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Influence of Abiotic and Environmental Factors on the Density and Infection Prevalence of *Ixodes pacificus* (Acari: Ixodidae) With *Borrelia burgdorferi*

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ABSTRACT The abiotic and biotic factors that govern the spatial distribution of Lyme disease vectors are poorly understood. This study addressed the influence of abiotic and biotic environmental variables on *Ixodes pacificus* Cooley & Kohls (Acari: Ixodidae) nymphs, because it is the primary vector of *Borrelia burgdorferi* Johnson, Schmidt, Hyde, Steigerwaldt & Brenner in the far-western United States. Three metrics of Lyme disease risk were evaluated: the density of nymphs, the density of infected nymphs, and the nymphal infection prevalence. This study sampled randomly located plots in oak (*Quercus* spp.) woodland habitat in Sonoma County, CA. Each plot was drag-sampled for nymphal ticks and tested for *B. burgdorferi* infection. Path analysis was used to evaluate the direct and indirect relationship between topographic, forest structure and microclimatic variables on ticks. Significant negative correlations were found between maximum temperature in the dry season and the density of infected ticks in 2006 and tick density in 2007, but we did not find a significant relationship with nymphal infection prevalence in either year. Tick density and infected tick density had an indirect, positive correlation with elevation, mediated through temperature. This study found that in certain years but not others, temperature maxima in the dry season may constrain the density and density of infected *I. pacificus* nymphs. In other years, biotic or stochastic factors may play a more important role in determining tick density.

KEY WORDS abiotic limits, disease ecology, path analysis, spatial autocorrelation

Ixodes spp. ticks are long-lived relative to other arthropod vectors, with life cycles of 2–6 yr (Yuval and Spielman 1990, Padgett and Lane 2001). Because of this long life span, biotic and abiotic constraints are especially important in limiting their life history and the pathogens they transmit (Randolph 1998). This study evaluates the influence of several key biotic and abiotic factors on the distribution of *Ixodes pacificus* Cooley and Kohls (Acari: Ixodidae), the vector of *Borrelia burgdorferi* Johnson, Schmidt, Hyde, Steigerwaldt & Brenner, the pathogen that causes Lyme disease in the far western United States (Burgdorfer et al. 1985).

In the United States, Lyme disease is caused by bacterium *Borrelia burgdorferi* s.s. (hereafter *B. burgdorferi*) where it is the most prevalent vector-borne disease (CDC 2008). The distribution of Lyme disease is largely coincident with the distribution of the vectors of the disease (Kurtenbach et al. 2002). However, there is a great deal of spatial and temporal variability in the prevalence of Lyme disease within their distribution (Lane et al. 1992, Talleklint-Eisen and Lane 1999, Eisen et al. 2003). In the eastern United States, *Ixodes scapularis* Say is the primary vector and in the far-western United States, the vector is *I. pacificus*. *I. pacificus* has a 3-yr minimum life cycle with three postegg stages: larva, nymph, and adult (Padgett and Lane 2001). Each of these life stages takes a single bloodmeal from a vertebrate host before molting into the next stage, or in the case of adult females, producing eggs (Padgett and Lane 2001). The remainder of the time, *I. pacificus* is inactive on the ground or in the leaf litter (Padgett and Lane 2001). During this off-host time, *I. pacificus* is vulnerable to a suite of daily and seasonal abiotic constraints (Randolph 1998). Both *I. pacificus* and *I. scapularis* are affected by similar abiotic factors that can impact their growth and survival. Differences in their life cycle and phenology are largely attributed to local abiotic conditions. Molting success and survival of *I. scapularis* has been found to be limited by minimum temperature in the winter (Ogden et al. 2004, Ogden et al. 2006). However, in the western United States, *I. pacificus* molting rate and survival is most likely limited by summer drought conditions (Padgett and Lane 2001, Eisen et al. 2003). Summer drought conditions are believed to be so

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detrimental to *I. pacificus* survival that although larvae hatch in the early summer, they remain in behavioral diapause until the following spring to avoid activity during dry, summer conditions (Padgett and Lane 2001).

Availability of vertebrate tick hosts is another important factor that affects the abundance of *Ixodes* spp., although this has been difficult to validate with field data (Killilea et al. 2008). There is some limited evidence that presence of deer is positively associated with larval and adult stages of *I. scapularis*, but the data are equivocal (Wilson et al. 1988, Rand et al. 2003, Ostfeld et al. 2006, Perkins et al. 2006). Tick hosts also may be affected by habitat features such as plant diversity, overstory cover, and resource availability. All of these factors can indirectly affect the abundance of ticks and their infection prevalence of *B. burgdorferi*.

I. pacificus does not pass the Lyme disease pathogen vertically from adult to larvae and must acquire *B. burgdorferi* from a pathogen reservoir during a bloodmeal. *I. pacificus* nymphs are the principal vector of *B. burgdorferi* because larvae are uninfected and adult ticks have lower infection prevalence due to the ability of lizards to reduce the infection prevalence of feeding nymphs by killing *B. burgdorferi* through the alternative pathway of their protein complement system (Lane and Quistad 1998, Kuo et al. 2000). This is consistent with the coincident peaks of reported human cases with nymphal activity (Clover and Lane 1995). The main reservoirs of *B. burgdorferi* for *I. pacificus* are the dusky-footed woodrat (*Neotoma fuscipes*), California kangaroo rat (*Dipodomys californicus*), deer mice (*Peromyscus maniculatus*), and western gray squirrels (*Sciurus griseus*) (Lane and Brown 1991; Brown and Lane 1992, 1996; Peavey and Lane 1995; Lane et al. 2005). Infection prevalence of *I. pacificus* is indirectly affected by the biotic and abiotic factors that determine the distribution of reservoir species as well as nonreservoir tick hosts such as the western fence lizard (*Sceloporus occidentalis*) and Columbian black-tailed deer (*Odocoileus hemionus columbianus*).

To jointly address the direct effects of microclimate and habitat features on tick density and infection prevalence, this study conducted tick surveys to determine the density and infection prevalence of *I. pacificus* across a range of biotic and abiotic conditions. This study took place on a network of randomly selected plots in oak woodland habitat (Meentemeyer et al. 2008b) to examine the relationship between microclimatic factors and habitat features on tick abundance and infection prevalence in Sonoma County, CA.

Materials and Methods

This study was conducted within a 275-km^2 region in Sonoma County, north of San Francisco, CA (Fig. 1). The habitat in this region is a heterogeneous mixture of vegetation and topography types, including oak (*Quercus* spp.) woodlands, redwood–tanoak, chaparral, grassland, agricultural areas, and residential developments. Climactic conditions are Mediterranean with cool, wet winters and warm, dry summers. Field sampling of ticks was conducted in several state parks and reserves in Sonoma County, CA (Fig. 1), including Jack London State Historic Park (38.35670° N, 122.54400° W), Annadel State Park (38.45200° N, −122.63400° W), Sugarloaf Ridge State Park (38.43786° N, 122.51432° W), Sonoma State University Fairfield Osborn Preserve (38.348923° N, −122.595711° W), and Audubon Canyon Ranch Bouverie Preserve (38.364359° N, −122.509971° W). Field sites were randomly selected in oak woodlands. For specific plot selection methods see Meentemeyer et al. (2008a). In brief, oak woodland habitat was identified from georectified multispectral aircraft imagery (ADAR, Positive Systems, Whitefish, MT; Meentemeyer et al. 2008a). Imagery was collected as four spectral bands of data (red, green, blue, and near infrared) at 1- by 1-m pixels and classified as oak woodland habitat with unsupervised ISODATA image processing (Erdas Imagine 8.7, Leica Geosystems, Norcross, GA). Geographic Information System was then used to randomly select sites from public land. All plots were spaced at least 300 m apart.

Data on site-specific biotic and abiotic variables were collected in 2005 and 2006 to predict their impacts on larvae in those years and therefore on nymphs the following year (2006 and 2007). Abiotic data were collected hourly on each site using microclimate data loggers located in the center of each site and mounted on a pole 1 m above the ground and housed within a protective solar shield (Onset Corporation, Bourne, MA). From the data loggers, we calculated the averages of daily maximum temperature and minimum relative humidity over the dry (1 May–30 October) and rainy seasons (1 November–30 April). We measured all stems that exceeded 5 cm in diameter at breast height (dbh) and 1.4 m in height. Species richness was calculated as the number of species identified from each plot and stem density was calculated as the number of stems (dbh >5 cm) per 225-m^2 plot.

Tick collections were conducted on each site once per year. Sampling for all sites took place over 2–3 wk to minimize temporal patterns in *I. pacificus* questing activity. Sampling was conducted from 18 to 28 April in 2006 and from 7 to 31 May in 2007. In 2006, 37 sites in total were visited. This sample was expanded to 53 sites in 2007. Ticks were collected by dragging a 1-m^2 white flannel cloth attached to a wooden dowel across the entire understory of each 15- by 15-m plot (225 m^2). Every 15 m, the cloth was checked thoroughly for ticks. All ticks were collected and preserved in 95% ethanol for species identification and tested in the lab for infection with *B. burgdorferi*.

DNA from all *I. pacificus* nymphs was extracted using a DNeasy extraction kit (QIAGEN, Valencia, CA) following manufacturer's instructions. All ticks samples were then screened for infection with *B. burgdorferi* s.s. by real-time polymerase chain reaction (PCR) (Swei et al. 2011). Positive samples were sequenced at the 5S-23S intergenic spacer region following Lane et al. (2004). Sequences were processed

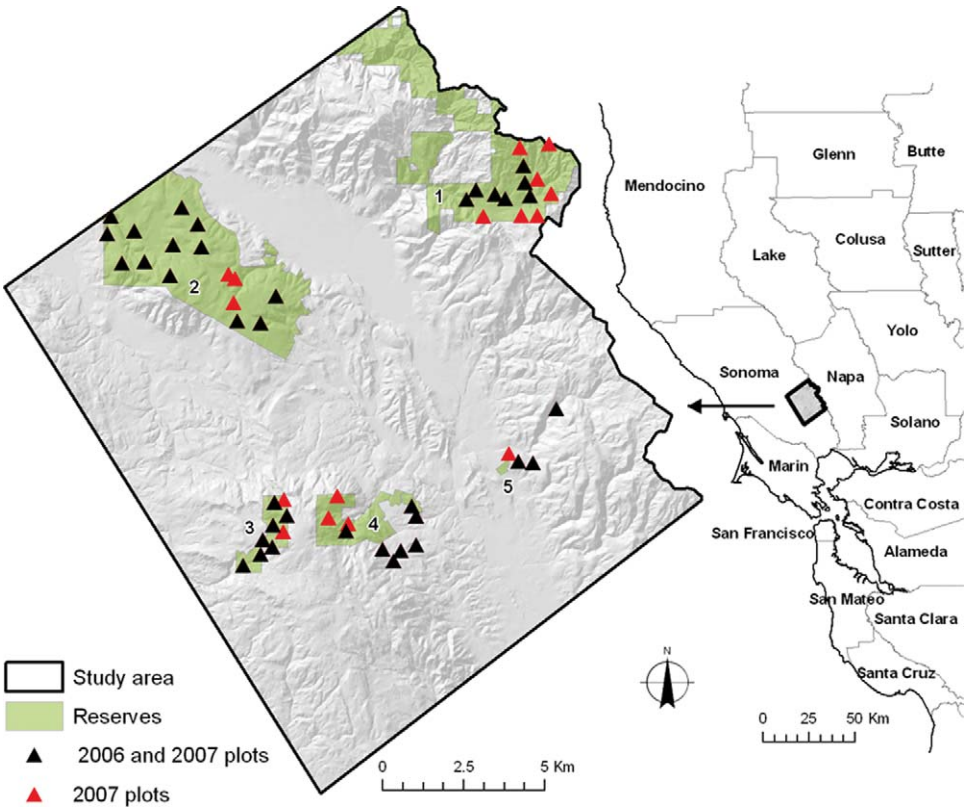

Fig. 1. Map of the plots sampled in this study. All plots were on public land and natural reserves indicated in green. Area one is Sugarloaf Ridge State Park, Area two is Annadel State Park, area three is Sonoma State University Fairfield Osborn Preserve, area four is Jack London Historic State Park, and area five is Audubon Canyon Ranch Bouverie Preserve. In 2006, 37 plots were sampled and in 2007 sampling was expanded to 53 plots. (Online figure in color.)

on an ABI 3770 (Applied Biosystems, Foster City, CA) and aligned using Sequencher (Gene Codes, Ann Arbor, MI).

Path analysis was used to assess the multivariate relationship between topography, microclimate, and forest structure on tick density, density of infected nymphs, and nymphal infection prevalence (Fig. 2). Plot-level variables that potentially impact the spatial distribution of ticks or tick infection prevalence were selected a priori based on previously published studies (Eisen et al. 2003, Rand et al. 2003, Diuk-Wasser et al. 2006, Prusinski et al. 2006). Plot elevation was used as the topographic variable in the model (Glass et al. 1995). Forest structure variables assessed were: total species richness, understory species richness, tree stem density, and *Quercus* spp. biomass (Prusinski et al. 2006). Microclimate variables that were most likely to limit *I. pacificus* also were evaluated and included minimum relative humidity in the dry season, and maximum temperature in the dry season (Estrada-Pena 2002, Brownstein et al. 2003, Diuk-Wasser et al. 2006). The path analysis tested the following hypotheses: 1) higher elevation determines microclimate conditions such as temperature and humidity as well as forest structure such as tree species composition and tree density, 2) forest structure also influences microclimate such that higher density of trees reduces temperatures and increases relative humidity, 3) lower temperatures and higher relative humidity in the summer increase tick survival and molting success, and 4) forest structure indirectly affects tick infection prevalence by influencing the distribution of tick hosts and reservoirs of *B. burgdorferi*.

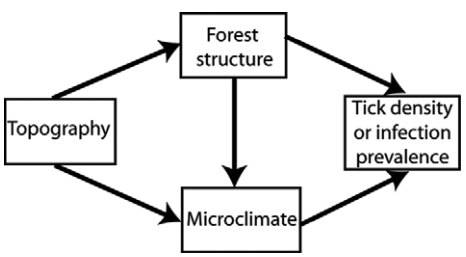

Fig. 2. Generalized structural equation model illustrating a priori hypotheses of pathways between topography, microclimate, forest structure, and tick abundance (DON) and infection prevalence (DIN and NIP).

Our sample size ($N = 37$ and 53 in 2006 and 2007, respectively) limited the number of variables that

Table 1. Density and infection prevalence of *I. pacificus* nymphs in Sonoma County parks and preserves

Land authority	Density per 100 m^2 (SE)		Infection prevalence (%)		Area sampled (m^2)	
	2006	2007	2006	2007	2006	2007
Annandale State Park	5.26 (1.89)	8.53 (1.71)	21.43	10.65	2,925	3,600
ACR Bouverie Preserve	3.70 (1.44)	5.33 (4.60)	20.00	1.16	675	900
Fairfield Osborn Preserve	5.44 (0.82)	11.56 (4.19)	17.07	3.09	900	900
Jack London State Historic Park	9.84 (1.97)	13.16 (3.36)	8.07	6.10	1,575	2,250
Sugarloaf Ridge State Park	13.60 (3.09)	8.58 (2.30)	21.49	5.79	2,250	4,275

Data are shown for 2006 and 2007.

could be used in path analysis to three predictor variables (McGarigal et al. 2000, Gotelli and Ellison 2004). As such, multiple regression analyses were used to reduce the number of variables. Microclimate (mean minimum relative humidity and mean maximum temperature in the dry season) and forest structure (species richness, understory richness, tree density, and cumulative *Quercus* spp. biomass) variables were selected by multiple regression to determine what variables to retain for path analysis (Meentemeyer et al. 2008b). Model selection was based on Akaike information criterion (AIC) scores (Burnham and Anderson 2002). Maximum temperature was a significantly better predictor than minimum relative humidity for the total density of nymphs so it was used in the path analysis model. Tree stem density was the most significant forest structure parameter when regressed against infected tick density and infection prevalence and when regressed against maximum temperature and was therefore included in the final path analysis model. To meet the assumption of multivariate normality, density of nymphs and density of infected nymphs were log-transformed. Nymphal infection prevalence was arcsine, square-root transformed. Maximum temperature was square root transformed for normality. Total tree stem count was square root transformed.

We assessed the assumption of statistical independence by determining whether there was significant spatial autocorrelation in the data. We used Moran's I statistic to evaluate spatial autocorrelation across our plots for each year. We found evidence of spatial autocorrelation in maximum temperature in the dry season in 2005 and 2006 ($I = 0.38$, SD $= 0.057$, $P < 0.001$ and $I = 0.37$, SD $= 0.039$, $P < 0.001$, respectively), stem density in 2006 ($I = 0.081$, SD $= 0.039$, $P = 0.005$) but not 2005 ($I = 0.066$, SD $= 0.056$, $P = 0.099$), and the density of nymphs in 2006 ($I = 0.28$, SD $= 0.056$, $P < 0.001$) but not 2007 ($I = 0.010$, SD $= 0.039$, $P = 0.456$). None of the other tick parameters were found to be spatially autocorrelated. As such, path analyses accounted for spatial autocorrelation with spatial autoregressive analyses, implemented in the program Spatial Analysis in Macroecology (SAM version 3, Rangel et al. 2010).

All explanatory variables were parameterized with plot-level data from the year before tick collection ($t - 1$) because density and infection prevalence of nymphal ticks in the current year is determined by survival and activity of tick larvae in the previous year.

Results

In total, 3,832 *I. pacificus* ticks were collected by drag sampling 37 plots in 2006. Of these, 3312 were larvae, 480 were nymphs, and 16 were adults. Other tick species collected but not evaluated in this analysis were western ticks (*Dermacentor occidentalis* Marx; $N = 33$) and rabbit ticks (*Haemaphysalis leporispalustris* Packard; $N = 141$). *I. pacificus* densities ranged from 0 to 26.67 nymphs per 100 m^2 and 0–351.56 larvae per 100 m^2. Mean nymphal density was 6.13 ± 0.95 (SE) nymphs per 100 m^2. Tick infection prevalence with *B. burgdorferi* ranged from 0 to 100%, with a mean nymphal infection prevalence of 10.0%. In 2007, 6,228 *I. pacificus* ticks in total were collected by drag sampling 53 plots; 5224 larvae, 996 nymphs, and eight adults. Mean nymphal density increased in 2007–9.41 ± 1.24 (SE) nymphs per 100 m^2. Tick infection prevalence ranged from 0 to 66.66% in 2007, and the mean nymphal infection prevalence was lower than the prevalence in 2006 at 7.1%.

Density and infection prevalence of *I. pacificus* nymphs with *B. burgdorferi* varied between the parks sampled and between years (Table 1; Supp. Table S1). Sugarloaf State Park had the highest density and highest infection prevalence (13.60 per 100 m^2, 21.49%; Table 1), whereas the Audubon Canyon Ranch Bouverie Preserve had the lowest densities of *I. pacificus* nymphs and had the lowest infection prevalence (1.16%) in 2007 but relatively high infection prevalence in 2006 (20.0%; Table 1).

Separate path analysis models were constructed for each tick response variable (Figs. 3 and 4). The path analysis found that the plot parameters elevation, stem density, and maximum temperature explained a small fraction of the variability in *I. pacificus* density, infected density, and infection prevalence ($r^2 < 0.14$; Table 2, 3). In both 2005 and 2006, elevation was negatively correlated with maximum temperature in the dry season in 2005 (path coefficient $= -0.005$, $P < 0.001$; Fig. 3) and 2006 (path coefficient $= -0.004$, $P < 0.001$; Fig. 4). Tree density was positively correlation with maximum temperature, but this relationship was not significant (Table 2). In both years, the direct and indirect effect of stem density had a negligible effect on the infection prevalence and density of *I. pacificus* (Tables 4 and 5). Maximum temperature was negatively correlated with the density of infected nymphs in 2006 (path coefficient $= -0.08$, $P = 0.049$). This relationship was again negative in 2007, but not significant (Table 3). The density of *I. pacificus* nymphs was negatively correlated with maxi-

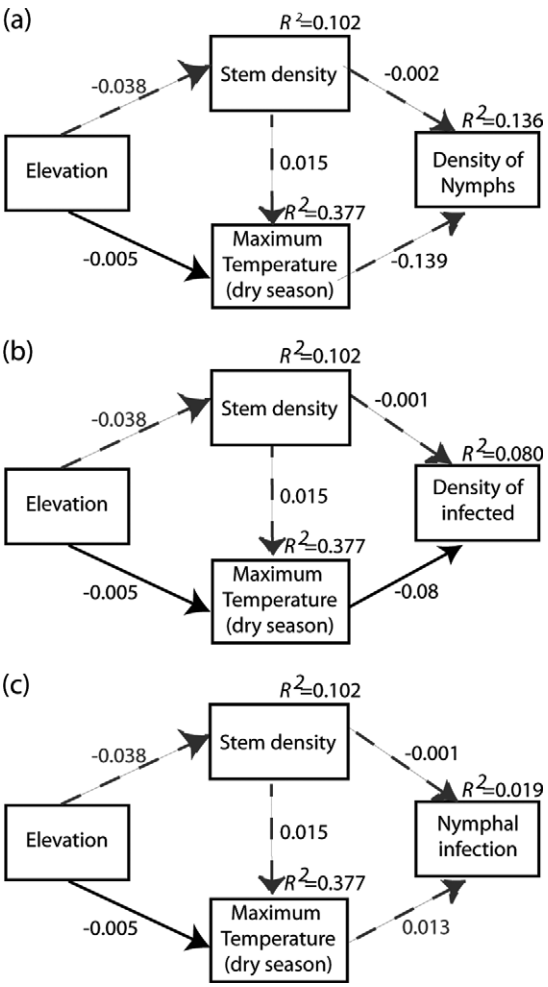

Fig. 3. Results of path analysis from 2006 data with density of nymphal ticks (DON; a), density of infected nymphs (DON; b), and nymphal infection prevalence (NIP; c) as response parameters. Elevation was selected as the topography parameter, maximum temperature in the dry season was the microclimate parameter, and tree stem density was selected as the forest structure parameter. Arrows illustrate pathways in the final SEM model. Pathways with statistical significance ($P < 0.05$) are shown in black and nonsignificant pathways are shown in dashed gray arrows. Standardized regression weights are shown alongside each arrow, and r^2 values are shown above each response parameter.

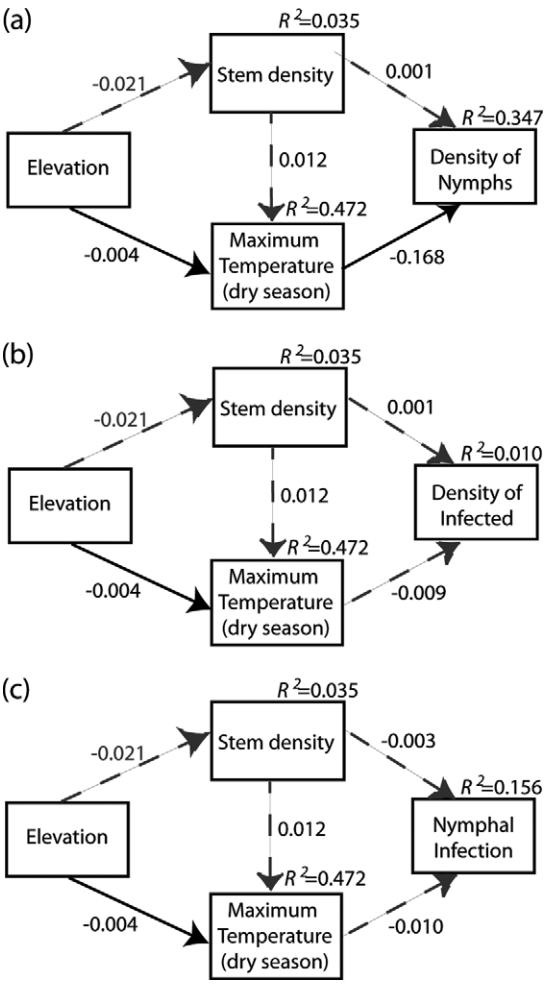

Fig. 4. Results of path analysis from 2007 data with density of nymphal ticks (DON; a), density of infected nymphs (DON; b), and nymphal infection prevalence (NIP; c) as response parameters. Elevation was selected as the topography parameter, maximum temperature in the dry season was the microclimate parameter, and tree stem density was selected as the forest structure parameter. Arrows illustrate pathways in the final SEM model. Pathways with statistical significance ($P < 0.05$) are shown in black and nonsignificant pathways are shown in dashed gray arrows. Standardized regression weights are shown alongside each arrow, and r^2 values are shown above each response parameter.

mum temperature but this relationship was not significant in 2006. However, the negative correlation between maximum temperature and the density of *I. pacificus* was significant in 2007 (path coefficient = −0.168, $P = 0.026$). Nymphal infection prevalence was not significantly correlated with any of the plot variables in either year (Tables 2 and 3).

Discussion

The importance of abiotic factors such as temperature and relative humidity on tick growth and survival has been demonstrated in the laboratory (Padgett and Lane 2001, Ogden et al. 2004), but how biotic habitat variables affect ticks is less clear. This study evaluated both microclimate characteristics and habitat features in a path analysis to explicitly test the direct and indirect paths between ticks and these types of parameters and found evidence of an indirect influence of elevation and a direct influence of maximum temperature in the dry season. These relationships were not consistent across years and only represent our findings in oak woodland habitat in Sonoma County, CA. However our sample of 37 plots in 2006

Table 2. Structural equation model results from 2006 showing path coefficients of all dependent variables and the influence of independent variables

Dependent variable and independent variable	r^2	Path coefficient	Critical ratio	*P*
Total stem density	0.102			
Elevation		−0.038	−1.93	0.062
Max temp (dry season)	0.377			
Elevation		−0.005	−3.718	<0.001
Total stem density		0.015	−0.015	0.988
Density of nymphs (DON)	0.136			
Max temp		−0.139	−1.371	0.180
Total stem density		−0.002	0.544	0.590
Density of infected nymphs (DIN)	0.136			
Max temp		−0.080	−2.047	0.049
Total stem density		<0.001	0.034	0.973
Nymphal infection prevalence (NIP)	0.139			
Max temp		−0.013	−0.197	0.847
Total stem density		−0.001	−0.234	0.817

Standardized regression weights are expressed as path coefficients and critical ratio values (the estimate divided by the standard error) and *P* values also are shown for path significance.

found that elevation was negatively correlated with maximum temperature in the dry season, which in turn was negatively correlated with the density of infected *I. pacificus* nymphs. We did not find this same relationship in 2007. In addition, we found that maximum temperature was negatively correlated with the density of *I. pacificus* nymphs in 2007 but not 2006. These results indicate that abiotic limitations on *I. pacificus* vary from year to year and may be jointly controlled by direct and indirect factors. However, there were many factors not included in this analysis that may help explain tick density and infection prevalence such as biotic variables.

Biotic factors such as host density may buffer the direct effects of temperature and explain the poor predictive ability of elevation and temperature in certain years. Our forest structure variable was supposed to be an indirect measure of the biotic influence on tick hosts but was not a significant component of the path model. Thus, we did not find that forest structure was a reliable predictor of the influence of tick host density on ticks. The scale of our sampling (15 by 15 m) was unlikely to capture the influence of biotic factors such as host abundance and remains an undetermined component of our path analysis that may explain the inconsistent relationships we found between the 2 yr of our study. However, there has been limited empirical evidence of the relationship between tick host abundance on tick density (Killilea et al. 2008) and the density of ticks, and it was beyond the scope of this study to explore this relationship in detail, particularly across a large geographic area.

Table 3. Structural equation model results from 2007 showing path coefficients of all dependent variables and the influence of independent variables

Dependent variable and independent variable	r^2	Path coefficient	Critical ratio	*P*
Total stem density	0.035			
Elevation		−0.021	−1.164	0.250
Max temp (dry season)	0.472			
Elevation		−0.004	−4.53	<0.001
Total stem density		0.012	0.291	0.772
Density of nymphs (DON)	0.347			
Max temp		−0.168	−2.301	0.026
Total stem density		0.001	0.537	0.594
Density of infected nymphs (DIN)	0.010			
Max temp		−0.009	−0.178	0.860
Total stem density		−0.001	−0.443	0.659
Nymphal infection prevalence (NIP)	0.055			
Max temp		−0.010	−0.205	0.838
Total stem density		−0.003	−1.317	0.194

Standardized regression weights are expressed as path coefficients and critical ratio values (the estimate divided by the standard error) and *P* values also are shown for path significance.

Table 4. Results of SEM from 2006 showing direct effects, indirect effects, and effect coefficients of *I. pacificus* density and infection prevalence

Dependent and independent variables	Direct effect	Indirect effect	Effect coefficient
Stem density			
Elevation (2006)	−0.038	0	−0.02
Max temp (dry season)			
Elevation (2006)	−0.005	−0.01	−0.60
Tree stem density (2006)	0.015	0	0.28
Density of nymphs (DON)			
Elevation	0	$8.5e^{-4}$	$8.5e^{-4}$
Tree stem density	−0.002	−0.002	−0.004
Max temp	−0.139	0	−0.139
Density of infected nymphs (DIN)			
Elevation	0	$4.5e^{-4}$	$4.5e^{-4}$
Tree stem density	0	−0.001	−0.001
Max temp	−0.08	0	−0.08
Nymphal infection prevalence (NIP)			
Elevation	0	$-3.4e^{-5}$	$-3.4e^{-5}$
Tree stem density	−0.001	0.0002	0.0001
Max temp	0.013	0	0.013

Table 5. Results of SEM from 2007 showing direct effects, indirect effects, and effect coefficients of *I. pacificus* density and infection prevalence

Dependent and independent variables	Direct effect	Indirect effect	Effect coefficient
Stem density			
Elevation (2006)	−0.004	0	−0.0045
Max temp (dry season)			
Elevation (2006)	−0.005	0	−0.005
Tree stem density (2006)	0.012	0	0.012
Density of nymphs (DON)			
Elevation	0	0.001	0.001
Tree stem density	0.001	−0.002	−0.001
Max temp	−0.168	0	−0.168
Density of infected nymphs (DIN)			
Elevation	0	$1.7e^{-5}$	$1.7e^{-5}$
Tree stem density	0.001	0	0.001
Max temp	−0.009	0	−0.009
Nymphal infection prevalence (NIP)			
Elevation	0	$1.1e^{-4}$	$1.1e^{-4}$
Tree stem density	−0.003	0	−0.003
Max temp	−0.010	0	−0.010

It has been proposed that *I. pacificus* is limited by summer drought conditions (Padgett and Lane 2001). Here, we find evidence that in some years maximum temperature may be a limiting factor on the density of *I. pacificus* nymphs and the density of infected *I. pacificus* nymphs. These results are in opposition to a recent study in Mendocino, CA, that found a positive correlation between temperature and nymph density (Eisen et al. 2010). However, our study focused on one habitat type, oak woodlands, whereas Eisen et al. (2010) conducted their study over a broader geographic area and across many more habitats including redwood–tanoak forests that are generally cooler and damper than oak woodlands and are associated with lower densities of *I. pacificus* (Eisen et al. 2003). As such, the negative relationship we cite in this study focuses on the upper portion of the temperature gradient in the Eisen et al. (2010) study. Although it is likely that *I. pacificus* is associated with warmer temperatures across a broad spatial scale that includes many habitat types, when focusing on the upper end of this distribution, we found evidence that in some years, temperature can ultimately limit the density of ticks and the density of infected ticks. Thus, we find some evidence in support of the limiting effect of summer drought temperatures on *I. pacificus.*

This study did not find significant predictors of nymphal infection prevalence. The factors that determine the host community composition of *B. burgdorferi* reservoirs were probably not captured by the variables examined in this study. Vertebrates are probably responding more to stochastic factors or biotic factors that were not explicitly included in the model. Our path analysis was limited to three predictor variables because although path analysis can be powerful, it also requires high sample sizes. As such, we used multiple regression to reduce the forest structure variables down to the best fit parameter, stem density. The role of tick host density and species composition on tick density or infection prevalence is a complex issue that warrants further study but was beyond the scope of this study. Furthermore, it remains to be seen to what extent *I. pacificus* is limited by hosts for adults or juveniles (Killilea et al. 2008).

Our analysis accounted for nonindependence of our response variables in our path analysis. Therefore, our path coefficients are not inflated by spatial autocorrelation. Spatial autocorrelations in tick density has been reported in *I. scapularis* in the eastern United States (Nicholson and Mather 1996, Kitron and Kazmierczak 1997) and is probably a common issue in vector distribution data. Here, we found spatial autocorrelation in the topographic data, forest structure data, abiotic data, and the tick density data in 2006. Because of the extensiveness of spatial autocorrelation, our analysis removed spatial association from all of our data. As a result, the results presented here reflect the unbiased relationships between plot variables and tick variables. By accounting for spatial autocorrelation, we significantly reduced the value of path coefficients in our model. For example, in a path analysis that does not consider spatial autocorrelation, the path coefficient between elevation and maximum temperature in 2006 is −0.59 (data unpublished) instead of −0.005 (Table 2) and maximum temperature and density of infected nymphs in 2006 is −0.33 (data unpublished) as opposed to −0.08 (Table 2). Therefore, the small path coefficients that we report in this study reflect the incorporation of an important spatial correction that is particularly critical for spatial data and analyses but is often not taken into account in analyses.

Laboratory studies have shown that tick molting rate is maximized at temperatures of ≈21°C for *I. pacificus* (Padgett and Lane 2001) and 28°C for *I. scapularis* (Ogden et al. 2004). Despite the importance of temperature extremes in limiting *Ixodes* spp. growth and survival (Brownstein et al. 2003; Eisen et al. 2003, 2006), few field-based studies have found a significant relationship between abiotic factors (e.g., temperature and relative humidity) and density of *Ixodes* spp. (Killilea et al. 2008). Our path analysis revealed a statistically significant relationship between maximum temperature and the density of nymphs as well as the density of infected nymphs although these findings were not consistent between the 2 yr of this study (Figs. 3 and 4). This inconsistency suggests that in some years other stochastic factors may play a more important role in determining tick density and the density of infected ticks. It should be noted that due to logistical constraints, our sampling of ticks in 2006 and 2007 took place 3–4 wk apart. Some of the interannual differences that we cite could be due to this difference in timing. However, in both years, sampling took place within a 2–3-wk window of time, and thus the relative relationship of the plots within each year should be consistent.

Habitat variables were found to be indirectly tied to tick density and the density of infected ticks through maximum temperature. The indirect effect of elevation on tick density and the density of infected nymphs suggest that they should increase with elevation. More traditional regression analyses would not have found a significant relationship between elevation and ticks because they do not explicitly incorporate theses weaker, indirect pathways between parameters. But such indirect pathways are important in regulating microclimate variables that directly affect tick abundance and survival and warrant explicit examination. The indirect relationship between elevation and tick density and the density of infected tick suggest that in those years where ticks are limited by temperature, they may be able to escape these constraints at higher elevation where maximum temperature in the dry season is cooler. In a more general sense, the indirect effect of elevation on tick density is most likely context dependent. In a similar study, Bunnell et al. (2003) found that at lower elevations, elevation is positively correlated with adult *I. scapularis* density. However, at higher elevations, the correlation between elevation and tick density reverses and becomes negative (Bunnell et al. 2003). These results suggest that tick abundance is maximized at

intermediate elevations where temperature and relative humidity are optimal for tick growth and survival.

There is a great deal of natural heterogeneity in tick distribution, abundance, and infection prevalence with *B. burgdorferi.* Here, we attempt to minimize the background noise exhibited in tick density and infection prevalence data to determine the influence of topographic, abiotic, and biotic factors in a particular habitat type. We found that in oak woodland in some years, elevation may indirectly influence tick density and the density of infected ticks through its impacts on maximum temperature in the dry season. In other years, the unevaluated influence of stochastic factors such as the composition or density of tick hosts may be more important.

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