Title

Spatial and Temporal Variation in Vector-borne Disease Risk; Influence of Land Cover, Irrigation, and Multiple Vector Species on West Nile Virus Transmission

Permalink

https://escholarship.org/uc/item/04v8d7zs

Author

Kovach, Tony

Publication Date

2018

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA
SANTA CRUZ

SPATIAL AND TEMPORAL VARIATION IN VECTOR-BORNE DISEASE RISK; INFLUENCE OF LAND COVER, IRRIGATION, AND MULTIPLE VECTOR SPECIES ON WEST NILE VIRUS TRANSMISSION

A dissertation submitted in partial satisfaction
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

ECOLOGY AND EVOLUTIONARY BIOLOGY

by

Tony J. Kovach

December 2018

The Dissertation of Tony J. Kovach
is approved:

Professor A. Marm Kilpatrick, Chair

Professor Pete Raimondi

Professor Eric Palkovacs

Professor William Walton

Lori Kletzer
Vice Provost and Dean of Graduate Studies

TABLE OF CONTENTS

SPATIAL AND TEMPORAL VARIATION IN VECTOR-BORNE DISEASE RISK; INFLUENCE OF LAND COVER, IRRIGATION, AND MULTIPLE VECTOR SPECIES ON WEST NILE VIRUS TRANSMISSION

Tony Kovach

OVERALL ABSTRACT

Vector-borne pathogens, such as Malaria, Dengue, West Nile and Zika virus, infect hundreds of millions of people each year and lead to widespread human morbidity and mortality, with enormous spatial and temporal variation in disease risk. The recent emergence of West Nile virus (WNV) into the developed world offers a unique opportunity to better understand ecological drivers that contribute to variation in disease risk, as a step toward more effective disease management through targeted interventions. In Chapter 1, we examined correlations between rice cultivation and WNV human disease incidence in rice-growing regions within the United States (US). We found WNV human disease incidence increased with the fraction of each county under rice cultivation in California, but not in the southern US. We show that this is likely due to regional differences in the mosquitoes transmitting WNV. These results illustrate how cultivation of particularly water-demanding agricultural crops can increase mosquito-borne disease risk and how spatial variation in vector ecology can alter the relationship between land cover and disease. In Chapter 2, we examined the effect of irrigation, climate and land cover on mosquito abundance and WNV human disease cases across California. Irrigation made up nearly a third of total water inputs to the region, with irrigation exceeding precipitation in some dry regions. Irrigation reduced seasonal variability in mosquito abundance by more than 40%, and increased abundance by more than an order of magnitude. In addition, irrigation increased human WNV cases and explained 33% of variation in WNV cases among California counties. These results suggest that irrigation can increase and decouple mosquito populations from natural precipitation variability, resulting in sustained and increased disease risk. In Chapter 3, we quantified the risk of WNV transmission to humans from 6 *Culex* mosquito species by integrating mosquito abundance, infection prevalence, vector competence, and blood feeding patterns, and examined correlations between risk indices and human disease cases. Human WNV cases were strongly correlated with the density of infectious vectors feeding on humans. However, different mosquito vector species contributed to transmission in different land use types and within seasons and across years. *Culex tarsalis* was more abundant in agricultural areas, whereas *Culex pipiens* and *Culex quinquefasciatus* were abundant in developed and agricultural areas, and *Culex erythrothorax* was abundant in wetland areas. As a result, WNV risk did not change substantially along either agricultural or urbanization land use gradients because the diversity of vectors maintained high disease risk across a range of habitats. These results show how a diversity of vectors can maintain an ecosystem disservice - vector borne disease – by their differential response to environmental disturbance.

ACKNOWLEDGEMENTS

I wanted to thank the many people that contributed to make this project happen. My adviser, A. Marm Kilpatrick for greatly contributing to make a much better overall project though insightful feedback and suggestions for data analysis. Thank you to my thesis committee members: Pete Raimondi, Eric Palkovacs, and William Walton for feedback on writing and suggestions for improvements in research design. Many mahalo's to members of the Kilpatrick lab including: Jordan Ruybal, Katherine McClure, and Tina Cheng for allowing me to clarify unfiltered, impractical thoughts and ideas into coherent scientific research. A special thanks to Stevie Scout Kovach for last minute inspiration to complete thesis writing.

I had the help of many field assistants including: Carrie De Jesus, Canyon Miller, Michael Bandy, Charles Farley Connelly, Eric Tymstra, Tim Bower, and Michelle Evans, who endured the hot dry summertime weather of the central valley to help me capture and identify >1.5 million mosquitoes and quantify the bird host community in the early morning hours. Further help with mosquito identification and testing of mosquitoes for West Nile virus was provided by University of California Santa Cruz undergraduate volunteers including: Ajuni Sohota, Alan Droege, Steven Flammer, Michael Laranjo, Christian Ring, and Amy Jeung. Special thanks to Rayna Ben-Zeev, an REU undergraduate that provided field assistance while completing a tangential mosquito dispersal project. Additional laboratory assistance was provided by high school students in the Science internship program at UCSC: Brian Zhong, Julia Wang, Ishani Shah. Thank you for putting up with my countless hours of ramblings about the importance of mosquito-host interactions while we dissected mosquitoes and performed DNA extractions.

I would like to thank our collaborators at UC Berkeley: Steve Beissinger, Lynn Huntsinger, Norm Miller, Nathan Van Schmidt with whom we continue to work with closely to examine links between human water use, conservation of threatened birds, and WNV risk. Many thanks to Nathan Van Schmidt and Laurie Hall for getting me acquainted with the study area and sharing resources to ensure a more successful project. Many thanks to the Sierra Foothill Research and Extension Center and all its employees for providing logistical support vital to the success of this project. Special thanks to all the private landowners that allowed us to conduct research on their property.

Mosquito trapping and WNV mosquito pools used in parts of this thesis were obtained from the California Vector-borne Disease Surveillance (CalSurv) System, and we acknowledge its contributors, including the member agencies of the Mosquito and Vector Control Association of California, California Department of Public Health, and the Davis Arbovirus Research and Training (DART) laboratory at the University of California, Davis.

Lastly, many thanks to the following funding sources for help with financing the many aspects of this project: NSF grants DEB-1115069 and EF-0914866, NIH grant 1R01AI090159 along with a post-graduate doctoral scholarship from NSERC.

OVERALL INTRODUCTION

A ubiquitous feature of vector-borne pathogen transmission is enormous spatial and temporal variation in transmission intensity at multiple scales (Gething et al. 2011; Kilpatrick 2011; Bhatt et al. 2013; Bejon et al. 2014). Multiple factors contribute to this heterogeneity including human land cover change affecting the distribution and abundance of key vector species (Bradley & Altizer 2007; Gottdenker et al. 2014; Hassell et al. 2017). Anthropogenic land use changes commonly associated with increased mosquito-borne disease include deforestation, urbanization and irrigated agricultural development (Gottdenker et al. 2014). Irrigation for agriculture has drastically altered the terrestrial water cycle (Vitousek et al. 1997; Vorosmarty et al. 2000; Pimentel et al. 2004; Oki 2006; Rost et al. 2008; Ellis 2011; Sterling et al. 2012) and climate change is predicted to further increase conversion of rain-fed agriculture to irrigation-based systems (Negri et al. 2005; Troy et al. 2015). Agricultural cropland currently covers ~12% of the earth's surface and flooded rice fields make up ~11% of these areas (Gong et al. 2013). Rice fields and other heavily irrigated crops can provide extensive larval habitat for particular mosquito species, increasing local mosquito populations and disease risk in surrounding regions (Diuk-Wasser et al. 2007; Richards et al. 2010). Agricultural and other land cover changes resulting in variation in abundance of vector species with differing traits can affect the magnitude and seasonality of vector borne disease risk (Lord 2011; Johnson et al. 2015; Park et al. 2016).

The recent emergence of West Nile virus into the developed world offers a unique opportunity to better examine ecological drivers that contribute to spatial and temporal changes in vector borne disease risk. West Nile virus (WNV) is a globally distributed multi-host mosquito-borne pathogen introduced into North America in 1999 that quickly spread westward and became established in California by 2004 (Kilpatrick 2011). WNV causes yearly epidemics in the United States (US) with an average of ~1200 neuro-invasive cases and 120 deaths per year, but with enormous year-to-year and spatial variation (CDC 2018). Mosquitoes in the genera *Culex* are the most important vectors for WNV bird-to-bird and bird-to-human transmission (Kilpatrick et al. 2005; Hamer et al. 2008a; Kramer et al. 2008) , but species within this genus differ substantially in many traits and are differentially affected by land use and climate (Farajollahi et al. 2011; Ciota et al. 2014). *Culex tarsalis* breeds in fresh water habitats and is frequently associated with agricultural land use (Surtees 1970; Reisen et al. 1992a; Wegbreit & Reisen 2000; Crowder et al. 2013; Schurich et al. 2014b; Kovach & Kilpatrick 2018), whereas *Culex pipiens* and *Culex quinquefasciatus* breeds in organically rich container habitats and is associated with urban habitats (Diuk-wasser et al. 2006; Trawinski & Mackay 2010; Andreadis 2012; Landau & Leeuwen 2012; Rochlin et al. 2016; McClure et al. 2018).

In Chapter 1, we examined how the relationship between land cover and disease risk can be altered by presence of certain vector species that can take advantage of expanded larval habitat. Many studies have examined correlations between land cover and WNV disease risk; with higher human disease cases commonly associated with increasing urbanization in central and eastern US (Ruiz et al. 2007; Bowden et al. 2011; Degroote & Sugumaran 2012; Degroote et al. 2014) and grassland/agricultural land covers in the Western US (Eisen et al. 2010; Bowden et al. 2011; Chuang et al. 2012; Degroote & Sugumaran 2012; Degroote et al. 2014). However, none of the broad-scale studies correlating land cover with WNV human disease incidence include quantitative mosquito data to support the different regional correlations and conclusions. In Chapter 1, we focused on the influence of one type of intensively irrigated agricultural land cover, rice fields, that provides larval habitat for some, but not all mosquito species. We tested the following hypotheses: rice cultivation would increase the abundance of *C. tarsalis* mosquitoes, human WNV disease incidence would increase with *C. tarsalis* abundance, and that, as a result, human WNV disease incidence would increase with rice cultivation in regions where *C. tarsalis* was a key vector of WNV, but not in areas where it is not.

In Chapter 2, we examined how irrigation amounts in different regions can affect seasonal variability and overall mosquito populations as well as human disease risk. Agricultural irrigation increases and stabilizes agricultural yields by buffering crops against natural variability associated with fluctuations in precipitation, and in doing so alters the timing and spatial availability of water across the landscape (Lo & Famiglietti 2013; Müller et al. 2018). Irrigated agriculture has previously been associated with increased mosquito abundance (Surtees 1970; Reisen et al. 1992a;

Wegbreit & Reisen 2000; Schurich et al. 2014a) and higher rates of human disease including malaria (Ijumba & Lindsay 2001; Jaleta et al. 2013), Japanese encephalitis (Keiser et al. 2005), lymphatic filariasis (Erlanger et al. 2005), Rift Valley fever (Bett et al. 2017), and West Nile virus encephalitis (Gates & Boston 2009; Eisen et al. 2010; Kovach & Kilpatrick 2018). However, the effects of rainfall on mosquito populations are often mixed, and far less is known about the effects of irrigation on variability in disease risk, despite the importance of irrigation in many arid regions (Baeza et al. 2011). In Chapter 2, we examine the effect of irrigation on the magnitude and variability in mosquito populations and WNV human disease incidence across California. First, we quantified water amounts coming from irrigation and precipitation across space and time. We then examined irrigation, climate, and land cover data as predictors of mosquito abundance, seasonal variability in mosquito populations, and WNV human disease incidence. We hypothesized that irrigation would increase mosquito abundance and WNV incidence and reduce seasonal variability in mosquito populations by stabilizing water availability for larval mosquito development.

In Chapter 3, we take a closer look at how multiple vector species contribute to spatial and temporal changes in disease risk. Species diversity plays a key role in maintaining ecosystem processes across space, time, and environmental gradients (Ives et al. 1999; Cardinale et al. 2006; Hooper et al. 2012). However, this pattern is usually studied for processes that are beneficial to humans, such as ecosystem services (Hooper et al. 2005; Cardinale et al. 2012). Few studies have examined the effects of diversity in maintaining ecosystem processes that are detrimental to humans, termed ecosystem disservices (Daszak & Kilpatrick 2009), such as disease transmission. Variation in the abundance of vector species with differing traits can result in variation in their contributions to the spread of pathogens among hosts (Lord 2011; Johnson et al. 2015; Park et al. 2016). Although large-scale differences in the importance of different vector species is well known (Sinka et al. 2011; Bowden et al. 2011; Kraemer et al. 2015), fine-scale spatial and temporal variation is less appreciated. A better understanding of how vector diversity contributes to spatial and temporal variation in human disease risk across environmental gradients offers opportunities for more effective disease management through targeted interventions (Bousema et al. 2010). In Chapter 3, we specifically quantify the role of different *Culex* mosquito species in the transmission of West Nile virus across California and examine whether the diversity of vectors in a relatively small region results in sustained transmission risk. We examine how the contribution of different mosquito species to human disease risk changes spatially (across agricultural and urbanization gradients) and temporally (within seasons and across years). We first examined correlations between a WNV risk index (the density of infectious vectors feeding on humans) and the number of reported WNV human cases to establish this index as a valid surrogate for WNV disease risk. We then examined variation in WNV risk across space and time, and the contribution of each vector species to risk. We hypothesized that the contributions to WNV transmission from different species would change with surrounding land use; with increased importance of *C. tarsalis* in agricultural areas as well as increased importance of *C. quinquefasciatus* and *C. pipiens* in urban areas. We also hypothesized that key vectors would alternate importance between years and within seasons due to differences in their response to climate (Reisen et al. 2010; Walton & Reisen 2013; Ciota et al. 2014; Paull et al. 2017).

CHAPTER 1

INCREASED HUMAN INCIDENCE OF WEST NILE VIRUS DISEASE NEAR RICE FIELDS IN CALIFORNIA BUT NOT IN SOUTHERN USA

ABSTRACT

Anthropogenic land use change, including agriculture, can alter mosquito larval habitat quality, increase mosquito abundance, and increase incidence of vector-borne disease. Rice is a staple food crop for over half of the world's population, with ~1% of global production occurring within the United States (US). Flooded rice fields provide enormous areas of larval habitat for mosquito species and may be hotspots for mosquito-borne pathogens, including West Nile virus (WNV). WNV was introduced into the Americas in 1999 and causes yearly epidemics in the US with an average of approximately 1400 neuro-invasive cases and 130 deaths per year. We examined correlations between rice cultivation and WNV disease incidence in rice-growing regions within the US. Incidence of WNV disease increased with the fraction of each county under rice cultivation in California, but not in the southern US. We show that this is likely due to regional variation in the mosquitoes transmitting WNV. *Culex tarsalis* was an important vector of WNV in California, and its abundance increased with rice cultivation, whereas in rice-growing areas of the southern USA the dominant WNV vector was *Culex quinquefasciatus* which rarely breeds in rice fields. These results illustrate how cultivation of particular crops can

increase disease risk and how spatial variation in vector ecology can alter the relationship between land cover and disease.

INTRODUCTION

Human land cover change can alter the spatial and temporal risk of vector-borne disease (Bradley & Altizer 2007; Gottdenker et al. 2014; Hassell et al. 2017). Anthropogenic land use changes commonly associated with increased mosquito-borne disease include deforestation, urbanization and agricultural development (Gottdenker et al. 2014). Agricultural cropland covers ~12% of the earth's surface and flooded rice fields make up ~11% of these areas (Gong et al. 2013). Rice fields can provide extensive larval habitat for particular mosquito species, increasing local mosquito populations and disease risk in surrounding regions (Diuk-Wasser et al. 2007; Richards et al. 2010).

West Nile virus (WNV) is a widespread mosquito-borne pathogen that was introduced to the Americas in 1999 and causes yearly epidemics in the US with an average of ~1400 neuro-invasive cases and 130 deaths (Kilpatrick 2011; CDC 2016). In addition, WNV has caused widespread mortality and substantial declines in populations of several bird species (LaDeau et al. 2007; Wheeler et al. 2009; Kilpatrick et al. 2013). *Culex* mosquitoes are considered to be the most important vectors for WNV transmission (Kilpatrick et al. 2005; Kramer et al. 2008) and the abundance of infected *Culex* mosquitoes is strongly correlated with the number of human WNV cases (Kilpatrick & Pape 2013; Paull et al. 2017).

Several studies have previously examined the effect of land use on several aspects of WNV transmission. Urbanization has been associated with higher WNV seroprevalence in wild bird and mammal populations (Gomez et al. 2008; Bradley et al. 2008), and higher human disease incidence on a county scale in the eastern and central regions of the US (Ruiz et al. 2007; Bowden et al. 2011; Degroote & Sugumaran 2012; Degroote et al. 2014). This is thought to be due to urbanization increasing larval habitat for container-breeding vectors of WNV including *Culex pipiens* and *Culex quinquefasciatus* (Trawinski & Mackay 2010; Landau & Leeuwen 2012; Rochlin et al. 2016). In the western US, grassland and agricultural land covers have been associated with higher human WNV disease incidence (Eisen et al. 2010; Bowden et al. 2011; Chuang et al. 2012; Degroote & Sugumaran 2012; Degroote et al. 2014). Grassland and agricultural habitats are thought to increase the abundance of another important WNV vector, *Culex tarsalis* (Crowder et al. 2013; Schurich et al. 2014a). Several studies at the national scale have argued that regional differences in land covers associated with increased WNV disease incidence roughly correspond to the distributions of major *Culex* vectors (Bowden et al. 2011; Degroote & Sugumaran 2012; Degroote et al. 2014; Skaff & Cheruvelil 2016). However, the broad classification of grassland and agriculture land cover encapsulates a wide diversity of crop types, each with variable effects on mosquito abundance and WNV risk

(Crowder et al. 2013). In addition, none of the broad-scale studies correlating land cover with WNV disease incidence include quantitative data on mosquito abundance or infection to support the different regional correlations and conclusions.

We examined the influence of a particular agricultural land cover, rice fields, that provides larval habitat for some, but not all mosquito species. We tested the following hypotheses: rice cultivation would increase the abundance of *C. tarsalis* mosquitoes; human WNV disease incidence would increase with *C. tarsalis* abundance; as a result, human WNV disease incidence would increase with rice cultivation in regions where *C. tarsalis* was a key vector of WNV, but not in areas where *C. tarsalis* is rare or absent and other mosquito species are the dominant WNV vectors.

MATERIALS AND METHODS

Land use, climate, and WNV disease incidence:

We obtained 30m land cover data from the USDA National Agricultural Statistics Service (NASS 2015). For each county, we summed the area of several land cover classes that could potentially be important for mosquitoes (rice fields, developed areas, wetlands, open water, forested areas). We averaged data from 7 different years available with 30m resolution (2008-2014) and calculated the percent cover of each land cover class using the area of each land cover class divided by total county area. Developed areas included a combination of low, medium, and high intensity, as well as open space developed. Wetland areas included a combination of woody and herbaceous wetlands and forested areas included all deciduous, mixed and evergreen forest types.

We calculated the percent of each county that was “irrigated agriculture” (including rice fields) from USGS MODIS at 250m resolution (USGS 2012). We estimated “non-rice irrigated areas” by subtracting the rice-growing areas from the total irrigated area within each county. In addition, we calculated mean annual temperature and precipitation (2003-2011) for each county using data from North America Land Data Assimilation System (NLDAS 2011).

We compiled reported human cases of West Nile virus for each county from CDC’s ArboNET program for the years 2004-2015 (CDC 2016). Average human WNV disease incidence was calculated as the mean number of all cases (fever and neuro-invasive cases combined) per year divided by the county’s population (Census 2010).

WNV vector identification:

We estimated the role of different *Culex* species mosquitoes in transmission of WNV in each county using the fraction of *Culex* WNV-positive pools reported to the CDC from 2004 to 2009. We used the fraction of positive pools to account for differences in sampling effort among counties; as long as each pool of *Culex* mosquitoes is equally likely to be tested and reported to the CDC, these data should provide relatively accurate estimates of the relative abundance of infected mosquitoes of each *Culex* species. We also examined the importance of *Culex* species in human WNV disease incidence across the US, by calculating a human-population weighted average of the county values of the fraction of WNV-positive mosquito pools attributed to each species. These estimates do not take into account differences in feeding preferences or the fraction of WNV-infected mosquitoes that transmit WNV between mosquito species (Kilpatrick et al. 2005).

Mosquito abundance:

We obtained mosquito trapping data from the CALSURV vector-borne disease surveillance system which includes trapping data from vector control districts across California. We used New Jersey light trap (NJLT) data from the summer months (June-Sept) from the years 2000-2015. This dataset consisted of >100,000 unique site visits across 1,284 locations spanning 34 counties in California. We estimated relative summer abundance of *Culex tarsalis* in each county by taking the mean # of mosquitoes caught per trap location and then averaging across all trap locations within each county over the period 2000-2015. We further estimated the mean summer (June-Sept) abundance of *Culex* mosquitoes at particular trap sites located within 10km of rice fields. This distance is well above the estimated average dispersal distance for *C. tarsalis* (Reisen et al. 2003). For these estimates, each trap site was included only if it had at least 5 years of NJLT data with at least 10 visits per year.

Statistics:

We summarized geographic data using ESRI ArcMap 10 and performed all statistical analyses using program R, version 3.1.3. We used generalized least squares (gls) to build least squares regression models to predict mean WNV disease incidence in each county using land cover and climate data (developed, water, wetland, rice, forest, irrigated areas, mean temperature and mean rainfall data). WNV disease incidence, land cover variables, and *Culex* abundance data were $\log_{10}$ transformed to equalize leverage and maintain adequate homogeneity of variance (see Table S1). We accounted for spatial autocorrelation in WNV disease incidence data using exponential correlation structure within the gls models. We used piecewise regression models (R package “segmented”) to examine relationships between mosquito abundance and distance to nearest rice field (Muggeo 2017). Piecewise regression uses an iterative process to reduce the residual sum of squares by fitting linear line segments across different rice distance intervals, and comparing models with multiple segments to models with fewer segments (Muggeo 2017).

RESULTS

The main rice-growing regions of the US were in California, the Mississippi river delta and southern Texas, with small additional areas in South Carolina and Florida (figure 1A). A total of 459 counties in the US had rice-growing regions during the years 2008-2014. The amount of rice grown in each county varied widely with some rice-intensive counties having up to 30% of the county area covered in rice fields. Mean WNV disease incidence (2004-2015) in rice-growing counties was 1.25 people /100,000 per year (95% CI= 1.08-1.42, SE=0.09) and ranged from 0-31 cases/100,000 people/year (figure 1B).

In California, incidence of WNV disease increased with the percent of the county growing rice (figure 1C). No other climate or land use variables contributed to an increase in WNV disease incidence (Table S2). In contrast, outside California incidence of WNV disease was uncorrelated with rice cover, and increased with developed area, and decreased with open water cover (figure 1D; table S3).

Out of 13 different *Culex* species found to test positive for WNV across the US, the vast majority (>93%) of WNV-positive mosquito pools came from only four species (*C. pipiens* (29%, SE=1.1), *C. tarsalis* (28.3%, SE=1.4), *C. restuans* (18.7%, SE=0.9) and *C. quinquefasciatus* (16.6%, SE=1.2) (figure S1). The population weighted analysis also identified the same four species (*C. pipiens* (33%, SE=0.7), *C. quinquefasciatus* (27%, SE=1.5), *C. restuans* (17%, SE=0.5) and *C. tarsalis* (15%, SE=0.5; figure S1). However, the importance of each mosquito species differed among counties and regions (figures 2, 3). In rice-growing regions of California, approximately 65% (SE=5.0) of all reported WNV positive mosquito samples were from *C. tarsalis*, 14% (SE=3.6) were *C. pipiens*, and 14% (SE=4.3) were *C. quinquefasciatus* (figure 3). In rice-growing regions outside of California the most important species were *C.*

quinquefasciatus 66.8% (SE=3.7), *C. pipiens* 20% (SE=2.8), and *C. restuans* 7.3% (SE=1.6), while *C. tarsalis* made up very few of the WNV pools 0.02% (SE=0.01) (figure 3).

In California, rice cultivation was linked to mosquito abundance, and mosquito abundance was correlated with WNV disease incidence. The relative abundance of *C. tarsalis* per NJLT-week increased with rice cover (figure 4A) and WNV disease incidence increased with *C. tarsalis* abundance (figure 4B). *C. tarsalis* abundance increased at trap sites (n=388) located near rice fields (figure 5). In contrast, we found no significant relationship between *C. pipiens* abundance and distance to rice fields (P=0.74, figure S2). In addition, zero *C. quinquefasciatus* were caught in >97% of trap sites (377/388) located within 10km of rice fields.

DISCUSSION

The larval ecology of mosquito vectors appears to play a key role in determining the effect of land use on mosquito-borne disease. Previous studies had found correlations between WNV disease incidence and agricultural land cover in the western USA, and urban land cover in eastern regions (Bowden et al. 2011; Degroote & Sugumaran 2012; Degroote et al. 2014). These studies attributed these regional differences in which land cover increased WNV disease incidence to differences in the distributions of mosquito vectors. We extend these results by showing that an important agricultural crop, rice, appears to play a key role in WNV transmission in the western USA by increasing the abundance of an important WNV vector in this region, *C. tarsalis*. We further showed that the effect of rice fields on WNV disease incidence depended on the relative importance of *C. tarsalis* in that region. Of the four *Culex* species that make more than 93% of reported WNV-positive mosquito pools in the USA (*C. pipiens*, *C. quinquefasciatus*, *C. restuans*, and *C. tarsalis*), only *C. tarsalis* breeds in flooded agricultural fields and grasslands, whereas the other three species breed in container habitats (Reisen 2012). As a result, in rice-growing regions outside of California, where the dominant WNV vector was *C. quinquefasciatus*, WNV disease incidence was no longer correlated with rice cover and was instead correlated with urban land cover, as in other studies (Calhoun et al. 2007; Chaves et al. 2009; Deichmeister & Telang 2011). These results provide a more detailed understanding of the mechanisms underlying some previous correlations with land use and land cover along with an evidentiary basis for the previously proposed hypotheses (Bowden et al. 2011; Degroote & Sugumaran 2012; Degroote et al. 2014). It is worth noting that these findings are limited to rice-growing regions in California and the US. In other regions of North America other species of mosquitoes are more important WNV vectors, including *C. pipiens* and *C. restuans* (Andreadis et al. 2004; Kilpatrick et al. 2005; Kilpatrick & Pape 2013). Our study further shows how rice fields specifically increased *C. tarsalis* abundance while having no effects on other important WNV vectors such as *C. pipiens* or *C. quinquefasciatus*. Previous studies in California have found *C. tarsalis* larva to be abundant in rice fields (Pitcairn & Wilson 1994) and the rice-growing region of northern California to have the highest overall abundance of adult *C. tarsalis* of anywhere in the state (Barker et al. 2010). Other studies outside of the US have also found that the extent of rice fields was uncorrelated with the abundance of *C. pipiens* and *C. quinquefasciatus* (Bisanzio et al. 2011; Thongsripong et al. 2013). We also show that the increased abundance of *C. tarsalis* in rice field areas extended outward 2km from rice field sites, well within the dispersal distance associated with this mosquito species (Reisen & Lothrop 1995). We observed a 7-fold increase in *C. tarsalis* abundance within 2km of rice fields. This suggests residential neighborhoods located within 2km of rice fields are likely to have higher WNV disease risk. Although rice cultivation is clearly important for *C. tarsalis*, other factors, such as blood meal hosts (Wood et al. 1991), and anthropogenic sources of light, also influence mosquito abundance, or abundance estimates using NJLT (McDermott & Mullens 2018).

Rice fields also appear to be important for other mosquito-borne diseases. Results from this study are similar to findings in another disease system, Japanese encephalitis (JE). JE is an important emerging infectious disease, endemic to many regions of SE Asia resulting in widespread morbidity (30,000-50,000 annual cases) and mortality (10,000-15,000 annual deaths) (Keiser et al. 2005; Erlanger et al. 2009). The abundance of one potentially important mosquito vector of Japanese encephalitis, *Culex tritaeniorhynchus*, closely tracks rice-growing in space

(Amerasinghe & Ariyasena 1991; Richards et al. 2010; Thongsripong et al. 2013) and time (Samuel et al. 2016), and *C. tritaeniorhynchus* abundance is correlated with Japanese encephalitis disease incidence (Miller et al. 2012; Guo et al. 2014; Tian et al. 2015).

Rice is grown in more than 100 countries worldwide, with extensive cultivation in Southeast Asia (figure S3)(FAO 2017). Our results illustrate how certain crops can increase disease risk and how spatial variation in vector ecology can alter the relationship between land cover and disease. Efforts to mitigate this increased disease risk, while supporting production of this key agricultural crop are needed to maximize human health and well-being (Kilpatrick et al. 2017).

CH 1- FIGURES

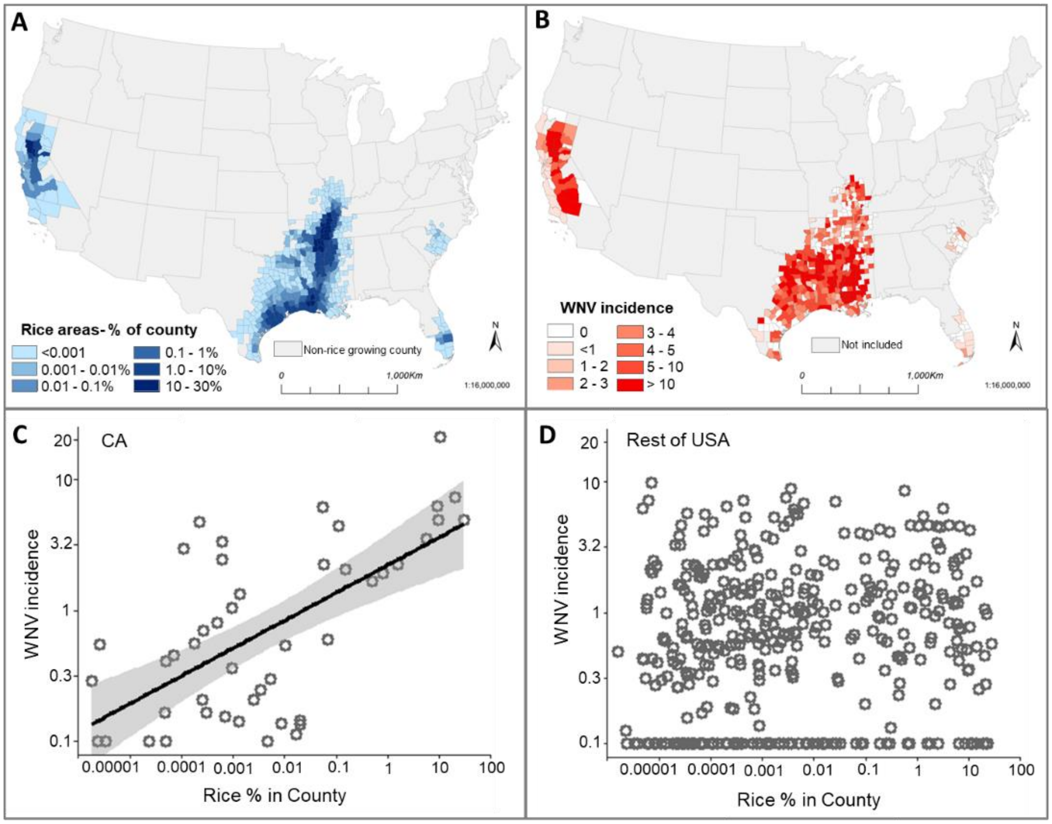

Figure 1: Rice cultivation and WNV incidence in rice-growing regions of the USA. A) Average percent of each county growing rice over the period 2008-2014. B) Average yearly human WNV incidence in reported cases per 100,000 people over the period in rice-growing counties 2004-2015. C) Yearly average WNV incidence plotted against average rice cover in California: Log$_{10}$ WNV incidence = 0.35 + 0.21 ($\pm$ SE = 0.039) * Log$_{10}$ Percent rice cultivation; R^2=0.41, n=46; general least squares model including spatial autocorrelation, P=0.04). D) Average yearly WNV incidence plotted against average rice cover in the rest of the US: Log$_{10}$ WNV incidence = -0.11 + 0.04 ($\pm$ SE =0.014) * Log$_{10}$ Percent rice cultivation; n=413; R^2 = 0.02; general least squares model including spatial autocorrelation, P=0.88). For panels (C) and (D) counties with no WNV cases are shown with an incidence of 0.1.

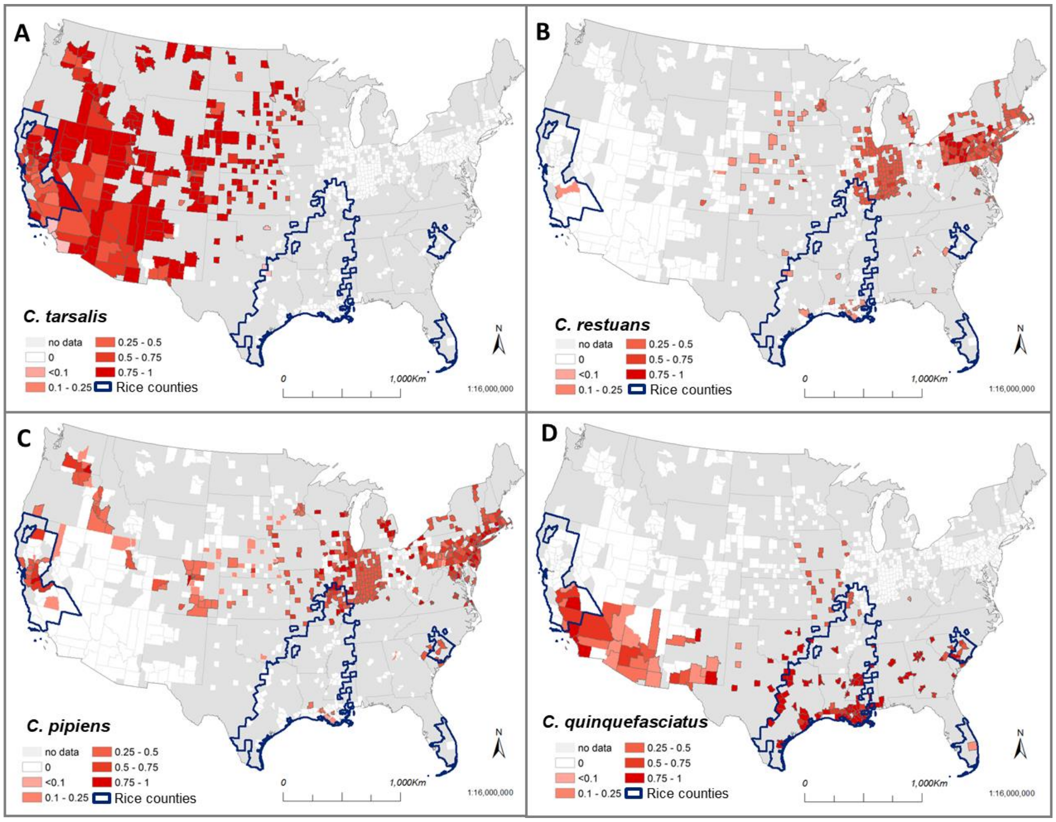

Figure 2: Spatial variation in West Nile virus infected mosquitos. Panels show the fraction of 51,650 reported *Culex* WNV-positive mosquito pools (of 1-50 mosquitoes) from each of four *Culex* species (A=*C. tarsalis*, B=*C. restuans*, C=*C. pipiens*, D=*C. quinquefasciatus*) for 821 counties across the USA between the years 2004-2009.

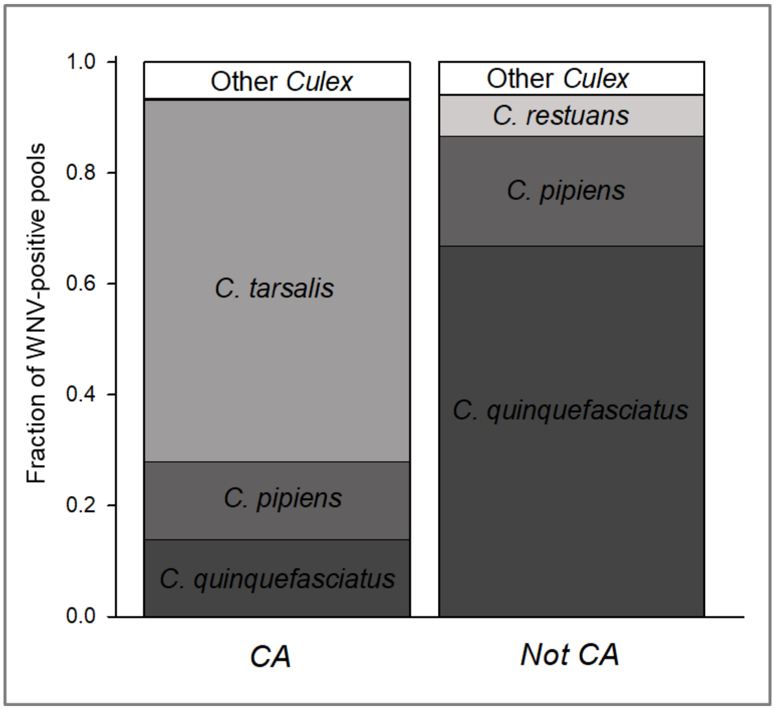

Figure 3: Relative contribution to WNV-infected *Culex* species in rice-growing areas across 2 different regions of the US, 2004-2009. Columns show average fraction of WNV-positive pools attributed to each mosquito species from California counties with rice fields (CA, n=44) and other rice field counties not in California (Not CA, n=106).

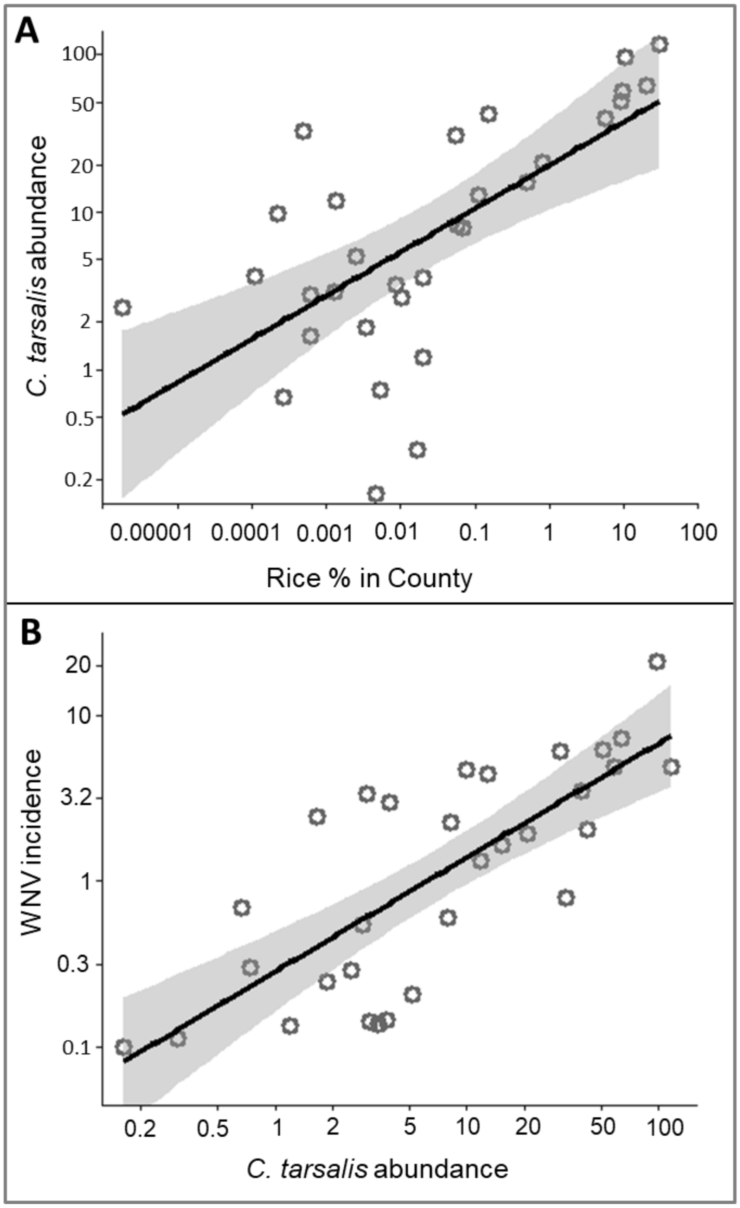

Figure 4*:* Rice cover, *Culex tarsalis* mosquito abundance, and human WNV incidence. A) *C. tarsalis* abundance (NJLT mosquitoes per trap-week) in California between June and September, over the period 2000-2015 plotted against the percent rice cover in each county: Log_{10} *C. tarsalis* abundance = 1.30 + 0.28 (±SE =0.06) * Log_{10} Percent rice cultivation; n=31, R^2=0.44, general least squares model including spatial autocorrelation, P=0.0004). B) Average yearly human WNV incidence per 100,000 people (2004-2014) plotted against *C. tarsalis* abundance in each county: Log_{10} WNV incidence = -0.54 + 0.69 (± SE = 0.11) * Log_{10} *C. tarsalis* abundance; n=31, R^2=0.59, general least squares model including spatial autocorrelation, P=0.0002). For panel B, counties with no WNV cases are shown with an incidence of 0.1.

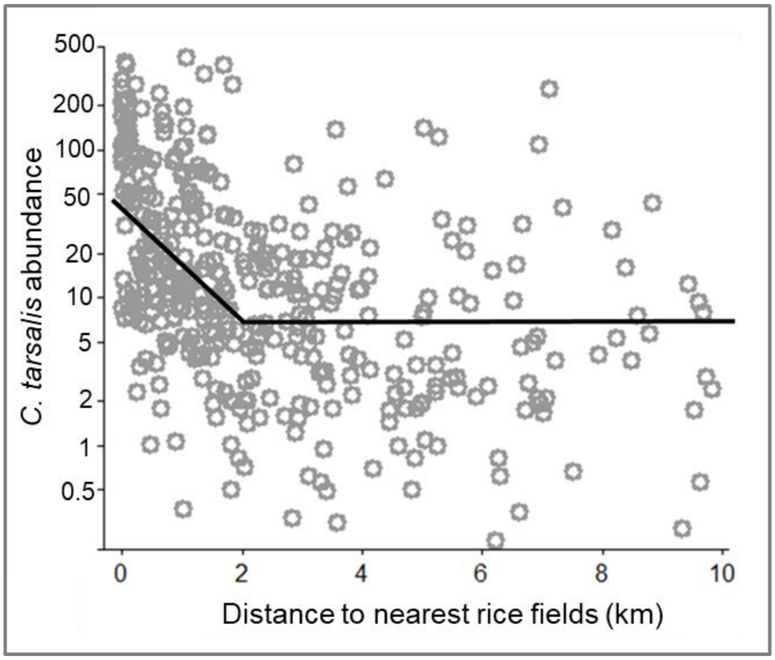

Figure 5*: Segmented regression analysis of *Culex tarsalis* mosquito abundance and distance to rice fields.* Average *C. tarsalis* abundance (mosquitoes per NJLT trap-week) in California between June and September, plotted against distance to nearest rice field. Initial segment: Log_{10} *C. tarsalis* abundance = 1.7 -0.43 (± SE = 0.067) * Distance to rice field, $R^2=0.27$, $P<0.0001$, n=388, estimated regression break point= 2.0 km (±SE=0.28). 2nd segment slope (Slope=-0.027, ±SE=0.021) was non-significant.

CH 1- SUPPLEMENTAL FIGURES

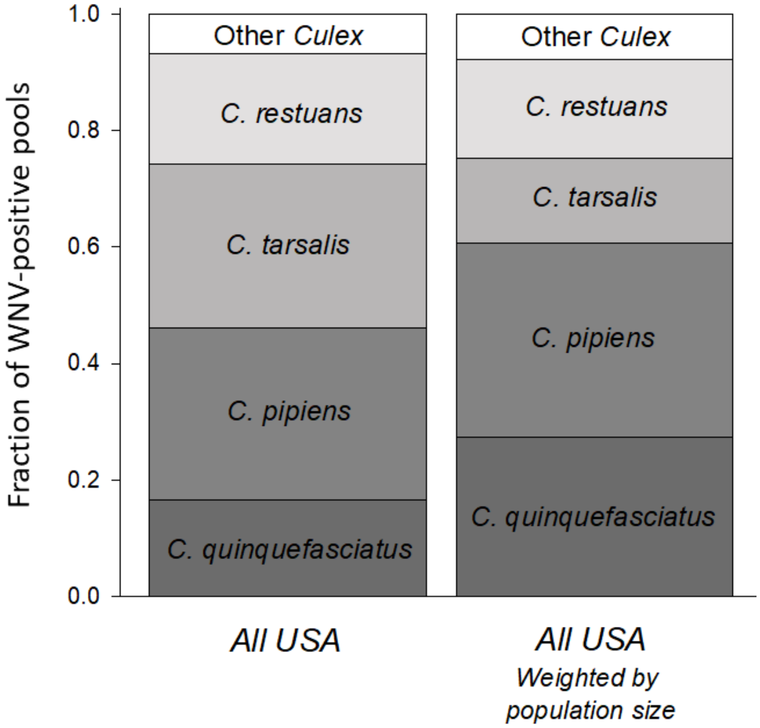

Figure S1: Relative contribution to WNV-infected *Culex* species across the US, 2004-2009. Columns show average fraction of WNV-positive pools attributed to each mosquito species (All USA, n=821), shown with and without adjustment for county human population sizes.

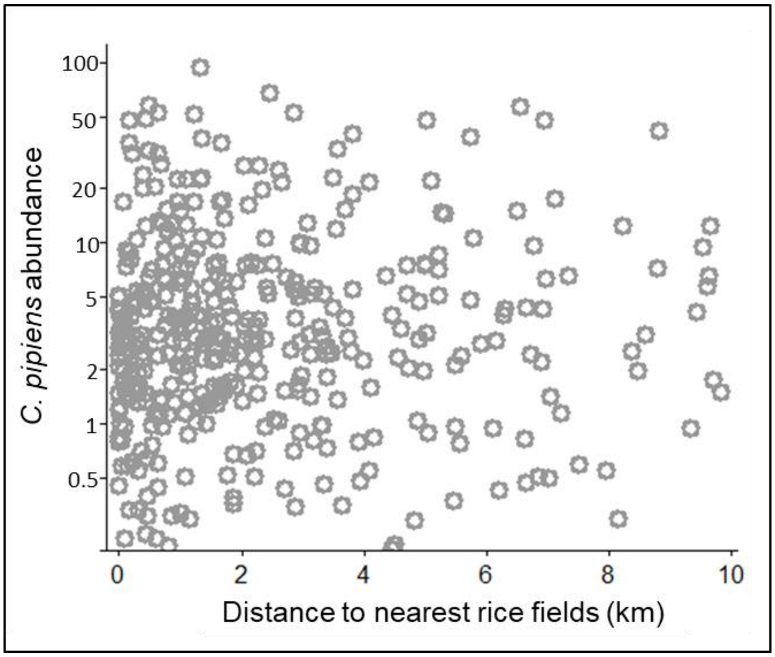

Figure S2*: Culex pipiens* mosquito abundance and distance to rice fields. *C. pipiens* abundance (mosquitoes per NJLT trap-week) in California between June and September, plotted against distance to nearest rice field (n=388). In contrast to *C. tarsalis*, we observed no significant relationship (P=0.74) between distance to rice field and abundance of *C. pipiens*.

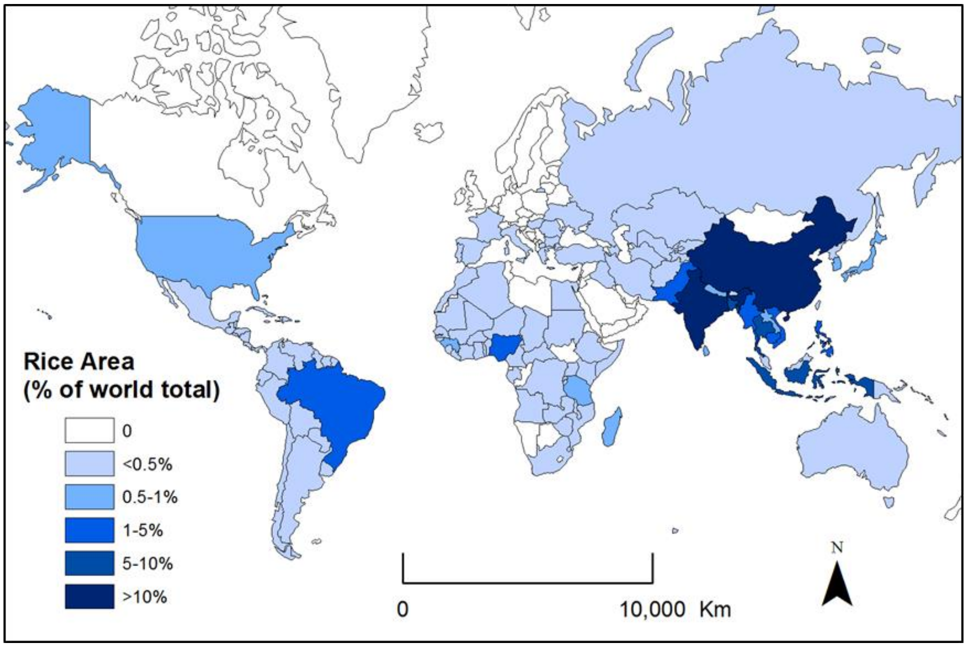

Figure S3: Rice-growing areas of the world. Map showing the percent contribution of each country to total rice area in the world. Estimated rice area for each country based on mean rice-growing region from years 2010-2014 (FAO 2017)

CH 1- SUPPLEMENTAL TABLES

Table S1: List of predictor and response variables, transformations, and sources.

Variables	**Transformations**	**Source**
WNV incidence	Log10 (WNV incidence+0.1)	CDC-cases by county 2004-2015
Irrigated (not-rice)	Log10 (Irrigated+0.1)	USGS Modis- 2012, 250m resolution (minus rice areas)
Developed	Log10 (Developed)	Cropland- NASS- 2008-2014, 30m resolution
Open water	Log10 (Open water+0.05)	Cropland- NASS- 2008-2014, 30m resolution
Wetland	Log10 (Wetland+0.05)	Cropland- NASS- 2008-2014, 30m resolution
Forest	None	Cropland- NASS- 2008-2014, 30m resolution
Rice	Log10 (RICE)	Cropland- NASS- 2008-2014, 30m resolution
County area	Area, used to normalize	US Census Bureau- 2010
Mean rainfall	Log10 (rain)	CDC Wonder NLDAS mean 2003-2011
Mean temperature	None	CDC Wonder NLDAS mean 2003-2011- 2m above ground
Human population	Used to calculate WNV	US Census Bureau- 2010
Mean *C. tarsalis*	Log10 (*C. tarsalis* mean +1)	NJLT data 2000-2015, Calsurv- vector control data

Table S2: Output from generalized least squares model predicting human WNV incidence on a county scale using land cover and climate predictors across California. Model includes exponential spatial autocorrelation, n=46.

Predictors	Coefficient/Intercept	SE	P-value
Irrigation (non-rice)	0.236	0.137	0.0943
Developed land	0.255	0.214	0.2419
Wetland	-0.304	0.150	0.0494
Open water	0.089	0.146	0.5458
Forest	0.001	0.006	0.9128
Rice fields	0.132	0.046	0.0072
Mean Temperature	0.023	0.031	0.478
Mean Rainfall	-0.403	0.649	0.5383
Intercept	-0.762	0.731	0.304

Table S3: Output from generalized least squares model predicting human WNV incidence on a county scale using land cover and climate predictors across rice-growing areas in the rest of the US (excluding California). Model includes exponential spatial autocorrelation, n=413.

Predictors	**Coefficient/Intercept**	**SE**	**P-value**
Irrigation (non-rice)	-0.01116	0.05282	0.8328
Developed land	0.534014	0.116767	<0.0001
Wetland	0.035156	0.072197	0.6266
Open water	-0.12248	0.062228	0.0497
Forest	-0.0026	0.002292	0.2575
Rice fields	0.029085	0.025097	0.2472
Mean Temperature	0.009563	0.025579	0.7087
Mean Rainfall	0.812468	0.775813	0.2956
Intercept	-1.223	0.882	0.1664

CHAPTER 2

IRRIGATION INCREASES MOSQUITO POPULATIONS, WEST NILE VIRUS INCIDENCE, AND REDUCES SEASONAL VARIABILITY

ABSTRACT

Humans have altered earth's terrestrial water cycle with the majority of fresh water being used for agriculture. Irrigation often changes spatial and temporal water availability and may thereby alter mosquito abundance and phenology. While irrigation has been shown to increase mosquito abundance and mosquito-borne disease in some regions, its effect is inconsistent and very little is known about how irrigation affects variability in mosquito abundance and disease risk. We examined the effect of irrigation, climate and land cover on mosquito abundance and WNV human disease cases across California. Irrigation made up nearly a third of total water inputs to the region, with irrigation exceeding precipitation in some dry regions. Irrigation reduced seasonal variability in mosquito abundance by more than 40%, and increased abundance by more than an order of magnitude. In addition, irrigation increased human West Nile virus cases and explained 33% of variation in WNV cases among California counties. These results suggest that irrigation can increase and decouple mosquito populations from natural precipitation variability, resulting in sustained and increased disease risk.

INTRODUCTION

Humans have altered many of the Earth's natural biogeochemical processes, including the terrestrial water cycle (Vitousek et al. 1997; Vorosmarty et al. 2000; Ellis 2011). The majority of fresh water is used for growing irrigated crops (Pimentel et al. 2004; Oki 2006; Rost et al. 2008; Sterling et al. 2012), and climate change is predicted to further increase conversion of rain-fed agriculture to irrigation-based systems (Negri et al. 2005; Troy et al. 2015). Irrigation increases and stabilizes agricultural yields by buffering crops against natural variability associated with fluctuations in precipitation, and in doing so alters the timing and spatial availability of water across the landscape (Lo & Famiglietti 2013; Müller et al. 2018). Agriculture has previously been associated with increased mosquito abundance (Surtees 1970; Reisen et al. 1992a; Wegbreit & Reisen 2000; Schurich et al. 2014a) and higher rates of human disease including malaria (Ijumba & Lindsay 2001; Jaleta et al. 2013), Japanese encephalitis (Keiser et al. 2005), lymphatic filariasis (Erlanger et al. 2005), Rift Valley fever (Bett et al. 2017), and West Nile virus encephalitis (Gates & Boston 2009; Eisen et al. 2010; Kovach & Kilpatrick 2018). However, the effects of irrigation per se are less well studied and far less is known about the effects of irrigation on variability in disease risk, despite the importance of irrigation in many arid regions (Baeza et al. 2011). Examining the effect of irrigation on mosquito populations and vector-borne disease requires simultaneously examining the effects of land use and climate (Bradley & Altizer 2007; Bowden et al. 2011; Gottdenker et al. 2014).

In California, one of the largest irrigated agricultural regions in the world (Maupin et al. 2010; Famiglietti et al. 2011), irrigation during dry periods (April-October) is likely to both increase mosquito abundance and decrease its seasonal variability (Barker et al. 2010; PRISM Climate Group 2018). Abundance of one mosquito species, *Culex tarsalis*, sometimes increases with irrigation water releases (Reisen et al. 1992a; Wegbreit & Reisen 2000; Schurich et al. 2014a). Irrigation also enables several species of mosquitoes to inhabit otherwise inhospitably dry regions (Reisen et al. 2008b; Barker et al. 2010; Grantham & Viers 2014). Finally, the effects of irrigation likely contribute to the effects of land use on mosquito abundance and vector borne disease in California. Agriculture has been associated with increased abundance of *C. tarsalis* (Reeves & Hammon 1962; Reisen et al. 1992a; Wegbreit & Reisen 2000; Kovach & Kilpatrick 2018) and higher human WNV disease incidence (Eisen et al. 2010; Bowden et al. 2011; Degroote & Sugumaran 2012; Degroote et al. 2014; Kovach & Kilpatrick 2018).

While these studies suggest that mosquito populations and mosquito-borne disease often increase with water availability, the effects of rainfall on mosquito populations and WNV human disease rates in the western USA have been mixed. Some studies have found positive associations between increased rainfall and *C. tarsalis* mosquito populations (Reisen et al. 2008b; Chuang et al. 2011) while others have found no effect (Pecoraro et al. 2007). WNV incidence has been associated with both increased and decreased rainfall (Degroote et al. 2014; Wimberly et al. 2014), decreased rainfall and drought conditions (Landesman et al. 2007; Crowder et al. 2013; Paull et al. 2017), and moderate rainfall amount (Wimberly et al. 2008). These inconsistent relationships with rainfall may result from unmeasured effects of irrigation or land cover that affect mosquito larval habitat.

Our goal was to examine the effect of irrigation on the magnitude and variability in mosquito populations and WNV human disease incidence across California. First, we quantified water amounts coming from irrigation and precipitation across space and time. We then examined irrigation, climate, and land cover data as predictors of mosquito abundance, seasonal variability in mosquito populations, and WNV human disease incidence. We hypothesized that irrigation would increase mosquito abundance and WNV incidence, and reduce seasonal variability in mosquito populations by stabilizing water availability for larval mosquito development.

METHODS

Mosquito abundance:

We obtained mosquito trapping data collected by vector control districts across California via the CALSURV vector-borne disease surveillance system (UC Davis 2016). These public health agencies focus mosquito trapping efforts near human population centers, in regions with urban and agricultural land use. We used CO_2 trapping data from the months April through November over a 13-year period during which irrigation data was also available (1998-2010). We included trap sites with at least 10 annual visits (n=1336 sites), for a total of ~80,000 unique site visits, in 78 detailed analysis units. We separated California into discrete "detailed analysis units" (DAU) to integrate with irrigation estimates from state governmental agencies (CDWR 2018). We quantified average *C. tarsalis* abundance estimates per CO_2 trap/night for each DAU by first by averaging across each site for each year (site-year average), then averaging across sites within an DAU (DAU-year), and then averaging all years. We spatial quantified variability in *C. tarsalis* abundance estimates per CO_2 trap/night for each DAU by first by calculating the percent coefficient of variation (CV = 100*sd/mean) for each site-year, and then averaged these CV values across sites and then averaged them across years.

<u>Irrigation estimates:</u>

We obtained annual irrigation estimates ("Applied water", acre-feet per acre) for each DAU in California from 1998-2010 from the California department of water resources (CDWR 2018). These annual irrigation estimates per DAU are based on the growing areas of 20 agricultural crop categories, each with specific crop coefficients corresponding to estimated annual water use (CDWR 2018). In each detailed analysis unit, we multiplied the growing area of each crop by estimates of the irrigation water for growing each crop and summarized annual estimates of total irrigated water for each detailed analysis area.

Temperature and Precipitation:

We obtained spatial estimates of average monthly temperature and precipitation data across California at 4 km resolution (PRISM Climate Group 2018). We used ArcGIS to calculate average annual estimates of temperature and precipitation for each detailed analysis unit across California (n=278), including the 78 detailed analysis regions with mosquito trapping data. We calculated the average annual precipitation (Dec-Nov, in mm) to include winter snowpack (Reisen et al. 2008b) and average temperature (°C) during each mosquito trapping season (Apr-Nov) in each DAU for the years 1998-2010. To make precipitation and irrigation amounts comparable, we converted precipitation (mm) into volumetric amounts of water per acre (acre-feet per acre).

Land Cover datasets:

Within each DAU, we summarized percent land cover of several land cover classes that past analyses indicated were important in mosquito abundance (developed areas, wetlands, open water; (Bowden et al. 2011; Skaff & Cheruvelil 2016; Kovach & Kilpatrick 2018)) using datasets available from 2010 USDA National Agricultural Statistics Service (30m resolution; (NASS 2015). Developed areas included a combination of low, medium, and high intensity, as well as open space developed. Wetland areas included a combination of woody and herbaceous wetlands. We did not include fraction agricultural land as an independent variable because the irrigation predictor was created using agricultural land use fractions of 20 different crops and was therefore highly correlated with irrigation ($r=0.94$, $N = 78$, $P <0.001$).

WNV human disease cases:

We compiled reported human cases of WNV for each California county from Centers for Disease Control and Prevention (CDC) ArboNET program for the years 2004–2010. Average human WNV disease incidence was calculated as the mean number of all cases (fever and neuro-invasive cases combined) per year divided by the county's population, multiplied by 100,000. We converted DAU estimates of irrigation to county estimates based on weighted averages of county area overlapping with DAU areas. Counties with <75% overlap with DAU areas were discarded.

Statistics:

All statistical analyses were done using the program R (v. 3.5.0) with spatial summaries and maps made using ArcGIS (v. 10.5). We used generalized least squares models (R package, "gls"), including exponential spatial autocorrelation, to examine the effects of climate, land cover, and irrigation on the abundance and seasonal variation in *C. tarsalis* mosquito populations, and human WNV disease incidence. We $\log_{10}$ transformed dependent and independent variables to linearize relationships and increase normality in residuals (table S1). We examined the potential for non-linear relationships between response variables and temperature by including a temperature^2 term in each analysis. However, these models all had higher AIC values than models without the quadratic term. We used Shapiro-Wilke tests to test for normality of residuals and used R package, “PiecewiseDEM” to output R^2 values.

RESULTS

Culex tarsalis abundance varied over four orders of magnitude across California (0.03-555 mosquitoes per trap/night), and seasonal variability of *C. tarsalis* populations (measured using percent coefficient of variation) varied five-fold (76-412%) (figure 1). Irrigation was also spatially variable (0-2.97 acre-feet per acre; figure 1), with irrigation making up 18.7% of total annual water (irrigation + precipitation) across the state and 30.9% of total water inputs in the 78 DAUs where there were mosquito data (figure 2a). Water from irrigation exceeded that from precipitation during the seasonally dry mosquito trapping season for all but one of 13 years of the study period (figure 2b).

C. tarsalis abundance increased with irrigation, higher average temperatures, greater amounts of wetland landcover, and lower amounts of developed land cover, but was not significantly correlated with precipitation or open water cover (figure 3, table S2). *C. tarsalis* mosquito abundance nearly doubled with each 10-fold increase in irrigation across the 300-fold range of irrigation amounts (Figure 3, table S2). *C. pipiens complex* abundance increased with irrigation and developed land cover but was uncorrelated with other climate and land use predictors (table S3). In contrast, *C. erythrothorax* abundance decreased with irrigation and rainfall, and increased with wetland land cover (table S4).

Spatial variation in seasonal variability in *C. tarsalis* abundance decreased with irrigation but was not significantly correlated with climate or land cover (figure 4; table S5). Seasonal variation in *C. tarsalis* mosquito abundance decreased by 50% across the range of irrigation observed (figure 4; table S5). The decrease in variability was due primarily to a sustained period of higher abundance in high-irrigation areas, rather than a single sharp peak in low-irrigation areas (figure 4a). Seasonal variation in *C. erythrothorax* abundance decreased with greater wetland land cover but was uncorrelated with irrigation or other variables (table S6), and variation in *C. pipiens complex* mosquito abundance was uncorrelated with any of the predictors (table S7).

Human WNV disease incidence also increased with irrigation but was not significantly correlated with climate or land cover (figure 5; table S8). Irrigation explained 33% of the variation in human WNV disease incidence among California counties (figure 5).

CH 2- FIGURES

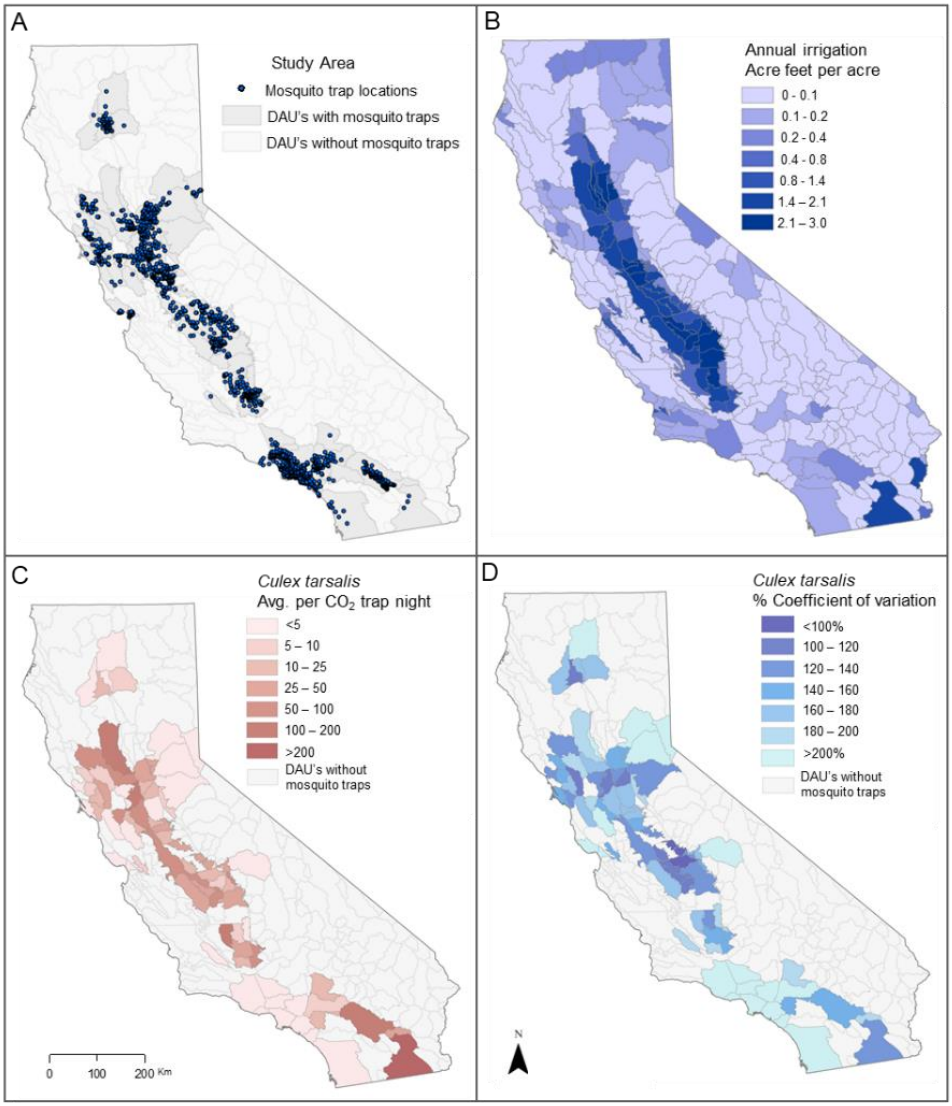

Figure 1: Mosquito trap locations, temperature, precipitation and irrigation shown across California for 1998-2010. A) Mosquito CO$_2$ trap locations (n=1336) and Detailed analysis units (DAU) with mosquito traps (n=78). B) Average annual irrigation (typically from April-Oct) for each DAU obtained from California Department of Water Resources (CDWR 2018). C) Average *C. tarsalis* per CO$_2$ trap-night in each detailed analysis unit {average across sites [average across years (average per trap site-year)]}. D) Average coefficient of variation in *C. tarsalis* abundance in each DAU {average across sites [average across years (CV per trap site-year)]}.

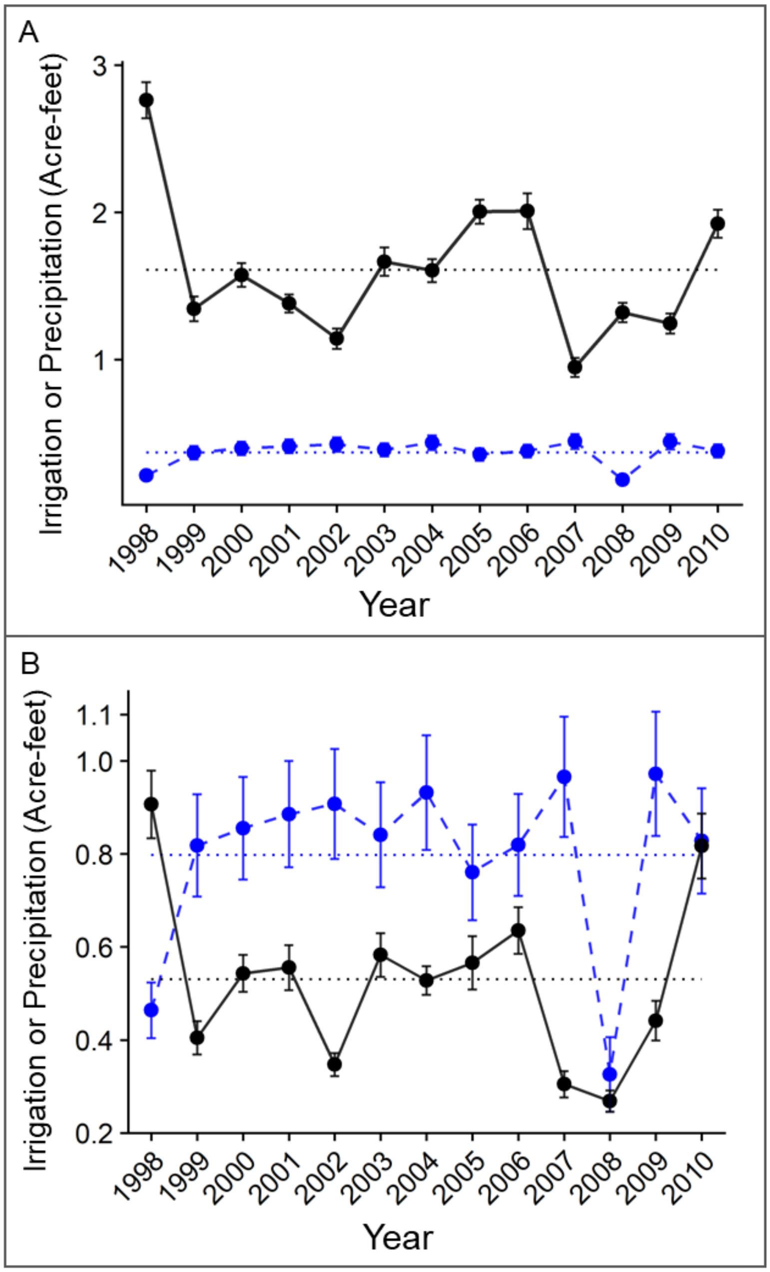

Figure 2: Yearly and seasonal precipitation and irrigation. A) Average yearly precipitation (Dec.-Nov., solid black line) and average yearly irrigation (dashed blue line) within study region (n=78 detailed analysis units). B) Average precipitation during mosquito trapping season (April-November) compared to irrigation within study region. Dotted lines display overall average across years.

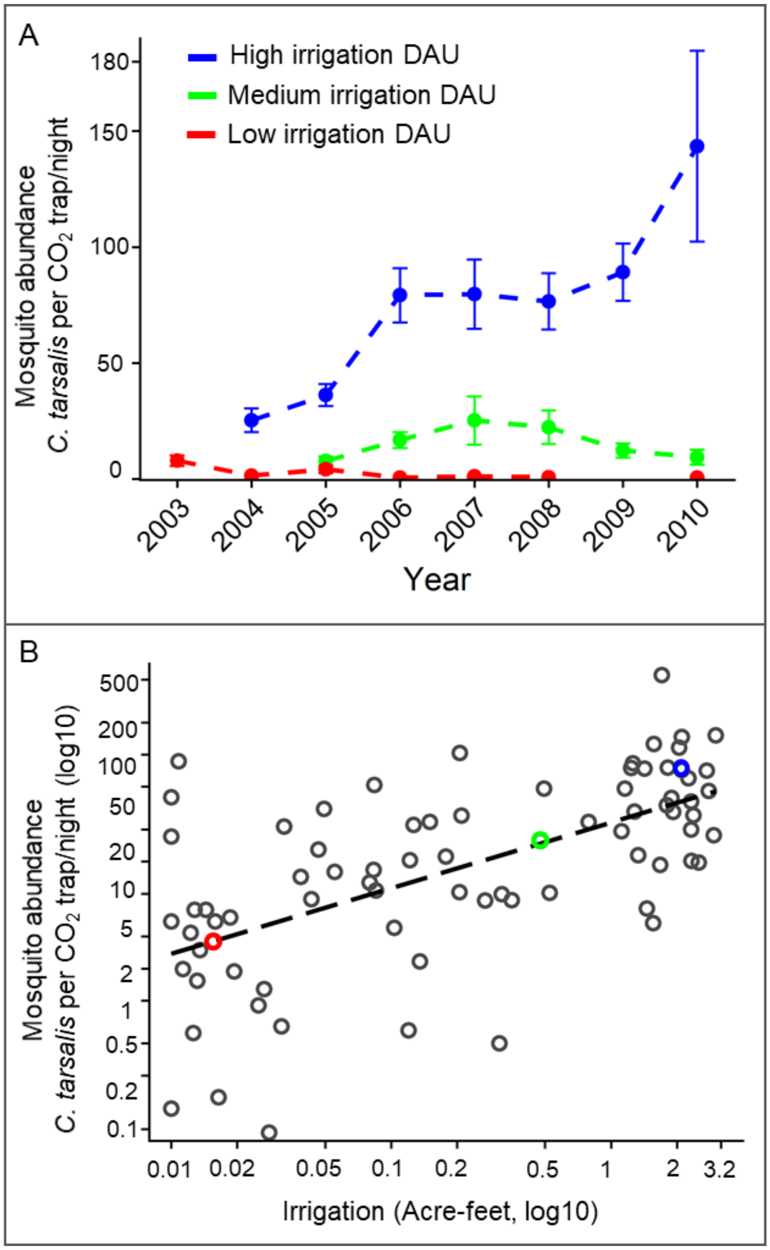

Figure 3: Irrigation and mosquito abundance. A) Time series for three DAU differing in irrigation amounts. The y-axis shows average *C. tarsalis* per CO_2 trap-night plotted across years (+/- SE). B) Average *C. tarsalis* per CO_2 trap-night in each detailed analysis unit plotted against average irrigation amount (Acre-feet/acre) in each DAU: General least squares model including spatial autocorrelation: Log_{10} *C. tarsalis* abundance = 1.37 + 0.59 ($\pm$ SE = 0.08) $*\log_{10}(\text{Irrigation}+0.01)$; $R^2 = 0.39$, n=78; normality of residuals: Shapiro-Wilk test P>0.63. The three colored points show the values for the time series shown in panel A.

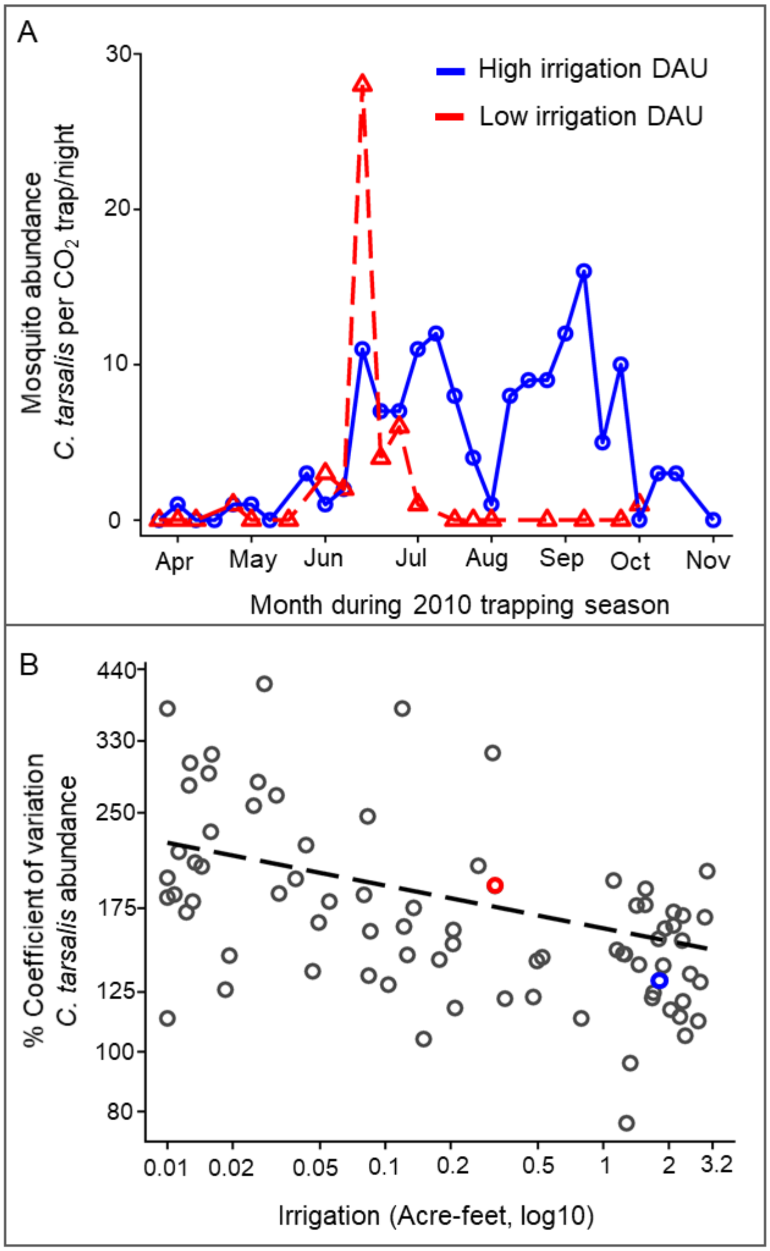

Figure 4: Irrigation effects on seasonal variation in mosquito abundance. A) Example time series for 2010 showing *C. tarsalis* per CO_2 trap-night at 2 trap sites located in adjacent DAU's which differed in irrigation amounts. B) Average coefficient of variation in *C. tarsalis* abundance in each DAU {average across sites [average across years (CV per trap site-year)]} plotted against average irrigation amount (Acre-feet/acre) in each detailed analysis unit. General least squares model including spatial autocorrelation: Log_{10} CV *C. tarsalis* abundance = 2.20 - 0.09 (±SE = 0.02) * $\log_{10}$(Irrigation+0.01); $R^2 = 0.27$, n= 78. normality of residuals: Shapiro-Wilk test P>0.16). The two colored points show the values for the time series in panel A.

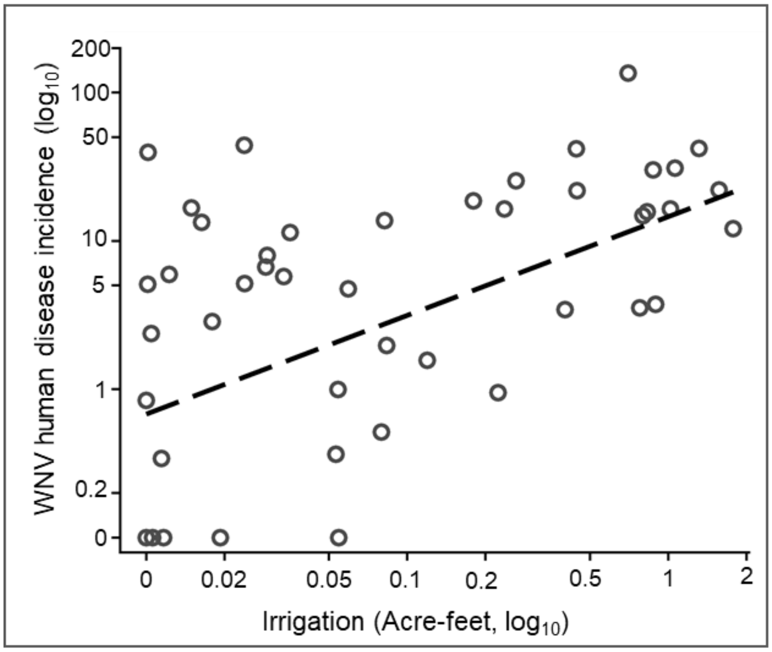

Figure 5: Irrigation effects on human WNV disease incidence in California counties. General least squares model including spatial autocorrelation: Log_{10} (Human WNV incidence + 0.1) = 1.16 + 0.67 (± SE = 0.15) * $\log_{10}$(Irrigation+0.01); $R^2 = 0.33$, n= 47. Normality of residuals: Shapiro-Wilk test $P>0.53$. WNV human disease incidence is average number of WNV cases per year/100,000 people.

DISCUSSION

We found that irrigation was correlated with increased mosquito abundance of two species, and reduced seasonal variability in one species, and increased human WNV incidence. The reduced variability in *C. tarsalis* populations resulted from irrigation increasing periods of elevated abundance, which is consistent with previously observed multi-modal seasonal peaks of *C. tarsalis* abundance in regions of California with heavy irrigation inputs (Barker et al. 2010). These relationships suggest that irrigation can partially decouple mosquito abundance of some species from natural precipitation patterns (Baeza et al. 2011) and result in elevated and sustained risk of mosquito-borne pathogens. The effect of irrigation on both *C. tarsalis* and *C. pipiens* abundance may result from irrigation increasing larval habitat for these species (Reisen 2012).

Irrigation also alters seasonal variation in mosquito abundance and transmission of other mosquito-borne diseases, such as malaria. In Africa irrigation both increased mosquito abundance and dampened seasonal variability, with the strongest effects occurring in dry regions (Ijumba & Lindsay 2001; Kibret et al. 2010, 2014). Irrigation, especially during the drier months, also prolonged elevated mosquito abundance and enabled year-round transmission (Kibret et al. 2010; Baeza et al. 2011; Ohta & Kaga 2014). As a result, as we found for mosquitoes in California, human malarial transmission in heavily irrigated regions is no longer tightly coupled with natural precipitation patterns (Baeza et al. 2011).

We found correlations between mosquito abundance and land use that match previous associations, but climate relationships were somewhat unexpected. Both *C. tarsalis* and *C. erythrothorax* abundance increased with wetland area and *C. pipiens* complex increased with developed land (Pitcairn & Wilson 1994; Andreadis et al. 2004; Thiemann et al. 2012; Walton 2012; Walton et al. 2013; Schurich et al. 2014a; Van Schmidt et al. 2018; Kovach & Kilpatrick 2018). Only *C. tarsalis* abundance increased with temperature, with little evidence for a non-linear relationship as would be expected based on the increase developmental rate and decreased survival rate with temperature (Reisen & Lothrop 1995; Ciota et al. 2014; Ruybal et al. 2016). Similarly, only *C. erythrothorax* abundance was correlated with precipitation and abundance decreased with increasing precipitation, which is somewhat counterintuitive. None of the climate predicators were correlated with seasonal variability in abundance of any of the three species. Overall, the data suggest that spatial variation in mosquito abundance and seasonal variability at this scale (and with this unit of analysis) is not tightly linked to climate and is more influenced by land use and irrigation.

More broadly, the patterns we observed suggest that manipulations to the water cycle increase the magnitude and reduce the variability of mosquito populations. Improvements in irrigation water delivery systems (e.g. fixing leaky irrigation canals), irrigation methods (e.g. sub-surface drip irrigation) and irrigation pricing schemes (farmers paying for actual amounts of water used rather than supply) might reduce the impacts of irrigation on mosquito populations by limiting potential breeding sites (Ayars et al. 1999; El Chami et al. 2011; Kibret et al. 2014). A key challenge in the coming decades will be findings ways to use irrigation to maintain food security, without increasing mosquito populations and disease risk.

CH 2- SUPPLEMENTAL TABLES

Table S1: List of predictor and response variables used in generalized least squares models along with transformations and descriptions. Each variable is summarized as an average per detailed analysis unit.

Variables	Transformation	Description
Temperature	none	Average temperature (Celsius), April-Nov, 1998-2010
Precipitation	$\log_{10}$	Average precipitation (Acre-feet-acre), Dec-Nov, 1998-2010
Irrigation	$\log_{10}$ + 0.01	Average irrigation (Acre-feet-acre), Apr-Oct, 1998-2010
Wetland	$\log_{10}$	Percent cover of wetland land cover (woody & herbaceous)
Developed	$\log_{10}$	Percent cover of developed land cover (High, medium, low intensity and open space developed)
Open Water	$\log_{10}$	Percent cover of open water land cover
C. tarsalis abundance	$\log_{10}$	CO_2 trap sites with>=10 visits, averaged per site-year, averaged per DAU-year, averaged per DAU
C. tarsalis CV	$\log_{10}$	CO_2 trap sites with>=10 visits, For each site-year calculated CV=100*(sd/mean), averaged per DAU-year, averaged per DAU
Human WNV disease incidence	$\log_{10}$ + 0.01	Average human disease incidence per county (2004-2010).

Table S2: Regression model predicting *C. tarsalis* abundance using climate, irrigation and land cover variables. *C. tarsalis* abundance estimates were averaged for each detailed analysis unit (n=78). Transformations were used to linearize relationships and improve normality in residuals; Shapiro-Wilk test, P>0.36. Generalized Least Squares model including spatial autocorrelation with exponential correlation structure, R^2=0.59.

GLS Model ~ $\log_{10}$(*C. tarsalis* abundance)	Coefficient	SE	T-value	P-value
Intercept	-1.073	0.856	-1.254	0.214
Temperature	0.144	0.041	3.471	0.001
Precipitation ($\log_{10}$)	-0.390	0.369	-1.055	0.295
Irrigation ($\log_{10}$+0.01)	0.247	0.095	2.617	0.011
Wetland ($\log_{10}$)	0.211	0.092	2.301	0.024
Developed ($\log_{10}$)	-0.291	0.139	-2.085	0.041
Open Water ($\log_{10}$)	0.247	0.137	1.808	0.075

Table S3: Regression model predicting *C. pipiens complex* abundance using climate, irrigation and land cover variables. *C. pipiens* complex is made up of both *C. pipiens* and *C. quinquefasciatus* and abundance estimates were averaged for each detailed analysis unit (n=78). Transformations were used to linearize relationships and improve normality in residuals; Shapiro-Wilk test, P>0.79. Generalized Least Squares model including spatial autocorrelation with exponential correlation structure, R^2=0.43.

GLS Model ~ $\log_{10}$(*C. pipiens* complex)	Coefficient	SE	t-value	P-value
Intercept	1.234	1.126	1.096	0.277
Temperature	-0.033	0.056	-0.590	0.557
Precipitation ($\log_{10}$)	-0.479	0.454	-1.055	0.295
Irrigation ($\log_{10}$+0.01)	0.531	0.130	4.094	<0.001
Wetland ($\log_{10}$)	-0.076	0.123	-0.617	0.539
Developed ($\log_{10}$)	0.454	0.191	2.381	0.020
Open Water ($\log_{10}$)	-0.369	0.177	-2.085	0.041

Table S4: Regression model predicting *C. erythrothorax* abundance using climate, irrigation and land cover variables. Abundance estimates were averaged for each detailed analysis unit (n=78). Transformations were used to linearize relationships and improve normality in residuals; Shapiro-Wilk test, P>0.003; some non-normality in residuals still present. Generalized Least Squares model including spatial autocorrelation with exponential correlation structure, R^2=0.37.

GLS Model ~ $\log_{10}$(*C. erythrothorax*+0.1)	Coefficient	SE	t-value	P-value
Intercept	0.997	1.339	0.744	0.459
Temperature	-0.056	0.066	-0.844	0.401
Precipitation ($\log_{10}$)	-1.801	0.528	-3.412	0.001
Irrigation ($\log_{10}$+0.01)	-0.546	0.148	-3.688	<0.001
Wetland ($\log_{10}$)	0.611	0.150	4.079	<0.001
Developed ($\log_{10}$)	0.353	0.230	1.537	0.129
Open Water ($\log_{10}$)	-0.109	0.224	-0.487	0.628

Table S5: Regression model predicting Coefficient of Variation (CV) of *C. tarsalis* abundance within each mosquito trapping season, using climate, irrigation and land cover variables. CV estimates were averaged for each detailed analysis unit (n=78). Transformations were used to linearize relationships and improve normality in residuals; Shapiro-Wilk test, P>0.97 Generalized Least Squares model including spatial autocorrelation with exponential correlation structure, R^2=0.35.

GLS Model ~ $\log_{10}$(CV *C. tarsalis*)	Coefficient	SE	T-value	P-value
Intercept	2.532	0.201	12.601	0.000
Temperature	-0.017	0.010	-1.717	0.090
Precipitation ($\log_{10}$)	0.035	0.108	0.321	0.749
Irrigation ($\log_{10}$+0.01)	-0.057	0.026	-2.230	0.029
Wetland ($\log_{10}$)	0.002	0.018	0.134	0.894
Developed ($\log_{10}$)	-0.008	0.032	-0.245	0.807
Open Water ($\log_{10}$)	-0.028	0.024	-1.166	0.248

Table S6: Regression model predicting Coefficient of Variation (CV) of *C. erythrothorax* within each mosquito trapping season, using climate, irrigation and land cover variables. CV estimates were averaged for each detailed analysis unit (n=78). Transformations were used to linearize relationships and improve normality in residuals; Shapiro-Wilk test, P>0.06. Generalized Least Squares model including spatial autocorrelation with exponential correlation structure, R^2=0.12.

GLS Model ~ $\log_{10}$(CV of *C. erythrothorax* +0.1)	Coefficient	SE	T-value	P-value
Intercept	2.184	0.279	7.838	<0.001
Temperature	0.013	0.014	0.923	0.360
Precipitation ($\log_{10}$)	0.191	0.116	1.640	0.107
Irrigation ($\log_{10}$+0.01)	0.038	0.030	1.295	0.201
Wetland ($\log_{10}$)	-0.068	0.034	-2.018	0.048
Developed ($\log_{10}$)	0.008	0.044	0.178	0.860
Open Water ($\log_{10}$)	0.030	0.053	0.573	0.569

Table S7: Regression model predicting Coefficient of Variation (CV) of *C. pipiens* complex within each mosquito trapping season, using climate, irrigation and land cover variables. *C. pipiens* complex is made up of both *C. pipiens* and *C. quinquefasciatus* and CV estimates were averaged for each detailed analysis unit (n=78). Transformations were used to linearize relationships and improve normality in residuals; Shapiro-Wilk test, P>0.13. Generalized Least Squares model including spatial autocorrelation with exponential correlation structure, R^2=0.08.

GLS Model ~ $\log_{10}$(CV of *C. pipiens complex*+0.1)	Coefficient	SE	T-value	P-value
Intercept	2.413	0.271	8.891	<0.001
Temperature	-0.004	0.014	-0.294	0.770
Precipitation ($\log_{10}$)	0.062	0.121	0.508	0.613
Irrigation ($\log_{10}$+0.01)	-0.017	0.033	-0.525	0.601
Wetland ($\log_{10}$)	0.030	0.026	1.176	0.244
Developed ($\log_{10}$)	-0.046	0.047	-0.973	0.334
Open Water ($\log_{10}$)	0.015	0.037	0.408	0.684

Table S8: Regression model predicting human WNV disease incidence within California counties, using climate, irrigation and land cover variables. General least squares model including spatial autocorrelation: $R^2 = 0.29$, n= 47. Normality of residuals: Shapiro-Wilk test P>0.12 WNV human disease incidence is average number of WNV cases per year/100,000 people.

GLS Model ~ $\log_{10}$(WNV human incidence+0.1)	Coefficient	SE	t-value	P-value
Intercept	0.857	0.530	1.618	0.114
Temperature	0.002	0.028	0.087	0.931
Precipitation ($\log_{10}$)	0.458	0.404	1.132	0.264
Irrigation ($\log_{10}$+0.01)	0.587	0.222	2.646	0.012
Wetland ($\log_{10}$)	-0.023	0.192	-0.119	0.906
Developed ($\log_{10}$)	0.200	0.229	0.874	0.387
Open Water ($\log_{10}$)	-0.508	0.365	-1.390	0.172

CHAPTER 3

VECTOR DIVERSITY MAINTAINS WEST NILE VIRUS TRANSMISSION ACROSS LAND USE GRADIENTS

ABSTRACT

Although species diversity is known to play a key role in maintaining ecosystem processes across environmental gradients, this pattern is usually studied for ecosystem services - processes that are beneficial to humans. We examined the role of different mosquito vectors in transmitting West Nile virus to humans across agricultural and urbanization land use gradients. We quantified the risk of West Nile virus (WNV) transmission to humans from 6 *Culex* species by integrating mosquito abundance, infection prevalence, vector competence, and blood feeding patterns, and examined correlations between risk indices and human cases. Human WNV cases were strongly correlated with the density of infectious vectors feeding on humans. However, different mosquito vector species contributed to transmission in different land use types and within seasons and across years. *Culex tarsalis* was more abundant in agricultural areas, whereas *C. pipiens* and *C. quinquefasciatus* were abundant in developed and agricultural areas, and *C. erythrothorax* was abundant in wetland areas. As a result, WNV risk did not change substantially along either agricultural or urbanization land use gradients because the diversity of vectors maintained high disease risk across a range of habitats. These results show how a

diversity of vectors can maintain an ecosystem disservice - vector borne disease – by their differential response to environmental disturbance.

INTRODUCTION

Species diversity plays a key role in maintaining ecosystem processes across space, time, and environmental gradients (Ives et al. 1999; Cardinale et al. 2006; Hooper et al. 2012). However, this pattern is usually studied for processes that are beneficial to humans, such as ecosystem services (Hooper et al. 2005; Cardinale et al. 2012). Few studies have examined the effects of diversity in maintaining ecosystem processes that are detrimental to humans, termed ecosystem disservices (Daszak & Kilpatrick 2009), such as disease transmission. Species diversity of hosts and vectors likely contributes to transmission across a range of habitats and time periods because of their differential response to environmental variation (Ives et al. 1999). Despite this, the contribution of vector diversity to pathogen transmission has rarely been examined. Although large-scale differences in the importance and contribution of different vector species is well known (e.g. various vector species in different parts of continents for malaria, dengue and West Nile virus (Hay et al. 2005; Sinka et al. 2011; Bowden et al. 2011; Bhatt et al. 2013; Kraemer et al. 2015), fine-scale spatio-temporal variation in their contribution is less appreciated. A better understanding of how vector diversity contributes to spatial and temporal variation in human disease risk across environmental gradients offers opportunities for more effective disease management through targeted interventions (Bousema et al. 2010).

The contribution of a vector species to human disease risk is often estimated using an index of entomological risk that includes a combination of four components, mosquito abundance, mosquito infection prevalence, mosquito feeding patterns, and vector competence (Kilpatrick et al. 2005; Bolling et al. 2009; Scott & Morrison 2010; Smith et al. 2010; Kilpatrick & Pape 2013). Land cover influences the abundance of different mosquito vectors by changing larval habitat availability, and composition of hosts interacting with vectors (Gimnig et al. 2001; Minakawa et al. 2002; Reisen 2012; Burkett-Cadena et al. 2013). Spatial and temporal changes in host abundance can result in infection prevalence differences among mosquito species (Kilpatrick et al. 2006, 2017, Hamer et al. 2008b, 2011). Seasonal or spatial asynchrony between mosquito abundance and mosquito infection prevalence can lead to differences among vector species contributions to pathogen transmission (Smith et al. 2004). Mosquito feeding patterns and vector competence also influence entomological risk, but are difficult to measure on a fine scale in space and time (Kilpatrick et al. 2006, 2010; Hamer et al. 2011). For zoonotic vector borne diseases, differences in vector traits can be especially important because the vector responsible for enzootic transmission may be different from the species transmitting the pathogen to humans. This is especially true for West Nile virus.

West Nile virus (WNV) is a globally distributed multi-host mosquito-borne pathogen introduced into North America in 1999 that quickly spread westward and became established in California by 2004 (Kilpatrick 2011). WNV causes yearly epidemics in the US with an average of ~1200 neuro-invasive cases and 120 deaths per year, but with enormous year-to-year and spatial variation (CDC 2018). Mosquitoes in the genera *Culex* are the most important vectors for WNV bird-to-bird and bird-to-human transmission (Kilpatrick et al. 2005; Hamer et al. 2008a; Kramer et al. 2008) , but species within this genus differ substantially in many traits and are differentially affected by land use and climate (Farajollahi et al. 2011; Ciota et al. 2014). *Culex tarsalis* breeds in fresh water habitats and is frequently associated with agricultural land use (Surtees 1970; Reisen et al. 1992a; Wegbreit & Reisen 2000; Crowder et al. 2013; Schurich et al. 2014b; Kovach & Kilpatrick 2018), whereas *Culex pipiens* and *Culex quinquefasciatus* breed in organically rich container habitats and is associated with urban habitats (Diuk-wasser et al. 2006; Trawinski & Mackay 2010; Andreadis 2012; Landau & Leeuwen 2012; Rochlin et al. 2016; McClure et al. 2018).

Our goal was to examine the role of different *Culex* mosquito species in the transmission of West Nile virus and whether the diversity of vectors in a relatively small region results in sustained transmission risk. We examine how the contribution of different mosquito species to human disease risk changes spatially (across agricultural and urbanization gradients) and temporally (within seasons and across years). We first examined correlations between a WNV risk index (the density of infectious vectors feeding on humans) and the number of reported WNV human cases to establish this index as a valid surrogate for WNV disease risk. We then examined variation in WNV risk across space and time, and the contribution of each vector species to risk. We hypothesized that the contributions to WNV transmission from different species would change with surrounding land use; with increased importance of *C. tarsalis* in agricultural areas as well as increased importance of *C. quinquefasciatus* and *C. pipiens* in urban areas. We also hypothesized that key vectors would alternate importance between years and within seasons due to differences in their response to climate (Reisen et al. 2010; Walton & Reisen 2013; Ciota et al. 2014; Paull et al. 2017).

METHODS

Study Area:

We used mosquito trapping data and West Nile virus mosquito testing conducted by vector control districts across California. Vector control districts throughout the state have extensive mosquito trapping programs to monitor mosquito populations and detect the presence of mosquito-borne pathogens, including West Nile virus, Western equine encephalomyelitis virus, and St. Louis encephalitis virus. These public health focused agencies focus mosquito trapping efforts near human population centers, in regions with urban and agricultural land uses.

California is approximately 400km x 1240km and encompasses a wide latitudinal range with extensive geographic and climatic variability. The state is characterized by an overall Mediterranean climate with hot dry summers and mild wet winters. We separated California into 10 discrete hydrological zones (largely based on river drainage) to examine differences in vectors in regions with different temperature and precipitation. To further divide up the latitudinal range and reflect trap location differences, we designated an 11th zone as "Shasta", in the northern part of the Sacramento hydrologic zone (Barker et al. 2010).

<u>Landcover</u>:

To examine how WNV vectors and human risk varied with land use, we quantified the amount of agricultural, developed, and wetland land covers within a 1 km buffer around each trap site (30 m resolution, (NASS 2015). For *C. tarsalis*, we expanded buffers around each trap site to 2 km based on previous studies suggesting a larger dispersal distance for this particular species (Reisen et al. 2003; Kovach & Kilpatrick 2018). Wetland land cover consisted of herbaceous and woody wetlands. Developed land cover consisted of open space, low, medium and high developed areas. Agricultural land cover consisted of any of the 75 crop types listed in the cropland 2015 dataset (NASS 2015).

Climate datasets:

To examine how inter-annual variation in climate can affect WNV risk, we estimated annual precipitation and non-winter air temperatures for each hydro-region. We used ArcGIS to obtain spatially explicit estimates across California (from (PRISM Climate Group 2018), 4 km resolution) into average annual values of precipitation (full year, beginning in the previous Dec: Dec-Nov) and temperature (mosquito season: May-Nov) for each hydro-region for years 2004-2015.

Quantifying WNV risk:

We estimated WNV risk at trap sites using the bridge vector index (Kilpatrick et al. 2005):

Risk = Mosquito abundance (daily estimates) * Mosquito West Nile virus prevalence (daily estimates based on trap site covariates) *Partial vector competence (species estimate) *Fraction of mosquitoes feeding on humans (species estimate).

Partial vector competence is the fraction of mosquitoes transmitting WNV divided by the fraction that were infected in vector competence studies, and accounts for differences in midgut and salivary barriers (Kramer 2015). We focused on six *Culex* species: *C. tarsalis*, *C. pipiens*, *C. quinquefasciatus*, *C. erythrothorax*, *C. stigmatosoma*, and *C. thriambus.*

Mosquito abundance:

We obtained mosquito trapping data from vector control districts across California via the CALSURV vector borne disease surveillance system (UC Davis 2016). We used CO_2 trapping data (obtained from CDC traps baited with dry ice) from the summer months (May-Nov) over a 12-year period following the arrival of WNV to the state (2004-2015). This dataset consisted of >140,000 unique site visits spanning 31 Californian counties.

Our goal was to describe temporal variation in the abundance of infected mosquitoes at each site. At trap sites with >=12 annual visits (n=1743), we used Generalized linear models (GLM) with a Poisson distribution to estimate daily mosquito abundance (per trap/night) for 6 *Culex* species. We fit GLM models separately for each species and each site-year. For site-years with >3 non-zero values we included up to a 4th order polynomial to allow for potential bimodal seasonal peaks in mosquito abundance (Mosquito count~$day+day^2+day^3+day^4$). For site-years having <=3 positive values we averaged mosquito abundance across season. We limited all predicted model estimates to actual date range sampled at each site-year. We inspected fitted models to ensure they mostly interpolated between observed data points and did not predict seasonal mosquito abundance patterns at sites where predicted values exceeded maximum observed values by >25%. For more detailed methods on mosquito abundance modelling see supplemental methods section.

WNV Prevalence:

We obtained data showing the presence/absence of West Nile virus in >200,000 *Culex* mosquito pools (containing >5,800,000 *Culex* mosquitoes) from 2004-2015.

For each mosquito species in each hydro region, we fit 7 generalized linear models with a binomial distribution, a logit link, and an offset for log (# of mosquitoes tested). We used the model with lowest AIC value to estimate daily prevalence estimates based on trap site covariates, sample day, and year.

1) WNV~1
2) WNV~ day + day^2
3) WNV~ day + day^2 + climate and land cover covariates
4) WNV~ day + day^2 + Year + climate and land cover covariates
5) WNV~ day + day^2 + day^3 + day^4 + Year + climate and land cover covariates
6) WNV~ day*Year + day^2*Year + climate and land cover covariates
7) WNV~ (day*Year) + (day^2 *Year) + (day^3*Year) + (day^4*Year) + climate and land cover covariates

We multiplied daily estimates of WNV prevalence with daily mosquito abundance estimates for each species at each trap location.

Vector competence:

We used species estimates of partial vector competence because finer scale data were not available.

For each mosquito species we divided the fraction of mosquitoes that transmitted WNV by the fraction that were infected from ten laboratory studies of vector competence from across the US (table S1, (Turell et al. 2000, 2001; Sardelis et al. 2001; Goddard et al. 2002; Ebel et al. 2005; Reisen et al. 2005, 2006, 2008a; Vaidyanathan & Scott 2007; Kilpatrick et al. 2010)). We only included datasets that reported both the total number of mosquitoes infected and the number transmitting 14 days post-infection from temperatures of 26-30°C and viral infection concentrations of $10^{6.5}$-$10^{7.3}$ plaque forming units/ml. We used the inverse of the standard error of the estimate of partial vector competence ($SE = (P*(1-P)/N)^{0.5}$ where P is partial vector competence, and N is the sample size) to calculate a weighted average vector competence across multiple studies with differing samples sizes.

__Fraction blood feeding on humans__:

For each mosquito species we estimated the fraction of mosquitoes that took bloodmeals from humans using fifteen studies from California (table S2) (Tempelis et al. 1965; Tempelis & Washino 1967; Gunstream et al. 1971; Reeves 1990; Reisen et al. 1992b, 2013; Wekesa et al. 1997; Lothrop & Reisen 2001; Molaei et al. 2010; Thiemann & Wheeler 2011; Montgomery et al. 2011; Thiemann et al. 2012, 2017;

Campbell et al. 2013; Nelms et al. 2016). As above, we used the inverse of the SE of each estimate to calculate a weighted average fraction feeding on humans across multiple studies with differing samples sizes. There were very limited data for feeding patterns of *C. thriambus* (one study with n=27, and 0 human bloodmeals) so we used the value for *C. stigmatosoma* for human blood-feeding fraction 0.0018.

Human WNV infection data:

We also examined whether the bridge vector index was correlated with spatial and temporal variation in WNV human risk. We obtained WNV human infection data for California counties for the years 2004-2009 (the last year CDC made this data easily available; (CDC 2018)). For each year, we summarized WNV human cases by county-week and limited our analysis to counties with >5 human cases total across years. For each county, we examined the relationship between total WNV vector index and number of human cases occurring 2 weeks later (based on the ~3-14 day delay between infection and illness; (CDC 2018)).

Statistics:

We used program R (v. 3.5.0) to perform all statistical analyses and ArcGIS (v. 10.5) to summarize temperature and precipitation data for each hydro-region. We used generalized linear models to model mosquito abundance (Poisson distribution) and West Nile prevalence (Binomial distribution with offset for # of mosquitoes tested). For analyses on the trap scale, we used generalized least squares models (R package, “gls”), including exponential spatial autocorrelation, to examine the effects of land cover on WNV human risk indices, and used R package, “PiecewiseDEM” to output R^2 values. We used Log_{10} transformations on mosquito abundance data and arcsine square root transformations on proportion land cover datasets to linearize relationships and increase normality in residuals. We examined relationships between the number of human cases per county-week and our combined WNV risk index (with a 2-week time lag) using generalized least squares including temporal autocorrelation with a moving average correlation structure grouped within years (corARMA). On several graphs, for display purposes, we separated land cover (developed, agricultural, wetland) into 10 groups of equal sizes. However, due to rarity of wetland cover surrounding trap sites, we included one group with zero wetland land cover and then separated positive wetland values into 9 equal categories.

RESULTS

Overall WNV risk and the primary vectors associated with human WNV transmission varied substantially across California (figure 1) and at smaller scales, depending on land cover surrounding trap sites (figure 2). Total WNV risk to humans from all *Culex* species was significantly correlated with number of human WNV cases 2 weeks later and explained 19% of the variation in all human cases statewide and up to 67% of variation within specific counties (figures 3 & S1, table S3). Four of the six *Culex* species studied contributed nearly all (99.8%) of WNV human risk; *C. tarsalis* (45.9%), *C. pipiens* (22.5%), *C. quinquefasciatus* (19.9%), and *C. erythrothorax* (11.4%; table S4). In addition, each of these four species were the most important WNV vector in at least one hydro-region (figures S2-S5, table S4). Extremely low mosquito abundance coupled with low human blood-feeding rates resulted in minimal contributions to WNV human risk from *C. stigmatosoma* (0.2%) and *C. thriambus* (0.03%; table S4).

The four most important vectors showed differential responses to land use gradients. *C. tarsalis* increased with agricultural land cover*, C. erythrothorax* increased with wetland land cover, and *C. pipiens* and *C. quinquefasciatus* increased with developed and agricultural land cover (figures 2 & S2-S8, tables S5-S7). The presence of multiple vector species contributing to WNV risk resulted in risk being essentially constant across nearly the entire range of urbanization and agricultural land use gradients (table S4, figures 1b, 2) in California. Only in the most agriculturally intensive areas (with >68% agricultural land cover, which were the same as those with the least (<5%) urban cover) was WNV risk different than along the rest of the range of land use. Similarly, in subregions with multiple vectors, WNV risk did not substantially change along most of the land use gradients primarily because the diversity of vectors maintained transmission across the land use gradient (figures 2, S2-S5; table S8).

Similarly, multiple mosquito species contributed to WNV risk across years (figure 4) and within seasons (figures S6-S9). Variation in both mosquito abundance and infection prevalence among species resulted in differential contributions to WNV risk at different times (figure 4). For example, in a year in which the normally dominant vector in a region (Sacramento hydro region in 2005), *C. tarsalis*, was at lower abundance two other species, *C. pipiens*, and *C. erythrothorax* had higher than average mosquito abundance and infection prevalence, resulting in sustained WNV risk (figure 4b). Different vectors also contributed to WNV risk at different times within seasons (figures S6-S9). For example, in the San Joaquin River hydro region in 2014 and 2015, early season transmission was dominated by *C. tarsalis*, midseason transmission dominated by *C. pipiens*, and late season transmission dominated by *C. erythrothorax* (figure S8).

CH 3- FIGURES

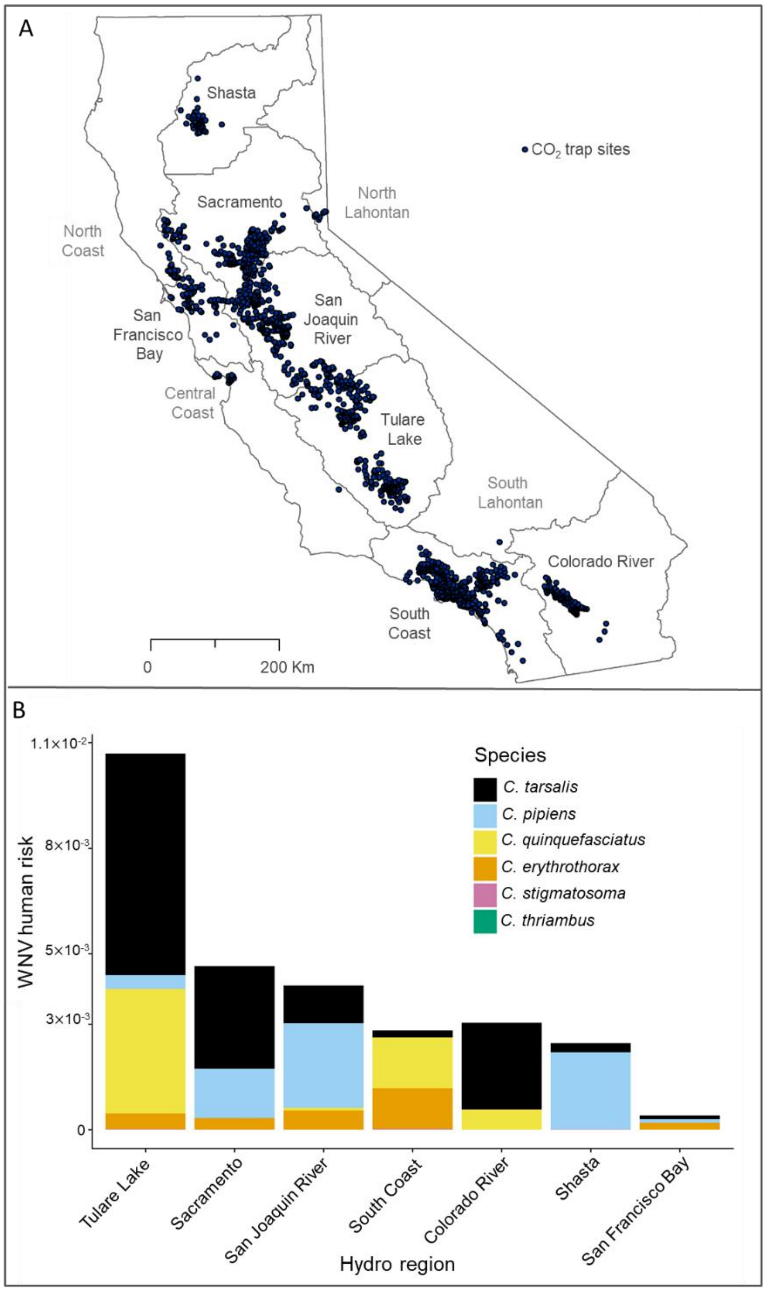

Figure 1: Mosquito trap locations and human West Nile risk across hydro regions in California. A) CO$_2$ mosquito trap locations (n=1743) and hydro-regions (n=11) in California. **B)** Average daily WNV human risk plotted for seven hydro-regions across all years (2004-2015). Risk is the number of WNV-infectious mosquitoes feeding on humans per CO$_2$ trap/night. Hydro-regions are sorted by overall risk values. Four hydro-regions (North Lahontan, South Lahontan, North Coast, and Central Coast) with $<5*10^{-4}$) are not shown.

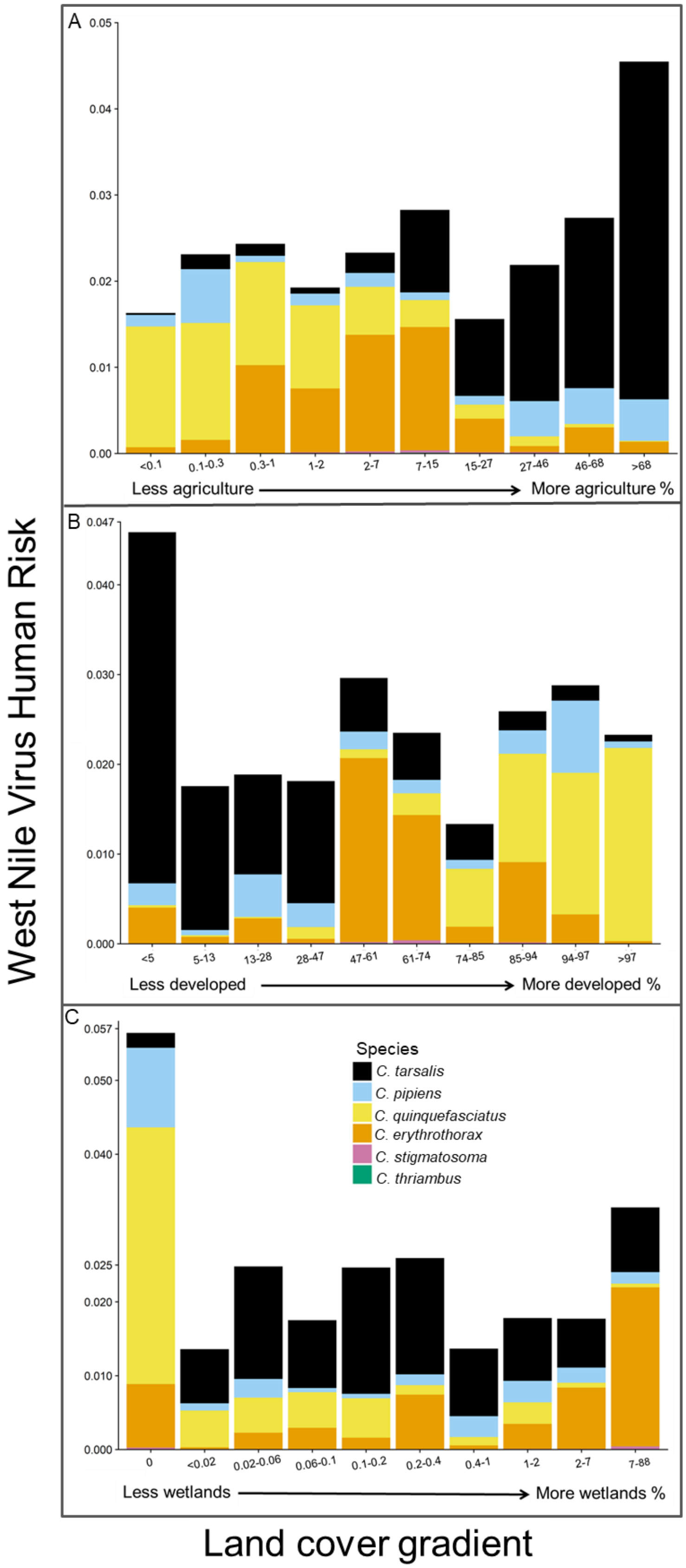
A
0.05
0.04
0.03
0.02
0.01
0.00
<0.1
0.1-0.3
0.3-1
1-2
2-7
7-15
15-27
27-46
46-68
>68
Less agriculture
More agriculture %
B
0.047
0.040
0.030
0.020
0.010
0.000
<5
5-13
13-28
28-47
47-61
61-74
74-85
85-94
94-97
>97
Less developed
More developed %
C
0.057
0.050
0.040
0.025
0.020
0.010
0.000
0
<0.02
0.02-0.06
0.06-0.1
0.1-0.2
0.2-0.4
0.4-1
1-2
2-7
7-88
Less wetlands
More wetlands %
Species
C. tarsalis
C. pipiens
C. quinquefasciatus
C. erythrothorax
C. stigmatosoma
C. thriambus
West Nile Virus Human Risk
Land cover gradient

Figure 2: Human West Nile risk across agricultural, urbanization, and wetland gradients. Trap sites are grouped into 10 intervals with similar sample sizes. For display purposes to show differences within *C. pipiens* complex, we excluded trap sites located in hydro-regions where both *C. pipiens* and *C. quinquefasciatus* overlap and hybridize (Central Coast, Tulare Lake, and San Joaquin River). n=1029 trap sites.

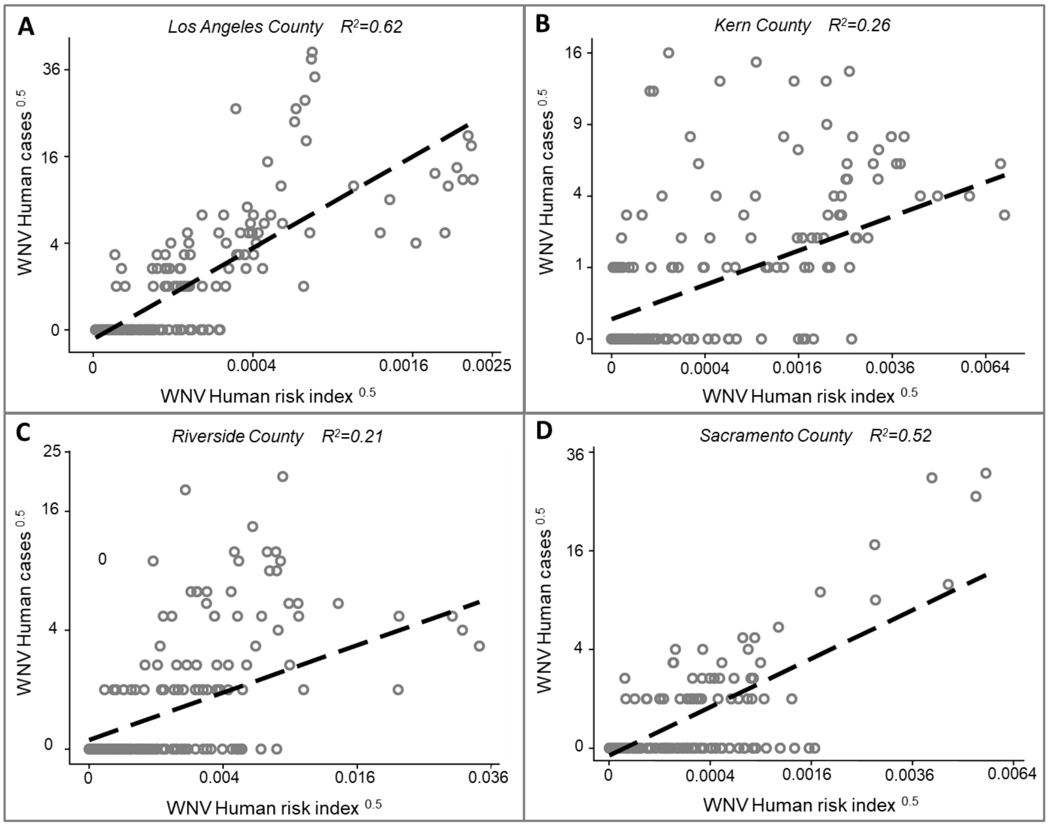

Figure 3: Human cases per county-week-year predicted by WNV human risk index per county week-year. WNV human cases reported to CDC during years 2004-2009. WNV human risk estimated using the mean number of infectious mosquitoes feeding on humans per county-year-week. Counties listed in order from highest to lowest overall number of total human WNV cases. Includes a 2-week lag between variables- i.e. Human cases per county-year predicted by mosquito index 2 week prior in same county-year. Generalized least square models including temporal autocorrelation with moving average correlation structure (corARMA) grouped within years.

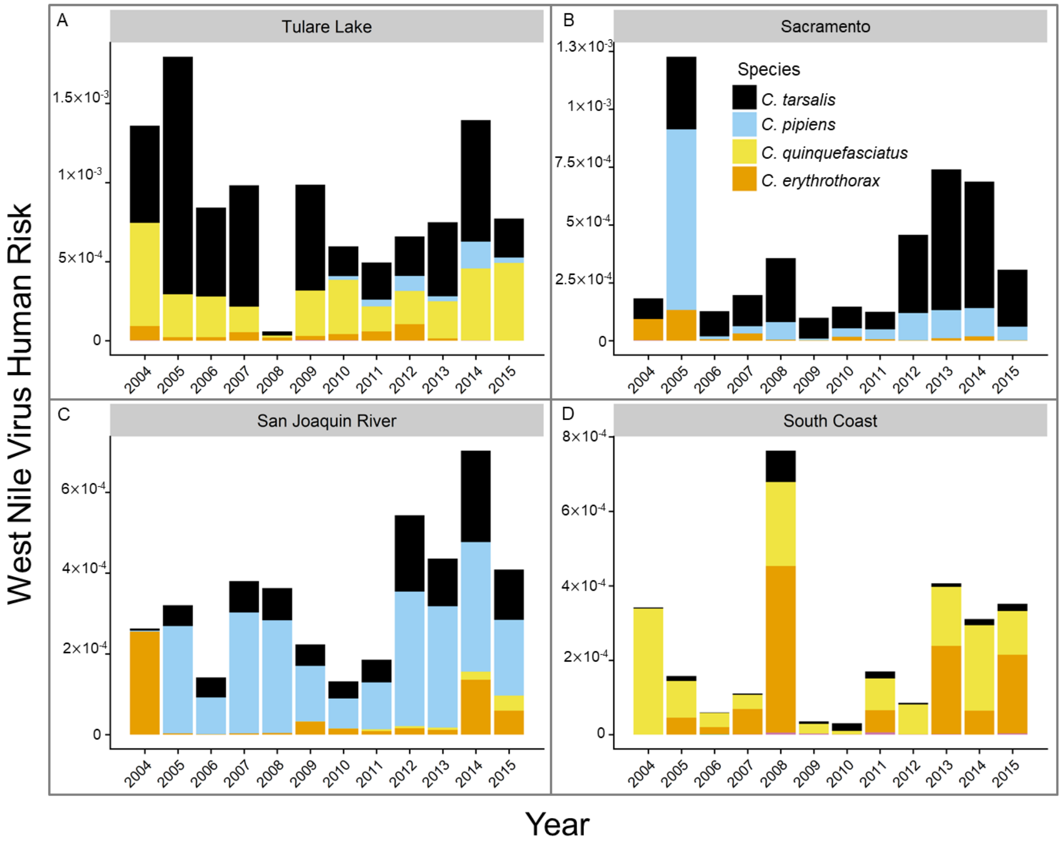

Figure 4: Inter-annual variation in human West Nile risk across hydro regions in California. Hydro-regions listed in order from highest to lowest overall risk values. WNV risk estimated using the mean number of infectious mosquitoes feeding on humans per trap/night. Other hydro regions in supplemental.

DISCUSSION

Biodiversity plays a key role in the provision of ecosystem services across a range of environmental conditions (Cardinale et al. 2012; Hooper et al. 2012). We found that the presence of multiple vector species had a similar effect in maintaining pathogen transmission across a range of habitats and over time. This was because each mosquito vector responded differently to land use cover, with *C. tarsalis* increasing with agricultural land use (as in other studies; (Surtees 1970; Reisen et al. 1992a; Wegbreit & Reisen 2000; Crowder et al. 2013; Schurich et al. 2014b; Kovach & Kilpatrick 2018)), *C. pipiens* and *C. quinquefasciatus* increasing with developed land use and somewhat with agricultural land use (Diuk-wasser et al. 2006; Trawinski & Mackay 2010; Andreadis 2012; Landau & Leeuwen 2012; Rochlin et al. 2016; McClure et al. 2018), and *C. erythrothorax* increasing with wetland cover (Walton & Workman 1998; Workman & Walton 2000; Sanford et al. 2005; Reisen & Lothrop 2008; Walton et al. 2012, 2013; Rey et al. 2012). Similarly, mosquito species abundance peaked in different years and in different periods seasonally, which resulted in much more sustained WNV risk across space and time than would have been the case had only a single species been present across this region.

As with biodiversity and ecosystem services, the role of vector diversity in maintaining high WNV transmission across space and time was driven by the specific traits of the vector species (Tilman 1999). The four key vectors in California (many of which also occur in other parts of the Western USA (Kilpatrick & Pape 2013)) maintain high WNV risk because they are abundant (each in different habitats or at different times), are competent vectors for WNV (the virus replicates within them and can escape their midgut and reach and infect their salivary glands (Turell et al. 2001), and they feed both on birds from which they acquire the virus and on humans to which they transmit the virus (Kilpatrick et al. 2005, 2007; Farajollahi et al. 2011). In other regions of the USA there is a diversity of vector species, but all but one or two species have traits that make them poor vectors for WNV. For example, in forested areas in the northeastern USA the most abundant mosquito species are often in the *Aedes* genus and feed primarily on mammals, resulting in inefficient transmission of WNV. The two important WNV vectors in this region (*C. pipiens* and *C. restuans*) have the key traits described above, but are rare in forested areas with low development (Andreadis et al. 2004; Kilpatrick et al. 2005).

The differential use of larval habitats by these species minimizes competition among them, creates challenges for control, and results in species complementarity, as has been found for plant species diversity increasing productivity (Cardinale et al. 2007). *C. pipiens* and *C. quinquefasciatus* are container-breeding vectors with water that is highly rich in organic matter, whereas *C. tarsalis* breeds in newly created water pools surrounded with vegetation that are not excessively polluted, and *C.*

erythrothorax are found in wetland areas containing emergent vegetation (Workman & Walton 2000; Reisen 2012). This presence of a diverse set of mosquito vectors emphasizes the need for diverse and adaptive vector control efforts to minimize human WNV outbreaks. A similar idea has recently been proposed for mosquito vectors of malaria (Killeen & Reed 2018).

Vector borne pathogens are responsible for infecting more than 1 billion people annually resulting in over 1 million deaths worldwide (Lozano et al. 2012; Bhatt et al. 2013; World Health Organization 2014).

We found that a diversity of vector species can promote an ecosystem disservice, the sustained transmission of WNV, over space and time which creates challenges for effectively limiting the spread of vector borne pathogens. The introduction of vectors to new regions (Tatem et al. 2006) only compounds this challenge and is a key threat to human health.

CH 3- SUPPLEMENTARY TABLES

Table S1: Summary of vector competence studies for 6 *Culex* species. Partial vector competence values used in this study (#transmitting/#infected) highlighted in bold. Species abbreviations: TAR= *C. tarsalis*, PIP=*C. pipiens*, QNX=*C. quinquefasciatus*, ERY=*C. erythrothorax*, THR=*C. thriambus*. We used inverse of binomial SE to calculate a weighted average across multiple studies with different samples sizes.

Species	Partial Vector Competence	# tested	# infected	# transmitting	PFU/ml	Strain	Locations	Reference
ERY	**0.64**	25	25	16	$10^{7.1}$	WNV strain 35211	CA:Orange county	Goddard et al. 2002
PIP	0.33	1873	399	132	10^{7}	WNV strain 3356	MA, NY, + Colony popn's	Kilpatrick et al. 2010
PIP	0.19	795	112	21	$10^{6.7}$	WNV strain 3356	NY: Albany, Richmond, Suffolk counties	Ebel et al. 2005
PIP	0.25	95	77	19	$10^{7.2}$		NY	Turell et al. 2001
PIP	0.18	83	67	12	$10^{7.2}$	Crow 397–99 strain	NY	Turell et al. 2000
PIP	0.29	103	59	17	$10^{6.8}$	WNV strain 35211	CA	Reisen et al. 2008
PIP	0.71	31	31	22	$10^{7.1}$	WNV strain 35211	CA: Shasta county	Goddard et al. 2002
PIP	0.57	58	14	8	$10^{6.8}$		CA: Shasta and Merced counties	Vaidyanathan & Scott 2007
PIP AVG	**0.31**							
QNX	0.22	412	279	61	$10^{6.8}$	WNV strain 35211	CA	Reisen et al. 2008
QNX	0.71	158	78	55	$10^{7.1}$	WNV strain 35211	CA: Kern, Riverside, Orange counties	Goddard et al. 2002
QNX	0.23	78	71	16	10^{7}	WNV strain 35211	FL Sebring	Sardelis et al. 2001
QNX	0.23	30	26	6	$10^{7.8}$	WNV strain 35211	CA: Whittier	Reisen et al. 2006
QNX	0.04	30	23	1	$10^{6.4}$	WNV strain 35211	CA: San Gabriel dam	Reisen et al. 2006
QNX	0.11	25	19	2	$10^{7.3}$	WNV strain 35211	CA: LA-metro	Reisen et al. 2005
QNX	0.12	24	16	1.92	$10^{7.3}$	WNV strain 35211	CA: LA-Machado	Reisen et al. 2005
QNX	0.18	25	11	2	$10^{7.3}$	WNV strain 35211	CA:Coachella	Reisen et al. 2005
QNX	0.18	25	11	2	$10^{7.3}$	WNV strain 35211	CA:LA-San	Reisen et al. 2005
QNX	0.63	43	8	5	$10^{6.8}$		CA: San Bernadino	Vaidyanathan & Scott 2007
QNX AVG	**0.26**							
STG	0.24	48	37	9	$10^{7.1}$	WNV strain 35211	CA:San Bernadino county	Goddard et al. 2002
STG	0.45	22	20	9	$10^{6.5}$	WNV strain 35211	CA	Reisen et al. 2006
STG	0.18	19	17	3.04	$10^{5.8}$	WNV strain 35211	CA:LA-Panorama	Reisen et al. 2005
STG AVG	**0.28**							
TAR	0.33	509	356	117	$10^{6.8}$	WNV strain 35211	CA	Reisen et al. 2008
TAR	0.76	91	74	56	$10^{7.1}$	WNV strain 35211	CA:Yolo,Kern,Riverside counties	Goddard et al. 2002
TAR	0.54	30	24	13	$10^{6.5}$	WNV strain 35211	CA	Reisen et al. 2006
TAR	0.12	18	16	1.98	$10^{6.6}$	WNV strain 35211	CA: COAV-c	Reisen et al. 2005
TAR	0.47	22	15	7	$10^{6.8}$	WNV strain 35211	CA: Coachella-WWDC	Reisen et al. 2005
TAR	0.14	25	14	2	$10^{6.6}$	WNV strain 35211	CA: KNWR-c	Reisen et al. 2005
TAR	0.33	19	9	3	$10^{6.6}$	WNV strain 35211	CA: Yolo county	Reisen et al. 2005
TAR	0.71	15	7	5	$10^{6.8}$	WNV strain 35211	CA: Kern-Bakersfield	Reisen et al. 2005
TAR AVG	**0.40**							
THR	**0.46**	16	13	6	$10^{6.5}$	WNV strain 35211	CA	Reisen et al. 2006

Table S2: Summary of blood-feeding studies for 6 *Culex* species. Human blood-feeding values used in this study highlighted in bold. Species abbreviations: TAR= *C. tarsalis*, PIP=*C. pipiens*, QNX=*C. quinquefasciatus*, ERY=*C. erythrothorax*, THR=*C. thriambus*. To estimate WNV risk index for *C. thriambus* we substituted in *C. stigmatosoma* human blood feeding fraction due to small sample size for *C. thriambus*. We used inverse of binomial SE to calculate a weighted average across multiple studies with different samples sizes.

Species	**Fraction Human**	**Fraction Mammal**	**Fraction Bird**	**N**	**# human**	**# mammal**	**# bird**	**Locations**	**References**
ERY	0.015	0.39	0.61	67	1	26	41	Orange and Riverside county	Molaei et al. 2010
ERY	0.015	0.42	0.58	67	1	28	39	Riverside county	Gunstream et al. 1971
ERY	0.000	0.83	0.17	24	0	20	4	Sacramento Valley	Reeves 1990
ERY	0.048	0.88	0.12	334	16	293	41	Orange and Los Angeles counties	Reisen et al. 1992
ERY	0.000	0.97	0.03	984	0	955	29	Kern county	Reeves 1990
ERY AVG	**0.0273**								
PIP	0.000	0	1	29	0	0	29	Yolo county	Thiemann et al. 2012
PIP	0.000	0.01	0.99	330	0	3	327	Sacramento and Yolo counties	Montgomery et al. 2011
PIP	0.013	0.01	0.99	151	2	2	149	Sutter county	Campbell et al. 2013
PIP	0.019	0.02	0.98	107	2	2	105	Sutter county	Thiemann et al. 2012
PIP	0.000	0.25	0.75	28	0	7	21	Sacramento Valley	Reeves 1990
PIP AVG	**0.0136**								
QNX	0.000	0.01	0.99	343	0	3	340	Kern county	Reeves 1990
QNX	0.000	0.06	0.94	162	0	9	153	Kern county	Thiemann et al. 2012
QNX	0.007	0.05	0.92	273	2	13	250	Los Angeles county	Thiemann et al. 2012
QNX	0.000	0.09	0.91	397	0	34	363	Riverside county	Thiemann et al. 2012
QNX	0.019	0.12	0.88	424	8	49	375	Orange, Riverside, and San Bernadino counties	Molaei et al. 2010
QNX	0.006	0.37	0.63	521	3	195	326	Orange and Los Angeles counties	Reisen et al. 1992
QNX AVG	**0.0088**								
STG	0.000	0	1	11	0	0	11	Orange, Riverside, and San Bernadino counties	Molaei et al. 2010
STG	0.000	0	1	58	0	0	58	Kern county	Reeves 1990
STG	0.000	0	1	206	0	0	206	Lake county	Theiman et al. 2017
STG	0.002	0.03	0.97	539	1	16	523	Sacramento Valley	Reeves 1990
STG	0.000	0.16	0.84	56	0	9	47	Orange and Los Angeles counties	Reisen et al. 1992
STG AVG	**0.0018**								
TAR	0.000	0	1	116	0	0	116	Yolo county	Thiemann & Wheeler 2011
TAR	0.000	0.04	0.96	459	0	18	441	Kern county- Hart Park	Tempelis et al. 1965
TAR	0.000	0.03	0.96	90	0	3	86	Kern county	Thiemann et al. 2012
TAR	0.000	0.12	0.88	8888	0	1067	7821	Kern county	Tempelis et al. 1965
TAR	0.008	0.11	0.88	132	1	15	116	Lake county	Theiman et al. 2017
TAR	0.000	0.13	0.88	168	0	21	147	Yolo county	Thiemann & Wheeler 2011
TAR	0.051	0.15	0.85	39	2	6	33	Orange and Riverside counties	Molaei et al. 2010
TAR	0.000	0.17	0.83	102	0	17	85	Orange and Los Angeles counties	Reisen et al. 1992
TAR	0.000	0.17	0.83	89	0	15	74	Yolo county	Thiemann et al. 2012
TAR	0.004	0.18	0.82	1413	5	259	1154	Kern county- Jerry Slough	Tempelis et al. 1965
TAR	0.011	0.18	0.77	265	3	48	203	Riverside county	Reisen et al. 2013
TAR	0.011	0.26	0.74	93	1	24	69	Riverside county	Thiemann et al. 2012
TAR	0.000	0.27	0.73	231	0	63	168	Sutter county	Thiemann et al. 2012
TAR	0.002	0.28	0.72	601	1	168	433	Sutter county	Campbell et al. 2013
TAR	0.017	0.29	0.71	939	16	269	670	Sutter county	Wekesa et al. 1997
TAR	0.000	0.30	0.70	603	0	182	421	Riverside county	Lothrop & Reisen 2001
TAR	0.000	0.41	0.58	951	0	394	556	Butte, Sutter, Yolo, and West Valley foothills	Tempelis & Washino 1967
TAR	0.020	0.48	0.50	247	5	119	124	Riverside county	Gunstream et al. 1971
TAR	0.000	0.70	0.30	822	0	578	244	Kern county- Poso Creek	Tempelis et al. 1965
TAR AVG	**0.0054**								
THR	**0**	0	1	27	0	0	27	Lake county	Nelms et al. 2016

Table S3: Generalized least square models predicting human cases per week in 15 California counties using bridge vector index. We used square root transformations on WNV human cases and bridge vector index to linearize relationships and improve normality in residuals. Generalized Least Squares models including temporal autocorrelation with moving average correlation structure (corARMA) grouped within years; for "all counties" analysis temporal autocorrelation grouped within county-years.

Response= Human cases per county-week$^{0.5}$, Predictor= bridge vector index$^{0.5}$ (2-week lag)	Intercept	Slope	Slope SE	R^2	N
Los Angeles	-0.22	105.33	11.08	0.62	182
Kern	0.28	23.95	5.27	0.26	168
Riverside	0.15	39.84	9.60	0.21	180
Sacramento	-0.15	49.13	5.62	0.52	164
Stanislaus	0.17	16.44	7.25	0.08	153
Orange	-0.21	95.15	9.91	0.67	90
San Joaquin	-0.07	31.72	7.51	0.24	149
San Bernardino	-0.17	44.63	5.79	0.52	122
Yolo	0.06	11.39	6.21	0.06	137
Fresno	0.13	16.50	3.95	0.21	94
Placer	-0.08	29.23	4.04	0.36	140
Merced	-0.05	25.60	8.16	0.22	99
Kings	0.01	7.15	1.48	0.18	88
Contra Costa	-0.09	31.69	9.69	0.14	78
Shasta	-0.16	13.31	7.58	0.14	33
All Counties	0.06	27.50	2.08	0.19	1877

Table S4: Summary table showing percent contribution to overall WNV human risk from 6 *Culex* species across hydro-regions.

Hydro-region	*Culex tarsalis*	*Culex pipiens*	*Culex quinquefasciatus*	*Culex erythrothorax*	*Culex stigmatosoma*	*Culex thriambus*
Tulare Lake	58.9	3.7	33.0	4.2	0.1	0
Sacramento	62.7	30.2	0	6.9	0.1	0.1
San Joaquin River	26.2	58.7	1.8	13.3	0.01	<0.001
South Coast	7.1	0	51.2	40.8	0.7	0.1
Colorado River	81.1	0	18.9	<0.001	<0.001	0
Shasta	10.4	89.2	0	<0.001	0.3	0.1
San Francisco Bay	25.9	25.9	0	48.2	0.1	0.002
All of California	45.9	22.5	19.9	11.4	0.2	0.03

Table S5: Regression model predicting *C. tarsalis* WNV vector index using land cover data. WNV vector index is averaged across years at each trap site (n=1743). We used $\log_{10}$ transformation on mosquito index and arc sine square root transformations on proportion of each land cover (2 km buffer) to linearize relationships and improve normality in residuals. Generalized Least Squares model including spatial autocorrelation with exponential correlation structure, R^2=0.32.

Response= log10(*C. tarsalis* +10^{-6})	Coefficient	SE	t-value	p-value
Intercept	-5.886	0.126	-46.859	<0.001
Agricultural land (arcsin)	1.540	0.089	17.322	<0.001
Developed (arcsin)	0.071	0.098	0.722	0.470
Wetland (arcsin)	-0.021	0.197	-0.107	0.915

Table S6: Regression model predicting *C. pipiens and quinquefasciatus* WNV vector index using land cover data. WNV vector index is averaged across years at each trap site (n=1743). We used $\log_{10}$ transformations on mosquito abundance and arc sine square root transformations on proportion of each land cover (1 km buffer) to linearize relationships and improve normality in residuals. Generalized Least Squares model including spatial autocorrelation with exponential correlation structure, R^2=0.15. These species were combined into same analysis to include all trap sites across California.

Response= $\log_{10}$(*C. pipiens/C. quinquefasciatus*+10^{-6})	Coefficient	SE	t-value	p-value
Intercept	-7.363	0.224	-32.880	<0.001
Agricultural land (arcsin)	1.651	0.156	10.590	<0.001
Developed (arcsin)	1.934	0.172	11.238	<0.001
Wetland (arcsin)	0.0143	0.309	0.046	0.9631

Table S7: Regression model predicting *C. erythrothorax* WNV vector index using land cover data. WNV vector index is averaged across years at each trap site (n=1743). We used $\log_{10}$ transformation on mosquito abundance and arc sine square root transformations on proportion of each land cover (1km buffer) to linearize relationships and improve normality in residuals. Generalized Least Squares model including spatial autocorrelation with exponential correlation structure, R^2=0.10.

Response= $\log_{10}$(*C. erythrothorax* +10^{-6})	Coefficient	SE	t-value	p-value
Intercept	-6.628	0.137	-48.393	<0.001
Agricultural land (arcsin)	0.089	0.096	0.924	0.356
Developed (arcsin)	-0.040	0.104	-0.381	0.703
Wetland (arcsin)	**2.230**	**0.186**	**12.004**	**<0.001**

Table S8: Regression model predicting overall combined WNV vector index using land cover data. WNV vector index is averaged across years at each trap site. We used $\log_{10}$ transformations on mosquito index and arc sine square root transformations on proportion of each land cover (2 km buffer) to linearize relationships and improve normality in residuals. Generalized Least Squares model including spatial autocorrelation with exponential correlation structure.

Hydro-region	GLS Model ~ log10(all CX+10^{-6})	Coefficient	SE	t-value	P-value
Tulare Lake, n=340, R^2=0.13	Intercept	-2.150	1.143	-1.882	0.061
	Agricultural land (arcsin)	-0.821	0.731	-1.123	0.262
	Developed (arcsin)	-1.497	0.757	-1.979	0.049
	Wetland (arcsin)	0.702	0.908	0.774	0.440
Sacramento, n=295, R^2=0.33	Intercept	-5.771	0.190	-30.355	<0.001
	Agricultural land (arcsin)	1.421	0.155	9.184	<0.001
	Developed (arcsin)	1.142	0.162	7.041	<0.001
	Wetland (arcsin)	1.352	0.611	2.214	0.028
San Joaquin River, n=332, R^2=0.04	Intercept	-4.659	0.825	-5.649	<0.001
	Agricultural land (arcsin)	0.679	0.527	1.287	0.199
	Developed (arcsin)	0.521	0.549	0.948	0.344
	Wetland (arcsin)	-0.131	0.495	-0.265	0.791
South Coast, n=432, R^2=0.16	Intercept	-6.165	0.246	-25.069	<0.001
	Agricultural land (arcsin)	1.405	0.313	4.490	<0.001
	Developed (arcsin)	1.505	0.178	8.450	<0.001
	Wetland (arcsin)	1.003	0.395	2.543	0.011
Colorado River, n=117, R^2=0.31	Intercept	-4.956	0.303	-16.369	<0.001
	Agricultural land (arcsin)	0.222	0.278	0.798	0.427
	Developed (arcsin)	0.163	0.313	0.520	0.604
	Wetland (arcsin)	10.595	2.180	4.860	<0.001
Shasta, n=50, R^2=0.27	Intercept	-4.551	0.313	-14.533	<0.001
	Agricultural land (arcsin)	0.166	0.450	0.369	0.714
	Developed (arcsin)	-0.619	0.360	-1.719	0.092
	Wetland (arcsin)	2.320	1.369	1.695	0.097
San Francisco Bay, n=81, R^2=0.27	Intercept	-7.720	0.590	-13.095	<0.001
	Agricultural land (arcsin)	0.613	0.493	1.243	0.218
	Developed (arcsin)	2.142	0.490	4.368	<0.001
	Wetland (arcsin)	2.170	0.615	3.526	0.001
All of California, n=1743, R^2=0.16	Intercept	-5.760	0.111	-51.733	<0.001
	Agricultural land (arcsin)	1.405	0.079	17.833	<0.001
	Developed (arcsin)	1.079	0.087	12.453	<0.001
	Wetland (arcsin)	0.059	0.174	0.338	0.735

CH 3- SUPPLEMENTAL FIGURES

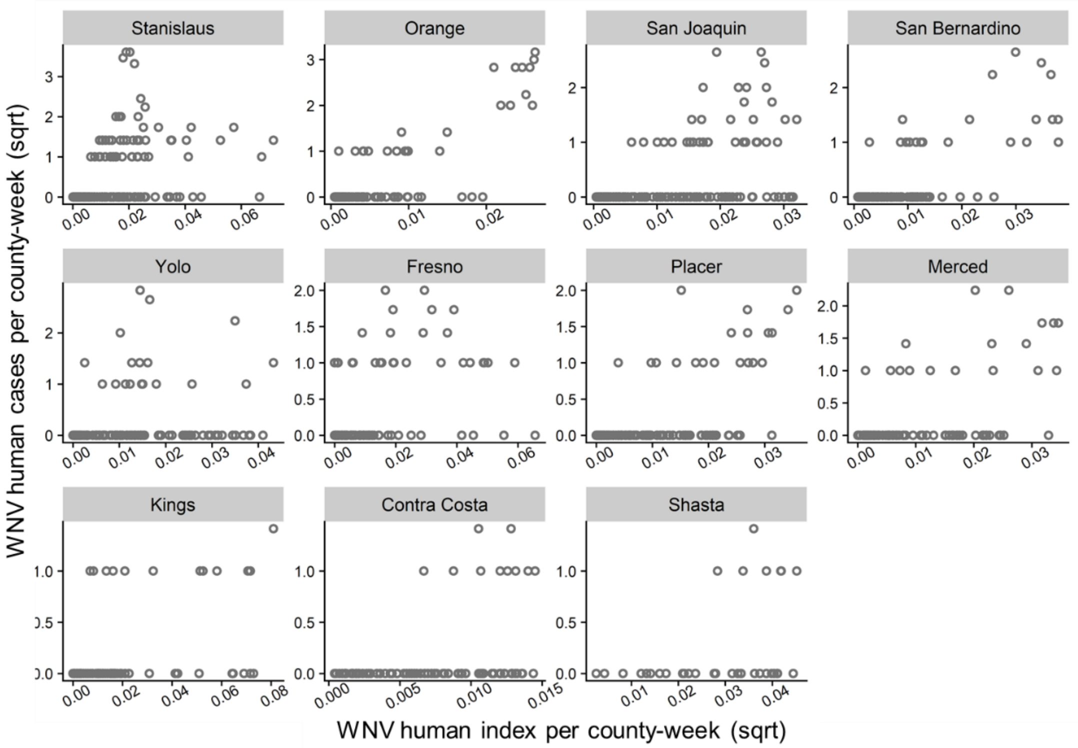

Figure S1: Human cases per county-week-year predicted by WNV human risk index per county week-year. WNV human cases reported to CDC during years 2004-2009. WNV human risk estimated using the mean number of infectious mosquitoes feeding on humans per county-year-week. Counties listed in order from highest to lowest overall number of total human WNV cases. Includes a 2-week lag between variables- i.e. Human cases per county-year predicted by mosquito index 2 week prior in same county-year. Generalized least square models including temporal autocorrelation with moving average correlation structure (corARMA) grouped within years. Other counties shown in main figures.

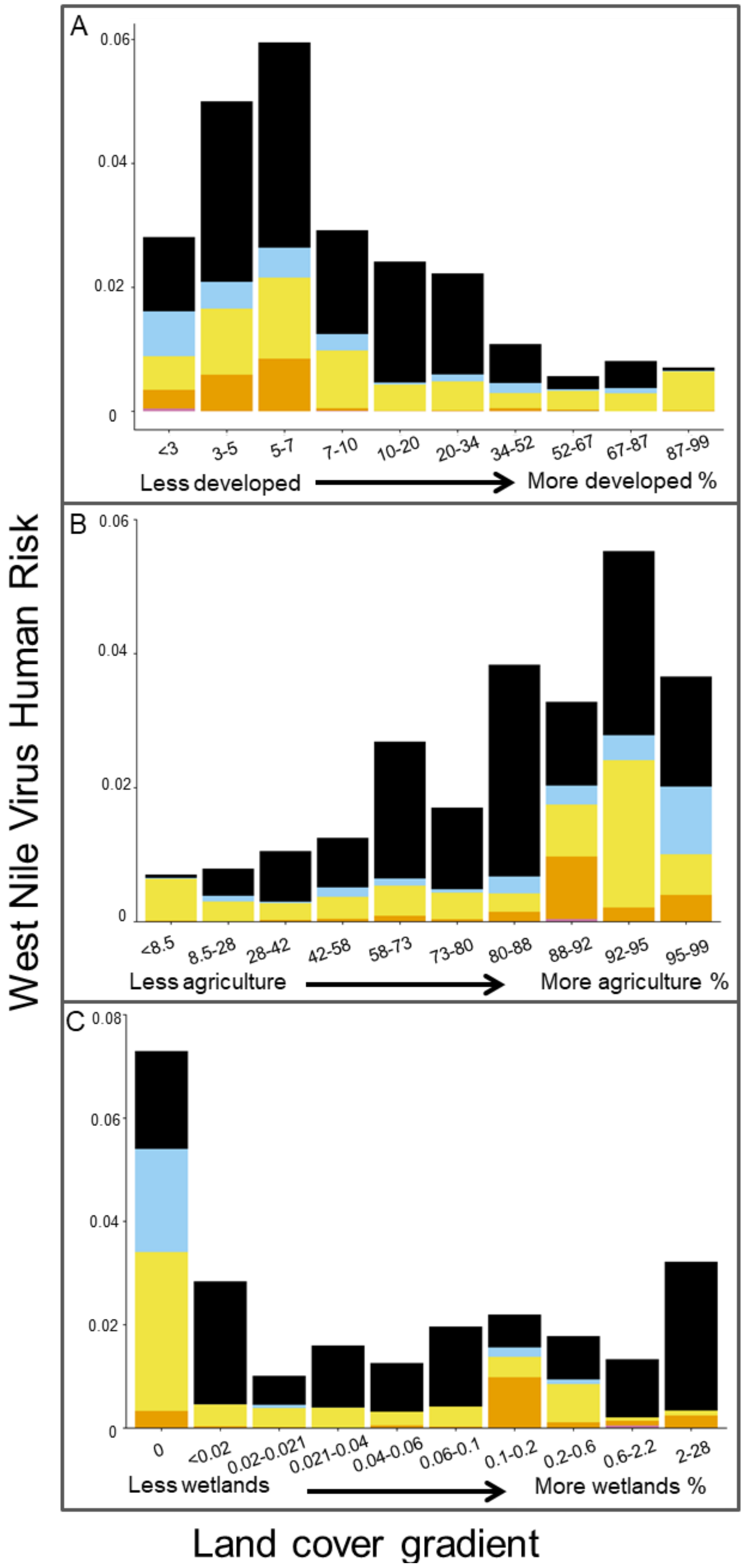
West Nile Virus Human Risk
A
0.06
0.04
0.02
0
<3
3-5
5-7
7-10
10-20
20-34
34-52
52-67
67-87
87-99
Less developed
More developed %
B
0.06
0.04
0.02
0
<8.5
8.5-28
28-42
42-58
58-73
73-80
80-88
88-92
92-95
95-99
Less agriculture
More agriculture %
C
0.08
0.06
0.04
0.02
0
0
<0.02
0.02-0.021
0.021-0.04
0.04-0.06
0.06-0.1
0.1-0.2
0.2-0.6
0.6-2.2
2-28
Less wetlands
More wetlands %
Land cover gradient

Figure S2: Human West Nile risk across land use gradients in Tulare Lake hydro region. We separated trap sites into 10 categories of similar sample sizes (using quantiles) along a developed, agricultural, and wetland gradient. WNV risk index estimated using the mean number of infectious mosquitoes feeding on humans per trap/night. Percent landcover occurring within 1 or 2 km buffer around trap sites (2 km buffer used for *C. tarsalis*, 1 km buffer for all other *Culex* species. n=340 trap sites.

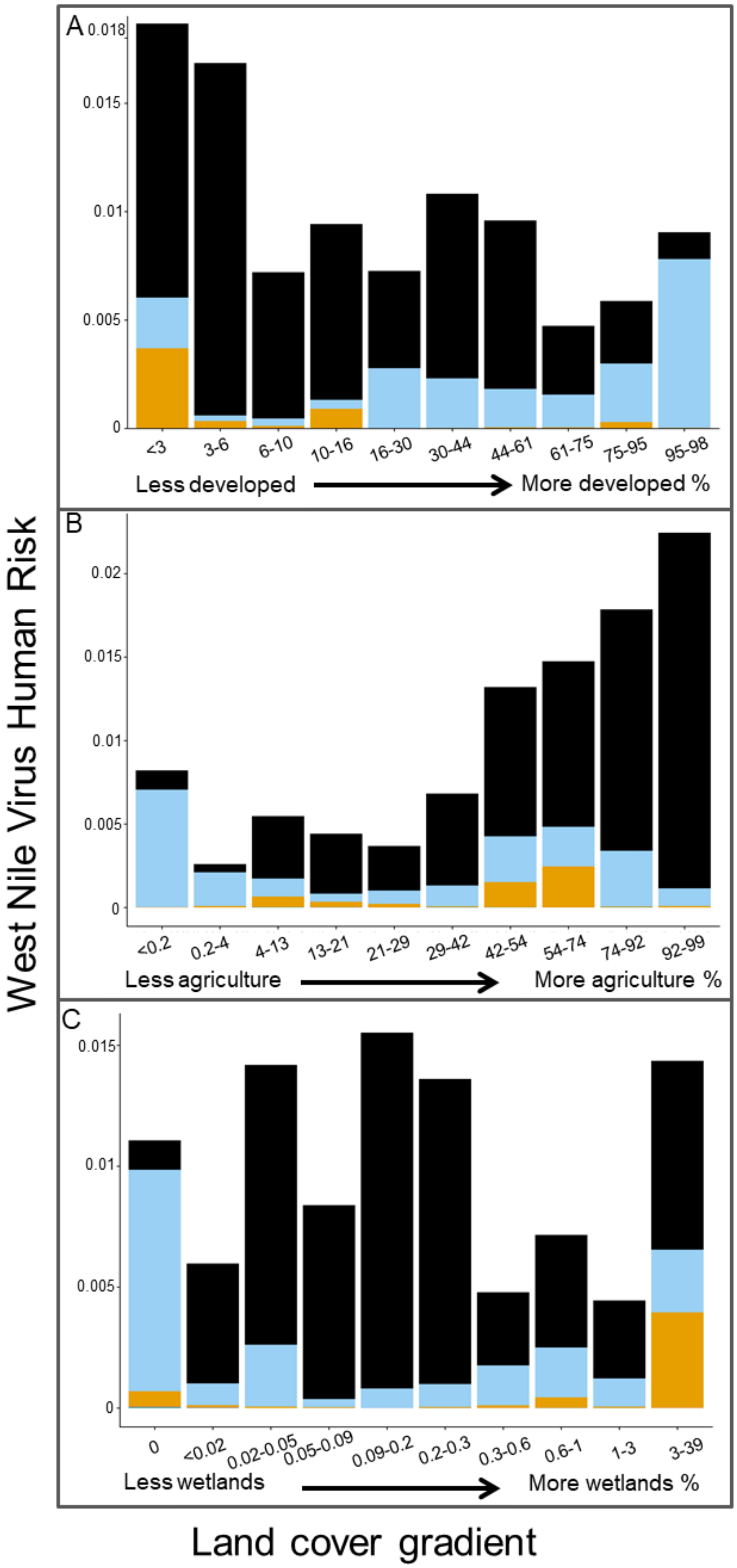
West Nile Virus Human Risk
A
0.018
0.015
0.01
0.005
0
<3
3-6
6-10
10-16
16-30
30-44
44-61
61-75
75-95
95-98
Less developed
More developed %
B
0.02
0.015
0.01
0.005
0
<0.2
0.2-4
4-13
13-21
21-29
29-42
42-54
54-74
74-92
92-99
Less agriculture
More agriculture %
C
0.015
0.01
0.005
0
0
<0.02
0.02-0.05
0.05-0.09
0.09-0.2
0.2-0.3
0.3-0.6
0.6-1
1-3
3-39
Less wetlands
More wetlands %
Land cover gradient

Figure S3: Human West Nile risk across land use gradients in Sacramento hydro region. We separated trap sites into 10 categories of similar sample sizes (using quantiles) along a developed, agricultural, and wetland gradient. WNV risk index estimated using the mean number of infectious mosquitoes feeding on humans per trap/night. Percent landcover occurring within 1 or 2 km buffer around trap sites (2 km buffer used for *C. tarsalis*, 1 km buffer for all other *Culex* species. n=295 trap sites.

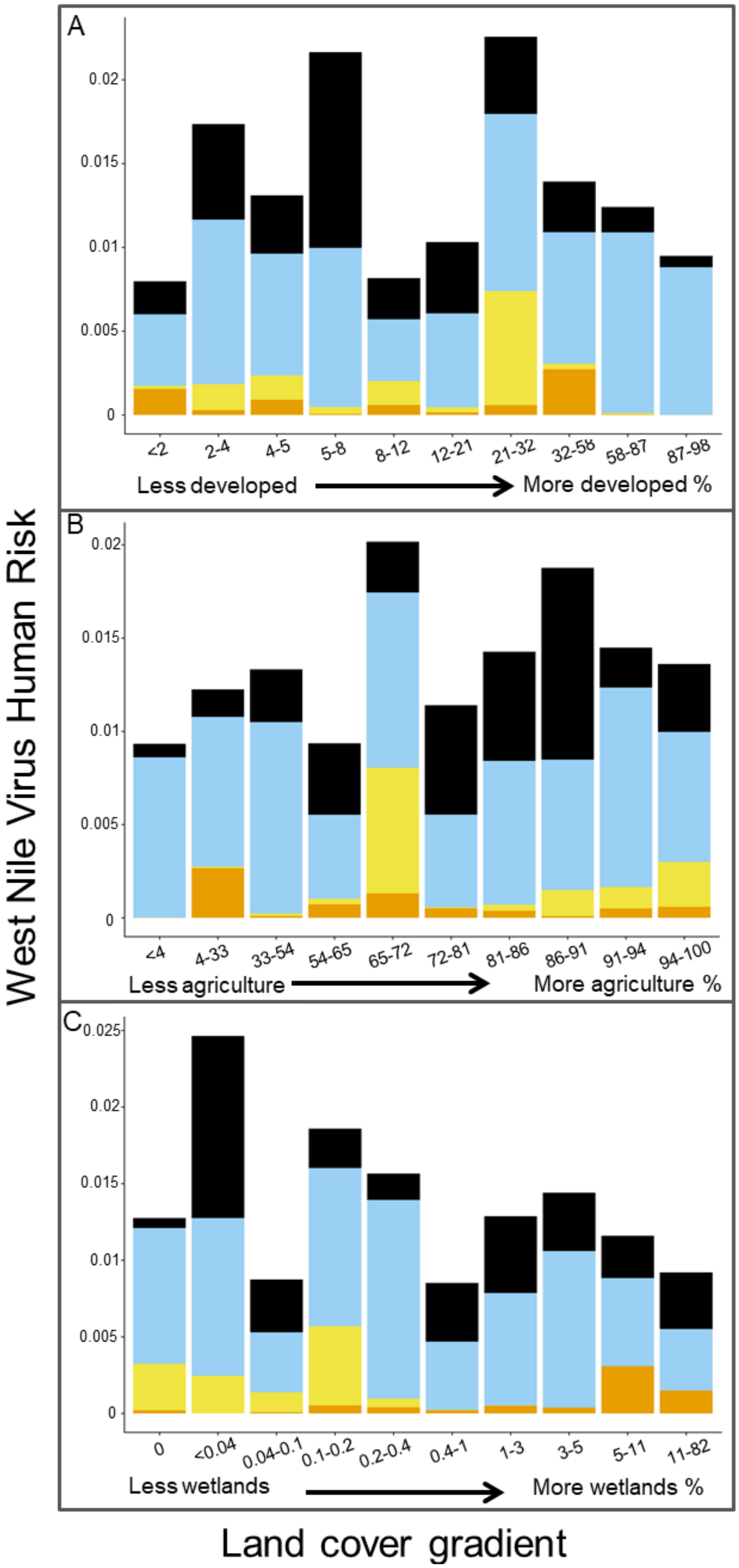
A
0.02
0.015
0.01
0.005
0
<2
2-4
4-5
5-8
8-12
12-21
21-32
32-58
58-87
87-98
Less developed
More developed %
B
0.02
0.015
0.01
0.005
0
<4
4-33
33-54
54-65
65-72
72-81
81-86
86-91
91-94
94-100
Less agriculture
More agriculture %
C
0.025
0.02
0.015
0.01
0.005
0
0
<0.04
0.04-0.1
0.1-0.2
0.2-0.4
0.4-1
1-3
3-5
5-11
11-82
Less wetlands
More wetlands %
West Nile Virus Human Risk
Land cover gradient

Figure S4: Human West Nile risk across land use gradients in San Joaquin River hydro region. We separate trap sites into 10 categories of similar sample sizes (using quantiles) along a developed, agricultural, and wetland gradient. WNV risk index estimated using the mean number of infectious mosquitoes feeding on humans per trap/night. Percent landcover occurring within 1 or 2 km buffer around trap sites (2 km buffer used for *C. tarsalis*, 1 km buffer for all other *Culex* species. n=332 trap sites.

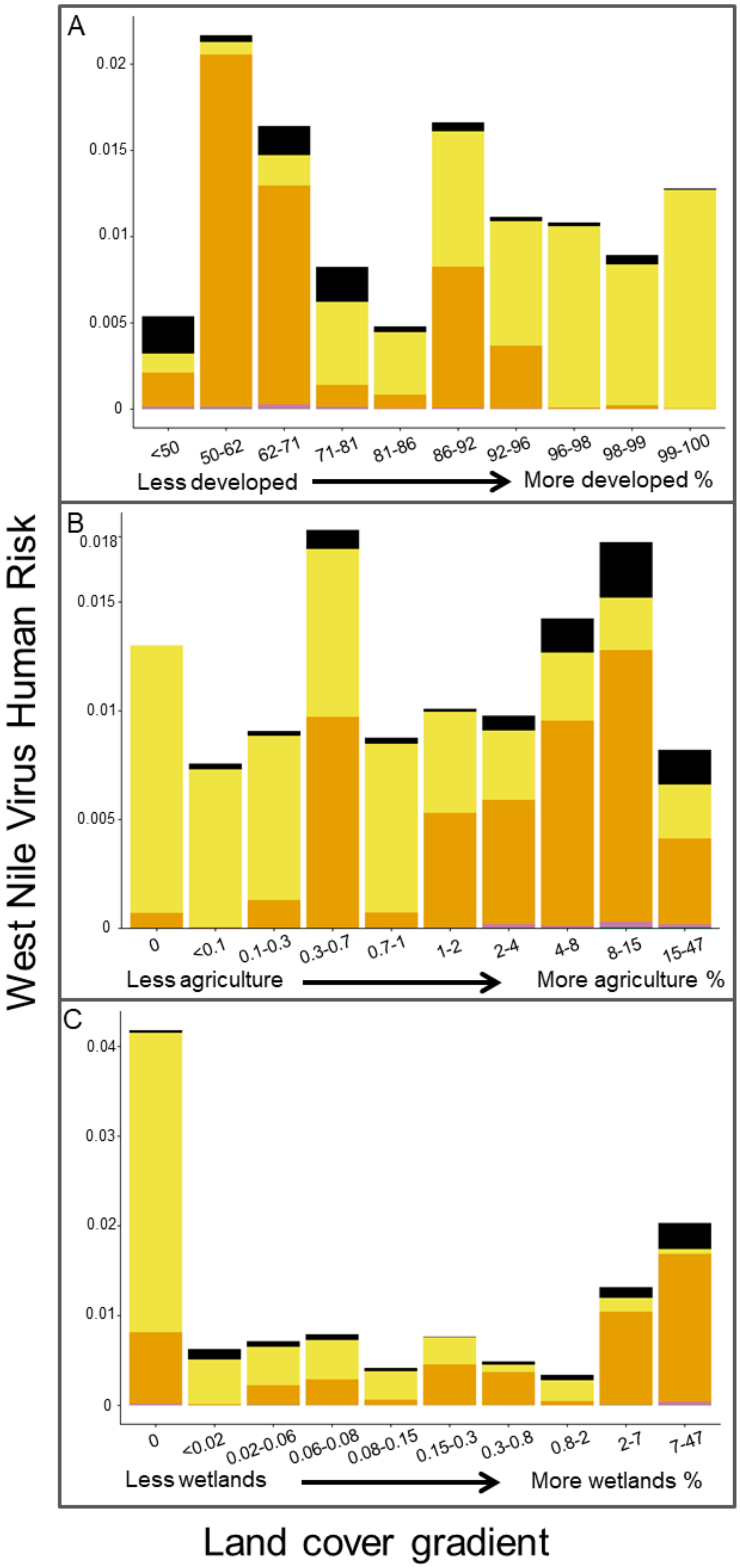
West Nile Virus Human Risk
A
0.02
0.015
0.01
0.005
0
<50
50-62
62-71
71-81
81-86
86-92
92-96
96-98
98-99
99-100
Less developed
More developed %
B
0.018
0.015
0.01
0.005
0
0
<0.1
0.1-0.3
0.3-0.7
0.7-1
1-2
2-4
4-8
8-15
15-47
Less agriculture
More agriculture %
C
0.04
0.03
0.02
0.01
0
0
<0.02
0.02-0.06
0.06-0.08
0.08-0.15
0.15-0.3
0.3-0.8
0.8-2
2-7
7-47
Less wetlands
More wetlands %
Land cover gradient

Figure S5: Human West Nile risk across land use gradients in South Coast hydro region. We separated trap sites into 10 categories of similar sample sizes (using quantiles) along a developed, agricultural, and wetland gradient. WNV risk index estimated using the mean number of infectious mosquitoes feeding on humans per trap/night. Percent landcover occurring within 1 or 2 km buffer around trap sites (2 km buffer used for *C. tarsalis*, 1 km buffer for all other *Culex* species. n=432 trap sites.

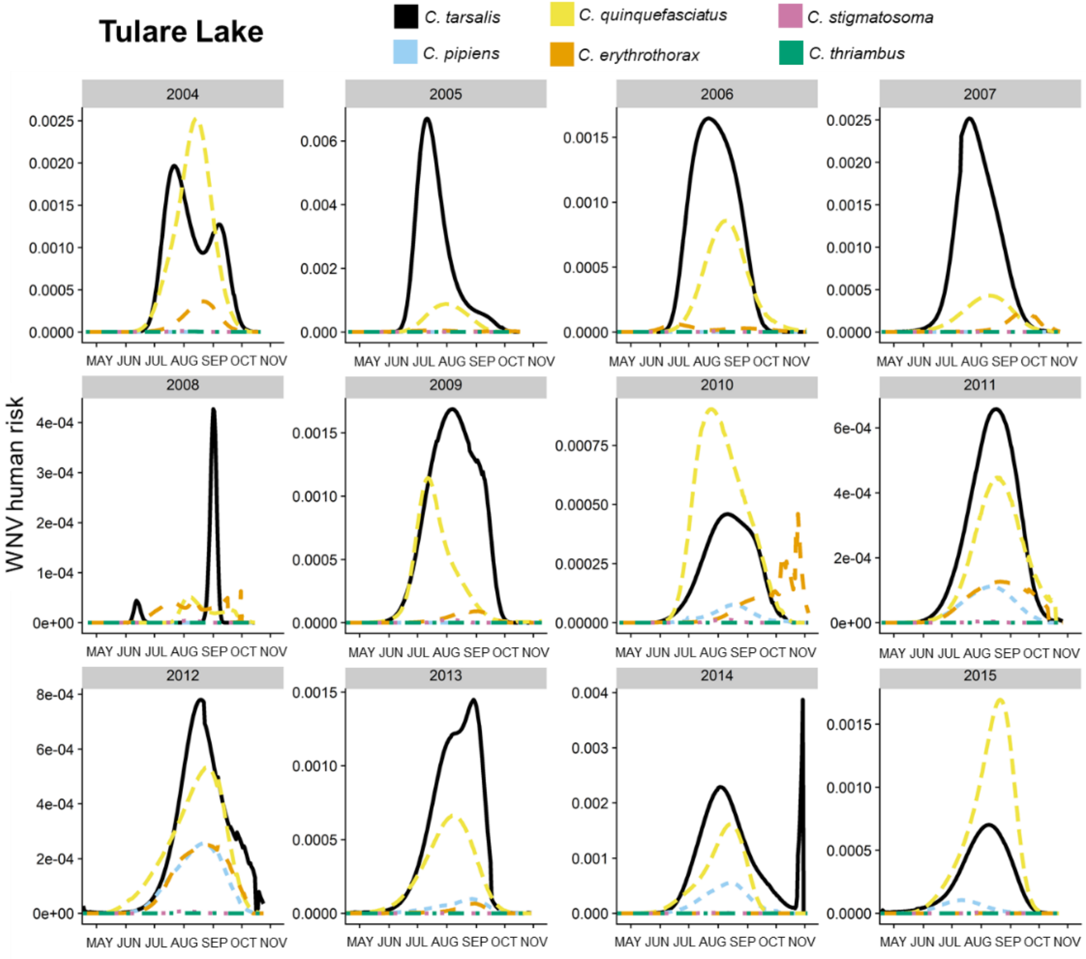

Figure S6: Seasonality of human West Nile risk for <u>Tulare Lake</u> hydro region across years. Lines show the average seasonal pattern from all trap sites located within hydro region-year.

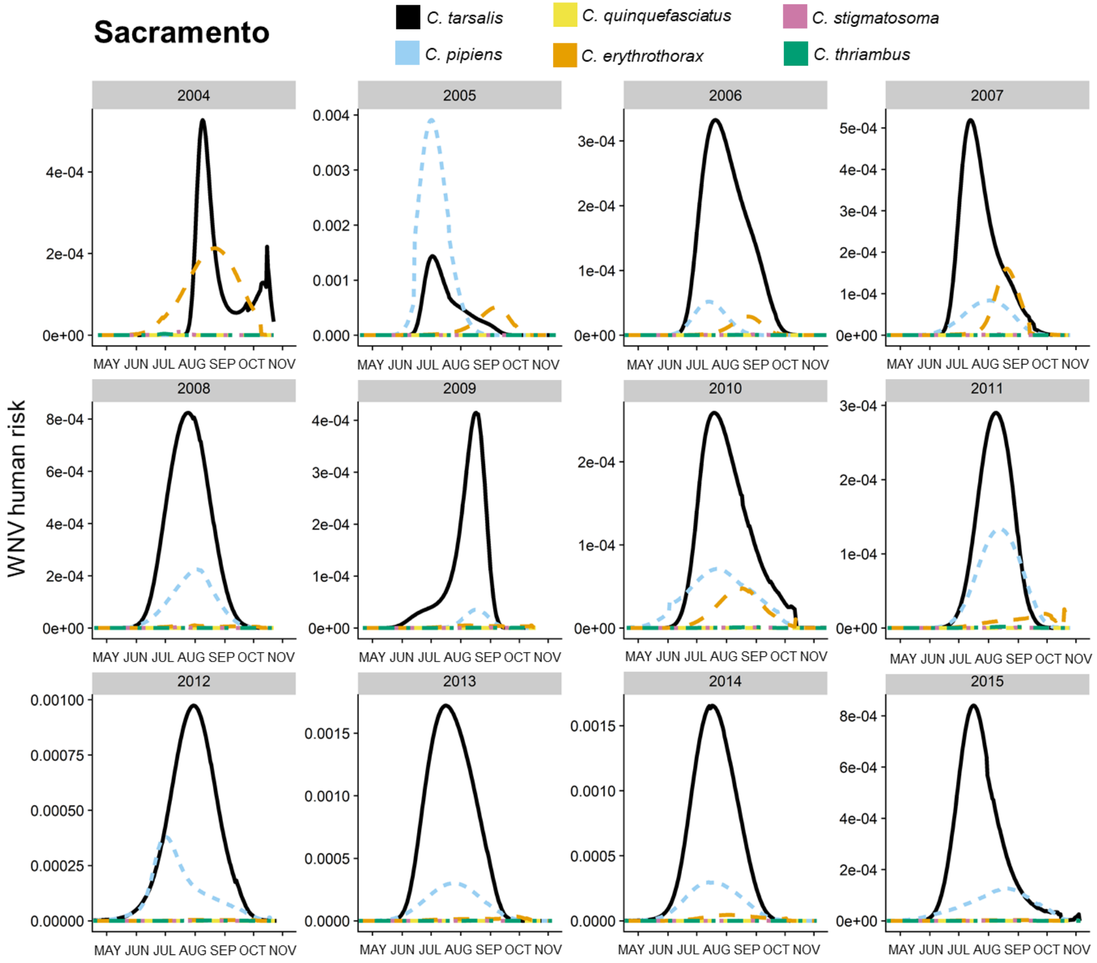

Figure S7: Seasonality of human West Nile risk for Sacramento hydro region across years. Lines show the average seasonal pattern from all trap sites located within hydro region-year.

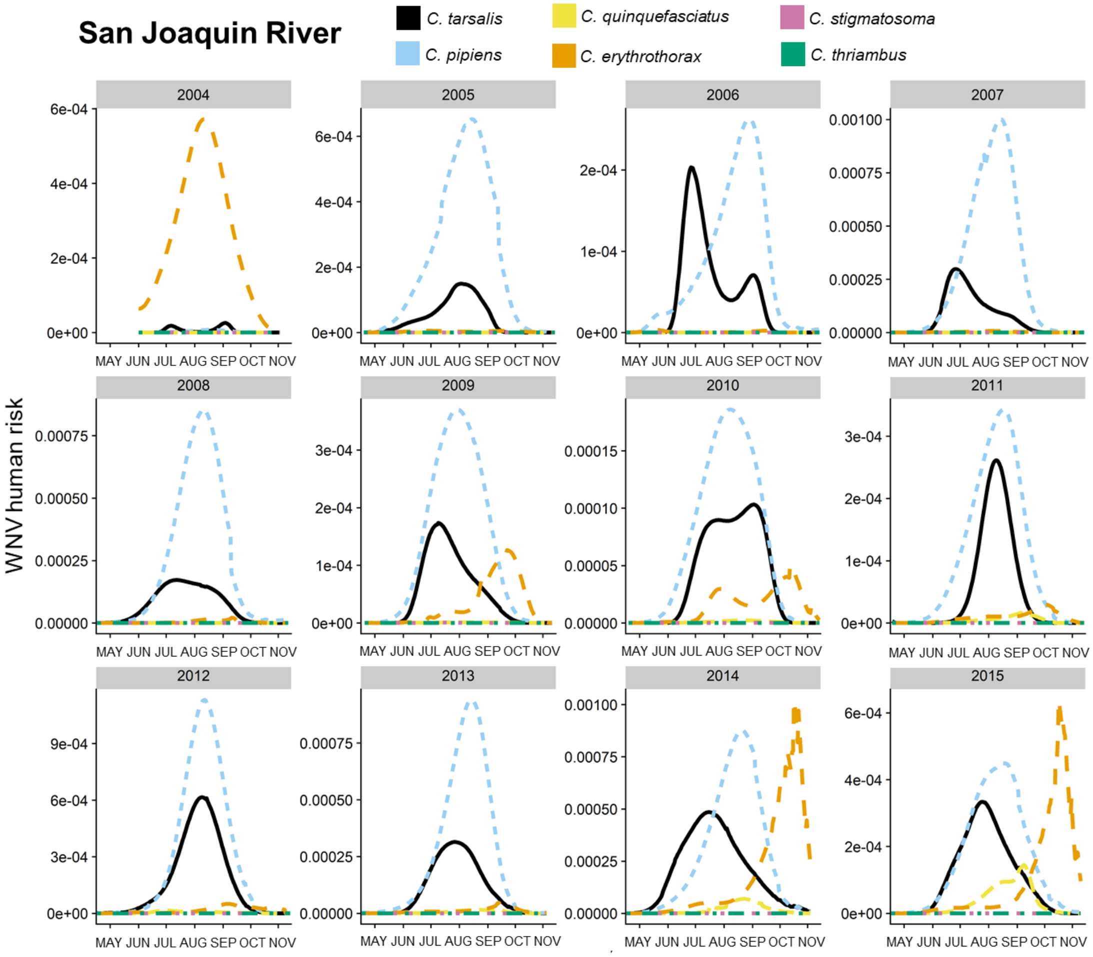

Figure S8: Seasonality of human West Nile risk for San Joaquin River hydro region across years. Lines show the average seasonal pattern from all trap sites located within hydro region-year.

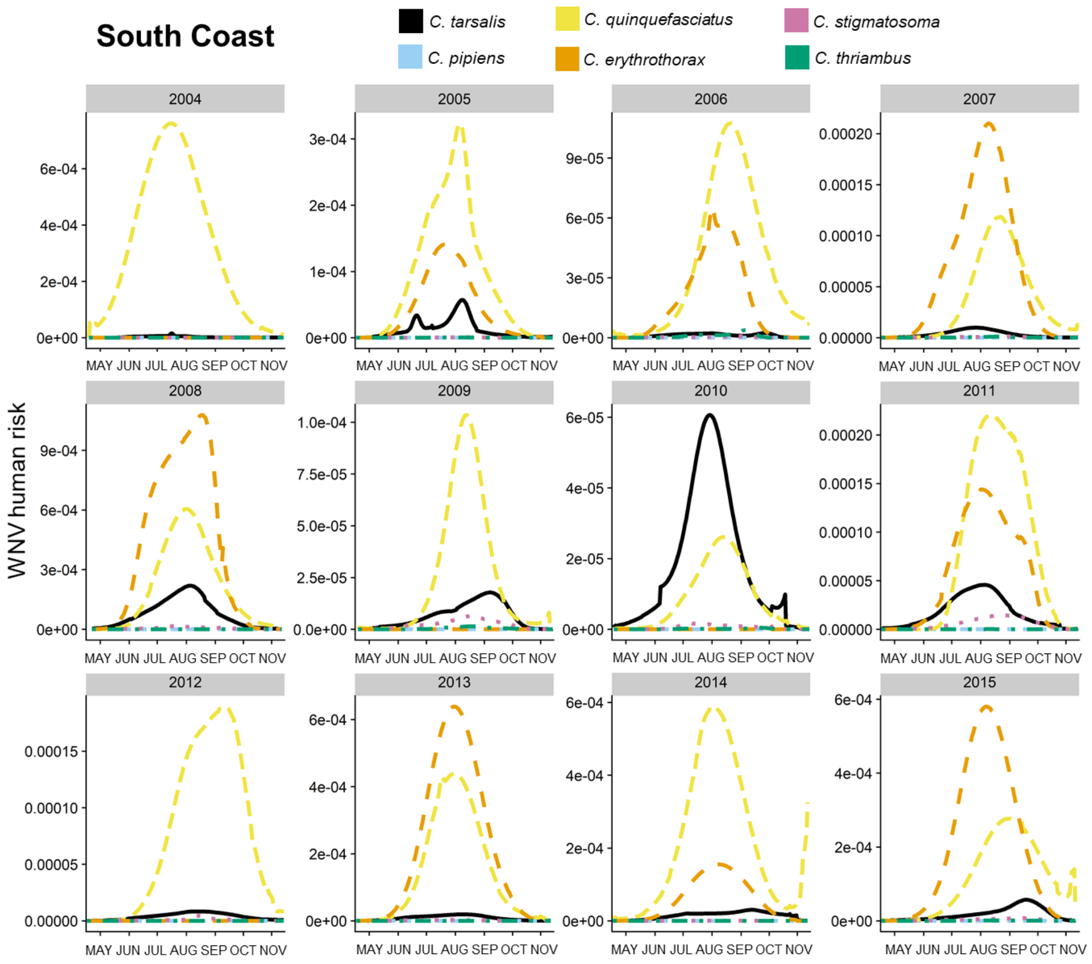

Figure S9: Seasonality of human West Nile risk for South Coast hydro region across years. Lines show the average seasonal pattern from all trap sites located within hydro region-year.

CH 3- SUPPLEMENTAL METHODS

Process for fitting mosquito abundance seasonal patterns for 6 *Culex* species:

- Included all site-years with >=12 visits.
- Generalized linear models with poisson distribution predicting mosquito counts per trap visit.
- At site-years with <3 positive mosquito count values, there was not enough data to fit seasonal pattern, so we fit intercept only model assuming constant value across season. Most of these site-years generally had really low overall mosquito counts- e.g. all 0's and 1 or 2 times where 1 mosquito was caught.
- At all other site-years; >=3 positive mosquito count values:
- First attempted to fit model including: Day+ Day^2+ Day^3 + Day^4
- If that model failed to converge- then fit simpler model including: Day + Day^2
- If that model also failed to converge- then fit intercept only model assuming constant value across season.
- Next- I plotted all site-years raw data compared to predicted model fitted values- Most of the site-years had predicted line that matched the raw data well, but I separated out the few with obvious mismatches associated with an overfitting model and/or where the predicted values were >25% of the maximum observed data.

- For these trap sites that failed the visual inspection- I fit simpler Day+ Day^2 model.
- If that model failed to converge or still failed to pass visual inspection - then fit intercept only model.
- Repeated process for each of 6 Culex species.

OVERALL CONCLUSIONS

Overall this thesis focused on understanding spatial and temporal differences in vector borne disease risk. Developing effective strategies to control the spread of emerging infectious diseases is one of the greatest challenges facing humanity. Identifying and understanding heterogeneity in pathogen transmission offers opportunities for better management and control through targeted interventions.

In Chapter 1; ***"Increased human incidence of West Nile virus near rice fields in California but not in Southern USA"***, we show how the cultivation of particular irrigation-intensive crops can increase disease risk and how spatial variation in vector ecology can alter the relationship between land cover and disease. Specifically, we found that in California, rice field areas led to an increased abundance of *C. tarsalis* and correlated with an increased number of human West Nile virus disease cases. In contrast, in other rice-growing regions in the USA outside of the distribution of *C. tarsalis*, we found no association between rice and increased WNV human cases. These results illustrate how the relationship between land use and disease risk can change depending on the mosquito species present. With climate change altering the potential range of vectors (Lafferty 2009) and global trade and travel introducing new vector species into new areas (Kilpatrick & Randolph 2012), there is a growing importance of understanding how links between land use and disease risk may be altered with the presence of different vector species.

In Chapter 2; ***"Irrigation increases mosquito populations, West Nile virus incidence, and reduces seasonal variability",*** we found irrigation amounts on the same order of magnitude as annual precipitation, with irrigation exceeding precipitation in some dry regions of California. Irrigation both increased mosquito abundance and reduced seasonal variability, by increasing periods of elevated mosquito abundance. This consistent elevated mosquito abundance likely contributed to the positive relationship we observed between irrigation and human WNV disease incidence. These results suggest that irrigation can partially decouple mosquito abundance from natural precipitation patterns (Baeza et al. 2011) and result in elevated and sustained risk of mosquito-borne pathogens.

In Chapter 3, **"*Vector diversity maintains West Nile virus transmission across land use gradients*"**, we found significant variation in the bridge vectors likely responsible for human West Nile virus transmission across California, within seasons and across years. Changes in species contributions resulted both from changes to mosquito abundance and mosquito infection prevalence. Across California counties, human WNV cases were strongly correlated with the density of infectious vectors feeding on humans. As expected, *C. tarsalis* was more important in agricultural areas, whereas *C. pipiens* and *C. quinquefasciatus* were important in developed and agricultural areas, and *C. erythrothorax* was important in wetland areas. In some regions having several important vectors, overall WNV risk did not substantially change along land cover gradients because *C. tarsalis* replaced *C.*

pipiens/quinquefasciatus contributions in low developed or agricultural areas. As a result, WNV risk did not change substantially along either agricultural or urbanization land use gradients because the diversity of vectors maintained high disease risk across a range of habitats. These results show how a diversity of vectors can maintain an ecosystem disservice - vector borne disease – by their differential response to environmental disturbance.

Collectively these results illustrate the importance of land cover relationships with different vector species driving spatial and temporal differences in vector borne disease risk. In addition, we highlight the value that long-term vector control datasets have in contributing better understanding of drivers of vector borne disease risk. While many vector control agencies across the US have spent exhaustive years trapping, identifying, counting and testing mosquitoes for various pathogens, to date we still have only a crude understanding of how the relative abundance of different mosquito species varies across the US. Future studies should aim to synthesize and standardize vector control datasets to help characterize the distribution and relative abundance of mosquitoes important for pathogen transmission. A better understanding of the spatial and temporal patterns of mosquito abundance can be highly informative in predicting areas of greatest risk from current vector borne diseases, as well as understanding where new emerging infectious diseases may have greatest impact.

LITERATURE CITED

Amerasinghe FP, Ariyasena TG. 1991. Survey of adult mosquitoes (Diptera: Culicidae) during irrigation development in the Mahaweli Project, Sri Lanka. Journal of Medical Entomology **28**:387–393.

Andreadis T, Anderson J, Vossbrinck C, Main A. 2004. Epidemiology of West Nile virus in Connecticut: a five-year analysis of mosquito data 1999-2003. Vector-Borne & Zoonotic Diseases **4**:360–378.

Andreadis TG. 2012. The contribution of *Culex Pipiens* complex mosquitoes to transmission and persistence of West Nile Virus in North America. Journal of the American Mosquito Control Association **18**:137–151.

Ayars JE, Phene CJ, Hutmacher RB, Davis KR, Schoneman RA, Vail SS, Mead RM. 1999. Subsurface drip irrigation of row crops: a review of 15 years of research at the Water Management Research Laboratory. Agricultural Water Management **42**:1–27.

Baeza A, Bouma MJ, Dobson AP, Dhiman R, Srivastava HC, Pascual M. 2011. Climate Forcing and Desert Malaria: The Effect of Irrigation. Malaria journal **10**:190-200.

Barker CCM, Eldridge BBF, Reisen WWK. 2010a. Seasonal Abundance of *Culex tarsalis* and *Culex pipiens complex* Mosquitoes (Diptera: Culicidae) in California. Journal of Medical Entomology **47**:759–768.

Bejon, P., Williams, T.N., Nyundo, C., Hay, S.I., Benz, D., Gething, P.W., Otiende, M., Peshu, J., Bashraheil, M., Greenhouse, B. and Bousema, T. 2014. A micro-epidemiological analysis of febrile malaria in coastal Kenya showing hotspots within hotspots. eLife **2014**:1–13.

Bett B et al. 2017. Effects of flood irrigation on the risk of selected zoonotic pathogens in an arid and semi-arid area in the eastern Kenya. PLoS ONE **12**:1–15.

Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., Drake, J.M., Brownstein, J.S., Hoen, A.G., Sankoh, O. and Myers, M.F. 2013. The global distribution and burden of dengue. Nature **496**:504–7.

Bisanzio D, Giacobini M, Bertolotti L, Mosca A, Balbo L, Kitron U, Vazquez-Prokopec GM. 2011. Spatio-temporal patterns of distribution of West Nile virus vectors in eastern Piedmont Region, Italy. Parasites & Vectors **4**:230.

Bolling B, Barker C, Moore C. 2009. Seasonal patterns for entomological measures of risk for exposure to Culex vectors and West Nile virus in relation to human disease cases in northeastern Colorado. Journal of Medical Entomology **46**:1519–1531.

Bousema, T., Drakeley, C., Gesase, S., Hashim, R., Magesa, S., Mosha, F., Otieno, S., Carneiro, I., Cox, J., Msuya, E. and Kleinschmidt, I. 2010. Identification of hot spots of malaria transmission for targeted malaria control. The Journal of infectious diseases **201**:1764–1774.

Bowden SE, Magori K, Drake JM. 2011. Regional differences in the association between land cover and West Nile virus disease incidence in humans in the United States. The American Journal of Tropical Medicine and Hygiene **84**:234–8.

Bradley C, Gibbs S, Altizer S. 2008. Urban land use predicts West Nile virus exposure in songbirds. Ecological applications **18**:1083–92.

Bradley CA, Altizer S. 2007. Urbanization and the ecology of wildlife diseases. Trends in Ecology & Evolution **22**:95–102.

Burkett-Cadena ND, McClure CJW, Estep LK, Eubanks MD. 2013. What drives the spatial distribution of mosquitoes? Ecosphere **4**:1–16.

Calhoun LM, Avery M, Jones LA, Gunarto K, King R, Roberts J, Burkot TR, Fox M. 2007. Combined sewage overflows (CSO) are major urban breeding sites for Culex quinquefasciatus in Atlanta, Georgia. American Journal of Tropical Medicine and Hygiene **77**:478–484.

Campbell R, Thiemann TC, Lemenager D, Reisen WK. 2013. Host-Selection Patterns of Culex tarsalis (Diptera: Culicidae) Determine the Spatial Heterogeneity of West Nile Virus Enzootic Activity in Northern California. Journal of Medical Entomology **50**:1303–1309.

Cardinale BJ et al. 2012. Biodiversity loss and its impact on humanity. Nature **486**:59–67.

Cardinale BJ, Srivastava DS, Duffy JE, Wright JP, Downing AL, Sankaran M, Jouseau C. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature **443**:989–992.

Cardinale BJ, Wright JP, Cadotte MW, Carroll IT, Hector A, Srivastava DS, Loreau M, Weis JJ. 2007. Impacts of plant diversity on biomass production increase through time because of species complementarity. Proceedings of the National Academy of Sciences **104**:18123–18128.

CDC. 2016. Centers for Disease Control and Prevention. Available from https://diseasemaps.usgs.gov/mapviewer/. (Accessed on May 17, 2018)

CDC. 2018. Centers for Disease Control and Prevention. Available from https://wwwn.cdc.gov/arbonet/maps/ADB_Diseases_Map/index.html. (Accessed on August 3,2018)

CDWR. 2018. Estimates of applied water for each detailed analyis unit. Available from http://wdl.water.ca.gov/landwateruse/anlwuest.cfm (Accessed December 6, 2018).

Census U. 2010. US Census Bureau. Available from https://www.census.gov/2010census/data/ (Accessed August 11,2018).

Chaves LF, Keogh CL, Vazquez-Prokopec GM, Kitron UD. 2009. Combined sewage overflow enhances oviposition of Culex quinquefasciatus (Diptera: Culicidae) in urban areas. Journal of Medical Entomology **46**:220–226.

Chuang T-W, Hildreth MB, Vanroekel DL, Wimberly MC. 2011. Weather and Land Cover Influences on Mosquito Populations in Sioux Falls, South Dakota. Journal of Medical Entomology **48**:669–679.

Chuang T-W, Hockett CW, Kightlinger L, Wimberly M. 2012. Landscape-Level Spatial Patterns of West Nile Virus Risk in the Northern Great Plains. American Journal of Tropical Medicine and Hygiene **86**:734–731.

Ciota AT, Matacchiero AC, Kilpatrick a M, Kramer LD. 2014. The effect of temperature on life history traits of Culex mosquitoes. Journal of Medical Entomology **51**:55–62.

Crowder DW, Dykstra EA, Brauner JM, Duffy A, Reed C, Martin E, Dutilleul P, Owen JP, Peterson W, Carrie Y. 2013. West Nile Virus Prevalence across Landscapes Is Mediated by Local Effects of Agriculture on Vector and Host Communities. PLOS one **8:** 1-8.

Daszak P, Kilpatrick AM. 2009. The Princeton Guide to Ecology. Page (Levin SA, Carpenter SR, Godfray HCJ, Kinzig AP, Loreau M, Losos JB, Walker B, Wilcove DS, Morris CG, editors). Princeton University Press, NJ.

Degroote J, Degroote JP, Sugumaran R, Ecker M. 2014. Landscape, demographic and climatic associations with human West Nile virus occurrence regionally in 2012 in the United States of America. Geospatial Health **9**:153-168

Degroote J, Sugumaran R. 2012. National and Regional Associations Between Human West Nile Virus Incidence and Demographic, Landscape, and Land Use Conditions in the Conterminous United States. Vector-Borne and Zoonotic Diseases **12**:657–665.

Deichmeister JM, Telang A. 2011. Abundance of West Nile Virus Mosquito Vectors in Relation to Climate and Landscape Variables Abundance of West Nile virus mosquito vectors in relation to climate. Journal of Vector Ecology **36**:75–85.

Diuk-wasser MA, Brown HE, Andreadis TG, Fish D. 2006. Modeling the Spatial Distribution of Mosquito Vectors for West Nile Virus in Connecticut. Vector Borne and Zoonotic Diseases **6**:283:295.

Diuk-Wasser MA, Touré MB, Dolo G, Bagayoko M, Sogoba N, Sissoko I, Traoré SF, Taylor CE. 2007. Effect of rice cultivation patterns on malaria vector abundance in rice-growing villages in Mali. American Journal of Tropical Medicine and Hygiene **76**:869–874.

Ebel GD, Rochlin I, Longacker J, Kramer LD. 2005. Culex restuans (Diptera: Culicidae) relative abundance and vector competence for West Nile Virus. Journal of Medical Mntomology **42**:838–843.

Eisen L, Barker CM, Moore CG, Pape WJ, Winters AM, Cheronis N. 2010. Irrigated Agriculture Is an Important Risk Factor for West Nile Virus Disease in the Hyperendemic Larimer-Boulder-Weld Area of North Central Colorado. Journal of Medical Entomology **47**:939–951.

El Chami D, Scardigno A, Malorgio G. 2011. Impacts of Combined Technical and Economic Measures on Water Saving in Agriculture under Water Availability Uncertainty. Water Resources Management **25**:3911–3929.

Ellis EC. 2011. Anthropogenic transformation of the terrestrial biosphere. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences **369**:1010–1035.

Erlanger TE, Keiser J, Caldas De Castro M, Bos R, Singer BH, Tanner M, Utzinger J. 2005. Effect of water resource development and management on lymphatic filariasis, and estimates of populations at risk. The American Journal of Tropical Medicine and Hygiene **73**:523–533.

Erlanger TE, Weiss S, Keiser J, Utzinger J, Wiedenmayer K. 2009. Past, present, and future of Japanese encephalitis. Emerging Infectious Diseases **15**:1–7.

Famiglietti JS, Lo M, Ho SL, Bethune J, Anderson KJ, Syed TH, Swenson SC, De Linage CR, Rodell M. 2011. Satellites measure recent rates of groundwater depletion in California's Central Valley. Geophysical Research Letters **38**.

FAO. 2017. Food and Agricultural Organization of the United Nations. Available from http://www.fao.org/faostat/en/#home. (Accessed on December 25 2017)

Farajollahi A, Fonseca DM, Kramer LD, Marm Kilpatrick A. 2011. "Bird biting" mosquitoes and human disease: a review of the role of Culex pipiens complex mosquitoes in epidemiology. Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases **11**:1577–85.

Gates MC, Boston RC. 2009. Irrigation linked to a greater incidence of human and veterinary West Nile virus cases in the United States from 2004 to 2006. Preventive Veterinary Medicine **89**:134–137.

Gething PW, Patil AP, Smith DL, Guerra C a., Elyazar IRF, Johnston GL, Tatem AJ, Hay SI. 2011. A new world malaria map: *Plasmodium falciparum* endemicity in 2010. Malaria Journal **10**:378.

Gimnig JE, Ombok M, Kamau L, Hawley W a, Gimnig JE, Ombok M, Kamau L, Hawley W a. 2001. Characteristics of Larval Anopheline (Diptera : Culicidae) Habitats in Western Kenya Characteristics of Larval Anopheline (Diptera : Culicidae) Habitats in Western Kenya. Journal of Medical Entomology **38**:282–288.

Goddard LB, Roth AE, Reisen WK, Scott TW. 2002. Vector competence of California mosquitoes for West Nile virus. Emerging infectious diseases **8**:1385–91.

Gomez A, Kilpatrick A, Kramer LD, Ii APD, Maffei JG, Goetz SJ, Marra PP, Daszak P, Aguirre AA. 2008. Land Use and West Nile Virus Seroprevalence in Wild Mammals. Emerging Infectious Diseases **14**:962–965.

Gong, P., Wang, J., Yu, L., Zhao, Y., Zhao, Y., Liang, L., Niu, Z., Huang, X., Fu, H., Liu, S. and Li, C. 2013. Finer resolution observation and monitoring of global land cover: first mapping results with Landsat TM and ETM+ data. International Journal of Remote Sensing **34**:2607–2654.

Gottdenker N, Streicker D, Faust C, Carroll C. 2014. Anthropogenic land use change and infectious diseases : a review of the evidence. EcoHealth **11**:619–632.

Grantham TE, Viers JH. 2014. 100 years of California's water rights system: Patterns, trends and uncertainty. Environmental Research Letters **9**.

Gunstream S, Chew R, Hagstrum D, Tempelis C. 1971. Feeding patterns of six species of mosquitoes in arid Southeastern California. Mosquito News **31**:99–101.

Guo S, Ling F, Hou J, Wang J, Fu G, Gong Z. 2014. Mosquito surveillance revealed lagged effects of mosquito abundance on mosquito-borne disease transmission: A retrospective study in Zhejiang, China. PLoS ONE **9**.

Hamer GL, Chaves LF, Anderson TK, Kitron UD, Brawn JD, Ruiz MO, Loss SR, Walker ED, Goldberg TL. 2011. Fine-scale variation in vector host use and force of infection drive localized patterns of West Nile virus transmission. PloS one **6**:e23767.

Hamer GL, Kitron UD, Brawn JD, Loss SR, Ruiz MO, Goldberg TL, Walker ED. 2008a. *Culex pipiens* (Diptera: Culicidae): a bridge vector of West Nile virus to humans. Journal of Medical Entomology **45**:125–8.

Hamer GL, Walker ED, Brawn JD, Loss SR, Ruiz MO, Goldberg TL, Schotthoefer AM, Brown WM, Wheeler E, Kitron UD. 2008b. Rapid amplification of West Nile virus: the role of hatch-year birds. Vector-Borne and Zoonotic Diseases **8**:57–67.

Hassell JM, Begon M, Ward MJ, Fèvre EM. 2017. Urbanization and disease emergence: dynamics at the wildlife-livestock-human interface. Trends in Ecology & Evolution **32**:55–67.

Hay SI, Guerra CA, Tatem AJ, Atkinson PM, Snow RW. 2005. Tropical Infectious Diseases: Urbanization, malaria transmission and disease burden in Africa. Nature Reviews Microbiology **3**:81–90.

Hooper DU et al. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecological Monographs **75**:3–35.

Hooper DU, Adair EC, Cardinale BJ, Byrnes JEK, Hungate BA, Matulich KL, Gonzalez A, Duffy JE, Gamfeldt L, Connor MI. 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature **486**:105–108.

Ijumba JN, Lindsay SW. 2001. Impact of irrigation on malaria in Africa: Paddies paradox. Medical and Veterinary Entomology **15**:1–11.

Ives AR, Gross K, Klug JL. 1999. Stability and variability in competative communities. Science **286**:542–544.

Jaleta KT, Hill SR, Seyoum E, Balkew M, Gebre-Michael T, Ignell R, Tekie H. 2013. Agro-ecosystems impact malaria prevalence: large-scale irrigation drives vector population in western Ethiopia. Malaria journal **12**:350.

Johnson PTJ, de Roode JC, Fenton A. 2015. Why infectious disease research needs community ecology. Science **349**:1259504.

Keiser J, Maltese MF, Erlanger TE, Bos R, Tanner M, Singer BH, Utzinger J. 2005. Effect of irrigated rice agriculture on Japanese encephalitis, including challenges and opportunities for integrated vector management. Acta Tropica **95**:40–57.

Kibret S, Alemu Y, Boelee E, Tekie H, Alemu D, Petros B. 2010. The impact of a small-scale irrigation scheme on malaria transmission in Ziway area, Central Ethiopia. Tropical Medicine and International Health **15**:41–50.

Kibret S, Wilson G, Tekie H, Petros B. 2014. Increased malaria transmission around irrigation schemes in Ethiopia and the potential of canal water management for malaria vector control. Malaria Journal **13**:360.

Killeen GF, Reed TE. 2018. The portfolio effect cushions mosquito populations and malaria transmission against vector control interventions. Malaria Journal **17**:1–7. BioMed Central.

Kilpatrick a M. 2011. Globalization, land use, and the invasion of West Nile virus. Science **334**:323–7.

Kilpatrick a M, Daszak P, Jones MJ, Marra PP, Kramer LD. 2006. Host heterogeneity dominates West Nile virus transmission. Proceedings of the Royal Society of London B: Biological Sciences **273**:2327–33.

Kilpatrick a M, Kramer LD, Campbell SR, Alleyne EO, Dobson AP, Daszak P. 2005. West Nile virus risk assessment and the bridge vector paradigm. Emerging infectious diseases **11**:425–9.

Kilpatrick a M, Pape WJ. 2013. Predicting human West Nile virus infections with mosquito surveillance data. American journal of epidemiology **178**:829–35.

Kilpatrick a M, Peters RJ, Dupuis AP, Jones MJ, Marra PP, Kramer LD. 2013. Predicted and observed mortality from vector-borne disease in small songbirds. Biological conservation **165**:79–85.

Kilpatrick AM, Fonseca DM, Ebel GD, Reddy MR, Kramer LD. 2010. Spatial and temporal variation in vector competence of Culex pipiens and Cx. restuans mosquitoes for West Nile virus. American Journal of Tropical Medicine and Hygiene **83**:607–613.

Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P, Fonseca DM. 2007. Genetic influences on mosquito feeding behavior and the emergence of zoonotic pathogens. American Journal of Tropical Medicine and Hygiene **77**:667–671.

Kilpatrick AM, Randolph SE. 2012. Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. The Lancet **380**:1946–1955.

Kilpatrick AM, Salkeld DJ, Titcomb G, Hahn MB. 2017. Conservation of biodiversity as a strategy for improving human health and well-being. Philosophical Transactions of the Royal Society of Biological Sciences **372**:20160131.

Kovach TJ, Kilpatrick AM. 2018. Increased Human Incidence of West Nile Virus Disease near Rice Fields in California but Not in Southern United States. The American Journal of Tropical Medicine and Hygiene **99:**222-228.

Kraemer, M.U., Sinka, M.E., Duda, K.A., Mylne, A.Q., Shearer, F.M., Barker, C.M., Moore, C.G., Carvalho, R.G., Coelho, G.E., Van Bortel, W. and Hendrickx, G. 2015. The global distribution of the arbovirus vectors Aedes aegypti and Ae. Albopictus. eLife **4**:1–18.

Kramer LD. 2015. Dissecting vectorial capacity for mosquito-borne viruses. Current Opinion in Virology **14**:871–882.

Kramer LD, Styer LM, Ebel GD. 2008. A global perspective on the epidemiology of West Nile virus. Annual review of entomology **53**:61–81.

LaDeau SL, Kilpatrick a M, Marra PP. 2007. West Nile virus emergence and large-scale declines of North American bird populations. Nature **447**:710–3.

Lafferty K. 2009. The ecology of climate change and infectious diseases. Ecology **90**:888–900.

Landau KI, Leeuwen WJD Van. 2012. Fine scale spatial urban land cover factors associated with adult mosquito abundance and risk in Tucson, Arizona. Journal of Vector Ecology **37**:407–418.

Landesman WJ, Allan BF, Langerhans RB, Knight TM, Chase JM. 2007. Inter-annual associations between precipitation and human incidence of West Nile virus in the United States. Vector borne and Zoonotic Diseases **7**:337–343.

Lo MH, Famiglietti JS. 2013. Irrigation in California's Central Valley strengthens the southwestern U.S. water cycle. Geophysical Research Letters **40**:301–306.

Lord CC. 2011. The Effectof Multiple Vectors on Arbovirus Transmission. Israel Journal of Ecology and Evolution **56**:371–392.

Lothrop HD, Reisen WK. 2001. Landscape Affects the Host-Seeking Patterns of *Culex tarsalis* (Diptera : Culicidae) in the Coachella Valley of California. Journal of Medical Entomology **38**:325–332.

Lozano R et al. 2012. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the Global Burden of Disease Study 2010. The Lancet **380**:2095–2128.

Maupin MA, Kenny JF, Hutson SS, Lovelace JK, Barber NL, Linsey KS. 2010. Estimated Use of Water in the United States in 2010. Circular 1405. US Geological Survey.

McClure KM, Lawrence C, Kilpatrick AM. 2018. Land Use and Larval Habitat Increase *Aedes albopictus* (Diptera: Culicidae) and *Culex quinquefasciatus* (Diptera: Culicidae) Abundance in Lowland Hawaii. Journal of Medical Entomology **55**:1509-1516.

McDermott E, Mullens B. 2018. The Dark Side of Light Traps. Journal of Medical Entomology **55**:251–261.

Miller RH, Masuoka P, Klein TA, Kim HC, Somer T, Grieco J. 2012. Ecological niche modeling to estimate the distribution of japanese encephalitis virus in asia. PLoS Neglected Tropical Diseases **6**.

Minakawa N, Seda P, Yan G. 2002. Influence of host and larval habitat distribution on the abundance of African malaria vectors in Western Kenya. American journal of tropical medicine and hygiene **67**:32–38.

Molaei G, Cummings RF, Su T, Armstrong PM, Williams G a, Cheng M-L, Webb JP, Andreadis TG. 2010. Vector-host interactions governing epidemiology of West Nile virus in Southern California. The American Journal of Tropical Medicine and Hygiene **83**:1269–82.

Montgomery MJ, Thiemann T, Macedo P, Brown D a., Scott TW. 2011. Blood-Feeding Patterns of the Culex pipiens Complex in Sacramento and Yolo Counties, California. Journal of Medical Entomology **48**:398–404.

Muggeo MVMR. 2017. Package ' segmented .' Biometrika **58**:525–534.

Müller C et al. 2018. Global patterns of crop yield stability under additional nutrient and water inputs. PLoS ONE **13**:1–14.

NASS. 2015. USDA National Agricultural Statistics Service. Available from http://www.nass.usda.gov/Research_and_Science/Cropland/SARS1a.php (Accessed January 11, 2017).

Negri DH, Gollehon NR, Aillery MP. 2005. The effects of climatic variability on US irrigation adoption. Climatic Change **69**:299–323.

Nelms BM, Thiemann TC, Bridges DN, Williams AE, Koschik ML, Ryan BM, Scott JJ. 2016. Bionomics and Vector Potential of *Culex thriambus* (Diptera: Culicidae) Mosquitoes in Lake County, California. Journal of Medical Entomology **53**:1473-1481.

NLDAS. 2011. North America Land Data Assimilation System. Available from http://wonder.cdc.gov/nasa-nldas.html. (Accessed on August 8, 2016)

Ohta S, Kaga T. 2014. Effect of irrigation systems on temporal distribution of malaria vectors in semi-arid regions. International Journal of Biometeorology **58**:349–59.

Oki T. 2006. Global Hydrological Cycles and World Water Resources. Science **313**:1068–1072.

Park AW, Cleveland CA, Dallas TA, Corn JL. 2016. Vector species richness increases haemorrhagic disease prevalence through functional diversity modulating the duration of seasonal transmission. Parasitology **143**:874–879.

Paull SH, Horton DE, Ashfaq M, Rastogi D, Kramer LD, Diffenbaugh NS, Kilpatrick AM. 2017. Drought and immunity determine the intensity of West Nile virus epidemics and climate change impacts. Proceedings of the Royal Society of Biological Sciences **284**.

Pecoraro HL, Day HL, Reineke R, Stevens N, Withey JC, Marzluff JM, Meschke JS. 2007. Climatic and landscape correlates for potential West Nile virus mosquito vectors in the Seattle region. Journal of Vector Ecology **32**:22–8.

Pimentel D, Berger B, Filiberto D, Newton M, Wolfe B, Clark S, Poon E, Abbett E, Nandagopal S. 2004. Water Resources:Agricultural and Environmental Issues **54**:909–918.

Pitcairn M, Wilson L. 1994. Spatial patterns of *Anopheles freeborni* and *Culex tarsalis* (Diptera: Culicidae) larvae in California rice fields. Journal of Medical Entomology **31**:545–553.

PRISM Climate Group. 2018. Temperature and precipitation datasets. Available from: http://prism.oregonstate.edu/ (Accessed on Nov 22, 2017)

Reeves WC. 1990. Epidemiology and Control of Mosquito-borne Arboviruses in California, 1943-1987. California Mosquito and Vector Control Association.

Reeves WC, Hammon W. 1962. Epidemiology of the arthropod-borne viral encephalitides in Kern County, California, 1943-1952. University of California Berkeley Public Health **4**:1–257.

Reisen W. 2012. The Contrasting Bionomics of Culex Mosquitoes in Western North America. Journal of the American Mosquito Control Association **28**:82–91.

Reisen W, Barker C, Fang Y, Martinez V. 2008a. Does variation in Culex (Diptera: Culicidae) vector competence enable outbreaks of West Nile virus in California? Journal of Medical Entomology **45**:1126–1138.

Reisen W, Lothrop H. 1995. Population Ecology and Dispersal of Culex tarsalis (Diptera:Culicidae) in the Coachella Valley of California. Journal of Medical Entomology **32**:490–502.

Reisen W, Lothrop H. 2008. Persistent West Nile Virus transmission and the apparent displacement St. Louis encephalitis virus in southeastern California, 2003–2006. Journal of Medical Entomology **45**:2003–2006.

Reisen WK, Cayan D, Tyree M, Barker CM, Eldridge B, Dettinger M. 2008b. Impact of climate variation on mosquito abundance in California. Journal of Vector Ecology **33**:89–98.

Reisen WK, Fang Y, Martinez VM. 2005. Avian host and mosquito (Diptera: Culicidae) vector competence determine the efficiency of West Nile and St. Louis encephalitis virus transmission. Journal of Medical Entomology **42**:367–375.

Reisen WK, Fang Y, Martinez VM. 2006. Vector competence of Culiseta incidens and Culex thriambus for West Nile virus. Journal of the American Mosquito Control Association **22**:662–665.

Reisen WK, Lothrop HD, Lothrop B. 2003. Factors influencing the outcome of mark-release-recapture studies with Culex tarsalis (Diptera: Culicidae). Journal of Medical Entomology **40**:820–829.

Reisen WK, Milby MM, Meyer RP. 1992a. Population dynamics of adult *Culex* mosquitoes (Diptera: Culicidae) along the Kern River, Kern County, California, in

1990. Journal of Medical Entomology **29**:531—543.

Reisen WK, Milby MM, Presser SB, Hardy JL. 1992b. Ecology of mosquitoes and St. Louis encephalitis virus in the Los Angeles Basin of California, 1987–1990. Journal of Medical Entomology **29**:1987–1990.

Reisen WK, Thiemann T, Barker CM, Lu H, Carroll B, Fang Y, Lothrop HD. 2010. Effects of Warm Winter Temperature on the Abundance and Gonotrophic Activity of Culex (Diptera: Culicidae) in California. Journal of Medical Entomology **47**:230–237.

Reisen WKWWK, Lothrop HDH, Thiemann T. 2013. Host Selection Patterns of *Culex tarsalis* (Diptera: Culicidae) at Wetlands Near the Salton Sea, Coachella Valley, California, 1998-2002. Journal of Medical Entomology **50**:1071–1076.

Rey JR, Walton WE, Wolfe RJ, Connelly CR, O'Connell SM, Berg J, Sakolsky-Hoopes GE, Laderman AD. 2012. North American wetlands and mosquito control. International Journal of Environmental Research and Public Health **9**:4537–605.

Richards EE, Masuoka P, Brett-Major D, Smith M, Klein TA, Kim HC, Anyamba A, Grieco J. 2010. The relationship between mosquito abundance and rice field density in the Republic of Korea. International Journal of Health Geographics **9**:32.

Rochlin I, Faraji A, Ninivaggi D V, Barker CM, Kilpatrick AM. 2016. Anthropogenic impacts on mosquito populations in North America over the past century. Nature Communications **7**:1–14. Nature Publishing Group.

Rost S, Gerten D, Bondeau A, Lucht W, Rohwer J, Schaphoff S. 2008. Agricultural green and blue water consumption and its influence on the global water system. Water Resources Research **44**:1–17.

Ruiz MO, Walker ED, Foster ES, Haramis LD, Kitron UD. 2007. Association of West Nile virus illness and urban landscapes in Chicago and Detroit. International Journal of Health Geographics **6**:10.

Ruybal JE, Kramer LD, Kilpatrick AM. 2016. Geographic variation in the response of *Culex pipiens* life history traits to temperature. Parasites & Vectors.**9**:116-125

Samuel P, Ramesh D, Thenmozhi V, Nagaraj J, Muniaraj M, Arunachalam N. 2016. Japanese Encephalitis vector abundance and infection frequency in Cuddalore District, Tamil Nadu, India: a five-year longitudinal study. Journal of Entomological and Acarological Research **48**:366.

Sanford M, Chan K, Walton W. 2005. Effects of inorganic nitrogen enrichment on mosquitoes (Diptera: Culicidae) and the associated aquatic community in constructed treatment wetlands. Journal of Medical Entomology **42**:766–776.

Sardelis MR, Turell MJ, Dohm DJ, O'Guinn ML. 2001. Vector competence of selected

North American *Culex* and *Coquillettidia* mosquitoes for West Nile virus. Emerging Infectious Diseases **7**:1018–1022.

Schurich JA, Kumar S, Eisen L, Moore CG. 2014a. Modeling Culex tarsalis Abundance on the Northern Colorado Front Range Using a Landscape-Level Approach. Journal of the American Mosquito Control Association **30**:7–20.

Schurich JA, Kumar S, Eisen L, Moore CG, Moore, Chester G. 2014b. Modeling Culex tarsalis Abundance on the Northern Colorado Front Range Using a Landscape-Level Approach. Journal of the American Mosquito Control Association **30**:7–20.

Scott TW, Morrison AC. 2010. Dengue virus. Vector dynamics and transmission of dengue virus: implications for dengue surveillance and prevention strategies. in: Dengue Virus (pp. 115-128). Springer, Berlin, Heidelberg

Sinka, M.E., Bangs, M.J., Manguin, S., Chareonviriyaphap, T., Patil, A.P., Temperley, W.H., Gething, P.W., Elyazar, I.R., Kabaria, C.W., Harbach, R.E. and Hay, S.I. 2011. The dominant anopheles vectors of human malaria in the Asia-Pacific region: Occurrence data, distribution maps and bionomic précis. Parasites and Vectors **4**.

Skaff NK, Cheruvelil KS. 2016. Fine-scale wetland features mediate vector and climate- dependent macroscale patterns in human West Nile virus incidence. Landscape Ecology **31**:1615–1628.

Smith DL, Drakeley CJ, Chiyaka C, Hay SI. 2010. A quantitative analysis of transmission efficiency versus intensity for malaria. Nature Communications **1**:108.

Smith DL, Dushoff J, McKenzie FE. 2004. The risk of a mosquito-borne infectionin a heterogeneous environment. PLoS Biology **2**.

Sterling SM, Ducharne A, Polcher J. 2012. The impact of global land-cover change on the terrestrial water cycle. Nature Climate Change **3**:385–390.

Surtees G. 1970. Effects of irrigation on mosquito populations and mosquito-borne diseases in man, with particular reference to ricefield extension. International Journal of Environmental Studies **1**:35–42.

Tatem AJ, Hay SI, Rogers DJ. 2006. Global traffic and disease vector dispersal. Proceedings of the National Academy of Sciences **103**:6242–6247.

Tempelis C, Washino R. 1967. Host-feeding patterns of Culex tarsalis in the Sacramento Valley, California, with notes on other species. Journal of Medical Entomology **4**:315–318.

Tempelis CH, Reeves WC, Bellamy RE, Lofy MF. 1965. A Three-Year Study of the Feeding Habits of Culex tarsalis in Kern County, California. American Journal of Tropical Medicine and Hygiene **14**:170–177.

Thiemann T, Wheeler S. 2011. Mosquito host selection varies seasonally with host availability and mosquito density. PLoS neglected tropical diseases **5**:1–9.

Thiemann TC, Lemenager DA, Kluh S, Carroll BD, Lothrop HD, Reisen WK. 2012. Spatial variation in host feeding patterns of *Culex tarsalis* and the *Culex pipiens* complex (Diptera: Culicidae) in California. Journal of medical entomology **49**:903–16.

Thiemann TC, Woodward DL, Fang Y, Ryan BM, Nelms BM, Scott JJ, Reisen WK. 2017. Abundance and bloodfeeding patterns of mosquitoes (Diptera: Culicidae) in an oak woodland on the eastern slope of the Northern Coast Range of California. Journal of Medical Entomology **54**:1344–1353.

Thongsripong P, Green A, Kittayapong P, Kapan D, Wilcox B, Bennett S. 2013. Mosquito Vector Diversity across Habitats in Central Thailand Endemic for Dengue and Other Arthropod-Borne Diseases. PLoS Neglected Tropical Diseases **7**.

Tian HY et al. 2015. How environmental conditions impact mosquito ecology and Japanese encephalitis: An eco-epidemiological approach. Environment International **79**:17–24. Elsevier B.V.

Tilman D. 1999. The Ecological Consequences of Changes in Biodiversity: A Search for General Principles. Ecology **80**:1455–1474.

Trawinski PR, Mackay DS. 2010. Identification of Environmental Covariates of West Nile Virus Vector Mosquito Population Abundance Identification of Environmental Covariates of West Nile Virus Vector Mosquito Population Abundance. Vector borne and Zoonotic Diseases **10**:515–526.

Troy TJ, Kipgen C, Pal I. 2015. The impact of climate extremes and irrigation on US crop yields. Environmental Research Letters **10**.

Turell M, O'Guinn M, Oliver J. 2000. Potential for New York mosquitoes to transmit West Nile virus. The American Journal of Tropical Medicine and Hygiene **62**:413–414.

Turell MJ, Guinn MLO, Dohm DJ, Jones JW. 2001. Vector Competence of North American Mosquitoes (Diptera:Culicidae) for West Nile Virus. Journal of Medical Entomology **38**:130–134.

UC Davis. 2016. California Vector-borne Disease Surveillance System (CALSURV). Available from https://gateway.calsurv.org/ (Accessed January 1, 2016).

USGS. 2012. Moderate Resolution Imaging Spectroradiometer (MODIS) Irrigated Agriculture Dataset for the United States. Available from http://earlywarning.usgs.gov/USirrigation. (Accessed on May 17, 2018)

Vaidyanathan R, Scott TW. 2007. Geographic Variation in Vector Competence for

West Nile Virus in the *Culex pipiens* (Diptera: Culicidae) Complex in California. Vector-Borne and Zoonotic Diseases **7**:193–198.

Van Schmidt N, Oviedo J, Hruska T, Huntsinger L, Kovach T, Kilpatrick A, Miller N, Beissinger S. 2018. Impacts of social-ecological diversity on resilience in a coupled human and natural system. Nature Sustainability **In Review**.

Vitousek PM, Mooney H a, Lubchenco J, Melillo JM. 1997. Human Domination of Earth' s Ecosystems. Science **277**:494–499.

Vorosmarty C, Sahagian D, Vörösmarty CJ, Analysis E, That D, Engineering H, Produced HAS, Impacts S, The ON, Cycle TW. 2000. Anthropogenic Disturbance of the Terrestrial Water Cycle. BioScience **50**:753–765.

Walton W. 2012. Design and management of free water surface constructed wetlands to minimize mosquito production. Wetlands Ecology and Management **20**:173–195.

Walton W, Popko D, Dam A Van. 2012. Width of planting beds for emergent vegetation influences mosquito production from a constructed wetland in California (USA). Ecological Engineering **42**:150–159.

Walton W, Reisen W. 2013. Influence of Climate Change on Mosquito Development and Blood-feeding Patterns. Viral Infections and Global Change 33-56

Walton W, Workman P. 1998. Effectiveness of control measures against mosquitoes at a constructed wetland in southern California. Journal of Vector Ecology **23**:149–160.

Walton WE, Popko DA, Van Dam AR, Merrill A. 2013. Distribution of Culex species in vegetation bands of a constructed wetland undergoing integrated mosquito management. Journal of the American Mosquito Control Association **29**:69–73.

Wegbreit J, Reisen WK. 2000. Relationships among weather, mosquito abundance, and encephalitis virus activity in California: Kern County 1990-98. Journal of the American Mosquito Control Association **16**:22–7.

Wekesa JW, Yuval B, Washino RK, de Vasquez AM. 1997. Blood feeding patterns of *Anopheles freeborni* and *Culex tarsalis* (Diptera: Culicidae): effects of habitat and host abundance. Bulletin of Entomological Research **87**:633–641.

Wheeler SS, Barker CM, Fang Y, Armijos MV, Carroll BD, Husted S, Johnson WO, Reisen WK. 2009. Differential Impact of West Nile Virus on California Birds. The Condor **111**:1–20.

Wimberly MC, Hildreth MB, Boyte SP, Lindquist E, Kightlinger L. 2008. Ecological niche of the 2003 west nile virus epidemic in the northern great plains of the United

States. PloS one **3**:e3744.

Wimberly MC, Lamsal A, Giacomo P, Chuang TW. 2014. Regional variation of climatic influences on West Nile virus outbreaks in the United States. American Journal of Tropical Medicine and Hygiene **91**:677–684.

Wood B, Washino R, Beck L, Hibbard K. 1991. Distinguishing high and low anopheline-producing rice fields using remote sensing and GIS technologies. Preventive Veterinary Medicine **11**:277–288.

Workman PD, Walton WE. 2000. Emergence Patterns of Culex Mosquitoes at an Experimental Constructed Treatment Wetland in Southern California. Journal of the American Mosquito Control Association **16**:124–130.

World Health Organization. 2014. A global brief on vector-borne diseases. World Health Organization:9. Available from http://apps.who.int/iris/bitstream/10665/111008/1/WHO_DCO_WHD_2014.1_eng.pdf. (Accessed on May 17, 2018)