week in Dubai, United Arab Emirates [54]. Without untreated comparison locations, it is hard to directly compare these results to those of our study.

Our model structure most closely compares to the study design used by Elnaiem et al. [21] where a timescale of one week before and after a spray event with a pyrethroid pesticide was chosen when assessing mosquito abundance (Table 1-1). Our estimates for reduction for *Cx. pipiens* (-52.4%) and *Cx.*

tarsalis (-30.7%) are lower than the observed reductions (*Cx. pipiens*: -75%; *Cx. tarsalis*: -48.7%). While qualitatively similar, the differences in magnitude may be due to differences in analytical methods, shifts from pyrethrin to pyrethroids over time, or the longer twelve-year time period of our study that could have yielded a more conservative estimate of average spray effects.

Our approach to causal inference using observational data builds upon earlier contributions from the fields of environmental science and epidemiology. Mulla's formula can be considered an extension of the Before/After and Control/Impact (BACI) analysis framework. Originally defined by Green [55] and extended and applied by others [56–59], BACI originated in environmental science literature to distinguish natural variability from the impact of an anthropogenic disturbance and has been applied to mosquito larvicide evaluations [60,61]. The BACI methodology compares an impact and at least one separate control location, sampled at various time points before and after the impact, to detect changes in the natural history of the environment due to the impacts [56–58]. An ANOVA test is used to detect a significant difference in the trajectories before vs. after the disturbance in the impact area as compared to the control area. Location and timing of sampling in each is chosen to ensure each are independent across space and time and increase the evidence that a detected change was attributable to the disturbance itself [56,59]. Our GAM framework extends BACI using a three-dimensional spatio-temporal function and other covariates to capture entire spatio-temporal context as a way to estimate the expected mosquito abundance in the absence of spraying. The functions also capture trends in the impact over spatial and temporal combinations, while the BACI framework may miss significant changes due to the sampling timeframe chosen [62]. Additionally, our methods do not depend on the ANOVA assumptions of independence and homoscedasticity of samples [63], as the spatial and temporal correlation and the non-normal distribution of trap collections are accounted for through the covariates in the negative binomial GAMs.

Our statistical approach for estimating the effects of mosquito control on abundance relies on counterfactual theory that has been applied in the field of epidemiology as a conceptual basis for understanding measures of effect [64–66]. Counterfactual theory as a basis for causal inference is

premised on the idea that for any unit being observed, there are multiple potential exposures but only one actually occurs, and outcomes under other alternative exposures exist only as potential outcomes that would have occurred if an alternative exposure had been applied. Because the alternative exposures did not occur, these are contrary to fact, or counterfactual. In this study, our units of study are trapping locations, and we are seeking to understand the effect of aerial spraying by statistically relating the observed mosquito abundance following spray events to the mosquito abundance in the same place and time that would have been observed in the absence of the spray. BACI and Mulla's formula approaches utilize untreated control sites to establish expectations for the unsprayed condition. Our approach instead aims to estimate the counterfactual expectation for mosquito abundance directly at the same place and time using spatio-temporal trends and contextual variables (i.e., weather and land use). This approach offers two clear advantages for estimating the effects of public-health pesticide use: (1) it allows for use of rich observational data sets that exist already and capture pesticide usage in real operational contexts, as opposed to experimental settings that are often closer to ideal conditions and (2) it does not rely on pre-selected, untreated control sites, which is helpful because vector management programs are rarely willing to withhold treatments in experimental control sites if their public-health action thresholds are met.

The smooth functions associated with land-use categories in the final GAMs accurately captured known seasonal and population dynamics. Cultivated crops were the primary source of *Cx. tarsalis* with smaller contributions from other non-urban land types during the warmest months of the year, accurately representing the presence of highly productive larval habitats in clean, recently created water sources characteristic of cultivated crops [35,48,67]. Urbanized areas did not produce large numbers of *Cx. tarsalis* during the WNV season as they contain few suitable larval habitats for this species. The estimated peak in abundance occurred in late July, but remained high through September, capturing the variation in timing of the peak across the years of the study. Populations of *Cx. tarsalis* in the Sacramento Valley are greatest from July-September [35,68,69]. The steep slope of the curve up to the peak mimicked the rapid increase in *Cx. tarsalis* observed at the start of the planting season [35,69]. For *Cx. pipiens*, urbanized areas largely contributed to abundance throughout the year with additional contributions in natural areas

(aka non-cultivated croplands) later in the season, reflecting the presence of larval habitats in artificial structures like storm drains or dairy wastewater lagoons [70,71]. Crops generally had lower *Cx. pipiens* abundance across the season, reflecting the general lack of high quality suitable larval habits in these areas.

Temperature anomalies during trapping and the two-week average antecedent temperature prior to trapping contributed to the overall abundance of both species, albeit relatively weakly in the presence of the other spatio-temporal terms. Their inclusion in the model was required to fully account for mosquito dynamics and night-to-night fluctuations in trapping success. Concordant with previous experiments, extremes in the average temperatures reduced abundance for both species illustrating the negative impacts on mosquito developmental rates and adult survivorship [32,33]. The estimated region of positive contribution to abundance for both species (*Cx. pipiens*: 19.2-25.4°C; *Cx. tarsalis*: 18.9-27.0°C) was narrower than the thermal tolerance of the species, but contains the observed regions of rapid developmental and high reproduction rates and the typical temperature ranges during the summer. As expected, small anomalies in average temperature on the night of trapping made relatively small contributions to change in abundance while extreme deviations result in much more marked change, highlighting the non-linear relationship underlying temperature and trap success.

It is interesting to note the additional marked reduction in *Cx. pipiens* abundance when at least one organophosphate product was used, especially as compared to the lack of a similar difference in *Cx. tarsalis* populations. As mentioned above, the class of product used may be more important for *Cx. pipiens* due to their focal distributions and more limited dispersal [35,72]. As an aerial spray will likely impact a large proportion of the localize *Cx. pipiens* population at once, there will be limited immigration from unsprayed segments of the population in the nearby proximity. Therefore, the full effect of an aerial spray is discernable. In contrast, the dispersed nature of *Cx. tarsalis* populations facilitates rapid immigration from surrounding unsprayed locations [35,36,38], thus diluting any difference in effect between product classes; any difference is not discernable against the background population dynamics accounted for in our modeling framework. Another factor contributing to the difference by species could

be insecticide resistance, as resistance to pyrethroids and organophosphates have been reported for both species in California [14,73]. If *Cx. pipiens* populations in the study area were more resistant to pyrethroids than *Cx. tarsalis* as has been previously reported in the Central Valley [74–76], this could explain the increased efficacy of organophosphates for *Cx. pipiens*. However, since we found a stronger effect of pyrethroids on *Cx. pipiens* as compared to *Cx. tarsalis*, resistance does not fully explain the observed difference. Alternatively, a single organophosphate spray may be insufficient to produce a marked difference in *Cx. tarsalis* populations; a repetition may be required. The underlying shape of the smooth function of spatio-temporal impacts of aerial spraying for either species likely differs between product classes and the specific timing and number of different products used, but sparse data prevented us from including an interaction to assess these dynamics.

A potential population rebound effect occurred for both species at more distant time lags from spraying where abundance was estimated to be higher than expected two to four (*Cx. pipiens* under pyrethrin and pyrethroid sprays) and three to four (*Cx. tarsalis*) weeks post-spray. Appropriately spaced treatments in time may be required to maintain a long-term reduction in population abundance. However, such a rebound does not negate the potential value of aerial treatments for achieving short-term reductions in the abundance of WNV-infected adult mosquitoes during periods of epidemic risk.

The increase in abundance at low spatial coverages of sprays for both species could reflect excito-repellency of pesticides at the fringes of targeted areas. Excito-repellency, a form of behavioral avoidance, combines two forms of sub-lethal exposure that results in mosquito movement away from a chemical source; contact excitation (increased activity upon contact) and non-contact spatial repellency [77–79]. These non-toxic behavioral impacts of pesticides were first identified in *Anopheles* mosquitoes in response to DDT and later with insecticide-treated bed nets and indoor residual spraying [80–82]. Populations of *Cx. quinquefasciatus*, another species in the *Cx. pipiens* complex, exhibit strong contact excitation and poor spatial repellency to pyrethroid, organophosphate, and carbamate pesticides [83,84]. No study has investigated excito-repellency in *Cx. tarsalis* populations. The behavioral avoidance of *Culex* to pesticides could be pushing mosquitoes out of the spray zones, resulting in an increase in

abundance around the fringes of sprays, consistent with our estimates for spatial coverages < 40% for *Cx. pipiens* following sprays with pyrethrin or pyrethroids only in the previous week.

A limitation of choosing a GAM framework is that we were only able to capture the average effects of covariates on nightly mosquito trap counts and unable to fully account for large stochastic fluctuations inherent to mosquito populations. However, GAMs easily allowed us to incorporate nonlinear relationships between abundance and covariates without having to constrain relationships with a priori knowledge. In particular, we were able to capture the higher-order relationships and correlation across space and time with the three-dimensional spatio-temporal function. The form of the smooth relationships in the final model did appear to approximate what is observed in nature. Other strengths of our modeling approach are that it takes into account regional differences in mosquito populations, population dynamics and seasonality in different land use types, and the impacts of short-term (night) and longer-term (two week) weather, resulting in robust estimation of the baseline expected abundance in the absence of spray effects, allowing us to isolate the deviations in abundance due to aerial spraying. This counterfactual basis of the model enables estimation in the absence of an independent control. However, even with the large amount of data available, data were inadequate to estimate the impact of aerial spraying reliably for certain time lags or spatial coverages that were rare or absent in the data. This is primarily due to typical SYMVCD spraying and trapping practices due to logistical and financial constraints. SYMVCD concentrated their mosquito collections efforts near urban areas to maximize the sensitivity for assessing the risk in proximity to human populations while minimizing time and costs associated with large-scale mosquito surveillance. Additionally, in an effort to control mosquitoes in known problem areas (highly productive larval habitats in proximity to the margins of urban areas) and reduce aerial applications over urban areas, the majority of areas receiving repeated sprays across the WNV season are in more rural areas where the mosquito trapping is sparser. This limited the data and statistical power to quantify the full range of spatial overlap with aerial sprays.

We were also unable to account explicitly for drift outside the target zones during sprays, as has been previously described [20]. Our use of a continuous variable to measure spray coverage within the

5km-radius collection areas surrounding each trap partially accounts for this effect. As such, however, we are unable to fully parse out the effect of aerial spraying on populations outside aerial spray zones and limited our analysis to only assess spatial coverage of traps within targeted spray zones.

Additionally, we were unable to estimate the relative effects of different lengths of multi-night spray events (one vs two vs three consecutive nights) due to data limitations and our analytical choice to aggregate all sprays on the weekly scale to achieve the balance between robust estimates and operationally relevant information for vector control districts. These limitations of observational studies such as this one could be addressed in future experimental field trials.

Conclusions

Aerial adulticides were shown to achieve short-term reductions in the abundance of the primary West Nile virus vectors, *Cx. tarsalis* and *Cx. pipiens*. A greater reduction was estimated for *Cx. pipiens*, likely due to its focal distribution in urbanizes areas and limited dispersal. The use of organophosphate products versus a combination of pyrethrins and pyrethroids increased the magnitude of reduction estimated for *Cx. pipiens* while the difference by broad insecticide class was not significant for *Cx. tarsalis*. The effects of aerial sprays on *Cx. tarsalis* populations were likely moderated by the species broad dispersal ability, large population sizes, and vast expanses of productive larval habitat in the study area. Therefore, the best control of *Cx. tarsalis* would be achieved in areas with isolated or highly spatially segmented populations. For both species, aerial spraying reduced abundance at high spatial coverage while reductions were also estimated at lower spatial coverage, at albeit greatly reduced magnitudes, indicating that aerial sprays had some impacts beyond the target zone. There was also evidence for population rebounds at periods of two to four weeks post-spraying. Our modeling approach allowed us to utilize observational data to isolate aerial treatment effects while taking into account contextual factors like spatio-temporal relationships, weather, and habitat that contribute to stochastic variation in nightly trap counts. This is an important advance that complements experimental trials and expands upon conventional observational approaches that summarize population changes following aerial

treatments at individual time points. Further work should expand upon these methods to estimate the change in WNV transmission potential and resulting human infections following aerial spray events.

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Chapter 2: Reduced West Nile virus seroconversions and mosquito parity following ivermectin-treatment of backyard chickens to reduce local West: Nile virus transmission

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Abstract:

Vector control strategies typically rely on pesticides to target mosquitoes involved in enzootic and zoonotic transmission of West Nile virus (WNV) but increasing insecticide resistance and a desire to reduce pesticide usage provide the impetus for developing alternative strategies. Ivermectin (IVM), an antiparasitic drug which is widely used in human and veterinary medicine, is a potential alternative for targeted control as *Culex* mosquitoes experience increased mortality following ingestion of IVM in bloodmeals. We investigated the impact of treating backyard chicken flocks in urban neighborhoods across Davis, California on mosquito populations and WNV transmission dynamics. We observed a reduction in WNV seroconversion in treated vs. untreated chickens, suggesting a reduction in WNV transmission intensity around treated flocks. We also detected a reduction in parity rates of *Cx. tarsalis*

near treated vs. untreated flocks and increased mortality in wild mosquitoes following a bloodmeal on treated chickens (IVM concentration 17-32 ng/mL) vs. untreated chickens, pointing to a reduction in WNV transmission due to the impact of IVM on *Culex* mosquito populations. Our results support the on-going investigation of oral administration of IVM to birds for local control of WNV transmission.

Introduction:

West Nile virus (WNV) is a zoonotic mosquito-borne pathogen that can cause a potentially fatal, neuroinvasive disease in humans [1]. It is maintained in an enzootic cycle between birds [2,3] and bird-biting mosquitoes (predominantly in the genus *Culex*) [4], but can spill over to infect horses and humans, both of which are dead-end hosts susceptible to disease following infection [5]. WNV is the most widespread flavivirus with evidence of transmission on all continents except Antarctica [6] and the leading cause of mosquito-borne disease in the US [7]. While 80% of human infections are asymptomatic, approximately 20% result in a febrile illness and 1% in a neuroinvasive disease with manifestations including encephalitis, meningitis, and acute flaccid paralysis [8]. The severe form of the disease has an approximately 10% case fatality rate and often results in long-term physical and mental sequelae [9]. From 1999-2018, >50,000 cases and >2,300 associated deaths were reported in the US [10], but the total number of infections is estimated to be >7 million [11]. The highest incidence occurs along the Great Plains, with a similar rate reported in California [12], where irrigated agriculture provides ample habitat for *Cx. tarsalis*, the primary WNV vector in the western United States [13], in proximity to avian hosts and humans [14,15].

No WNV vaccine exists for human so infection prevention relies on mosquito control and personal protective measures (i.e., wearing long sleeves, avoiding dawn/dusk periods when *Culex* mosquitoes are active, and using insect repellent) [16,17]. Control strategies primarily utilize chemical or microbial insecticides to manage mosquito populations in the larval or adult stages [18]. Larval control measures are generally preferred as a proactive strategy to directly target developing mosquitoes [16]. Previous studies support the effectiveness of larviciding catch basins, a common larval *Culex* habitat, to reduce the abundance of larvae [19,20], but environmental conditions and sub-optimal catch basin design

can significantly reduce the efficacy [21,22] and larviciding alone is insufficient to control mosquito populations and curb WNV transmission [23]. While costly [24], aerial applications of insecticides have been shown to rapidly reduce the abundance of WNV vectors [25] and the abundance of infectious mosquitoes [26–30] and have been linked to a reduction in human WNV cases in a treated area versus an untreated area [30]. However, efficacy varies widely due to differences in environmental conditions [31,32]. Ground-based adulticide applications can reduce target mosquito populations under ideal conditions, but estimates of the effects on WNV transmission are less consistent [33–36]. Overall, adulticide applications have limited precision to target mosquitoes involved in enzootic and zoonotic transmission without disseminating pesticides over large areas, thereby increasing the possibility of off-target effects despite careful tuning of applications to times of peak activity of target mosquitoes [18,37–40]. Widespread insecticide applications also face increasing levels of insecticide resistance in mosquito populations which render control measures ineffectual [41–43], prompting the development of alternative products and strategies.

Ivermectin (IVM), a widely used antiparasitic drug in human and veterinary medicine [44,45], provides the potential for targeted control by increasing the mortality of bird-feeding mosquitoes. Mosquitoes that ingest IVM experience increased mortality [46,47] so few will likely survive long enough to take another bloodmeal at which disease transmission could occur, thus preventing future mosquito bites and blocking subsequent transmission events. The mosquitocidal properties of IVM were first characterized in *Anopheles* mosquitoes in conjunction with mass drug administration campaigns and resulted in a reduction in malarial incidence [48,49]. Recently, the mosquitocidal applications of IVM have been investigated in *Culex* mosquitoes for controlling WNV transmission with the hypothesis that targeting the common avian species that account for the majority of *Cx. tarsalis* bloodmeals during the WNV transmission season could act as an effective WNV control strategy [46]. The authors, using laboratory and a pilot field-based trial, demonstrated the feasibility and effectiveness of developing IVM-treated birdfeed as a novel WNV transmission control strategy.

IVM use in birds is primarily off-label, but it has been used effectively to treat a variety of avian parasites, including in falcons, budgerigars, and chickens [50–53]. Nguyen et al. observed no toxicity or abnormal blood chemistry in chickens and doves fed exclusively on IVM-treated feed (200 mg IVM/kg feed) for 3-10 days and demonstrated the mosquitocidal activity of the blood of these orally treated birds [46]. While not directly involved in the enzootic transmission cycle of WNV, chickens are a common bloodmeal source, being preferentially bitten over other species within 50 m of flocks [54,55].

Our study, conducted in suburban neighborhoods across Davis, California, aimed to determine whether IVM delivered through backyard chicken flocks can suppress the abundance of WNV-infected mosquitoes and transmission of WNV as measured by chicken seroconversions. This study expanded upon the pilot study and paralleled a simultaneous study by collaborators in northern Colorado.

Methods:

Colony mosquito membrane feeding assays

To confirm previous findings of the susceptibility of *Cx. tarsalis* to IVM, we performed artificial membrane feedings over a range of oral IVM doses using the Kern National Wildlife Refuge (KNWR) colony established in 2002 from *Cx. tarsalis* collected at the Kern National Wildlife Refuge (35.7458° N, 118.6179° W), in Kern County, California. *Cx. tarsalis* were reared under consistent insectary conditions (temperature 24°C, relative humidity 40-60%, photoperiod 14L:10D). Larvae were reared in plastic trays with approximately 300-400 larvae in approximately 750 mL of water and fed ground Tetramin fish food daily until pupation. Adults were housed at approximately 300 per cage with constant access to 10% sucrose solution until allocation into 3.97 L (1 gal) plastic cartons with screen tops for bioassays. For mosquito bioassays, we added IVM (Sigma Aldrich 18898, PubChem Substance ID: 24278497) in heparinized sheep blood (HemoStat Laboratories, Dixon, CA, USA) at serial dilutions (600, 300, 150, 75, 37.5, 0 ng IVM/mL) for artificial membrane feeding. Approximately 70 adults were allocated into each treatment group. Following blood feeding, fully-engorged females were collected with a hand aspirator and held for nine days in the same insectary conditions. Mosquito mortality was recorded every 24 hours.

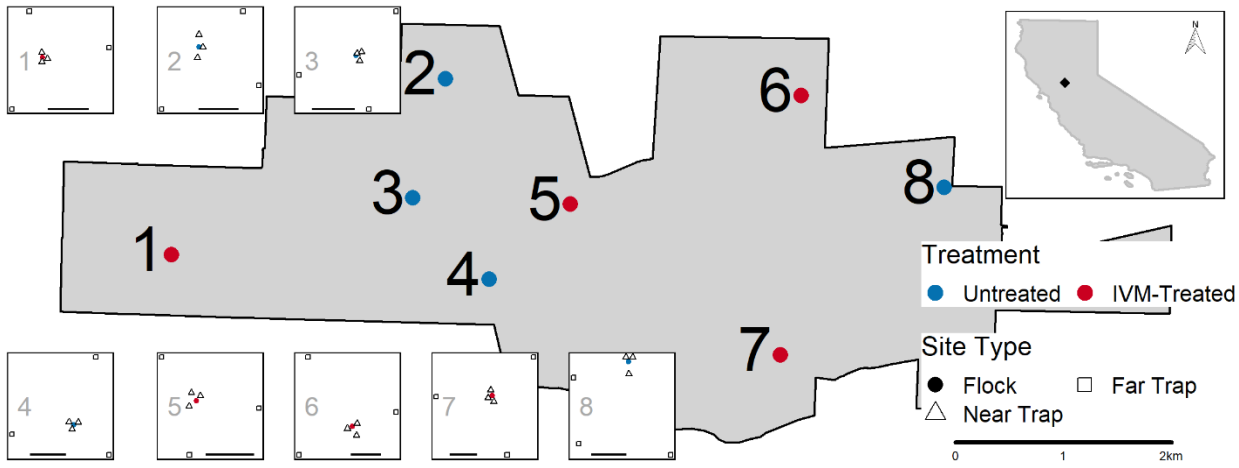

Figure 2-1: Location of ivermectin (IVM)-treated (red) and untreated (blue) chicken flocks and dry-ice baited mosquito traps in Davis, California. *Numbered insets illustrate the arrangement of dry-ice baited CO_2-traps around each flock, with three traps within 10m (triangle) and three traps approximately 150m from the flock (square). Bar in each inset indicates 100m. Top right inset illustrates the location of the study site in relation to the state of California.*

The lethal concentration resulting in 50% mortality (LC_{50}) was calculated using probit regression analysis (R statistical software, version 4.0.2 [56]).

Chicken flock field sites

We placed eight flocks—four IVM-treated and four untreated controls—of six chickens each in coops in backyards across Davis, California from June 28 - Sept 20, 2019 (Fig 2-1). Locations were chosen to achieve broad spatial coverage of the differing environments in suburban areas of Davis and in backyards of homeowners willing to host chickens for the duration of the study. We randomly assigned treatment status to flocks. We also placed 24 chickens in three coops at the UC Davis south campus facilities >2 km south of Davis city limits as an untreated reserve flock. We used 16-month-old female Lohmann Brown chickens in the Innovation Pet Chicken Homestead Coop (Tractor Supply, Brentwood, TN, USA), replacing the original 1.27 cm mesh sides with 2.54 x 2.54 cm welded wire mesh (YardGard, North Plains, OR, USA) to permit mosquitoes to access the chickens freely for blood-feeding and exclude predators.

Chicken care and monitoring

Treated flocks received IVM daily in their feed (200 mg IVM/kg feed) during routine husbandry from July 8 - Sept 18, 2019. We mechanically mixed powdered IVM (Sigma Aldrich 18898, PubChem Substance ID: 24278497) into chicken feed daily (1:40 ratio of DuMOR 16% Poultry Layer Crumbles and DuMOR grit, Tractor Supply, Brentwood, TN, USA). All flocks daily received a total of 0.907 kg feed (1/3 lb feed per chicken). All procedures were approved by the University of California Davis animal care and use committee (protocol #20980).

We monitored for WNV seroconversions in all chickens and IVM levels in the blood of treated chickens every 1-2 weeks throughout the study, taking all blood samples at similar times in the morning. We obtained blood samples from a comb prick and/or the brachial vein. We initially used a comb prick to obtain a small blood sample for WNV surveillance, but a brachial bleed reduced the handling time so all blood samples after week four were via brachial bleeds. For WNV serology, we soaked a 1.27 cm wide filter paper strip with blood, either from the comb following piercing with a standard lancet [17] or from the blood obtained from the brachial bleed. For quantification of IVM, we collected whole blood samples into serum tubes (Greiner Bio-One Serum Clot Activator Tubes, Fisher Scientific, Pittsburgh, PA, USA), gently inverted three to five times, and held at ambient temperature to coagulate. Following coagulation, we centrifuged the blood at 1800 RPM for 10 minutes at 4°C and removed the serum. Serum was stored at -80°C until testing to quantify IVM using high-performance liquid chromatography (HPLC)-fluorescence. IVM was first extracted from serum and derivatized after methanol precipitation, following [46,57,58] with the modification that 50 µl of serum was added to 400 µl of methanol prior to vortexing. A Waters 700 autosampler system was then used for quantification as previously described [46]. Briefly, a mobile phase of acetonitrile/water (3:1, v/v) was pumped through a C8 column (Waters, XBridge BEH C8 XP, 130 Å, 2.5 µm, 3.0x100 mm) at a rate of 0.45 mL/min and 50 µL of derivatized sample was injected by the autosampler. Excitation and emission spectra were 365 and 470 nm, respectively.

Blood samples on filter paper were submitted to the California Department of Public Health for testing for IgG antibodies to flaviviruses (West Nile, western equine encephalomyelitis, and St. Louis

encephalitis viruses) using an enzyme immunoassay [59,60]. A western blot was used to differentiate positive samples due to the presence of WNV antibodies. WNV seroconversions in IVM-treated vs. untreated flocks was analyzed using Kaplan-Meier survival curves and compared using the Mantel-Haenszel test in R [56] (version 4.0.2; survival package version 3.1-12 [61]).

Mosquito monitoring and indices

We collected mosquitoes weekly to estimate *Culex* abundance, infection prevalence, and parity near (≤10 m) and far (~150 m) from each coop location using an array of six CO_2-baited EVS traps per location, three near and three far (Fig 2-1). Traps were placed in yards, greenbelts, and parks, aiming to maximize similarity of environmental contexts of trap sites across coop locations. We placed traps between the hours of 1430-1800 and picked them up following morning between 0730-1000. Each week, half were run Mon-Tue and the other half Wed-Thu. Collected mosquitoes were immobilized with triethylamine [62] and identified by species and sex [63]. *Cx. tarsalis* and *Cx. pipiens* females were pooled separately for WNV testing (pools up to ~50 each). If >50 *Cx. tarsalis* females were collected from a trapping site, we removed 20% (up to 30 females total) for parity dissection. We calculated vector index [64], which combines abundance and infection prevalence, to estimate the number of infectious mosquitoes present at each distance for treated and untreated locations. We used ANCOVA to compare total abundance, infection prevalence, and vector index separately between near and far trap locations for treated and untreated locations across the study period (car package version 3.0-8 [65] in R software [56], version 4.0.2).

Following previously described ovarian tracheation techniques [66,67], we dissected mosquito ovaries in a drop of deionized (DI) water on a glass slide under a stereomicroscope and mounted them on slides, allowing them to dry before storing them in slide boxes. If we were unable to complete mosquito dissections on the day of trap collections, they were stored at 4°C for up to two days until dissections could be completed. After drying, slides were stored at room temperature until they could be examined. All slides were read using a compound microscope independently by two researchers (KMH and ETL) who were blinded to the treatment status. Any discrepancies in grading were resolved by mutual consent

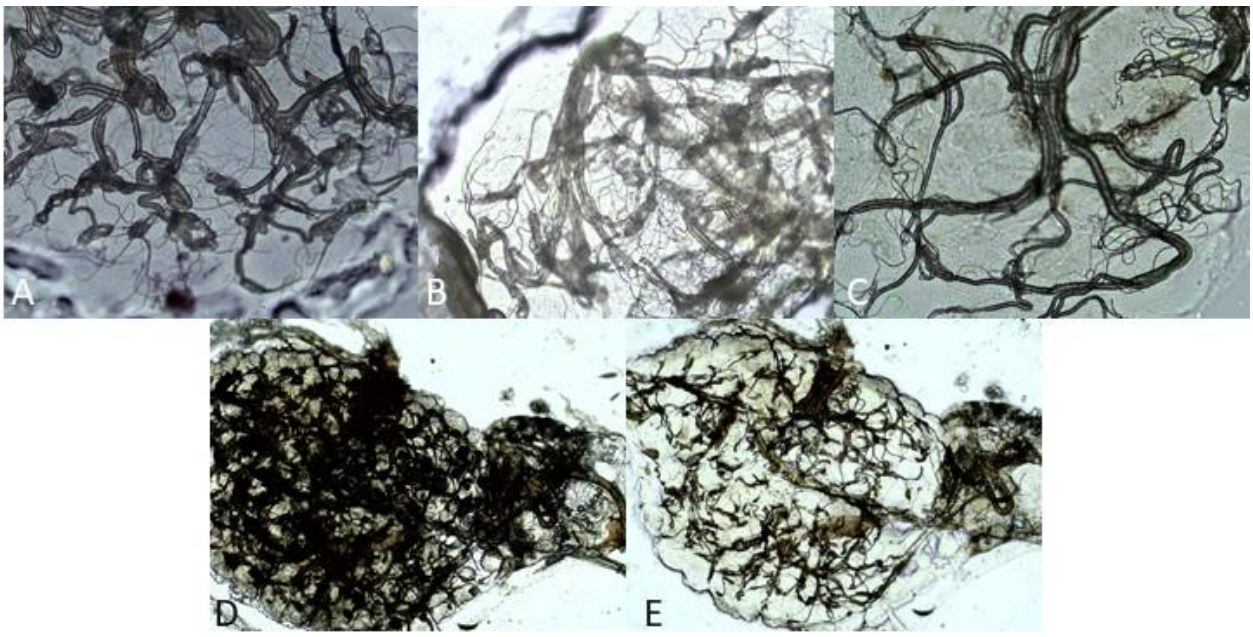

Figure 2-2: Categories used for ovarian grading in Cx. tarsalis. *Parity status graded as A) nulliparous with all tightly wound skeins, B) intermediate with a combination of tight and loose skeins, or C) parous with all unwound skeins. D) Dark mass observed in some ovaries, likely due to egg protein, which is E) removed with washing with deionized water. Ovarian mounts presented at 400x magnification (A-C) or 100x magnification (D-E).*

following re-observation. If a reticulated pattern or dark mass (Fig 2-2D) [68], likely due to egg protein [69], obscured the ovary, we washed slides briefly with DI water and air-dried before re-examining (Fig 2-2E). We classified ovaries as nulliparous when all skeins were tightly coiled (Fig 2-2A), parous when all skeins were completely unwound (Fig 2-2C), and intermediate when a combination of tight and unwound skeins were observed or all skeins appeared loose, but not fully unwound (Fig 2-2B) [68–70]. We included this third category due to high rates of autogeny in *Cx. tarsalis* previously reported in California's Central Valley [71,72]; autogenous females lay a smaller than average egg batch prior to the first bloodmeal, resulting in an intermediate appearance that cannot be accurately assigned prior to the first bloodmeal [69]. Following subsequent bloodmeals, ovaries of autogenous and anautogenous mosquitoes are indistinguishable. We first compared the overall parity rates (i.e., proportion of parous mosquitoes) across distances for each treatment group using Z-tests (R software [56], version 4.0.2) and then examined the number of parous vs. nulliparous or intermediate mosquitoes between treatment groups and distances with mixed effects logistic regression, using week as a random intercept (lme4 package

version 1.1-23 [73] in R version 4.0.2 [56]). Comparisons between all treatment-distance pairings were based on the Wald test (aod package version 1.3.1 [74] in R version 4.0.2 [56]). For both the Z-test and mixed effects model, we included the intermediate category to obtain a conservative estimate on change in age structure.

At the termination of the study, we fed wild-caught adult *Cx. tarsalis* females on one, randomly selected chicken from each flock to assess the mosquitocidal activity of the blood of treated chickens vs. untreated chickens for wild mosquitoes. *Cx. tarsalis* were collected using CO_2-baited EVS traps from the nearby Yolo Bypass Wildlife Area, a 65-km^2 area consisting primarily of rice (*Oryza sativa* L.) fields and managed wetlands. We allowed approximately 700 wild-caught *Cx. tarsalis* to feed on each chicken overnight. Each chicken was placed within a lidded plastic bin (73.03 x 40.64 x 46.36 cm) inside a secondary 120 x 60 x 60 cm mesh enclosure (BugDorm-6M60, Taiwan), set within the same backyard as the flock from which it was taken. The sides of the plastic bin were cut out, leaving 2.5-5 cm borders around all sides, and the open plastic bins were wrapped in 2.54 cm-square chicken wire that was secured to the bin's frame with zip ties. Our design allowed mosquitoes introduced into the container to have free access to the chicken, but also have ample resting locations within the BugDorm out of reach of the chicken. In the morning, all mosquitoes were collected from the BugDorms and sorted in the lab; blood-fed mosquitoes from each flock location were placed together in an individual 3.79 L (1 gal) plastic carton with screen top, provided a sugar water-soaked cotton ball or wick, and held in standard insectary conditions for eleven days. We recorded mortality and removed dead mosquitoes every 24 hours. We analyzed the survival of wild mosquitoes fed on treated vs. untreated chickens using Kaplan-Meier survival curves and compared using the Mantel-Haenszel test. A Cox proportional-hazards model was used to compare mosquito survival stratified by the IVM serum concentration in treated chickens (at or above the limit of quantification). Survival analyses were performed using the survival package version 3.1-12 [61] in R (version 4.0.2 [56]).

Safety of IVM ingestion

To assess any effects of prolonged oral exposure to IVM in birds, we necropsied twelve chickens upon completion of the field study; three randomly selected per flock from two treated and two untreated flocks. The pathologist was blinded to the treatment status of the chickens. Samples were taken for histological exam (brain, peripheral nerves, skeletal muscle, heart, lungs, trachea, liver, kidney, ovary, pancreas, and intestines) and to quantify the level IVM (liver, brain, lung, intestines, fat, blood, feces, kidney, and trachea). During the necropsy, a gross examination was made to assess the overall health, tissue status, and presence of parasites. Fisher's exact test was used to compare the distribution of pathological and histological findings at necropsy between treated and untreated groups (R software, version 4.0.2 [56]).

Results:

Colony mosquito bioassay

Cx. tarsalis mosquitoes (KNWR colony) are susceptible to IVM. We observed dramatic reductions in survivorship following ingestion and up to 100% mortality within three days (Appendix 3: Fig A2-1), supporting results previously obtained with the Bakersfield colony [46]. We estimated an LC_{50} at three days post-bloodmeal of 66.03 ng/mL.

Chicken WNV seroconversions

Due to the late timing of chicken availability, we obtained baseline blood samples from all chickens on the same day the chickens were placed in backyards. After serological testing, chickens that were initially seropositive at baseline were replaced in the untreated flocks with immunologically naïve chickens from our reserve flock, but seven seropositive chickens in the treated flocks could not be rehoused following treatment. Therefore, seven seropositive chickens remained across all treated flocks (3-5 seronegative chickens per treated flock).

At the end of the WNV season, accounting for the timing of replacement of seropositive chickens, fewer chickens seroconverted in treated flocks (3/17, 18%) than in untreated flocks (11/24,

46%) and these seroconversions occurred later in the season compared to untreated flocks, pointing to a lower WNV transmission to chickens at treated locations (Fig 2-3, $\chi 2 = 4.7$, $P = 0.03$).

IVM serum concentrations in chickens

In treated chickens, serum concentrations ranged from 0-155.2 ng/mL, with an average concentration of 33.1 ng/mL (Fig 2-4). Serum concentration generally peaked early during the study (max 155.2 ng/ml) and decreased to a lower level during the remainder of the study period (average final concentration 22.6 ng/ml). All the samples with zero concentrations (five samples from four chickens) were from a single flock (coop 7). All these chickens did have non-zero levels of IVM at different times in the study. The limit of quantification (LOQ) of the assay was 5 ng/mL.

Serum samples from untreated chickens across the study period had no IVM present, confirming there was no accidental cross-contamination between flock locations.

Mortality in field-collected adult *Cx. tarsalis* following blood-feeding on IVM-treated chickens

Serum concentrations (ng/mL) obtained from the four randomly selected treated chickens on the morning prior to the bioassay were 32.3, 23.2, 17.0, and at the limit quantification (5 ng/mL). During the

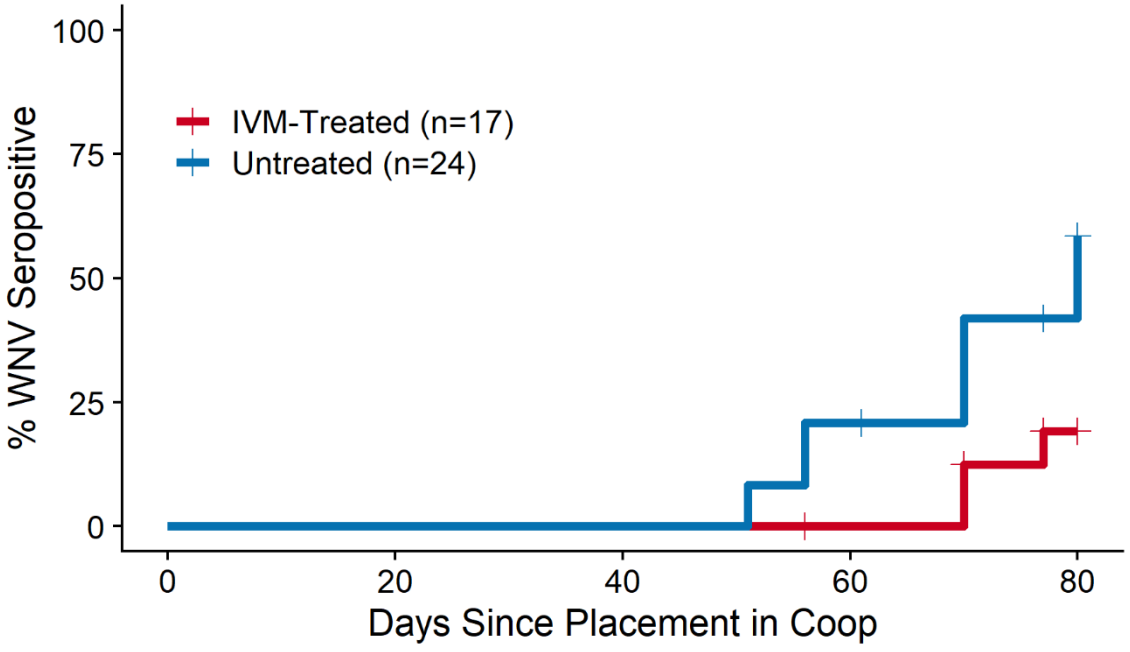

Figure 2-3: Reduced West Nile virus (WNV) seroconversions in ivermectin (IVM)-treated vs. untreated chicken flocks. *Four flocks per treatment group*.

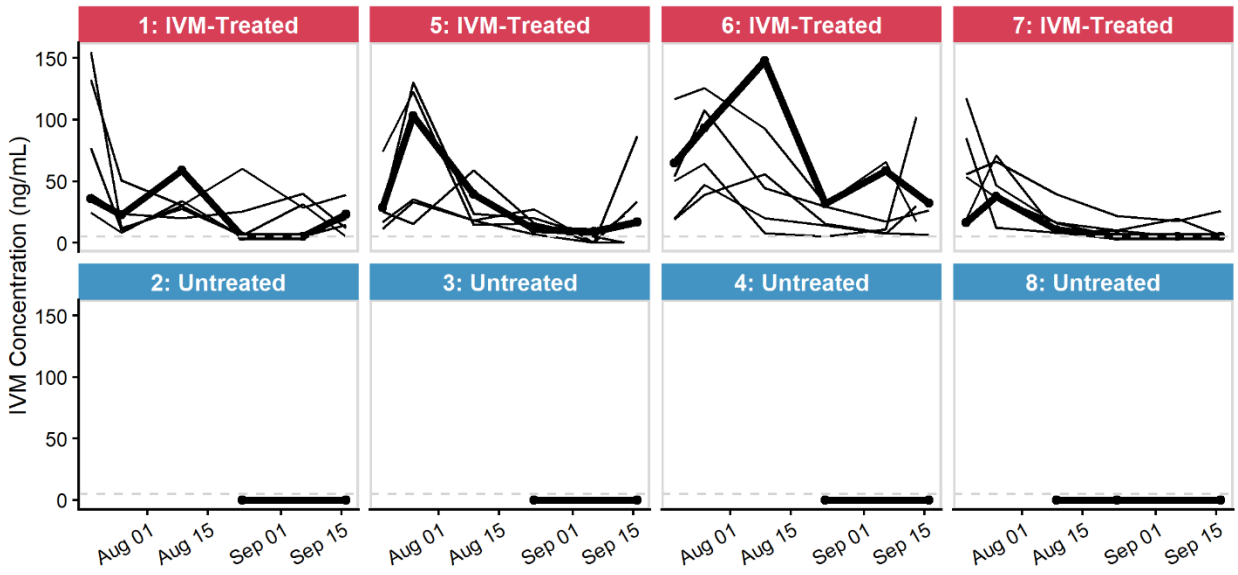

Figure 2-4: Ivermectin (IVM) serum concentrations (ng/mL) in treated and untreated chickens. *Grey dashed line indicates the limit of quantification (LOQ, 5 ng/mL). Bold line indicates chicken used in mosquito bioassay at end of study.*

three days post-bloodmeal when IVM-related effects were expected to occur [46], the higher mortality observed in wild *Cx. tarsalis* feeding on a randomly chosen IVM-treated chicken vs. an untreated chicken was approaching significance (Fig 2-5A, $\chi 2 = 3.09$, $P = 0.079$). When stratified by the final serum concentration in treated chickens (Fig 2-5B), we observed a significant difference in the mortality of wild mosquitoes feeding on a treated chicken with a serum concentration above the LOQ (5 ng/mL) vs. on an untreated chicken (hazard ratio = 1.82, Z = 4.84, $P < 0.0001$). We observed a 45.6% morality in mosquitoes within three days post-bloodmeal on a chicken with a concentration above the LOQ. This was 16.3% greater than mortality observed during this period in mosquitoes following a bloodmeal on an untreated chicken. There was no difference in mortality between mosquitoes feeding on the chicken with a concentration at the limit of quantification vs. an untreated chicken or (hazard ratio = 0.79, Z = -1.39, $P = 0.16$).

When considering the full 11-day course mosquitoes were held for post-bloodmeal, *Cx. tarsalis* fed on an IVM-treated chicken had a significantly higher mortality than those fed on an untreated chicken ($\chi 2 = 10.71$, $P = 0.001$). Additionally, the survival curves remained approximately parallel from day 3-11

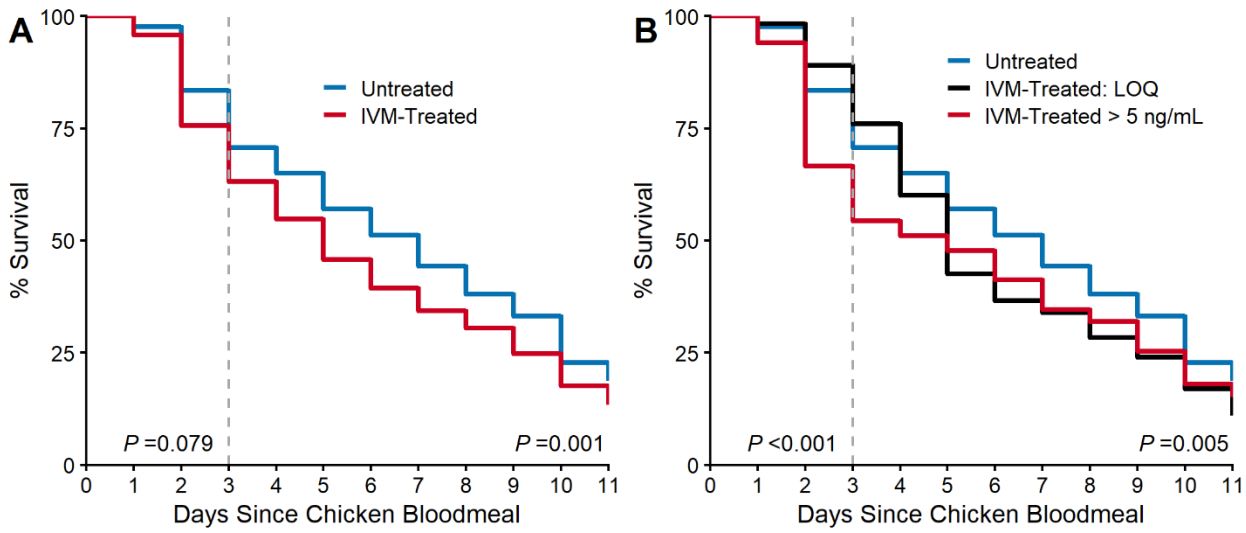

Figure 2-5: Blood feeding on ivermectin (IVM)-treated chickens above limit of quantification increased wild Culex tarsalis mortality. *Wild-caught Cx. tarsalis survival following blood feeding on a randomly chosen chicken from each coop A) by treatment group and B) stratified by serum concentration at or above the limit of quantification (LOQ, 5 ng/mL). Mantel-Haenszel chi-square (A) or likelihood ratio test (B) P-value indicated for comparison of Kaplan-Meier survival curves for 1-3 and 1-11 days. IVM-related effects expected to occur within 3 days post-bloodmeal (vertical dashed line).*

post-bloodmeal, supporting the conclusion that the majority of the difference in survival between groups occurred in the expected three-day period when IVM-related effects occur.

Effect of IVM on parity of *Cx. tarsalis*.

We observed an overall reduction in parity rates near vs. far IVM-treated flocks, but parity rates varied significantly between weeks (Fig 2-6). Of the 3,665 total dissections, we removed 139 that contained eggs as completely developed eggs prevented visualization of the tracheoles. The nine weeks with observations in each of the four groups (i.e., weeks 29-37) encompassed 3,342 dissections. Of these, 2,748 were graded as either parous, nulliparous, or intermediate and 594 could not be evaluated due to damage to tracheoles or because ovaries were obscured by fat or reticulation. We observed a highly significant reduction in the overall parity rates near treated flocks compared to corresponding far sites (43.5% vs. 50.7%; Z = 90.72, $P = 0$), in contrasted to nearly equal, yet still significant, rates near and far from untreated flocks (near: 47.9%; far: 47.5%; Z = 6.02, $P < 0.0001$) (Fig 2-6C). Large sample sizes resulted in high power to detect small difference in parity rates between groups.

Table 2-1. Odds ratio (95% CI) of a mosquito being parous across distances from ivermectin (IVM)-treated and untreated flocks.

Comparison	Odds Ratio	95% CI	*P*-value^
Control-near vs. Control-far	1.09	0.89, 1.33	0.407
Control-near vs. IVM-near	0.74	0.61, 0.90	0.002
Control-near vs. IVM-far	0.88	0.73, 1.06	0.184
IVM-near vs IVM-far	0.84	0.68, 1.03	0.096
IVM-near vs. Control-far	0.68	0.55, 0.85	< 0.001
IVM-far vs. Control-far	0.81	0.66, 1.00	0.046

^ Wald test *P-value*

When adjusting for the effect of weeks on parity, mosquitoes near IVM-treated flocks had reduced odds for being parous as compared to mosquitoes near untreated control flocks (Table 2-1; OR = 0.74, $P = 0.002$). However, there was no difference in the odds of a mosquito being parous near vs. far within each treatment group (Table 2-1; IVM-treated: $P = 0.18$; Control: $P = 0.71$) and parity rates at both distances of IVM-treated coops were relatively lower than at corresponding distances from untreated control coops. See Appendix 3: Table A2-1 for full model regression results, including random effects.

Cx. tarsalis abundance, infection prevalence, and vector index

We observed a temporal pattern in *Cx. tarsalis* abundance typical of the Sacramento Valley [75,76] with trap counts increasing sharply across all groups to a peak in early August and decreasing into

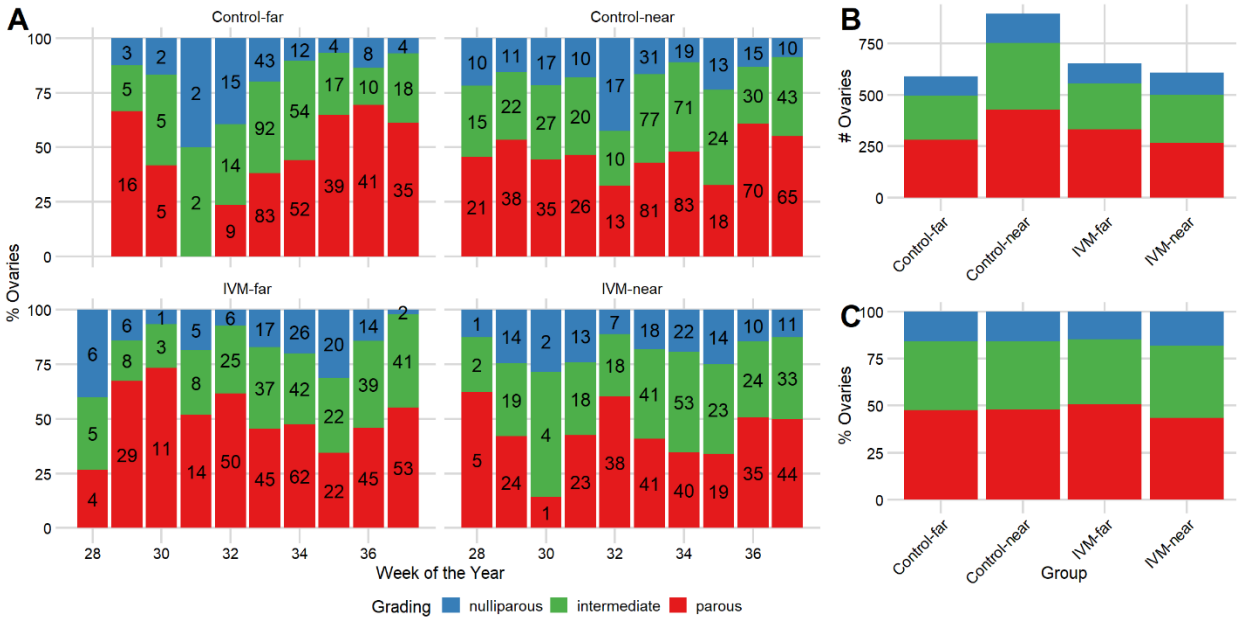

Figure 2-6: Parity rates in Culex tarsalis around ivermectin (IVM)-treated and untreated chicken flocks. *Parity grading during the West Nile virus (WNV) season near (≤10m) and far (~150m) from treated and untreated flocks for (A) individual weeks and (B-C) collapsed by group for weeks with observations in each group (weeks 29-37). Number of ovaries in each category by week indicated in bars in A.*

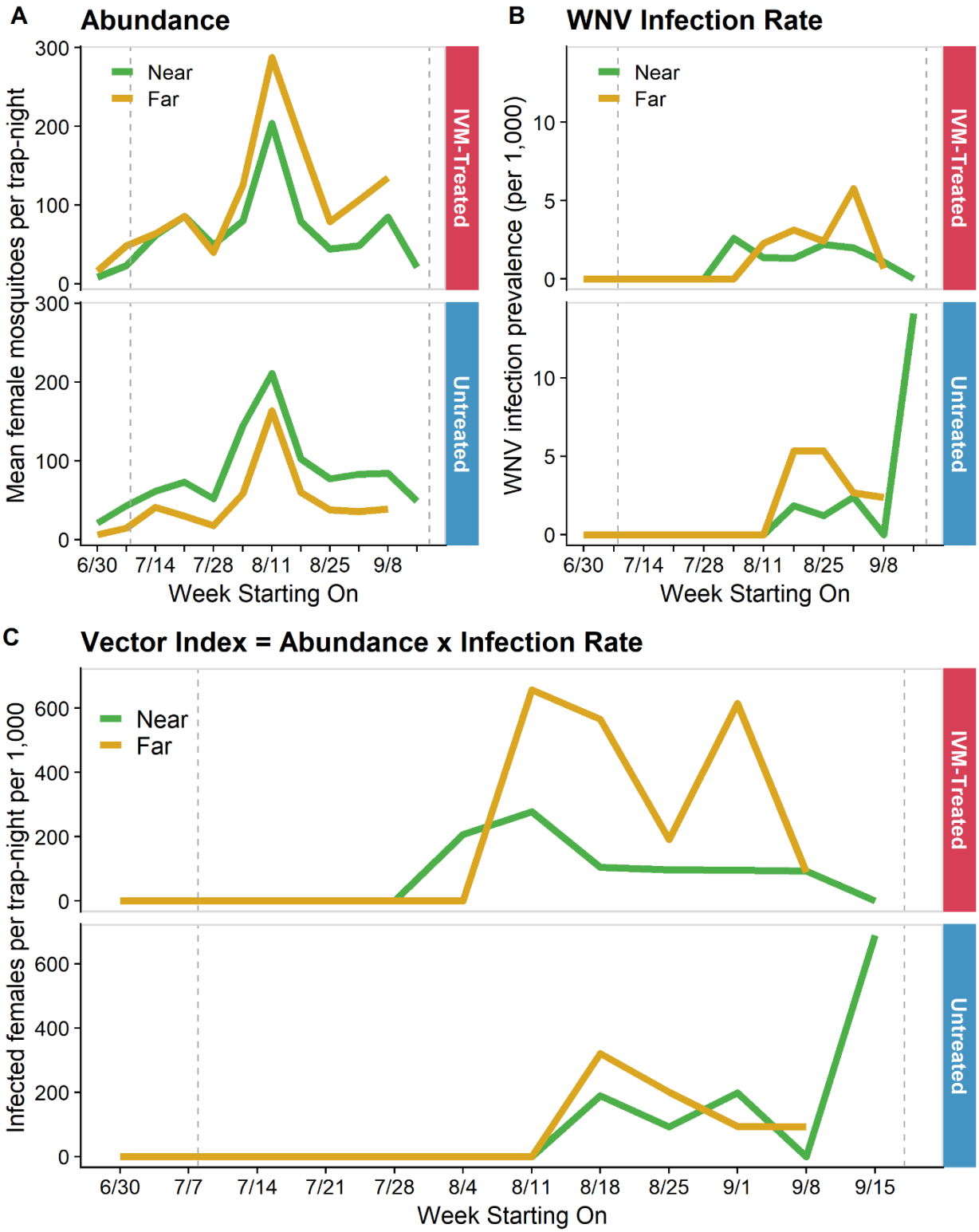

Figure 2-7: Entomological indices of Culex tarsalis around ivermectin (IVM)-treated and untreated chicken flocks. *Weekly (A) abundance, (B) infection prevalence per 1,000, and (C) vector index (VI) near (≤ 10m) and far (~150m) from IVM-treated and untreated flocks. VI is a risk metric that approximates the number of infectious mosquitoes present as the product of abundance and infection prevalence. Vertical dashed lines indicate the first and last day IVM-treated feed was provided.*

September (Figure 2-7A). Higher average trap-counts were observed at sites closer to irrigated agriculture and overall in the eastern portion of Davis (A2). We observed a small increase in abundance in early September at both near and far distances of IVM-treated flocks. Excluding the final week due to small sample sizes, abundance varied significantly across weeks ($F(10, 162) = 3.661$, $P < 0.001$), but not across distance-treatment groups (i.e., near-treated, far-treated, near-control, far-control; $F(3, 162) = 2.144$, $P = 0.097$).

In terms of infection rates in mosquitoes, we observed a lower and later than average WNV season in Davis; typically, WNV is detected in late June and peaks in late August [77]. The initial detection of WNV occurred in early August at a near site to an IVM-treated flock and subsequently WNV was detected at both distances of flocks in both treatment groups for the remainder of the study (Fig 2-7B). Qualitatively, around IVM-treated flocks, infection prevalence increased over time at far distances, peaking in early September, while remaining similar or decreasing at near distances. We observed an opposite relationship in untreated flocks where infection prevalence peaked in far traps in August and subsequently declined while infection prevalence remained similar or slightly increasing at near traps; small sample sizes in the final week resulted in a dramatic increase in infection prevalence for the final week. Comparing infection prevalence across weeks with equal sample size (i.e., not the final week), there was no difference by week ($F(10, 162) = 1.421$, $P = 0.175$) or distance-treatment group ($F(3, 162) = 2.336$, $P = 0.076$).

Qualitatively, we observed a sustained reduction in the number of infected mosquitoes, as estimated by vector index, near vs. far from IVM-treated coops while the number of infected mosquitoes appeared very similar across distances for untreated locations (Fig 2-7C). However, there was no significant difference in vector index by week ($F(10, 162) = 1.709$, $P = 0.083$) or distance-treatment group ($F(3, 162) = 1.589$, $P = 0.194$), excluding the final week due to small sample sizes.

Abundance, infection prevalence, and VI varied across individual flock location (Appendix 3: Fig A2-2).

Table 2-2. Pathological and histological findings at necropsy of ivermectin-treated (*n* = 6) and untreated (*n* = 6) chickens.

Tissue or Condition	Pathological or Histological Finding	Untreated	Treated	*P*-value^
Liver	Healthy	4	3	1
	Hemorrhage	2	3	
Tapeworms	None	2	1	0.318
	Few to some	4	2	
	Moderate to many	0	3	
Ascarids	None	1	5	0.080
	Few to some	4	1	
	Moderate	1	0	
Leiomyoma	Present	1	1	1
	Absent	5	5	
Lymphocytes in peripheral nerves†	None	0	2	0.455
	Rare to small numbers	6	4	
Lymphocytic infiltrations‡	Multi-systemic	6	6	1
Chronic egg yolk peritonitis§	Mild to marked	6	6	1
Pneumoconiosis and/or BALT⁋ hyperplasia	Minimal to moderate	6	6	1

^ Fisher's exact *P*-value

† Few lymphocytes in sciatic nerves likely associated with Marek's disease. No other significant lesions in nervous system.

‡ Lymphofollicular formations in heart (*n* = 7), mesentery (*n* = 12), and oviduct wall (*n* = 1) suggestive of possible mycoplasma infection.

§ Common condition in older layer hens.

⁋ BALT: bronchus-associated lymphoid tissue

Chicken necropsies

Findings at necropsy did not indicate any pathological differences due to sustained IVM treatment at higher doses than previously used nor differences between treated and untreated control chickens (Table 2-2). The main findings on gross and histological exams were acute liver hemorrhages in both treated and untreated chickens, likely indicative of the early stages of hemorrhagic liver syndrome of unknown etiology. All birds regardless of treatment status also had chronic yolk peritonitis which is common in older layer hens [78,79].

Discussion:

We assessed the efficacy of IVM delivered via backyard chicken flocks to suppress the abundance of WNV-infected mosquitoes and transmission of WNV as measured by chicken

seroconversions using a randomized trial. Taken together, our results suggest that IVM administration altered local *Cx. tarsalis* populations and potentially the resulting WNV transmission dynamics. We found reduced WNV transmission to treated chickens compared to untreated controls as measured by chicken seroconversions. Wild *Cx. tarsalis* had increased mortality following a bloodmeal on chickens with a serum concentration between 17-32 ng/mL as compared to untreated chickens or a chicken with a concentration at the limit of detection (5 ng/mL). Additionally, we detected a significant reduction in mosquito parity near treated flocks vs. near untreated flocks. We did not find a difference in abundance or infection prevalence in mosquitoes between treatment groups.

The estimated LC_{50} for colony *Cx. tarsalis* at three days post-bloodmeal from our single trial (66.03 ng/ml) was higher than previously reported (49.94 ng/ml, 95% CI: 39.71-59.93) [46], but qualitatively similar. The previous work used mosquitoes from a different colony of the same species (Bakersfield Field Station, BFS), potentially indicating a difference in susceptibility between these long-established strains. However, we used a single replicate of much smaller sample sizes than the previous work as our goal was confirmation instead of estimation so differences in magnitude should not be emphasized.

The average IVM concentration observed in the treated chickens was 33.1 ng/mL, lower than the target LC_{50} values, but we did observe a 45.6% morality in wild mosquitoes within three days of a bloodmeal on treated chickens (serum concentration of 17-32 ng/mL), indicating that we did achieve mosuitocidal levels in the chickens. We did not observe any difference in mortality following a bloodmeal on a treated chicken with a concentration at the LOQ (5 ng/mL) and an untreated chicken, indicating a minimum concentration >5 ng/mL is required to achieve mosquitocidal effects in the field.

Serum concentrations in chickens ranged widely over the study, so the exact dose biting mosquitoes ingested at each timepoint is unclear. However, previous work indicates that even low levels of IVM may exert strong mosquitocidal effects in the field; in a serum-replacement assay, 100% mortality in two days was observed in wild *Cx. tarsalis* that ingested serum from a wild-caught grackle (5.7 ng/mL) as compared to control calf serum [46].

Previous studies in chickens indicate rapid elimination of IVM from plasma following oral treatment. Peak plasma concentration (10.2 ng/mL) occurred 3.36 hours after a single dose of 0.2 mg/kg administered orally via crop feeding tube (IVM diluted (1:5 v/v) with propylene glycol) [80]. IVM was not detected in plasma after three days. One day following a five-day treatment course of 0.4 mg/kg dosing in available drinking water, a peak plasma concentration of 1.07 ng/ml was observed and was no longer detected after seven days [81]. However, no studies assessed concentrations during repeated IVM administration. Given the rapid elimination of IVM from plasma, the timing of treatment is important to determine the level of IVM exposure to biting insects and indicate that sustained treatment would be required to maintain the mosquitocidal activity in the blood of treated chickens. In our study, blood samples were taken in the morning around the time chickens were fed so serum levels could feasibly have been lower in the evening when mosquitoes are host-seeking. Therefore, the reported serum concentrations may represent the upper range of IVM to which mosquitoes were exposed. Additionally, given that these concentrations were lower than the estimated LC_{50} values and yet we observed significant increase in mortality approaching 50%, achieving the laboratory derived concentrations may not be necessary.

Successful mosquito control would be expected to produce a shift towards a lower mean age of the population due to elimination of extant adult mosquitoes that are replaced by newly emerged individuals. Parity was reduced significantly in *Cx. tarsalis* collected near treated flocks vs. near untreated controls, which, considered alone, suggested a possible elimination of older female mosquitoes attributable to IVM. Also, comparisons between distances within each treatment group showed that overall parity rates were lower at sites near vs. far from treated flocks, whereas rates remained very similar between distances for the untreated flocks. However, these distance-based comparisons were not significant for either treatment group, leaving open the possibility that some of the parity differences could have been due to chance differences in background mosquito population dynamics unrelated to treatment. Detecting a change in population age structure in natural setting following control is fraught with difficulties. Following aerial applications of adulticides, shifts in population age structure were not

discernable in highly connected areas or those with high autogeny rates, but were detected in semi-isolated areas with low autogeny [31,82]. In our study, we found a relatively high parity rate (44-51%), likely indicative of immigration of older female mosquitoes that could have diluted any effect of IVM on age structure. Also, the rate of autogeny in the Sacramento Valley is high (54-92%) [71,72], thus further increasing the difficulty to detect shifts in age structure due to IVM.

The increased mortality of wild mosquitoes feeding on treated chickens paired with the reduction in parity near treated flocks potentially indicates that reduced seroconversions resulted from the impact of IVM on mosquito populations. Additionally, as chicken seroconversions track human infections [83], IVM administration could have reduced zoonotic WNV transmission risk around treated flocks. However, we did not detect a difference in abundance or infection prevalence in *Cx. tarsalis* populations to fully connect the impact of IVM on mosquito populations to observed differences in WNV transmission. We found average trap-counts at sites closer to irrigated agriculture and overall in the eastern portion of Davis, as previously observed [84], highlighting immigration of *Cx. tarsalis* into Davis from areas with abundant larval habitats. Continual immigration of newly emerged *Cx. tarsalis* from these productive larval sites likely obscured any reduction in abundance due to IVM-induced mortality; the use of IVM is unlikely to cause sustained reduction in abundance in highly connected populations. Additionally, the lower-than-average WNV infection prevalence in Davis may have also impaired our ability to detect differences in mosquito infection rates, resulting in similar patterns across sites and treatments, and contributed to an inability to identify a difference in vector index across distances and treatment groups. We did not detect any negative health effects that were attributable to the sustained IVM treatment in chickens; typical IVM dosing schemes for parasites in birds involve a limited number and duration of treatment (1-2 doses over 7-14 days) [52,53]. Previously reported side effects of IVM toxicity in birds include slight somnolence, listlessness, ataxia, and death [51]. None of these were observed during daily checks. All birds regardless of treatment status did have chronic yolk peritonitis, but this is common in older layer hens [78,79].

While oral, subcutaneous, and intramuscular administrations of IVM are used to treat nematode infestations in birds, including chickens [53], we did not observe a significant reduction in ascarid loads at necropsy ($P = 0.08$), potentially due to small sample sizes and qualitative measures of loads used. Similarly, a previous study found that while highly effective against reducing experimental *Ascaridia galli* infections in chickens (89.8-95% reduction), two subcutaneous ivermectin doses of 0.3 mg/kg two weeks apart was not totally effective in eliminating the parasite [52]. Thus, while a side benefit of IVM treatment may be a reduction, complete elimination of any nematode loads may not be expected.

Our decision to randomization treatment status among the eight flock sites resulted in some limitations of our statistical power to detect treatment effects. Abundance of and WNV infection rate in *Cx. tarsalis* exhibited clear spatial patterns from east to west across the city of Davis during the study period, like Nielsen et al. reported [84]. Therefore, blocking flocks spatially into treated and untreated pairs to ensure equal representation of treatment groups across the gradient in entomological indices would have increased our statistical power.

Additionally, the unexpected number of seropositive chickens at the start of our study resulted in smaller than expected and unequal group sizes between treatment status. Even though the chickens had been housed outdoors during the previous year, we did not expect many seropositive chickens based on low annual seroconversions in previous sentinel flocks in Davis [85]. However, despite the resulting sample sizes, we were still able to detect a significant difference in seroconversion rates between groups. Future studies employing a larger sample size of spatially paired flock sites would be needed to further support these findings.

Homeowners enjoyed hosting chicken flocks, but this led to the unintended consequence that they supplemented our study diet with occasional food scraps, contrary to our instructions. We did observe five instances of 0 ng/mL IVM concentrations in chickens in one of our treated coops and this might be attributable to this supplemental feeding. While all feed was ingested each day, some chickens may have refrained from eating our provided food, preferring the supplemental items, and thus stopped self-medicating for a period, resulting in some degree of IVM washout. We observed evidence of

supplemental feeding occurring in other flocks as well, including other treated flocks, thus reducing the IVM concentrations in treated chickens and potentially biasing our results towards no effect of IVM on mosquito populations. We did not track the timing or identity of items provided for supplemental feeding, so we were unable to confirm supplemental feeding was the cause of this observation or quantify the potential extent of the impact on our findings.

Another limitation encountered was the relatively large number of ovarian mounts that could not be classified. Similar to previous evaluations using the tracheation method to age-grade mosquitoes (8-25%) [68,69], a portion of our mounts (19.2% overall and 17.8% in weeks 29-37) were unsatisfactory for classification due to obscuring dark masses likely attributable to egg yolk protein, loss of tracheation during dissection, and presence of debris and fat occluding tracheas. Despite this, we did detect a difference in parity, but the number of ungradable mounts prolonged the processing time required. This high level of un-gradable specimens across studies highlights the need for improved age-grading techniques. An alternative method, outlined by Polovodova [86] and applied to *Cx. tarsalis* by Nelson [87], uses dilatation in follicular tubes following development and deposition of an egg batch to successfully differentiates nulliparous and parous *Cx. tarsalis* females, removing the ambiguity of intermediate classifications resulting from the tracheation method [70]. However, this method still requires dissection and a relatively long processing time per mosquito [88]. A recent modification to a method suggested by Perry [89] based on wing wear uses the number of scales along the distal edge of wings of *Anopheles gambiae* to determine the relative age and can be automated [90]. If a similar relationship of scale loss and age holds for *Cx. tarsalis* populations, this automatable method would provide rapid and fine-scale resolution to relative age, but loss of scales due to passage through the fan and time spent in the collection container may still result in inappropriate grading of trap collected mosquitoes.

Use of backyard chickens as the means for exposing wild mosquitoes to IVM was intended as a first step toward potential future uses of IVM in backyard bird feeders as a way of achieving targeted WNV control near human residences. Compared to starting with wild birds, chickens had the advantage

of remaining in a single location and are fed upon frequently by *Cx. tarsalis* where they are present [54,55]. We anticipated that these factors would give the greatest chance at identifying IVM's spatial effects on mosquitoes and WNV transmission. For long-term considerations, IVM, a lipophilic drug [91], is known to accumulate in eggs [81,92], which is almost certain to limit appeal of a chicken-based control strategy among homeowners who typically eat the chickens' eggs. The FDA has not set a minimal allowable level of IVM in eggs, and homeowners were advised not to ingest eggs from treated chickens during this study. Assessing IVM concentrations in chickens and eggs over time, and the human health ramifications of ingesting such eggs was outside the scope of this study.

Conclusions:

Following oral administration of IVM to backyard chickens, we detected evidence of a reduction in WNV transmission due to the impact of IVM on *Culex* mosquito populations. We observed fewer WNV seroconversions in treated chickens than untreated chickens, a reduction in parity rates of *Cx. tarsalis* near treated vs. untreated flocks, and increased mortality in wild mosquitoes following a bloodmeal on treated chickens vs. untreated chickens. Serum concentrations resulting in increased mortality ranged between 17-32 ng/mL while a concentration at the limit of detection (5 ng/mL) did not increase mortality compared to untreated chickens, indicating that a certain threshold may be required to cause significant mosquitocidal impacts. IVM concentrations varied widely across the season with a mean of 33.1 ng/mL (range: 0-155.2 ng/mL). We did not observe a difference in either abundance or WNV infection prevalence in *Cx. tarsalis* populations between treated and untreated sites, potentially due to sustained immigration of newly emerged individuals and lower-than-average WNV activity in the study area. Sustained oral ingestion of IVM did not result in any adverse events highlighting the safety of this method. Taken together, oral administration of IVM provides a potential avenue for specifically targeted control of WNV in local areas. Future work aims to transition to wild birds and develop a commercial treated birdfeed for homeowner use to reduce WNV risk on the local neighborhood scale.

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Chapter 3: Feasibility of deploying ivermectin-treated birdfeeders for local control of West Nile virus transmission

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Abstract

Ivermectin (IVM)-treated birds provide the potential for targeted control of *Culex* mosquitoes to reduce West Nile virus (WNV) transmission by increasing the mortality of bird-feeding mosquitoes involved in the enzootic maintenance and amplification of WNV. Therefore, this strategy provides an alternative control strategy that is not hampered by increasing levels of insecticide resistance nor the logistics of large-scale pesticide applications. To assess the feasibility of deploying IVM-treated bird feed in neighborhoods to reduce WNV transmission, we aimed to characterize the bird feeder usage and nocturnal roost locations of six common backyard species (blue jays, common grackles, house sparrows, house finches, mourning doves, and red-winged blackbirds) and develop a spatially implicit compartmental patch model of WNV transmission in the presence of IVM-treated birdfeed. We tracked 105 birds using radio telemetry and radio frequency identification to monitor their feeder usage at and location of nocturnal roosts in relation to five feeder sites in a neighborhood in Fort Collins, Colorado.

Using these results, we modified a compartmental model of WNV transmission to account for the impact of IVM on mosquito mortality and spatial movement of birds and mosquitoes. Parameters with the largest influence on the estimated reduction in infection intensity were the competence of birds to infect biting mosquitoes with WNV, the level of IVM-induced mortality, and number of treated lots in a neighborhood while there was no significant difference in the reduction based on arrangement of treated lots. Increasing the proportion of treated birds, regardless of the WNV competency status, had the largest reduction in infection dynamics. Our results can guide the design and implementation of future field trials of this control strategy.

Introduction

West Nile virus (WNV), the leading cause of mosquito-borne disease in the United States [1], is maintained in an enzootic cycle between *Culex* mosquitoes and birds [2,3], but can spill over to infect other hosts like horses and human during a bloodmeal by an infectious mosquito [4]. Common bird species involved in maintenance and amplification of WNV include American crows and other corvids like jays, as well as house sparrows, house finches, and American robins [3,4]. In western North America, the predominant WNV vector mosquitoes are *Culex* (*Cx.*) *tarsalis* and those in the *Cx. pipiens* complex [5,6]. While 80% of infections in humans are asymptomatic, approximately 20% result in a febrile illness and around 1% in a potentially fatal neuroinvasive form of the disease with manifestations including encephalitis, meningitis, and acute flaccid paralysis [7]. Long-term physical and mental sequelae resulting from infection represent a considerable source of morbidity in patients long after recovery from the illness [8]. Average annual incidence is highest in the Great Plains region [9] due to the quantity of suitable larval habitat for *Cx. tarsalis* in areas of irrigated agriculture in proximity to avian amplification hosts and humans [10,11].

Current mosquito control strategies can reduce the risk of zoonotic WNV transmission but face several limitations. There is no licensed WNV vaccine for humans, and prevention strategies focus on the use of personal protective measures (i.e., applying insect repellent, wearing long pants and shirts, and avoiding dusk when mosquitoes are host-seeking) [12] and the control of mosquito populations through

removal of larval habitats and application of biological or chemical treatments to kill the larvae or adult stages [13]. While the use of larvicides can reduce the abundance of *Cx. pipiens* larva in catch basins [14,15], a common larval habitat for *Culex* in urban areas, larviciding alone is insufficient to control mosquito populations to prevent WNV transmission [16]. Response to imminent outbreak risk requires rapid elimination of adult mosquitoes. Ground-based adulticide applications can reduce target mosquito populations under ideal conditions, but estimates of the effects on WNV transmission are inconsistent [17–20]. Aerial applications of insecticides have greater costs [21], but during periods with high zoonotic transmission risk can rapidly reduce the abundance of WNV vectors [22] and infectious mosquitoes [23–27], and have been linked to a reduction in human WNV cases in a treated area versus an untreated area [27]. However, efficacy varies widely due environmental conditions [28,29] and adulticide spraying has limited ability to target the bird-biting mosquitoes responsible for WNV maintenance and zoonotic transmission. Additionally, applications of insecticides often face resistance by some members of the public [30] and the increasing development of insecticide resistance in mosquito populations can render current vector control strategies ineffective [31,32].

Orally administered ivermectin (IVM) represents a promising alternative control strategy that could overcome some of the limitations of current vector control strategies. It has a different mode of action than that of currently used insecticides, alleviating selection pressure driving the current development of insecticide resistance [33,34]. IVM decreased the survival of *Anopheles* mosquitoes that fed on treated humans [35,36], reducing cumulative malaria incidence following mass drug administrations of IVM in Burkina Faso [37,38]. Preliminary lab and field data indicated increased mortality in *Culex* mosquitoes that fed on IVM-treated chickens and Eurasian collared doves [39]. Pilot trials of IVM-treated wild birds and backyard chickens provided support for the efficacy and feasibility of IVM-treated birds to act as a WNV control strategy, pointing to a reduction in the level of WNV transmission near treated feeders and chicken flocks due to IVM (Chapter 2). However, the full impact on mosquito populations and WNV transmission was unclear as small sample sizes and low WNV infection rates in *Cx. tarsalis* limited statistical power.

Treating wild birds commonly fed upon by *Cx. tarsalis* with IVM can reduce WNV transmission without wide-scale applications of insecticides. House sparrows *(Passer domesticus*), house finches (*Haemorhous mexicanus*), and mourning doves (*Zenaida macroura*) are common bloodmeal hosts for *Cx. tarsalis* [6,40,41] that often visit backyard birdfeeders [42,43]. Additionally, blue jays (*Cyanocitta cristata*), common grackles (*Quiscalus quiscula*) and red-winged blackbirds (*Agelaius phoeniceus*) also often visit birdfeeders [42] and are occasionally fed upon by *Cx. tarsalis* [6]. Thus, these species could effectively disseminate IVM to bird-biting mosquitoes involved in enzootic WNV transmission, preventing subsequent bloodmeals at which WNV transmission could occur.

The effect of IVM-treated feeders on WNV transmission depends on birdfeeder usage and spatial distribution of nocturnal roosting of backyard birds, but these habits are largely unknown during the WNV season (Jun-Sep). Feeder usage patterns influences the attainment and duration of mosquitocidal levels of IVM in the blood of birds. Nocturnal roost constancy, or the probability a bird uses the same roost on two consecutive nights [44], and proximity of roosts to birdfeeders influence the potential spatial extent of this control strategy by modifying the distribution and number of potentially fatal mosquito bloodmeals present in the local area. Breeding season and fall migration are intensively studied by ornithologists, but the intervening period, which coincides with the WNV season, has received less attention [45]. Previous studies on feeder usage patterns investigated species-specific seed preferences [42], the structure and health of avian communities around feeders [43,46,47], and assessed seasonal feeder usage patterns across a gradient of greenspace fragmentation in urban areas [48]. Assessment of nocturnal roosts is generally performed in conjunction with seasonal changes in communal roost composition [44,49] or reproductive strategies [50]. To our knowledge, no study has assessed nocturnal roost locations of backyard birds in relation to bird feeders.

Modeling can aid in establishing data-based theoretical expectations for the effects of IVM-treated birdfeed on neighborhood-level WNV transmission accounting for uncertainty in avian population dynamics. We can also use a modeling framework to design effective deployment strategies.

In this study, we assessed the feasibility of deploying IVM-treated bird feed in neighborhoods to reduce WNV transmission through two interrelated aims: (1) characterization of birdfeeder usage and nocturnal roost locations of common backyard species and (2) estimation of the optimal deployment strategy of IVM-treated birdfeeders. For the first aim, we monitored birds using a combination of radio telemetry, radio frequency identification, motion-activated cameras, and point counts in a neighborhood with untreated birdfeeders. To address the second aim, we developed a spatially implicit compartmental patch model of WNV transmission on the neighborhood-scale in the presence of IVM-treated birdfeeders, using results from fieldwork to parameterize the bird populations. Using the model, we assessed the density and spatial arrangement of treated feeders required for effective WNV control and evaluated whether treating bird populations broadly obtained larger reductions in infection dynamics than treating birds based on WNV competence.

Methods

Methods overview

To characterize birdfeeder usage and nocturnal roosting habits, we tagged and monitored six species of common backyard birds [i.e., blue jays (*Cyanocitta cristata*), common grackles (*Quiscalus quiscula*), house finches (*Haemorhous mexicanus*), house sparrows (*Passer domesticus*), mourning doves (*Zenaida macroura*), and red-winged blackbirds (*Agelaius phoeniceus*)]. We used radio telemetry to determine the spatial distribution of nocturnal roosts on sequential nights. We used radio frequency identification to assess birdfeeder usage patterns. Comparing motion-activated camera pictures with point counts, we determined the proportion of WNV competent and incompetent species visiting birdfeeders.

We also developed a spatially implicit patch model to capture WNV infection dynamics with self-medication of birds at IVM-treated feeders and dispersal of birds and mosquitoes across lots in a neighborhood. We used our field-derived nocturnal roost locations to parameterize Gaussian dispersal kernels for avian dispersal across lots. In each patch (IVM-treated and untreated), WNV dynamics between mosquitoes and birds proceeded according to a compartmental model, accounting for increased mosquito mortality following a bloodmeal on an IVM-treated bird.

Field study on avian birdfeeder usage and nocturnal roosting habits

Birdfeeder site selection and monitoring

We selected five locations in eastern Fort Collins, Colorado (Figure 3-1) comprising neighborhoods adjoining a central natural area (River Bend Ponds Natural Area) to place bird feeders during the summer of 2020 (June-September). We selected sites which provided spatial coverage of the area and in locations where we observed our six target species. We arranged four of the sites approximately 700 m away from our main site and placed a single tube feeder (classic tube feeder, New Hyde Park, NY, USA) on a shepherd hook at each of these sites. Sites were in neighborhoods (n = 3) and

Figure 3-1: Bird feeder placement sites in Fort Collins, Colorado. *The main study site (site 1) contained two sub-sites on opposites ends of the property with two tube feeders at 1a and two tube and one platform feeder at 1b. The remainder of the sites had a single tube feeder. Green outlines indicate borders of the natural areas. Grey lines trace roads.*

in the natural area (n = 2) to represent the land use types present. At the main site (site 1), we established two sub-sites at opposite ends of the property (approximately 37 m apart) to represent movement of birds within the property. We placed five total feeders (four tube and one platform) at this site with two tube feeders on a pole in the northwestern section (site 1a; placed Jun 22 and 24) and two tube and one platform feeder suspended on clothesline in the southeastern section of the yard (site 1b; tube feeders placed Jul 1 and platform placed Jul 9). We established the other sites on Jun 24 (sites 2, 3, and 5) and Jul 9 (site 4).

At all locations, we hung each tube feeder under a squirrel baffle (Perky-Pet, Denver, CO, USA) and attached a 5-cm diameter metal spring toy (Slinky, Bedwina, New York, NY, USA) to the pole to minimize squirrel access to feeders. We visited the feeders every 1-2 days to refill seed [manually mixed Royal Wing Classic Mix (white millet, milo, wheat, black oil sunflower seed, and cracked corn) and Royal Wing Nyjer seed from Tractor Supply Company, Brentwood, TN, USA].

We placed a motion-activated trail camera (Trophy Cam HD Essential E2, Bushnell, Overland Park, KS, USA) at each feeder location (n = 6). Cameras were placed on the same day or day after the feeder was placed except at site 4 where there was a lag of 13 days due to a delay in acquiring the final camera. At the end of the season, we inspected images to visually identify avian species interacting with the feeder each day. We noted any issue with the camera or feeder limiting or preventing us from categorizing the full range of species present at a feeder location each day.

We performed weekly point counts at each site for five consecutive weeks starting Aug 7 to document the species present in the area. At each site, visual or auditory detections of species during a 10-minute interval were recorded using an eBird list [51]. Detections were used to characterize the species composition of the area around each site and used to determine the proportion of WNV competent or incompetent bird species present in the area that interacted with the feeder, as determined by images from the motion-activated cameras at each site. Competency of birds for WNV was based on results from previous experimental studies [52,53]. Comparing the species detected with motion-activated camera and

point counts, we also determined the proportion of the target species that were detected in the area that interacted with the feeder at each site.

We removed feeders and cameras from the study sites on a single day at the end of the study (Sep 8) except site 1a where the feeders and camera were removed on Aug 31 following squirrel damage to the antennas on the radio frequency identification readers.

Mist netting and tagging birds

We collected birds of our six target species using 38 mm polyester mist nets (Avinet Inc, Portland, ME, USA) at our main site over the course of nine days during the period of Aug 3-17, 2020. Nets were closed and secured when researchers were not present at the site. Each captured bird was identified to species, weighed, and wing cord and tail measurements were taken. We also determined the age (i.e., hatch year, after hatch year, second year, after second year, or unknown) and sex (female, male, or unknown) of each bird according to Pyle [54] based on measurements, molt patterns, plumage, and presence of cloacal protrusion or brood patch.

We attached a radio transmitter (PicoPip Arg376; mass = 0.83-0.94 g, Lotek, Newmarket, Ontario, Canada) to a subset of the captured birds using the backpack harness technique [55] using 1-mm fabric-covered elastic cord. We aimed to tag five individuals from each of the six target species. Prior to release, all birds were placed in a flight cage for observation to confirm that neither the harness nor tag interfered with flight and ambulation.

All birds were marked with numbered USFWS aluminum leg bands (U.S. Department of Interior Bird Banding Laboratory) and the majority also received a passive integrated transponder (PIT) tag affixed to a colored plastic leg band (Darvic leg bands, Avinet Inc, Portland, ME, USA) on the opposite leg if the combined weight of the tags would not exceed 3% of their body weight. We affixed the PIT tag to the plastic leg bands initially using Super Glue (Gorilla Glue, Cincinnati, OH, USA) and then transitioned to using a UV resin (Solarez, Vista, CA, USA) because super glue resulted in a brittle finish and PIT tags were easily knocked off during handling. During the last half of tagging, we encased individual PIT tags in heat shrink tubing (Electro Insulation Corporation, Arlington Heights, IL, USA)

prior to attaching to the leg band with resin to improve adhesion, similar to [56]. Following placement on the bird, the ends of the colored leg band were sealed together using a handheld thread burner (Beadsmith Thread Zap II, Carteret, NJ, USA) to prevent un-joined ends snagging on objects and impairing the bird or resulting in the loss of the band.

All procedures were approved by the University of California Davis institutional animal care and use committee (#20980) and with an inter-institutional agreement with the Division of Vector-Borne Diseases of the Centers for Disease Control and Prevention. Work in the natural areas was approved by the City of Fort Collins (permit #4919647-43). Capture and auxiliary tagging of birds approved by the federal bird banding permit (#22866) and Colorado Parks and Wildlife permit (#TRb3531).

Radio telemetry

We performed radio telemetry using a Yagi antenna and a Biotrack receiver (Lotek Wireless Inc, Newmarket, Ontario, Canada) to determine the nocturnal roosting location of tagged birds from Aug 5-Sep 8, 2020. Telemetry began after dusk (starting at 20:00 PM) to ensure birds had settled into their nocturnal roosting location and was performed at unique locations (range 3-16 total locations per night) across the broad area to obtain at least two to three bearings per detected bird. At each telemetry location, a GPS point (e-trex 30x, Garmin, Schaffhausen, Switzerland) was taken and bearings were obtained for signals using a compass. Relative signal strength and notes on unusual sounds (i.e., strong/loud, weak/quiet, distorted) were also recorded. In some instances, the exact roost was located and was visited on subsequent nights to record the presence of tagged birds. The exact location could not be ascertained for all detected birds as roosting locations often fell within private property.

We used the triangulate function in the radiotrack package (version 0.0.0.9000) [57] in R statistical software (version 4.0.2) [58] to estimate the location of each of the detected birds using the maximum likelihood estimator. Any estimated locations that were implausible (i.e., large distances away from the study site beyond the detection ability of the receiver) or for calculations that did not converge, bearings were re-examined and corrected to produce plausible estimates (i.e., based on previous and future locations for that bird and comparison of bearings with field notes to correct errors).

For species that had at least two individuals with at least three re-sighting locations, we calculated the probability a bird remained in the same roosting location on sequential nights, previously termed roost constancy [44], and the mean distance between nocturnal roosting locations on sequential nights. To estimate the widest area a bird utilized for nocturnal roosting sites, we calculated the maximum Euclidean distance between any two locations for each individual and averaged by species to estimate a species' average "nocturnal home range".

Radio frequency identification

To monitor visitation to feeders by PIT-tagged birds and characterize feeder usage patterns, we placed an Arduino-based radio frequency identification reader (RFID) [59] on all feeders. Due to delays in shipping, we placed three RFID readers on Aug 13 and the remaining six on Aug 20. Readers were removed concurrently with feeder removal (i.e., Aug 31 or Sep 8). For tube feeders, we secured the 100-mm diameter loop antenna around the tube at the level of the upper openings to provide perching locations for birds visiting the feeder, thereby increasing the probability of successful detection of the tag. For the platform feeder, we placed the antenna in the bottom of the feeder with a small amount of seed on top so as not to cause a visual anomaly to deter usage. The microprocessor was housed in a plastic container with a snap-on lid (KLIP IT sandwich containers, Sistema, Moscow, Russia) to protect it from the elements and attached to a 12V battery through a voltage regulator to maintain a constant supply of 5V. We set the reader to poll for tags every eight seconds from 05:00 AM to 22:00 PM daily to balance energy consumptions and temporal scale of detection.

We calculated the duration of individual visits, number of visits, and total duration of visits during each day by each detected PIT tagged bird.

<u>WNV transmission model with the impact of IVM-treated birdfeed</u>

Spatial parameterization of patch model

We set our model in a neighborhood of 1,500 m in length which was subdivided into 75 individual properties (20 m long) each representing a typical homeowner's lot. We categorized each property as treated if it had an IVM-treated feeder or untreated otherwise. We captured dispersal of *Cx.*

tarsalis mosquitoes and birds (WNV competent or incompetent) across the properties in the neighborhood with Gaussian kernels. The group-specific kernels were parameterized based on mean dispersal distances from literature (*Cx. tarsalis*) [60–63] and our radio telemetry results (birds). We used data for house sparrows *(Passer domesticus*) to parameterize the dispersal of WNV competent birds and data for mourning doves (*Zenaida macroura*) to parameterize the dispersal of WNV incompetent birds. We constrained bird movement spatially by truncating the tails of the dispersal kernels beyond a species' "nocturnal home range" (i.e., observed area encompassing nocturnal roosting locations), similar to [64], to account for movement of species within their home range. We parameterized the width of the "nocturnal home range" as the mean maximum distance between nocturnal roosting locations for all individuals of that species (see Mathematical Details in Appendix 4; Table A3-2). Note our use of "home range" does not correspond to the oft-used term in ecology to characterize the area utilized by an individual during normal activities of gathering food, mating, and caring for young [65–67], but rather chosen to capture the area a bird utilizes for nocturnal roosting only.

We simplified the neighborhood-level model to a two patch model with an IVM-treated and an untreated patch. Group-specific movement parameters were obtained with integrodifference equations [68,69]. That is, integrating and summing dispersal kernel over the arrangement of treated and/or untreated properties in our neighborhood resulted in the movement rates within and between treated and untreated patches for each species following [70]. We used the integral2 functions in the pracma package (version 2.2.9) [71] in R (version 4.0.2) [58] to perform integration.

West Nile virus compartmental model structure in patches

We modified a previous compartmental model by Hartley et al. 2012 [72] to capture WNV transmission dynamics in IVM-treated and untreated patches (Figure 3-2). We simplified the previous SEIR model to include only two avian hosts (WNV competent and incompetent hosts) and added IVM-treated and untreated host classes for both host competencies. The assumptions of the model are as follows:

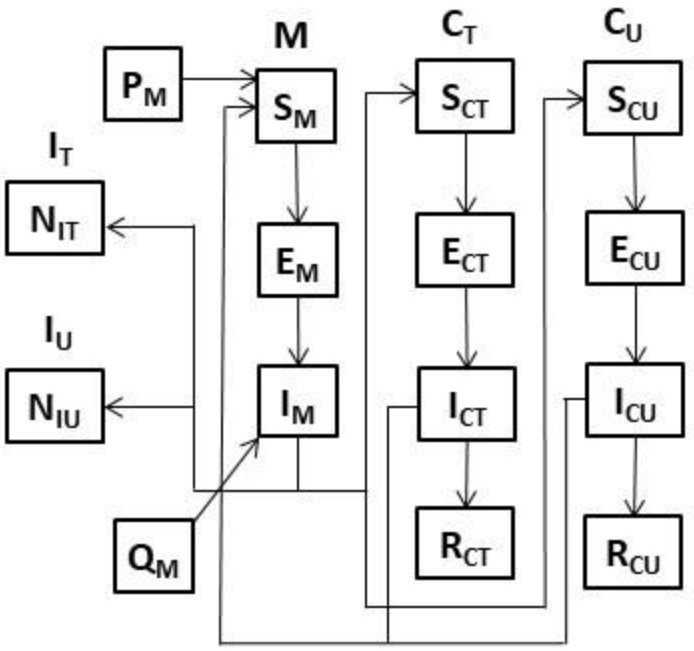

Figure 3-2: Schematic of SEIR model developed for West Nile virus (WNV) transmission with ivermectin (IVM)-treated birdfeeders. *Birds are categorized as WNV competent (C) or incompetent (I) and can be either treated (T) or untreated (U). Adult mosquitoes (M) emerge from either uninfected (P) or vertically infected (Q) eggs. See methods for complete explanation.*

1. Avian hosts can be either WNV competent or incompetent. Following infection, a competent species developed a moderate to high viremia, had a high probability of infecting feeding vectors, and sometimes succumbed to the infection. Incompetent species did not achieve high enough viremias to infect vectors, thus acting as dead-end hosts for the virus and diverting bites from competent hosts [52,73]. Mathematically, the competent and incompetent hosts could represent any avian host fed upon by mosquitoes, but we chose to use house sparrows and house finches to parameterize competent hosts and mourning doves to parameterize incompetent hosts.
2. All avian hosts were either treated or untreated. Regardless of WNV competency status, birds could feed at IVM-treated feeders in treated patches and self-medicate. The ingested IVM rapidly entered the bloodstream [74,75] and biting vectors had a relatively high probability of dying following a bloodmeal on a treated bird [39]. Unless a bird subsequently visited a treated feeder, IVM was removed from the plasma over time, transitioning the bird to the untreated status where biting vectors did not ingest sufficient or any amount of IVM to cause mortality. Each treated lot in the neighborhood contained a treated feeder, but birds did not always visit it when in that lot. Birds in untreated lots did not have access to IVM-treated feed.

3. At a given time, avian hosts were either susceptible (S) to infection, infected, but not yet infectious (E, during intrinsic incubation period), infectious (I), or immune for life after recovering from infection (R) [76,77].
4. At a given time, adult mosquitoes (M) were either susceptible (S) to infection, infected, but not yet infectious (E, during extrinsic incubation period), infectious (I). Mosquitoes remain infectious for life [78] and feed on hosts in proportion to abundance in the patch [79]. We used *Cx. tarsalis* as vectors, the predominant WNV bridges in Western North America [11].
5. The gonotrophic period (GP), or the number of days between bloodmeals for mosquitoes, was three days (see Mathematical Details in Appendix 4 for selection).
6. Mosquito eggs were rarely vertically infected [80,81]. Adults emerged from uninfected (P) or infected (Q) eggs as susceptible (S) or infectious (I), respectively.
7. The growth of mosquito and bird populations was logistic with respective rates of birth and non-disease death. Environmental carrying capacity for avian hosts was constant across the season.

As done in the previous compartmental model [72], extrinsic incubation period (EIP) and environmental mosquito carrying capacity (K_M) were temperature dependent parameters. We used loess smoothed daily mean temperature data (Jun 1-Sep 15, 2007) extracted from the PRISM Climate Group database [82] for Fort Collins, Colorado to model these parameters according to the following relationships that have been outlined previously [72,83]. Briefly, the extrinsic incubation period, or number of days for an exposed mosquito to become infectious, was modeled as EIP=1/(-0.132+0.0092 x temperature) based on a previously published regression of median extrinsic incubation rates for *Cx. tarsalis* [78]. We truncated the EIP at temperatures below the thermal minimum (14.3°C) observed for *Cx. tarsalis*. As the environmental carrying capacity for *Cx. tarsalis* cannot be explicitly measured we approximated it following [83] based on a generalized time series of trap counts six days later using the following assumptions that allowed us to capture the observed timing of increasing and decreasing mosquito populations. Starting with the observed average number of *Cx. tarsalis* per trap-night from Fort Collins for 2007 on a weekly scale [84], we used linear interpolation to obtain a generalized average

nightly trap count abundance. We multiplied this by the gonotrophic period to scale the proportion of female mosquitoes that would be host-seeking each night to the total population size and by a scaled number of hosts (i.e., (competent + incompetent birds) / 5), based on the assumption that a trap would represent multiple hosts because birds often roost in the near vicinity of others and thus each does not have the same "attractiveness" as an individual trap (see Mathematical Details in Appendix 4; Table A3-1). We set the scale factor for hosts to reproduce a realistic magnitude of mosquitoes present in a neighborhood. We used abundance measures six days later to account for the delay between peak carrying capacity and observed abundance in order to match the timing of observed peak abundance. We scaled the nightly trap count by gonotrophic period because, on average, only 1/(gonotrophic period) of female mosquitoes would be host-seeking on a given night, but the model required an estimate for total number of females in all stages of the gonotrophic cycle (e.g., laying eggs or resting).

In order to capture realistic infection dynamics observed at the city level [84] on the neighborhood level, we forced a rate of 0.75 per 1,000 exposed mosquitoes in week 25 (Jun 18-24) in each patch and introduced a total of 2.5 exposed competent birds across the patches at the start of week 28 (Jul 9) (see Mathematical Details in Appendix 4).

WNV transmission intensity with ivermectin-treated feeders

We implemented the full model (see Mathematical Details in Appendix 4; Tables A1-2) of differential equations in each patch and dispersal kernels for the nightly movement of birds and mosquitoes between patches based on the number and spatial arrangement of IVM-treated properties in a neighborhood using R (version 4.0.2) [58].

We used total infectious mosquito-days in the full neighborhood as the metric of WNV transmission intensity and assessed the change in this metric under two scenarios. In the first scenario, we varied the number and spatial arrangement of treated patches in the neighborhood to determine the optimal spatial design for deploying IVM-feeders. We compared two placement strategies of treated properties to represent potential deployment or adoption strategies: all treated properties in a single cluster randomly placed in the neighborhood (contiguous) or in multiple, smaller clusters randomly placed in the

neighborhood (random). In this scenario, we also assessed the change in infectious mosquito-days based on a range of daily probability of mosquito mortality following a bloodmeal on a treated bird (25, 50, and 100%) to explore a range of potential outcomes. We fit a linear regression model to the change in infectious mosquito-days by number of treated lots to calculate the average percent reduction in infection with increased number of treated lots. In the second scenario, we independently varied the probability that competent and incompetent hosts visited IVM-treated feeders (0-100%) to detect any differential impacts on transmission intensity by bird group treated and assess if feeder design targeting a certain type of bird species reduced infectious mosquito-days over widely treating a variety of species.

For both scenarios, we compared the change in transmission intensity to account for the forcing used to recapitulate observed infection dynamics, presenting the mean (95% confidence interval) from 300 iterations of each combination of parameters explored.

Sensitivity Analysis

We applied stochastic sampling from ranges of parameter values (Appendix 4, Table A3-3) to perform a global sensitivity analysis of the model [85]. We generated 300 sets of sampled parameter values for our 26 uncertain variables using Sobol sampling (sobol.samp function in the randtoolbox package version 1.30.1) [86] in R (version 4.0.2) [58]), following the suggestion of Matala [87]. We chose Sobol sampling for efficacy and efficiency in sampling [88,89]. We used a random forest model (randomForest function in the randomForest package version 4.6-14 [90] in R (version 4.0.2) [58] to assess the importance of each parameter with respect to the total infectious mosquito-days. Importance was assessed based on the parameter's explanatory value for predicting the total infectious mosquito-days, which was calculated as the difference in prediction error (mean squared error, MSE) using the out-of-bag (OOB) portion of the data with the parameter of interest permuted vs. using the un-permuted OOB portion of the data. This difference was averaged over all trees [90]. In the random forest analysis, eight variables were randomly sampled as candidates at each split, the terminal node size was five, and we grew 500 trees.

Results

Tagged birds

We tagged 105 birds of our six target species with radio tags and/or PIT tags (Table 3-1). Few blue jays and common grackles were captured, and all house finches weighed too little to allow for carrying a radio tag (average weight 20.6 g). Therefore, we increased the number of tagged red-winged blackbirds and mourning doves for a total of 28 tagged birds. Due to diminishing returns and to maximize the number of relocations per tagged bird acquired prior to the end of the WNV season, we stopped mist netting before achieving our goal of 30.

A total of 101 birds received a PIT tag mounted on a colored leg band (Table 3-1). Three house sparrows did not receive a PIT tag because the combined weight of the radio and PIT tags would have exceeded the 3% body weight threshold set for tagging birds. None of the mourning doves received a PIT tag leg band because this species would not visit tube feeders and had not been observed on the platform feeder.

Table 3-1. Radio telemetry and radio frequency identification tagging of six common backyard bird species in Fort Collins, Colorado during August 2020.

Species	Radio tag only	Radio + PIT^ tag	PIT^ tag only	Total tagged
Blue jay (*Cyanocitta cristata*)	0	2	0	2
Common grackle (*Quiscalus quiscula*)	0	3	0	3
House finch (*Haemorhous mexicanus*)	0	0	53	53
House sparrow (*Passer domesticus*)	3	2	0	5
Mourning dove (*Zenaida macroura*)	6	0	0	6
Red-wing blackbird (*Agelaius phoeniceus*)	0	12	24	36
Total	9	19	77	105

^ Passive integrated transponder (PIT) tag for radio frequency identification system

Radio telemetry

We performed a total of 29 nights of telemetry and obtained 252 unique nocturnal roost locations (Figure 3-3). Of these, 60.6% (n = 149) of roosting locations were within 400 m of where birds were tagged, and 5.2% (n = 13) were > 2.5 km away from the tagging location.

Tagged red-winged blackbirds could not be located within the study area after Aug 12. We engaged in an intensive search in the surrounding neighborhoods and on feeding grounds in the surrounding agricultural areas of Weld county in which red-winged blackbirds had been previously observed, but were unable to relocate any, presumably because they had moved to different foraging and roosting areas. Similarly, we were unable to relocate either of the tagged blue jays after Aug 6.

We investigated a tag that had been detected in the same location during both the day and night for several days and reclaimed it from the mud near a roost utilized by a great horned owl *(Bubo virginianus*). It is possible that the tag became detached from the bird during an encounter with the owl, although it is unlikely that tagging had made the bird more vulnerable to predation. We could not be

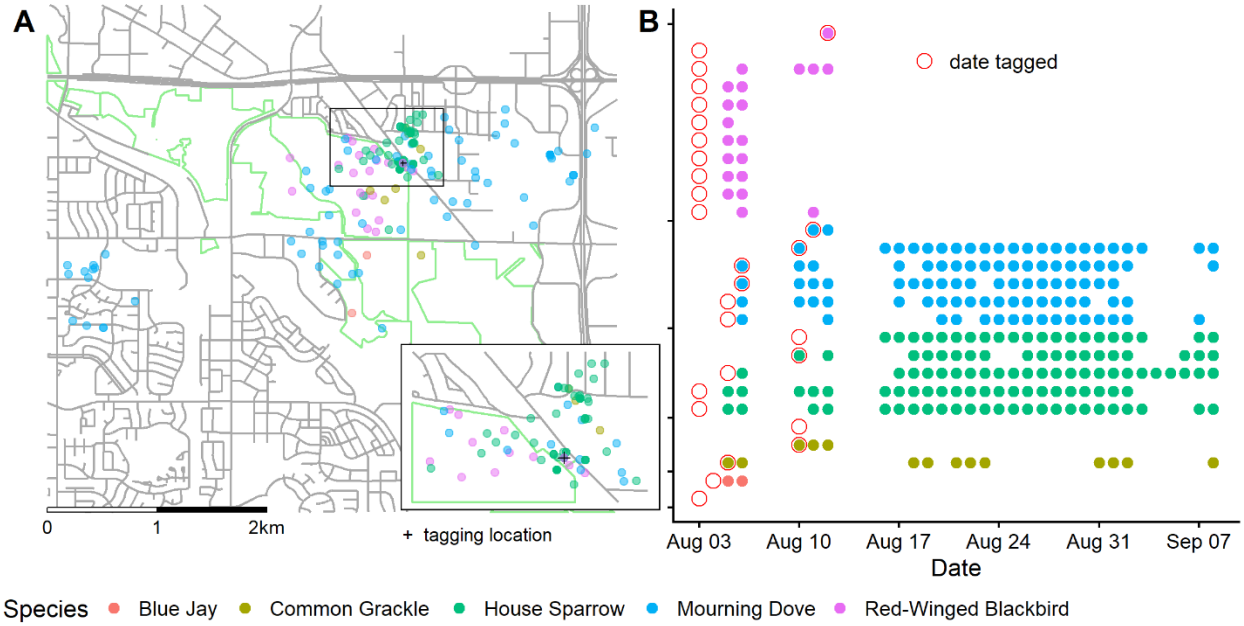

Figure 3-3: Location and date of estimated nocturnal roosting sites from radio telemetry and visual location. *(A) Location of nocturnal roosting sites determined from triangulation of radio telemetry data or visual location. Inset of area around tagging location. Green outlines indicate borders of the natural areas. Grey lines trace roads. (B) Date of tagging and relocation of species using radio telemetry or visual location. Ticks on y-axis group individuals of same species.*

certain about the date this tag was lost because other red-winged blackbirds were roosting in the vicinity over the same period, so we conservatively excluded all telemetry data related to this bird (n = 6), resulting in 246 nocturnal roost locations in the final analysis.

For all birds that were located on consecutive nights [i.e., house sparrows (n = 5), mourning doves (n = 5), and common grackles (n = 2)], we calculated the distances between nocturnal roosting locations on subsequent nights (Table 3-2). House sparrows had a smaller mean dispersal distance between roost locations than either mourning doves or common grackles and were found in the same roost on consecutive nights more often than these other two species.

Radio frequency identification

We detected fourteen PIT-tagged house finches at three of the five sites on a total of 27 different days (Figure 3-4). No detections occurred at sites 3 and 4. Of the 2,082 detections, 1,045 (50.1%) were less than 8 secs (polling interval set on microprocessor) so the exact duration could not be ascertained.

Table 3-2. Mean and standard deviation of distances between nocturnal roosting locations on consecutive nights and percent of roosts that were the same as the previous night.

Species	Total nocturnal locations	Sequential nocturnal locations	% roosts same as previous^	Mean (m)†	Standard deviation (m) †
House sparrow (*Passer domesticus*)	25	21	57.1	223.7	153.5
	23	20	45.0	316.4	152.0
	24	23	60.9	165.7	129.3
	19	14	71.4	91.3	55.0
	21	19	68.4	200.6	133.1
		Average	**60.6**	**219.3**	**149.4**
Mourning dove (*Zenaida macroura*)	16	12	8.3	394.5	511.3
	19	15	6.7	939.5	703.4
	19	15	0.0	1,246.0	773.9
	20	15	40.0	755.5	303.8
	22	19	63.2	205.4	131.5
	2	1	0.0	88.6	NA
		Average	**19.7**	**780.9**	**685.5**
Common grackle (*Quiscalus quiscula*)	11	6	66.7	141.7	168.2
	3	2	0.0	778.0	202.0
		Average	**33.3**	**459.9**	**397.5**

^ Percent of roosting locations that did not change from that of the previous night

† Mean and standard deviation of distance between consecutive nocturnal roosts if roost location changed.

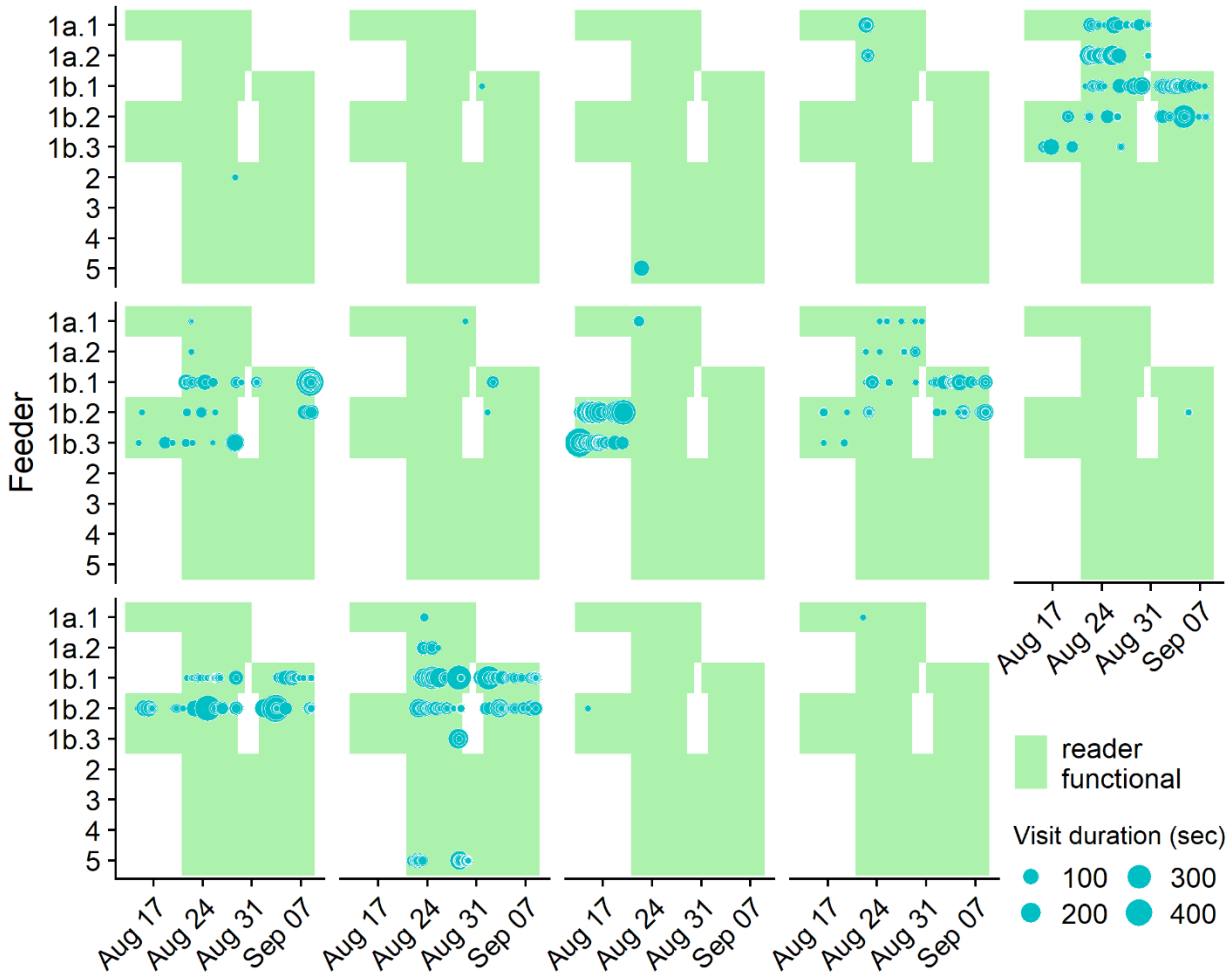

Figure 3-4: Duration of individual bird visits to feeders with radio frequency identification (RFID) readers. *Individual feeders in the main site, site 1a (1a.1 and 1a.2) and 1b (1b.1, 1b.2, and 1b.3, indicated. Date ranges with functional RFID readers in place indicated in green shading.*

Conservatively assuming a duration of 4 secs for these visits, the average duration per visit was 20.7 secs (max 471 secs) and the daily average total duration of all visits was 398.8 secs (range: 4-3,191 secs). On average, an individual was detected 19.3 times per day (range: 1-78).

Due to issues with the backup batteries maintaining time on the RFID readers, we had to adjust the timing of detections for 98 readings (4.7%) based on camera detections and RFID readers on adjacent feeders. Thunderstorms between Aug 28-31 resulted in moisture accumulation on the RFID microprocessor boards and/or batteries, causing the readers to overload, but we were able to replace the inoperable boards by Sep 1. Squirrels chewed the antenna wires on both readers at site 1a on Aug 31 so these feeders and readers were removed from the study.

Avian species composition in local area and at feeder sites

A total of 264,910 images from the motion-activated cameras were inspected visually and the species of birds interacting with each feeder per day were recorded (Figure 3-5). Due to issues with battery life and maintaining the correct time on cameras, a finer resolution than species per day was no possible. Other camera issues that precluded complete documentation of the full range of species at the feeders included the camera falling or only part of the feeder visible in the camera's field of view.

The comparison of species identified from motion-activated camera pictures and during point counts (Appendix 5, Table A3-4) indicated that approximately 82% of bird species in the area were WNV competent and 18% were incompetent (Table 3-3). Excluding flyover observations (i.e., birds observed in flight across the area, but not utilizing the habitat), approximately 27% of competent species and 4% of incompetent species identified during point counts were detected with motion-activated cameras visiting the feeder in that area. Of the target species detected in the area during point counts, an average of 37% of those species were also detected at the corresponding feeder using motion-activated cameras.

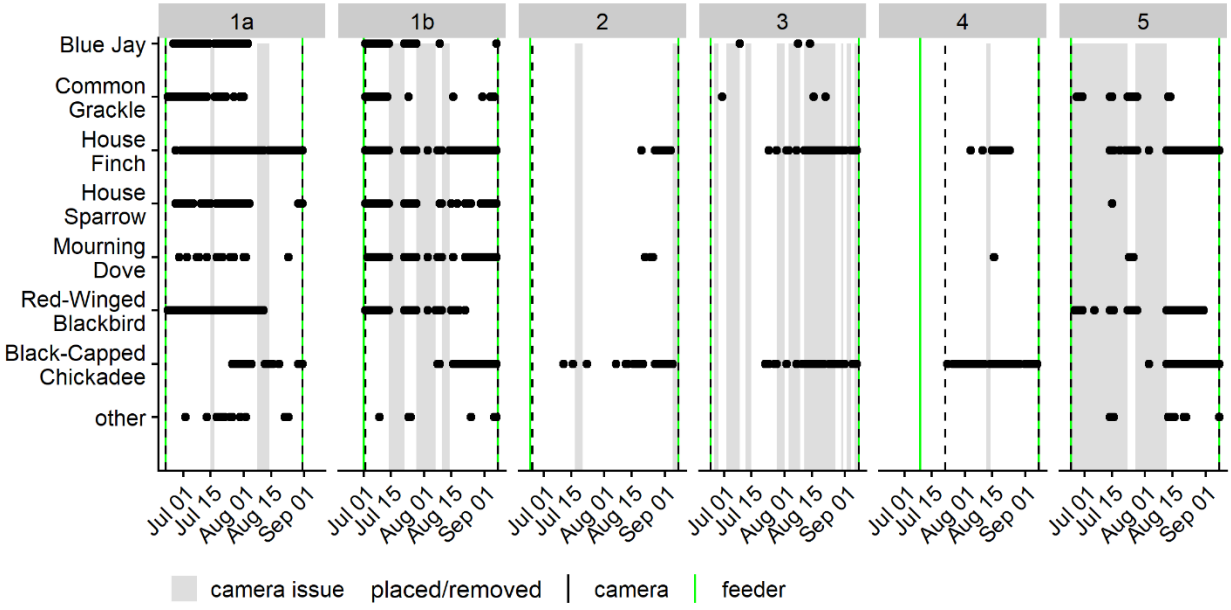

Figure 3-5: Daily detections of species interacting with feeders based on motion-activated cameras. *Date feeder and camera placed at site indicated. Camera issues included dead batteries, fallen cameras, and not all of feeder in camera's field of vision that prevented fully documenting full range of species present. Other species observed included American robin (Turdus migratorius), orchard oriole (Icterus spurius), American goldfinch (Spinus tristis), brown-headed cowbird (Molothrus ater), and Northern flicker (Colaptes auratus).*

Table 3-3. Detection of WNV competent and incompetent bird species through point counts* and motion-activated cameras.

Date	Site	Total competent species	Competent species at feeder (%)	Total incompetent species	Incompetent species at feeder (%)	% competent species[#]	Target species[+]	Target species[+] at feeder (%)
Aug 7	1[‡]	9	2 (22.2)	3	0 (0.0)	75.0	4	2 (50.0)
	2	5	1 (20.0)	1	0 (0.0)	83.3	5	0 (0.0)
	3[‡]	10	2 (20.0)	2	0 (0.0)	83.3	6	1 (16.7)
	4	6	1 (16.7)	0	0 (NaN)	100.0	3	0 (0.0)
	5[‡]	8	0 (0.0)	2	0 (0.0)	80.0	3	0 (0.0)
Aug 14	1[‡]	7	2 (28.6)	5	0 (0.0)	58.3	4	1 (25.0)
	2	6	0 (0.0)	1	0 (0.0)	85.7	3	0 (0.0)
	3[‡]	10	3 (30.0)	1	0 (0.0)	90.9	5	2 (40.0)
	4[‡]	6	1 (16.7)	0	0 (NaN)	100.0	2	0 (0.0)
	5	12	5 (41.7)	0	0 (NaN)	100.0	3	3 (100.0)
Aug 21	1	12	2 (16.7)	3	0 (0.0)	80.0	4	1 (25.0)
	2	8	1 (12.5)	2	0 (0.0)	80.0	6	0 (0.0)
	3[‡]	6	2 (33.3)	2	0 (0.0)	75.0	3	1 (33.3)
	4	2	1 (50.0)	2	0 (0.0)	50.0	2	1 (50.0)
	5	8	4 (50.0)	3	0 (0.0)	72.7	3	2 (66.7)
Aug 28	1	7	2 (28.6)	5	1 (20.0)	58.3	5	2 (40.0)
	2	6	2 (3.33)	1	0 (0.0)	85.7	2	1 (50.0)
	3	8	2 (25.0)	0	0 (NaN)	100.0	4	1 (25.0)
	4	3	1 (33.3)	0	0 (NaN)	100.0	1	0 (0.0)
	5	8	3 (37.5)	2	0 (0.0)	80.0	2	2 (100.0)
Sep 4	1	11	3 (27.3)	3	1 (33.3)	78.6	4	3 (75.0)
	2	7	2 (28.6)	2	0 (0.0)	77.8	2	1 (50.0)
	3[‡]	5	1 (20.0)	1	0 (0.0)	83.3	3	1 (33.3)
	4	5	1 (20.0)	2	0 (0.0)	71.4	1	0 (0.0)
	5	8	2 (25.0)	1	0 (0.0)	88.9	2	1 (50.0)
Mean %			27.4^		4.1^	81.9^		37.2^

* Excluding flyover detections

[#] Percent of bird species detected that were competent.

[+] House sparrow, house finch, red-winged blackbird, mourning dove, blue jay, and common grackle.

[‡] Camera malfunction (dead batteries or incomplete view of feeder) for part of day.

NaN: divide by zero

^ Mean percentage of given category for days without camera malfunction

Baseline WNV infection dynamics in mosquitoes

In the absence of ivermectin, modeled mosquito population and WNV transmission dynamics (Figure 3-6) reproduced dynamics previously reported in Fort Collins [84]. WNV infection peaked at 11.2 per 1,000 infected and infectious female mosquitoes in week 34 (Aug 20-26). Mosquito abundance peaked in week 27 (July 2-8), decreasing throughout the rest of the season, except for a brief increase in week 32 (Aug 6-12).

Impact of number and spacing of ivermectin-treated lots on WNV infection dynamics

Contiguous and random arrangement of IVM-treated lots resulted in nearly identical effects on WNV transmission risk, as measured by total infectious mosquito-days (Figure 3-7A). At a realistic level of involvement by homeowners (five treated lots), contiguous placement resulted in a reduction of 6.49% (95% CI: 6.18-6.08%) and random placement resulted in a reduction of 6.53% (95% CI: 6.21-6.86%). With twenty treated lots (27% of the neighborhood), a realistic level that could be obtained by a targeted public-health intervention, reductions of 22.45% (contiguous; 95% CI: 22.13-22.76%) to 23.04% (random; 95% CI: 22.72-23.37%) were obtained.

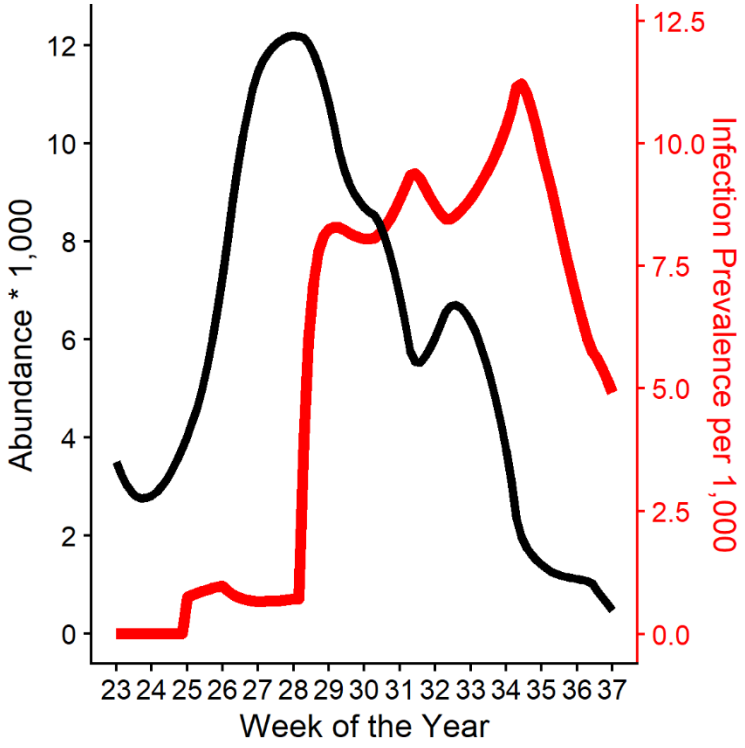

Figure 3-6. Baseline West Nile virus (WNV) infection dynamics in Cx. tarsalis in absence of ivermectin. *WNV infection prevalence (number of infected and infectious mosquitoes per 1,000) and abundance of Cx. tarsalis.*

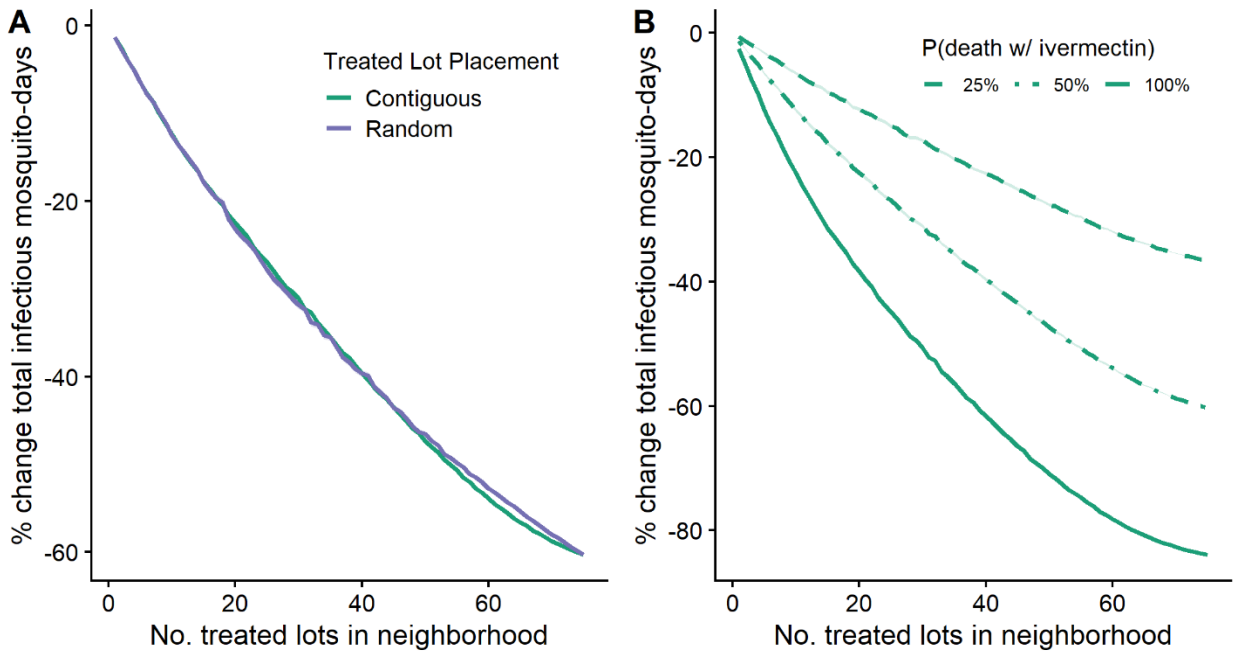

Figure 3-7: Reduced infectious mosquito-days with increased ivermectin-treated lots and daily probability of mosquito death following a bloodmeal from an ivermectin-treated bird. *Percent change in infectious mosquito-days with A) contiguous or random placement of ivermectin-treated lots in neighborhood, assuming a 50% daily probability of mosquito death following a bloodmeal on an ivermectin-treated bird, and B) contiguous placement of ivermectin-treated lots in neighborhood with 25, 50, and 100% daily probability of mosquito death following a bloodmeal on an ivermectin-treated bird. 95% CI indicated with shaded region.*

If all neighborhood lots could be treated, we estimated a maximum reduction of 83.9% in infectious mosquito-days if 100% of mosquitoes died following a bloodmeal on a treated bird (Figure 3-7B); complete elimination of infection did not occur because treated feeders were assumed to represent only a small proportion of birds' daily food sources, leaving untreated birds even if treated feeders were present in all lots. If the daily probability of mosquito death was 50%, we estimated a reduction of 60.3% and if the daily probability was 25%, we estimated a 36.6% reduction. With complete spatial coverage, IVM deployment reduce the total number of infectious mosquito-days by 1,106 (100% probability), 795 (50% probability), or 483 (25% probability), based on the daily probability of IVM-induced mosquito mortality (Figure A3-1 in Appendix 5).

Using a linear regression model, we estimated that each addition of one treated lot results in an average reduction of 0.49% (25% mortality; 95% CI: 0.48-0.50; $R^2 = 0.99$), 0.80% (50% mortality; 95% CI: 0.78-0.82; $R^2 = 0.99$), and 1.06% (100% mortality; 95% CI: 1.01-1.11; $R^2 = 0.96$) in total infectious

mosquito-days in the neighborhood, depending on the level of IVM-induced mortality achieved in the mosquito population. Similarly, with increased randomly placed lots, we estimated an average reduction of 0.48% (25% mortality; 95% CI: 0.47-0.49; $R^2 = 0.99$), 0.78% (50% mortality; 95% CI: 0.76-0.80; $R^2 = 0.98$), and 1.03% (100% mortality; 95% CI: 0.97-1.09; $R^2 = 0.94$) in total infectious mosquito-days in the neighborhood.

With 50% IVM-induce mortality in mosquitoes, we estimated that IVM-treated birdfeeders would prevent up to 5.2 infections in competent birds (40.7% reduction in infections) with 100% spatial coverage of the neighborhood (Appendix 5, Figure A3-2).

Variation in infection intensity with treated feeder usage by WNV competent and incompetent birds

At the estimated visitation rates of birds (i.e., 27% competent and 4% incompetent birds), we estimated a reduction in infectious mosquito-days of 5.33% (5 contiguous treated lots; 95% CI: 5.21-

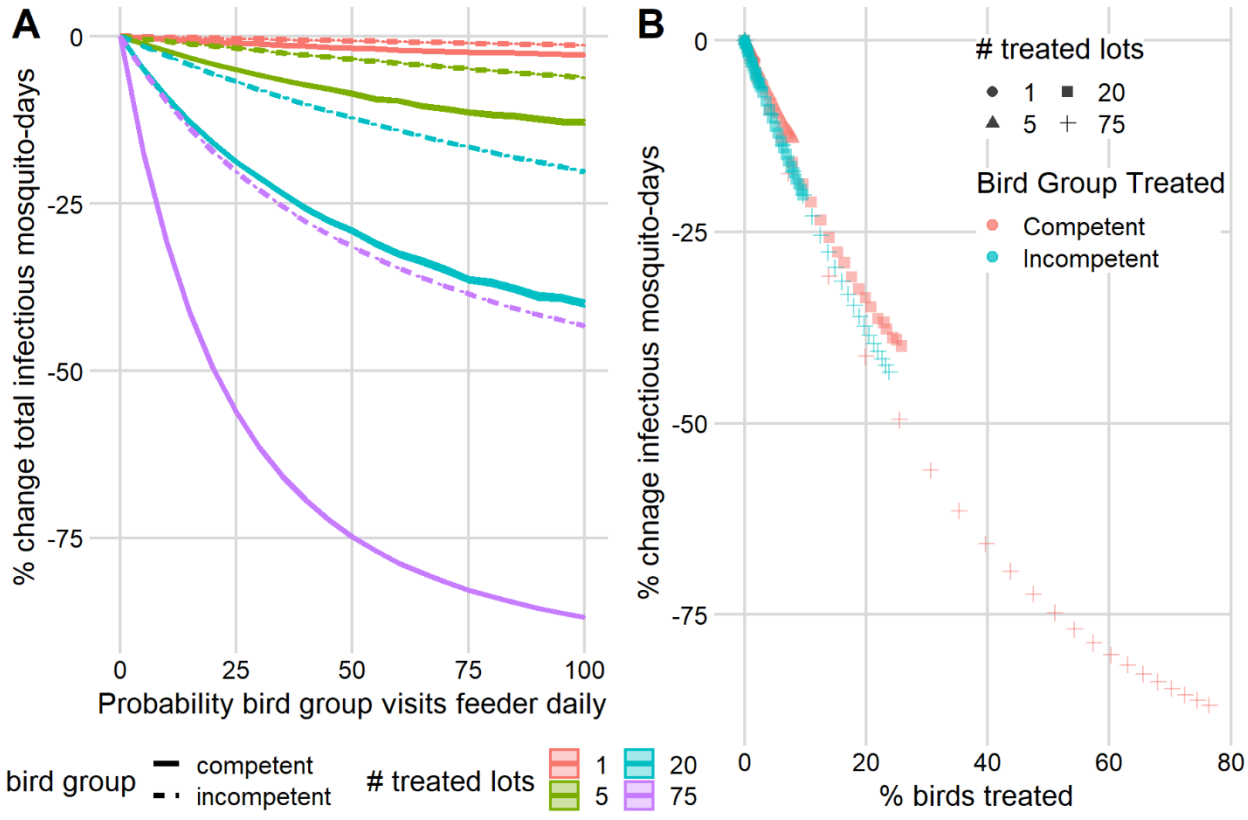

Figure 3-8: Reduced infectious mosquito-days with increased treated feeder visitation by competent and incompetent birds. *A) Change in infectious mosquito-days resulting from an increase in the daily visitation rates by each group of birds in four deployment scenarios of contiguous treated lots. B) Change in infectious mosquito-days relative to total proportion of birds treated under each visitation rate. In each calculation, probability of daily feeder visitation held at 0 for other group. 95% CI indicated by the shaded regions.*

5.46%), 19.9% (20 contiguous treated lots; 95% CI: 19.7-20.1%), or 58.5% (75 treated lots).

Increasing the daily probability competent birds visit treated feeders resulted in a larger reduction in the total infectious mosquito-days than for the same increase of daily visitation for incompetent birds (Figure 3-8A). For example, with five treated patches, 100% daily visitation by competent birds (0% for incompetent birds) resulted in a reduction of 12.9% (95% CI: 12.9-12.8%) while the reverse (i.e., feeder visitation by 0% of competent birds and 100% by incompetent birds) resulted in a reduction of 6.17% (95% CI: 6.12-6.22%).

The magnitude of difference between groups increased as the number of treated patches increased. Since 80% of the birds in the neighborhood were competent, a larger resulting proportion of the population is treated as the daily visitation rate for competent birds increased, resulting in larger reductions in infections in mosquitoes (Figure 3-8B).

No variation in estimated change was observed with 75 lots because the entire neighborhood is treated at this level. Thus, there was no movement of birds and mosquitoes between patches nor variation in the placement of treated lots influencing these movement probabilities.

Sensitivity analysis

The top four most important parameter were the probability of successful WNV transmission from competent bird to mosquito, birth/death rate of mosquitoes, daily IVM-induced mosquito mortality, and number of treated patches (Figure 3-9 and Table A3-3 in Appendix 4). Together, these parameters explained 64.4% of the increase in prediction error when parameters were permuted in the random forest analysis.

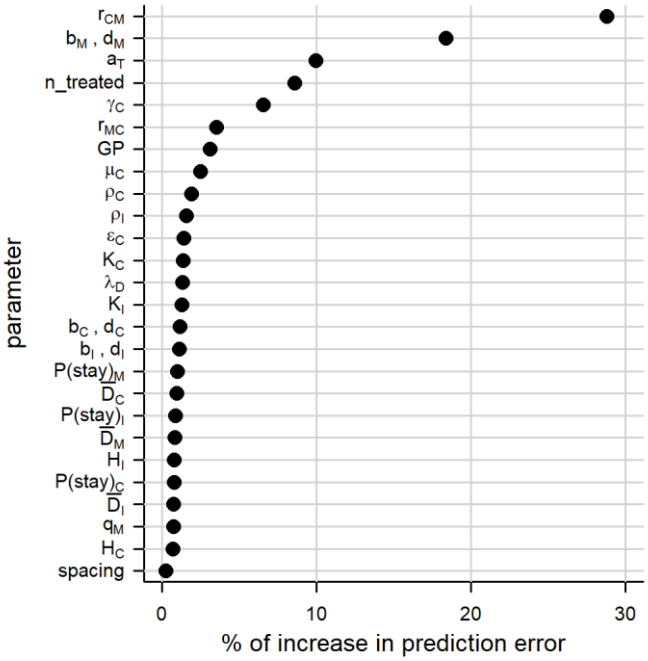

Figure 3-9. Contribution of parameter to increased prediction error. *Increase in mean squared error of prediction when parameter permuted in random forest analysis. See Tables A1-2 in Appendix 4 for symbol definitions.*

All parameters related to the spatial movement of birds and mosquitoes (i.e., bird nocturnal home ranges, daily probability of staying in the roost (birds) or lot (mosquitoes), and standard deviation of the Gaussian dispersal kernel) were among the least important parameters, each contributing < 1% of the increase in total prediction error when permuted.

The random forest model had a mean pseudo R-squared value of 0.906, indicating good model fit and performance.

Discussion

Our combined field and model-based investigation on the impact of IVM-treated birdfeeders indicated that this strategy could reduce the WNV transmission intensity in local neighborhoods, as measured by total infectious mosquito-days. Both the probability of IVM-induced mosquito mortality and number of treated lots highly impacted the magnitude of reduction while the spatial distribution of treated lots within a neighborhood did not. Increasing the total number of treated birds in a neighborhood, irrespective of WNV competency, resulted in a larger reduction in WNV transmission intensity than selectively increasing treatment rates among competent species.

The reduction in infectious mosquito-days achieved depended largely on the number of treated lots and mosquito mortality following a bloodmeal on a treated bird. The arrangement of treated lots in the neighborhood did not significantly alter the estimated reduction. If the plasma level of IVM in the blood of birds results in 50% mosquito mortality and all 75 lots in the neighborhood are treated, we estimated a 60.3% reduction in total infectious mosquito-days and a 40.7% reduction in infections in competent birds. However, this level of coverage represents a significant investment by homeowners that is unlikely to occur. If a targeted public health campaign could recruit around ¼ of the neighborhood (20 homeowners) to have treated feeders for the summer, we estimate a reduction of 22.45% (contiguous placement) to 23.04% (random placement) in infectious mosquito-days could be obtained. Also, at 20 treated lots, we estimate a 13.7% (contiguous) to 14.1% (random) reduction in WNV infections in competent birds. Overall, with each additional treated lot, we estimate an additional reduction of 0.78%

(random) or 0.80% (contiguous) in total infectious mosquito-days. Thus, a large investment would be needed to achieve meaningful reductions in infection transmission.

Under maximal assumptions (i.e., 100% IVM-induced mortality, 100% spatial coverage of neighborhood), we estimate a reduction of 83.9% in infectious mosquito-days could be achieved. This was accompanied by a 61.3% reduction in number of infections in competent birds (7.8 infections prevented). Complete elimination of mosquito infections did not occur as only about 25% (WNV competent) and 5% (WNV incompetent) of birds visit a feeder daily and IVM has a relatively fast washout period in plasma [39,75] so only an average of 34.4% of the total bird population was treated under this scenario.

The level of IVM in plasma achievable in wild birds is largely unknown. However, this level has a significant impact on the resulting level of mosquito infections. Additionally, the relationship of IVM concentration to mosquito mortality is unclear. Lab studies with colony mosquitoes point to an LC_{50} of 49.9 ng/mL [39] to 60.6 ng/mL (Chapter 2). However, a strong mosquitocidal effect from the serum of a wild-caught common grackle during a pilot trial vs. control calf serum was observed for *Cx. tarsalis* in a serum-replacement assay (100% mortality within two days), even with an IVM blood concentration of 5.7 ng/mL [39]. Mortality of 45.6% in three days was observed following a bloodmeal on IVM-treated chickens with serum concentrations of 17-32 ng/mL; concentrations were obtained several hours prior to blood-feeding so the dose mosquitoes were exposed to could have been lower (Chapter 2). Further investigation of the impact of IVM on wild mosquitoes is required to refine estimates of achievable reductions.

Feeder usage data from RFID readers provides an initial indication at the dose birds could achieve. While the average house finch spent around six minutes per day at the feeders, the maximum daily duration was >50 minutes. If ingestion rates were constant across visits, a wide variation in dose between birds could occur. Care should be taken to balance the IVM:feed ratio with a potentially wide range of ingestion rates to reduce the potential for accidental toxicity, as this level is known to vary across

bird species [91–93]. Feeder usage data may not be representative of usage across species or for house finches across the full season, but provide an initial estimate.

We found that increasing the daily feeder visitation rates by competent birds reduced infectious mosquito-days more than the same increase for incompetent birds. This difference increased as the number of treated lots increased, but the effect was not attributable to the competency of the birds, but rather the relative abundance of each group. During fieldwork, we found around 80% of the bird community around our feeder sites were competent, and since we assumed that mosquitoes bite hosts in proportion to their presence in the environment [79], targeting competent birds increased the total proportion of treated birds more rapidly than targeting the smaller subset of incompetent birds, thus resulting in larger impacts in the mosquito population. Comparisons of change in mosquito infections by proportion of the total bird population treated illustrated that targeting WNV competent or incompetent birds did not result in meaningfully different reductions; the increased IVM-induced mortality in WNV vector mosquitoes was a more important driver of reduced infection transmission than the WNV competence of the treated bird. We assumed that mosquito biting was opportunistic (i.e., directly proportional to the fraction of each species present), but there is some evidence that mourning doves account for a large proportion of the bloodmeals by *Cx. tarsalis* in Colorado during the WNV season (range 12-43%) and may be preferentially bitten [6,41,94]. Thus, the importance of treating preferred bloodmeal hosts, even if incompetent, could have a higher impact than estimated.

The current feeder design appears to have low attractiveness to even target species or may have been competing with other natural food sources or more attractive or established feeder locations in the neighborhood. We estimated that only 27% of competent species and 4% of incompetent species present in the area visited a feeder and only 37% of our target species present in the vicinity visited a feeder. Quantification and monitoring of other feeders in the area was not feasible so we could not estimate the total number of birds visiting any feeder from our data. Also, various camera issues limited our field and reduced our ability to document the full daily range of species visiting a feeder, meaning our study may have underrepresented the total species diversity that visited study feeders. Nonetheless, at the estimated

visitation rates of birds, a reduction in infectious mosquito-days of only 5.33% (5 contiguous treated lots), 19.9% (20 contiguous treated lots), or 58.5% (75 treated lots) could be expected. Increasing the attractiveness of feeders to a wide proportion of the bird population would likely be needed to achieve a meaningful reduction in WNV transmission at feasible deployment levels.

The transmission probability of WNV from birds to mosquitoes, or the competence of birds, was the single most important parameter from the random forest analysis, accounting for over 25% of the increase in prediction error when permuted. WNV competence values for house sparrows and house finches derived from lab studies (i.e., 0.3-0.5) [3,52] were insufficient to produce realistic infection dynamics consistent with those observed previously in the study area, so we used a higher competence value of corvids to reproduce expected dynamics. This suggests that our modeling framework might not have captured factors or heterogeneity in biting preference or competence that drive infection in nature. One such factor could be the contribution of hatch-year birds to WNV amplification [95]. While we included a birth term in our populations of birds, we did not have differential competencies based on age as this would have increased the complexity and reduced tractability of our model. This and other heterogeneities in competence between species and individual birds could be driving infection dynamics in nature while our modeling framework only captured the average effect.

Of the most important parameters identified in the random forest analysis, the number of treated patches and birth/death rates of mosquitoes are the most management-relevant parameters. Vector control or public health agencies deploying IVM-treated birdfeeders presumably would be able to enlist larger numbers of households in an intervention to increase the number of neighborhood lots with treated feeders. Also, using IVM-treated feeders in concert with other control strategies that affect mosquito birth or death rates (e.g., removal of breeding locations or application of larvicides or adulticides) might enhance the efficacy of IVM.

Due to delays in permitting, we were unable to capture and tag birds until early August, almost two months after our intended start date. Results from the motion-activated cameras indicated that the species composition around some of our feeder sites shifted around this time with a decrease in daily

visitation by red-winged blackbirds and common grackles and an increase in visits by house finches and black-capped chickadees. This shift was corroborated by telemetry; tagged red-winged blackbirds could not be relocated after Aug 11 despite intensive searching and it was supposed that red-winged blackbirds and common grackles were shifting from breeding sites into larger, communal flocks in preparation for fall migration [96,97]. After tagging, the blue jays did not return, potentially due to avoidance of the study site following the tagging event, and they could not be relocated. Pairing the observed shift in feeder visitation with reported shifts in bloodmeal hosts of *Cx. tarsalis* from American robins to house sparrows and doves [6,41], highlights the potential for variation in the impacts of IVM over the season as the composition of the local avian community changes. However, further work is needed to elucidate the magnitude, if any, in change in total treated birds from which a mosquito may take a bloodmeal based on changes in feeder usage across the season to determine if there is the potential for differential impacts of IVM throughout the season.

Based on the random forest analysis, all parameters used to characterize the spatial movement of birds and mosquitoes across the landscape had low relative importance in terms of the total infectious mosquito-days. This could indicate that the spatial aspects of the model were less important than the direct impact of IVM on mosquitoes. Alternatively, the method we used to translate the observed spatial aspects of bird movement into the model may have failed to capture realistic dynamics that would modify potential impact of IVM-treated feeders. In particular, our use of dispersal kernels, even truncated to represent "nocturnal home ranges" and used in tandem with the probability of remaining in the same nocturnal roost, introduced a Markov process to bird movement, ignoring the role memory and habitat quality may play in roost selection and allowed avian populations to diffuse across the neighborhood over the season. With this diffusion, our estimates are likely conservative, representing a spatial averaging of the effect on transmission intensity and not capturing heterogeneity we might expect due to localized bird movement. Methods utilized in other systems to model movement in a spatial context have included likelihood procedures with habitat-dependent dispersal kernels [98] or mechanistic modeling approaches [99,100] to mirror natural processes more closely. These methods or agent-based models may improve

spatial accuracy, but would add computational and mathematical complexity, reducing the tractability of the model.

A better quantification of *Cx. tarsalis* movements in urban areas is also needed to refine estimates of expected reduction in WNV infection transmission. Previous mark-release-recapture studies estimate mosquito dispersal in largely agricultural settings that may not reflect those in more urbanized locations. Habitat and land use is known to influence *Culex* dispersal and mosquitoes tend to disperse longer distances in agricultural settings [62,101,102], potentially to find bloodmeal hosts, resting locations, and suitable larval habitats. Our choice to use a Gaussian kernel for mosquito dispersal may have allowed unrealistically high probabilities of long-distance dispersal events and artificially elevated movement between patches. Estimating mosquito dispersal in such a way as to reproduce mark-recapture studies is difficult. A previous model estimating the dispersal of *Aedes albopictus* in urban areas, simulated the probability of movement between parcels within 200 m (radius of reported daily recapture of released mosquitoes) based on the quality of the parcels with inverse-distance weighting so that movement to nearby parcels was more likely than to more distant ones, modified by the suitability of the new parcel relative to the current one [103]. An approach of this nature that takes into account landscape features and explicitly truncates dispersal distances could more accurately capture the factors driving the movement of *Cx. tarsalis* in neighborhoods and refine our estimates.

While the previous compartmental model effectively reproduced seasonal infection dynamics on the scale of 20 km-square grids [72], we had to include forcing parameters (i.e., rate of exposed mosquitoes and introduced exposed birds) to capture realistic infection dynamics observed at the city level on the neighborhood level, indicating that the modeling assumptions and disease processes do not translate directly across spatial scales. Focusing our model on the neighborhood level enabled us to estimate the effect on the scale on which deployment would occur, but did not consider the spatial structure and surroundings of the neighborhood that may influence pathogen transmission dynamics and the efficacy of the intervention. Additionally, our decision to use least-squares optimization to select these forcing values resulted in a fractional number of introduced birds, a highly artificial construct. However,

as we needed to introduce birds into the system to cause the dramatic increase in infection, this could indicate that immigration may play a role in initiating or sustaining local infection dynamics.

Despite the limitation imposed by delays in fieldwork, our approach of pairing fieldwork with model development allowed us to collect and incorporate firsthand data into our modeling framework, thus improving the biological validity of our modeling approach.

Conclusion

Deployment of IVM-treated birdfeed in neighborhoods could reduce the local WNV transmission intensity, as measured by total infectious-mosquito days. Both the probability of IVM-induced mosquito mortality and number of treated lots highly impact the magnitude of reduction while the spatial distribution of treated lots within a neighborhood did not. Further work is needed to estimate the daily probability of IVM-induced mortality in mosquitoes to refine the achievable reduction in WNV transmission under field conditions. Increasing the total number of treated birds in a neighborhood, irrespective of WNV competency, reduced WNV transmission intensity, indicating that effective IVM deployment would target a wide variety of backyard bird species. Our fieldwork and modeling results are a step towards designing effective randomized controlled trials of IVM-treated birdfeed. They also provide a view of the intersection of feeder usage and nocturnal roosting habits of common backyard birds during the period of typical WNV amplification following the breeding season. Our results also open the possibility that IVM treatment of the maintenance hosts for other mosquito-borne disease systems could act as a viable control strategy.

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Summary and Conclusions

This dissertation addressed current gaps in the knowledge of the spatial and temporal impacts of adult mosquito control on population dynamics of *Culex* mosquitoes and WNV transmission risk, using both computational and field-based approaches.

First, we developed an improved method of analysis to estimate the effect of aerial applications of insecticides. Aerial spraying is used to reduce transmission of WNV to humans during periods of epidemic risk. However, estimates of the reduction in abundance following these treatments typically focus on single events, rely on pre-defined, untreated control sites, and can vary widely due to stochastic variation in population dynamics and trapping success unrelated to the treatment. Our modeling approach allowed us to utilize the large observational datasets of surveillance and control data from vector control districts to isolate aerial treatment effects while accounting for contextual factors like spatio-temporal relationships, weather, and habitat that contribute to stochastic variation in nightly trap counts. Using these contextual factors to capture the baseline expected dynamics of mosquito populations enabled counterfactual assessment of the abundance in the absence of aerial spraying to identify deviation attributable to treatment alone without the need of an independent control. This is an important advance that complements experimental trials and expands upon conventional observational approaches that summarize population changes following aerial treatments at individual time points under field conditions.

In summary, a greater reduction was estimated for *Cx. pipiens*, likely due to its focal distribution in urbanized areas and limited dispersal. The use of organophosphate products versus a combination of pyrethrins and pyrethroids increased the magnitude of reduction estimated for *Cx. pipiens* while the difference by broad insecticide class was not significant for *Cx. tarsalis*. The effects of aerial sprays on *Cx. tarsalis* populations were likely moderated by the species broad dispersal ability, large population sizes, and vast expanses of productive larval habitat in the study area. Therefore, the most effective control of *Cx. tarsalis* would be expected in areas with isolated or highly spatially segmented populations. For both species, aerial spraying reduced abundance at high spatial coverage while reductions were also

estimated at lower spatial coverage, at albeit greatly reduced magnitudes, indicating that aerial sprays had some impacts beyond the target zone. There was also evidence for population rebounds at periods of two to four weeks post-spraying, but this does not negate the immediate public health benefit of rapid reduction in abundance. For example, *Cx. pipiens* abundance was reduced by a mean of 52.4% (pyrethrin and/or pyrethroids) to 76.2% (≥1 organophosphate) and *Cx. tarsalis* was reduced by 30.7% (any combination of products) one-week post-treatment with full coverage. We also detected increases in abundance at the margins of the spray zones, potentially indicating the presence of excito-repellency in *Culex* due to spraying. We detected short-term reductions in the abundance of the primary WNV vectors, *Cx. tarsalis* and *Cx. pipiens*, following aerial spraying and identified species-specific variation in the reduction as well as eluciating gaps in current data collection and assessment methods preventing the estimation of the full spatio-temporal impacts of aerial sprays.

We then transitioned to investigating the efficacy and feasibility of using bird-delivered IVM as a WNV control strategy. Current vector control strategies rely primarily on pesticides to target mosquitoes involved in enzootic and zoonotic transmission of WNV, but increasing insecticide resistance and a desire to reduce pesticide usage provide the impetus for developing alternative strategies. Additionally, current control strategies lack specificity for targeting mosquitoes actively involved in enzootic maintenance and amplification of WNV without large-scale applications of pesticides that are time-intensive and often need to be repeated for sustained control. Through IVM treatment of avian hosts that account for a large proportion of *Culex* bloodmeals, we hypothesized we could achieve effective control of WNV transmission and in a manner that is more accepted by the general public, thus increasing potential collaboration and participation.

We first investigated the spatial impact of IVM on *Culex* populations and WNV transmission under field conditions through a randomized field trial of IVM-treated backyard chicken flocks in Davis, California. Compared to initially starting with wild birds, chickens had the advantage of remaining in a single location and are known to be fed upon frequently by *Cx. tarsalis* where they are present. Thus, increasing our ability to detect an effect. Following oral administration of IVM to backyard chickens, we

observed fewer WNV seroconversions in treated chickens than untreated chickens, a reduction in parity rates of *Cx. tarsalis* near treated vs. untreated flocks, and increased mortality in wild mosquitoes following a bloodmeal on treated chickens vs. untreated chickens, pointing to a reduction in WNV transmission due to the impact of IVM on *Culex* mosquito populations. Increased mosquito mortality was detected in chickens with a serum concentration between 17-32 ng/mL while there was no difference in mortality for mosquitoes taking a bloodmeal from an untreated chicken or one with a serum concentration at the limit of quantification (5 ng/mL), supporting previous findings that a certain concentration must be obtained to achieve significant mosquitocidal impacts. IVM concentrations varied widely across the study with a mean of 33.1 ng/mL (range: 0-155.2 ng/mL) so the exact doses mosquitoes were exposed to is uncertain. However, sustained oral ingestion of IVM did not result in any adverse events in chickens highlighting the safety of this method. We did not observe a difference in either abundance or WNV infection prevalence in *Cx. tarsalis* populations between treated and untreated sites, potentially due to sustained immigration of newly emerged individuals and lower-than-average WNV activity in the study area. Taken together, our results indicate that IVM does have mosquitocidal impacts on wild *Cx. tarsalis* and deployment could thereby have beneficial impacts b reducing WNV transmission.

Further work is needed to estimate the expected reduction in WNV transmission due to the impact of IVM on mosquitoes, especially with movement of treated birds spatially, as well as to identify areas with gaps in knowledge that highly impact the efficacy of deployment; this will guide future studies to address these gaps and the design of efficient field trials for deployment of treated birdfeed.

To identify the impact of these unknown relationships and to fill some gaps, we performed a combination of fieldwork and modeling to assess the feasibility of deploying IVM-treated bird feed in neighborhoods to reduce WNV transmission. We aimed to characterize the bird feeder usage and nocturnal roost locations of six common backyard species (blue jays, common grackles, house sparrows, house finches, mourning doves, and red-winged blackbirds) and develop a spatially implicit compartmental patch model of WNV transmission in the presence of IVM-treated birdfeed. We tracked a total of 105 birds using radio telemetry and radio frequency identification to monitor their feeder usage at

and location of nocturnal roosts in relation to five feeder sites in a neighborhood in Fort Collins, Colorado. Using these results, we modified a compartmental model of WNV transmission to account for the impact of IVM on mosquito mortality and spatial movement of birds and mosquitoes. We identified the competence of birds to infect biting mosquitoes with WNV, the level of IVM-induced mosquito mortality, and number of treated lots in a neighborhood as the parameters with the largest influence on the estimated reduction in infection intensity. There was no significant difference in the reduction based on arrangement of treated lots. Our results also indicated that the feeder design and deployment strategy that results in the largest proportion of treated birds, regardless of the WNV competency status of the birds, will achieve the largest reduction. Overall, we estimated reductions up to 83.9% in infectious mosquito-days and 61.3% in infections in competent birds could be obtained under ideal conditions, but these circumstances are unlikely to occur under field conditions. Balancing estimated mosquito mortality due to IVM and the achievable spatial coverage with treated feeders will inform the expected level of control.

Fieldwork allowed us an interesting ecological investigation into birdfeeder usage and nocturnal roosting habits of common backyard birdfeeder birds. We found that house sparrows remain in the same nocturnal roosting site on sequential nights 61% of the time while mourning doves were only found in the same location 20% of the time. When house sparrows disperse to new nocturnal roosts, the average dispersal distance is 219 m while mourning doves disperse an average of 780 m. The average duration of daily feeder visitation by house finches was 398 sec (6.6 mins). Due to delays, we were unable to characterize the feeder usage and nocturnal roosting habits of the full suite of targeted birds nor assess changes in these parameters across the season, but our work provided a glimpse into the feeder usage and nocturnal habits of common backyard birds during the WNV season.

Taken together, the results of this dissertation elucidate the effects of existing and novel control strategies on the dynamics of WNV and its mosquito vectors. These findings can be used by vector control and public health professionals to design, implement, and evaluate future effective vector control strategies. The counterfactual-based modeling framework we developed for aerial spraying can be applied to other districts and control strategies to improve evaluation of vector control methods and identify areas,

spatially or temporally, that are lacking sufficient data for estimation. The field-based studies and modeling efforts support the ongoing investigation of bird-delivered IVM as a novel WNV control strategy, potentially resulting in an effective alternative vector control strategy to improve overall vector management and disease prevention.

Appendices

Appendix 1: Supplemental figures and tables (Chapter 1)

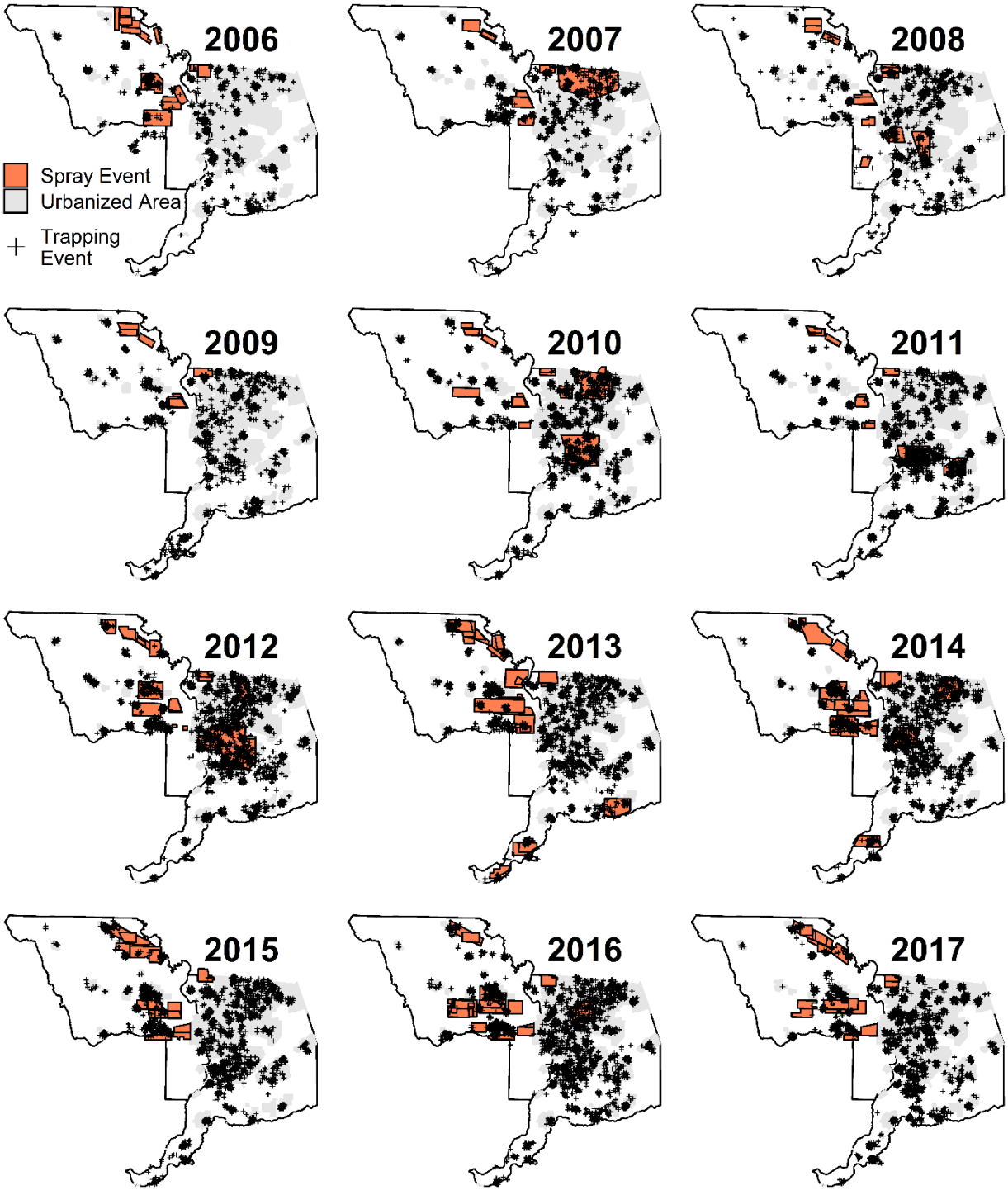


Figure A1-1: Location of CO_2-baited mosquito trapping events and aerial spray events stratified by year (2006-2017). *A random jitter of ≤ 1 km applied to trapping locations for visualization of repeated events at the same site. Each spray event polygon represents the area targeted during a single aerial spray application.*

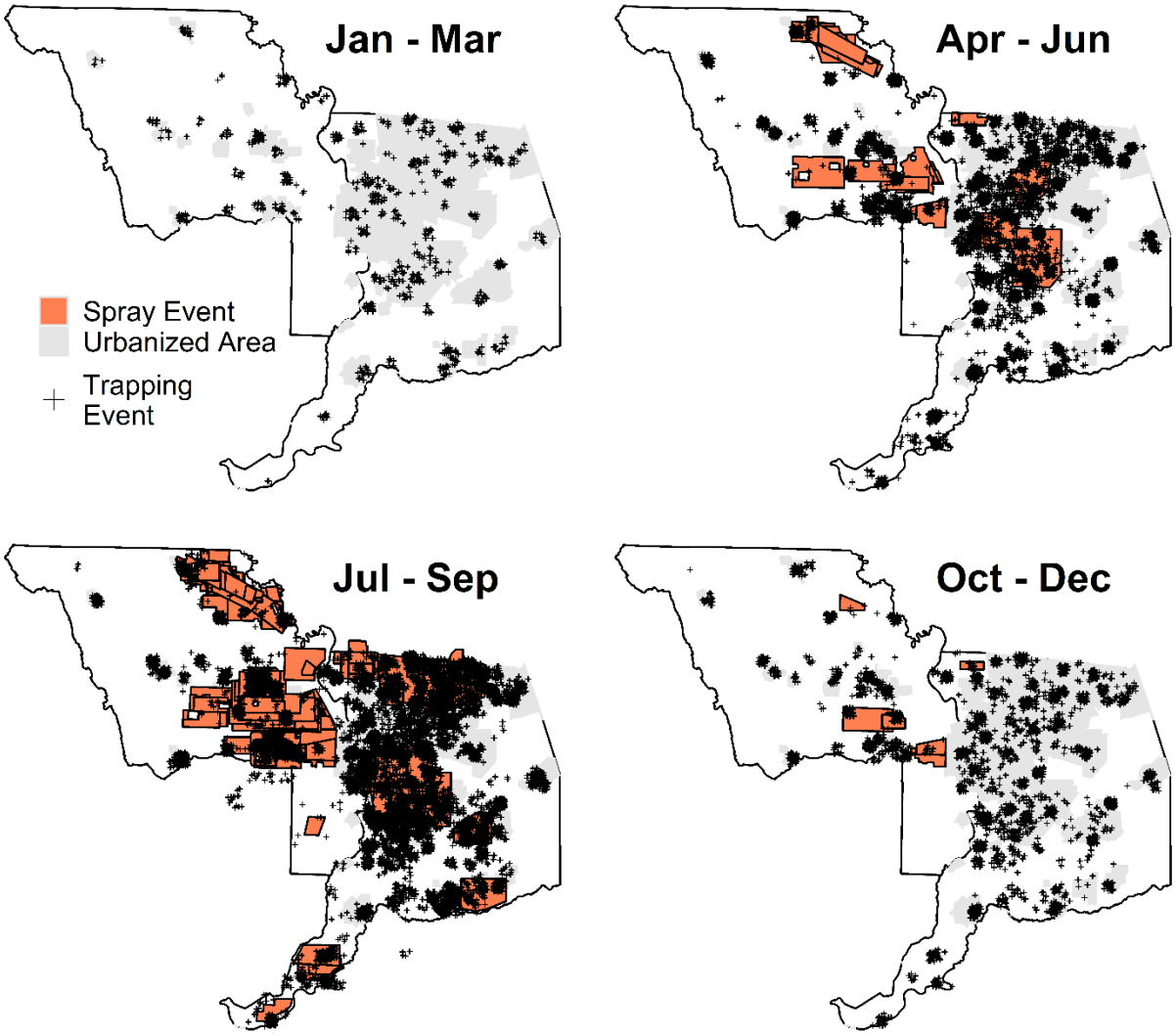


Figure A1-2: Location of CO_2-baited mosquito trapping events and aerial spray events stratified by season. *Season defined into three-month intervals. A random jitter of ≤ 1 km applied to trapping locations for visualization of repeated events at the same site. Each spray event polygon represents the area targeted during a single aerial spray application.*

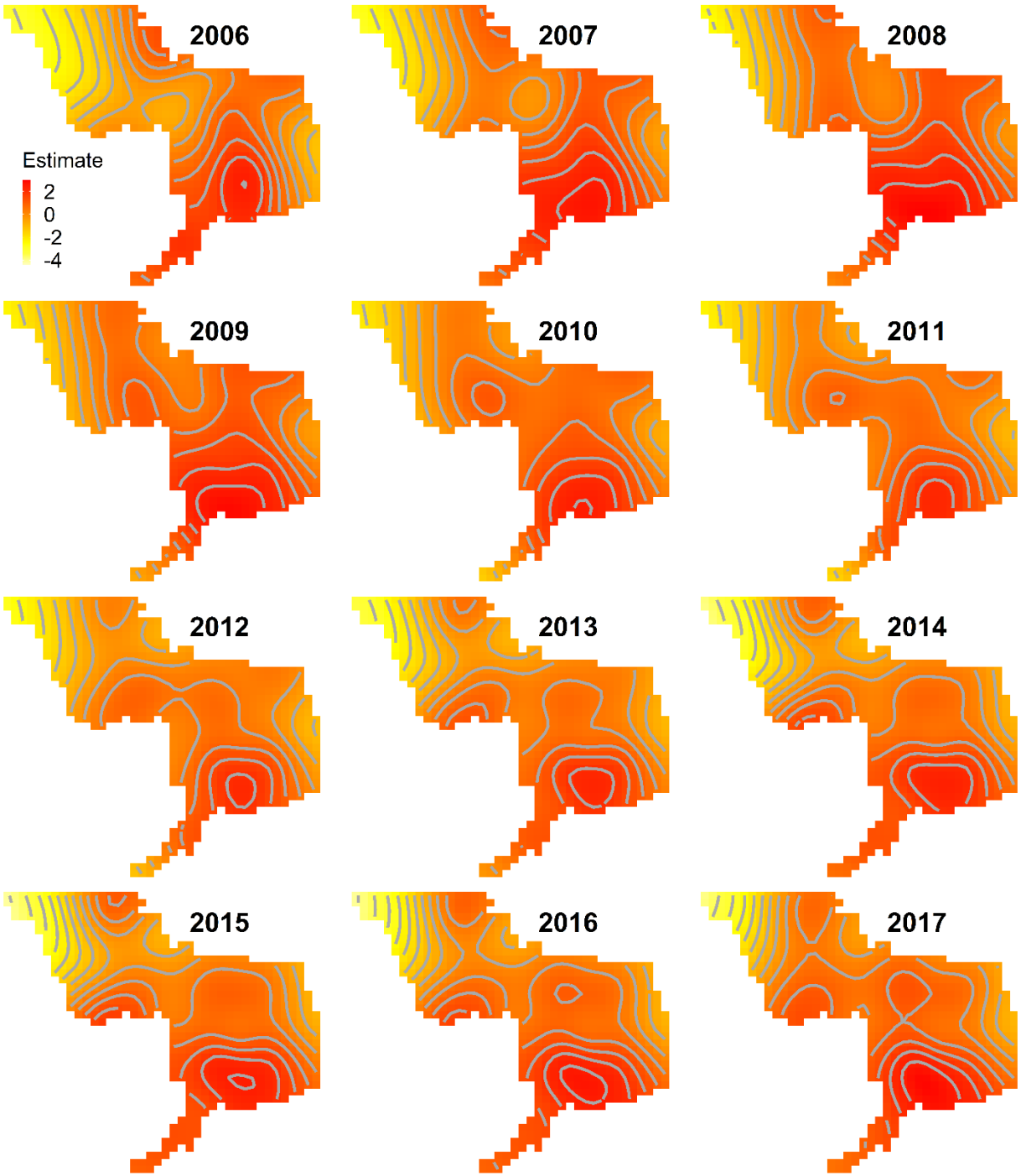


Figure A1-3: Spatial surface for Cx. pipiens at midpoint of the typical WNV season (week of Aug 1). *Surface presented for each year (2006-2017) reflects the relative abundance of the species and is a slice from the three-dimensional spatio-temporal smoothed function. Contours applied for visualization of estimates.*

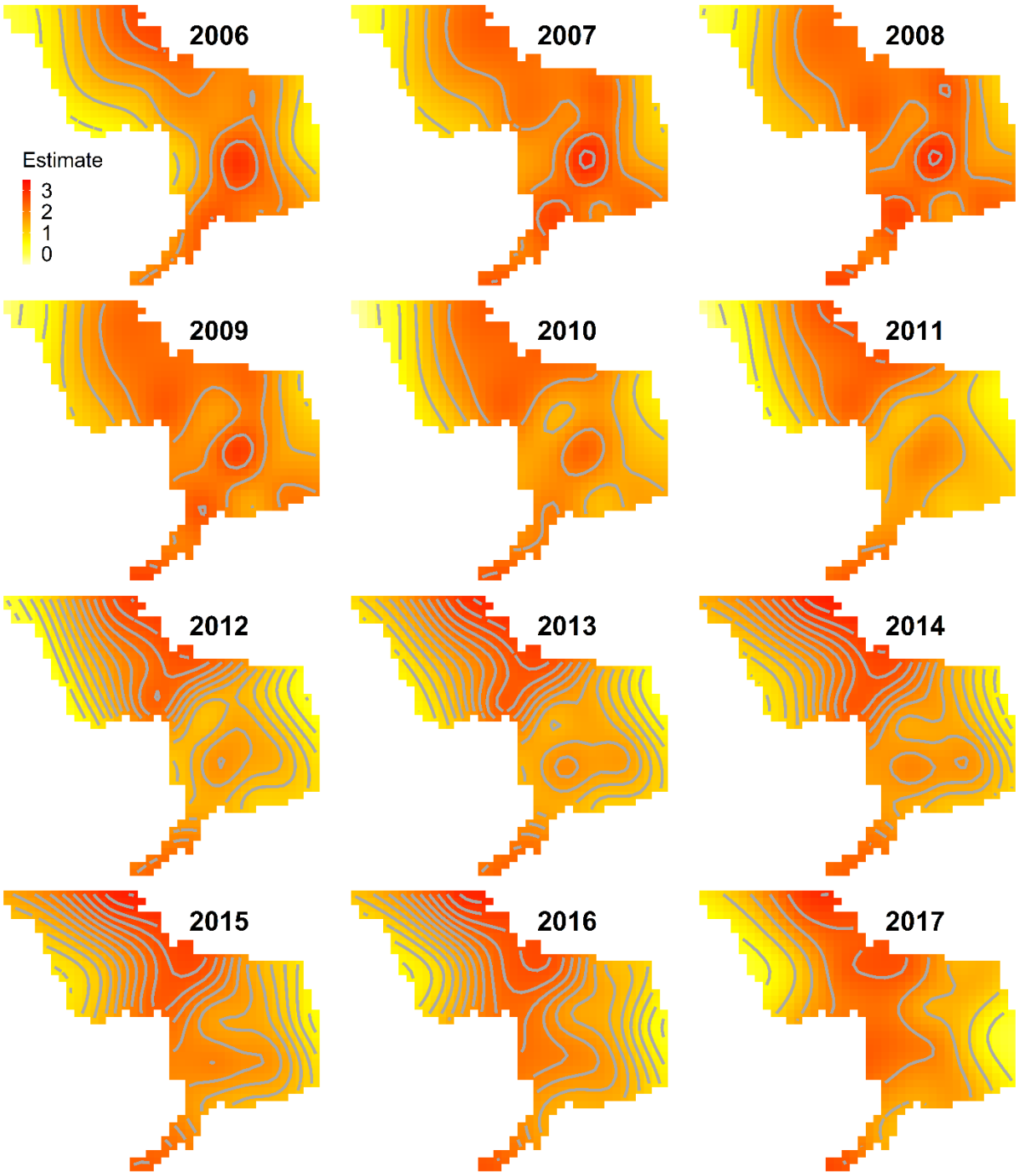


Figure A1-4: Spatial surface for Cx. tarsalis at midpoint of the typical WNV season (week of Aug 1). *Surface presented for each year (2006-2017) reflects the relative abundance of the species and is a slice from the three-dimensional spatio-temporal smoothed function. Contours applied for visualization of estimates.*

Table A1-1. Smooth functions included in the final generalized additive models (GAMs) for both *Cx. tarsalis* and *Cx. pipiens*.

Variable(s) included	Biological interpretation	Spline chosen	Basis dimensions[+]
Longitude, latitude, Week[#]	Spatial relationships across Sacramento and Yolo counties allowed to vary on the weekly scale	Tensor product of 2D thin plate regression spline (long/lat) and cubic regression spline (time)	125
Day[†] by 'urban' land use	Seasonal curve in urban areas	Cyclic cubic regression spline	10
Day[†] by 'crops' land use	Seasonal curve in cultivated crop lands	Cyclic cubic regression spline	10
Day[†] by 'natural' land use	Seasonal curve in non-urban non-crop areas	Cyclic cubic regression spline	10
Temperature deviation from monthly average on night of trapping (°C)	Impact of warmer/colder than normal temperatures on activity of mosquitoes on night of collection	Thin plate regression spline	10
Average temperature during previous 2 weeks (°C)	Developmental rates of mosquitoes	Thin plate regression spline	10
Spatial overlap of spray, temporal sequence of sprays	Spatio-temporal impacts of aeial spraying	Tensor product of 2 thin plate regression splines	25
Collection site	Random intercept for each collection site location	Penalized coefficient spline (identity penalty matrix)	1,065[‡]

[+] The dimension of the basis used to represent the smooth function. One degree of freedom is lost to the identifiability constraint of the smooth and the remainder sets the upper limit on the degrees of freedom associated with the smooth and was selected using the method outlined by Wood [1].

[#] Continuous time variable indicating the number of weeks from the start of the study period (range 1-626).

[†] Day of the year (range 1-365).

[‡] Number of unique collection sites.

Reference Cited:

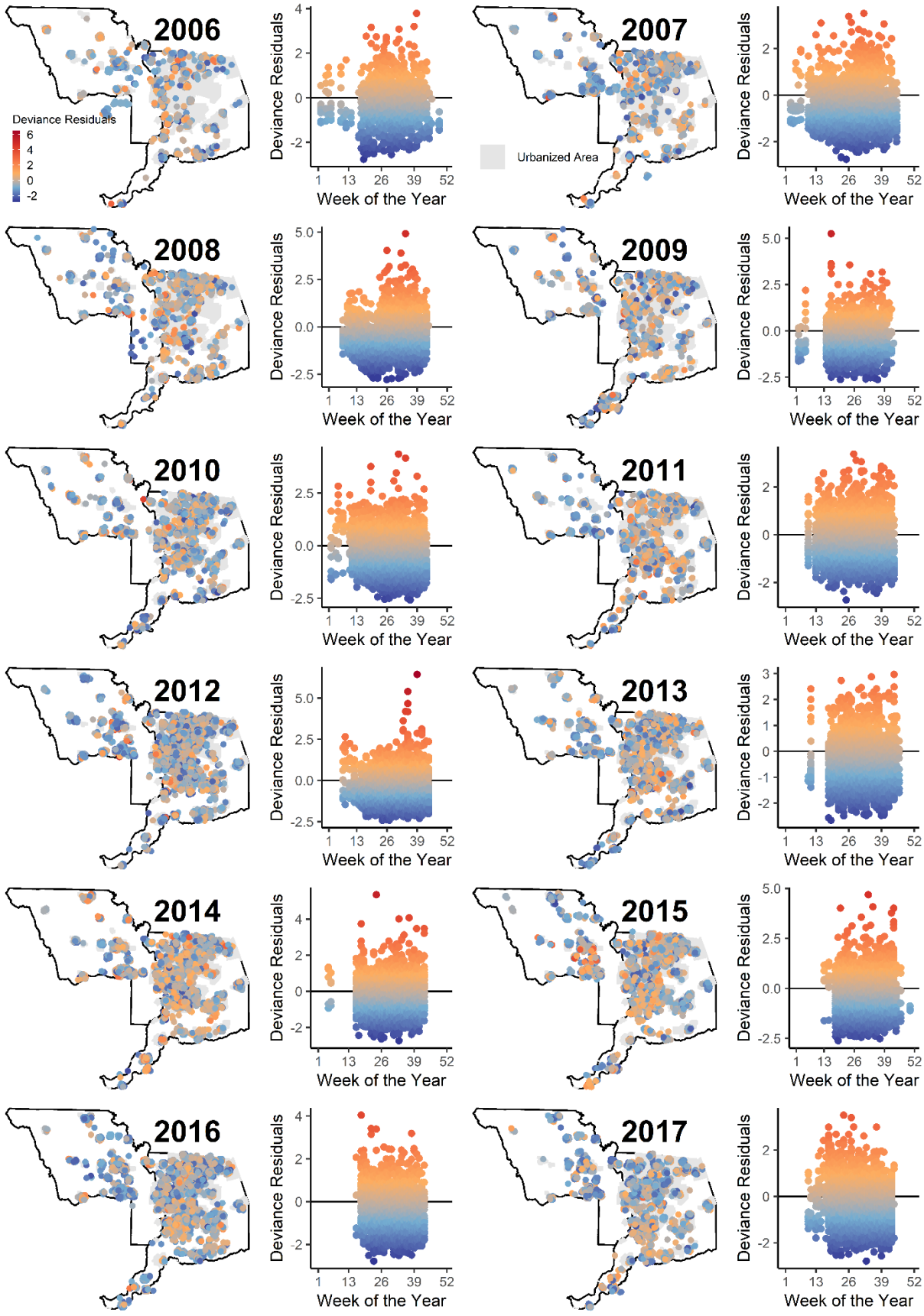
1. Wood SN. Generalized additive models: An introduction with R. Boca Raton, FL: Chapman & Hall/CRC; 2006.

In order to capture dramatic deviation from 'normal' mosquito abundance that partially characterize high-risk periods and precipitate an aerial spray response, we considered parameters indicating the presence of an aerial spray in the following one to four weeks after a collection event in the GAMs. In other words, if a collection event precedes a spray, it would have higher abundance than expected because there is higher than average abundance and this was a factor precipitating the following spray event. These parameters captured the dramatic deviation in a way that the smooth functions describing expected abundance could not. Estimated change in abundance for retained covariates (based on reduction in AIC) in the final models for collections one to four weeks before an aerial spray are outlined in Table A1-2 below

Table A1-2. Change (%) in nightly abundance from expected for collections preceding aerial spraying.

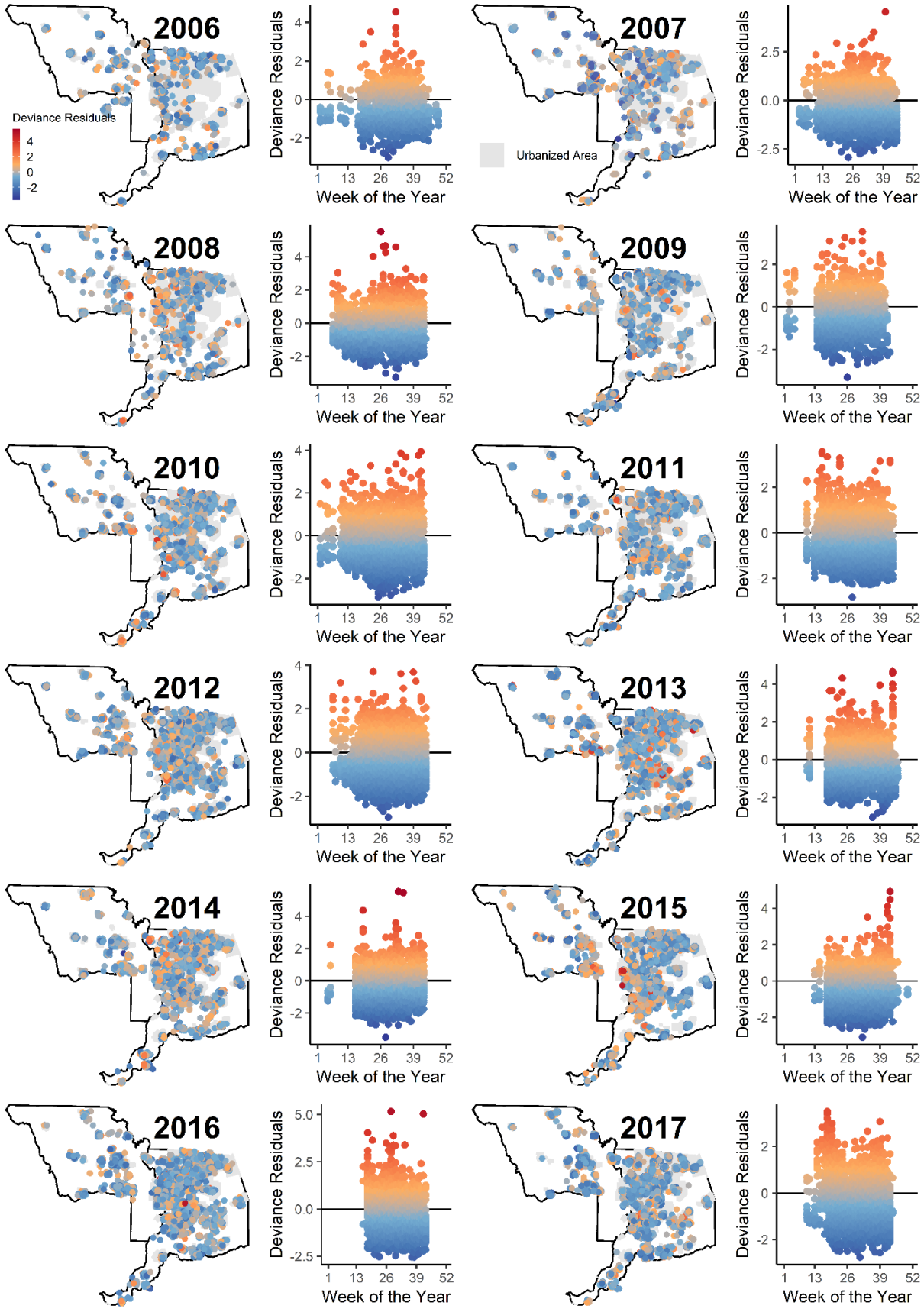
Species	**PreSpray1**‡	**PreSpray2**‡	**PreSpray3**‡	**PreSpray4**‡
Cx. pipiens	21.9***	NA	NA	23.9**
Cx. tarsalis	13.8*	29.0***	21.0**	NA

Parameter significance: *$P<0.05$; **$P<0.005$; ***$P<0.0001$
NA: term not retained through backward selection so not present in final model.
‡ PreSpray#: presence of a spatially overlapping aerial spray in the indicated 1, 2, 3, or 4 weeks following a trap collection.


2006
Deviance Residuals
6
4
2
0
-2
Deviance Residuals
Week of the Year
2007
Urbanized Area
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017

(figure is on the previous page)

Figure A1-5: Spatial and temporal distribution of model deviance residuals for Cx. pipiens for 2006-2017. *Residuals presented spatially at the associated trapping location (random jitter of ≤ 1 km applied for visualization of repeated events).*


2006
Deviance Residuals
4
2
0
-2
Deviance Residuals
Week of the Year
2007
Urbanized Area
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017

(figure is on the previous page)

Figure A1-6: Spatial and temporal distribution of model deviance residuals for Cx. tarsalis for 2006-2017. *Residuals presented spatially at the associated trapping location (random jitter of ≤ 1 km applied for visualization of repeated events).*

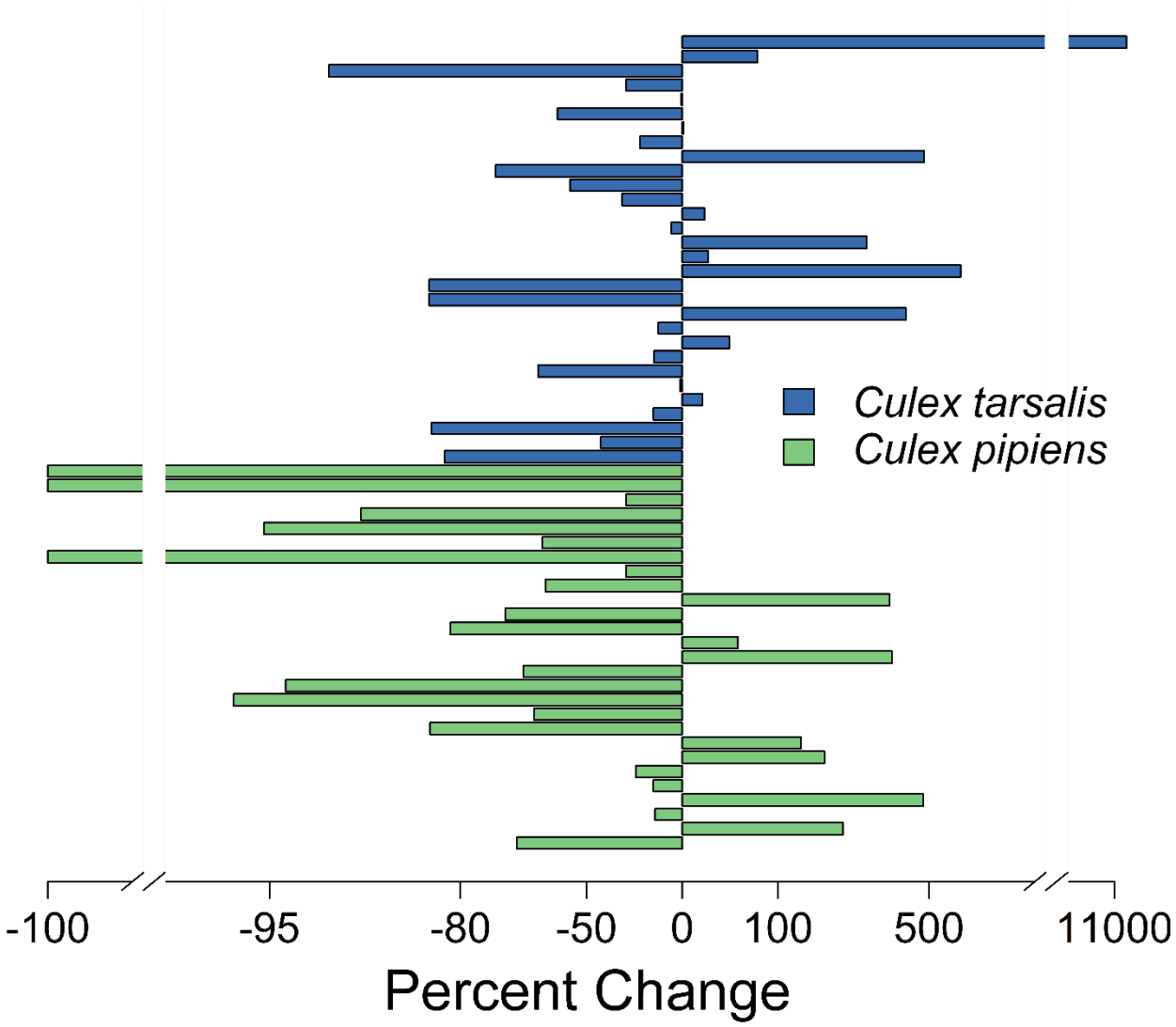


Figure A1-7: Estimated percent change in Cx. pipiens and Cx. tarsalis populations with Mulla's formula. *Change estimated for the 36 aerial sprays in Sacramento and Yolo counties, CA (2006-2017) with associated trap collections within the targeted zone (treated) and an adjacent 5km buffer (control) within one-week before and one-week following spraying.*

Appendix 2: Generalized R script of generalized additive model (GAM) fitting and estimation (Chapter 1).

Script outlines our workflow of covariate development, GAM model fitting, and estimated change in abundance. Code presented is not necessarily the most efficient, but provides the logic of our methodology to guide future use of this method.

```
#####
# Author: Karen Holcomb (kmholcomb@ucdavis.edu)
# Last Updated: 12/03/20
####
# Generalized version of workflow developed for estimation of the effect of aerial spraying
#   on abundance of Culex tarsalis and Culex pipiens populations in Sacramento and Yolo county, CA
#
# Note: Code presented is compiled over many iterations and likely not the most efficient way
#
# Flow of code: pre-process data, spatial join of traps and spraying data, fit GAMs, estimate change in abundance
# Terminology of components:
# spray.spdf - Spatial Polygons DataFrame with shapefiles for each spray event and dataframe with
#              information on each spray event (each row is a different spray; need column for date,
#              name of spray event, and product used (code assumes 1 polygon per of row of spraying data)
# trap.data - dataframe with trapping record (each row is different trap event);
#              need columns with date, number of traps involved per trapping event, lat/long, temperature,
#              number mosquitoes collected, DiffSpray (see below)
# DiffSpray - number that identifies which sprays are part of the same multi-night spray event;
#              same number means sprays are part of same event (my set-up method shown below)
####


## Set-up data frames (trap.data and spray.spdf) ################
library(mgcv) #GAM package
library(sp)
library(rgdal)
library(rgeos)


## Load the mosquito collection data (data.raw)
```

```
# Subset trapping data to CO2 traps run w/o issue; columns for long/lat, date of collection, number of traps
#  run at that event, number of nights traps run, ID for collection location, total tarsalis and pipiens caught,
#   and ID number for collection
Keep <- which(data.raw$trap_problem == "N" & data.raw$trap_type == "CO2")
trap.data <- data.raw[Keep,c("longitude","latitude","collection_date","num_trap","trap_nights",
                             "site_code","tarsalis_females","pipiens_females","collection_id")]
trap.data <- trap.data[trap.data$trap_nights == 1, ] #exclude any CO2 traps run longer than 1 night

## Set-up date-related variables for trap data ('collection_date' is date when trap was picked up)
trap.data$collection_date <- as.Date(trap.data$collection_date, format="%m/%d/%Y")
trap.data$Day <- strptime(trap.data$collection_date,format="%Y-%m-%d")$yday+1
trap.data$Year <- as.numeric(format(trap.data$collection_date,"%Y"))
trap.data$Week <- as.numeric(as.character(format(trap.data$collection_date, "%V"))) #week of the year
trap.data$YearWeek <- as.numeric(as.character(trap.data$Year)) + trap.data$Week/52 #continuous time
trap.data$Year <- as.factor(trap.data$Year)

## Set-up date-related variables for spray data ('date' is date aerial spray application occurred)
spay.spdf$date <- as.Date(spay.spdf$date, format="%m/%d/%Y")
spay.spdf$Day <- strptime(spay.spdf$date,format="%Y-%m-%d")$yday+1
spay.spdf$Year <- as.numeric(format(spay.spdf$date,"%Y"))
spay.spdf$Year <- as.factor(spay.spdf$Year)

## Set-up DiffSpray numbers to indicate which sprays are part of same multi-night spray event (based on name, date)
# assumes dates are oldest to newest
# 'name' is name of spray location in spray.data dataframe
diffy = vector() #holding vector for below loop, gathering info before putting all in new col together at end
for (i in 1:length(levels(spray.spdf$name))) {
  ord <- cumsum(c(1, diff(spray.spdf$date[spray.spdf$name == levels(spray.spdf$name)[i]]) != 1))
  loc <- which(spray.spdf$name == levels(spray.spdf$name)[i])
  diffy[loc] <- as.numeric(paste(i)) + ((ord-1) / length(ord))
```

```
}
spray.spdf$DiffSpray <- diffy #diff spray events by diff numbers

## Add in temperature data as columns (used 2 week average and deviation from average on night of trapping)
# trap.data$av.temp_2wk #average temperature in 2 weeks prior to trapping
# trap.data$Temperature #deviation in temperature from average
# Used Daymet's Single Pixel Extraction tool: https://daymet.ornl.gov/single-pixel/) based on lat/long of traps

## Add in land use data (% of 5km buffer around trap of each land use type) from https://www.mrlc.gov/data/nlcd-2011-land-cover-conus-0
# Extract land use based on lat/long of trap w/ 5km buffer around each trap
# Land use categories (NLCD codes): urban (21,22,23,24), crops (82), natural (31,41,42,43,52,71,81,11,90,95)
# trap.data$urban #proportion of buffer 'urban' land use
# trap.data$crops #proportion of buffer 'crops' land use
# trap.data$natural #proportion of buffer 'natural' land use

## Project trap location data (used NADM83)
coordinates(trap.data) <- ~ longitude + latitude
proj4string(trap.data) <- CRS("+proj=longlat")
trap.data <- spTransform(trap.data, CRS('+init=epsg:3310'))

## Make site_code factor for use as random effect
trap.data$site_code <- as.factor(trap.data$site_code)

## Spatial set up and join of trapping and spraying data ################
## Create circle polygons ('collection area') around each included traps (5 km radius) w/ unique IDs
col.area <- lapply(1:nrow(trap.data), function(j) gBuffer(trap.data[j,], width = 5000))
for(k in 1:length(col.area)) { col.area[[k]]@polygons[[1]]@ID <- as.character(as.numeric(k)) }

## Spatial join of traps with spray polygons
# Create start/end times (days) for weekly temporal windows pre-trapping (1,2,3,4 weeks)
```

```
c.lag.start = c(0,8,15,22)
c.lag.end = c(7,14,21,28)

## Create vectors indicating days for start/end of assessing pre-spray (if trap 1-4 weeks prior to spray event)
pre.spray_start <- -seq(1, max(c.lag.end), by=7)
pre.spray_end <- -seq(7, max(c.lag.end), by=7)

## Setting up spray related columns (spatial overlap of previous sprays, product used, and prior to future sprays)
name.vect <- c(sapply(1:length(c.lag.end), function(x) paste("c.lag", x, sep="")), #% of 5km buffer around trap sprayed in indicated week prior to trapping
        sapply(1:length(c.lag.end), function(x) paste("m.lag", x, sep="")), #product used in spray(s) in indicated week
        sapply(1:length(c.lag.end), function(x) paste("PreSpray.", x, sep=""))) #spraying (0/1) in indicated week post trapping

trap.data <- cbind(trap.data, lapply(1:(3*length(c.lag.start)), function(x) rep(NA, nrow(trap.data))))
names(trap.data2006)[-1*(1:(ncol(trap.data2006) - length(name.vect)))] <- name.vect


## Spatial and temporal join for each trap (collection area) and each spray
sprays = list() # list to hold DiffSpray numbers
t.spray = vector() # vector to hold 'time since last spray' variable created in loops below
pre.spray = vector() # vector to hold 'time to next spray' variable created in loops below
z = ncol(trap.data) #used for assigning calculations to appropriate column below

for (i in 1:nrow(trap.data)) { # for each trapping event
  for (j in 1:length(spray.spdf)) { # for each spray event polygon
    t.spray[j] <- ifelse(as.character(is.na(over(col.area[[i]], spray.spdf[j,])) == FALSE)[1],
                  ifelse(trap.data$Year[i] == spray.spdf$Year[j],
                    (trap.data$Day[i]-1) - spray.spdf$Day.2014[j], 400), 400)
    #if trap in spray area and same year, how long since last spray
    # (trap date adjusted back 1 day to when collecting mosquitoes vs when trap was collected)
```

```
   #if not in spray area or in different year, nonsense number (400)
  }

  pre.spray <- t.spray #save time to next spray for pre.spray assessment
  t.spray <- replace(t.spray, t.spray < 0, 400) #nonesense number for negative times

  # Calculate % of collection area sprayed in each week prior to trapping; gather all unique spray events in
time window
  for (m in 1:length(c.lag.start)) {
   trap.data[i, z+m] <- ifelse(any(t.spray >= c.lag.start[m] & t.spray <= c.lag.end[m]),
                               gArea(gIntersection(spray.spdf[which(t.spray >= c.lag.start[m] & t.spray <=
c.lag.end[m]),],
                                                   col.area[[i]])) / gArea(col.area[[i]]), 0)

   # Product (aka Material) used for each timeframe if sprayed; note if at least one organophosphate/naled
   #   Note 'Trumpet EC' and 'Dibrom' are organophosphate/naled products used
   mats <- levels(spay.spdf$Material)

   trap.data[i, (z+length(c.lag.end))+m] <- ifelse(trap.data@data[i, z+m] == 0, as.character('None'),
                                                   ifelse(length(mats) == 1, as.character(mats),
                                                          ifelse("Trumpet EC" %in% mats | "Dibrom" %in% mats,
as.character("Mixed.n"),
                                                                 as.character("Mixed"))))

   # Indicate if trap within 1-4 weeks prior to next spray (prespray)
   trap.data[i,(z+2*length(c.lag.end))+m] <- ifelse(any(pre.spray <= pre.spray_start[m] & pre.spray >=
pre.spray_end[m]), 1, 0)
   }
}



## Set up spatial and temporal effects of aerial spraying #################
## Make PreSpray columns as factor
trap.data@data[,c(((z+2*length(c.lag.end))+1) : (z+3*length(c.lag.end)))] <-
```

```
  lapply(trap.data@data[,c(((z+2*length(c.lag.end))+1) : (z+3*length(c.lag.end)))], factor)

## Spatial effect: sum of proportion sprayed over lags (average coverage per week with spray)
trap.data$c.lag1 <- replace(trap.data$c.lag1, trap.data$c.lag1 > 1, 1) #max 100%
trap.data$c.lag2 <- replace(trap.data$c.lag2, trap.data$c.lag2 > 1, 1) #max 100%
trap.data$c.lag3 <- replace(trap.data$c.lag3, trap.data$c.lag3 > 1, 1) #max 100%
trap.data$c.lag4 <- replace(trap.data$c.lag4, trap.data$c.lag4 > 1, 1) #max 100%

for(i in 1:nrow(trap.data)) {
  props <- c(trap.data$c.lag1[i], trap.data$c.lag2[i], trap.data$c.lag3[i], trap.data$c.lag4[i])
  trap.data$Control_space[i] <- ifelse(sum(props)==0, 0, mean(props[props > 0]))
}
trap.data$Control_space <- replace(trap.data$Control_space, trap.data$Control_space > 1, 1) #100%+
coverage -> 100%

## Temporal sequence: create factor for time (each combo of sprayed weeks as unique factor)
# left = 4 wks ago, right = 1 week ago
factor_time <- sapply(1:nrow(trap.data),
                      function(x) as.factor(paste(c(ifelse(trap.data$c.lag1[x] > 0, 1, 0),
                                                    ifelse(trap.data$c.lag2[x] > 0, 1, 0),
                                                    ifelse(trap.data$c.lag3[x] > 0, 1, 0),
                                                    ifelse(trap.data$c.lag4[x] > 0, 1, 0)),
                                                  collapse="")))

# Control time as factor (can use as numeric version later)
trap.data$Control_time <- factor(factor_time, levels(factor_time)[order(levels(factor_time))])
trap.data$Control_time_n <- as.numeric(trap.data$Control_time) #numeric verrsion

## Create variable indicating if at least 1 spray used during spray history in last 4 weeks
e <- length(c.lag.start) #last cumulative lag length
all.cum.mats <- lapply(1:nrow(trap.data@data), function(x) {
  levels(as.factor(as.character(trap.data@data[x,(z+e+1):(z+e+e)])))})
```

```
trap.data$naled <- as.factor(sapply(all.cum.mats, function(y) ifelse("Trumpet EC" %in% y | "Dibrom" %in% y |
                                                                       "Mixed.n" %in% y,
                                                                     1,0)))

## Fit GAMs for each species ################
## set species of interest
sp = '*tarsalis*' #options: '*pipiens*', '*tarsalis*'

## Fit GAMs; covariates chosen based on AIC (see Methods)
gam.mod_df <- if(sp == "*tarsalis*") { #*tarsalis* GAMs
  dat_cut_fin <- trap.data[0,] #set-up
  for(i in 1:length(unique(trap.data$Week))) {
    dat_cut <- NULL
    abund <- trap.data$*tarsalis*_females[trap.data$Week == unique(trap.data$Week)[i]] /
      trap.data$num_trap[trap.data$Week == unique(trap.data$Week)[i]] #*tarsalis* per trap for that unit
    cutoff <- mean(abund) + 2*sd(abund)
    dat_cut <- trap.data[trap.data$Week == unique(trap.data$Week)[i],][-c(which(abund > cutoff)),]
    if(!identical(dat_cut, dat_cut_fin)) {
      dat_cut_fin <- rbind(dat_cut_fin, dat_cut) }
  }
  trap.data <- dat_cut_fin #removed top 5% of collections per week (improves fitting)
  *tarsalis*.gam <- gam(*tarsalis*_females ~ offset(log(num_trap)) +
                          te(longitude,latitude,YearWeek, d=c(2,1), bs=c("tp","cr")) +
                          s(Day, bs="cc", by=urban) +
                          s(Day, bs="cc", by=natural) +
                          s(Day, bs="cc", by=crops) +
                          s(Temperature) +
                          s(av.temp_2wk) +
                          s(site_code, bs="re") +
                          PreSpray.1 + PreSpray.2 + PreSpray.3 +
                          te(Control_space, Control_time_n),
                        data = trap.data, family = nb ,method = "REML")
  return(list(*pipiens*.gam, trap.data)) #return fitted GAM and data used in fitting
```

```
} else { #pipiens GAMs
 dat_cut_fin <- trap.data[0,] #set-up
 for(i in 1:length(unique(trap.data$Week))) {
  dat_cut <- NULL
  abund <- trap.data$pipiens_females[trap.data$Week == unique(trap.data$Week)[i]] /
   trap.data$num_trap[trap.data$Week == unique(trap.data$Week)[i]] #pipiens per trap for that unit
  cutoff <- mean(abund) + 2*sd(abund)
  dat_cut <- trap.data[trap.data$Week == unique(trap.data$Week)[i],][-c(which(abund > cutoff)),]
  if(!identical(dat_cut, dat_cut_fin)) {
   dat_cut_fin <- rbind(dat_cut_fin, dat_cut) }
 }
 trap.data <- dat_cut_fin #removed top 5% of collections per week (improves fitting)
 pipiens.gam <- gam(pipiens_females ~ offset(log(num_trap)) +
                    te(longitude,latitude,YearWeek, d=c(2,1), bs=c("tp","cr")) +
                    s(Day, bs="cc", by=urban) +
                    s(Day, bs="cc", by=natural) +
                    s(Day, bs="cc", by=crops) +
                    s(Temperature) +
                    s(av.temp_2wk) +
                    s(site_code, bs="re") +
                    PreSpray.1 + PreSpray.4 +
                    naled +
                    te(Control_space, Control_time_n),
                   data = trap.data, family = nb ,method = "REML")
 return(list(pipiens.gam, trap.data)) #return fitted GAM and data used in fitting
}

## See Appendix 1: Table A1-1 for details on choice of splines and basis dimensions
## See R documentation for GAMs for further information on fitting, checking, and plotting


## Calculate change in abundance across spatio-temporal grid ################
```

```
## control_data is a function that returns a dataframe with the mean change in abundance for each spatio-temporal
#   combination in the data along with 95% CI and indication if estimated change is significant
## model: list with GAM object and dataframe used in fitting (i.e. gam.mod_df returned above)
## step: unit used in estimation of spatial coverage (0-1); 0.01 = 1% change in spatial coverage
## naled: 0/1 indicator if to estimate change assuming at least 1 organophosphate used;
#   1 can be used only if covariate present in GAM

est.change_data <- function(model = gam.mod_df, step = 0.01, naled = 0) {
  # Set-up data to use for prediction
  new.dat <- expand.grid("Control_space" = seq(0,1,by=step),
                         "Control_time_n" = 1:length(levels(model[[2]]$Control_time))) #spatio-temporal grid

  new.dat <- new.dat[-c(2:length(seq(0,1,by=step))),] #rm control_space > 0 for no spray control_time (don't need these calcs)

  new.dat <- data.frame(new.dat, num_trap = 1,
                        longitude = unique(model[[2]]$longitude[model[[2]]$site_code == 233001]),
                        latitude = unique(model[[2]]$latitude[model[[2]]$site_code == 233001]),
                        YearDay = median(model[[2]]$YearDay),
                        Day = (median(model[[2]]$YearDay) - floor(median(model[[2]]$YearDay))) * 365,
                        YearWeek = median(model[[2]]$YearWeek),
                        urban = unique(model[[2]]$urban[model[[2]]$site_code == 233001]),
                        non.urban = unique(model[[2]]$non.urban[model[[2]]$site_code == 233001]),
                        natural = unique(model[[2]]$natural[model[[2]]$site_code == 233001]),
                        crops = unique(model[[2]]$crops[model[[2]]$site_code == 233001]),
                        Temperature = mean(model[[2]]$Temperature),
                        av.temp_2wk = mean(model[[2]]$av.temp_2wk),
                        site_code = 233001, naled = 0,
                        PreSpray.1 = 0, PreSpray.2 = 0, PreSpray.3 = 0, PreSpray.4 = 0)
  # site code 233001 used most in our data, used across years, and rather central spatially

  if(naled == 1) new.dat$naled[2:nrow(new.dat)] <- 1 # replace all sprayed with 1 (no spray still 0)
```

```
Xp <- predict(model[[1]], new.dat ,type="lpmatrix") #linear predictor matrix

# simulate from posterior distribution to estimate mean and variance of % change in abundance
rmvn <- function(n,mu,sig) { ## multivariante normal random deviates (need mgcv lib)
  L <- mgcv::mroot(sig); m <- ncol(L);
  t(mu + L%*%matrix(rnorm(m*n),m,n))
}

set.seed(04232019) #seed so same sampling each run and resulting plot
reps <- 10000 #number of samples from posterior distribution
br <- rmvn(reps, coef(model[[1]]), model[[1]]$Vc) ## 10000 replicate param. vectors,
#Note: Vc is corrected vcov for uncertainty in smooths (since using REML est method)
res <- array(0, dim=c(nrow(new.dat), 1, reps))

for (i in 1:reps) {
  #for each iteration of predictions, calculate rate ratio (#/night spray/no spray) and % change
  pr <- Xp %*% br[i,] ## replicate predictions
  res[,,i] <- (exp(pr)/exp(pr[1])) #ratio of trap-counts/night (compared to no spray)
}

# For each cell in array (est % change), calc mean and var/SD
resp_change <- data.frame(Control_space = new.dat[,1], Control_time_n = new.dat[,2])

# Mean %change, calculated on ratio scale so symmetric around 0 and then converted to %change
resp_change$mean.chng <- sapply(1:nrow(res[,,]), function(x) mean(res[x,,])-1)

# 95% credible interval of change from posterior draws (ratio scale)
resp_change$CI_low <- sapply(1:nrow(res[,,]), function(x) quantile(res[x,,], 0.025))
resp_change$CI_up <- sapply(1:nrow(res[,,]), function(x) quantile(res[x,,], 0.975))

# Remove estimates outside the range of control_space for each sequence of sprays (control_time)
rng <- lapply(levels(model[[2]]$Control_time),
       function(x) range(model[[2]]$Control_space[model[[2]]$Control_time == x])) #range of coverage
for each seq of sprays
```

```
  rng.ext <- lapply(rng, function(x) c(min(x) - 0.05, max(x) + 0.05)) #add little extra buffer on either side of range for plotting
  rng.rnd <- lapply(rng.ext, function(x) round(replace(x, x > 1, 1), 2)) #2 digit round
  rng.rnd[[1]] <- NULL #remove first entry (no spray scenario)
  rng.rnd <- lapply(rng.rnd, function(x) if(-Inf %in% x) c(0,0) else x) #change -inf to inf ranges to 0,0
  cov.seq <- unlist(lapply(rng.rnd, function(x) {
    if(0 %in% x) { c(0,0,101) #correct assignment of in/out for control time w/o any data points
    } else {
      c(length(seq(0,x[1], by = 0.01))-1,
        length(seq(x[1], x[2], by =0.01)),
        length(seq(x[2], 1, by = 0.01))-1)
    }} ))
  in.cov <- which(rep(rep(c(0,1,0), time = 15), times = cov.seq) == 0) + 1 #which inside data coverage, accounting for 0 time
  resp_change[in.cov, c("mean.chng","CI_low","CI_up")] <- NA #set combinations outside coverage as NA

  # Number of different signs in estimated confidence interval limits to determine if crosses 0
  num_sign <- sapply(1:nrow(resp_change),
                     function(x) length(unique(unlist(sign(resp_change[x,c("CI_low","CI_up")]-1)))))
  resp_change$sig_chng <- replace(resp_change$mean.chng, num_sign == 2, NA) #NA for est with CI crossing 0

  return(resp_change) #output dataframe for model with mean change, CI, and significance for spatio-temporal combinations
}
```

Appendix 3: Supplemental figures and table (Chapter 2)

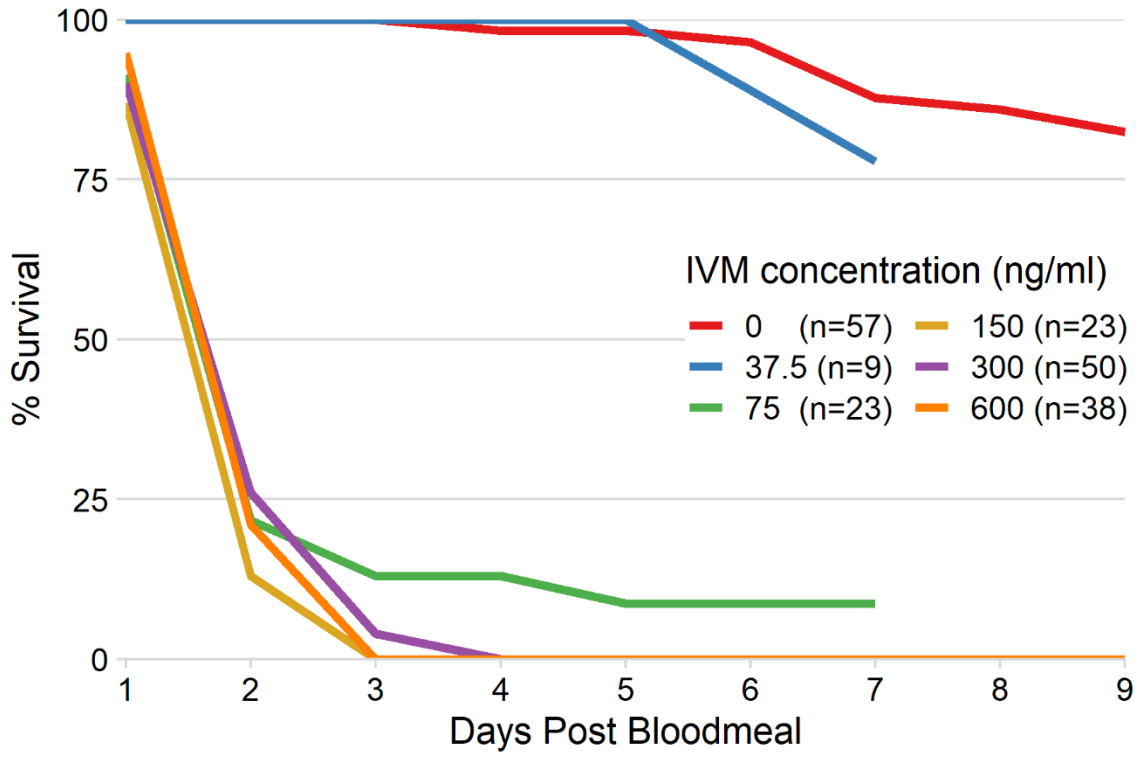
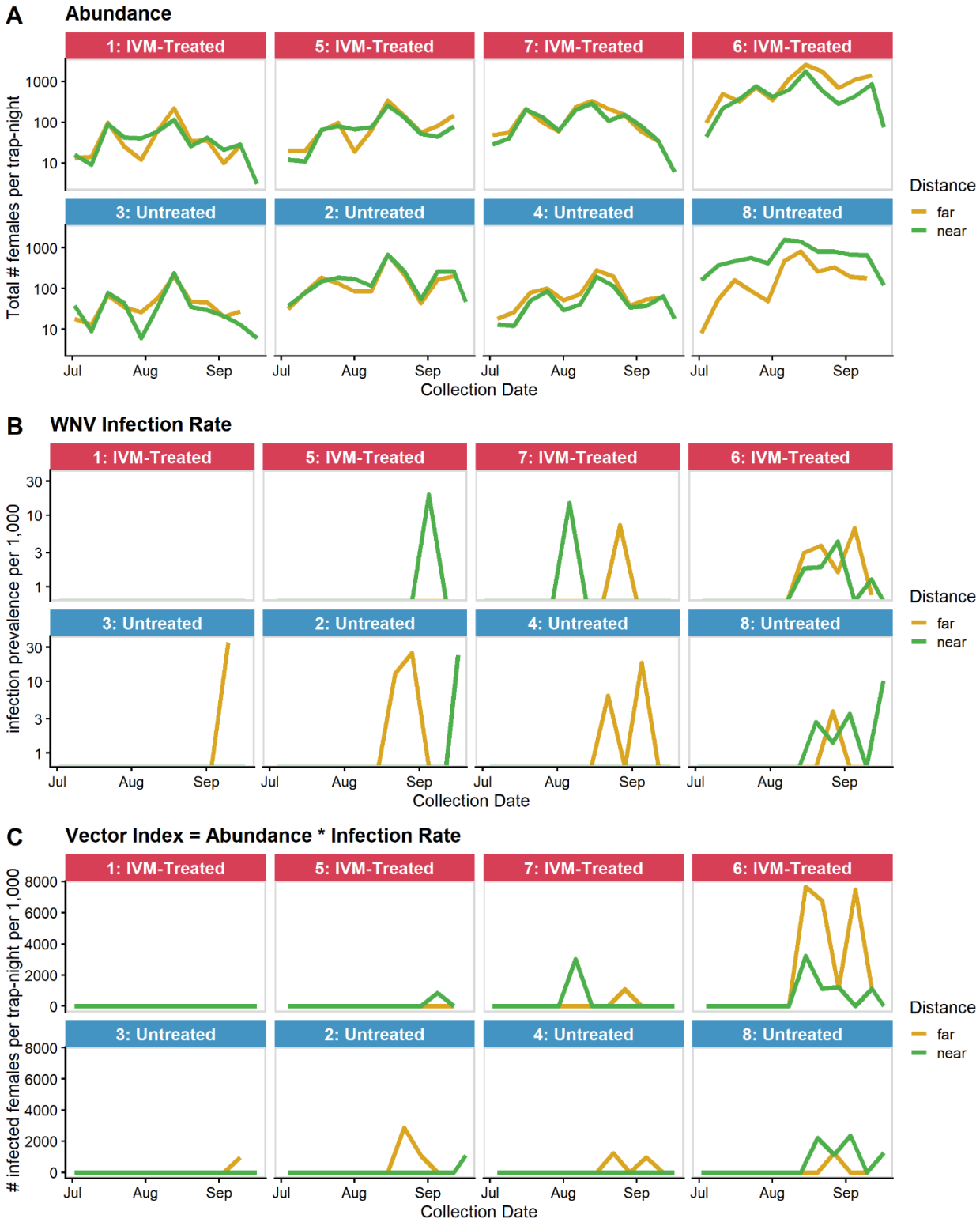


Figure A2-1: Culex tarsalis survival in bioassay with ivermectin. *Cx. tarsalis (Kern Natural Wildlife Reserve colony) survival following a membrane bloodmeal containing serial dilutions of IVM. Number of blood-fed female mosquitoes at each concentration indicated.*


A
Abundance
1: IVM-Treated
5: IVM-Treated
7: IVM-Treated
6: IVM-Treated
3: Untreated
2: Untreated
4: Untreated
8: Untreated
Total # females per trap-night
1000
100
10
Jul
Aug
Sep
Collection Date
Distance
far
near
B
WNV Infection Rate
infection prevalence per 1,000
30
10
3
1
C
Vector Index = Abundance * Infection Rate
infected females per trap-night per 1,000
8000
6000
4000
2000
0

(figure on previous page)

Figure A2-2: Entomological indices of Culex tarsalis by study site and treatment status. *Weekly (A) abundance, (B) infection prevalence per 1,000, and (C) vector index (VI) near (≤ 10m) and far (~150m) from ivermectin (IVM)-treated and untreated flocks. VI is a risk metric that approximates the number of infectious mosquitoes present as the product of abundance and infection rate. Individual plot headers indicate site number (see Figure 2-1) and treatment status and are ordered by spatial location west to east (L to R).*

Table A2-1. **Final model estimates.** Fixed and random effect estimates from mixed effects logistic regression for parity in *Cx. tarsalis* mosquitoes at near and far distances from ivermectin (IVM)-treated and untreated control flocks.

		Estimate	Standard Deviation	*P*-value
Fixed Effects	**Intercept**	-0.475	0.162	0.003
	Control-far	0.085	0.103	0.407
	IVM-far	-0.127	0.095	0.184
	IVM-near	-0.301	0.099	0.002
Random Effects*	**Week 29**	0.036	0.118	NA
	Week 30	-0.456	0.150	
	Week 31	-0.348	0.139	
	Week 32	-0.419	0.108	
	Week 33	-0.414	0.073	
	Week 34	0.267	0.085	
	Week 35	-0.0004	0.122	
	Week 36	0.670	0.104	
	Week 37	0.679	0.102	

* Random intercept by week of the year

Appendix 4: Mathematical details (Chapter 3)

The model equations and details shown below for the compartmental model are modified from a previous model [1] which was developed to assess the impact of temperature on the emergence and seasonality of West Nile virus in California. Symbols and values are summarized in Tables A3-1 & A3-2. Sensitivity analysis presented in Table A3-3.

In each patch in the spatially implicit framework, our model consists of a mosquito vector species and two bird species, a WNV competent and a WNV incompetent species, which can be either ivermectin-treated or untreated. The initial number of mosquitoes and both competencies of birds are equally distributed across the neighborhood such that the initial number in each patch corresponds to the fraction of the neighborhood in the respective treatment status. Each group *X* has a respective birth (b_X) and non-disease related mortality (d_X) and follows a logistic growth curve with a carrying capacity for the neighborhood (K_X). The carrying capacity for treated and untreated patches of the neighborhood is weighted by the proportion of the neighborhood in each treatment status.

The adult mosquito vector population contains susceptible (S_M), exposed (i.e., infected, but not infectious) (E_M), and infectious (I_M) individuals as well as uninfected (P_M) and infected (Q_M) eggs. The probability of vertical transmission of WNV to eggs is q_M. Adult mosquito population size is described as $N_M = S_M + E_M + I_M$. The competent bird population consists of individuals that are susceptible, exposed, infectious, and recovered, and either treated (S_{CT}, E_{CT}, I_{CT}, R_{CT}) or untreated (S_{CU}, E_{CU}, I_{CU}, R_{CU}). The total population size of treated, competent birds is described as $N_{CT} = S_{CT} + E_{CT} + I_{CT} + R_{CT}$ and the total population size of untreated, competent birds is described as $N_{UT} = S_{UT} + E_{UT} + I_{UT} + R_{UT}$. When infected, the incompetent birds do not achieve high enough viral titers in blood to re-infect mosquitoes so the susceptible, exposed, infected, and recovered categories are not applicable, but incompetent birds can be either treated or untreated such that the total population size is denoted as N_{IT} and N_{IU}, respectively. Infections in competent birds occur at rate β_{MC} and in mosquitoes at rate β_{CM}. Exposed individuals become infectious at rate ε_C (competent birds) and ε_M (mosquitoes). Infectious competent birds succumb to WNV at rate μ_C and recover at rate γ_C.

Untreated birds in group *X* visit an ivermectin-treated feeder with probability ρ_X and become treated. Ivermectin is removed from the blood of treated birds at rate λ_D. IVM-induced mortality in mosquito populations occurs at rate β_D. In addition to these continuous-time dynamics, we added a discrete, group-specific movement probability derived with integrodifference equations [2,3] to capture the dispersal between nocturnal roosts (birds) and host-seeking behavior (mosquitoes) each night, similar to [4]. We used a normal dispersal kernel, $\kappa_X(x, y)$, to describe the movement of birds between nocturnal roosts and mosquitoes across the neighborhood.

Considering all combinations of treated (d_i) and untreated (c_j) properties in the neighborhood, movement for group *X* (competent birds, incompetent birds, and mosquitoes) originating from *x* in treated lot *i* and ending at *y* in untreated lot *j* is defined as

$$\sum_{i,j} \int_{c_j} \int_{d_i} \kappa_X(x, y) dx dy.$$

Dividing this by all movements in the neighborhood (*D*) originating from treated lots, the movement probability for group X from treated to untreated patches is:

$$m_X = \frac{\sum_{i,j} \int_{c_j} \int_{d_i} \kappa_X(x,y) dx dy}{\int_D \int_{d_i} \kappa_X(x,y) dx dy}.$$

To represent a "nocturnal home range" (H_X) or the full area utilized for nocturnal roosting by competent and incompetent birds,

$$\kappa_X(x, y) = \begin{cases} \frac{1}{\sigma_X \sqrt{2\pi}} e^{-(x-y)^2 / 2\sigma_X^2}, & if\ y \geq x - \frac{H_X}{2}\ and\ y \leq x + \frac{H_X}{2} \\ 0, & otherwise \end{cases}.$$

For mosquitoes,

$$\kappa_X(x, y) = \frac{1}{\sigma_X \sqrt{2\pi}} e^{-(x-y)^2 / 2\sigma_X^2}.$$

Similarly, the movement probability for group X from untreated to treated patches is:

$$n_X = \frac{\sum_{i,j} \int_{d_i} \int_{c_j} \kappa_X(x,y) dx dy}{\int_D \int_{c_j} \kappa_X(x,y) dx dy}.$$

Following the semi-discrete model notation of Mailleret and Lemesle [5], and incorporating the probability that birds and mosquitoes remain in the same roost or resting location each evening (time τ), the full movement probability for each group from the treated to untreated patch is:

$$M_x = p_X m_X$$

and from the untreated to treated patch is:

$$N_x = p_X n_X,$$

where

$$p_X = \begin{cases} 1, & if\ r > P(stay)_X \\ 0, & otherwise \end{cases}.$$

For this, we compared a random draw from a uniform distribution on [0,1] (r) with the observed roost constancy for house sparrows (competent birds) and mourning doves (incompetent birds) (Table 3-2) and $1 - 1/(gonotrophic\ period)$ for mosquitoes because, on average only a proportion of the female mosquitoes would be host-seeking on a given night (i.e., $1/(gonotrophic\ period)$), with the rest in other stages of the gonotrophic cycle (e.g., laying eggs or digesting bloodmeal).

Thus, the resulting system of ordinary differential equations in each patch ($t \neq \tau$) is as follows:

For the vector mosquitoes,

$$\frac{dP_M}{dt} = \frac{b_M K_M}{N_M}(N_M - q_M I_M - P_M)$$

$$\frac{dQ_M}{dt} = \frac{b_M K_M}{N_M}(q_M I_M - Q_M)$$

$$\frac{dS_M}{dt} = \frac{b_M K_M}{N_M} P_M - \beta_{CM} S_M \frac{I_{CT} + I_{CU}}{N_{CT} + N_{CU}} - \frac{d_M N_M}{K_M} S_M - \beta_D S_M$$

$$\frac{dE_M}{dt} = \beta_{CM} S_M \frac{I_{CT} + I_{CU}}{N_{CT} + N_{CU}} - \frac{d_M N_M}{K_M} E_M - \varepsilon_M E_M - \beta_D E_M$$

$$\frac{dI_M}{dt} = \frac{b_M K_M}{N_M} Q_M + \varepsilon_M E_M - \frac{d_M N_M}{K_M} I_M - \beta_D I_M$$

$$\frac{dN_M}{dt} = \frac{b_M K_M}{N_M}(P_M + Q_M) - \frac{d_M N_M}{K_M} N_M - \beta_D N_M$$

For the treated competent bird species,

$$\frac{dS_{CT}}{dt} = b_C N_{CT} - \beta_{MC} S_{CT} \frac{I_M}{N_M} - \frac{d_C N_{CT}}{K_C} S_{CT} + \rho_C S_{CU} - (1-\rho_C)\lambda_D S_{CT}$$

$$\frac{dE_{CT}}{dt} = \beta_{MC} S_{CT} \frac{I_M}{N_M} - \frac{d_C N_{CT}}{K_C} E_{CT} - \varepsilon_C E_{CT} + \rho_C E_{CU} - (1-\rho_C)\lambda_D E_{CT}$$

$$\frac{dI_{CT}}{dt} = \varepsilon_C E_{CT} - (\gamma_C + \mu_C) I_{CT} - \frac{d_C N_{CT}}{K_C} I_{CT} + \rho_C I_{CU} - (1-\rho_C)\lambda_D I_{CT}$$

$$\frac{dR_{CT}}{dt} = \gamma_C I_{CT} - \frac{d_C N_{CT}}{K_C} R_{CT} + \rho_C R_{CU} - (1-\rho_C)\lambda_D R_{CT}$$

$$\frac{dN_{CT}}{dt} = b_C N_{CT} - \frac{d_C N_{CT}}{K_C} N_{CT} - \mu_C I_{CT} + \rho_C N_{CU} - (1-\rho_C)\lambda_D N_{CT}$$

For the untreated competent bird species,

$$\frac{dS_{CU}}{dt} = b_C N_{CU} - \beta_{MC} S_{CU} \frac{I_M}{N_M} - \frac{d_C N_{CU}}{K_C} S_{CU} - \rho_C S_{CU} + (1-\rho_C)\lambda_D S_{CT}$$

$$\frac{dE_{CU}}{dt} = \beta_{MC} S_{CU} \frac{I_M}{N_M} - \frac{d_C N_{CU}}{K_C} E_{CU} - \varepsilon_C E_{CU} - \rho_C E_{CU} + (1-\rho_C)\lambda_D E_{CT}$$

$$\frac{dI_{CU}}{dt} = \varepsilon_C E_{CU} - (\gamma_C + \mu_C) I_{CU} - \frac{d_C N_{CU}}{K_C} I_{CU} - \rho_C I_{CU} + (1-\rho_C)\lambda_D I_{CT}$$

$$\frac{dR_{CU}}{dt} = \gamma_C I_{CU} - \frac{d_C N_{CU}}{K_C} R_{CU} - \rho_C R_{CU} + (1-\rho_C)\lambda_D R_{CT}$$

$$\frac{dN_{CU}}{dt} = b_C N_{CU} - \frac{d_C N_{CU}}{K_C} N_{CU} - \mu_C I_{CU} - \rho_C N_{CU} + (1-\rho_C)\lambda_D N_{CT}$$

For the treated incompetent bird species,

$$\frac{dN_{IT}}{dt} = b_I N_{IT} - \frac{d_I N_{IT}}{K_I} N_{IT} + \rho_I N_{IU} - (1-\rho_I)\lambda_D N_{IT}$$

For the untreated incompetent bird species,

$$\frac{dN_{IU}}{dt} = b_I N_{IU} - \frac{d_I N_{IU}}{K_I} N_{IU} - \rho_I N_{IU} + (1-\rho_I)\lambda_D N_{IT}$$

At the moment immediately following τ, that is the end of the night (τ^+), the total number of mosquitoes (M) in each patch (*T* = treated, *U* = untreated) in compartment *Y* is:

$$Y_{M_T}(\tau^+) = N_M * Y_{M_U}(\tau) - M_M * Y_{M_T}(\tau)$$

$$Y_{M_U}(\tau^+) = M_M * Y_{M_T}(\tau) - N_M * Y_{M_U}(\tau),$$

and the total number of birds in each patch (*T* = treated, *U* = untreated) in group *X* in compartment *Y* with treatment status *k* is:

$$Y_{Xk_T}(\tau^+) = N_X * Y_{Xk_U}(\tau) - M_X * Y_{Xk_T}(\tau)$$

$$Y_{Xk_T}(\tau^+) = M_X * Y_{Xk_T}(\tau) - N_X * Y_{Xk_U}(\tau).$$

In order to capture realistic infection dynamics observed at the city level on the neighborhood level, we forced a rate of 0.75 exposed mosquitoes per 1,000 in week 25 (Jun 18-24) in each patch and introduced a total of 2.5 exposed competent birds to the whole neighborhood at the start of week 28 (Jul 9); the number of exposed birds introduced per patch was based on the proportion of the total competent birds (treated and untreated) present in each patch at that time. These forcing parameters and the gonotrophic period (GP) (i.e., time between bloodmeals) were chosen together to reduce mean squared error between predicted infection dynamics and observed infection dynamics in Fort Collins for 2007 2007 [6] using the optim function in R [7].

Table A3-1. Symbols, parameters, and sources for parameterization of West Nile virus (WNV) transmission in spatially implicit patch model of West Nile virus (WNV) transmission with ivermectin-treated birdfeeders.

Symbol	Meaning	Value	Units	Reference
b_M	Birth rate of mosquitoes	d_M	day^{-1}	
b_C	Birth rate of competent birds	d_C	year^{-1}	
b_I	Birth rate of incompetent birds	d_I	year^{-1}	
K_M	Carrying capacity of mosquitoes	$\frac{Culex\ females}{CO_2\ trap-night_{time+6}}\ x\ GP\ x\ (N_{CT}+N_{CU}+N_{IT}+N_{IU})/5$	mosquitoes	[8]
K_C	Carrying capacity of competent birds	500	birds	arbitrary
K_I	Carrying capacity of incompetent birds	500	birds	arbitrary
$1/d_M$	Lifespan of mosquitoes	14	days	[9-11]
$1/d_C$	Lifespan of competent birds	4	years	[12]
$1/d_I$	Lifespan of incompetent birds	1	years	[12]
q_M	Probability of vertical transmission	0.003	-	[13-15]
β_{CM}	Adequate contact: competent bird to mosquito	$\frac{f_C\ x\ r_{CM}}{GP}$	day^{-1}	
β_{MC}	Adequate contact: mosquito to competent bird	$\frac{f_C\ x\ r_{MC}}{GP}$	day^{-1}	

β_D	Death rate of mosquitoes from ivermectin	$\frac{f_T \, x \, a_T}{GP}$	day^{-1}	
f_T	Probability of feeding on an ivermectin-treated bird	$\frac{N_{CT} + N_{IT}}{N_{CT} + N_{CU} + N_{IT} + N_{IU}}$	-	
f_C	Probability of feeding on a competent bird	$\frac{N_{CT} + N_{CU}}{N_{CT} + N_{CU} + N_{IT} + N_{IU}}$	-	
a_T	Probability of death from biting a treated bird per bite	0.5	-	[16]
r_{CM}	Probability of successful WNV transmission from competent bird to mosquito per bite	0.8	-	[17,18]
r_{MC}	Probability of successful WNV transmission from mosquito to competent bird per bite	1	-	[19]
$1/\varepsilon_M$	Extrinsic incubation period of mosquito	See text	days	[20]
$1/\varepsilon_C$	Intrinsic incubation period of competent birds	1	days	[18,19]
$1/\gamma_C$	Infectious period of competent birds	5.5	days	[18,19]
μ_C	Disease-related mortality of competent birds	1/7	day^{-1}	[19]
ρ_C	Probability of competent bird feeding at an ivermectin-treated feeder per day	0.27 in treated patches 0 in untreated patches	-	Table 3-4
ρ_I	Probability of incompetent bird feeding at an ivermectin-treated feeder per day	0.04 in treated patches 0 in untreated patches	-	Table 3-4
$1/\lambda_D$	Washout period of ivermectin in birds (from plasma)	2	days	[16,21]
GP	Gonotrophic period (period between bloodmeals)	3	days	see details

Table A3-2. Symbols, parameters, and sources for parameterization of movement of birds and mosquitoes between ivermectin-treated and untreated patches in spatially implicit patch model of West Nile virus (WNV) transmission with ivermectin-treated birdfeeders.

Symbol	Meaning	Value	Units	Reference
$P(stay)_M$	Daily probability of mosquito staying in lot	1 – 1/GP	-	see details
$P(stay)_C$	Daily probability of competent bird remaining in same nocturnal roost	0.61	-	Table 3-2
$P(stay)_I$	Daily probability of incompetent bird remaining in same nocturnal roost	0.20	-	Table 3-2
$\overline{D}_M$	Mean daily dispersal distance of mosquitoes	110	m	[22-25]
$\overline{D}_C$	Mean daily dispersal distance (nocturnal roost locations) of competent birds	219.3	m	Table 3-2
$\overline{D}_I$	Mean daily dispersal distance (nocturnal roost locations) of incompetent birds	780.9	m	Table 3-2
$\sigma^2{}_M$	Variance in daily dispersal distance for mosquitoes (for normal kernel)	$\frac{\pi}{2} x\, \overline{D}^2{}_M$	m	[26]
$\sigma^2{}_C$	Variance in daily dispersal distance for competent birds (for normal kernel)	$\frac{\pi}{2} x\, \overline{D}^2{}_C$	m	[26]
$\sigma^2{}_I$	Variance in daily dispersal distance for incompetent birds (for normal kernel)	$\frac{\pi}{2} x\, \overline{D}^2{}_I$	m	[26]
H_C	Mean max distance between nocturnal roost locations of competent birds	779	m	fieldwork
H_I	Mean max distance between nocturnal roost locations of incompetent birds	3,132	m	fieldwork

Sensitivity Analysis

Table A3-3. Sensitivity analysis. Parameter ranges used in global sensitivity analysis and mean change in accuracy. Sensitivity analysis performed using random forest approach by regressing total infectious mosquito-days against listed parameters.

Symbol*	Range	total infectious mosquito-days		
		%IncMSE†	Contribution^	Relative Importance=
r_{CM}	[0.05, 1]	18,695.70	28.82	1
b_M, d_M	[1/21, 1/7]	11,931.40	18.39	2
a_T	[0.01, 1]	6,457.10	9.95	3
n_treated#	[1, 75]	5,577.80	8.60	4
γ_C	[1/7, 1/2]	4,248.20	6.55	5
GP	[3, 7]	2,272.60	3.50	6
r_{MC}	[0.8, 1]	2,004.70	3.09	7
μ_C	[1/8, 1/3]	1,617.80	2.49	8
ρ_C	[0.1, 1]	1,241.70	1.91	9
ε_C	[0.5, 1]	1,009.20	1.56	10
K_I	[100, 500]	908.80	1.40	11
λ_D	[1/5, 1/1]	865.6	1.33	12
K_C	[100, 500]	846.3	1.30	13
b_C, d_C	[(1/6)/365, (1/0.75)/365]	827.4	1.28	14
ρ_I	[0, 0.8]	755.4	1.16	15
b_I, d_I	[(1/3)/365, (1/0.75)/365]	710.7	1.10	16
$\bar{D}_C$	[11, 545]	632.5	0.97	17
$P(stay)_M$	[0.1, 0.75]	620.9	0.96	18
H_I	[1,580, 4,400]	566.1	0.87	19
q_M	[0.001, 0.008]	517.2	0.8	20
$P(stay)_I$	[0, 0.65]	500.1	0.77	21
$\bar{D}_M$	[20, 1,000]	497.1	0.77	22
H_C	[420, 1,178]	487.5	0.75	23
$\bar{D}_I$	[63, 3,335]	477.6	0.74	24
$P(stay)_C$	[0.45, 0.75]	495.1	0.71	25
Spacing+	Contiguous or random	150.4	0.23	26
K_M	Not evaluated‡	-	-	-

* See Tables A3-1 & A3-2 for symbol definitions.

† Percent increase in prediction error (mean squared error) of outcome when parameter permuted in out-of-bag sample vs. not permuted, averaged over all trees.

^ Percent of total increase in mean squared error due to permuting this parameter.

= Order of relative importance based on contribution to total increase in prediction error.

Number of backyards with treated feeder in neighborhood.

+ Spatial arrangement of treated lots in neighborhood (see text for details).

‡ Not evaluated in sensitivity analysis because form developed in modeling framework to produce realistic dynamics (see text for details).

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Appendix 5: Supplemental figures and tables (Chapter 3)

Table A3-4. Bird species detected during weekly point counts around the five feeder sites deployed in Fort Collins, Colorado (Aug – Sep 2020) for study on birdfeeder usage and nocturnal roosting habits of six common backyard species in neighborhoods. WNV competency and residence status of detected species indicated.

Date	Site Name	Site Number	Start Time	Species	Species status	#	Notes	WNV Competency
7-Aug	BC	1	622	American goldfinch	Resident	4		Competent
7-Aug	BC	1	622	American kestrel	Resident	2		Competent
7-Aug	BC	1	622	American robin	Resident	1		Competent
7-Aug	BC	1	622	black-capped chickadee	Resident	1		Competent
7-Aug	BC	1	622	blue jay	Resident	2		Competent
7-Aug	BC	1	622	Eurasian collared-dove	Resident	1		Incompetent
7-Aug	BC	1	622	European starling	Resident	3		Incompetent
7-Aug	BC	1	622	house finch	Resident	1		Competent
7-Aug	BC	1	622	house wren	Resident	1		Competent
7-Aug	BC	1	622	mourning dove	Resident	5		Incompetent
7-Aug	BC	1	622	red-tailed hawk	Resident	1		Competent
7-Aug	BC	1	622	red-winged blackbird	Resident	5		Competent
7-Aug	BC	1	622	unidentified egret	unknown	1 flyover		Competent
7-Aug	SV	2	737	black-capped chickadee	Resident	3		Competent
7-Aug	SV	2	737	blue jay	Resident	2		Competent
7-Aug	SV	2	737	Cooper’s hawk	Resident	3		Competent
7-Aug	SV	2	737	house finch	Resident	1		Competent
7-Aug	SV	2	737	mourning dove	Resident	1		Incompetent
7-Aug	SV	2	737	red-winged blackbird	Resident	2		Competent
7-Aug	SV	2	737	snowy egret	Transient	1 flyover		Competent
7-Aug	LD	3	751	American crow	Resident	1 flyover		Competent
7-Aug	LD	3	751	American goldfinch	Resident	1		Competent
7-Aug	LD	3	751	American robin	Resident	2		Competent
7-Aug	LD	3	751	barn swallow	Resident	1		Competent
7-Aug	LD	3	751	blue jay	Resident	5		Competent

Date	Site Name	Site Number	Start Time	Species	Species status	#	Notes	WNV Competency
7-Aug	LD	3	751	common grackle	Resident	1		Competent
7-Aug	LD	3	751	downy woodpecker	Resident	1		Incompetent
7-Aug	LD	3	751	house finch	Resident	5		Competent
7-Aug	LD	3	751	house sparrow	Resident	3		Competent
7-Aug	LD	3	751	mourning dove	Resident	2		Incompetent
7-Aug	LD	3	751	red-winged blackbird	Resident	3		Competent
7-Aug	CC	4	706	black-capped chickadee	Resident	1		Competent
7-Aug	CC	4	706	blue jay	Resident	2		Competent
7-Aug	CC	4	706	Cooper’s hawk	Resident	1		Competent
7-Aug	CC	4	706	house finch	Resident	3		Competent
7-Aug	CC	4	706	red-breasted nuthatch	Transient	1		Competent
7-Aug	CC	4	706	red-winged blackbird	Resident	1		Competent
7-Aug	RBP	5	645	American goldfinch	Resident	2		Competent
7-Aug	RBP	5	645	barn swallow	Resident	1		Competent
7-Aug	RBP	5	645	belted kingfisher	Resident	1		Incompetent
7-Aug	RBP	5	645	black-capped chickadee	Resident	2		Competent
7-Aug	RBP	5	645	common grackle	Resident	2		Competent
7-Aug	RBP	5	645	killdeer	Resident	1		Competent
7-Aug	RBP	5	645	mallard	Resident	2		Incompetent
7-Aug	RBP	5	645	mourning dove	Resident	1		Incompetent
7-Aug	RBP	5	645	osprey	Resident	1		Competent
7-Aug	RBP	5	645	red-winged blackbird	Resident	1		Competent
14-Aug	BC	1	742	American goldfinch	Resident	2		Competent
14-Aug	BC	1	742	American robin	Resident	4		Competent
14-Aug	BC	1	742	American white pelican	Resident	1		Incompetent
14-Aug	BC	1	742	black-capped chickadee	Resident	1		Competent
14-Aug	BC	1	742	blue jay	Resident	3		Competent
14-Aug	BC	1	742	Canada goose	Resident	7 flyover		Incompetent
14-Aug	BC	1	742	common grackle	Resident	1		Competent

Date	Site Name	Site Number	Start Time	Species	Species status	#	Notes	WNV Competency
14-Aug	BC	1	742	downy woodpecker	Resident	2		Incompetent
14-Aug	BC	1	742	Eurasian collared-dove	Resident	2		Incompetent
14-Aug	BC	1	742	European starling	Resident	24		Incompetent
14-Aug	BC	1	742	house finch	Resident	9		Competent
14-Aug	BC	1	742	house sparrow	Resident	2		Competent
14-Aug	BC	1	742	northern flicker	Resident	2		Incompetent
14-Aug	SV	2	638	American robin	Resident	2		Competent
14-Aug	SV	2	638	black-capped chickadee	Resident	7		Competent
14-Aug	SV	2	638	blue jay	Resident	7		Competent
14-Aug	SV	2	638	house finch	Resident	9		Competent
14-Aug	SV	2	638	mallard	Resident	5		Incompetent
14-Aug	SV	2	638	northern flicker	Resident	1		Incompetent
14-Aug	SV	2	638	red-winged blackbird	Resident	6		Competent
14-Aug	LD	3	623	American goldfinch	Resident	2		Competent
14-Aug	LD	3	623	American robin	Resident	1		Competent
14-Aug	LD	3	623	barn swallow	Resident	2		Competent
14-Aug	LD	3	623	blue jay	Resident	6		Competent
14-Aug	LD	3	623	common grackle	Resident	2	plus 7 flyover	Competent
14-Aug	LD	3	623	downy woodpecker	Resident	1		Incompetent
14-Aug	LD	3	623	house finch	Resident	5		Competent
14-Aug	LD	3	623	house sparrow	Resident	1		Competent
14-Aug	LD	3	623	red-breasted nuthatch	Transient	1		Competent
14-Aug	LD	3	623	red-winged blackbird	Resident	1		Competent
14-Aug	CC	4	721	black-capped chickadee	Resident	4		Competent
14-Aug	CC	4	721	blue jay	Resident	1		Competent
14-Aug	CC	4	721	house finch	Resident	1		Competent
14-Aug	CC	4	721	house wren	Resident	1		Competent
14-Aug	CC	4	721	killdeer	Resident	1		Competent
14-Aug	CC	4	721	mallard	Resident	3	labelled RBP in eBird	Incompetent

Date	Site Name	Site Number	Start Time	Species	Species status	#	Notes	WNV Competency
14-Aug	RBP	5	701	American goldfinch	Resident	1		Competent
14-Aug	RBP	5	701	American robin	Resident	1		Competent
14-Aug	RBP	5	701	barn swallow	Resident	4		Competent
14-Aug	RBP	5	701	black-capped chickadee	Resident	3		Competent
14-Aug	RBP	5	701	cedar waxwing	Resident	11		Competent
14-Aug	RBP	5	701	eastern kingbird	Resident	2		Competent
14-Aug	RBP	5	701	great egret	Resident	1 flyover		Competent
14-Aug	RBP	5	701	house finch	Resident	1		Competent
14-Aug	RBP	5	701	mallard	Resident	2	labelled CC in eBird	Incompetent
14-Aug	RBP	5	701	osprey	Resident	2		Competent
14-Aug	RBP	5	701	red-winged blackbird	Resident	10		Competent
14-Aug	RBP	5	701	snowy egret	Transient	1 flyover		Competent
14-Aug	RBP	5	701	yellow warbler	Transient	1		Competent
21-Aug	BC	1	706	American goldfinch	Resident	2		Competent
21-Aug	BC	1	706	American kestrel	Resident	1		Competent
21-Aug	BC	1	706	barn swallow	Resident	1		Competent
21-Aug	BC	1	706	black-capped chickadee	Resident	3		Competent
21-Aug	BC	1	706	blue jay	Resident	3		Competent
21-Aug	BC	1	706	cedar waxwing	Resident	2		Competent
21-Aug	BC	1	706	common grackle	Resident	3		Competent
21-Aug	BC	1	706	Eurasian collared-dove	Resident	1		Incompetent
21-Aug	BC	1	706	European starling	Resident	50		Incompetent
21-Aug	BC	1	706	house finch	Resident	2		Competent
21-Aug	BC	1	706	house sparrow	Resident	1		Competent
21-Aug	BC	1	706	northern flicker	Resident	2		Incompetent
21-Aug	BC	1	706	western kingbird	Transient	1		Competent
21-Aug	BC	1	706	western wood-pewee	Transient	1		Competent
21-Aug	BC	1	706	white-breasted nuthatch	Resident	1		Competent
21-Aug	SV	2	721	American robin	Resident	3		Competent

Date	Site Name	Site Number	Start Time	Species	Species status	#	Notes	WNV Competency
21-Aug	SV	2	721	black-capped chickadee	Resident	1		Competent
21-Aug	SV	2	721	blue jay	Resident	3		Competent
21-Aug	SV	2	721	common grackle	Resident	1		Competent
21-Aug	SV	2	721	Eurasian collared-dove	Resident	2		Incompetent
21-Aug	SV	2	721	house finch	Resident	5		Competent
21-Aug	SV	2	721	house sparrow	Resident	3		Competent
21-Aug	SV	2	721	mallard	Resident	1		Incompetent
21-Aug	SV	2	721	mourning dove	Resident	1		Incompetent
21-Aug	SV	2	721	red-winged blackbird	Resident	3		Competent
21-Aug	LD	3	737	American goldfinch	Resident	1		Competent
21-Aug	LD	3	737	black-capped chickadee	Resident	2		Competent
21-Aug	LD	3	737	blue jay	Resident	6		Competent
21-Aug	LD	3	737	Eurasian collared-dove	Resident	1		Incompetent
21-Aug	LD	3	737	great-tailed grackle	Resident	3 flyover		Competent
21-Aug	LD	3	737	house finch	Resident	6		Competent
21-Aug	LD	3	737	house sparrow	Resident	1		Competent
21-Aug	LD	3	737	northern flicker	Resident	1		Incompetent
21-Aug	CC	4	623	blue jay	Resident	4		Competent
21-Aug	CC	4	623	Canada goose	Resident	3	labelled RBP in eBird	Incompetent
21-Aug	CC	4	623	downy woodpecker	Resident	1		Incompetent
21-Aug	RBP	5	646	American goldfinch	Resident	1		Competent
21-Aug	RBP	5	646	barn swallow	Resident	4		Competent
21-Aug	RBP	5	646	belted kingfisher	Resident	1		Incompetent
21-Aug	RBP	5	646	blue jay	Resident	1		Competent
21-Aug	RBP	5	646	eastern kingbird	Resident	1		Competent
21-Aug	RBP	5	646	European starling	Resident	4		Incompetent
21-Aug	RBP	5	646	house finch	Resident	2		Competent
21-Aug	RBP	5	646	mallard	Resident	1	labelled CC in eBird	Incompetent
21-Aug	RBP	5	646	rock pigeon	Resident	1		Incompetent

Date	Site Name	Site Number	Start Time	Species	Species status	#	Notes	WNV Competency
21-Aug	RBP	5	646	unidentified duck	unknown	1 flyover		Incompetent
28-Aug	BC	1	629	American goldfinch	Resident	2		Competent
28-Aug	BC	1	629	black-capped chickadee	Resident	2		Competent
28-Aug	BC	1	629	blue jay	Resident	4		Competent
28-Aug	BC	1	629	cedar waxwing	Resident	1		Competent
28-Aug	BC	1	629	common grackle	Resident	10		Competent
28-Aug	BC	1	629	downy woodpecker	Resident	1		Incompetent
28-Aug	BC	1	629	Eurasian collared-dove	Resident	3		Incompetent
28-Aug	BC	1	629	European starling	Resident	19		Incompetent
28-Aug	BC	1	629	house finch	Resident	4		Competent
28-Aug	BC	1	629	house sparrow	Resident	3		Competent
28-Aug	BC	1	629	mourning dove	Resident	9		Incompetent
28-Aug	BC	1	629	northern flicker	Resident	1		Incompetent
28-Aug	BC	1	629	unidentified passerine	unknown	1 flyover		Competent
28-Aug	SV	2	642	American crow	Resident	3		Competent
28-Aug	SV	2	642	American robin	Resident	13		Competent
28-Aug	SV	2	642	barn swallow	Resident	2		Competent
28-Aug	SV	2	642	black-capped chickadee	Resident	3		Competent
28-Aug	SV	2	642	blue jay	Resident	3		Competent
28-Aug	SV	2	642	European starling	Resident	2		Incompetent
28-Aug	SV	2	642	house finch	Resident	2		Competent
28-Aug	LD	3	655	American goldfinch	Resident	2		Competent
28-Aug	LD	3	655	blue jay	Resident	6		Competent
28-Aug	LD	3	655	common grackle	Resident	1		Competent
28-Aug	LD	3	655	green-winged teal	Resident	1 flyover		Incompetent
28-Aug	LD	3	655	house finch	Resident	5		Competent
28-Aug	LD	3	655	house sparrow	Resident	1		Competent
28-Aug	LD	3	655	unidentified passerine	unknown	1		Competent
28-Aug	LD	3	655	white-breasted nuthatch	Resident	2		Competent

Date	Site Name	Site Number	Start Time	Species	Species status	#	Notes	WNV Competency
28-Aug	CC	4	748	black-capped chickadee	Resident	3		Competent
28-Aug	CC	4	748	house finch	Resident	1		Competent
28-Aug	CC	4	748	unidentified egret	unknown	1 flyover	labelled RBP in eBird	Competent
28-Aug	CC	4	748	Wilson's warbler	Transient	2		Competent
28-Aug	RBP	5	728	American goldfinch	Resident	1		Competent
28-Aug	RBP	5	728	barn swallow	Resident	8		Competent
28-Aug	RBP	5	728	belted kingfisher	Resident	1		Incompetent
28-Aug	RBP	5	728	Canada goose	Resident	4	labelled CC in eBird	Incompetent
28-Aug	RBP	5	728	chipping sparrow	Transient	15		Competent
28-Aug	RBP	5	728	house finch	Resident	5		Competent
28-Aug	RBP	5	728	lark sparrow	Transient	1		Competent
28-Aug	RBP	5	728	osprey	Resident	2		Competent
4-Sep	BC	1	630	American goldfinch	Resident	1		Competent
4-Sep	BC	1	630	American robin	Resident	7		Competent
4-Sep	BC	1	630	barn swallow	Resident	2		Competent
4-Sep	BC	1	630	black-capped chickadee	Resident	3		Competent
4-Sep	BC	1	630	blue jay	Resident	3		Competent
4-Sep	BC	1	630	European starling	Resident	21		Incompetent
4-Sep	BC	1	630	green-tailed towhee	Transient	1		Competent
4-Sep	BC	1	630	house finch	Resident	1		Competent
4-Sep	BC	1	630	mallard	Resident	1 flyover		Incompetent
4-Sep	BC	1	630	mourning dove	Resident	6		Incompetent
4-Sep	BC	1	630	northern flicker	Resident	1		Incompetent
4-Sep	BC	1	630	unidentified bunting	Transient	1		Competent
4-Sep	BC	1	630	unidentified hummingbird	unknown	2		Competent
4-Sep	BC	1	630	Wilson's warbler	Transient	3		Competent
4-Sep	SV	2	758	American goldfinch	Resident	1		Competent
4-Sep	SV	2	758	American robin	Resident	1		Competent
4-Sep	SV	2	758	barn swallow	Resident	2		Competent

Date	Site Name	Site Number	Start Time	Species	Species status	#	Notes	WNV Competency
4-Sep	SV	2	758	black-capped chickadee	Resident	2		Competent
4-Sep	SV	2	758	blue jay	Resident	8		Competent
4-Sep	SV	2	758	Eurasian collared-dove	Resident	3		Incompetent
4-Sep	SV	2	758	house finch	Resident	1		Competent
4-Sep	SV	2	758	northern flicker	Resident	3		Incompetent
4-Sep	SV	2	758	unidentified passerine	unknown	1		Competent
4-Sep	LD	3	743	blue jay	Resident	5		Competent
4-Sep	LD	3	743	common grackle	Resident	3		Competent
4-Sep	LD	3	743	house finch	Resident	2		Competent
4-Sep	LD	3	743	mallard	Resident	1		Incompetent
4-Sep	LD	3	743	northern flicker	Resident	1		Incompetent
4-Sep	LD	3	743	white-breasted nuthatch	Resident	1		Competent
4-Sep	CC	4	714	American goldfinch	Resident	1		Competent
4-Sep	CC	4	714	belted kingfisher	Resident	1	labelled RBP in eBird	Incompetent
4-Sep	CC	4	714	black-capped chickadee	Resident	2		Competent
4-Sep	CC	4	714	blue jay	Resident	1		Competent
4-Sep	CC	4	714	downy woodpecker	Resident	2		Incompetent
4-Sep	CC	4	714	Wilson's warbler	Transient	3		Competent
4-Sep	CC	4	714	yellow warbler	Transient	1		Competent
4-Sep	RBP	5	653	American goldfinch	Resident	1		Competent
4-Sep	RBP	5	653	barn swallow	Resident	2		Competent
4-Sep	RBP	5	653	blue jay	Resident	1		Competent
4-Sep	RBP	5	653	Canada goose	Resident	8	labelled CC in eBird	Incompetent
4-Sep	RBP	5	653	European starling	Resident	1 flyover		Incompetent
4-Sep	RBP	5	653	killdeer	Resident	4		Competent
4-Sep	RBP	5	653	mallard	Resident	4		Incompetent
4-Sep	RBP	5	653	osprey	Resident	3		Competent

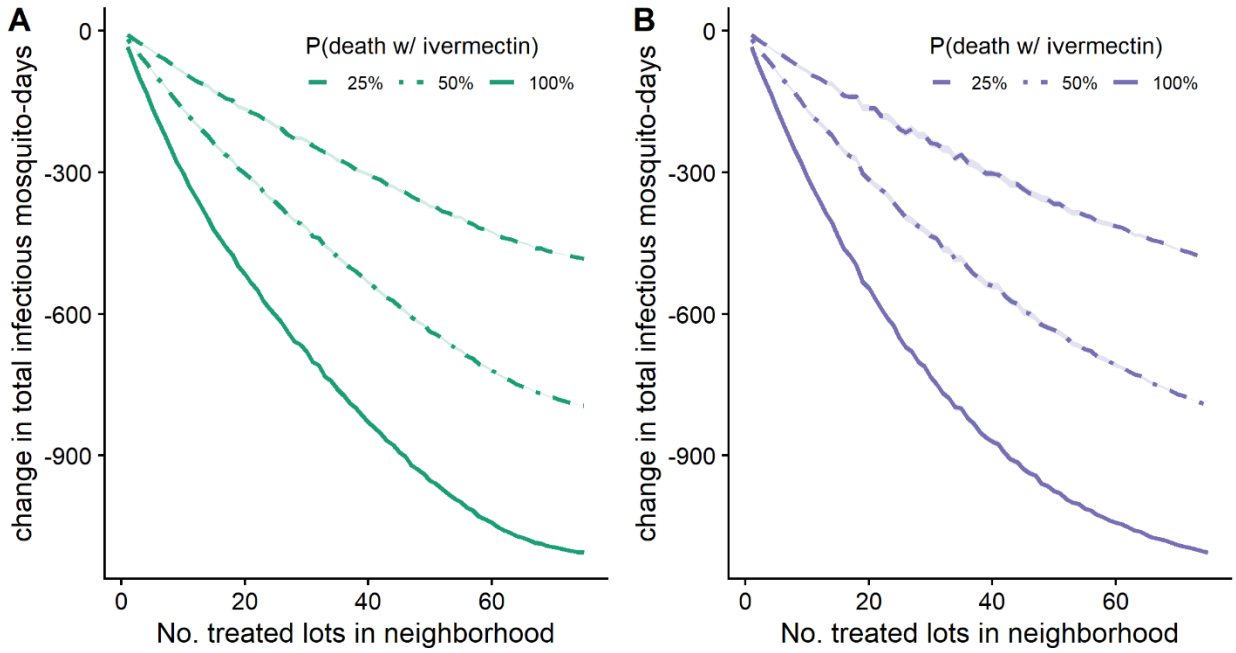

Figure A3-1. *Change in number of infectious mosquito-days for A) contiguous and B) random placement of treated plots in neighborhood with varying daily probability of mosquito death following a bloodmeal on an ivermectin-treated bird. 95% CI indicated by shaded region.*

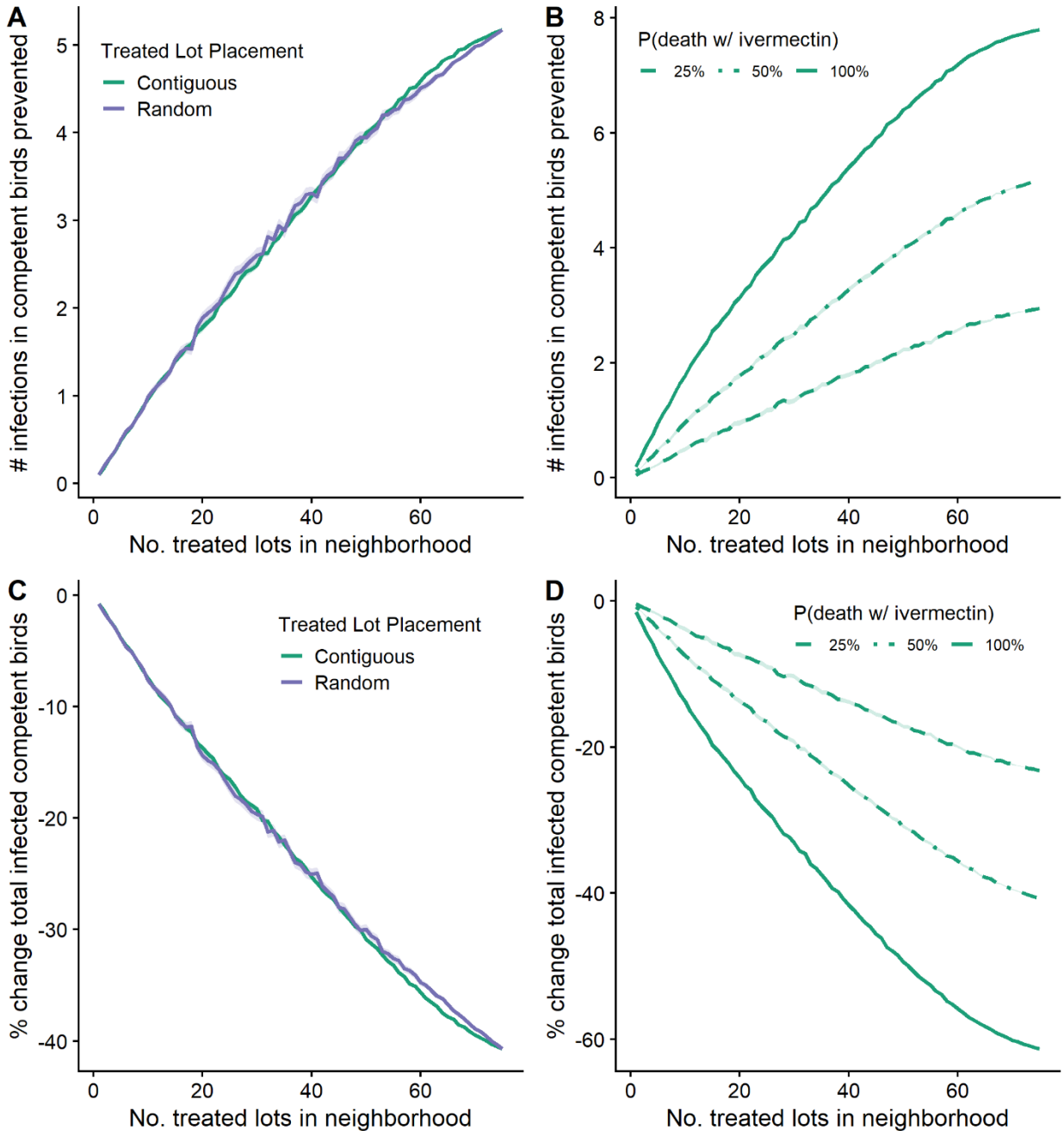

Figure A3-2. *Impact of IVM deployment on WNV infections in competent birds. Number of infections in competent birds avoided (A, B) and corresponding percent change in number of infections (C, D). 95% CI indicated by shaded region. Comparison of number and percent change in infections for contiguous vs. random placement of treated lots with 50% probability of death with IVM (A, C). Comparison of number and percent change in infections for contiguous placement across a range of values for IVM-induced mosquito mortality (B, D).*