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Tick-borne disease ecology in a changing world: The influence of climate, habitat and abiotic conditions, and forest disturbance on Lyme disease ecology and risk in California

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Tick-borne disease ecology in a changing world: The influence of climate, habitat and abiotic conditions, and forest disturbance on Lyme disease ecology and risk in California

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy
in Ecology, Evolution, and Marine Biology

by

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ABSTRACT

Tick-borne disease ecology in a changing world: The influence of climate, habitat and abiotic
conditions, and forest disturbance on Lyme disease ecology and risk in California

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Globally, the rate of emergence of infectious diseases has increased significantly over time. Vector-borne diseases in particular present one of the biggest threats to public health globally. Many of these diseases are zoonotic in nature, meaning they cycle in animal populations but can spillover to infect humans. As such, risk to humans of acquiring a zoonotic disease depends in large part on the distribution and abundance of the reservoir hosts, the species of animals that the pathogen naturally infects, as well as of the vector species. The ecology of many reservoir hosts and vectors is rapidly changing due to ongoing environmental change, which will fundamentally alter human disease risk in the future. To understand how disease risk may be forecasted to change requires an understanding of the drivers of the distribution and abundance of pathogens, disease vectors and reservoir hosts.

This research uses Lyme disease in California as a model system to understand the drivers of zoonotic disease risk on a rapidly changing planet, particularly for vector-borne diseases. Specifically, it investigates the following questions: 1) are there latitudinal differences in the seasonal activity patterns of the primary tick vector, *Ixodes pacificus*, in California, and might these differences be driven by climate? 2) What abiotic and environmental factors drive tick abundance and infection prevalence with the causative agent of Lyme disease, *Borrelia burgdorferi* sensu stricto, in southern California? 3) What are the effects of large-scale, intense ecological disturbances, namely wildfire, on the ecology of Lyme disease in the far western US?

To address these questions, I use a combination of field, laboratory and statistical methods including field collection of samples (ticks and tissue samples from host animals), DNA extraction and polymerase chain reaction (PCR) in the laboratory for analysis of infection, and statistical and GIS-based analysis of field-derived and public data sets.

I find that tick vector activity patterns in southern California are highly truncated, and tick density is significantly lower than in northern California where human Lyme disease incidence is higher. This suggests much lower tick-borne disease risk in southern California, which I broadly attribute to the hotter, drier climate in this region. This has implications for reduced tick-borne disease risk under future climate change, in contrast with the predictions made for numerous other emerging diseases (Chapter 1). Additionally, I identify dense oak woodlands as the highest risk habitats for *I. pacificus* tick encounter in southern California. I also find densities of small vertebrate hosts to be significant predictors of tick abundance, though found a shift in the relative importance of host availability and habitat characteristics in predicting juvenile tick abundance as California's historic drought intensified. This finding suggests that habitat providing suitable microclimates for tick survivorship became centrally important to patterns of abundance in the face of adverse abiotic conditions. This further suggests that predicted climate change in California may act as an important limiting factor on *I. pacificus* populations. And, despite the low risk of human Lyme disease infection posed by *I. pacificus* in southern California, comparatively high rates of infection were found in other tick species, suggesting that enzootic transmission of tick-borne borreliae may be more

common in southern California than previously suspected (Chapter 2).

Finally, I find that wildfire disturbance, which is projected to become more severe under climate change, initially increases tick-borne disease risk in the year following fire, with this effect reversing in subsequent years. Tick abundance was elevated in plots sampled within the perimeter of a recent wildfire in Santa Barbara County, California in the year following the fire, though declined precipitously in following years. Also notable was the finding that populations of one of the primary reservoir hosts for the Lyme bacteria, dusky-footed woodrats (*Neotoma fuscipes*), were eliminated within the perimeter of the fire. In contrast, populations of western fence lizards (*Sceloporus occidentalis*), considered to be "non-competent hosts" for the bacteria because they cleanse feeding ticks of infection and do not carry the pathogen, were unaffected by the wildfire. Taken together with significantly reduced tick populations within the fire perimeter following the fire, this evidence suggests that wildfire disturbance may ultimately reduce tick-borne disease risk for multiple years following wildfire in California.

Ongoing environmental change has already led to disease emergence across the globe, with consequences for human health. Further, the way in which environmental change is expected to impact the ecology of infectious diseases is challenging to predict and poorly understood for many disease systems. My dissertation research informs some of these key gaps in our understanding of tick-borne disease in the western US under environmental change.

I. Truncated seasonal activity patterns of the western blacklegged tick (*Ixodes pacificus*) in central and southern California

This chapter appeared as a manuscript in February 2016 in Ticks and Tick-Borne Diseases, volume 7, issue 1, pages 234-242. The doi is: 10.1016/j.ttbdis.2015.10.016. Authorship on the published manuscript is as follows: Andrew J. MacDonald and Cheryl J. Briggs.

A. Introduction

Globally, there has been an increase in the rate of emergence of vector-borne zoonotic diseases in recent decades, presenting new challenges and threats to public health (Jones et al. 2008, Kilpatrick and Randolph 2012). A number of large-scale anthropogenic changes, such as land use and climate change, are contributing to the amplification of emerging infectious zoonotic diseases. For example, the distribution of vector species may shift or expand as a result of climate change, and lead to subsequent shifts in vector-borne disease burden (Bounoua et al. 2013, Ogden et al. 2008b). In the case of pathogens with complex transmission cycles involving multiple hosts and vector life stages, changing host ecology resulting from land use or environmental change may also alter human disease risk through vector abundance (Ogden et al. 2014), infection prevalence with the pathogen (Allan et al. 2003, Patz et al. 2004), or vector activity patterns (Ogden et al. 2008a). Thus, understanding when and where vector species are active and how these patterns may be expected to change given ongoing climate or environmental change is crucial to prevention and control of vector-borne diseases.

Lyme disease is the most commonly reported vector-borne disease in the United States, and is increasing in incidence and geographic range (Bacon et al. 2008). In the United

States, Lyme disease is caused by an infection with *Borrelia burgdorferi*, a spirochete that is transmitted to humans by blacklegged ticks—*Ixodes scapularis* in the eastern United States and *Ixodes pacificus* in the western United States. In addition to *B. burgdorferi*, both blacklegged and western blacklegged ticks vector a number of other emerging pathogens including the causative agents of tick-borne relapsing fever (*Borrelia miyamotoi*), anaplasmosis (*Anaplasma phagocytophilum*), and babesiosis (*Babesia* spp.). *Ixodes* spp. ticks have a four-stage life cycle, comprised of the egg stage and the parasitic larval, nymphal and adult stages, and maintain enzootic transmission of *B. burgdorferi* in complex cycles involving many different vertebrate hosts (Gray et al. 2002, Kurtenbach et al. 2006). *Borrelia burgdorferi* is not transmitted transovarially and can be acquired by larval and nymphal ticks only through blood meals taken from infected hosts, and thus infections may be transmitted only by infected nymphal or adult female ticks (Clover and Lane 1995, Falco et al. 1999, Gray et al. 2002, Kurtenbach et al. 2006). Seasonal activity and density of potentially infectious tick life stages are thus critical components of Lyme disease risk.

In the eastern US where human Lyme disease is most common, larval *I. scapularis* peak in activity in the early fall (August-October) in the northeastern US, and in the summer months (June-August) in the upper Midwestern US, while nymphal *I. scapularis* peak in activity during the summer months (June-August) and adult *I. scapularis* have bimodal peaks in activity during the fall and spring (Falco et al. 1999, Gatewood et al. 2009, Hamer et al. 2012, Ostfeld et al. 1996). Consequently, because nymphal *Ixodes* spp. are the primary vector, peak Lyme disease transmission in the eastern US occurs during the summer months when nymphal ticks are most active (Falco et al. 1999), and Lyme disease risk is absent during the winter months when much of the northeast and upper Midwest is blanketed in snow or experiencing temperatures consistently below 0° C. In contrast, in western North America, the area of highest risk for acquiring Lyme disease is northwestern California, where vector ticks have been found to be active throughout the year, presenting a year-round risk of Lyme disease transmission (Salkeld et al. 2014).

In central and southern California, infected *I. pacificus* ticks have been identified, but transmission of *B. burgdorferi* to humans is less common (Padgett et al. 2014). A handful of previous studies suggest that infection prevalence in vector tick populations in central and southern California—with both *B. burgdorferi* as well as the relapsing fever spirochete, *B. miyamotoi*—is low (Lane et al. 2013, Padgett et al. 2014), which is likely contributing significantly to the low rate of transmission to humans in this region. However, the underlying mechanism producing low infection prevalence in southern California tick populations is not well understood. Here we examine one possible mechanism, namely the seasonal activity patterns of the western blacklegged tick, and investigate whether this vector species exhibits a truncated period of seasonal activity in southern California. We report on seasonal activity patterns of *I. pacificus* in sites in Santa Barbara County and Los Angeles County, California in which weekly to monthly tick collection was undertaken over multiple years. We show that *I. pacificus* activity patterns, particularly of the juvenile stages, are truncated relative to those observed in northwestern California. We discuss possible causes of these observed patterns, implications for human tick-borne disease risk in central and southern California, as well as implications for tick-borne disease risk under projected climate change in the western US.

B. Methods

I. pacificus ticks were collected at three sites in Santa Barbara and Los Angeles County, California to determine seasonal activity patterns and timing of peak density in central and southern California. Santa Barbara County collection locations included Sedgwick Reserve, part of the University of California Natural Reserve System and located in the Santa Ynez Valley, and Paradise Reserve located in the Los Padres National Forest on the north side of the Santa Ynez Mountains (Figure 1). Collection sites in Sedgwick Reserve were characterized by oak woodland, consisting of coast live oak (*Quercus agrifolia*), blue oak (*Quercus douglasii*) and occasional valley oak (*Quercus lobata*). The understory was dominated by introduced grasses including brome (*Bromus* spp.), wild oats (*Avena* spp.) and occasional native bunch grasses, as well as common vetch (*Vicia sativa*) and California sagebrush (*Artemisia californica*). Collection sites in Paradise Reserve were characterized by similar plant communities, notably coast live oak woodland with occasional California bay-laurel (*Umbellaria californica*) and an understory dominated by introduced grasses and western poison-oak (*Toxicodendron diversilobum*). In Los Angeles County, ticks were collected from Stunt Ranch Reserve, also a part of the University of California Natural Reserve System, in the Santa Monica Mountains (Figure 1). Collection sites in Stunt Ranch Reserve were also characterized by coast live oak woodland with an understory dominated by introduced grasses and western poison-oak.

1. Tick Sampling for all parasitic life stages: Southern California

Western blacklegged ticks were collected at Sedgwick, Paradise and Stunt Ranch Reserves using the flagging method, in which a 1m^2 white flannel cloth is dragged over understory vegetation and leaf litter, and attached, questing ticks are counted and removed (e.g. Daniels et al. 2000). Adult ticks were primarily encountered on understory shrubs and grasses, and juvenile ticks were only encountered in patches of leaf litter, so two distinct habitat types were sampled for the different stages. Adult ticks were collected from understory shrubs and grasses along established transects at each reserve, and an area of 500 m^2 of oak woodland was sampled during each sampling event. Adult ticks were collected weekly to biweekly from sites established at Sedgwick Reserve from December 2012 to December 2013, and monthly from January 2014 through June 2014. Adult ticks were collected from Paradise Reserve weekly to monthly from November 2013 through June 2015, and from Stunt Ranch Reserve approximately monthly from December 2013 through June 2014, and from December 2014 through June 2015.

Larval and nymphal ticks were collected using the same flagging method as adult ticks, with flagging effort focused on dense patches of leaf litter. Juvenile ticks were collected at each reserve on the same sampling schedule as adult ticks, and an area of 200 m^2 of leaf litter was sampled in oak understory each time (a smaller area was sampled for juvenile ticks than adults due to the general rarity and patchiness of dense leaf litter habitat in these southern California oak woodland sites). Juvenile ticks were not found to be questing on vegetation above the surface of the leaf litter, so no juvenile ticks were encountered or collected on adult tick transects. Adult ticks were, however, occasionally collected in patches of leaf litter in which juvenile tick sampling took place. These adult ticks were not included in the analysis in order to maintain consistency in area sampled for the various parasitic life stages for the duration of the study.

2. Tick seasonality data: Northwestern California

We compared our data from southern California collection sites to previously published data from northern California. Northwestern California tick data are from China

Camp State Park in Marin County and were published in a recent study by Salkeld and colleagues (2014). In this study, all parasitic life stages were sampled concurrently over multiple years, producing estimates of tick density through time. This is the only published study from northern California with comparable data to the present study for all parasitic life stages, and data were provided in raw form to be re-analyzed. Details of the sampling methodology are described in Salkeld and colleagues (2014). Sampling methodology in southern California sites matched the methodology used in northern California, though total area sampled over the duration of the studies differed between sites (e.g., due to the number of times each site was sampled). For comparison between sampling locations, density of ticks was calculated for each stage over time for each site, and standardized between sites.

3. Weather station data

Weather station data (precipitation, temperature and relative humidity) were obtained from the nearest weather station to each sampling location in this study—from the Point San Pedro weather station (http://www.ipm.ucdavis.edu/WEATHER/wxactstnames.html) adjacent to China Camp State Park in Marin County, from the UCSB department of geography weather station (http://www.geog.ucsb.edu/ideas/) at Sedgwick Reserve for both Sedgwick and Paradise Reserves in Santa Barbara County, and from the Stunt Ranch Reserve weather station in Los Angeles County (http://www.wrcc.dri.edu/weather/ucsr.html). The variables chosen—seasonal temperature, relative humidity and timing of precipitation events—are thought to regulate the life cycle of *I. pacificus* (Padgett and Lane 2001, Salkeld et al. 2014), and are included to illustrate broad climatic differences between the two regions that may be driving seasonal activity patterns of questing *I. pacificus* ticks.

4. Statistical analyses

The Welch's *t*-test (for unequal variances and unequal sample sizes) was employed to determine whether mean peak density of each parasitic life stage of *I. pacificus* was significantly lower at the southern California sampling locations than at China Camp State Park. The Welch's *t*-test was chosen due to the unequal number of samples taken from each study site over the duration of the two years of sampling.

C. Results

1. Adult tick activity (Santa Barbara and Los Angeles Counties)

At Sedgwick Reserve in the Santa Ynez Valley, the earliest observation of questing adult *I. pacificus* was in mid-December—12/15/2013, and peak density occurred in February-March (Figure 2c; Tables 1 and 2). At Paradise Reserve on the north side of the Santa Ynez Mountains, earliest observation was in late November—11/23/2013, and peak density occurred in February-March, though there appeared to be bimodal peaks in January and May of 2014 (Figure 2b; Tables 1 and 2). At Stunt Ranch Reserve in the Santa Monica Mountains, adult ticks became active in late December 2013 and 2014, peaked in density in February-March, and were no longer active by late April to early May (Figure 2d; Tables 1 and 2). Peak density of adult ticks was significantly lower at Sedgwick Reserve ($t(17.118)=4.6154$, $p < 0.001$), Paradise Reserve ($t(17.583)=4.1437$, $p < 0.001$) and Stunt Ranch Reserve ($t(17.956)=4.4816$, $p < 0.001$) than at China Camp State Park in northern California. As in previous studies (Salkeld et al. 2014), adult tick activity generally began following the first substantial rains of the wet season, with adult ticks becoming rare or absent following the last major rain events of the season. For this study, adult *I. pacificus* became rare or absent by late April to early May at all sites sampled in central and southern

California, which mirrored patterns of precipitation, with final major rain events occurring in late March to early April.

2. Immature tick activity (Santa Barbara and Los Angeles Counties)

Nymphal *I. pacificus* were first observed in late February and absent by mid-April at Sedgwick Reserve (Figure 3c). At Paradise Reserve, patterns were similar with first observations occurring in early March, and questing nymphs absent by mid-May (Figure 3b). At Stunt Ranch Reserve, nymphs were first observed in early March and active through early June (Figure 3d). Density of nymphal ticks was significantly lower at Sedgwick ($t(26.994)=2.3209$, $p < 0.05$), Paradise ($t(24.248)=2.2309$, $p < 0.05$), and Stunt Ranch ($t(18.827)=3.861$, $p < 0.001$) Reserves than at China Camp State Park in northern California. Questing nymphal ticks were rare throughout the entire season at all southern California sites (Table 2), with no clear peaks in activity when density of ticks was substantially higher than at other times of the year.

Patterns of larval tick activity were similar to those of nymphal ticks, with first observations occurring in late February and questing larval ticks absent by mid-May at Sedgwick Reserve (Figure 4c). At Paradise Reserve, questing larval ticks were first observed in early March and were absent by mid-May (Figure 4b). At Stunt Ranch Reserve, larval ticks were first active by early to late February and absent by early to late May (Figure 4d). Peak density of larval ticks was significantly lower at Sedgwick ($t(15.051)=1.9921$, $p < 0.05$), Paradise ($t(10.042)=3.0555$, $p < 0.01$), and Stunt Ranch ($t(12.848)=2.4215$, $p < 0.05$) Reserves than at China Camp State Park in northern California. Timing of peak larval activity varied between years and sampling locations, though consistently fell within the months of March and April (Table 1).

Abiotic conditions differed substantially between northern and southern California collection locations, and weather station data show that average relative humidity was ~10% higher at China Camp State Park (71.48%) in Marin County than at Sedgwick Reserve (61.71%) in Santa Barbara County, and ~23% higher at China Camp State Park than Stunt Ranch Reserve (48.70%), throughout the duration of the tick sampling periods at each site (Figure 5a). Similarly, average maximum temperature was ~5° C lower at China Camp State Park (20.32° C) than at Sedgwick Reserve (25.01° C), and ~6° C lower at China Camp State Park than at Stunt Ranch Reserve (26.76° C), over the same period (Figure 5b). Further, seasonal trends of relative humidity and temperature illustrate that the differences between northwestern and southern sites are particularly apparent during the seasonal summer drought. Finally, timing between precipitation events was shorter at China Camp State Park than at Sedgwick or Stunt Ranch Reserves over the duration of each respective study period (Figure 6a-c). The first rains of the season occurred earlier and last rains of the season occurred later at China Camp State Park than at either Sedgwick or Stunt Ranch Reserves, which experience a more protracted summer drought.

D. Discussion

In this study, the season for questing adult *I. pacificus* ticks in Los Angeles and Santa Barbara counties began in late November to late-December, depending on the sampling location, peaked between January and May, and ended by late April to early May. Differences between sampling locations are likely due to the microclimatic conditions within each site, which have previously been shown to drive local-scale differences in tick density (Eisen et al. 2003). The observed patterns of adult tick activity generally follow those reported from northwestern California (Salkeld et al. 2014), though activity in central and southern California consistently begins later in the season and ends earlier than in northwestern California (Figure 2), displaying a truncated pattern. Furthermore, density of adult ticks is much lower at sites sampled in Santa Barbara and Los Angeles County than in sites sampled in northwestern California (Salkeld et al. 2014) (Figure 2; Table 2).

Seasonal activity patterns of juvenile *I. pacificus* were also found to be truncated relative to those reported for China Camp State Park, as well as other study sites in northwestern California (Salkeld et al. 2014) (Figures 3 and 4; Table 1). Nymphal tick activity reported in northwestern California began as early as the beginning of February, lasting throughout the summer months and in some cases as late as October (Salkeld et al. 2014). In southern California sites, nymphal ticks were found to be active only from late February through early June. Larval *I. pacificus* displayed a similarly abbreviated pattern of seasonal activity as nymphal ticks in southern California sites. In a previous study conducted in southern California (Lane et al. 2013) during the months of March, April and May of 2010, juvenile *I. pacificus* activity was found to be broadly similar to seasonal patterns of activity reported for northern California, based on flagging and tick removal from western fence lizards (*Sceloporus occidentalis*), which are a primary host for juvenile *I. pacificus* in northern California (Lane and Loye 1989). However, due to the short duration of sampling for juvenile ticks in this study, seasonal trends could not easily be discerned.

Ixodid ticks, especially those species that spend a significant proportion of their life-cycle off-host, are highly susceptible to adverse abiotic conditions (e.g. high temperatures and low humidity), and avoid such conditions by entering states of inactivity or behavioral diapause (Needham and Teel 1991, Padgett and Lane 2001). *I. pacificus*, which spends >90% of its three year life cycle off-host, has been found to be particularly susceptible to high temperatures and low humidity and precipitation, which likely drive seasonal activity patterns of this tick (Eisen et al. 2002, Eisen et al. 2003, Padgett and Lane 2001, Swei et al. 2011). Thus, while the variation in patterns of seasonal activity between the sites sampled in northwestern and southern California may be due to interannual differences in weather and abiotic conditions (Figures 5 and 6; Table 1), especially given the drought conditions that California experienced during the course of the present study, broader climatic differences between northern and southern California are consistent. Therefore, they may be expected to produce consistent differences in tick density and seasonal activity between the two regions (Eisen et al. 2003). This effect of climate could manifest as a direct negative effect on tick survivorship during off-host periods throughout the protracted summer drought, or could be indirect through a negative effect on the densities of vertebrate hosts resulting in fewer successful juvenile tick blood meals in southern California.

The observed patterns of seasonal activity in central and southern California thus have potential implications for tick-borne disease risk in the western US under future climate change. In California, temperatures are expected to increase by 1.5 °C to 5.8 °C, depending on emissions scenarios and the climate model used, by the end of the century (Cayan et al. 2008, Hayhoe et al. 2004). Much of this warming is expected to occur during the summer months (Hayhoe et al. 2004). Additionally, average precipitation is expected to decline in California, primarily in the winter months (Hayhoe et al. 2004). These projected impacts of climate change in California are predicted to be more pronounced in northern and north pacific coastal regions of the state (Cayan et al. 2008, Hayhoe et al. 2004), where tick-borne disease risk is currently higher (Eisen et al. 2006b, Salkeld et al. 2014), than elsewhere in the state. If climate change leads to hotter, longer summers and drier winters in northwestern

California, more closely approximating the current climate in southern California, this could lead to reduced tick-borne disease risk in northwestern California via climate impacts on tick density and seasonal activity (Eisen et al. 2006b, 2003, 2006a). This prediction contrasts with those made for tick-borne disease in the eastern US (e.g., Ogden et al. 2014), as well as with the often reported result that climate change will exacerbate infectious disease burden and increase risks to human health (Patz et al. 2005, Altizer et al. 2013). Interactions between climate change and infectious disease are complex, and impacts will vary regionally and by disease agent (Lafferty 2009, Holt et al. 2009).

However, given the uncertainties surrounding the magnitude and direction of climate change impacts, as well as uncertainty surrounding species adaptation to changing climate—in this case ticks and their vertebrate hosts, particularly reservoir hosts for *B. burgdorferi* and other pathogens—impacts of climate change on tick-borne disease risk in California remain challenging to predict. Future studies should examine patterns of tick activity and density, host-feeding, and infection prevalence across California's extensive latitudinal and climate gradients, to better inform our understanding of current, as well as predictions of future, tick-borne disease risk in California.

The truncated period of seasonal activity of the juvenile stages in particular, also has important implications for enzootic pathogen transmission dynamics in southern California. *I. pacificus* has three parasitic life stages, and one opportunity for pathogen acquisition during the larval stage before molting into the epidemiologically important nymphal stage. Given the highly reduced period of activity of the larval stage in this study, there exists a shorter period of time and fewer opportunities for larval ticks to feed successfully on an infected host. This could lead to lower infection rates in the nymphal stage, as observed in previous studies (Lane et al. 2013, Padgett et al. 2014), than in northwestern California as well as lower risk of human infection. However, to determine whether this mechanism is indeed operating, additional studies are needed that explore the seasonality of host feeding across various species of hosts including reservoir hosts as well as western fence lizards, a dilution host in this system (Lane and Quistad 1998). Additionally, the proportion of blood meals coming from reservoir hosts may differ between southern and northern California, which may also be playing an important role in the ecology of tick-borne pathogens in this region.

The low number of nymphs retrieved in the central and southern California study sites may be due to regional behavioral differences in tick questing. For example, other studies have yielded similar results in which nymphal *I. scapularis* in the southeast exhibit different questing behavior than *I. scapularis* in the northeast (Arsnoe et al. 2015), and nymphal *I. pacificus* in southern California were found at similarly low densities by flagging, which did not track densities of host feeding nymphs on western fence lizards (Lane et al. 2013). Thus, while southern California nymphal ticks may be active below the surface of the leaf litter or exhibit more nidicolous questing behavior than in northern California, they are not found at high densities using the drag method, which closely approximates human risk of tick encounter. Such low encounter rates using the drag method with nymphal ticks, the most epidemiologically important tick life stage for pathogen transmission to humans, coupled with low rates of enzootic pathogen transmission and tick infection prevalence (Lane et al. 2013, Padgett et al. 2014) suggests that human risk of tick-borne disease is exceedingly low in central and southern California. If human cases of Lyme disease are acquired in southern California, dates of onset are expected to fall within a brief window between early March to early June, given observed nymphal *I. pacificus* questing activity in this region, and the average delay in occurrence of 10 days following tick bite of erythema migrans rash, the characteristic skin lesion associated with human Lyme disease (Nadelman et al. 1996).

In conclusion, based on this and other recent studies (Lane et al. 2013, Padgett et al. 2014), risk of *I. pacificus* tick encounter and human tick-borne disease appear to be comparatively low in southern California. The period of seasonal activity of questing *I. pacificus*, particularly the juvenile stages, was truncated in this study in the three sites sampled in southern California relative to activity patterns reported from one recent study, as well as historical data and long-term monitoring of sites in northwestern California (Salkeld et al. 2014). Additionally, density of questing nymphal ticks—the most important life stage for human disease risk—collected using the flagging method, which is a good proxy for risk of human tick encounter, was found to be very low in various sites across central and southern California (Lane et al. 2013, and the present study). Given the potential role of weather and climate, including seasonal precipitation patterns, relative humidity and temperature, in producing the observed differences in density and seasonal activity between regions in California (Eisen et al. 2006b, 2006a, 2003), these results also have implications for reduced tick-borne disease risk under future climate change in the western United States.

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G. Figure Captions

Figure 1. Map of tick sampling sites in northwestern, and southern California. California hillshade data layer was obtained from Cal-Atlas (www.atlas.ca.gov/download.html) through https://koordinates.com.

Figure 2. Seasonal activity patterns of adult western blacklegged ticks, *Ixodes pacificus*, in California sampling sites. Polygons represent tick density through time, and points represent sampling events. (a) Adult *I. pacificus* ticks per 100m^2 collected at China Camp State Park, 2011-2012; (b) adult *I. pacificus* ticks per 100m^2 collected at Paradise Reserve, 2014-2015; (c) adult *I. pacificus* ticks per 100m^2 collected at Sedgwick Reserve, 2013-2014; and (d) adult *I. pacificus* ticks per 100m^2 collected at Stunt Ranch Reserve, 2014-2015. Note that the y-axes are scaled differently, illustrating marked differences in tick density between the northern site and the southern sites. Data are not available from China Camp State Park after March 19, 2012, where density appears to drop precipitously to zero in panel (a) of the figure.

Figure 3. Seasonal activity patterns of nymphal western blacklegged ticks, *Ixodes pacificus*, in California sampling sites. Polygons represent tick density through time, and points represent sampling events. (a) Nymphal *I. pacificus* ticks per 100m^2 collected at China Camp State Park, 2010-2012; (b) nymphal *I. pacificus* ticks per 100m^2 collected at Paradise Reserve, 2014-2015; (c) nymphal *I. pacificus* ticks per 100m^2 collected at Sedgwick Reserve, 2013-2014; and (d) nymphal *I. pacificus* ticks per 100m^2 collected at Stunt Ranch Reserve, 2014-2015. Note that the y-axes are scaled differently, illustrating marked differences in tick density between the northern site and the southern sites. Data are not available from China Camp State Park after May 2, 2012, where density appears to drop precipitously to zero in panel (a) of the figure.

Figure 4. Seasonal activity patterns of larval western blacklegged ticks, *Ixodes pacificus*, in California sampling sites. Polygons represent tick density through time, and points represent sampling events. (a) Larval *I. pacificus* ticks per 100m^2 collected at China Camp State Park, 2010-2012; (b) larval *I. pacificus* ticks per 100m^2 collected at Paradise Reserve, 2014-2015; (c) larval *I. pacificus* ticks per 100m^2 collected at Sedgwick Reserve, 2013-2014; and (d) larval *I. pacificus* ticks per 100m^2 collected at Stunt Ranch Reserve, 2014-2015. Note that the y-axes are scaled differently, illustrating marked differences in tick density between the northern site and the southern sites.

Figure 5. Weather station data from Sedgwick, Stunt Ranch and China Camp State Park collection sites. (a) Average weekly relative humidity (%) from 2008-2015; solid lines represent a smoothing loess for each site illustrating seasonal patterns. (b) Average weekly temperature (°C) from 2008-2015; solid lines represent a smoothing loess for each site illustrating seasonal patterns. Shaded boxes overlying the data in each panel of the figure illustrate the dates over which tick sampling took place at each of the three sites. Weather station data is not available prior to 2013 at Stunt Ranch, and no data is available for Paradise Reserve.

Figure 6. Seasonal precipitation (mm) from 2008-2015: (a) China Camp State Park, (b) Sedgwick Reserve, and (c) Stunt Ranch Reserve. Timing between rain events is longer at both Sedgwick and Stunt Ranch Reserves producing a more protracted summer drought at these sites than is experienced at China Camp State Park in northwestern California. Data are not available for Stunt Ranch Reserve prior to 2013, and no data are available for Paradise Reserve.

Figure 1.

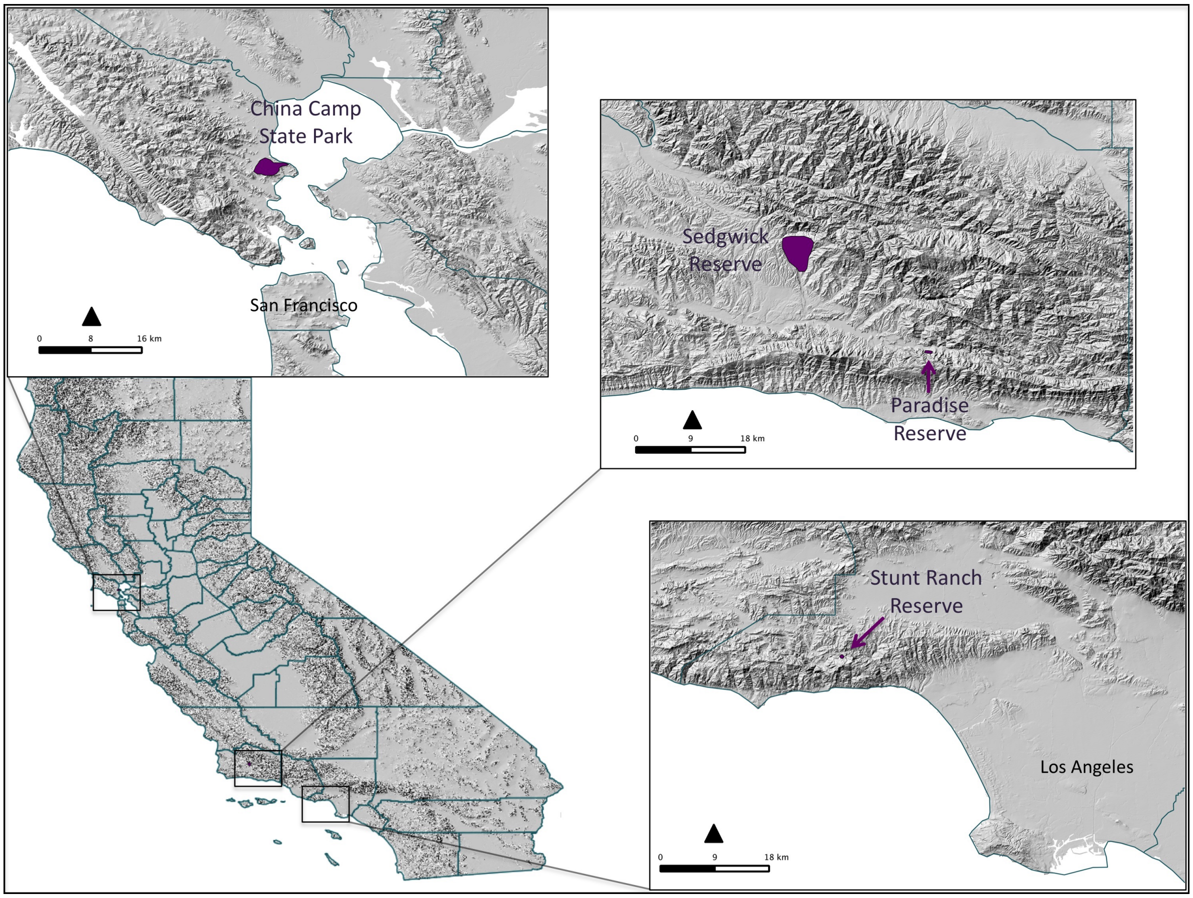
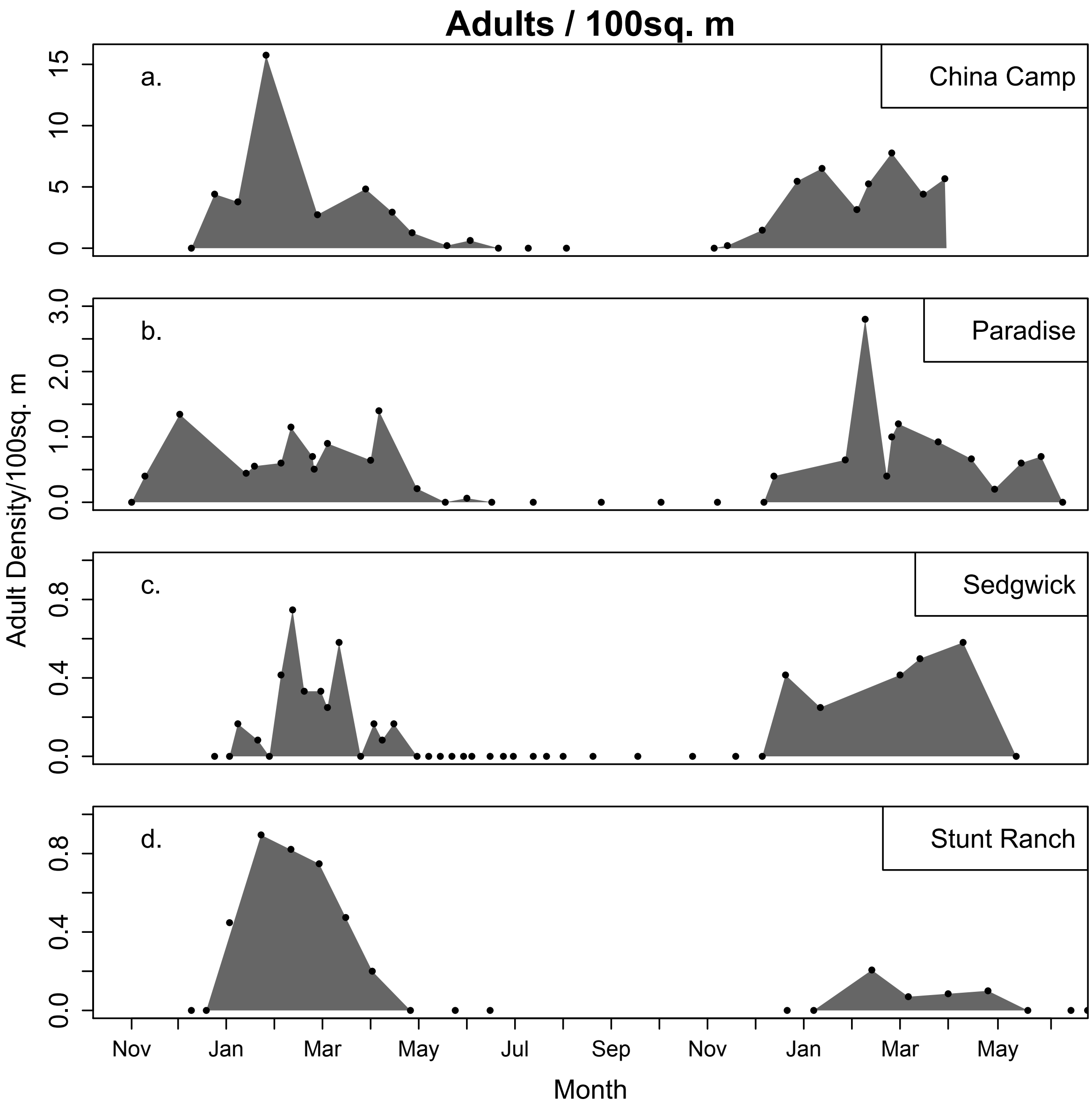

China Camp State Park
San Francisco
0 8 16 km
Sedgwick Reserve
Paradise Reserve
0 9 18 km
Stunt Ranch Reserve
Los Angeles
0 9 18 km

Figure 2.



Adults / 100sq. m
Adult Density/100sq. m
a.
China Camp
0
5
10
15
b.
Paradise
0.0
1.0
2.0
3.0
c.
Sedgwick
0.0
0.4
0.8
d.
Stunt Ranch
0.0
0.4
0.8
Nov
Jan
Mar
May
Jul
Sep
Nov
Jan
Mar
May
Month

Figure 3.

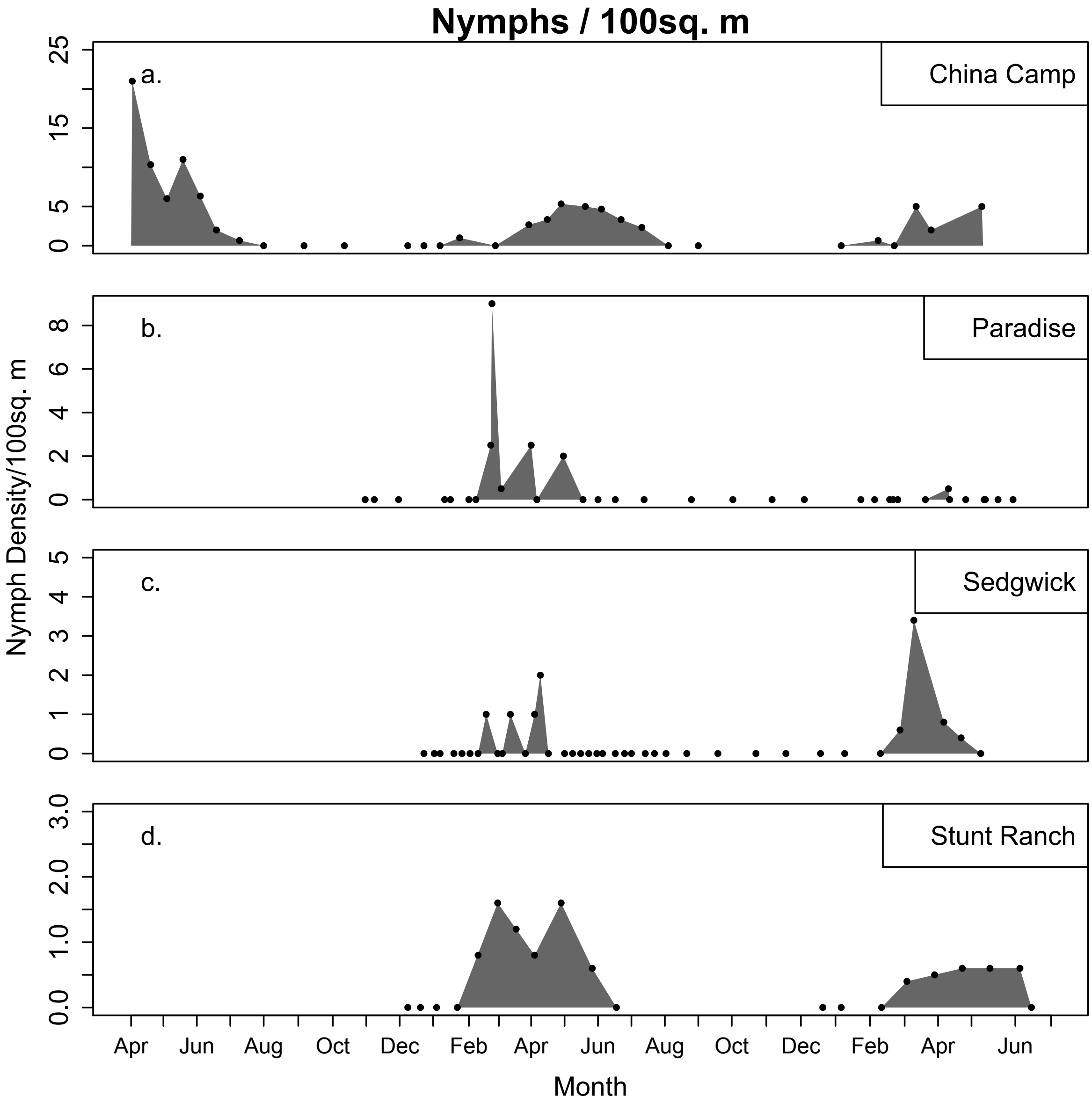

Nymphs / 100sq. m
a.
China Camp
b.
Paradise
c.
Sedgwick
d.
Stunt Ranch
Nymph Density/100sq. m
0
5
15
25
0
2
4
6
8
0
1
2
3
4
5
0.0
1.0
2.0
3.0
Apr
Jun
Aug
Oct
Dec
Feb
Apr
Jun
Aug
Oct
Dec
Feb
Apr
Jun
Month

Figure 4.

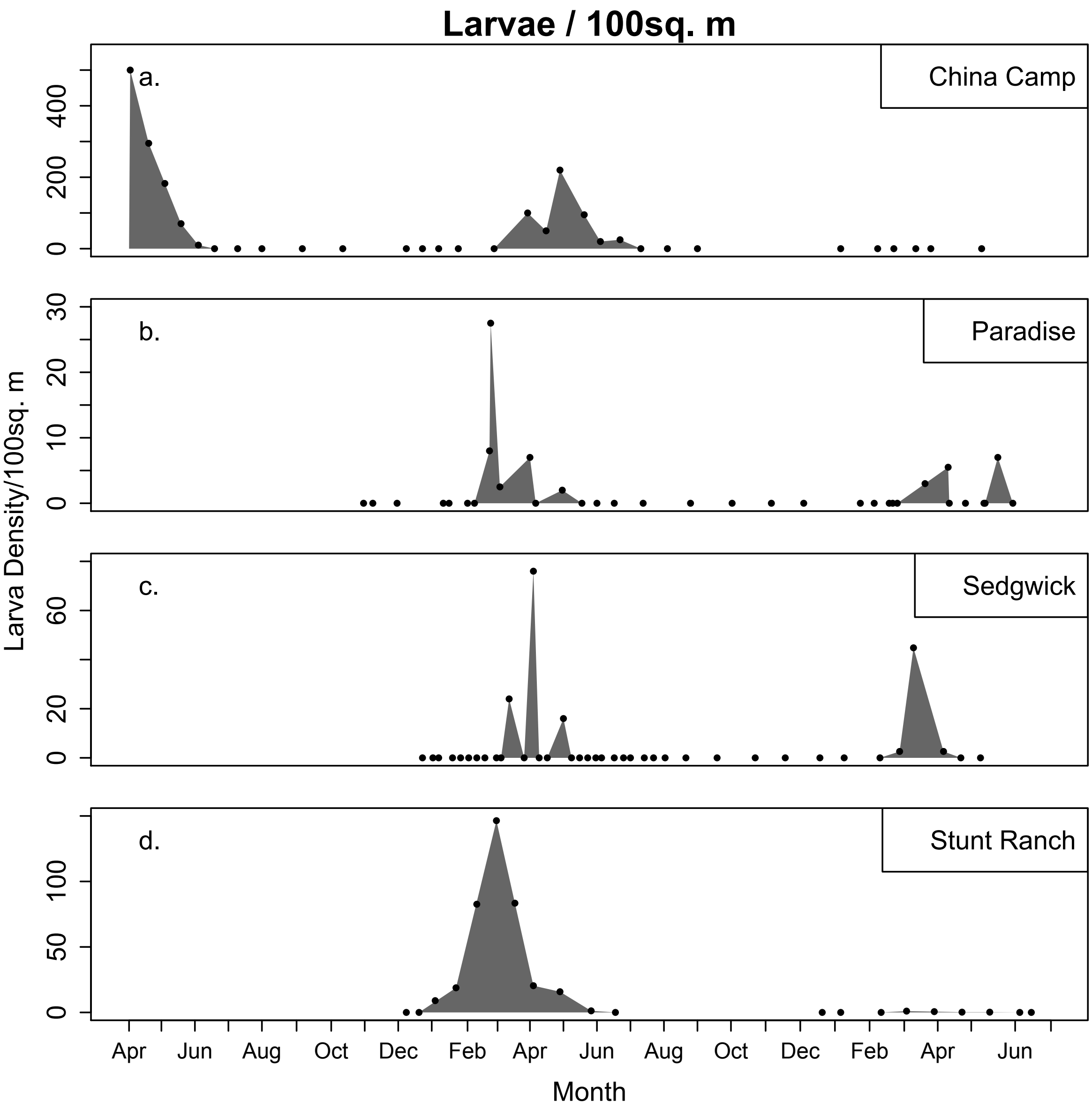

Larvae / 100sq. m
Larva Density/100sq. m
a.
China Camp
0
200
400
b.
Paradise
0
10
20
30
c.
Sedgwick
0
20
60
d.
Stunt Ranch
0
50
100
Apr
Jun
Aug
Oct
Dec
Feb
Apr
Jun
Aug
Oct
Dec
Feb
Apr
Jun
Month

Figure 5.

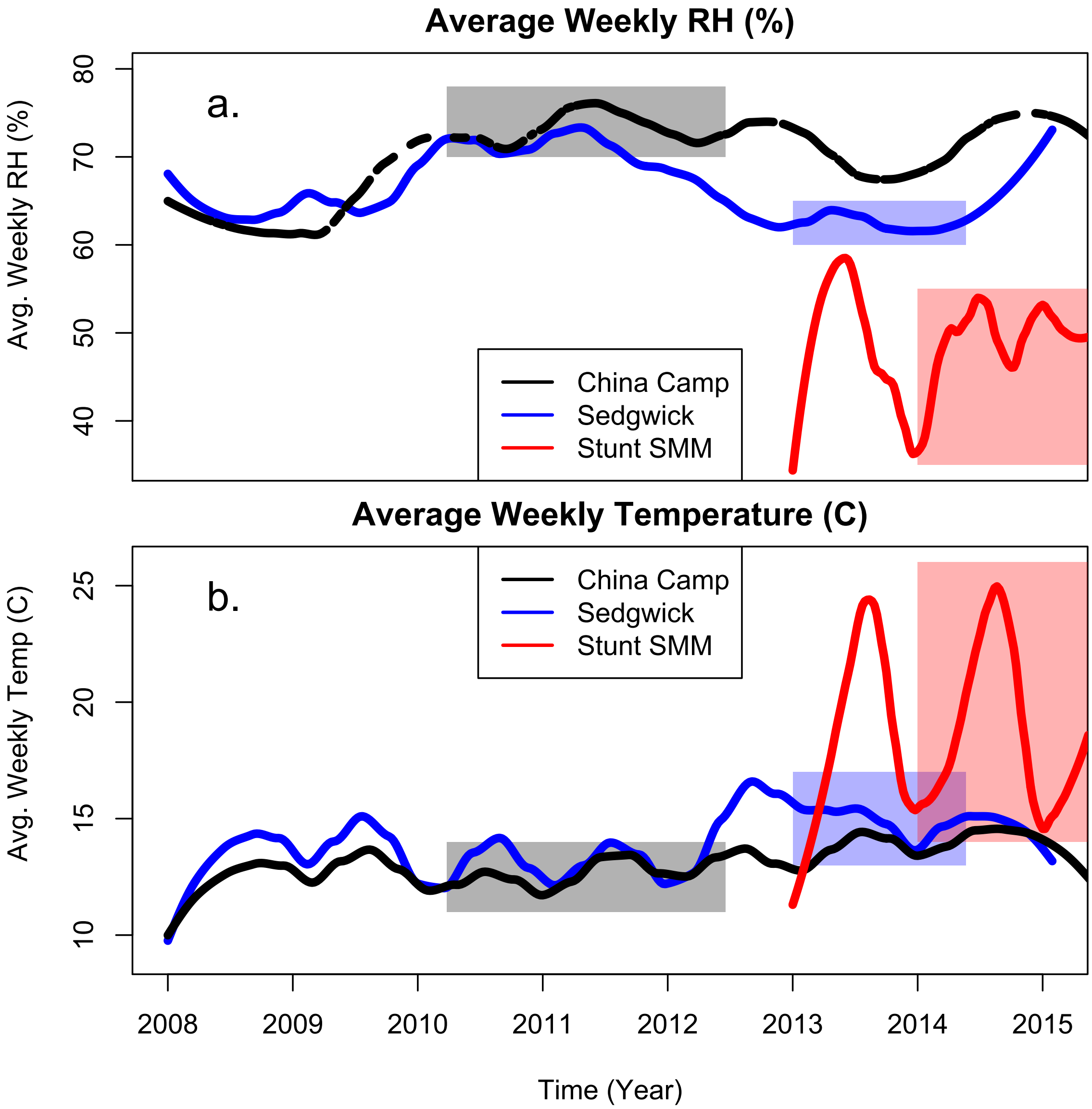

Average Weekly RH (%)
a.
Avg. Weekly RH (%)
80
70
60
50
40
China Camp
Sedgwick
Stunt SMM
Average Weekly Temperature (C)
b.
Avg. Weekly Temp (C)
25
20
15
10
China Camp
Sedgwick
Stunt SMM
2008
2009
2010
2011
2012
2013
2014
2015
Time (Year)

Figure 6.

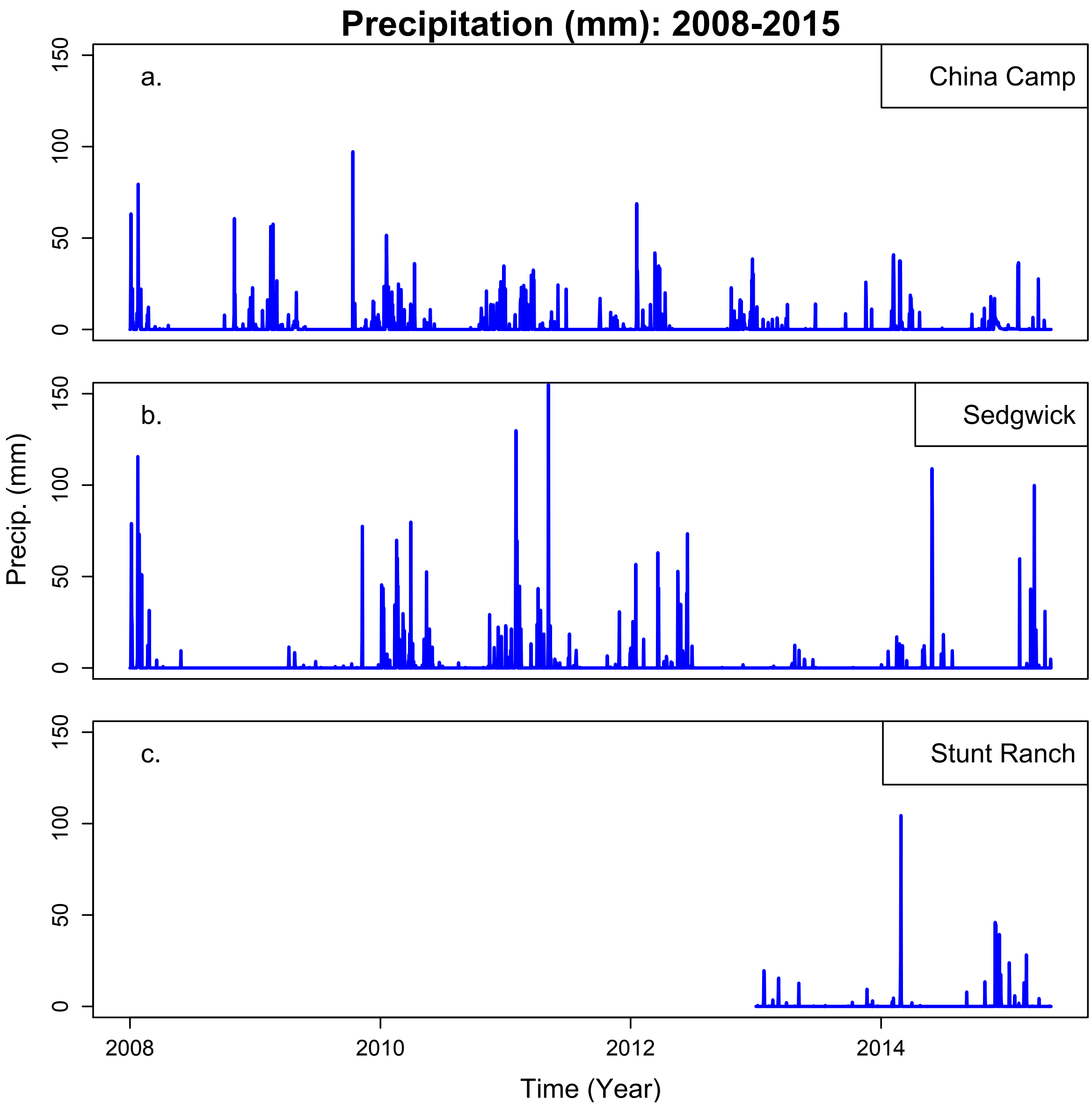

Precipitation (mm): 2008-2015
a.
China Camp
b.
Sedgwick
c.
Stunt Ranch
Precip. (mm)
0
50
100
150
2008
2010
2012
2014
Time (Year)

Table 1. Summary of observed seasonal patterns of questing *Ixodes pacificus* in southern California sites, and China Camp State Park in northwestern California. Length of season for each stage is presented including number of days that each season lasted at each site, as well as dates of peak density—sites sampled over multiple seasons have two numbers in parentheses, indicating the number of days each season lasted.

Sampling Location	Date of Investigation	Adult Season (# of days)	Nymphal Season (# of days)	Peak Nymphal Density	Larval Season (# of days)	Peak Larval Density
China Camp State Park (Marin Co.)	April 2010 – March 2012	November – Mid June (210)	Early February – August (175, 158)	April – June	April – June (81, 80)	May
Paradise Reserve (Santa Barbara Co.)	November 2013 – June 2015	Late November – April (194, 161)	Early March – Mid May (63, 34)	March – April	Early March – Mid May (63, 62)	March
Sedgwick Reserve (Santa Barbara Co.)	December 2012 – June 2014	Mid December – April (94, 107)	Late February – Mid April (47, 38)	March – April	Late February – Mid May (47, 38)	March
Stunt Ranch Reserve (Los Angeles Co.)	December 2013 – June 2015	December – April (67, 70)	Early March – Early June (82, 98)	March – April	Early February – May (117, 72)	February – March

Table 2. Peak density per $100m^2$ of each parasitic life stage (mean and standard deviation over sampling events during seasonal peak activity are presented in parentheses), and total number of collected adults, nymphs and larvae per site over the course of the two years sampled for each site. Density of adults is expressed as average density per $100m^2$ of understory vegetation, and juvenile tick density is expressed as average density per $100m^2$ of leaf litter, following the sampling protocol employed. Total area sampled differs between sites, thus density and total number of ticks collected do not scale by the same factor across all sites sampled.

Sampling Location	Adult Peak Density/$100m^2$ (Mean, SD)	Total Adults Collected	Nymph Peak Density/$100m^2$ (Mean, SD)	Total Nymphs Collected	Larva Peak Density/$100m^2$ (Mean, SD)	Total Larvae Collected
China Camp State Park (Marin Co.)	15.75 (5.03, 3.46)	364	21 (8.33, 5.28)	336	500 (232.08, 151.38)	277
Paradise Reserve (Santa Barbara Co.)	2.8 (0.86, 0.54)	194	3.6 (1.18, 1.22)	34	27.5 (8.64, 8.57)	125
Sedgwick Reserve (Santa Barbara Co.)	0.75 (0.44, 0.15)	132	3.4 (2.86, 2.54)	58	76 (61.00, 83.42)	308
Stunt Ranch Reserve (Los Angeles Co.)	0.9 (0.51, 0.36)	52	1.6 (0.97, 0.50)	34	146.4 (46.65, 67.08)	1020

II. Lyme disease risk in southern California: abiotic and environmental drivers of *Ixodes pacificus* (Acari: Ixodidae) density and infection prevalence with *Borrelia burgdorferi*

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A. Introduction

In recent decades numerous vector-borne zoonotic diseases (VBZDs) have emerged, and endemic regions have experienced increases in human incidence and transmission intensity in vector populations and wildlife hosts (Weaver and Reisen 2010, Kilpatrick and Randolph 2012). Transmission of VBZDs to humans requires the interaction of human populations with natural pathogen transmission cycles between competent vectors and reservoir hosts at the human-animal interface (Gortazar et al. 2014). Understanding the factors influencing the distribution and abundance of vector species (Kilpatrick and Randolph 2012), as well as infection prevalence in vector populations is thus critical to an understanding of human risk of infection with VBZDs. For VBZDs, infection prevalence in vector populations, and thus risk of human transmission, are driven primarily by wildlife reservoirs and enzootic pathogen transmission cycles (Kilpatrick and Randolph 2012). Management and control of such zoonoses is quite difficult because vaccination or treatment of human populations has no effect on underlying enzootic transmission, and infection is determined largely by contact with vectors (Barrett and Higgs 2007, Kilpatrick and Randolph 2012). Strategies for disease control involving environmental management or land use planning (Jackson et al. 2006, Larsen et al. 2014) may be increasingly important due to development of resistance to insecticides in vector populations and the challenges associated with managing enzootic transmission cycles (Lindsay and Birley 2004, Beaujean et al. 2016). Such management strategies require an understanding of the abiotic and environmental conditions that promote both vector populations and elevated infection prevalence with zoonotic pathogens on the landscape.

In this study, the influence of environmental and abiotic factors on densities of the primary vector of Lyme disease in the western United States (US), *Ixodes pacificus*, as well as infection prevalence of vector ticks with the causative agent, *Borrelia burgdorferi* sensu stricto (s.s.), were investigated in southern California. Tick-borne diseases, such as Lyme disease, are particularly challenging to manage because tick populations are difficult to control (Beaujean et al. 2016) and environmental or landscape risk factors conducive to high tick abundance are often not well understood (Killilea et al. 2008). Lyme disease, which is caused by the spirochete bacterium *B. burgdorferi* s.s. and vectored by ticks in the genus *Ixodes*, is one of the most common tick-borne diseases globally. In North America, there are distinct Lyme disease foci in the eastern, upper midwestern and far western regions of the US and Canada. In the far western US, human incidence of Lyme disease is highest in northwestern California and investigations of the ecology of this disease system have largely focused on that region. In northern California, dense oak woodland habitats tend to harbor the highest densities of *I. pacificus* ticks, particularly for the larval and nymphal stages; they also experience higher nymphal infection prevalence with *B. burgdorferi* s.s., both key elements of entomological risk of human infection (Eisen et al. 2003, 2006a, 2009). Within oak woodland habitats, temperature—particularly maximum summer temperatures—relative humidity, elevation, aspect and presence of dense leaf litter have been found to be important predictors of tick density and/or infection prevalence (Padgett and Lane 2001, Eisen et al.

2010, Swei et al. 2011). These associations suggest that a combination of direct—e.g. accelerated molting and mortality due to high summer temperatures (Padgett and Lane 2001), and indirect—e.g. influences on densities of key tick hosts and pathogen reservoirs, effects of abiotic and habitat conditions determine tick density and infection prevalence in northwestern California (Swei et al. 2011).

However, there is evidence suggesting that these abiotic and environmental factors may not be predictive of entomological risk in central and southern California where nymphal *I. pacificus* questing behavior appears to differ markedly from that observed in Lyme-endemic northwestern California (Lane et al. 2013, MacDonald and Briggs 2016). Densities of questing nymphal *I. pacificus* are extremely low in oak woodland sites in southern California (Lane et al. 2013, MacDonald and Briggs 2016) relative to infestation of western fence lizards (*Sceloporus occidentalis*), a key host for juvenile *I. pacificus* and effective sentinel animal for detecting juvenile tick activity in this region (Lane et al. 2013). This suggests both that: 1) risk of human exposure to nymphal *I. pacificus* in southern California is relatively low and likely to be highly localized to areas with habitat types, abiotic conditions and host assemblages that promote nymphal questing activity; and 2) due to comparatively high densities of questing adult *I. pacificus* relative to nymphs, possibly due to differences in questing behavior in the nymphal stage (MacDonald and Briggs 2016), exposure to adult female ticks may present greater risk in central and southern California than exposure to nymphal ticks (Lane et al. 2013, MacDonald and Briggs 2016).

Thus, in this study we were interested in determining whether dense oak woodland habitats are associated with elevated densities of nymphal *I. pacificus* and whether different habitat and abiotic conditions might be better predictors of exposure to adult *I. pacificus*, and therefore aggregate entomological risk, in southern California. Given the low densities of nymphal *I. pacificus* observed in previous studies (Lane et al. 2013, MacDonald and Briggs 2016), we expected to find elevated densities of nymphal ticks to be narrowly associated with dense oak woodland habitat, higher in elevation, with dense leaf litter in the understory, which together promote microclimatic conditions found to be important to nymphal tick abundance in northern California (Eisen et al. 2003, 2006a, 2010, Swei et al. 2011). Similarly, we expected elevated adult tick density to be associated with dense oak woodland, or forest edge habitats where deer, important reproductive hosts for adult ticks, tend to forage. Additionally, we were interested in investigating whether these same abiotic and habitat conditions predict vector tick infection with *B. burgdorferi* s.s. on the landscape, or whether this pathogen may be associated with different habitats and environmental conditions in southern California. Again, we expected a similar pattern, with elevated infection prevalence associated with dense oak woodland habitats as in northern California (Eisen et al. 2003, 2010, Swei et al. 2011). To address these questions, tick surveys were conducted in plots representing a range of habitats, environmental and abiotic conditions in Santa Barbara County, California across two years to elucidate the habitat and abiotic factors associated with elevated entomological risk (e.g. elevated densities of infected ticks) for Lyme disease in this understudied region.

B. Methods

1. Field sites, tick sampling and environmental data collection

Questing ticks were collected in 24, 50 x 50m plots across three sites in Santa Barbara County, California. Climatic conditions in this region are Mediterranean with cool, wet winters and warm, dry summers with microclimatic variation driven largely by topography and habitat characteristics. The three sites selected were: 1) Sedgwick Reserve (34°42’04.38” N, 120°02’50.81” W), a 2,388 ha reserve that is part of the University of

California Natural Reserve System (UCNRS) and located in the Santa Ynez Valley (x10 plots); 2) Paradise Reserve (34°32’22.07” N, 119°47’51.89” W), a ~67 ha privately owned natural area located on the north side of the Santa Ynez Mountains in the Los Padres National Forest (x4 plots); and 3) Coal Oil Point Reserve (34°24’52.96” N, 119°52’48.59” W), a 69 ha coastal reserve that is part of the UCNRS and located west of the University of California, Santa Barbara campus (x10 plots) (Figure 1). These three sites were chosen because they represent a range of habitat types, abiotic conditions and degree of maritime influence common in this region. Coal Oil Point experiences a significant marine influence, which moderates temperature extremes and provides fog water subsidies, with habitat dominated by coastal scrub, grassland and patches of coast live oak (*Quercus agrifolia*). Sedgwick experiences less maritime influence, with generally warmer summers and colder winters, with habitats dominated by oak woodland on north facing slopes, oak savannah in valleys, as well as grassland and chaparral/scrub. Paradise Reserve is dominated by oak woodland with patches of open grassland and chaparral/scrub habitat interspersed, and experiences lower temperature extremes than Sedgwick, but less maritime influence than Coal Oil Point. Plots were chosen within each of these three sites using a stratified random design to ensure that different habitat types (oak woodland, oak savannah and chaparral/grassland) with a range of abiotic and environmental conditions were sampled equally across all three sites. Habitat types were classified based on satellite imagery from Google Earth and subsequently ground-truthed (see below). Plots were chosen randomly, and located at least 200 m apart, within each broad habitat type with the aid of Quantum GIS, an open source Geographic Information System (Quantum GIS Development Team 2016).

Data on plot-specific habitat, abiotic and environmental variables were collected each year of the study (2013-2015), and chosen based on previous studies (Padgett and Lane 2001, Eisen et al. 2003, Swei et al. 2011). Data loggers, placed in each plot just above ground level and protected from direct solar radiation, collected hourly temperature data during both summer and winter months (iButtons, Maxim Integrated, San Jose CA). From the data loggers, we calculated average maximum and minimum daily temperature over the dry (1 May-31 October) and rainy (1 November-30 April) seasons. We estimated overstory canopy cover in each plot using satellite imagery derived from Google Earth and processed in Quantum GIS (Quantum GIS Development Team 2016), which we subsequently ground-truthed with a densitometer. We estimated percent cover of leaf litter greater than 5cm in depth, grass/herbaceous vegetation, understory woody vegetation (e.g. *Artemisia californica*, *Baccharis pilularis*, *Toxicodendron diversilobum*) and bare ground following the same procedure as above. Additionally, we measured stem density (number of stems greater than 5 cm in diameter at breast height and greater than 1.5 m in height), slope and elevation at the center of each plot, as well as density of inhabited dusky-footed woodrat (*Neotoma fuscipes*) middens (Hamm et al. 2002). Woodrat middens were censused because *N. fuscipes* is an important reservoir for *B. burgdorferi* s.s. in California, the nests are conspicuous on the landscape and density of active nests may be indicative of small vertebrate host availability for the immature stages of *I. pacificus* more generally in this region (Bolger et al. 1997, Tietje et al. 1997). Measurements of relative humidity, while thought to be an important driver of tick survivorship during the summer dry season (Padgett and Lane 2001, Eisen et al. 2010), were not collected at the plot level. However, weather station data from the region indicate that high summer- and winter-time temperatures are negatively correlated with measures of relative humidity (MacDonald and Briggs 2016), suggesting that plot level temperature and habitat characteristics alone are a good proxy for this metric of microclimatic conditions.

Questing ticks were collected at each site using the flagging method, in which a 1 m^2 white flannel cloth is dragged over understory vegetation and leaf litter, and attached, questing ticks are removed (e.g. Daniels et al. 2000). This method is effective for collection of Ixodid ticks and provides a proxy for human risk of tick encounter. An area of 500 m^2 was sampled on each sampling event in each of the 24 plots, which were sampled weekly to bi-weekly from late November to early June, the period of seasonal activity for *I. pacificus* in southern California (MacDonald and Briggs 2016) in order to determine both peak and average density of each life stage of *I. pacificus* in each plot. All 24 plots across the three reserves were sampled during the 2013-14 and 2014-15 seasons. All ticks encountered were collected and preserved in 70% ethanol for species identification and subsequent testing in the lab for infection with *B. burgdorferi* s.s.

2. DNA extraction and pathogen detection

DNA from all *Ixodes* spp. ticks was extracted using a DNeasy blood and tissue kit (Qiagen, Valencia, CA) following the manufacturer's instructions. All tick samples were then screened for infection with spirochetes in the *B. burgdorferi* sensu lato (s.l.) complex, of which *B. burgdorferi* s.s. is a part, via nested polymerase chain reaction (PCR) targeting the 5S-23S rRNA spacer region of all borreliae belonging to this group, following the methods outlined in Lane et al. 2004 (Lane et al. 2004). PCR-positive samples were sequenced at the 5S-23S intergenic spacer region following Lane et al. 2004 (Lane et al. 2004), and sequenced on an AB 3100 (Applied Biosystems, CA).

While *I. pacificus* is the primary vector of *B. burgdorferi* s.s. to humans, other species of ticks in the genus *Ixodes* have been implicated in enzootic transmission of this pathogen (Brown and Lane 1992). Given the relative dearth of previous studies on Lyme disease ecology in central and southern California, and the low prevalence in vector populations from these few investigations (Lane et al. 2013, Padgett et al. 2014), all adult and nymphal *Ixodes* spp. ticks were extracted and tested individually for infection with *B. burgdorferi* s.l. complex spirochetes.

3. Statistical analyses

Habitat and abiotic predictor variables, which were selected a priori based on previous studies (Padgett and Lane 2001, Eisen et al. 2003, Swei et al. 2011), were highly collinear in this study, and a comparatively large number of predictor variables of interest were measured relative to the number of observations (i.e. 10 to 13 predictor variables, depending on the model, and 24 sampled plots). To address the problem of multicollinearity and sample size, data dimensionality reduction was necessary in the statistical analyses employed. Partial least squares regression (PLSR) is particularly well suited to addressing these problems and has been shown to perform better than multiple regression or principal components regression techniques in similar ecological data analysis contexts (Carrascal et al. 2009).

PLSR generalizes and combines features of principal component analysis and multiple regression to 1) eliminate the problem of multicollinearity in the independent variables ($\boldsymbol{X}$) that plagues the ordinary multiple regression approach, and 2) eliminate the problem of choosing an *optimum* subset of predictors that remains in the principal components regression approach. In principal components regression, orthogonal components are chosen that explain as much of the *variance* in $\boldsymbol{X}$ as possible, which does not guarantee that the components chosen are relevant to the dependent variable of interest ($\boldsymbol{Y}$) when subsequently used as predictors in a regression framework. In contrast, in PLSR a set of components, or *latent vectors*, are chosen that explain as much of the *covariance* between $\boldsymbol{X}$ and $\boldsymbol{Y}$ as possible. This is followed, as in principal components regression, by a regression step in which the set of orthogonal components chosen through the simultaneous decomposition of ***X*** and ***Y*** are used to predict ***Y***.

PLSR models were specified using the abiotic and environmental data described above as predictors, and both peak and average density of adult, nymphal and larval *I. pacificus* ticks as well as infection prevalence of *Ixodes* spp. ticks with *B. burgdorferi* s.l. as outcome variables. Environmental data measured in the year prior to tick collection were used in the models because abundance and infection prevalence of adult and nymphal ticks in year *t* are largely determined by survivorship and activity of nymphal and larval ticks in year *t* – 1, due to the 3-year lifecycle of *I. pacificus* (Padgett and Lane 2001). Concurrent temperature and relative humidity may also influence tick questing activity (Padgett and Lane 2001, Eisen et al. 2010), so average daily maximum rainy season temperature from the year of collection was also included in all models. Models specified for the first year of the study include only concurrent rainy season temperature, and no measure of lagged summer or winter temperature, because data were not available for the previous year. Models were specified independently for the different years of the study to both investigate the robustness of the core results, as well as to separate natural interannual variation in tick density that might be influenced by variation in environmental conditions from year to year. All statistical analyses were conducted in R 3.2.4 (R Core Team 2016), and PLSR models were run using the package 'plsdepot' (Sanchez 2012).

C. Results

1. Drivers of tick density

In total, 765 *I. pacificus* ticks—288 adults, 67 nymphs and 410 larvae—were collected over the duration of the study across all 24 plots. In addition, 9 *Ixodes spinipalpis*, 6 *Ixodes peromysci*, 178 *Ixodes brunneus*, 257 *Haemaphysalis leporispalustris* (rabbit tick), 544 *Dermacentor variabilis* (American dog tick), and 525 *Dermacentor occidentalis* (pacific coast tick) were collected over the course of the study. Both average and peak density of adult and nymphal *I. pacificus* declined slightly from the 2013-14 to the 2014-15 season, though not significantly (Table 1). In contrast, average and peak larval *I. pacificus* density increased slightly, though not significantly, from 2013-14 to the 2014-15 seasons (Table 1). Separate PLSR models were constructed for each tick response variable for each year of the study. Results were remarkably consistent between models predicting peak and average density of adult, nymphal and larval *I. pacificus* within a given year, due to significant positive correlation between these two measures, so multivariate PLSR models were run with both peak and average tick density as outcome variables in the same model. The results of the multivariate models of tick density, as well as the models predicting infection prevalence are presented here.

In the multivariate model predicting average and peak density of adult *I. pacificus* in 2013-14, 2 significant components were extracted that explained 71.8% of the original variance in the response variables (Table 2). The first component accounts for a reasonably large proportion of the overall variance (~45%), while the second component accounts for a smaller, but significant proportion (~27%). The underlying drivers of these two components can be deduced from the variable weights and variable importance in the projection (VIP) scores (Table 2). The sum of squared variable weights for each component is equal to one, so the relative contribution of individual variables to the meaning of each component, and thus to the interpretation of the factors driving tick density, can be estimated (Carrascal et al. 2009). In the multivariate model predicting average and peak density of adult *I. pacificus* in 2013-14, the first component is primarily determined by woodrat nest density (~43%), with stem density, canopy cover and elevation all contributing >10% of the information content of the first component (Table 2). These four variables are not independent, but rather define an environment/habitat type that can be characterized as dense oak woodland. The significance of woodrat nest density here also suggests that host availability is an important driver of this first component. The VIP scores—which are calculated as the weighted sum of squares of the PLS weights, taking into account the explained variance of each PLS component—confirm that these same four variables (woodrat density, stem density, canopy cover and elevation) are driving the relationship between the first component and adult tick density. The correlation between average and peak adult tick density in 2013-14 and the position of the 24 plots in the first component of this PLSR model are shown in Figure 2a and 2b ($r=0.592$, $p<0.01$; and $r=0.707$, $p<0.0001$, respectively). The second component is applied to the residual variation not explained by the first component, illustrated in Figure 2c and 2d ($r=0.742$, $p<0.0001$; and $r=0.592$, $p<0.01$, respectively). Variable weights and VIP scores indicate that the residual variation in adult tick density is primarily explained by woodrat density, as well as a negative association with slope, canopy cover and stem density (Table 2). This set of explanatory variables suggests an association with both forested and more open grassland/chaparral habitats, due to the woodland habitat requirements of dusky-footed woodrats (*N. fuscipes*) and the more open habitats indicative of the other set of significant predictor variables. This association with both woodland and more open habitats may be indicative of an association with the forest edge ecotone. Additionally, the significance of woodrat nest density may indicate the importance of juvenile tick host availability for predicting adult tick density.

The multivariate model of average and peak adult tick density the following year, in 2014-15, yielded 2 significant components explaining 72.8% of the variance in the outcome variables (~44% and ~29% for component 1 and 2, respectively). VIP scores indicate that woodrat density, stem density and a negative association with average daily maximum rainy season temperature in the year of tick collection are driving the relationship between the first component and adult tick density in 2014-15 (Table 2). Again, the residual variation is primarily explained by woodrat density, as well as a negative association with slope, stem density and canopy cover (Table 2). The multivariate PLSR models of average and peak adult *I. pacificus* density specified for each year of the study thus yielded remarkably consistent results.

Results of the multivariate models of average and peak nymphal *I. pacificus* density were not as strong as those predicting adult tick density and not as consistent between the two years of the study. In 2013-14, 2 extracted components explained 66% of the variation in the outcome variables (~48% and ~18% by component 1 and 2, respectively). VIP scores indicate that woodrat density, stem density and canopy cover are driving the relationship between the first component and nymphal tick density (Table 3; Figure 3). The residual variation is primarily explained by woodrat nest density, bare ground cover, as well as negative associations with stem density, slope and canopy cover. In the second year of the study, 2 extracted components explained only 54% of the variation in average and peak nymphal tick density (~30% and ~25% by component 1 and 2 respectively). The VIP scores indicate that woodrat density was no longer a significant predictor in 2014-15, instead percent leaf litter cover was the strongest predictor with stem density, canopy cover, elevation and a negative association with grass/herbaceous cover also contributing to the relationship between the first component and nymphal tick density (Table 3). Residual variation was explained primarily by leaf litter cover, as well as negative associations with woodrat density, stem density and canopy cover (Table 3).

The multivariate models of average and peak larval *I. pacificus* density show very similar patterns to models of nymphal ticks, suggesting immature stages responded similarly to abiotic conditions in this study. In 2013-14, 2 extracted components explained 48% of the variation in the outcome variables (~40% and ~8% by component 1 and 2, respectively). VIP scores indicate that woodrat density, stem density, canopy cover, leaf litter cover, as well as negative associations with grass cover are driving the relationship between the first component and larval tick density (Table 4; Figure 4). The residual variation is primarily explained by woodrat nest density, as well as negative associations with leaf litter cover, canopy cover, grass cover and stem density. In the second year of the study, 2 extracted components explained 63% of the variation in average and peak larval tick density (~36% and ~27% by component 1 and 2 respectively). The VIP scores again indicate that woodrat density was no longer a significant predictor in 2014-15, instead percent leaf litter cover was the strongest predictor with stem density, canopy cover, elevation and a negative association with grass/herbaceous cover also contributing to the relationship between the first component and larval tick density (Table 4). Residual variation was explained primarily by leaf litter cover, as well as negative associations with stem density, grass cover and canopy cover (Table 4).

2. Drivers of infection prevalence

Infection with *Borrelia burgdorferi* s.l. was extremely uncommon throughout the study area (Table 5). No *I. pacificus*, the primary vector of *B. burgdorferi* s.s. to humans, were infected with any spirochete in the *B. burgdorferi* s.l. complex out of 288 adults and 67 nymphs that were tested in this study. However, other *Ixodes* spp. ticks were positive for infection with *B. burgdorferi* s.s., the causative agent of Lyme disease, as well as *Borrelia bissettii*, another spirochete in the *B. burgdorferi* s.l. complex that is involved in Lyme borreliosis in central and southern Europe (Rudenko et al. 2008, 2009, Hulínská et al. 2009). *B. bissettii* has also been isolated from ticks and hosts throughout the US (Fedorova et al. 2014), and DNA resembling *B. bissettii* has been isolated from the serum of Lyme disease patients in northern California (Girard et al. 2011), so may be involved in Lyme borreliosis in the US. Specifically, in this study three of five (60%) *Ixodes peromysci* tested were positive for *B. burgdorferi* s.l., all from Coal Oil Point Reserve (Figure 1). *I. peromysci* is a specialist on deer mice (*Peromyscus maniculatus*) and other small rodents (Lane et al. 1982). One nymph was infected with *B. burgdorferi* s.s. and two nymphs were infected with *B. bissettii* (Table 5). In addition, one out of three (33%) *Ixodes spinipalpis* tested were positive for *B. burgdorferi* s.l., again from Coal Oil Point Reserve. *I. spinipalpis* is thought to play a role in the enzootic maintenance of *B. burgdorferi* s.l. in California (Brown and Lane 1992) and one adult female was found to be infected with *B. bissettii* in this study (Table 5).

Due to the lack of infection in ticks in 2013-14, PLSR models predicting infection were only run for the 2014-15 season. In addition to the environmental and habitat variables used in the models predicting tick density, tick species diversity (Shannon's H) was included in the set of predictors for the model of infection prevalence (Table 6). The two extracted components explained 62% of the variation in infection prevalence (~53% and ~9% by component 1 and 2, respectively). In contrast to models predicting density of *I. pacificus*, VIP scores indicate that infection prevalence in the tick community was most strongly influenced by vector diversity and both summer and winter temperature. There was a strong positive association between tick diversity and infection prevalence with *B. burgdorferi* s.l. spirochetes in the tick community (Table 6). In addition, there was a strong negative association with maximum summer temperature, and positive association with maximum winter temperature (Table 6).

D. Discussion

In order to better understand and manage risk of vector-borne and zoonotic disease an understanding of the environmental conditions that promote vector populations and infection prevalence with zoonotic pathogens on the landscape is increasingly necessary (Lindsay and Birley 2004, Jackson et al. 2006, Kilpatrick and Randolph 2012, Larsen et al. 2014, Ferreira and Castro 2016). In northwestern California, the region of western North America with the highest human incidence of Lyme disease (California Department of Public Health 2016), the abiotic, habitat and environmental conditions that might promote vector density and infection prevalence have been investigated in numerous studies (Padgett and Lane 2001, Eisen et al. 2002, 2003, 2006a, 2006b, Lane et al. 2007, Eisen et al. 2010, Swei et al. 2011). While there is substantial natural heterogeneity in density and infection prevalence of *I. pacificus* in California, both over space and through time (Killilea et al. 2008, Swei et al. 2011), these previous studies have largely identified dense oak woodlands with microclimates that maintain high relative humidity and small temperature fluctuations, particularly in the summer months, as high risk areas for Lyme disease (Padgett and Lane 2001, Eisen et al. 2002, 2003, 2006a, 2006b, Lane et al. 2007, Eisen et al. 2010, Swei et al. 2011). In southern California, one of the most densely populated regions of the US, Lyme disease ecology and environmental risk factors have not been well explored, though there is evidence to suggest the ecology and epidemiology of the disease differs from northwestern California (Lane et al. 2013, MacDonald and Briggs 2016).

In this study, density of *I. pacificus* ticks across all life stages—particularly the nymphal stage, and across all plots and years sampled was very low in comparison to previous density estimates from similar studies conducted in northwestern California (Swei et al. 2011, Salkeld et al. 2014). For example, previous estimates of peak adult density in northern California range from ~15 to ~380 ticks per $100m^2$ (Salkeld et al. 2014) compared to <1 in the present study to ~3 ticks per $100m^2$ in a recent study conducted in southern California (MacDonald and Briggs 2016), with similar patterns observed for nymphal and larval ticks. This may be due in part to the worsening drought conditions experienced in California over the course of the present study, but may also suggest actual differences in baseline density of *I. pacificus* between these two regions. For example, a previous study (Lane et al. 2013) found nymphal density to be extremely low in southern California in years prior to the recent drought. Furthermore, interannual patterns of nymphal and adult tick density in southern California suggest a significant difference in questing behavior of this life stage between northwestern and southern California. Adult tick density in year t should be determined by nymphal tick density in year $t - 1$, yet nymphal tick density in year $t - 1$ is substantially lower than adult tick density in year t in both the present study as well as in a recent study conducted in southern California (MacDonald and Briggs 2016). This hourglass-shaped demographic distribution, resulting from much higher densities of adult and larval ticks than nymphal ticks, contrasts with the expected pyramid-shaped distribution found in other studies in northern California (Salkeld et al. 2014). This curious demographic pattern suggests that nymphal ticks are not being captured at the same rate by the flagging method in southern California as they are in northern California, which may indicate a regional difference in questing behavior of this stage similar to patterns observed in the southeastern US with nymphal *I. scapularis* (Arsnoe et al. 2015).

In this study, the abiotic and environmental drivers of variation in tick density and infection prevalence in southern California were investigated using a partial least squares regression approach. Results from models predicting adult *I. pacificus* density were consistent across years and suggest that host availability for juvenile ticks was the most significant factor predicting adult tick density (i.e. VIP scores were substantially higher for woodrat density in both extracted components than for other significant predictors). Dense oak woodland and concurrent average daily maximum rainy season temperature were also found to be important drivers of adult tick density, as predicted, though not as strong as host availability for juvenile *I. pacificus*. The significance of rainy season temperature, concurrent with tick questing, for adult *I. pacificus* density suggests that high rainy season temperatures may preclude adult tick questing activity. Rainy season temperatures may be as significant as summer dry season conditions and average maximum daily temperature in the previous summer (Padgett and Lane 2001, Swei et al. 2011) in determining the likelihood of encountering adult *I. pacificus* ticks in southern California, and suggests that winter temperatures may also be an important limitation on adult tick populations.

Results from models predicting both nymphal and larval *I. pacificus* density were less consistent between years, and explained less of the variation in tick density than did models for adult tick density. In the first year of the study, results suggest that host availability for juvenile ticks was the strongest predictor of both nymphal and larval density, with variables characteristic of dense oak woodland also found to be significant predictors. However, in the second year of the study leaf litter cover was the strongest predictor of both nymphal and larval density, and host availability for juvenile ticks was no longer significant. This may suggest that as drought conditions worsened in California from 2013 through 2015, habitat characteristics—like dense leaf litter and overstory canopy—that promote the microclimatic conditions necessary for tick survivorship (i.e. lower temperatures and higher relative humidity) became more important than host availability as predictors of larval and nymphal tick density.

While the finding that no *I. pacificus* ticks were infected with *B. burgdorferi* s.l. spirochetes in this study suggests that human risk of Lyme disease in this region is exceedingly low, the identification of infected *I. spinipalpis* and *I. peromysci* ticks is significant. This finding suggests that despite enzootic transmission of the pathogen, populations of *I. pacificus* in southern California are avoiding infection, leading necessarily to lower human risk. Perhaps this lack of infection in *I. pacificus* populations is the result of low rates of blood feeding on reservoir hosts relative to non-competent hosts like western fence lizards (*S. occidentalis*). The exact mechanism behind this pattern of infection warrants further investigation, but suggests that pathogen transmission to humans through the bite of infected *I. pacificus* ticks in this region is highly unlikely. This pattern of infection also suggests that enzootic transmission of *B. burgdorferi* s.l. in natural transmission cycles involving alternative vector species may be more common than studies focusing solely on the role of *I. pacificus* in the ecology of tick-borne borreliae in California would suggest. This may be particularly relevant in the comparatively understudied central and south coastal regions of the state.

Furthermore, PLSR model results suggest that diversity in the vector community may predict infection prevalence in tick populations, and perhaps in the host community as a result, though further investigation is necessary to explore this possible relationship. In this study, low diversity vector communities were comprised of common generalist species like *I. pacificus* and *D. occidentalis*, while higher diversity communities included both common generalist species as well as less common specialists like *I. brunneus*, *I. spinipalpis* and *I. peromysci*. The higher diversity communities thus included vector species thought to be involved in enzootic maintenance of *B. burgdorferi* s.l., explaining the relationship between vector diversity and infection prevalence. Additionally, due to the lack of explanatory power of dusky-footed woodrat density for infection prevalence in ticks, this suggests that alternative reservoir hosts may be playing a more important role in the enzootic transmission of tick-borne borreliae in southern California. The significant negative relationship between maximum summer temperature and infection prevalence further corroborates the results of earlier studies suggesting that microclimates characterized by high relative humidity and small temperature fluctuations are more likely to harbor infected ticks in California. This also suggests that the protracted drought in California is negatively impacting tick populations and disease risk.

These results, in addition to identifying possible abiotic and environmental risk factors for Lyme disease in southern California, have implications for tick-borne disease risk in the western US under climate change. Evidence suggests that the geographic range of the vector in the eastern US, *Ixodes scapularis*, is increasing as a result of climate change, driving increases in regional Lyme disease risk (Ogden et al. 2005, 2006, 2008, 2014, Levi et al. 2015). This is likely due to increases in the basic reproductive number, R_0, of the vector, resulting from increased molting success and survivorship and accelerated phenology of *I. scapularis* due to milder winters, for example (Ogden et al. 2014). However, it is currently unknown whether Lyme disease foci in the western US will experience similar range shifts and changes in Lyme disease risk due to climate change. While growth and survival of *I. scapularis* appears to be limited primarily by winter conditions, *I. pacificus*, the primary Lyme disease vector in the western US, in contrast is largely limited by abiotic conditions in the summer dry season (Padgett and Lane 2001, Eisen et al. 2003, Swei et al. 2011). The results of this study further substantiate the importance of habitat and abiotic conditions that create microclimates that protect desiccation prone ticks from high temperatures and low relative humidity, as well as promote the necessary host populations for maintenance of *B.*

burgdorferi s.l. in enzootic transmission cycles. Moreover, in this study it appears that these factors were increasingly important as California's historic drought progressed. In California, temperatures are expected to increase, and precipitation decrease into the future, particularly in northern coastal California (Hayhoe et al. 2004, Cayan et al. 2008), exacerbating seasonal drought. These expected climate change impacts will likely further directly limit *I. pacificus* populations, leading to reduced human risk. However, the response of the pathogen to climate change, through altered interactions between reservoir hosts and competent vectors, remains uncertain.

While this study corroborates results of earlier studies conducted in northern California, identifying dense oak woodlands with abundant small vertebrate hosts to present the highest risk of *I. pacificus* tick encounter, particularly the nymphal stage, overall density of *I. pacificus* was found to be quite low in comparison to well studied sites in northwestern California (Swei et al. 2011, Salkeld et al. 2014). Furthermore, habitat types and microclimatic conditions thought to buffer juvenile ticks from desiccation over the summer dry season were found to be increasingly important as the study, and California's historic drought, progressed. These results suggest that climate change in California may act as an important limiting factor on *I. pacificus* populations. Additionally, infection prevalence with *B. burgdorferi* s.l. was exceedingly low or zero across all plots sampled in this study, in contrast with northwestern California. However, despite the low risk of human Lyme disease infection posed by *I. pacificus* in southern California, comparatively high rates of infection were detected in other *Ixodes* spp. ticks. Further, infection prevalence was not associated with habitats characterized by oak woodland nor with the presence of dusky-footed woodrats, which suggests both that enzootic transmission of tick-borne borreliae may be more common in southern California than suspected, and that different vector species and reservoir hosts may be playing a key role in the ecology of the transmission cycle in this region.

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G. Figure captions

Figure 1: Map of study sites. State of California is inset on the bottom left; Santa Barbara County is in the main frame with study site boundaries included. California hillshade data layer was obtained from Cal-Atlas (www.atlas.ca.gov/download.html) through https://koordinates.com.

Figure 2: Density (a) and peak density (b) of adult *I. pacificus* in 2013-14 plotted against the position of each sampled plot in the first PLSR component; and residual variation in density (c) and residual variation in peak density (d) plotted against the position of each sampled plot in the second PLSR component. Correlation coefficients and p-values are presented in each panel.

Figure 3: Density (a) and peak density (b) of nymphal *I. pacificus* in 2013-14 plotted against the position of each sampled plot in the first PLSR component; and residual variation in density (c) and residual variation in peak density (d) plotted against the position of each sampled plot in the second PLSR component. Correlation coefficients and p-values are presented in each panel.

Figure 4: Density (a) and peak density (b) of larval *I. pacificus* in 2013-14 plotted against the position of each sampled plot in the first PLSR component; and residual variation in density (c) and residual variation in peak density (d) plotted against the position of each sampled plot in the second PLSR component. Correlation coefficients and p-values are presented in each panel.

Figure 1.

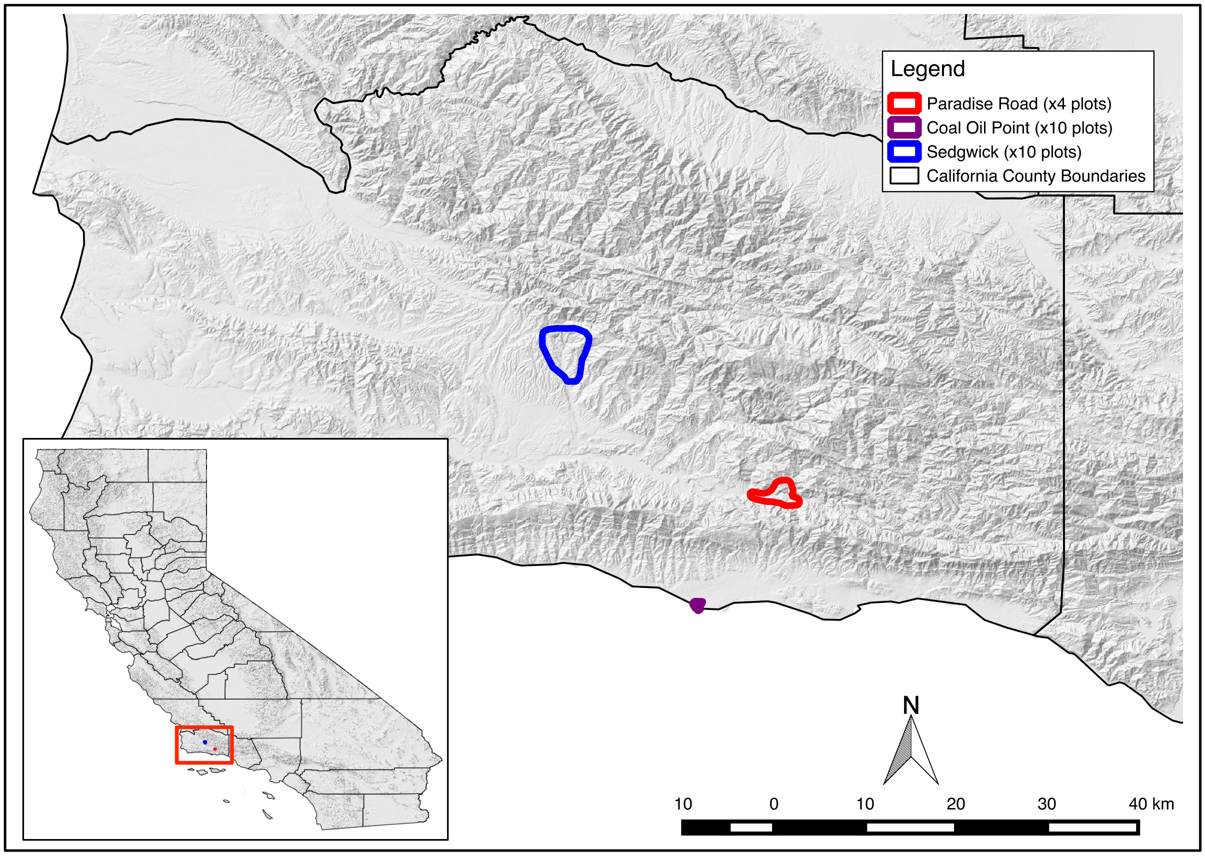
Legend
Paradise Road (x4 plots)
Coal Oil Point (x10 plots)
Sedgwick (x10 plots)
California County Boundaries
N
10
0
10
20
30
40 km

Figure 2.

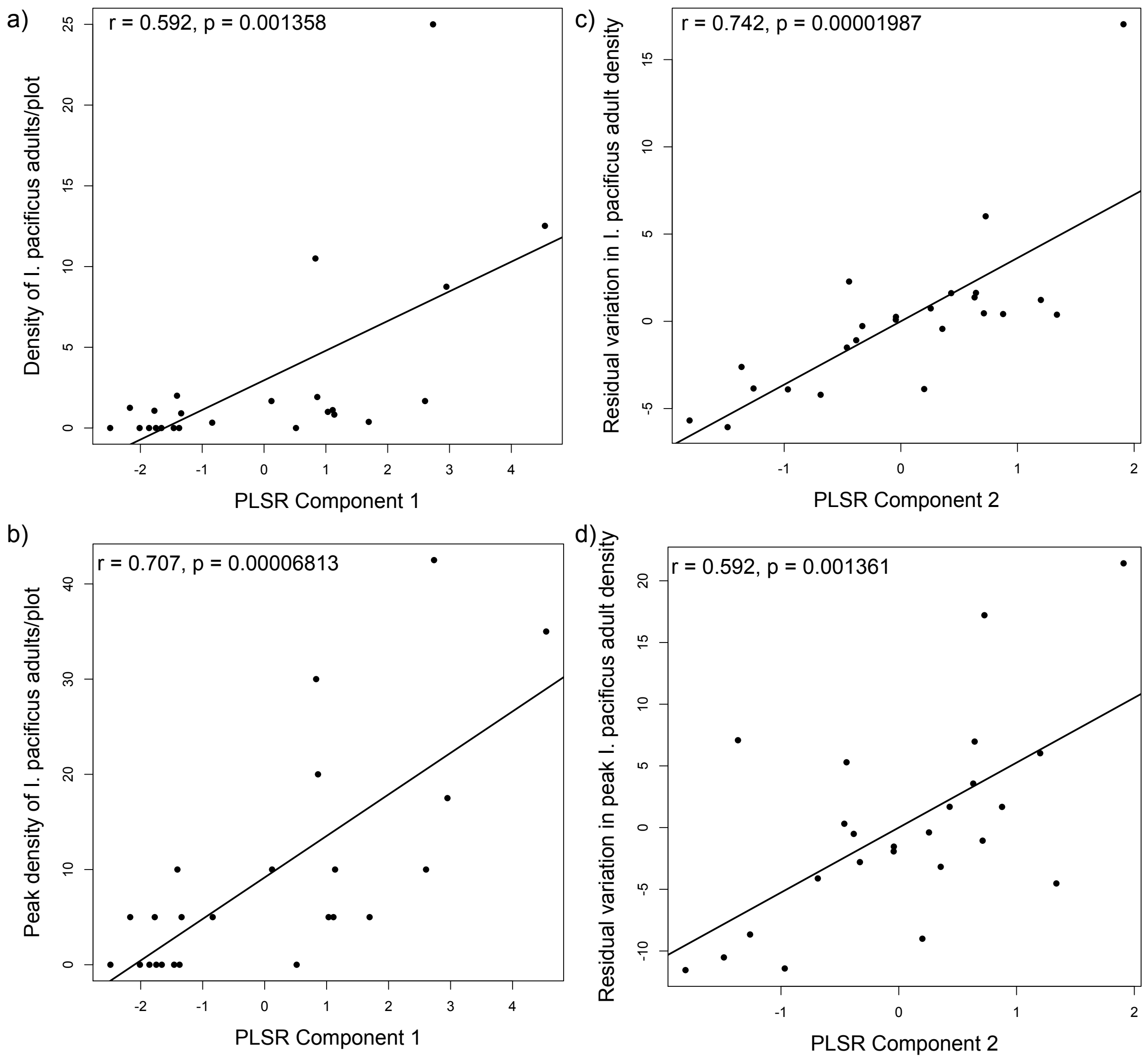
a)
r = 0.592, p = 0.001358
Density of I. pacificus adults/plot
0
5
10
15
20
25
-2
-1
0
1
2
3
4
PLSR Component 1
b)
r = 0.707, p = 0.00006813
Peak density of I. pacificus adults/plot
0
10
20
30
40
-2
-1
0
1
2
3
4
PLSR Component 1
c)
r = 0.742, p = 0.00001987
Residual variation in I. pacificus adult density
-5
0
5
10
15
-1
0
1
2
PLSR Component 2
d)
r = 0.592, p = 0.001361
Residual variation in peak I. pacificus adult density
-10
-5
0
5
10
15
20
-1
0
1
2
PLSR Component 2

Figure 3.

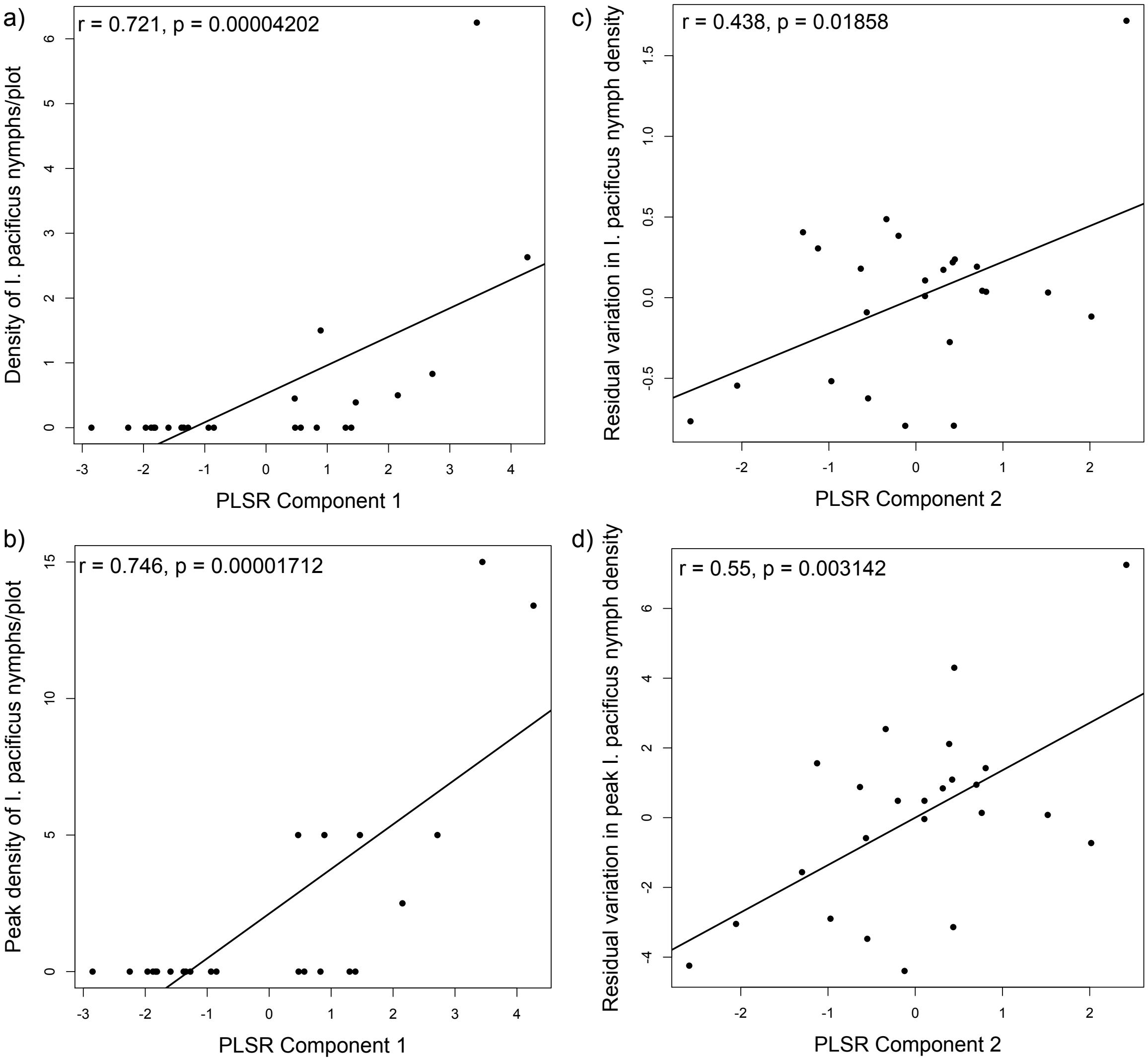
a)
r = 0.721, p = 0.00004202
Density of I. pacificus nymphs/plot
0
1
2
3
4
5
6
-3
-2
-1
0
1
2
3
4
PLSR Component 1
b)
r = 0.746, p = 0.00001712
Peak density of I. pacificus nymphs/plot
0
5
10
15
-3
-2
-1
0
1
2
3
4
PLSR Component 1
c)
r = 0.438, p = 0.01858
Residual variation in I. pacificus nymph density
-0.5
0.0
0.5
1.0
1.5
-2
-1
0
1
2
PLSR Component 2
d)
r = 0.55, p = 0.003142
Residual variation in peak I. pacificus nymph density
-4
-2
0
2
4
6
-2
-1
0
1
2
PLSR Component 2

Figure 4.

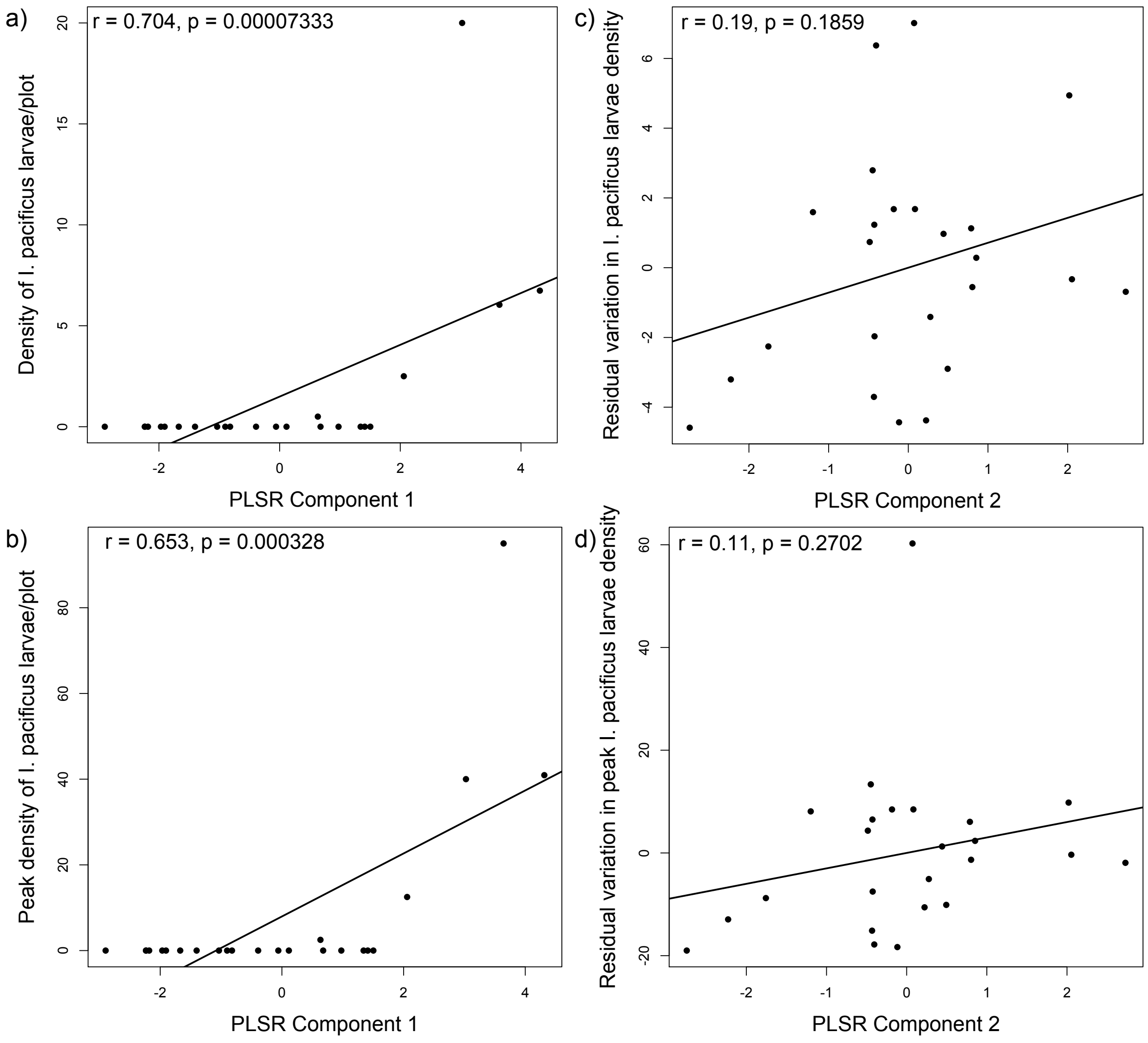
a)
r = 0.704, p = 0.00007333
Density of I. pacificus larvae/plot
0
5
10
15
20
-2
0
2
4
PLSR Component 1
b)
r = 0.653, p = 0.000328
Peak density of I. pacificus larvae/plot
0
20
40
60
80
-2
0
2
4
PLSR Component 1
c)
r = 0.19, p = 0.1859
Residual variation in I. pacificus larvae density
-4
-2
0
2
4
6
-2
-1
0
1
2
PLSR Component 2
d)
r = 0.11, p = 0.2702
Residual variation in peak I. pacificus larvae density
-20
0
20
40
60
-2
-1
0
1
2
PLSR Component 2

Table 1: Average and peak density of *I. pacificus* adults, nymphs and larvae in 2013-14 and 2014-15. Densities are presented as number of ticks per 100m^2 with standard errors in parentheses (SE).

	Average Density/100m^2 (SE)		Peak Density/100m^2 (SE)	
Life Stage	2013-14	2014-15	2013-14	2014-15
Adults	0.118 (0.047)	0.115 (0.053)	0.367 (0.096)	0.328 (0.122)
Nymphs	0.021 (0.011)	0.012 (0.008)	0.085 (0.034)	0.067 (0.044)
Larvae	0.06 (0.036)	0.148 (0.089)	0.318 (0.178)	0.914 (0.564)

Table 2: PLSR Model results for multivariate adult *I. pacificus* average and peak density in 2013-14 and 2014-15. VIP scores greater than 1 indicate significant contributions of those variables to the variation explained by each component; variable weights indicate the direction of the effect; " * " indicates the variable contributing most significantly to the variation in the component, and the strongest predictor of tick density. The second component acts on residual variation not explained by the first component.

	2013-14		2014-15	
	Weights (VIP) Comp. 1	Weights (VIP) Comp. 2	Weights (VIP) Comp. 1	Weights (VIP) Comp. 2
Avg. Max. Winter Temp. 2013-14	-0.313 (0.990)	-0.152 (0.837)	**-0.298 (1.033)**	0.015 (0.806)
Elevation (M)	**0.337 (1.067)**	-0.136 (0.885)	0.272 (0.944)	-0.089 (0.761)
Slope	0.144 (0.454)	**-0.490 (1.012)**	0.109 (0.379)	**-0.499 (1.122)**
Canopy Cover (%)	**0.355 (1.122)**	**-0.298 (1.058)**	**0.298 (1.032)**	**-0.306 (1.043)**
Litter Cover (%)	0.246 (0.777)	-0.389 (0.972)	0.229 (0.792)	-0.330 (0.946)
Shrub Cover (%)	0.021 (0.067)	0.077 (0.158)	0.041 (0.143)	0.066 (0.181)
Grass Cover (%)	-0.136 (0.431)	0.092 (0.384)	-0.147 (0.508)	0.063 (0.419)
Bare Ground Cover (%)	0.023 (0.071)	0.082 (0.167)	0.021 (0.074)	0.146 (0.321)
Stem Density (#/Plot)	**0.367 (1.160)**	**-0.310 (1.097)**	**0.351 (1.216)**	**-0.255 (1.098)**
Woodrat Density	**0.654 (2.067)***	**0.601 (2.007)***	**0.631 (2.187)***	**0.615 (2.165)***
Avg. Max. Winter Temp. 2014-15	NA	NA	**-0.350 (1.211)**	0.036 (0.947)
Avg. Max. Summer Temp. 2014	NA	NA	-0.128 (0.444)	0.257 (0.656)
R-squared	0.45	0.268	0.442	0.285

Table 3: PLSR Model results for multivariate nymphal *I. pacificus* average and peak density in 2013-14 and 2014-15. VIP scores greater than 1 indicate significant contributions of those variables to the variation explained by each component; variable weights indicate the direction of the effect; " * " indicates the variable contributing most significantly to the variation in the component, and the strongest predictor of tick density. The second component acts on residual variation not explained by the first component.

	2013-14		2014-15	
	Weights (VIP) Comp. 1	Weights (VIP) Comp. 2	Weights (VIP) Comp. 1	Weights (VIP) Comp. 2
Avg. Max. Winter Temp. 2013-14	-0.187 (0.590)	0.081 (0.522)	0.021 (0.073)	0.377 (0.882)
Elevation (M)	0.312 (0.986)	-0.237 (0.929)	**0.313 (1.083)**	-0.162 (0.885)
Slope	0.071 (0.223)	**-0.637 (1.059)**	0.228 (0.789)	-0.238 (0.806)
Canopy Cover (%)	**0.386 (1.220)**	**-0.225 (1.107)**	**0.307 (1.063)**	**-0.308 (1.065)**
Litter Cover (%)	0.299 (0.946)	-0.278 (0.929)	**0.706 (2.445)***	**0.403 (2.036)***
Shrub Cover (%)	0.055 (0.173)	0.116 (0.241)	0.007 (0.026)	0.005 (0.023)
Grass Cover (%)	-0.235 (0.742)	-0.082 (0.649)	**-0.317 (1.098)**	-0.184 (0.918)
Bare Ground Cover (%)	0.249 (0.788)	**0.534 (1.104)**	-0.068 (0.235)	-0.025 (0.183)
Stem Density (#/Plot)	**0.470 (1.485)**	**-0.043 (1.273)**	**0.365 (1.266)**	**-0.234 (1.082)**
Woodrat Density	**0.533 (1.686)***	**0.310 (1.529)***	0.121 (0.419)	**-0.413 (1.013)**
Avg. Max. Winter Temp. 2014-15	NA	NA	-0.063 (0.218)	0.372 (0.885)
Avg. Max. Summer Temp. 2014	NA	NA	0.017 (0.059)	0.346 (0.809)
R-squared	0.484	0.177	0.295	0.247

Table 4. PLSR Model results for multivariate larval *I. pacificus* average and peak density in 2013-14 and 2014-15. VIP scores greater than 1 indicate significant contributions of those variables to the variation explained by each component; variable weights indicate the direction of the effect; " * " indicates the variable contributing most significantly to the variation in the component, and the strongest predictor of tick density. The second component acts on residual variation not explained by the first component.

	2013-14		2014-15	
	Weights (VIP) Comp. 1	Weights (VIP) Comp. 2	Weights (VIP) Comp. 1	Weights (VIP) Comp. 2
Avg. Max. Winter Temp. 2013-14	-0.178 (0.561)	0.112 (0.533)	-0.017 (0.057)	0.395 (0.895)
Elevation (M)	0.281 (0.890)	-0.258 (0.878)	**0.304 (1.054)**	-0.123 (0.849)
Slope	0.124 (0.391)	-0.647 (0.901)	0.214 (0.741)	-0.254 (0.804)
Canopy Cover (%)	**0.351 (1.111)**	**-0.281 (1.078)**	**0.311 (1.078)**	**-0.293 (1.052)**
Litter Cover (%)	**0.446 (1.411)**	**-0.048 (1.292)**	**0.674 (2.335)***	**0.443 (2.032)***
Shrub Cover (%)	0.155 (0.491)	0.287 (0.580)	0.056 (0.194)	0.049 (0.184)
Grass Cover (%)	**-0.356 (1.126)**	**-0.315 (1.106)**	**-0.343 (1.189)**	**-0.238 (1.049)**
Bare Ground Cover (%)	0.046 (0.145)	0.381 (0.504)	-0.088 (0.306)	-0.054 (0.261)
Stem Density (#/Plot)	**0.438 (1.385)**	**-0.046 (1.268)**	**0.372 (1.289)**	**-0.215 (1.090)**
Woodrat Density	**0.455 (1.438)***	**0.305 (1.371)***	0.176 (0.609)	-0.365 (0.946)
Avg. Max. Winter Temp. 2014-15	NA	NA	-0.101 (0.351)	0.377 (0.895)
Avg. Max. Summer Temp. 2014	NA	NA	-0.043 (0.150)	0.312 (0.716)
R-squared	0.398	0.078	0.359	0.267

Table 5. Summary of infection results by tick species.

Tick Species	Number Tested	Number Infected	*B. burgdorferi* s.l. Type	Species-Level Prevalence
I. pacificus	288 adults; 67 nymphs	0	NA	NA
I. brunneus	2 adults; 4 nymphs	0	NA	NA
I. spinipalpis	1 adult; 2 nymphs	1 adult	*B. bissettii*	33.33%
I. peromysci	0 adults; 5 nymphs	3 nymphs	*B. burgdorferi* s.s. (1); *B. bissettii* (2)	60%

Table 6. PLSR Model results for infection prevalence with *Borrelia burgdorferi* s.l. in *Ixodes* spp. ticks, 2014-15. VIP scores greater than 1 indicate significant contributions of those variables to the variation explained by each component; variable weights indicate the direction of the effect; " * " indicates the variable(s) contributing most significantly to the variation in the component, and the strongest predictor of infection. The second component acts on residual variation not explained by the first component.

	2014-15	
	Weights (VIP) Comp. 1	Weights (VIP) Comp. 2
Avg. Max. Winter Temp. 2013-14	-0.040 (0.144)	**0.442 (1.594)***
Elevation (M)	**-0.278 (1.001)**	-0.025 (0.092)
Slope	-0.103 (0.373)	-0.186 (0.670)
Canopy Cover (%)	-0.091 (0.328)	-0.239 (0.861)
Litter Cover (%)	0.123 (0.443)	-0.211 (0.761)
Shrub Cover (%)	0.156 (0.561)	**-0.373 (1.344)**
Grass Cover (%)	-0.144 (0.518)	**0.381 (1.375)**
Bare Ground Cover (%)	**-0.296 (1.068)**	**0.311 (1.120)**
Stem Density (#/Plot)	-0.065 (0.234)	-0.202 (0.729)
Woodrat Density	-0.144 (0.518)	-0.185 (0.667)
Tick Diversity (Shannon's)	**0.603 (2.174)***	0.224 (0.807)
Avg. Max. Winter Temp. 2014-15	0.012 (0.044)	**0.401 (1.446)**
Avg. Max. Summer Temp. 2014	**-0.605 (2.181)***	-0.034 (0.124)
R-squared	0.529	0.086

III. Boon and bust: Wildfire initially increases Lyme disease risk in California, but subsequently leads to persistent risk reduction

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A. Introduction

Understanding the role of disturbance in shaping ecological communities remains a central aim in ecology, and is particularly salient in zoonotic disease systems on an increasingly altered and human-dominated planet (Bellard et al. 2012, Cardinale et al. 2012, Kilpatrick and Randolph 2012, Lawler et al. 2014). While disturbances, such as habitat fragmentation (Brownstein et al. 2005) or species invasions (Benedict et al. 2007) have been extensively studied in zoonotic and vector-borne disease systems, far less is understood about how the ecology of disease responds to catastrophic disturbance events such as hurricanes or wildfires.

In the fire-prone western United States (US), ticks vector dozens of pathogens causing a range of debilitating diseases including Lyme disease, tick-borne relapsing fever, babesiosis and Rocky Mountain spotted fever. Many of these disease cycles, including Lyme disease, rely on transmission between tick vectors and a suite of animal hosts. While the spirochete bacteria that causes Lyme disease, *Borrelia burgdorferi* sensu stricto (s.s.), is amplified and transmitted most efficiently by small mammal hosts such as rodents (Brown and Lane 1992, Salkeld et al. 2008, Swei et al. 2012), maintenance of populations of the primary tick vector, *Ixodes pacificus*, is tied to large, wide ranging mammals like deer (Lane and Burgdorfer 1986). Further, host preference is specific to tick life stage, where larval and nymphal ticks feed primarily on small vertebrate hosts living and feeding in the leaf litter, while the more robust adult ticks seek large mammal hosts from understory vegetation (Eisen et al. 2001, Casher et al. 2002, Eisen et al. 2003, Lane et al. 2009, MacDonald and Briggs 2016). Due to the variable habitat requirements and spatial ranges of these different groups of key hosts, and the catastrophic impacts of large-scale, intense disturbance events like wildfire on important host and tick habitat, the effects of such disturbances on the Lyme disease system will strongly depend on how each group of hosts and each tick life stage responds to the disturbance event.

In the western US, wildfire is one of the most important and significant natural, as well as anthropogenic, disturbances and plays a central role in the ecology of Californian forests and oak woodlands. In California, wildfire activity has increased in recent years and is expected to increase into the future (Westerling and Bryant 2007, Westerling et al. 2011). Wildfires have cascading impacts on entire communities from direct effects of mortality associated with fire itself (Smith et al. 2012), to post-fire succession and associated impacts on herbivores and their predators (Swanson et al. 2011). These impacts may have important implications for tick populations, both directly from mortality during wildfire events or associated with changes in environmental conditions following fire resulting from loss of vegetation, as well as indirectly through effects on vertebrate hosts (Lawrence 1966, Alverson et al. 1988, Allan 2009). Given the widespread distribution and frequency of wildfire disturbance in California, and expectations that wildfire frequency and intensity are likely to increase under climate change (Westerling and Bryant 2007, Westerling et al. 2011), better understanding the impacts of fire on ticks and their vertebrate hosts will be critical to predicting changing patterns of human disease risk associated with tick-borne pathogens throughout California and the West more generally.

While investigating the effects of fire on tick abundance and tick-borne disease risk has been of interest to disease ecologists and public health practitioners in its possible application as a control strategy or intervention measure for the prevention of human disease (Stafford et al. 1998, Padgett et al. 2009), these effects have only been explored utilizing controlled burns. Studies of the impacts of controlled burns largely have found minimal effects on tick populations or disease risk (Hoch et al. 1972, Scifres et al. 1988, Spickett et al. 1992, Davidson et al. 1994, Stafford et al. 1998, Cully 1999, Horak et al. 2006, Allan 2009, Padgett et al. 2009). However, such fires are relatively small in geographic area as well as low in intensity and severity. Wildfires, on the other hand, can burn very large areas of forest and other habitat, and have the potential to burn at higher temperatures leading to more destruction of leaf litter, duff and below ground biomass (Neary et al. 1999). Wildfires thus have the potential to have greater impacts on tick abundance, both directly as well as indirectly through effects on vertebrate hosts, than do controlled or prescribed burns. Yet, the impact of wildfire on vector ticks and the community of key hosts in the transmission of Lyme disease has not been investigated.

In this study we investigated the effect of wildfire on tick and vertebrate host abundance in southern California using the 2013 White Fire in the Los Padres National Forest of Santa Barbara County as a natural experiment. Given the comparatively large area (~800 ha) of oak woodland that burned in this wildfire and the destruction of both understory and overstory habitat, we expected that ticks would experience direct mortality from the fire due to destruction of leaf litter and duff, but that this effect may differ by life stage. Similarly, small vertebrate hosts like dusky-footed woodrats (*Neotoma fuscipes*)—important amplification hosts, and western fence lizards (*Sceloporus occidentalis*)—important dilution hosts, were expected to decrease in abundance in response to the fire due to direct mortality and destruction of nests and burrows (Lawrence 1966). On the other hand, deer (*Odocoileus hemionus californicus*) which are important reproductive hosts for adult ticks were expected to increase in abundance following the fire due to an attraction to new vegetation for forage and reduced predation risk associated with more open habitats (Allan 2009). Thus, in aggregate, we expected initial reductions in tick populations and tick-borne disease risk in the year following the fire, with tick populations rebounding due to increased deer activity and recovery of vegetation providing necessary microclimates for tick survivorship in subsequent years (i.e. two years post-fire and beyond). To test these hypotheses, we sampled tick populations and vertebrate host communities within and adjacent to the White Fire burn extent for three years following the fire in order to track the response of each stage of the vector, and key vertebrate hosts in the transmission cycle of *B. burgdorferi* s.s., to wildfire disturbance.

B. Methods

1. Study site and habitat characterization

Field sampling was conducted in Santa Barbara County, California, northwest of greater Los Angeles. This region is characterized by a Mediterranean climate with relatively cool, wet winters and warm, dry summers. With the onset of the summer dry season in California comes elevated wildfire risk, when live fuel moisture declines, increasing the chance of ignition (Roberts et al. 2006). This study took advantage of the May 2013 White Fire, which burned ~800 ha of oak woodland and oak savannah habitat in Los Padres National Forest while leaving neighboring oak woodland habitat unaffected (Figure 1).

Within the Los Padres National Forest, six 1-hectare sites were chosen, three in oak woodland habitat within the burn extent and three in oak woodland habitat adjacent to the burn to sample abundance of ticks and hosts. These six sites were located within a ~4 km^2

area and were chosen to minimize variation in background (pre-fire) microclimate or habitat differences between sites. We selected only dense oak woodland sites because this habitat type has been found to be associated with elevated densities of *I. pacificus* (Eisen et al. 2003, 2006, Swei et al. 2011a), as well as with higher abundances of competent reservoir hosts for *B. burgdorferi* s.s. (as evidenced by higher infection prevalence in *I. pacificus* tick populations found in these habitat types) (Eisen et al. 2003).

Site-specific habitat, abiotic and environmental data were collected each year of the study (2014-2016), and chosen based on previous studies (Padgett and Lane 2001, Eisen et al. 2003, Swei et al. 2011a), in order to track environmental conditions and recovery of vegetation following the fire. Data loggers, placed in each site just above ground level and protected from direct solar radiation, collected hourly temperature data during both summer and winter months (iButtons, Maxim Integrated, San Jose CA). From the data loggers, we calculated average maximum and minimum daily temperature over the dry (1 May-31 October) and rainy (1 November-30 April) seasons. We also measured overstory canopy cover, stem density (number of stems greater than 5 cm in diameter at breast height and greater than 1.5 m in height), slope and elevation, percent cover of dense leaf litter (>5cm in depth), grass/herbaceous vegetation, understory woody vegetation (e.g. *Artemisia californica*, *Toxicodendron diversilobum*) and bare ground microhabitats in each site. Site-specific data on abiotic and habitat characteristics were included in models of the effect of wildfire on tick and host abundance (see below) to control for habitat and abiotic factors that have previously been shown to influence tick and host populations (Padgett and Lane 2001, Eisen et al. 2003, Swei et al. 2011a).

2. Tick and vertebrate host sampling

Ticks were sampled within each of the 1-ha sites approximately monthly between early February and early June of 2014-15, and between early March and early May of 2016. A total area of 4,000 m^2 was sampled at each of the six sites in 2014 and 2015, and 2,000 m^2 each in 2016. Sampling in 2016 was interrupted by flooding of roads, which limited access to the field sites early in the season, so a smaller total area was sampled over fewer months in 2016 than in 2014-15. Ticks were collected using the flagging method (e.g. Daniels et al. 2000) in which a 1m x 1m white flannel cloth is dragged along the ground and understory vegetation and periodically checked for attached ticks. Collected ticks were stored in 70% EtOH for later identification, DNA extraction and pathogen testing by polymerase chain reaction (PCR).

Small mammals like dusky-footed woodrats (*Neotoma fuscipes*) and western gray squirrels (*Sciurus griseus*) are important reservoir hosts for *B. burgdorferi* s.s., while other common species like deer mice (*Peromyscus maniculatus*) can act as pathogen reservoirs, even though they are less competent (Lane and Brown 1991, Brown and Lane 1992, Lane et al. 2005, Salkeld et al. 2008, Salkeld and Lane 2010, Swei et al. 2012). A 10 x 10 trapping grid was established at each of the six sites to estimate relative abundance of small mammal hosts. Each trap station was located 10 m apart from adjacent trap stations and included two extra-large Sherman live traps (7.6 x 9.5 x 30.5 cm; H.B. Sherman Traps, Tallahassee, FL, USA), for a total of 200 traps per grid. Mark-recapture trapping took place for three consecutive nights at each of the six sites between mid-March and mid-May, 2014. Captured mammals were anesthetized in order to collect tissue samples and attached ectoparasites, as well as tagged with an individually numbered eartag (National Band and Tag Company) for identification of recaptured animals.

Western fence lizards (*Sceloporus occidentalis*) are important hosts for immature *I. pacificus* (Casher et al. 2002). They also act as dilution hosts for *B. burgdorferi* s.s. in California, by cleansing ticks of infection with the pathogen (Lane and Quistad 1998). Lizard abundance was estimated using a “sight-re-sight” protocol in which five of the ten transects of each of the six trapping grids were surveyed for lizards between mid-March and mid-May, 2014. Lizards were marked with a diluted latex paint mixture using a tree-marking gun (Swei et al. 2011b). Three different colors of paint were used, one for each of three consecutive days to determine lizard encounter history (Swei et al. 2011b). Lizards were also captured at each of the six sites between early March and early May, 2014 in order to estimate tick burdens.

Because small vertebrate host sampling was undertaken in only the first year following the fire (2014), density of inhabited dusky-footed woodrat (*Neotoma fuscipes*) middens (Hamm et al. 2002) was also surveyed as a proxy for small vertebrate host abundance (Bolger et al. 1997, Tietje et al. 1997) to include as a covariate in models of tick abundance (see below). Density of *N. fuscipes* middens was used because it has been found to be a good proxy for small vertebrate host abundance both in this (Spearman’s correlation coefficient between sampled small vertebrate host abundance in 2014 and density of inhabited woodrat middens, $\rho = 0.64$) and in previous studies (Bolger et al. 1997, Tietje et al. 1997). In addition, *N. fuscipes* is an important reservoir for *B. burgdorferi* s.s. in California, and a previous study found that controlled burns in northern California removed *N. fuscipes* from the system for at least one year following the burn (Padgett et al. 2009).

Finally, deer are important reproductive hosts for adult *I. pacificus* (Lane and Burgdorfer 1986). California mule deer (*Odocoileus hemionus californicus*) use of each site was estimated using standardized pellet-group counts in which five, 4 x 22 m, randomly assigned sub-plots within each site were surveyed for pellet-groups (>4 pellets/group) and subsequently cleared. Each of these sub-plots was then re-surveyed and new pellet groups quantified (White and Eberhardt 1980, Rowland et al. 1984). Pellet-group data were summed across all subplots within a site to obtain relative measures of deer use. Pellet-groups were surveyed and cleared in fall 2013, spring 2014, fall 2014, spring 2015, fall 2015 and spring 2016.

3. Infection with *Borrelia burgdorferi*

DNA was extracted from collected ticks and small mammal tissue samples using a Qiagen DNeasy extraction kit (Qiagen, Valencia, CA) following manufacturers instructions. All tick and tissue samples were then screened for infection with *B. burgdorferi* sensu lato (s.l.) spirochetes via nested PCR targeting the 5S-23S rRNA spacer region of all borreliae belonging to this group, which includes *B. burgdorferi* s.s., following the methods outlined in Lane and colleagues (2004).

4. Statistical analysis

Generalized linear mixed-effects models (GLMMs) were used to determine the effect of wildfire on abundance of *I. pacificus* of each life stage. Counts of *I. pacificus* per collection date, per site were the outcome variables, regressed against whether the site was burned or not, year of the study (2014-2016, 1 year post-fire to 3 years post-fire), Julian day^2 (to account for temporal autocorrelation), site-specific habitat characteristics, as well as density of inhabited woodrat middens (as a proxy for small vertebrate host abundance) all included as fixed effects. Site was included as a random effect to control for repeated measures in each of the six sites sampled in each year of the study. An interaction term between burn status and year was included to determine whether the effect of being burned changed from year to year as the system recovered following the fire. In addition, lagged relative deer activity was included in the model predicting larval tick abundance. Adult female *I. pacificus* ticks lay eggs in the spring at the site where they drop off of deer and other hosts following blood meals; these eggs hatch into larval ticks in the summer and emerge to seek hosts the following spring (Padgett and Lane 2001). Thus, deer activity in the previous spring would be expected to predict larval tick abundance in the current year, which has been found in similar systems (Ostfeld et al. 2006). In contrast, nymphal and adult tick abundance would be expected to respond more directly to small vertebrate host abundance (Ostfeld et al. 2006), because small vertebrate hosts account for the majority of blood meals taken by larval and nymphal ticks, respectively (Ostfeld et al. 2006). Thus, measures of deer activity would not be expected to be predictive of nymphal and adult tick abundance (Ostfeld et al. 2006), and were not included in these models. The full model of tick abundance for each life stage included each of these fixed and random effects described above. Subsequent candidate models were constructed that included combinations of subsets of covariates from the full model that did not display high levels of collinearity (determined by calculation of variance inflation factors), and model selection was based on Akaike weights derived from AICc and WAIC.

The effect of wildfire on key hosts in the transmission of *B. burgdorferi* s.s. (e.g. dusky-footed woodrats) as well as keys hosts in the maintenance of tick populations (e.g. deer and western fence lizards) was also of interest, in order to understand and predict potential longer-term impacts of wildfire on Lyme disease risk. Thus, GLMMs were also used independently to determine the effect of wildfire on relative abundance of deer in each of the six sites in each year of the study. The measure of relative deer activity was regressed against whether the site was burned or not, year of the study (2014-2016, 1 year post-fire to 3 years post-fire) and site-specific habitat characteristics (with the exception of temperature for which there was no a priori argument for inclusion) all included as fixed effects, with site included as a random effect to control for repeated measures in each of the six sites sampled in each year of the study. An interaction term between burn status and year was included to determine whether the effect of being burned changed from year to year as the system recovered following the fire. Again, candidate models were constructed that included combinations of subsets of covariates from the full model that did not display high levels of collinearity and model selection was based on AICc and WAIC.

GLMMs with Poisson error distributions for count data were implemented using the package ‘lme4’ (Bates et al. 2015) in R (R Core Team 2016) for adult *I. pacificus* abundance and relative abundance of deer, because they produced the best fit to the data. GLMMs with zero-inflated negative binomial error distributions were implemented using the package ‘pscl’ (Zeileis et al. 2008) in R (R Core Team 2016) for nymphal and larval *I. pacificus* abundance, due to overdispersion in the data as well as the relatively low probability of encountering these life stages in southern California (MacDonald and Briggs 2016), resulting in excess zeroes in the data.

In order to estimate relative abundance of small vertebrate hosts in the year following the fire (2014) within and adjacent to the burn extent, a Huggins closed population mark-recapture model was used (Huggins 1989). Each species (*P. maniculatus*, *N. fuscipes* and *S. occidentalis*) was analyzed individually by site, for each of the six sampling sites using the ‘mra’ package (McDonald 2015) in R (R Core Team 2016). Population estimates for small vertebrate hosts were only produced for the year following the fire (2013-14), because host sampling was conducted only in this year. Due to the resulting small sample size (6 population estimates per host species, 3 in each treatment), and the comparatively large number of predictor variables, a partial least squares regression (PLSR) approach (Carrascal et al. 2009) was utilized to determine the effect of wildfire, and other abiotic and habitat covariates, on populations of important host species in the year following the fire. PLSR is particularly well suited to data analysis problems involving larger numbers of predictor variables than data points, as well as collinearity if it is present in the set of predictors, outperforming multiple regression and principal components regression techniques (Carrascal et al. 2009). Separate PLSR models were specified for each host species to determine whether wildfire had a significant impact on relative abundance of each species, while controlling for other abiotic and habitat characteristics that might also be influencing small vertebrate host populations. PLSR models were similarly used to determine whether wildfire had a significant impact on average tick burdens for each species of small vertebrate host. PLSR models were run in R (R Core Team 2016) using the package 'plsdepot' (Sanchez 2012).

C. Results

1. Tick responses to wildfire

Tick communities sampled in this region were dominated by western blacklegged ticks (*I. pacificus*), which made up ~94.4% of all 551 ticks collected. Pacific coast ticks (*Dermacentor occidentalis*) were also encountered, making up the other ~5.6% of ticks collected. In 2014, the first year post-fire, adult *I. pacificus* abundance was higher within the burn extent than in adjacent unburned woodland (Figure 2). However, this pattern reversed in the second (2015) and third (2016) years post-fire (Figure 2). GLMM model results further indicate a significant positive effect of wildfire on adult tick abundance, but the interaction of year and wildfire reveals a significant negative effect of wildfire on adult tick abundance in the second (2015) and third (2016) years post-fire (Table 1). Nymphal *I. pacificus* display a similar pattern, with abundance elevated within the burn extent in the first year following the fire, relative to adjacent unburned oak woodland (Figure 3). In the second and third years post-fire, nymphal tick abundance dropped to zero within the burn extent, yet remained low and stable in adjacent unburned sites (Figure 3). GLMM model results indicate a significant positive effect of wildfire on nymphal tick abundance, but no significant interaction between burn status and year (Table 1), despite the absence of nymphal ticks from the burned sites in 2015 and 2016. Larval *I. pacificus* on the other hand appeared to be more negatively impacted by the wildfire with reduced abundance within the burn extent relative to adjacent unburned sites in the first year following the fire (Figure 4). Similar to the pattern observed for nymphal ticks, larval abundance dropped to zero in years two and three post-fire (Figure 4). In the case of larval *I. pacificus*, GLMM model results indicate a significant negative effect of wildfire on larval tick abundance, though again no significant interaction between burn status and year (Table 1). Unexpectedly, abundance of deer in the previous year was not found to be a significant driver of larval tick abundance.

2. Host responses to wildfire

Deer herds in the Los Padres National Forest of Santa Barbara County are comprised primarily of California mule deer (*Odocoileus hemionus californicus*) (USDA Forest Service 2016). Relative abundance of deer in the year following the fire was substantially lower within the burn extent than in adjacent sites, though rebounded in subsequent years to levels similar to those observed in adjacent unburned sites (Figure 5). GLMM model results indicate a significant negative effect of wildfire on the relative abundance of deer (Table 2). However, the interaction of burn status and year indicates that deer activity was less negatively impacted in the second year post-fire, and recovered to pre-fire levels by the third year post-fire (Table 2 and Figure 5).

Small vertebrate host communities were dominated by western fence lizards (*S. occidentalis*), deer mice (*P. maniculatus*), and dusky-footed woodrats (*N. fuscipes*), though southern alligator lizards (*Elgaria multicarinata*), Merriam's chipmunks (*Tamias merriami*), and California ground squirrels (*Otospermophilus beecheyi*) were also captured over the course of the study. In the year following the fire, abundance of deer mice was indistinguishable between burned and unburned sites (Figure 6), and PLSR model results indicate no significant contribution of wildfire to the observed patterns of abundance across the six sites sampled (Table 3). Similarly, abundance of fence lizards was not impacted by wildfire (Figure 6, Table 3). However, wildfire did have a substantial impact on the abundance of dusky-footed woodrats, with populations being reduced to zero within the fire extent (Figure 6) and PLSR model results indicating a significant negative contribution of wildfire to patterns of woodrat abundance across the six sites sampled (Table 3). Woodrat nests were also censused each year of the study, with little evidence of post-fire recolonization of burned sites until the third and final year of the study. Even so, recolonization and construction of new nests was extremely limited by the end of the three year study, representing only a small fraction of all inhabited nests across all study sites (~5%). The distribution of tick blood meals largely followed this same pattern of host abundance, with no detectable effect of wildfire on deer mouse tick burdens, but a small positive effect of wildfire on the residual variation in fence lizard tick burdens (Table 4), though tick burdens appeared to be slightly lower in sites impacted by wildfire on these two host species (Figure 7). However, due to the absence of dusky-footed woodrats from the sites impacted by wildfire, no tick blood meals came from individuals of this species within the burn extent (Figure 7, Table 4). The reduced deer activity, as well as lower tick burdens on small vertebrate hosts and loss of woodrat hosts observed in sites impacted by wildfire may explain the elevated abundance of questing adult and nymphal *I. pacificus*, respectively, in the year immediately following wildfire in this study.

3. Infection results

A total of 397 adult and 52 nymphal *I. pacificus* ticks, as well as 72 *P. maniculatus* and 31 *N. fuscipes* were assayed for infection with *B. burgdorferi* s.l. None of these tick or host samples screened for *B. burgdorferi* s.l. were PCR-positive in this study.

D. Discussion

Wildfire is one of the most significant and important natural disturbances in the western US, with impacts on everything from patterns of biodiversity to nutrient cycling and carbon storage (North and Hurteau 2011, Steel et al. 2015). Moreover, wildfire risk, extent and severity are expected to increase in California into the future under projected climate change, with particularly apparent impacts on forested habitats of northern California (Westerling and Bryant 2007, Westerling et al. 2011). These same habitats support the tick and host populations necessary for maintenance and transmission of numerous tick-borne diseases including babesiosis, anaplasmosis, relapsing fever and Lyme disease. Using the White Fire, which burned ~800 ha of oak woodland and oak savannah habitats in the Los Padres National Forest of Santa Barbara County, California, as a natural experiment, our study found that, in contrast with expectations, wildfire initially amplified tick-borne disease risk, with subsequent dampening of risk through time. Specifically, our study investigated the effect of wildfire on important host species, as well as on populations of *I. pacificus*, the primary vector of the causative agent of Lyme disease, for three years following the 2013 White Fire in Santa Barbara County to determine the aggregate effect of fire on tick-borne disease ecology and disease risk.

Notably, abundance of both adult and nymphal *I. pacificus*, which can both carry and transmit *B. burgdorferi* s.s., was amplified by wildfire in the year immediately following the White Fire. Because this fire occurred during the last week of May of 2013, at the very end of the period of seasonal activity of *I. pacificus* in southern California (MacDonald and Briggs 2016), it is unlikely that a significant number of ticks were transported into the burn extent by wide ranging hosts, such as deer, prior to tick sampling in winter and spring of 2013-14. Due to the timing of the fire, this suggests that any questing ticks collected in winter and spring of 2013-14 had survived the fire, likely in soil refugia (Padgett et al. 2009). Increases in abundance of questing adult and nymphal ticks may have been due to these life stages failing to successfully locate hosts (e.g. Perkins et al. 2006), because of the reduced abundance of deer and small vertebrate hosts within the burn extent. This increased abundance of questing adult and nymphal ticks on the landscape translates into elevated tick-borne disease risk in the winter and spring immediately following summer wildfires.

In contrast, adult and nymphal tick abundance declined substantially within the burn extent in the second and third years following the fire, suggesting a decrease in tick-borne disease risk through time. Reduced adult tick populations in years two and three following the fire could have been the result of lower nymphal tick survivorship over the summer dry season, due to adverse abiotic conditions resulting from the fire, or to fewer successful nymphal tick blood meals resulting from reduced host populations. Given comparable small vertebrate host populations and immature tick burdens between burned and unburned sites in the year following the fire, adult tick population reduction in subsequent years was more likely the result of reduced survivorship over the summer dry season. Nymphal tick declines in the second and third years post-fire may have also been due to adverse abiotic conditions and reduced larval tick populations immediately following the fire. Larval ticks appear to have been most directly impacted by the fire, with decreased abundance within the fire extent in every year of the study, leading necessarily to lower nymphal tick abundance in subsequent years. Again, these effects may have been the result of adverse microclimate and abiotic conditions created by the fire, leading to reduced survivorship over the summer dry season, or direct mortality resulting from the wildfire itself.

California mule deer (*O. hemionus californicus*), which are important reproductive hosts for adult *I. pacificus* (Lane and Burgdorfer 1986), showed patterns of reduced activity and abundance within the fire extent in the first two years following the fire, recovering to levels comparable to adjacent unburned woodland habitat by the third year of the study. This lagged recovery was unexpected, as new growth following fire has been shown to provide additional forage and attract deer (Allan 2009). The pattern observed may have been due to the interaction of wildfire and severe drought, which was impacting California throughout the course of this study, leading to a more protracted recovery of pre-fire vegetation. The ongoing drought may have also served to reduce interannual tick survivorship within the burn extent due to lack of suitable microclimates resulting from loss of soil organic matter, duff and herbaceous vegetation cover (Padgett and Lane 2001, Lane et al. 2009). Due to the lower deer activity observed within the fire extent, fewer successful adult tick blood meals and lower tick reproductive success would be expected to result from wildfire, serving to reduce local tick populations, as observed.

Furthermore, despite the lack of a significant effect of wildfire on abundance of *P. maniculatus* and *S. occidentalis*, or on tick burdens on these two host species, the loss of *N. fuscipes*, an important pathogen reservoir in California (Lane and Brown 1991, Brown and Lane 1992, Swei et al. 2012), from sites impacted by wildfire is significant and corroborates earlier results from studies of prescribed burns (Padgett et al. 2009). Coupled with significantly reduced tick populations within the burn extent, the loss of this relatively long-lived pathogen reservoir could serve to reduce tick-borne disease risk for multiple years within California oak woodlands impacted by wildfire. While no ticks or hosts were infected with *Borrelia burgdorferi* s.s. in this study, similar to the results of previous studies conducted in southern California (Lane et al. 2013, Padgett et al. 2014), the effect of wildfire on tick populations and abundance of key hosts in the Lyme disease transmission cycle is expected to be consistent throughout the far western US. Thus, in aggregate, based on patterns of tick and host abundance that were monitored for three years following wildfire, tick-borne disease risk is expected to increase in the winter and spring immediately following summer wildfires in California and subsequently decline for a minimum of three additional years.

Much of our understanding of the effects of disturbance on tick-borne disease systems comes from recent research focused on the impacts of forest fragmentation on Lyme disease ecology in the northeastern United States (LoGiudice et al. 2003, Allan et al. 2003, Brownstein et al. 2005, Jackson et al. 2006, Larsen et al. 2014, Seukep et al. 2015). In the northeast, forest fragmentation may have an indirect positive effect on the density of infected nymphal blacklegged ticks (*Ixodes scapularis*) through its effect on the community of hosts, namely through increases in abundance of highly competent hosts like white footed mice and loss of dilution hosts in more highly fragmented forests (LoGiudice et al. 2003, 2008). However, the evidence for this effect of fragmentation is mixed (e.g. Zolnik et al. 2015) and may be scale and context-dependent (Cohen et al. 2016). The ecology of Lyme disease in the far western United States is also influenced by perturbations resulting from disturbance events. For example, Swei and colleagues (2011b) found that the invasion of the plant pathogen *Phytophthora ramorum*, causing sudden oak death, leads to increases in the density of nymphal *I. pacificus* ticks, the primary Lyme vector in the western US, and an increase in Lyme disease risk in more heavily impacted forests (Swei et al. 2011b).

However, unlike forest fragmentation resulting from human development or sudden oak death, destruction of forested habitats by wildfire is much more sudden and catastrophic, which may have very different effects on the ecology of Lyme disease. Investigating the effects of fire on tick-borne disease systems has been of interest to disease ecologists and the public health community as a potential control strategy to protect human health (Stafford et al. 1998, Padgett et al. 2009). As such these effects have been explored utilizing controlled burns initiated for management purposes. Previous studies have found that controlled burns tend to reduce tick populations only temporarily (Hoch et al. 1972, Stafford et al. 1998, Cully 1999), but that these effects do not last beyond the year of the controlled burn (Stafford et al. 1998, Cully 1999). Other studies have even found an amplification of tick populations following fire (Scifres et al. 1988, Spickett et al. 1992, Horak et al. 2006), however, burn frequency may modify these effects (Davidson et al. 1994, Allan 2009). In one of the only studies of the effects of controlled burns on tick-borne disease ecology in the fire-prone western US, Padgett and colleagues (2009) found that controlled burns in northern California reduced populations of rodent hosts, but had no real effect on density of *I. pacificus*. In that study, adult *I. pacificus* were found to have survived in soil refugia and immature stages of *I. pacificus* were found infesting rodent hosts at similar, or even higher densities in the controlled burns as compared to unburned control plots suggesting that controlled burns have little impact on tick-borne disease risk in California (Padgett et al. 2009).

Given the remarkable consistency of the results of these studies exploring the effects of controlled burns on tick and host populations, the logical expectation is that the effects of wildfire should follow the same pattern, namely that wildfire should have little effect on tick populations and tick-borne disease risk. In this study wildfire was found to have similar effects on tick and host populations in the year immediately following fire; namely, tick abundance was elevated within the burn extent and small vertebrate host populations were unaffected with the exception of dusky-footed woodrats, an important pathogen reservoir in California. However, in contrast with expectations from studies of prescribed burns, in this study wildfire was found to substantially reduce tick populations and tick-borne disease risk in subsequent years, with potential long-term reductions in risk due to loss of amplification hosts (*N. fuscipes*) from the system, no change in populations of dilution hosts (*S. occidentalis*), slow recovery of reproductive hosts (*O. hemionus californicus*) and substantial reductions in populations of the primary tick vector (*I. pacificus*).

Large-scale disturbances resulting from human activity and climate change are increasing in frequency and intensity (Dale et al. 2001). As the pace of environmental change increases, resulting from land use and climate change as well as globalization, so too may the rate of emergence of zoonotic and vector-borne infectious disease (Patz et al. 2000, Jones et al. 2008, Lambin et al. 2010, Kilpatrick and Randolph 2012, Jones et al. 2013). It is thus increasingly important for ecologists to study the impacts of disturbance and environmental change on human disease emergence and risk. In California, Lyme disease is most common in the northwestern region of the state, where dense oak woodlands and mixed forests provide suitable habitat and microclimates for the tick vector, as well as key hosts in the maintenance and transmission of the pathogen. The forests of this region are also expected to experience the greatest impacts of climate change and increasing wildfire frequency and intensity of any region in the state (Hayhoe et al. 2004, Westerling and Bryant 2007, Westerling et al. 2011). Thus, increasing understanding of the effects of wildfire, and potential interactions with climate change, on tick-borne disease risk is of critical importance.

In this study, we find that wildfire results in elevated tick-borne disease risk immediately following fire, but that risk is subsequently reduced substantially for multiple years into the future.

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G. Figure captions

Figure 1: Map of study area, including 6, 1 ha. plots within and adjacent to the perimeter of the White Fire (May 2013), Santa Barbara County, CA. White Fire perimeter data layer was obtained from Cal Fire (http://frap.cdf.ca.gov/data/frapgisdata-sw-fireperimeters_download), and the California hillshade data layer was obtained from Cal-Atlas (www.atlas.ca.gov/download.html) through https://koordinates.com.

Figure 2: Adult *I. pacificus* abundance by year, by burn status. Data represented as box plots with standard errors and individual sample points.

Figure 3: Nymphal *I. pacificus* abundance by year, by burn status. Data represented as box plots with standard errors and individual sample points.

Figure 4: Larval *I. pacificus* abundance by year, by burn status. Data represented as box plots with standard errors and individual sample points.

Figure 5: Deer (*O. hemionus californicus*) plot use/activity by year, by burn status. Data represented as box plots with standard errors.

Figure 6: Small vertebrate host (*P. maniculatus*, *N. fuscipes* and *S. occidentalis*) population estimates/abundance in 2014 (1 Yr. post-fire), by burn status. Data represented as box plots with standard errors.

Figure 7: Small vertebrate host (*P. maniculatus*, *N. fuscipes* and *S. occidentalis*) immature *I. pacificus* tick burdens in 2014 (1 Yr. post-fire), by burn status. Data represented as box plots with standard errors.

Figure 1.

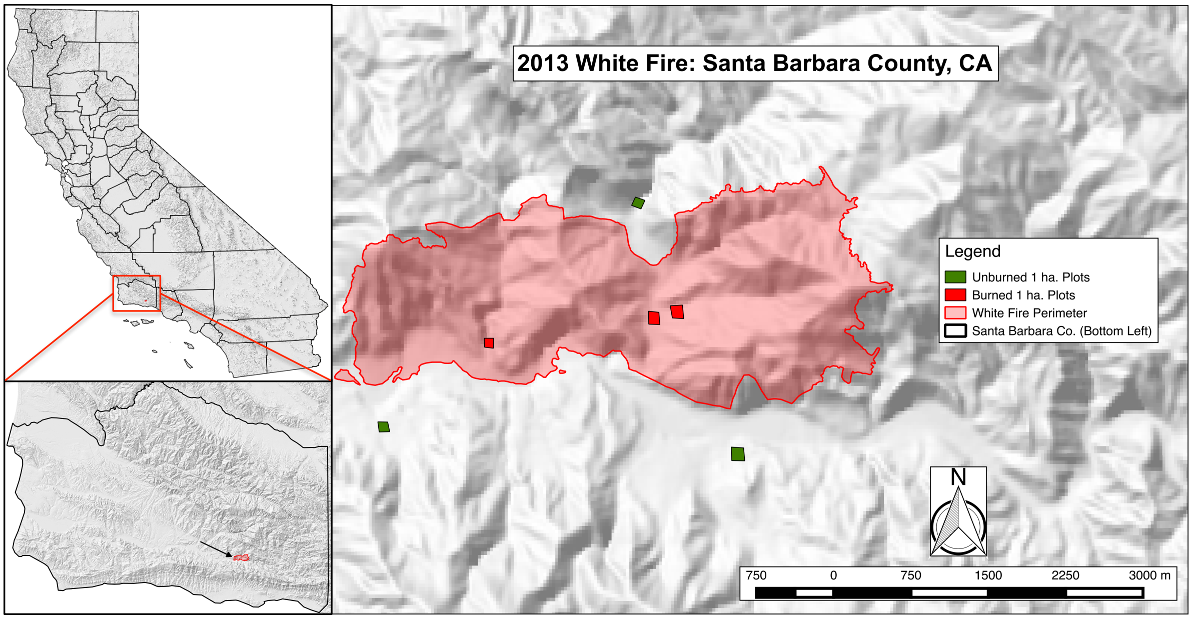

2013 White Fire: Santa Barbara County, CA
Legend
Unburned 1 ha. Plots
Burned 1 ha. Plots
White Fire Perimeter
Santa Barbara Co. (Bottom Left)
N
750
0
750
1500
2250
3000 m

Figure 2.

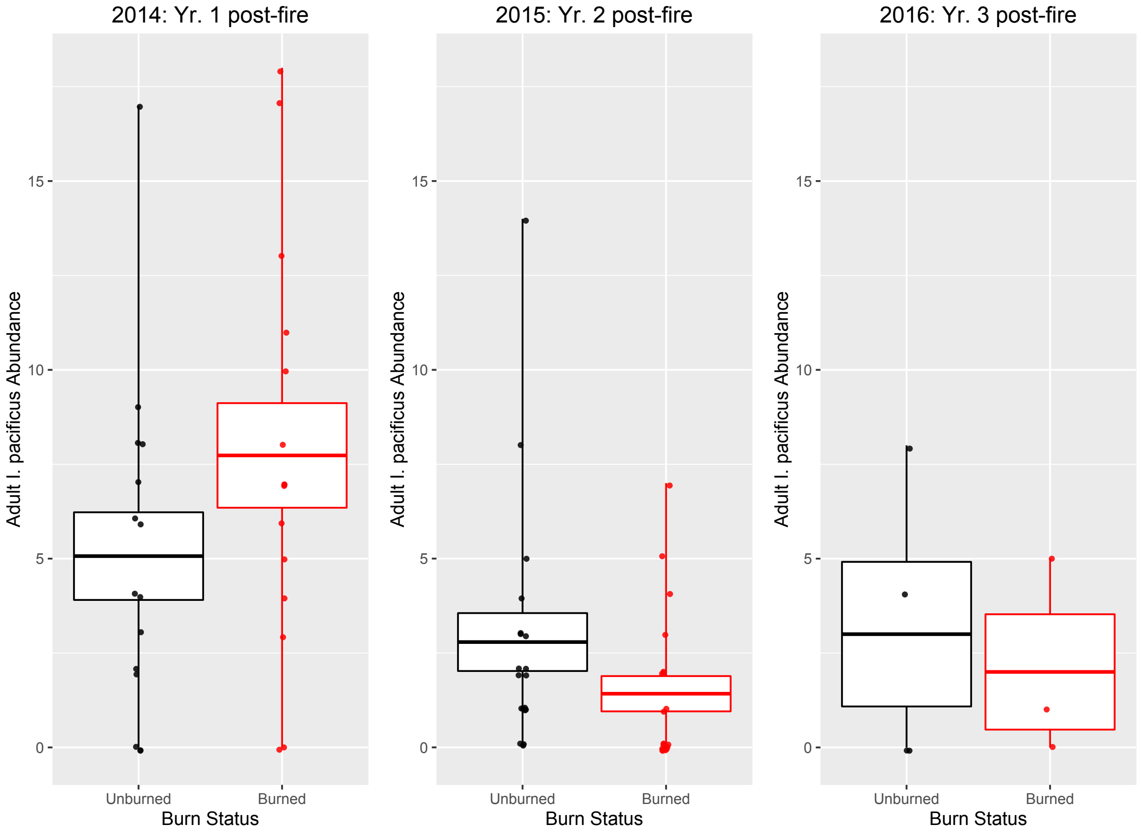
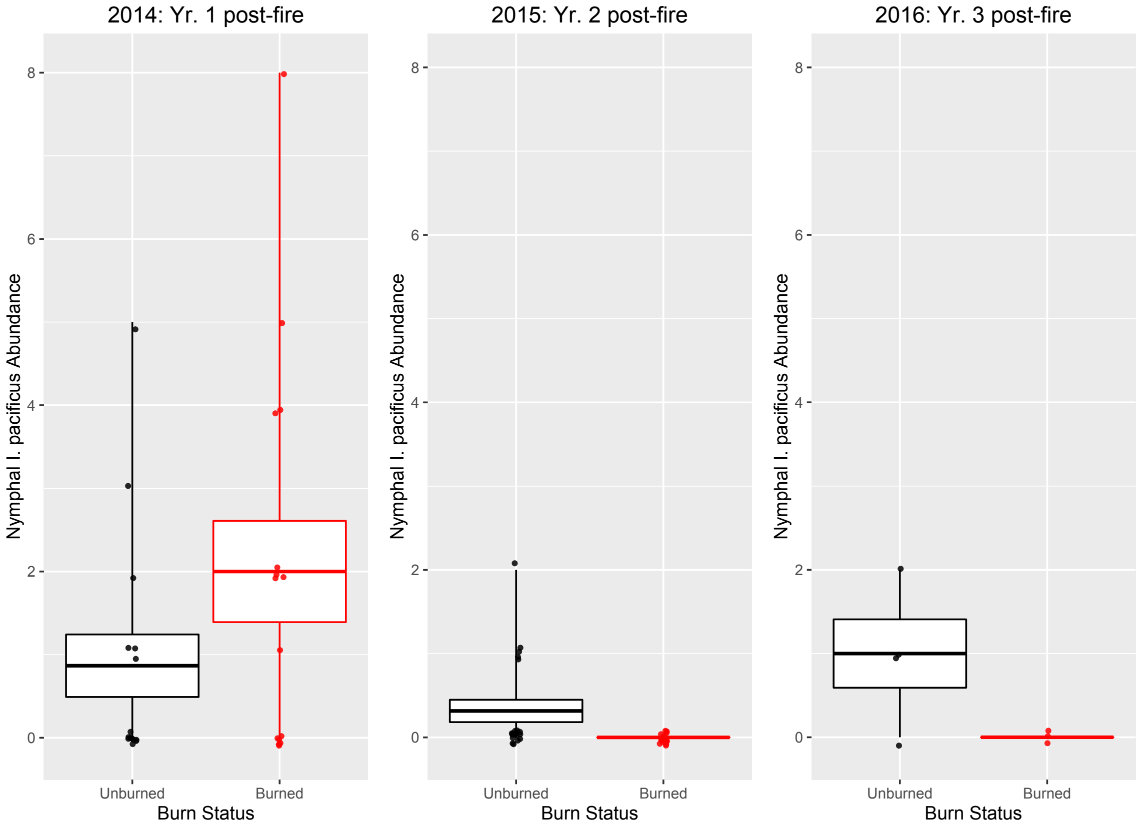

2014: Yr. 1 post-fire
2015: Yr. 2 post-fire
2016: Yr. 3 post-fire
Adult I. pacificus Abundance
0
5
10
15
Unburned
Burned
Burn Status

Figure 3.



2014: Yr. 1 post-fire
2015: Yr. 2 post-fire
2016: Yr. 3 post-fire
Nymphal I. pacificus Abundance
0
2
4
6
8
Unburned
Burned
Burn Status

Figure 4.

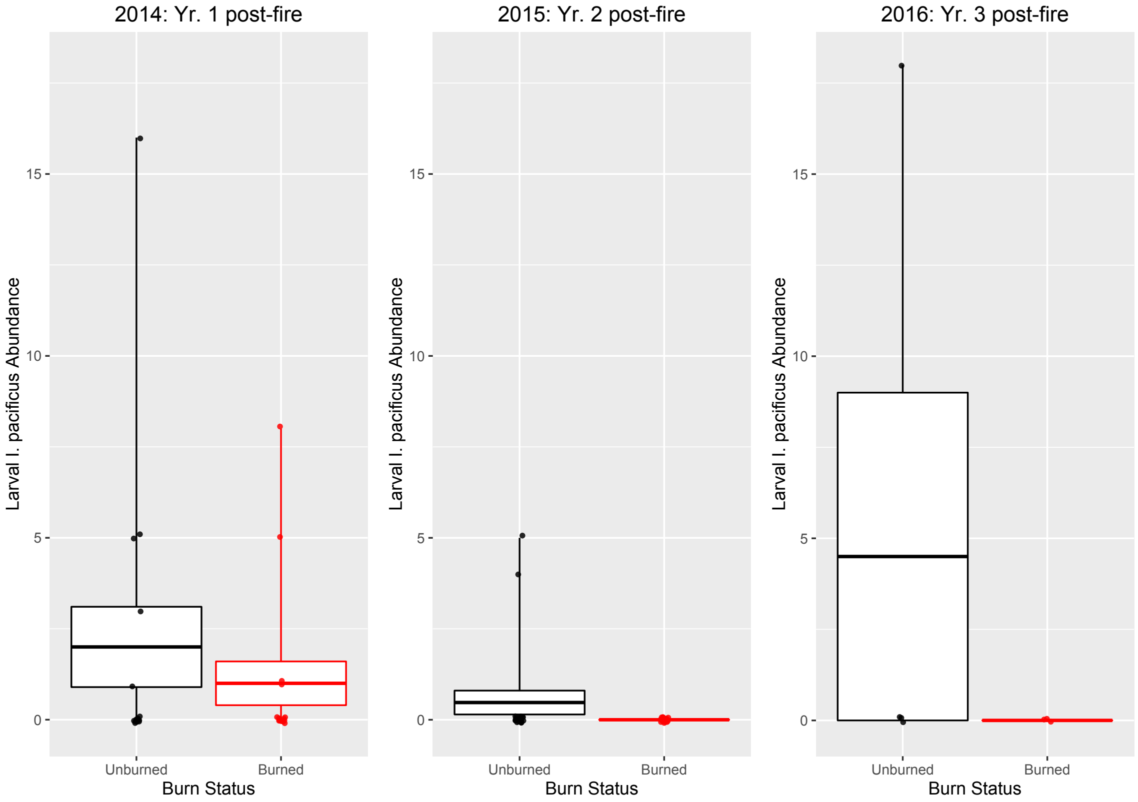

2014: Yr. 1 post-fire
2015: Yr. 2 post-fire
2016: Yr. 3 post-fire
Larval I. pacificus Abundance
15
10
5
0
Unburned
Burned
Burn Status

Figure 5.

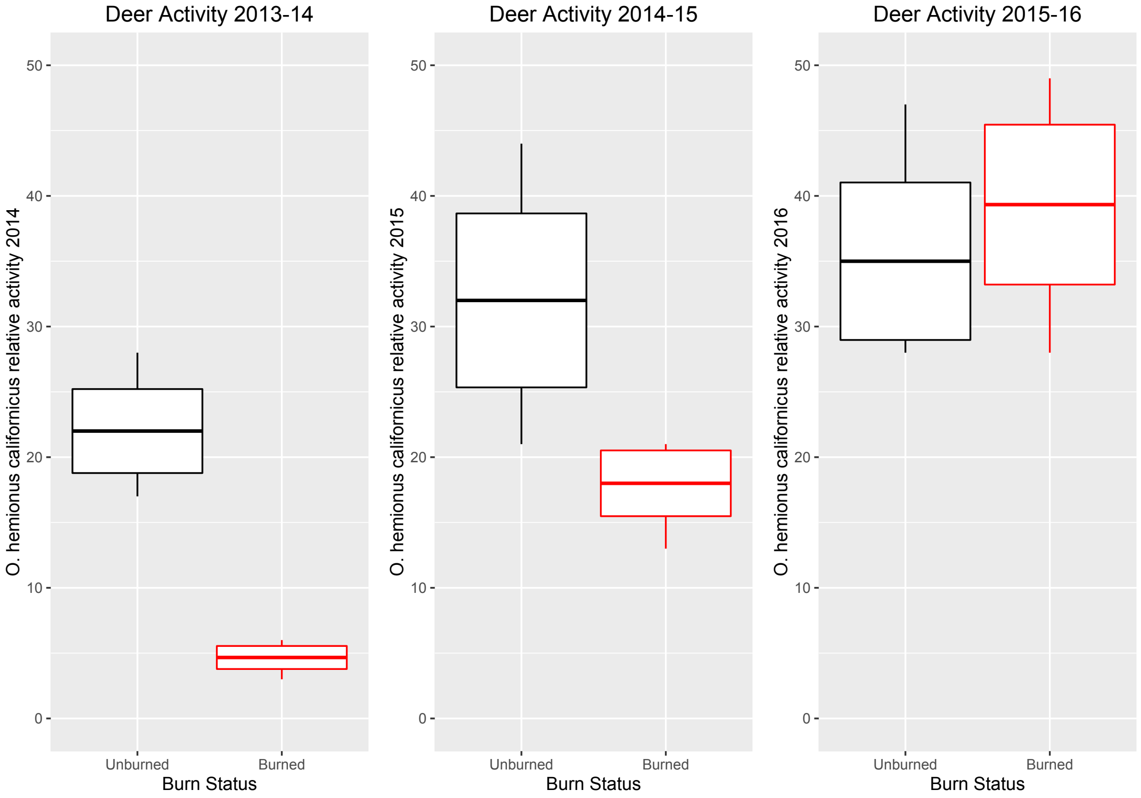
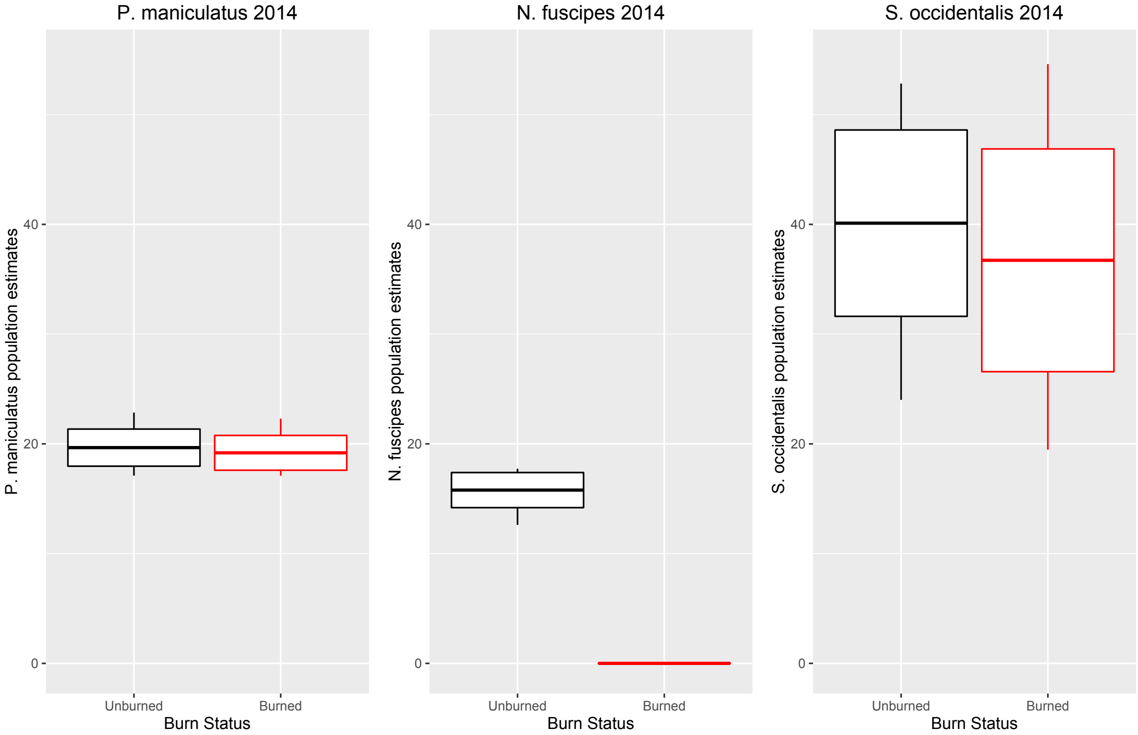

Deer Activity 2013-14
Deer Activity 2014-15
Deer Activity 2015-16
O. hemionus californicus relative activity 2014
O. hemionus californicus relative activity 2015
O. hemionus californicus relative activity 2016
0
10
20
30
40
50
Unburned
Burned
Burn Status

Figure 6.



P. maniculatus 2014
N. fuscipes 2014
S. occidentalis 2014
P. maniculatus population estimates
N. fuscipes population estimates
S. occidentalis population estimates
0
20
40
Unburned
Burned
Burn Status

Figure 7.

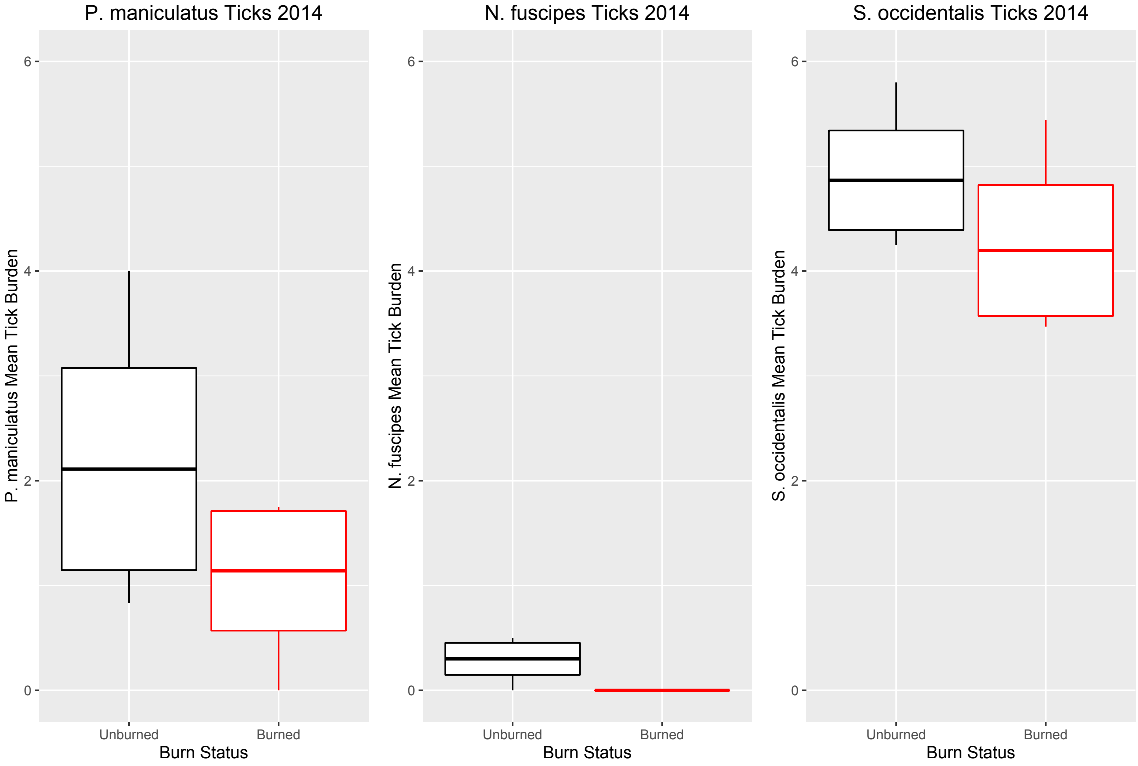

P. maniculatus Ticks 2014
N. fuscipes Ticks 2014
S. occidentalis Ticks 2014
P. maniculatus Mean Tick Burden
N. fuscipes Mean Tick Burden
S. occidentalis Mean Tick Burden
0
2
4
6
Unburned
Burned
Burn Status

Table 1: GLMM results for tick abundance for the 2013-14 through 2015-16 seasons (1-3 Yrs. post-fire). Bold results indicate significant effects of wildfire on tick abundance.

	Adult *I. pacificus* estimate (*P*)	Nymphal *I. pacificus* estimate (*P*)	Larval *I. pacificus* estimate (*P*)
Intercept	0.49 (P=0.047)*	-2.07 (P=0.004)**	7.14 (P<0.001)***
Burned	**2.57 (P<0.001)*** **	**4.87 (P<0.001)*** **	**-16.69 (P<0.001)*** **
Year: 2015	-0.81 (P<0.001)***	-0.82 (P=0.114)	-2.33 (P<0.001)***
Year: 2016	-0.11 (P=0.72)	0.08 (P=0.889)	-0.74 (P=0.187)
Julian Day Squared	-0.50 (P<0.001)***	0.53 (P=0.006)**	-0.43 (P=0.018)*
Max. Summer Temp.	NA	-1.70 (P<0.001)***	NA
Max. Winter Temp.	NA	NA	-1.40 (P<0.001)***
Bare Ground Cover	NA	NA	6.08 (P=0.001)**
Leaf Litter Cover	NA	NA	-1.04 (P=0.013)*
Stem Density	NA	NA	0.72 (P<0.001)***
Woodrat Nest Density	1.08 (P<0.001)***	NA	NA
Burned*Year: 2015	**-1.10 (P<0.001)*** **	-0.20 (P=0.996)	-10.63 (P=0.997)
Burned*Year: 2016	**-1.12 (P=0.03)* **	-0.24 (P=0.998)	-0.32 (P=0.999)

GLMM results for Adult (Poisson, log link), Nymphal (negative binomial, log link) and Larval (negative binomial, log link) *I. pacificus* abundance. Results are shown as model estimates and *P* values. Levels of significance are: * ≤ 0.05, ** ≤ 0.01, and *** ≤ 0.001

Table 2: GLMM results for Deer plot use for the 2013-14 through 2015-16 seasons (1-3 Yrs. post-fire). Bold results indicate significant effects of wildfire on deer activity.

	O. hemionus californicus estimate (*P*)
Intercept	3.12 (P<0.001)***
Burned	**-1.62 (P<0.001)*** **
Year: 2015	0.38 (P<0.001)***
Year: 2016	0.38 (P<0.001)***
Canopy Cover	0.15 (P<0.001)***
Burned*Year: 2015	**0.96 (P<0.001)*** **
Burned*Year: 2016	**1.75 (P<0.001)*** **

GLMM (Poisson, log link) results for *O. hemionus californicus* plot use. Results are shown as model estimates and *P* values. Levels of significance are: * ≤ 0.05, ** ≤ 0.01, and *** ≤ 0.001

Table 3: PLSR results for small vertebrate host abundance in 2014 (1 Yr. post-fire). Weights and VIP scores indicate that wildfire ("Burned") had a significant negative effect on abundance of *N. fuscipes*, but no measurable effect on abundance of *P. maniculatus* or *S. occidentalis*. Other variables that contributed significantly to host abundance are in bold. R-squared values indicate the amount of variation explained by each component, and the second component acts on residual variation not explained by the first component.

	P. maniculatus		*N. fuscipes*		*S. occidentalis*	
	Comp. 1 wts. (VIP)	Comp. 2 wts. (VIP)	Comp. 1 wts. (VIP)	Comp. 2 wts. (VIP)	Comp. 1 wts. (VIP)	Comp. 2 wts. (VIP)
Canopy Cover	-0.20 (0.63)	**0.62 (1.96)**	0.03 (0.11)	**0.47 (1.49)**	**0.58 (1.84)**	0.17 (0.55)
Shrub Cover	-0.29 (0.93)	0.20 (0.65)	**0.39 (1.24)**	0.16 (0.50)	**0.39 (1.25)**	**-0.33 (1.05)**
Herbaceous Cover	0.25 (0.81)	-0.13 (0.42)	**0.35 (1.10)**	-0.18 (0.56)	0.03 (0.11)	-0.17 (0.55)
Bare Ground Cover	-0.13 (0.40)	0.07 (0.22)	**-0.39 (1.22)**	0.11 (0.33)	-0.12 (0.36)	0.24 (0.77)
Litter Cover	-0.21 (0.66)	-0.25 (0.78)	0.29 (0.91)	-0.001 (0.00)	-0.14 (0.43)	**-0.52 (1.66)**
Stem Density	**-0.70 (2.21)**	0.19 (0.59)	0.16 (0.49)	**0.37 (1.16)**	**0.55 (1.74)**	-0.004 (0.01)
Woodrat Nest Density	0.30 (0.96)	0.22 (0.69)	**0.43 (1.36)**	0.22 (0.69)	0.21 (0.67)	-0.27 (0.85)
Max. Summer Temp.	0.09 (0.27)	0.01 (0.02)	**-0.36 (1.13)**	0.13 (0.43)	-0.06 (0.21)	**0.47 (1.48)**
Max. Winter Temp.	**-0.38 (1.21)**	**-0.64 (2.01)**	0.05 (0.17)	**-0.71 (2.23)**	**-0.34 (1.06)**	**-0.33 (1.03)**
Burned	-0.11 (0.36)	0.05 (0.17)	**-0.39 (1.23)**	0.11 (0.34)	-0.09 (0.28)	0.31 (0.98)
R-Squared	0.73	0.18	0.88	0.08	0.89	0.04

PLSR results for *P. maniculatus*, *N. fuscipes* and *S. occidentalis* abundance in 2014 (1 Yr. post-fire). Results are presented as variable weights, indicating direction of the effect, and VIP scores in parentheses. Significant variables (VIP > 1) are in bold.

Table 4: PLSR results for small vertebrate host tick burdens in 2014 (1 Yr. post-fire). Weights and VIP scores indicate that wildfire ("Burned") had a significant negative effect on *N. fuscipes* tick burdens (due to absence of *N. fuscipes* from burned plots), but no measurable effect on tick burdens of *P. maniculatus* or *S. occidentalis*, with the exception of a marginally significant contribution to the residual variation in tick burdens on *S. occidentalis*. Other variables that contribute significantly to host tick burdens are in bold. R-squared values indicate the amount of variation explained by each component, and the second component acts on residual variation not explained by the first component.

	P. maniculatus Ticks		*N. fuscipes* Ticks		*S. occidentalis* Ticks	
	Comp. 1 wts. (VIP)	Comp. 2 wts. (VIP)	Comp. 1 wts. (VIP)	Comp. 2 wts. (VIP)	Comp. 1 wts. (VIP)	Comp. 2 wts. (VIP)
Canopy Cover	0.16 (0.52)	**0.40 (1.28)**	**-0.34 (1.07)**	**-0.35 (1.11)**	**-0.51 (1.61)**	**-0.34 (1.09)**
Shrub Cover	**0.50 (1.59)**	0.17 (0.55)	0.08 (0.26)	**-0.53 (1.66)**	0.04 (0.12)	**-0.37 (1.16)**
Herbaceous Cover	0.12 (0.39)	**-0.58 (1.84)**	**0.48 (1.51)**	0.13 (0.40)	0.25 (0.79)	**-0.35 (1.10)**
Bare Ground Cover	-0.23 (0.73)	**0.43 (1.35)**	**-0.42 (1.31)**	0.03 (0.10)	-0.23 (0.72)	**0.36 (1.15)**
Litter Cover	0.31 (0.99)	0.02 (0.07)	0.22 (0.69)	-0.26 (0.83)	**0.43 (1.35)**	0.11 (0.34)
Stem Density	0.19 (0.60)	0.13 (0.41)	-0.25 (0.78)	**-0.48 (1.52)**	-0.19 (0.61)	-0.10 (0.33)
Woodrat Nest Density	0.24 (0.76)	**-0.34 (1.07)**	**0.38 (1.20)**	-0.01 (0.04)	0.09 (0.28)	**-0.47 (1.49)**
Max. Summer Temp.	**-0.53 (1.69)**	-0.22 (0.70)	-0.22 (0.69)	**0.43 (1.37)**	-0.26 (0.83)	0.25 (0.79)
Max. Winter Temp.	0.29 (0.92)	0.14 (0.43)	0.15 (0.49)	-0.29 (0.93)	**0.52 (1.63)**	0.26 (0.83)
Burned	-0.30 (0.95)	0.29 (0.93)	**-0.39 (1.24)**	0.11 (0.34)	-0.25 (0.78)	**0.34 (1.09)**
R-Squared	0.36	0.28	0.64	0.24	0.63	0.27

PLSR results for *P. maniculatus*, *N. fuscipes* and *S. occidentalis I. pacificus* burdens in 2014 (1 Yr. post-fire). Results are presented as variable weights, indicating direction of the effect, and VIP scores in parentheses. Significant variables (VIP > 1) are in bold.