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Effects of Fire on Tick-borne Disease Ecology and Risk in Northern California

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Effects of Fire on Tick-borne Disease Ecology and Risk in Northern California

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Abstract

The current status of ticks and tick-borne diseases in California has been impacted by over a century of fire suppression and irruptions of catastrophic wildfires exacerbated by fire suppression and ongoing anthropogenic climate change. Important tick-borne diseases include Lyme disease caused by *Borrelia burgdorferi*, granulocytic anaplasmosis caused by *Anaplasma phagocytophilum*, and spotted fever rickettsioses caused by several rickettsial species. Their vectors in this ecological system are *Ixodes pacificus*, *Dermacentor occidentalis,* and *D. similis* ticks. Published research has focused typically on direct effects on tick populations immediately and shortly after fires. However, California fire management is transitioning from a regime of fire suppression to controlled burning, which will alter interactions among tick-borne pathogens, vectors, hosts, and those fire managers who conduct the burns, albeit in ways that are not yet fully understood. This dissertation comprises three chapters dedicated to exploring the indirect and long-term effects of fire on tick-borne diseases in California. Chapter 1 compares public awareness, knowledge, and preventive practices regarding tick-borne diseases with those of individuals engaged in fire-related forest management. Chapter 2 investigates the effects of ash on these tick species in the laboratory. Finally, Chapter 3 presents a predictive model analyzing longitudinal trends in nymphal *I. pacificus* abundance in a region affected by a severe wildfire, aiming to disentangle the effects of fire from broader population dynamics over time.

Individuals involved in managing forests to prevent wildfires, such as firefighters, foresters, inmate laborers, and indigenous burn crews, may be at heightened risk of acquiring tick-borne diseases. Blood samples from 55 forest workers and 58 members of the public near sites of documented elevated risk for *Ixodes pacificus*-transmitted pathogens were tested for the presence of bacterial DNA and antibodies for the three pathogens. Although more workers were positive for *B. burgdorferi* antibodies than the public (5.5% compared with 1.7%), the difference was not statistically significant. A questionnaire administered to 54 workers and 84 members of the public identified that tick bites were common (reported by 67.4% of participants) and that important gaps in knowledge and prevention practices exist including tick identification and safe removal practices, despite many workers having received training on tick-borne disease prevention. These findings underscore the risk of tick-borne diseases faced by fire management workers in tick-borne disease–endemic regions and highlight the need for enhanced training programs to minimize these risks.

The second chapter investigates the effects of ash produced from burning common California vegetation on the three tick species. Ticks may be exposed to ash when their wildlife hosts bathe in ash, after wildfires or controlled burns, and through the global ethnoveterinary practice of using ash to control ectoparasites. Effects on survival and behavior of ash from coast redwood, California bay laurel, and Tasmanian blue gum eucalyptus were compared to diatomaceous earth and kaolin clay (active controls), and a negative control. Bay and eucalyptus ash are lethal to adult *I. pacificus* and larval *D. occidentalis*, rivaling the tested active controls, while redwood ash influenced the ability of adult *I. pacificus* to orient. These findings provide an alternative management strategy to chemical acaricides and broaden our understanding of the effects of fire on tick-borne disease beyond direct impacts.

The final chapter addresses a critical gap in understanding the long-term effects of fire and other environmental dynamics on tick-borne diseases. Unlike studies that assess the impacts of fire in short intervals of time, this chapter considers longitudinal variations in tick abundance to understand the effects of wildfire and climatic variables such as drought on nymphal *I. pacificus*. Two long-term datasets documenting nymphal abundance and questing activity before and after a severe wildfire were analyzed. Nymphal tick activity exhibited significant year-to-year variation, with densities predicted by temperatures from early spring of the prior year, and the timing of questing predicted by late spring temperatures and relative humidity from the prior year. After adjusting for drought and climatic variables, the model was able to successfully predict that low nymphal abundances would occur in the years that ultimately followed a severe wildfire. Furthermore, the model helps quantify how much of the decrease in tick abundance that observed post-wildfire was likely due to the wildfire versus underlying climate conditions. This study underscores the necessity of considering long-term trends when evaluating the effects of fire on ticks and tick-borne diseases, and highlights limitations of short-term studies that may overlook long trends and other ecological factors.

Together the three studies advance our understanding of this monumental shift in the state’s fire policy on tick-borne disease. In particular, they can help inform those who conduct burns about their risk levels, where they have gaps in knowledge about prevention, and how specific features of their burn efforts can influence tick-borne disease.

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Chapter 2

Chapter 1

Prevalence and Knowledge of Tick-borne Disease Among Forest Management Workers in Santa Cruz, California

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Abstract.

In Lyme-endemic areas, limited research has investigated the risk of tick-borne diseases among frontline workers in fire management. This study aimed to compare the exposure histories to ticks and tick-borne pathogens, knowledge, and prevention practices between individuals engaged in fire-related forest management and those participating in recreational activities within Santa Cruz County, CA in an area of high risk of tick exposure.

Methods: Blood samples from 55 forest workers and 58 members of the public were tested for bacterial DNA of and antibodies to *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, and *Rickettsia* species. Additionally, a questionnaire was administered to 54 workers and 84 members of the public to identify gaps in knowledge and prevention practices.

Results: Although workers had a higher percentage of positive *B. burgdorferi* antibodies than the public (5.5% compared to 1.7%), the difference was not statistically significant. Conversely, rickettsial antibodies were more prevalent among the public (17.2% versus 3.6% for workers), but specificity to pathogenic bacteria could not be confirmed. No DNA for the three pathogens or antibodies against *Anaplasma phagocytophilum* were detected. Many workers and members of the public reported tick bites (67.4% of participants), with a notable 11% increase among workers in the odds of being bitten for each additional year spent working in forests. While workers took greater precautions, significant knowledge and practice gaps were identified among both populations, such as an inability to distinguish tick species from common arthropods (mites, spiders, fleas), overestimating the size of ticks, and inappropriate tick-removal techniques.

Conclusion: This study underscores the risk of tick-borne diseases faced by fire management workers in Lyme disease endemic regions. The findings emphasize the necessity for future studies of Lyme disease within this population, and highlight the urgent need for enhanced training programs to minimize these risks.

KEYWORDS: Fire, forest management, ticks, *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Rickettsia* species

Introduction

Individuals involved in managing forests to prevent wildfires, such as firefighters, foresters, inmate laborers, and indigenous burn crews, may be at elevated risk of tick-borne diseases. Studies indicate increased risk among individuals working in forests (Magnavita et al, 2022; Tokarska-Rodak et al, 2014; Zając et al, 2013) and higher rates of co-infections (De Keukeleire et al, 2018; Grzeszczuk et al, 2004; Kiewra et al, 2018; Rath et al, 1996). However, their awareness of prevention practices may be insufficient (Cisak et al, 2012; St. Pierre et al, 2020). These issues are understudied in California where under-served populations like inmate firefighters and indigenous burners are at the front lines of fire management.

The paucity of data on human exposure to tick-borne disease in California limits the ability of fire crews to understand risks they face. In California, the western black-legged tick, *Ixodes pacificus* transmits the bacterial agents of Lyme disease (*Borrelia burgdorferi*), and anaplasmosis (*Anaplasma phagocytophilum*). Dermacentor tick species such as *Dermacentor similis* (formerly *variabilis*, the American dog tick) and *D. occidentalis* (the Pacific Coast tick) transmit several species of spotted fever group rickettsiae such as the etiologic agents of Rocky Mountain spotted fever (*Rickettsia rickettsii*) and, by the latter tick, Pacific coast tick fever (*Rickettsia* sp. 364D). Seroprevalence studies of tick-borne diseases in California are few and have suggested low exposure in populations ranging from less than 1% to *B. burgdorferi* (Brummitt et al, 2020), to 3.7% of *Anaplasma phagocytophilum* (Fritz et al, 2005), and up to 6.8% against at least one of multiple tick-borne pathogens (Pascoe et al, 2019). In the specific region on the current study, 3.8% and 3.1% of ticks in the Soquel Demonstration State Forest have been found to carry *B. burgdorferi* and *A. phagocytophilum*, respectively (López-Pérez et al, 2021). However, the seroprevalence among forestry workers remains unquantified.

Here we sought to compare the presence of bacterial DNA and prevalence of antibodies to *B. burgdorferi*, *A. phagocytophilum*, and *Rickettsia* species among individuals engaged in fire-related forest management and individuals who recreate in Santa Cruz, CA forests. Additionally, we assessed knowledge among both groups to help identify gaps in knowledge and current practices.

Methods

Purposive sampling of whole blood and serum was conducted in Santa Cruz County, CA during winter of 2021 and spring of 2022 and 2023. Individuals working in fire management including members of CAL FIRE, California Conservation Corps (CCC), and the Amah Mutsun tribal band, were invited to participate via email and in person, and inmate workers were invited via the California Department of Corrections and Rehabilitation. Surveys were offered at local fire stations, Soquel Demonstration State Forest, and Wilder Ranch State Forest. The public was recruited in person, via flyers placed throughout the Soquel Demonstration State Forest (SDSF), and through SDSF and Santa Cruz Mountain Trail Stewardship's social media. SDSF was chosen due to previous studies confirming the presence of ticks and tick-borne disease. Following heavy storms between 2022-2023, Wilder Ranch State Forest was added when SDSF became inaccessible.

Consenting participants provided a 3mL blood sample. A *B. burgdorferi* VIsE-OspC ELISA IgG/IgM Test kit was used to test for *B. burgdorferi* antibodies (Gold Standard Diagnostics, Davis, CA). Per the manufacturer's protocol, samples were classified as positive, equivocal or negative. Equivocal and positive results for *B. burgdorferi* antibodies were grouped together. Remaining DNA and serum testing followed Pascoe et al. (2019). In brief, DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) and tested for pathogen DNA using real-time PCR (Barbour et al, 2009; Drazenovich et al, 2006; Stenos et al, 2005). Serum samples were tested by indirect immunofluorescence for *A. phagocytophilum* antibodies at a 1:50 dilution and *Rickettsia* species at a 1:64 dilution. Indeterminate results by indirect immunofluorescence were considered negative. We employed a highly sensitive screening method similar to Pascoe et al. (2019), focusing solely on the first step of the modified two-tier testing process for *B. burgdorferi* and using a low cut-off for immunofluorescence to improve sensitivity given the low expected prevalence of antibodies.

We conducted a questionnaire in English and in Spanish to assess knowledge of ticks, means of prevention, actions taken when bites occur, methods of removal, and self-reported bites (Supplementary Materials). Workers were asked their job, how long they had worked in forests, and whether they recalled training on tick-borne disease prevention. Jobs were categorized as: fire, forestry, CCC's, or office/other work. The study received approval from the UC Davis Institutional Review Board (protocol # 1609273-2).

Univariable analyses were conducted in R v4.1.2 on associations between working in forest management and risks, knowledge, and practices. All questions except number of preventative measures were dichotomized. Fisher's exact test (two-sided) was used to compare exposure to tick-borne diseases, bites, knowledge, and removal between populations. A comparison of the number of preventative measures taken on average among each group was analyzed using a Wilcoxon rank sum test with continuity correction. Associations between the length of employment with tick removal and number of preventative measures taken were evaluated visually, while the relationship to knowledge about ticks was evaluated using logistic regression.

Results

In total, 113 individuals consented to blood draws: 55 workers representing CAL FIRE and CCC's, and 58 members of the public. Twenty-six members of the public consented to the questionnaire but not blood sampling. One worker did not participate in the questionnaire. One participating worker was also a member of an indigenous burn crew in another state, but no members from the local indigenous burn crew nor inmate laborers participated. Participating workers reported a 5-year (2.5 months to 43 years) median time working in forests.

Seroprevalence of Tick-borne Diseases

Overall seroprevalence for the three tick-borne pathogens was 14.2% (Table 1). All DNA samples were PCR-negative for the three pathogens, seronegative for anaplasmosis, and there were no co-exposures. The Lyme seroprevalence was 3.54% including two strong positives in workers, and weak/near equivocal positives in one worker and a member of the public. Workers had 3.26 times higher odds of testing positive for antibodies against *B. burgdorferi*, however the association was not statistically significant (p-value = 0.36). Three of these four *Borrelia*-positive participants reported a tick bite; none saw a health care provider after being bitten. One seronegative member of CAL FIRE reported a previous Lyme disease diagnosis. Rickettsial antibodies were detected in 10.6% of participants (3.6% of workers and 17.2% of the public). An additional twelve samples were indeterminate. The odds of testing positive were lower for workers (OR=0.18 (0.02-0.92), p=0.03). Nine (75%) of the 12 with rickettsial antibodies reported a tick bite; four saw a doctor after being bitten.

Tick Bites and Medical Access

Tick bites were common (67.4%) and not statistically different between the two groups (OR=0.83 (0.38-1.83), p=0.7). Among those who had been bitten, 70% did not visit a doctor, often stating that they weren't worried (89.2%). Lack of health insurance, money, access, transportation, nor time were cited as reasons for not seeing a doctor. One member of the public stated that conditions during the COVID pandemic prevented them from seeking care.

Tick Identification

Identification skills did not differ significantly different between workers and the public (Table 2). Most (89.1%) participants identified an image of a female adult *D. occidentalis* as a tick, declining to 57.2% for an image of a female adult *I. pacificus*. However, most participants (76.1%) struggled to distinguish between these common tick species and other arthropods, either failing to identify at least one of the two tick species or incorrectly classifying a spider, mite, or flea as a tick. The latter three were identified as ticks by 10.9%, 38.4%, and 10.1% of participants, respectively.

Tick Size

Participants were presented with five ovals from 1-23 mm and asked to identify all that represent the size of a tick. Over half (64.5%) of participants correctly identified that ticks could be at least as small as 1 mm; however, 16.7% overestimated the size of a tick as >12 mm (Figure 1, Table 2). Workers tended to be slightly better at selecting appropriate upper ranges, with 50% lower odds of including >12 mm options compared to the public (p=0.2).

Tick Removal

Participants were presented with six possible ways to safely remove a tick and asked to select all that apply (Table 2). Grasping the tick with tweezers and pulling straight back was considered the optimal answer (CDC, 2022). Overall, knowledge of the proper way to safely remove a tick was lacking. While the correct answer was the most prevalent, only 64.5% of participants included it in their responses, and this issue was slightly more pronounced among workers (OR=0.55, 95% CI: 0.25-1.16, p=0.1). The second most common response was to grasp the tick with tweezers and twist; workers had 2.41(1.11-5.33) times the odds of choosing this compared to the public (p=0.02).

Prevention

Most participants (89.9%) reported taking some preventative action to avoid tick bites, most commonly checking oneself after being in the woods (69.6%), bathing/showering after being in the woods (64.5%), and wearing long sleeves/pants (60.9%, Table 3). Workers had a higher odds of reporting wearing long sleeves/pants (OR=3.47, 95% CI: 1.53- 8.30, p=0.001) and using insect repellent (OR= 3.62, 95% CI: 1.61-8.35, p<0.001). CAL FIRE and CCC uniforms have long sleeves and pants with a buckle around the heel that likely provide some protection. They also reported a median of 3.5 precautions compared to 2 among the public (Wilcoxon rank sum test statistic=1620, p=0.004). The number of preventative actions was not associated with recalling having been bitten (Wilcoxon rank sum test statistic=2255, p=0.50). There were noted discrepancies between preventative actions stated versus those observed during sampling, for example in clothing.

Training

Only 22 workers (41.5%) recalled training on how to prevent tick-borne diseases. No statistically significant associations were identified between recalling training and either level of knowledge about tick-borne disease or prevention practices (Table 4). Those that recalled training trended towards being better at identifying the image of *Ixodes pacificus* as a tick (1.63 (0.47-5.88), p=0.4) but worse at differentiating between images of ticks, spiders, mites, and fleas (0.55 (0.08-2.81), p=0.5). They also trended towards lower odds of correctly identifying the recommended way to remove a tick (OR=0.41 (0.09-1.58), p=0.2). They did, however, trend towards taking slightly more precautionary measures (median 4 vs 3, Wilcoxon rank sum test statistic=298, p=0.4). Training was not associated with reduction odds of reporting a tick bite (OR=0.96 (0.27-3.54), p=1).

Nuances among Workers

Individuals who worked in forestry most often gave the correct responses about ticks while members of the CCC's tended to demonstrate that they would benefit from additional training (Table 5). Tick bites were prevalent among all CAL FIRE occupational categories, with those involved in forestry management most likely to report being bitten (88.9%). The association was not analyzed statistically by job classification due to small sample size. Job length was statistically associated with tick identification and the odds of being bitten, with odds of being bitten increasing by 11% for each year of employment (OR=1.11, 1.02-1.22, p=0.01). The ability to correctly differentiate both tick species from the other three arthropods was slightly, albeit non-significantly, higher among employees that had worked in forests for a longer period (1.05, 0.99-1.11, p=0.08). The ability to identify that ticks could be at least as small as 1mm increased with more years spent working in forests (OR=1.07, 1.00-1.44, p= 0.05). No trends were detected between length of employment and tick removal, nor number of preventative measures taken.

Discussion

We present findings and analysis, from an area of documented Lyme disease risk, and regular tick and pathogen exposure despite a gap in knowledge, training, and practice to allow people to protect themselves while working and recreating. Santa Cruz County is considered Lyme-endemic with average annual rates at 4/100,000 (Sohi et al, 2021). We documented considerable Lyme disease seroprevalence (3.54%), in contrast to no seroprevalence reported in 2019 (Pascoe et al, 2019), but similar to results from the first tier C6 Elisa screening of blood donors in other regions of the state (3.76%) Mendocino, Napa, San Mateo, Santa Clara, Sonoma, Orange, San Luis Obispo, Solano, and Ventura (Brummitt et al, 2020). We documented more than three times higher odds of Lyme antibodies among workers compared to the public despite a small sample size, consistent with other regions and forest-related occupations for other tick-borne diseases (Magnavita et al, 2022; Tokarska-Rodak et al, 2014; Zając et al, 2013).

Rickettsial antibodies were unexpectedly high, especially among the public. Our detected seroprevalence of 10.6% was higher than the 3% documented in Humboldt County, CA (Pascoe et al, 2019; Stephenson et al, 2017). We used a low cut-off which increases the risk for false positives but is also more sensitive. A wide diversity of *Rickettsia* spp. is found in California, including but not limited to *R. rickettsii*, *Rickettsia* sp. 364D, *R. rhipicephali*, *R. felis*, and *R. typhi*. While some infections can be fatal, there are diagnostic challenges such as serological cross-reactivity that make surveillance and even diagnosis sometimes challenging. Additional research is still needed to identify which rickettsial pathogens may be affecting these populations. Rickettsia testing in ticks and improved serological testing may help identify to which rickettsial pathogens people in this area may be exposed.

There were gaps in knowledge and tick prevention practices both among those who work in forest management and the public. For instance, workers had over twice the odds of stating that twisting was a safe way to remove a tick, an approach that can sever tick mouthparts from its body which then remain embedded in the host, contributing to skin irritation or allergic symptoms (CDC, 2022). Participants' ability to identify the vector for Lyme disease and anaplasmosis, *I. pacificus*, was quite low. They were more familiar with *D. occidentalis*, perhaps because it is often found on dogs and is larger when engorged. Those who work in forest management did tend to take more precautions and had higher odds of reporting wearing long sleeves/pants as well as insect repellent compared to the public, and longer length of employment correlated with a better ability to identify ticks.

Nevertheless, we suggest that individuals conducting forest management work in this region would benefit from additional training and protection from tick-borne diseases, with over half of workers not recalling having received training on tick-borne disease and gaps in knowledge and practices. We acknowledge that low sample size and possible confounding due to the category of employment and length of time individuals were employed. CAL FIRE and CCC uniforms likely provide some protection against tick bites. Individuals whose work focused on forestry tended to have greater knowledge about ticks, perhaps due to greater exposure to ticks and some training. This suggests that for jobs that have a higher exposure, training may be more detailed or better retained. Active learning, effectiveness of types of training, and optimal frequencies of training reinforcement are all areas of important research. In this area, additional training efforts should be directed towards identification of *I. pacificus*, the size of subadult ticks that can serve as vectors of disease agents, proper tick removal, and preventative practices.

We had limited ability to identify where tick-borne disease exposure occurred and directionality of detected associations due to the cross-sectional design of the study. Individuals may have acquired antibodies or knowledge before beginning their employment or moving to the region. Sampling was impeded in summer and fall months by limited access to potential participants working on wildfires. Heavy rain and flooding during the early months of 2023 prevented sampling the following spring. The serological assays used in this study detect exposure but cannot confirm active infection and, in the case of *Rickettsia* spp., are not species-specific. The C6 ELISA used in this study is highly sensitive and specific, but the low abundance of *Borrelia* spp. DNA that circulates in human blood makes detection by PCR unlikely (Schutzer et al, 2019). Further reducing our ability to detect active infections, human antibodies to the C6 peptide of *B. burgdorferi* can wane within two years after infection (Brummitt et al, 2020). We used single-tier testing for antibodies against *B. burgdorferi*. This approach increases sensitivity for early infections and provides comparable sensitivity for later infections, with slightly lower specificity compared to two-tier testing (Wormser et al, 2013). We cannot discern where and when participants may have been exposed and thus the extent of occupational hazards. Participating members of the public may reflect some bias if they have reason to be particularly worried about or aware of tick-borne disease than the overall population. Finally, low sample size may have produced type 2 errors.

We documented frequent tick bites, exposure to *B. burgdorferi*, and insufficient protection among both individuals involved in managing forests to prevent wildfires and the public. By quantifying differences in exposure and knowledge between forest management workers and the public, this study helps increase our understanding of the direct public health consequences of the intersection between the shift in fire management in California and tick-borne diseases faced by the understudied communities on the frontlines of forest management. Although neither members of the indigenous nor inmate burn crews participated in the study, the documented risk and gaps in knowledge to prevent tick-borne diseases demonstrate the potential risk posed to these populations. Future participatory research with these communities is needed to understand the impact of shifting threats of tick-borne disease to those on the frontlines of forest management.

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REFERENCES

Barbour AG, Bunikis J, Travinsky B, et al. Niche partitioning of *Borrelia burgdorferi* and *Borrelia miyamotoi* in the same tick vector and mammalian reservoir species. *Am J Trop. Med Hyg* 2009;81(6):1120–1131; doi:10.4269/ajtmh.2009.09-0208.

Brummitt SI, Kjemtrup AM, Harvey DJ, et al. *Borrelia burgdorferi* and *Borrelia miyamotoi* seroprevalence in California blood donors. *PLoS One* 2020;15(12); doi: 10.1371/journal.pone.0243950.

Centers for Disease Control and Prevention. Tick removal. Atlanta, GA; 2022. Available from: https://www.cdc.gov/ticks/removing_a_tick.html [Last accessed: 1/18/24].

Cisak E, Zając V, Wójcik-Fatla A, et al. Risk of tick-borne diseases in various categories of employment among forestry workers in eastern Poland. *Ann Agric Environ Med* 2012;19(3):469–474.

De Keukeleire M, Robert A, Luyasu V, et al. Seroprevalence of *Borrelia burgdorferi* in Belgian forestry workers and associated risk factors. *Parasit Vectors* 2018;11:1-11; doi: 10.1186/s13071-018-2860-2.

Drazenovich N, Foley J, Brown RN. Use of real-time quantitative PCR targeting the msp2 protein gene to identify cryptic *Anaplasma phagocytophilum* infections in wildlife and domestic animals. *Vector Borne Zoonotic Dis* 2006;6(1):83–90; doi: 10.1089/vbz.2006.6.83.

Fritz CL, Bronson LR, Smith CR, et al. Clinical, epidemiologic, and environmental surveillance for ehrlichiosis and anaplasmosis in an endemic area of northern California. *J Vector Ecol* 2005;30(1):4-10.

Grzeszczuk A, Stańczak J, Kubica-Biernat B, et al. Human anaplasmosis in north-eastern Poland: seroprevalence in humans and prevalence in *Ixodes ricinus* ticks. *Ann Agric Environ Med* 2004;11(1):99–103.

Kiewra D, Szymanowski M, Zalewska G, et al. Seroprevalence of *Borrelia burgdorferi* in forest workers from inspectorates with different forest types in Lower Silesia, SW Poland: preliminary study. *Int J Environ Health Res* 2018;28(5):502–510; doi: 10.1080/09603123.2018.1489954.

López-Pérez AM, Plourde B, Smith K, et al. Impacts of timber harvest on communities of small mammals, ticks, and tick-borne pathogens in a high-risk landscape in Northern California. *J Med Entomol* 2021;58(3):1171–1187; doi: 10.1093/jme/tjaa297.

Magnavita N, Capitanelli I, Ilesanmi O, et al. Occupational Lyme disease: a systematic review and meta-analysis. *Diagnostics* 2022;12(2):296; doi: 10.3390/diagnostics12020296.

Pascoe EL, Stephenson N, Abigana A, et al. Human seroprevalence of tick-borne *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, and Rickettsia species in Northern California. *Vector-Borne Zoonotic Dis* 2019;19(12):871–878; doi: 10.1089/vbz.2019.2489.

Rath PM, Ibershoff B, Mohnhaupt A, et al. Seroprevalence of Lyme borreliosis in forestry workers from Brandenburg, Germany. *Eur J Clin Microbiol Infect Dis* 1996;15:372–377; doi: 10.1007/BF01690092.

Schutzer SE, Body BA, Boyle J, et al. Direct diagnostic tests for Lyme disease. *Clin Infect Dis* 2019;68(6):1052–1057; doi: 10.1093/cid/ciy614.

Sohi I, Djamba Y, Kjemtrup A, et al. Epidemiologic Summary of Lyme Disease in California, 2013-2019. *CDPH*; 2021. Available from: https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/LymeDiseaseEpiSummary2013-2019.pdf [Last accessed: 10/12/2023].

St. Pierre SE, Gould ON, Lloyd V. Knowledge and knowledge needs about Lyme disease among occupational and recreational users of the outdoors. *Int J Environ Res Public Health* 2020;17(1):355; doi: 10.3390/ijerph17010355.

Stenos J, Graves SR, Unsworth NB. A highly sensitive and specific real-time PCR assay for the detection of spotted fever and typhus group Rickettsiae. *Am J Trop Med Hyg* 2005;73(6):1083–1085.

Stephenson N, Blaney A, Clifford D, et al. Diversity of rickettsiae in a rural community in Northern California. *Ticks Tick-Borne Dis* 2017; 8(4):526–531; doi: 10.1016/j.ttbdis.2017.02.014.

Tokarska-Rodak M, Plewik D, Kozioł-Montewka M, et al. Risk of occupational infections caused by *Borrelia burgdorferi* among forestry workers and farmers. *Med Pr* 2014;65(1):109–117; doi: 10.13075/mp.5893.2014.017.

Wormser GP, Schriefer M, Aguero-Rosenfeld ME, et al. Single-tier testing with the C6 peptide ELISA kit compared with two-tier testing for Lyme disease. *Diagn Microbiol Infect Dis* 2013;75(1):9-15.

Zając V, Wójcik-Fatla A, Cisak E, et al. Study on tick-borne rickettsiae in eastern Poland. II. Serological response of the occupationally exposed populations. *Ann Agric Environ Med* 2013;20(2):280–282.

Table 1. Number of individuals that tested positive for antibodies (prevalence) to three tick-borne pathogens in Santa Cruz County, CA 2021-2023. All samples were PCR negative. Workers represent CAL FIRE and the California Conservation Corps and the public participants represents individuals who recreate in Santa Cruz, CA forests. None of the comparisons between the groups was statistically different.

Pathogen	**Workers (n=55)**	**Public (n=58)**
Positive for any pathogen	5 (9.1%)	11 (19.0%)
Anaplasma phagocytophilum	0 (0. 0%)	0 (0.0%)
Borrelia burgdorferi	3 (5.5%)	1 (1.7%)
***Rickettsia* spp.**	2 (3.6%)	10 (17.2%)

Table 2.

Ability to identify ticks to species and size and to identify best removal practices among those who work in forest management and the public in Santa Cruz County, CA 2021-2023. Manuscript text gives questions and options for answers. Workers represent CAL FIRE and the California Conservation Corps and the public represents individuals who recreate in Santa Cruz, CA forests.

Selected Answer	**Overall (n=138)**	**Workers (n=54)**	**Public (n=84)**	**OR (95% CI), p-value**
Identify Photo as a Tick (Select All that Apply)				
Ixodes pacificus	80 (58.0%)	31 (57.4%)	49 (58.3%)	0.96 (0.46-2.04), 1
Dermacentor occidentalis	123 (89.1%)	50 (92.6%)	75 (89.3%)	1.50 (0.38-7.01), 0.6
Only selected correct answer (both ticks and not spider, mite, or flea)	33 (23.9%)	11 (20.4%)	22 (26.2%)	0.71 (0.28-1.72), 0.5
Identify Size of a Tick (Select All that Apply)				
Included 1mm (approx. size of *I. pacificus* nymph)	89 (64.5%)	35 (64.8%)	54 (64.3%)	1.02 (0.47-2.24), 1
Included >12mm (overestimated upper range)	23 (16.7%)	6 (11.1%)	17 (20.2%)	0.50 (0.15-1.44), 0.2
Excluded <4mm (excluded appropriate lower range)	14 (10.1%)	6 (11.1%)	8 (9.5%)	1.19 (0.32-4.18), 0.8
Ways to Remove a Tick (Select All that Apply)				
Burn	21 (15.2%)	8 (14.8%)	13 (15.5%)	0.95 (0.32-2.70), 1
Apply nail polish	3 (2.17%)	1 (1.85%)	2 (2.38%)	0.78 (0.01-15.23), 1
Squish	3 (2.17%)	1 (1.85%)	2 (2.38%)	0.78 (0.01-15.23), 1
Grasp with tweezers & twist	47 (34.1%)	25 (46.3%)	22 (26.2%)	2.41 (1.11-5.33), 0.02

Grasp with tweezers & pull straight back	89 (64.5%)	31 (57.4%)	58 (69.0%)	0.61 (0.28-1.31), 0.2
Smother with oil or Vaseline	17 (12.3%)	7 (13.0%)	10 (11.9%)	1.10 (0.33-3.47), 1
Only correct answer (grasp with tweezers & pull straight back)	61 (37%)	19 (35.2%)	42 (50.0%)	0.55 (0.25-1.16), 0.1

Table 3: Responses to the question: "Do you try to prevent tick bites with any of the following methods?" among forest management workers and the public in Santa Cruz County, CA 2021-2023. Workers represent CAL FIRE and the California Conservation Corps and the public represents individuals who recreate in Santa Cruz, CA forests.

Selected Answer	**Overall (n=138)**	**Workers (n=54)**	**Public (n=84)**	**OR (95% CI)**
Wear long sleeves/pants	84 (60.9%)	42 (77.8%)	42 (50.0%)	3.47 (1.53- 8.30)
Tuck pants in socks	33 (23.9%)	15 (27.8%)	18 (21.4%)	1.41 (0.59-3.34)
Insect repellent	43 (31.2%)	26 (48.1%)	17 (20.2%)	3.62 (1.61-8.35)
Wear light-colored clothing	28 (20.3%)	14 (25.9%)	14 (16.7%)	1.74 (0.69-4.40)
Check oneself	96 (69.6%)	41 (75.9%)	55 (65.5%)	1.66 (0.73-3.93)
Bathing/showering	89 (64.5%)	38 (70.4%)	51 (60.7%)	1.53 (0.70-3.44)
None	14 (10.1%)	4 (7.4%)	10 (11.9%)	0.59 (0.13-2.21)

Table 4: Responses to knowledge and practice questions among forest management workers comparing those who stated yes versus no to the question: “Do you recall receiving training at your current employment on how to prevent tick-borne diseases”. Workers represent CAL FIRE and the California Conservation Corps and the public represents individuals who recreate in Santa Cruz, CA forests.

Selected Answer	**All (n=53*)**	**Recalled (n=22)**	**Did not recall (n=31)**	**OR (95% CI)**
<u>Identify Photo as a Tick (Select All that Apply)</u>				
Ixodes pacificus	30 (56.6%)	14 (63.6%)	16 (51.6%)	1.63 (0.47-5.88)
Dermacentor occidentalis	49 (92.5%)	20 (90.9%)	29 (93.5%)	0.70 (0.05-10.32)
Only correct answers	10 (18.9%)	3 (13.6%)	7 (22.6%)	0.55 (0.08-2.81)
(both ticks and not spider, mite, or flea)				
<u>Identify Size of a Tick (Select All that Apply)</u>				
Included 1mm	34 (64.2.%)	16 (72.7%)	18 (58.1%)	1.9 (0.52-7.64)
(approx. size of *I. pacificus* nymph)				
Included >12mm	3 (5.7%)	2 (9.1%)	1 (3.2%)	2.94 (0.14-182.55)
(overestimated upper range)				
Excluded <4mm	6 (11.3%)	2 (9.1%)	4 (12.9%)	0.68 (0.06-5.30)
(excluded appropriate lower range)				
<u>Ways to Remove a Tick (Select All that Apply)</u>				
Only correct answer	18 (34.0%)	5 (22.7%)	13 (41.9%)	0.41 (0.09-1.58)
(grasp with tweezers & pull straight back)				

**one worker was not eligible to receive training due to their role and thus was excluded from this section of the analysis.*

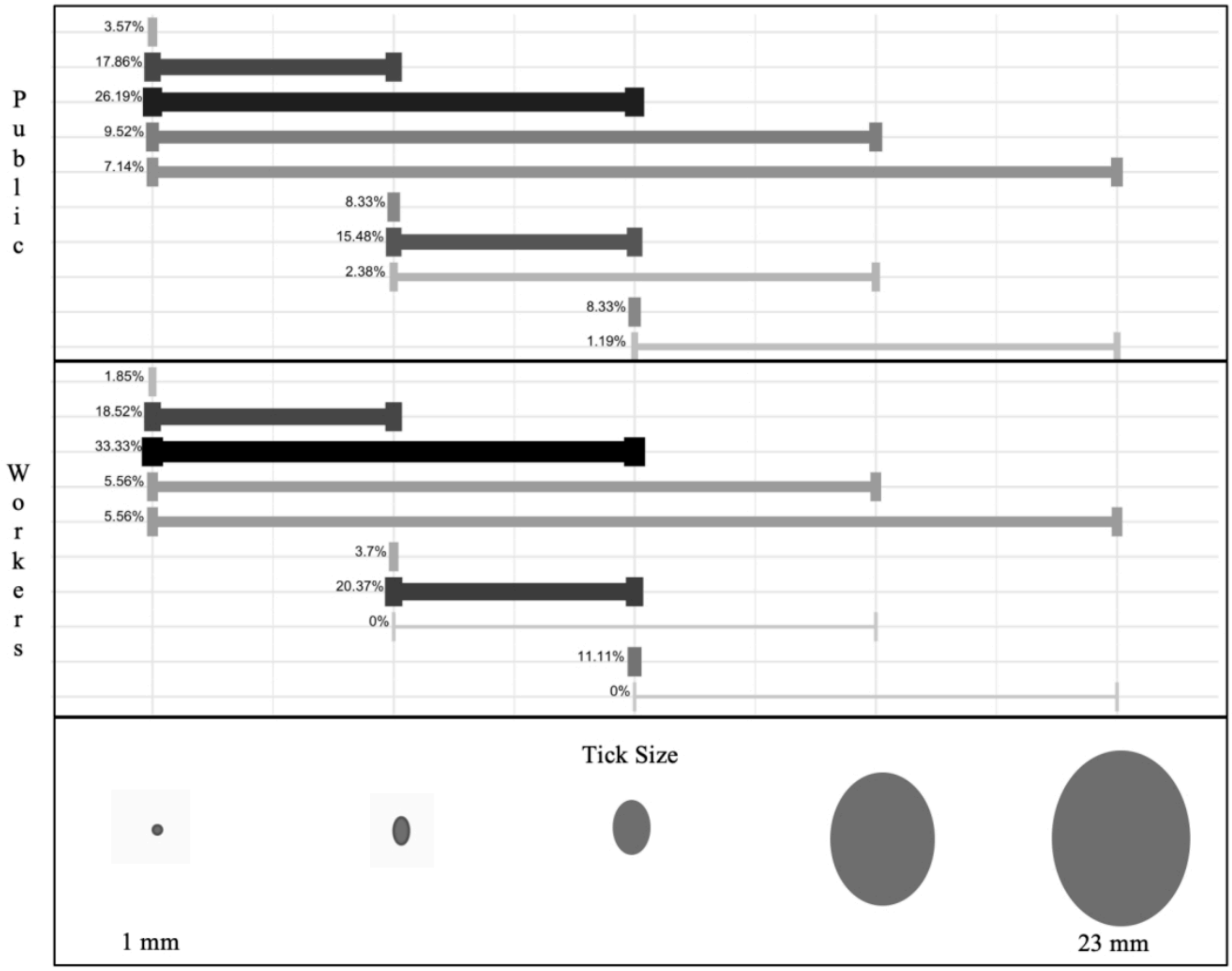
Table 5: Responses to knowledge and practice questions among forest management workers comparing across employment groups, categorized as CCC for members of the California Conservation Corps, fire-related, forestry-related, and office/other. "Other" included emergency medical response and apparatus engineers. Workers represent CAL FIRE and the California Conservation Corps and the public represents individuals who recreate in Santa Cruz, CA forests.

Selected Answer	**CCC (n=15)**	**Fire (n=25)**	**Forestry (n=9)**	**Office/other (n=6)**
<u>Identify Photo as a Tick (Select All that Apply)</u>				
Ixodes pacificus	7 (46.7%)	15 (60.0%)	7 (77.8%)	3 (50.0%)
Dermacentor occidentalis	13 (86.7%)	24 (96.0%)	9 (100%)	5 (83.3%)
Only selected correct answer (both ticks and not spider, mite, or flea)	2 (13.3%)	5 (20.0%)	3 (33.3%)	1 (16.7)
<u>Identify Size of a Tick (Select All that Apply)</u>				
Included 1mm	6 (40.0%)	17 (68.0%)	9 (100%)	4 (66.7%)
(approx. size of *I. pacificus* nymph)				
Included >12mm	2 (13.3%)	3 (12.9%)	2 (22.2%)	0 (0.0%)
(overestimated upper range)				
Excluded <4mm	4 (26.7%)	2 (8.0%)	0 (0.0%)	0 (0.0%)
(excluded appropriate lower range)				
<u>Ways to Remove a Tick (Select All that Apply)</u>				
Selected only grasp with tweezers & pull straight back	3 (20.0%)	8 (32.0%)	6 (66.7%)	3 (50.0%)
Reported being bitten by a tick				

Yes	6 (40.0%)	17 (68.0%)	8 (88.9%)	5 (83.3%)
Recalled receiving training at current employment on tick-borne disease prevention				
Yes*	7 (46.7%)	11 (44.0%)	3 (37.5%)	1 (20.0%)

*Two participants, one classified as office and as forestry-related, did not respond to the training question

Figure 1: Percentage of workers and the public in Santa Cruz County, CA 2021-2023 who identified each selected range in response to the question: “What is the size of a tick (check all that apply). The presented sizes ranged in diameter from 1 mm-23 mm on the long axis and are shown true to size. Workers represent CAL FIRE and the California Conservation Corps and the public represents individuals who recreate in Santa Cruz, CA forests.



Supplementary Materials.

SDSF Tick-Borne Disease Study Questionnaire

This survey is completely anonymous. Your answers will help us assess needs. Thank you!

1. Do you use the Soquel Demonstration Forest recreationally or occupationally?

    a. If occupational: what is your job?
        i. How long have you worked in jobs located in forests?
        ii. Do you recall receiving training at your current employment on how to prevent tick-borne diseases?

    b. If recreational, circle how frequently: daily, weekly, monthly or yearly

2. Do you identify as any of the following?
    a. Member of a Native American forestry management program
    b. An inmate

3.
    a. Which of the following do you feel confident in identifying as a tick (circle all that apply)?


A.


B.


C.


D.

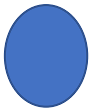

E.

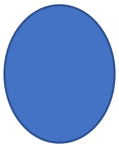
    b. What is the size of a tick (check all that apply)


A.


B.


C.


D.


E.

4. What are ways to safely remove a tick (circle all that apply)?
    a. Burn it
    b. Apply nail polish to the tick
    c. Squish it
    d. Grasp it with tweezers and twist
    e. Grasp it with tweezers and pull straight back
    f. Smother it with an oil or Vaseline

5. Have you ever been bitten by a tick?

   Yes / No / Unsure

   a. If yes, were you in the Soquel Demonstration Forest either the day or two days before you were bit?
      i. Yes
      ii. No
      iii. Not sure

6. Do you try to prevent tick bites with any of the following methods? (circle all that apply)
   a. Wear long sleeves and pants
   b. Tuck pants into socks
   c. Use insect repellent
   d. Wear light colored clothing
   e. Check yourself after being in the woods
   f. Bathe/shower after being in the woods
   g. Other, please explain:
   h. None of the above

7. Has a doctor ever told you that you have any of the following (circle all that apply)?
   a. Anaplasmosis/granulocytic anaplasmosis
   b. Lyme disease
   c. Spotted fever or Rickettsiosis
   d. Ehrlichiosis
   e. None of the above

8. After a tick bite have you ever seen a doctor?
   a. If not, why? Please check all that apply
      i. Not worried
      ii. Not sure what to do
      iii. Did not have health insurance/money
      iv. Couldn't get time off of work
      v. No access to a doctor
      vi. No access to transportation to see a doctor
      vii. Other:

Chapter 2

Effects of ash from different tree species on survival, orientation, and host-seeking of ticks

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Abstract.

Ticks are exposed to ash from wildlife hosts of tickborne diseases that bathe in ash, after wildfires or controlled burns, and through the global ethnoveterinary practice of using ash to control ectoparasites. Our study compared the effects of ash produced from Coast redwood, California bay laurel, and Tasmanian blue gum eucalyptus to diatomaceous earth, kaolin clay, and a negative control on survival and behavior of *Ixodes pacificus*, *Dermacentor occidentalis, D. similis, and D. albipictus*. We demonstrated that bay and eucalyptus ash are lethal to adult *I. pacificus* and larval *D. occidentalis*, rivaling some commercially available acaricidal dusts, while redwood ash influenced the tick's ability to orient and exhibited lethal effects on *D. albipictus* larvae. *Dermacentor* species showed less susceptibility to ash, with larvae more affected than adults. No preference was detected for hosts that did or did not bathe in ash. Our findings suggest that both the type of ash and the tick species and stage are important factors determining the effects of ash on ticks. This is the first study to show the effects of plant-produced ash on ticks, which may offer an alternative management strategy and broaden our understanding of the effects of fire on tickborne disease.

Introduction:

While ticks are among the most important vectors of human and non-human animal disease agents worldwide, numerous aspects of their ecology remain under studied. Research on influences from fire on ticks is still in its infancy, primarily focusing on the direct impacts of fire on ticks and their hosts (Gleim et al., 2019; MacDonald et al., 2018; Pascoe et al., 2020). Yet beyond directly killing ticks, removing substrate for molting, and eradicating hosts, fire leaves behind chemical and physical constituents of the incinerated material which can impact tick biology even after the fire is resolved.

Whether fire ash derives from wildfire or controlled burning, there are several reasons to expect it to impact ticks and their interactions with hosts. Many important hosts for tick-borne disease agents, such as birds, small mammals, and deer, engage in dust, mud, and ash baths, hypothetically to manage ectoparasites (Bracke, 2011; Clayton et al., 2010; Eisenberg, 1963; Mooring & Samuel, 1998; Oaster, 2020). Since at least the 2nd millennium B.C.E., civilizations worldwide have leveraged this common phenomenon to manage arthropod pests on domestic animals (Hakbijl, 2002). Indeed, ethnoveterinary surveys examining ectoparasite control methods frequently cite the use of ash (Banjo et al., 2009; Gabanakgosi et al., 2012; Hakbijl, 2002; Moyo et al., 2015; Wanzala, 2009; Wanzala, 2017). While the effects of some manufactured dusts have been explored on tick species including *Amblyomma americanum* (lone star tick) (Showler, Dorsey, et al., 2020; Showler, Flores, et al., 2020), *Ixodes scapularis* (black-legged tick) (Richardson et al., 2022), *Rhipicephalus sanguineus* (brown dog tick), and *Otobius megnini* (spinose ear tick) (Showler & Saelao, 2022), no studies have analyzed the effects of ash from wildfire or controlled burning on tick vectors of zoonotic agents.

Ash may influence ticks in multiple ways, including by impairing survival and changing behavior. Arthropods are lethally susceptible to some small particle-size inert dusts with abrasive and adsorptive qualities which induce lethal desiccation by damaging arthropods' protective epicuticular lipid layer (Tarshis, 1961; Wagner & Ebeling, 1959). Behavior appears to be affected as well; insects exposed to volcanic ash appear disoriented, followed by a decrease in spontaneous activity and responsiveness (Edwards & Schwartz, 1981). Fine ash particles absorbing host oils or masking host odors and CO_2 could also influence host-tick interactions.

This study sought to quantify the effects of ash on tick survival, ability to orient, and host-seeking. Specifically, we evaluated ash from three common trees in California (*Sequoia sempervirens* (coast redwood), *Umbellularia californica* (California bay laurel), and Tasmanian *Eucalyptus globulus* (blue gum)) on the common tick species *Ixodes pacificus* (western black-legged tick), *Dermacentor occidentalis* (Pacific Coast tick), *D. similis* (western American dog tick), and *D. albipictus* (winter tick). Ticks were exposed to one of these three types of ash, two active controls with documented acaricidal properties (diatomaceous earth and kaolin clay) (Showler & Saelao, 2022), or a negative control and observed for time to death and time to orient when placed upside down. We hypothesized that ash exposures would reduce survival times and lengthen time to orient as effectively as the two active controls. Additionally, we compared larval preference for hosts exposed to ash versus those exposed to a negative control. We expected larval ticks to prefer hosts that hadn't been exposed to ash. Validation of these predictions would not only support the ethnoveterinary use of ash for tick control but also elucidate other ways fire influences tick-borne disease.

Materials and methods

Ticks

The inclusion of ticks and stages was based on availability. Adult *I. pacificus, D. similis,* and *D. occidentalis* ticks were collected from field sites in Alameda and Santa Cruz Counties in California and maintained until experimentation at 2-4°C and >80% relative humidity. Larval *I. pacificus* were provided by BEI Resources, Centers for Disease Control and Prevention. *Dermacentor albipictus* from Tulare County and *D. similis* larvae from Santa Cruz County were acquired opportunistically from engorged adults and maintained at room temperature with >80% relative humidity until eclosion of ticks was observed; larval ticks were then maintained until experimentation at 2-4°C.

Treatment

Three ash exposures were selected for analysis based on tree genera with possible acaricidal properties common in California where *I. pacificus, D. similis and D. occidentalis* are found including coast redwood, California bay laurel, and Tasmanian blue gum (Adenubi et al., 2021; Clemente et al., 2010; Kim et al., 2003; Panella et al., 1997). Wood was collected from field sites in Alameda and Santa Cruz Counties, burned, and filtered twice through 500-micron sieves. We compared these products with two active controls with documented acaricidal properties: diatomaceous earth and kaolin clay. As a negative control, ticks and hosts were subjected to the same procedures as active controls and ash treatments but without a substrate. For survival and orientation experiments, to mimic the effects of dust-bathing, adult and larval ticks were rolled twice in their respective exposure material inside a petri dish. Negative controls were rolled twice against the petri dish.

Survival

A minimum sample size of 10 ticks for all combinations of each treatment, sex, and species was calculated based on accelerated failure time (AFT) analysis with an alpha of 0.05, 80% power, and an effect size of 2.5. A change in median survival from 10 to 2 days was chosen in order to assess whether ash has proactive effects, killing ticks within the period of time needed before pathogens are likely to be transmitted from the tick to a host (Eisen, 2018; Levin et al., 2020; Levin et al., 2021; Peavey & Lane, 1995). In all analyses, results from adult *D. occidentalis* and *D. similis* were grouped due to low male *D. similis* availability in the field.

For survival experiments, we placed adult and larval ticks in plastic tubes with mesh coverings allowing for temperature and humidity regulation. The tick tubes were then placed in sealed plastic tubs with humidity monitors and moisture. We observed daily whether the ticks were alive or dead for up to 11 days for adults and up to 5 days for larval ticks at 95% relative humidity and at 25° C with artificial overhead light provided from 9am-5pm.

Orientation

To measure physiological impacts of exposure before death that would impact fitness, adult ticks were placed upside down in a petri dish and observed for their ability orient themselves. The time until ticks righted themselves was observed. Ticks that failed to right themselves after 40 minutes were right-censored from the analysis.

Host Attraction

Attractiveness was defined as the number of ticks that approached a CD-1 lab mouse. To simulate ash bathing, ash was massaged into the fur of exposed mice with gloved hands, while negative control mice were massaged simply with gloved hands.

A repeated study design was used whereby a control mouse (A) was placed on one side of the set-up and a mouse exposed to ash (B) was placed on the other side. Trials were repeated before and after treatment. To reduce possible negative health effects on the mice due to the two active control exposures, only the effects of ash versus the negative control were compared. Ten mL of ash were applied to each of the twenty-four mice that were randomly assigned to each exposure. To reduce the total number of mice needed, the control mice (n=6) were each paired with an exposed mouse from each ash type (n=18). Mice were matched by sex within each comparison (i.e. a male control mouse was used for a male exposed mouse). A mouse assigned to an ash exposure was placed in a cage opposite the control mouse (Supplementary Figure 1). Ten larval *I. pacificus* ticks (for a total of 360 ticks) were placed using a paintbrush equidistant from both mice inside of a clear tube at the entry point. Fans pushed air at equal speeds from the mice towards the ticks to promote air flow from the host towards the tick. The tube was then sealed such that air flow was only available from the fans behind each mouse cage. The ten ticks were observed for 10 minutes. We counted the number of ticks that walked halfway from the entry point towards each cage. To account for inter-subject variability in attractiveness, a parallel study design was executed whereby attractiveness was tested for each mouse before and after exposure using the same ticks. In addition, to account for possible stimuli in the room, each trial was repeated, with the location of the mice switched. Thus, a total of four trials per exposed mouse was run. This research was approved by the UC Davis IACUC (Protocol #23210).

Statistical Analysis

Differences between treatments for orientation and survival were visualized using Kaplan-Meier survival curves. Analyses for survival were stratified by species and stage to account for differences in both the effects of treatment and baseline survival. Survival among different treatment groups exhibited a strong lack of proportionality. Thus, AFT models were used to quantify the decline in survival time associated with different treatments. Values are presented as how much faster death occurs among the treatment relative to the average rate among the negative controls. A series of AFT models were fitted under different distributions including: the log normal, log logistic, and Weibull distributions. Graphical analysis and Akaike information criteria indicated that the log logistic distribution best fit the data. The Gehan-Breslow-Wilcoxon test with Bonferroni's correction for multiple comparisons was used to compare the different effects of treatment, because it gives more weight to early events (Hazra & Gogtay, 2017), and we were particularly interested in whether ash successfully killed ticks within the first 24- and 48-hours, a time window when disease transmission risk is greatest (Eisen, 2018). In addition, Fisher’s exact test (2-tailed) was used to capture effects specifically within the first 24- and 48-hours after treatment.

The coxme and coxph packages for survival in R were used to quantify the effect of each treatment on orientation (Therneau, 2015a, 2015b). A random effect for each individual trial was used to account for possible environmental differences that may have occurred from the collection site, while ticks were maintained prior to experimentation, or during the experiment. No variation between trials was detected, and the random effect was dropped. Analyses were stratified by species and age to account for significant differences in both the effects of treatment and baseline hazards. Schoenfeld residuals and visual inspection were used to detect the varying effects of treatments over time. When such effects were detected, Cox models were fitted piecewise by dividing the follow-up period into intervals based on where the proportional hazards assumption was met.

Attraction was analyzed using mixed univariable logistic regression. The outcome was whether the tick preferred the control mouse (A) over the exposed mouse (B). A nested random effect was added to account for repeated measures from each treated and control mouse. However, model convergence could not be achieved with the nested random effect structure. As variance was only observed at the control mouse level, a random effect was only used to account for repeated measures from the control mouse.

Results

Survival

Female adult I. pacificus *survival:*

We examined changes in survival over the first 24 hours, 2 days, and 11 days (Figure 1). Within the first 24 hours, only a small fraction (3%) of ticks exposed to either the negative control or redwood ash died. In contrast, eucalyptus ash (39%), bay ash (39%), and the two active controls (58% for diatomaceous earth, 38% for kaolin clay) were all effective at killing ticks

(Supplementary Table 1) and showed equivalent effects comparatively ($p>0.05$, Supplementary Table 2).

Effects of bay, eucalyptus, and the active controls remained significant over 48 hours relative to the redwood and the negative control ($p>0.05$, Supplementary Table 1), with the percent of ticks killed by diatomaceous earth (95%) and kaolin clay (88%) only slightly higher than bay (85%) and eucalyptus (73%). Over the entire 11-day period, bay ash and eucalyptus ash demonstrated equivalent efficacy to kaolin clay and diatomaceous earth in killing female *I. pacificus* ticks while redwood ash remained ineffective, based on results from the Gehan-Breslow-Wilcoxon test (Supplementary Table 3). The AFT model (Table 1a) showed the largest effect on females was observed for diatomaceous earth (acceleration factor=7.66, $p<0.001$), followed by kaolin clay (acceleration factor=6.37, $p<0.001$), bay ash (acceleration factor=6.20, $p<0.001$), and eucalyptus ash (acceleration factor=5.46, $p<0.001$).

There was relatively high baseline mortality (76% after 11 days) among adult male *I. pacificus* negative controls (Figure 2). By 48 hours, eucalyptus (85%) and bay ash (82%) were successful at killing more ticks than the negative control (55%). The effects of bay and eucalyptus ash were comparable to both active controls, and all four remained more effective than redwood ash (Supplementary Tables 4-6). The AFT model (Table 1a) showed the largest effect on males was observed for diatomaceous earth (acceleration factor=1.95, $p=0.002$), followed by kaolin clay (acceleration factor=1.89, $p=0.005$), eucalyptus ash (acceleration factor=1.72, $p=0.006$), and bay ash (acceleration factor=1.67, $p=0.009$).

Adult Dermacentor occidentalis *and* Dermacentor similis *tick survival:*

None of the treatments were particularly effective at killing adult *D. occidentalis* and *D. similis* ticks (Figure 3) with only one male adult exposed to diatomaceous earth dying within the first 48 hours. Over ten days, none of the ticks exposed to eucalyptus or redwood ash died, and only two exposed to bay ash died. Given the low mortality rate, analysis could not be stratified by sex and data were not analyzed using a Fisher's Exact test. The results from the Gehan-Breslow-Wilcoxon test (Supplementary Table 7) and AFT showed lethal effects over the 11 days only from kaolin clay (acceleration factor SE: 4.25, p=0.02) compared to the negative control (Table 1b).

Larval Dermacentor *tick survival:*

All treatments, including redwood ash, effectively killed larval *D. occidentalis* ticks over the 5 days (Figure 4). Within the first 24 hours (Supplementary Table 8), redwood (28% mortality, p<0.001) and eucalyptus ash (20% mortality, p=0.002) were both effective at killing larvae compared to the negative control (7%), while bay ash was not (10%, p=0.43). By 48 hours, all treatments were significantly effective compared to the negative control. However, all three ash treatments were less effective than either active control in the first 24 and 48 hours (Supplementary Table 9), with drastically lower odds of mortality ranging from 0.01-0.07. The Gehan-Breslow-Wilcoxon test (Supplementary Table 10) and AFT model (Table 1b) showed that these effects persisted throughout the 5 days, with all three ashes showing efficacy, but less so than either active control.

Within the first 24 hours (Figure 5, Supplementary Table 11), only kaolin clay was effective at killing larval *D. albipictus* (36% mortality, p <0.001). By 48 hours, redwood ash (42% mortality) and diatomaceous earth (30% mortality) also become equally effective (p for both compared to negative control <0.001), although less so than kaolin clay (71% mortality, Supplementary Table 12). The Gehan-Breslow-Wilcoxon test (Supplementary Table 13) revealed that redwood ash and kaolin clay were the only effective treatments; they remained equally effective over the five days. Surprisingly, while early effects were observed from diatomaceous earth, overall, it was ineffective against larval *D. albipictus*. The AFT model (Table 1c) suggested that Kaolin clay led to mortality fastest (acceleration factor=2.76, p <0.001) followed by redwood ash (acceleration factor= 1.90, p<0.001) compared to the negative control.

Orientation

Orientation tests were performed on 497 ticks: 194 *Dermacentor* spp. ticks (73 female, 121 male) and 303 *I. pacificus* (167 female, 136 male). In brief, *I. pacificus* ticks were affected by the ash treatments while the *Dermacentor* spp. were not. Effects of treatment were time-dependent, with most ticks that ultimately righted themselves doing so within the first 12 minutes (Figure 6). Statistical analyses were therefore limited to the first 12 minutes (720 seconds) when proportional effects were observed.

Female *I. pacificus* (Figure 6a) righted themselves more slowly when exposed to redwood ash (HR= 2.08, 95% CI: 1.58-2.74, p=0.01), eucalyptus ash (HR=1.91, 95% CI: 1.45-2.52, p= 0.02), and bay ash (HR=1.86, 1.42-2.44) relative to negative controls during the first 12 minutes, but the effect of active controls was greater (HR kaolin clay vs negative control= 3.59, 95% CI:

2.44-5.27, p<0.001; diatomaceous earth vs negative control=2.48, 95% CI: 1.78-3.47, p=0.01). Strong effects of all treatments were observed among male *I. pacificus* (Figure 6b) compared to negative controls, with redwood ash (HR= 5.22, 95% CI: 3.74-7.29, p<0.001) outperforming eucalyptus (HR= 3.85, 2.82-5.26, p<0.001), kaolin clay (HR= 3.64, 95% CI: 2.53-5.25, p<0.001), and bay ash (HR= 3.41, 95% CI: 2.52-4.60, p<0.001). However, diatomaceous earth was the most effective at slowing male *I. pacificus* from righting (HR= 10.19, 95% CI: 6.42-16.19, p<0.001).

The only statistically significant slower time to right among *Dermacentor* spp. ticks was observed for those exposed to diatomaceous earth (HR= 2.64, 95% CI: 1.73-4.03, p=0.02, Figure 6c*)*. None of the treatments statistically slowed female *Dermacentor* ticks (Figure 6d), and bay ash was associated with a faster time to right compared to negative controls (HR= 0.36, 95% CI: 0.23-0.56, p=0.02).

Attraction

Attraction was measured using repeated sampling 727 times with larval *I. pacificus* ticks tested before and after mice were exposed to ash. The majority (n=169) of ticks showed no preference for either mouse before (n=169) or after exposure (n=174), remaining at the point of entry in the middle of the tube. The number of ticks that chose the exposed mouse was similar before (89) and after (90) exposure. After controlling for the repeated sampling, no effect on attraction was observed for any ash exposure (Supplementary Table 13).

Discussion

Survival and activity of ixodid ticks, including those that transmit human and zoonotic disease agents, are heavily influenced by environmental parameters including temperature, humidity, substrate particle structure, flora, fauna, and microbiome (Needham & Teel, 1991). With the rich ethnoveterinary history of using ash for parasite control and the increasing severity of wildfires from human-caused climate change, we sought to evaluate whether wood ash from three species of trees in California plant communities where ticks abound would influence tick survival, behavior, and host attraction. In general, eucalyptus and bay ash tended to have a more significant impact on ticks than redwood ash. However, there were dramatic differences across tick genera in the impacts of ash on survival and behavior, with *Ixodes* ticks being far more vulnerable to ash than *Dermacentor* ticks.

Many pathogens won't be transmitted to a host until after a tick has been attached for 24-48 hours, including the bacteria that cause rickettsioses transmitted by *D. occidentalis* and *D. similis* and the bacteria that cause Lyme disease and anaplasmosis transmitted by *I. pacificus* (Eisen, 2018; Levin et al., 2020; Levin et al., 2021; Peavey & Lane, 1995). In this key period before transmission would occur, bay and eucalyptus ash both reduced the survival of *I. pacificus* adults to levels comparable to impacts from recognized active controls, killing nearly 50% of male and female *I. pacificus* during the first 24 hours, and rising to over 75% by 48 hours. Redwood ash decreased male survival more slowly, with exposed ticks surviving an average of 3 days. Survival among male *I. pacificus* may have been particularly low since the ratio of their bodily surface area to volume is higher than those of the larger adult females.

Wood ash did not reduce the survival of either adult female or male *Dermacentor* spp. ticks. These species tolerate lower humidity than *I. pacificus*, which may explain the low mortality among active controls if the main mechanism for ash to induce mortality is dehydration (Estrada-Peña et al., 2013; Yoder et al., 2012). In contrast, however, diatomaceous earth and kaolin clay do induce mortality within 24 hours in other drought-tolerant species including *Amblyomma americanum* (Showler & Harlien, 2023b) and *Rhipicephalus microplus* (Showler & Harlien, 2023a). It is possible that mortality would be greater at relative humidities that were lower than used in our study or during regimes of fluctuating conditions. The critical equilibrium humidity above which ticks can actively absorb water and survive varies among species and likely confounded effects of ash on survival (Estrada-Peña et al., 2013). Indeed, even the effects of chemical acaricides like DDT are less effective on ticks at higher relative humidities (Darrow & Whetstone, 1972). Future research is needed to measure whether lethal effects of ash are stronger at different humidities and when ticks acquire moisture through other means like feeding.

Dusts may abrade and absorb wax from ticks' cuticles, causing desiccation (Showler & Harlien, 2023a). Alternatively, they may kill ticks by blocking respiration through the tick's spiracles (Richardson et al., 2022). These modes of action could differentially influence ticks in different life stages. Larval ixodid ticks lack spiracles and respire through the cuticle which plays an essential role in preventing desiccation, but also must be permeable enough for respiration. Larval ticks' more delicate cuticular layers may be particularly vulnerable to ash, preventing gas exchange and abrading the epicuticle, leading to desiccation. Indeed, acaricidal dusts and oils that are effective against larval ticks are less so against nymphal ticks, consistent with our finding that redwood ash affected *Dermacentor* spp. larvae but not *Dermacentor* spp. adults

(Panella et al., 1997; Showler et al., 2019). The decision was made early in the implementation to group *D. simils* and *D. occidentalis* adults to support a sufficient sample size. Although in retrospect, doing so unfortunately impaired our ability to differentiate possible differences between the two species. Nevertheless, we believe presenting this data is valuable because it demonstrates the considerable differences among genera. Additionally, adult ticks can be relatively easily acquired in the field, whereas larval and nymphal ticks are not. Immature stages play a critically important role in acquisition and transmission of pathogens. However, research on field-collected larvae and nymphs—which may differ from lab-raised ticks in survival, behavior, and host preference—is limited by difficulty in collecting them in large numbers. Additionally, the effects of acaricides vary in their impacts on ticks depending on time since molt (Darrow & Whetstone, 1972). Future work is needed to understand the lethal effects on each life stage and how effects vary over time.

I. pacificus ticks showed prolonged difficulty orienting themselves particularly when exposed to redwood ash while *Dermacentor* spp. did not. Changes in tick behavior may have large repercussions on survival and fitness as well. Increased energy expenditure if a tick is unable to orient, and the struggle and stress associated with attempts to right oneself may have significant impacts on physiology, depleting limited resources such as fat reserves, increase opening of the spiracle leading to water loss, and interfere with mating and oviposition. Exhaustion of limited energy reserves is a known cause of mortality in ticks (Nieto et al., 2010). Counter-intuitively, an ash that causes failure to orient could alternatively prolong survival as measured in our study by minimizing energy-expending movements. Ashes and dusts cause numerous behavioral changes in arthropods. For example, volcanic ash causes periodic bouts of exploratory activity followed by decreased spontaneous activity and responsiveness in crickets and cockroaches, likely due to disorientation (Edwards & Schwartz, 1981). Certain dust exposures cause decreased movement and avoidance behaviors in *Cimex lectularius* (common bed bugs) (Agnew & Romero, 2017), but no changes in behaviors in lone star ticks (Showler, Flores, et al., 2020). Future studies could explore numerous aspects of behavior of ticks exposed to ash, such as whether ticks may avoid areas post-burn that have ash, whether ash-bathing by host animals disrupts tick feeding, or the seasonal complexities of when both ticks are active and ash is available.

Naturally produced ash is complex— varying in size, charge, sorptive capacity, and chemical composition, all of which may influence its potency. While some species grow in monospecific grooves, ash produced in fires is frequently from an assortment of species. Ash composition depends not only on the wood burned but also on factors such as the temperature of the combustion process and duration of combustion (Bodí et al., 2014). All these factors may influence the ability of the ash to adhere and its effects on both the tick and host. Nevertheless, certain tree species found in habitat where ticks live clearly offer greater prospects for their acaricidal effects. Both eucalyptus and bay contain volatile constituents with known acaricidal properties, including 1,8-cineole in both and umbellulone in the latter (Adenubi et al., 2021; Alimi et al., 2021; Buttery et al., 1974; Clemente et al., 2010; Pålsson et al., 2008). Intriguingly, the dusky-footed wood rat (*Neotoma fuscipes*), an important local reservoir host of multiple tick-borne disease agents, often incorporates California bay laurel into its nests, thought to be a means to help control ectoparasites (Hemmes et al., 2002).

Additionally, burning Tasmanian blue gum eucalyptus and California bay laurel aligns with landscape goals to prevent wildfires and sudden oak death. Tasmanian blue gum eucalyptus is not only extremely invasive (Wolf & DiTomaso, 2016), it is also very fire-prone and a major focus for removal in some California fire-management plans (Boyd et al., 2006). California bay laurel on the other hand is native, but the main host for sudden oak disease; its removal followed by prescribed burning helps control the disease (Lee, 2009; Swiecki & Bernhardt, 2016). It may therefore be possible to coordinate efforts to control tick-borne diseases, devastating wildfires, and sudden oak death in California.

This is the seminal study to demonstrate the effects of plant-produced ash on ticks, which may offer an alternative management strategy, especially as some tick species show increasing levels of resistance to synthetic acaricides (Dzemo et al., 2022). Our findings add to our understanding of post-fire tick-borne disease dynamics and how fire management priorities of certain tree species align with tick-borne disease management. Furthermore, they suggest that the impacts of wildfires and prescribed burning on vertebrates go beyond direct effects and may provide wildlife a natural way of removing ectoparasites through ash-bathing.

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REFERENCES

Adenubi OT, Abolaji AO, Salihu T et al. 2021. Chemical composition and acaricidal activity of *Eucalyptus globulus* essential oil against the vector of tropical bovine piroplasmosis, *Rhipicephalus (Boophilus) annulatus*. Experimental and Applied Acarology 83:301-312.

Agnew JL, Romero A. 2017. Behavioral responses of the common bed bug, *Cimex lectularius*, to insecticide dusts. Insects 8(3):83.

Alimi D, Hajri A, Jallouli S et al. 2021. In vitro acaricidal activity of essential oil and crude extracts of *Laurus nobilis*,(Lauraceae) grown in Tunisia, against arthropod ectoparasites of livestock and poultry: *Hyalomma scupense* and *Dermanyssus gallinae*. Veterinary Parasitology 298:109507.

Banjo A, Otufale G, Awobajo O. 2009. Indigenous control of mite in poultry farming in Ogun State Nigeria. Journal Applied Science Research 5(10):1767-1771.

Bodí MB, Martin DA, Balfour VN et al. 2014. Wildland fire ash: production, composition and eco-hydro-geomorphic effects. Earth-Science Reviews 130:103-127.

Boyd D, Elliott T, Farres A et al. 2006. Fire management newsletter: eucalyptus: a complex challenge, National Park Service, https://home.nps.gov/pore/learn/nature/upload/firemanagement_fireeducation_newsletter_eucalyptus.pdf.

Bracke MB. 2011. Review of wallowing in pigs: Description of the behaviour and its motivational basis. Applied Animal Behaviour Science 132(1-2):1-13.

Buttery RG, Black DR, Guadagni DG et al. 1974. California bay oil. I. Constituents, odor properties. Journal of Agricultural and Food Chemistry 22(5):773-777.

Clayton DH, Koop JA, Harbison CW et al. 2010. How birds combat ectoparasites. H. W. Manter Laboratory Library Materials 40.

Clemente MA, de Oliveira Monteiro CM, Scoralik MG et al. 2010. Acaricidal activity of the essential oils from *Eucalyptus citriodora* and *Cymbopogon nardus* on larvae of *Amblyomma cajennense* (Acari: Ixodidae) and *Anocentor nitens* (Acari: Ixodidae). Parasitology Research 107:987-992.

Darrow D, Whetstone T. 1972. Age and susceptibility of nymphal lone star ticks to selected ixodicides. Journal of Economic Entomology 65(1): 156-158.

Dzemo WD, Thekisoe O, Vudriko P. 2022. Development of acaricide resistance in tick populations of cattle: A systematic review and meta-analysis. Heliyon 8(1).

Edwards JS, Schwartz LM. 1981. Mount St. Helens ash: a natural insecticide. Canadian Journal of Zoology 59(4):714-715.

Eisen L. 2018. Pathogen transmission in relation to duration of attachment by *Ixodes scapularis* ticks. Ticks and Tick-borne Diseases 9(3):535-542.

Eisenberg JF. 1963. A comparative study of sandbathing behavior in heteromyid rodents. Behaviour 22(1-2):16-23.

Estrada-Peña A, Gray JS, Kahl O et al. 2013. Research on the ecology of ticks and tick-borne pathogens—methodological principles and caveats. Frontiers in Cellular and Infection Microbiology 3(29).

Gabanakgosi K, Moreki J, Nsoso S et al. 2012. Ethnoveterinary medicine usage in family chickens in the selected four villages of Botswana. Journal of Veterinary Advances 2(12):586-594.

Gleim ER, Zemtsova GE, Berghaus RD et al. 2019. Frequent prescribed fires can reduce risk of tick-borne diseases. Scientific Reports 9(1):9974.

Hakbijl T. 2002. The traditional, historical and prehistoric use of ashes as an insecticide, with an experimental study on the insecticidal efficacy of washed ash. Environmental Archaeology 7(1):13-22.

Hazra A, Gogtay N. 2017. Biostatistics series module 9: survival analysis. Indian Journal of Dermatology 62(3):251-257.

Hemmes RB, Alvarado A, Hart BL. 2002. Use of California bay foliage by wood rats for possible fumigation of nest-borne ectoparasites. Behavioral Ecology 13(3):381-385.

Kim EH, Kim HK, Ahn YJ. 2003. Acaricidal activity of plant essential oils against *Tyrophagus putrescentiae* (Acari: Acaridae). Journal of Asia-Pacific Entomology 6(1):77-82.

Lee C. 2009. Sudden oak death and fire in California, California Oak Mortality Task Force, https://www.fs.usda.gov/wwetac/old/projects/PDFs/SOD_and_Fire_update_2009.pdf.

Levin ML, Ford SL, Hartzer K et al. 2020. Minimal duration of tick attachment sufficient for transmission of infectious *Rickettsia rickettsii* (Rickettsiales: Rickettsiaceae) by its primary vector *Dermacentor variabilis* (Acari: Ixodidae): duration of rickettsial reactivation in the vector revisited. Journal of Medical Entomology 57(2):585-594.

Levin ML, Troughton DR, Loftis AD. 2021. Duration of tick attachment necessary for transmission of *Anaplasma phagocytophilum* by *Ixodes scapularis* (Acari: Ixodidae) nymphs. Ticks and Tick-borne Diseases 12(6):101819.

MacDonald AJ, Hyon DW, McDaniels A et al. 2018. Risk of vector tick exposure initially increases, then declines through time in response to wildfire in California. Ecosphere 9(5):e02227.

Mooring M, Samuel W. 1998. Tick defense strategies in bison: the role of grooming and hair coat. Behaviour 135(6):693-718.

Moyo S, Masika P, Moyo B. 2015. A survey of external parasites of free-range chickens and their ethno-veterinary control remedies used by resource-limited farmers in Eastern Cape, South Africa. Scientific Journal of Veterinary Advances 4(2):13-20.

Needham GR, Teel PD. 1991. Off-host physiological ecology of ixodid ticks. Annual Review of Entomology 36(1):659-681.

Oaster BT. 2020. Native land management could save us from wildfires, experts say, Street Roots, https://www.streetroots.org/news/2020/10/21/native-land-management-could-save-us2020.

Pålsson K, Jaenson TG, Bæckström P et al. 2008. Tick repellent substances in the essential oil of *Tanacetum vulgare*. Journal of Medical Entomology 45(1):88-93.

Panella NA, Karchesy J, Maupin GO et al. 1997. Susceptibility of immature *Ixodes scapularis* (Acari: Ixodidae) to plant-derived acaricides. Journal of Medical Entomology 34(3):340-345.

Pascoe EL, Plourde BT, Lopéz-Perez AM et al. 2020. Response of small mammal and tick communities to a catastrophic wildfire and implications for tick-borne pathogens. Journal of Vector Ecology 45(2):269-284.

Pascoe EL, Vaughn CE, Jones MI, et al. 2023. Recovery of western black-legged tick and vertebrate populations after a destructive wildfire in an intensively-studied woodland in northern California. Journal of Vector Ecology 48(1):19-36.

Peavey CA, Lane RS. 1995. Transmission of *Borrelia burgdorferi* by *Ixodes pacificus* nymphs and reservoir competence of deer mice (*Peromyscus maniculatus*) infected by tick-bite. Journal of Parasitology:175-178.

Richardson EA, Ponnusamy L, Roe RM. 2022. Mechanical acaricides active against the blacklegged tick, *Ixodes scapularis*. Insects 13(8):672.

Showler AT, Saelao P. 2022. Integrative alternative tactics for ixodid control. Insects 13(3):302.

Showler AT, Harlien JL. 2023a. Lethal effects of Imergard WP, a perlite-based dust, on *Amblyomma americanum* (Ixodida: Ixodidae) larvae and nymphs. Journal of Medical Entomology 60(2):326-332.

Showler AT, Harlien JL. 2023b. Desiccant dusts, with and without bioactive botanicals, lethal to *Rhipicephalus (Boophilus) microplus* Canestrini (Ixodida: Ixodidae) in the laboratory and on cattle. Journal of Medical Entomology 60(2):346-355.

Showler AT, Dorsey BN, Caesar RM. 2020a. Lethal effects of a silica gel+ thyme oil (EcoVia) dust and aqueous suspensions on *Amblyomma americanum* (Ixodida: Ixodidae) larvae and nymphs. Journal of Medical Entomology 57(5):1516-1524.

Showler AT, Osbrink WL, Munoz E et al. 2019. Lethal effects of silica gel-based CimeXa and kaolin-based Surround dusts against ixodid (Acari: Ixodidae) eggs, larvae, and nymphs. Journal of Medical Entomology 56(1):215-221.

Showler AT, Flores N, Caesar RM et al. 2020b. Lethal effects of a commercial diatomaceous earth dust product on *Amblyomma americanum* (Ixodida: Ixodidae) larvae and nymphs. Journal of Medical Entomology 57(5):1575-1581.

Swiecki TJ, Bernhardt EA. 2016. Sudden oak death in California. Insects and Diseases of Mediterranean Forest Systems:731-756.

Tarshis IB. 1961. Laboratory and field studies with sorptive dusts for the control of arthropods affecting man and animal. Experimental Parasitology 11(1):10-33.

Wagner RE, Ebeling W. 1959. Lethality of inert dust materials to *Kalotermes minor* Hagen and their role as preventives in structural pest control. Journal of Economic Entomology 52(2):208-212.

Wanzala W. 2009. Ethnobotanicals for management of the brown ear tick *Rhipicephalus appendiculatus* in western Kenya [Ph. D. thesis]. Wageningen University, Wageningen, Netherlands 2(9).

Wanzala W. 2017. Potential of traditional knowledge of plants in the management of arthropods in livestock industry with focus on (Acari) ticks. Evidence-Based Complementary and Alternative Medicine 2017(1):8647919.

Wolf KM, DiTomaso J. 2016. Management of blue gum eucalyptus in California requires region-specific consideration. California Agriculture 70(1).

Yoder JA, Hedges BZ, Benoit JB. (2012). Water balance of the American dog tick, *Dermacentor variabilis*, throughout its development with comparative observations between field-collected and laboratory-reared ticks. International Journal of Acarology, 38(4), 334-343.

Table 1a: Hazard ratios for diatomaceous earth, kaolin clay, eucalyptus ash, bay ash, and redwood ash treatments compared to negative control on reducing survival of *Ixodes pacificus* according to accelerated failure time model with a log-logistic distribution (scale parameter for female model on the left= 0.45 and for male model on the right = 0.41).

	Females	**Males**
Treatment	Acceleration factor (s.e.), p	Acceleration factor (s.e.), p
Intercept	0.10 (0.16), <0.001	0.42 (0.16), <0.001
Diatomaceous earth	7.66 (0.23), <0.001	1.95 (0.23), 0.002
Kaolin clay	6.37 (0.24), <0.001	1.89 (0.24), 0.005
Bay ash	6.20 (0.20), <0.001	1.67 (0.20), 0.009
Redwood ash	1.36 (0.24), 0.19	1.39 (0.24), 0.12
Eucalyptus ash	5.46 (0.21), <0.001	1.72 (0.21), 0.006

Table 1b: Effect of diatomaceous earth, kaolin clay, and bay ash treatments compared to negative control treatment on survival of *Dermacentor occidentalis* and *Dermacentor similis* adults according to accelerated failure time model with a log logistic distribution (scale parameter for

adult model on the left= 0.49 and for larvae model on the right = 0.36). Insufficient deaths were observed among females exposed to redwood ash and eucalyptus ash to be analyzed.

	Adults	**Larvae**
Treatment	Acceleration factor (s.e.), p	Acceleration factor (s.e.), p
Intercept	0.02 (0.65), <0.001	0.12 (0.07). <0.001
Diatomaceous earth	2.12 (0.61), 0.21	7.54 (0.09), <0.001
Kaolin clay	4.25 (0.61), 0.02	7.53 (0.08), <0.001
Bay ash	1.69 (0.62), 0.40	2.34 (0.08), <0.001
Redwood ash	*NA*	1.70 (0.09), <0.001
Eucalyptus ash	*NA*	2.85 (0.09), <0.001

Table 1c: Effect of diatomaceous earth, kaolin clay, eucalyptus ash, bay ash, and redwood ash treatments compared to negative control treatment on survival of *Dermacentor albipictus* larvae according to accelerated failure time model with a log logistic distribution (scale parameter = 0.43).

Treatment	Acceleration factor (s.e.), p-value
Intercept	0.19 (0.10), <0.001
Diatomaceous earth	1.05 (0.15), 0.72
Kaolin clay	2.76 (0.13), <0.001
Bay ash	1.02 (0.14), 0.91
Redwood ash	1.90 (0.13), <0.001
Eucalyptus ash	0.75 (0.18), 0.11

Figure 1: Kaplan-Meier curves depicting proportion of *Ixodes pacificus* females surviving in each treatment group over duration of experiment.

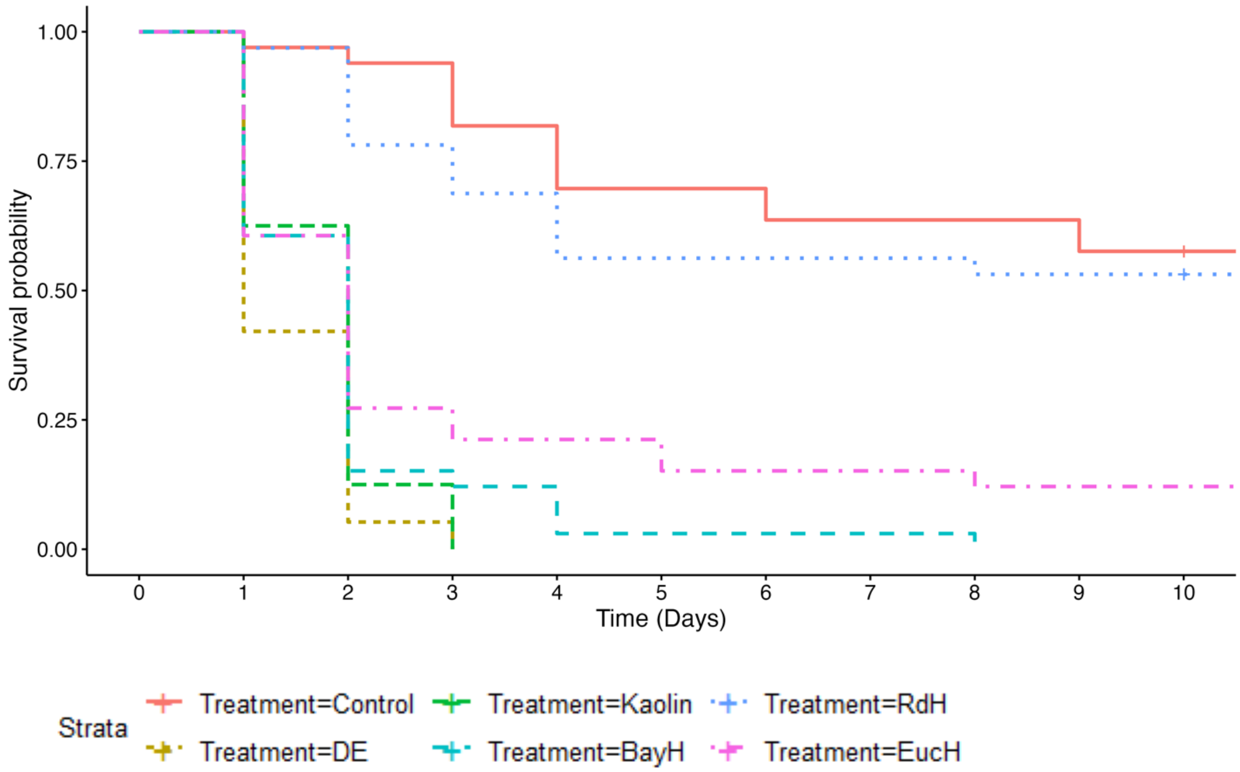


Figure 2: Kaplan-Meier curves depicting proportion of *Ixodes pacificus* males surviving in each treatment group over duration of experiment.

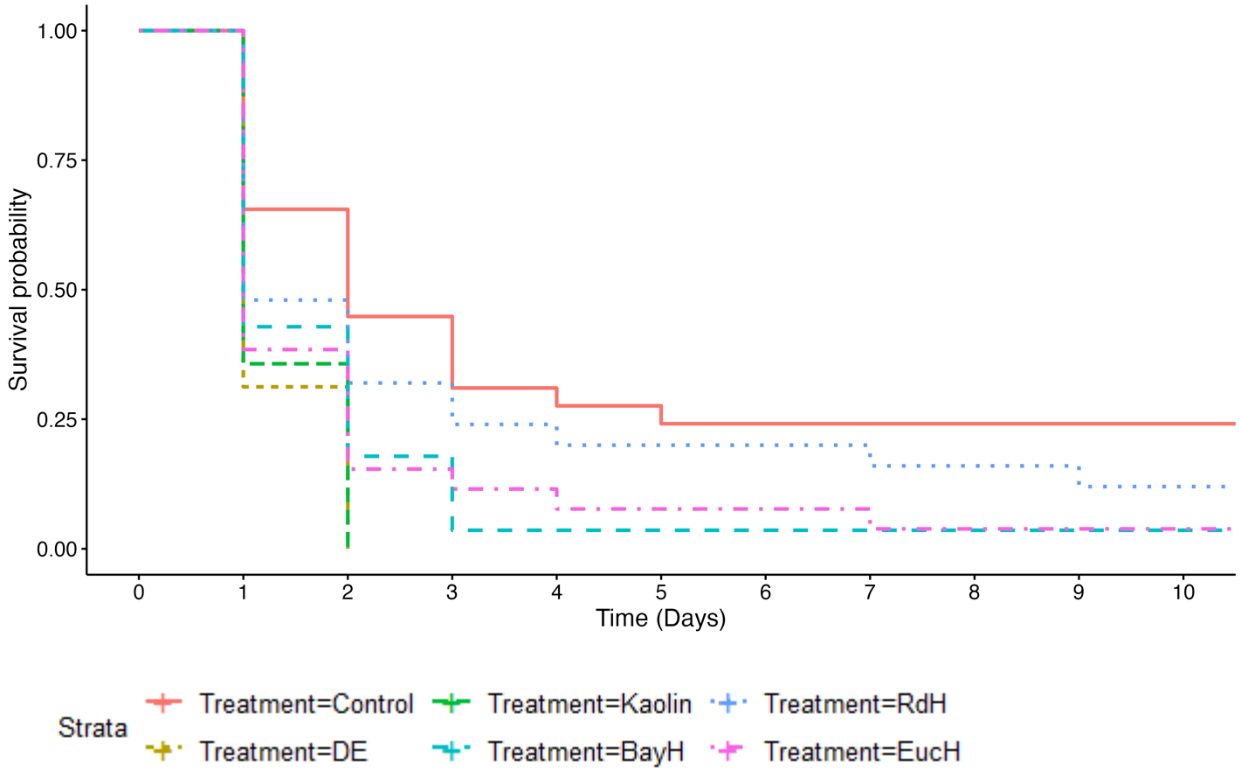


Figure 3: Kaplan-Meier curves depicting proportion of adult *Dermacentor occidentalis and Dermacentor similis* ticks (males and females grouped) surviving in each treatment group over duration of experiment.

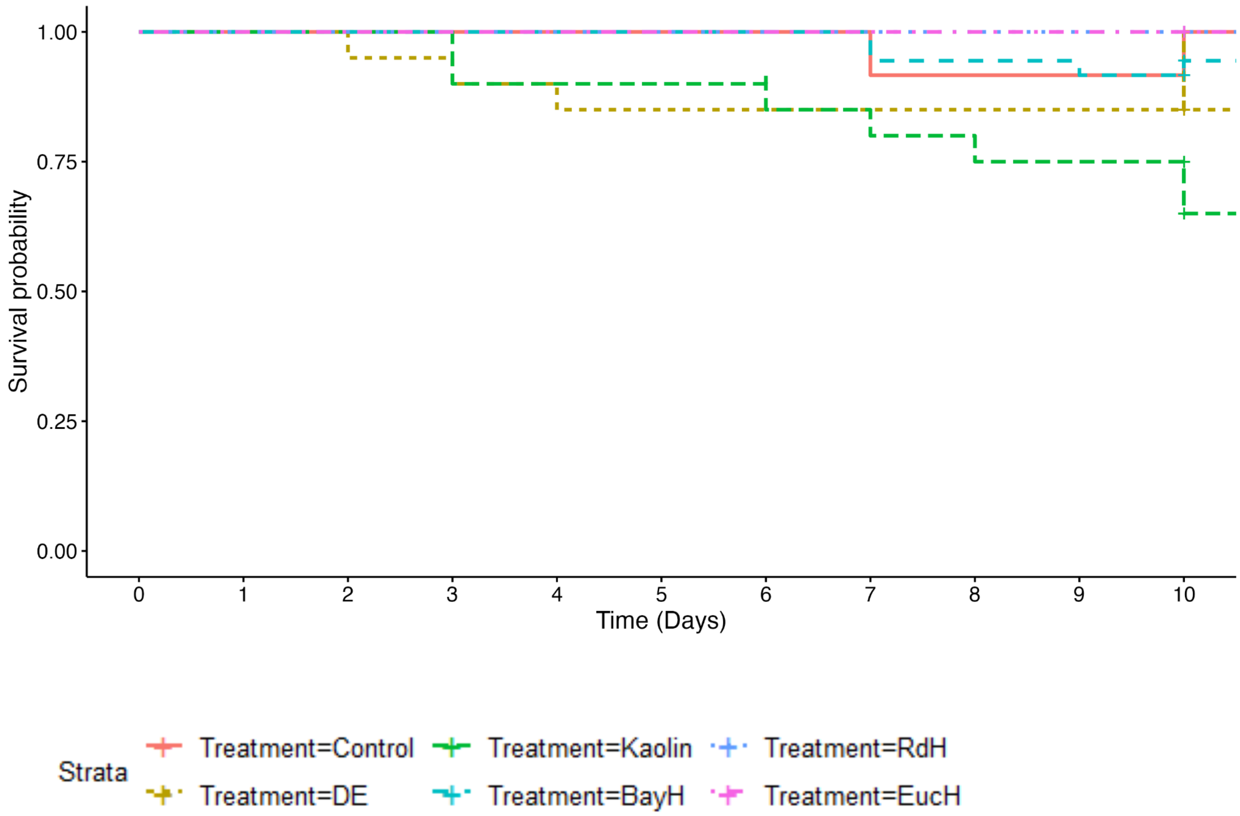


Figure 4: Kaplan-Meier curves depicting proportion of *Dermacentor occidentalis* larvae surviving in each treatment group over duration of experiment.

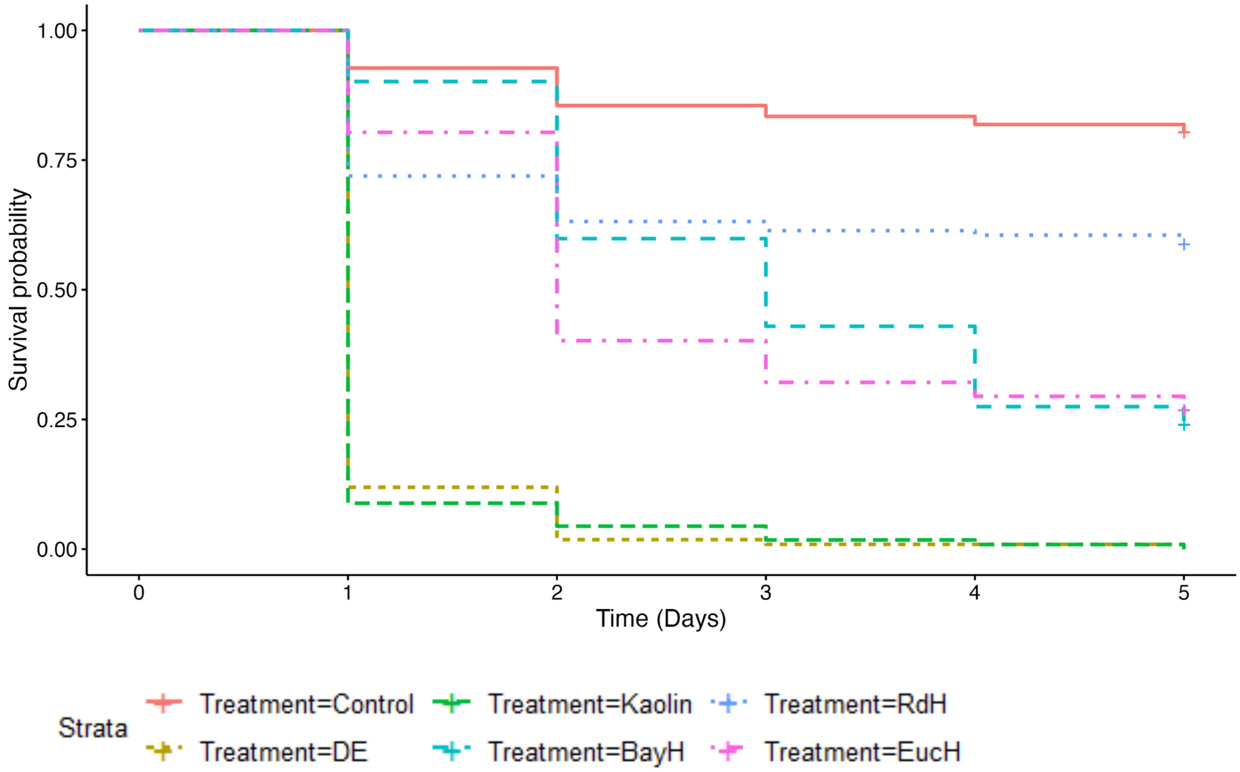


Figure 5: Kaplan-Meier curves depicting proportion of *Dermacentor albipictus* larvae surviving in each treatment group over duration of experiment.

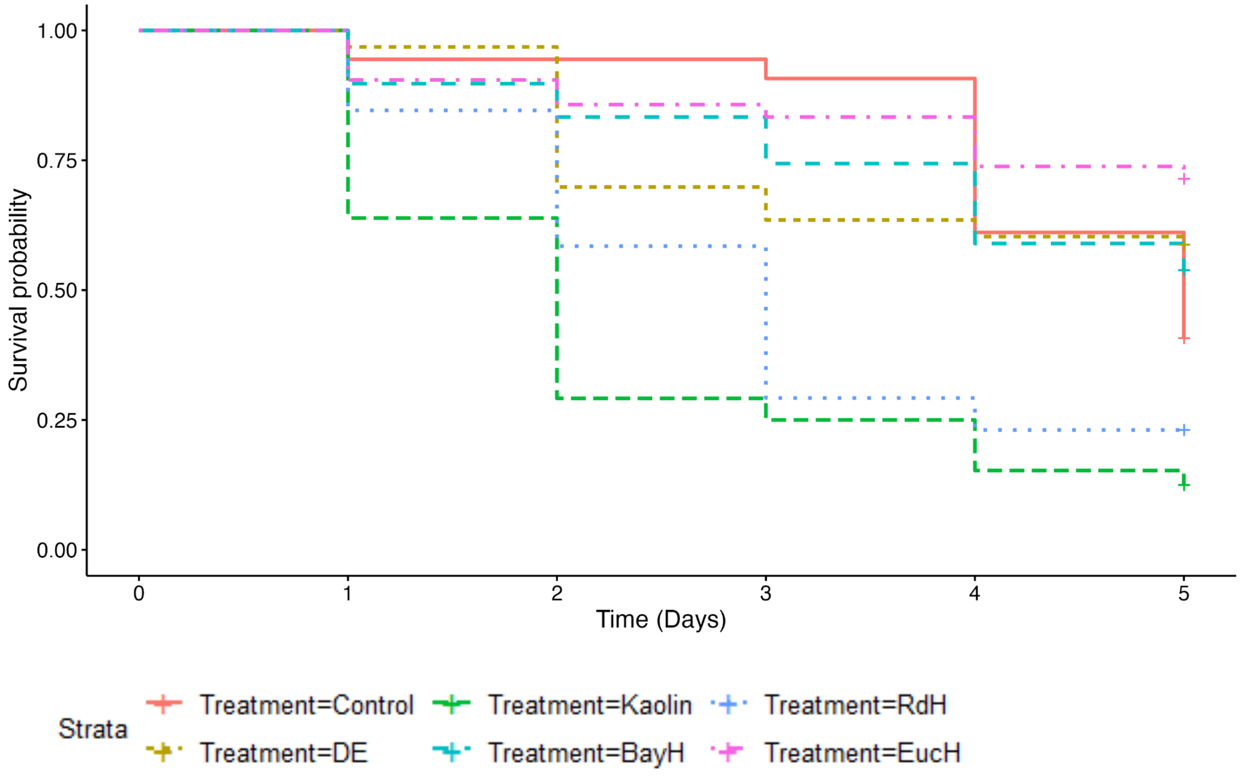


Figure 6a: Kaplan-Meier curves depicting cumulative probability of righting oneself among *Ixodes pacificus* females in each treatment group over duration of experiment.

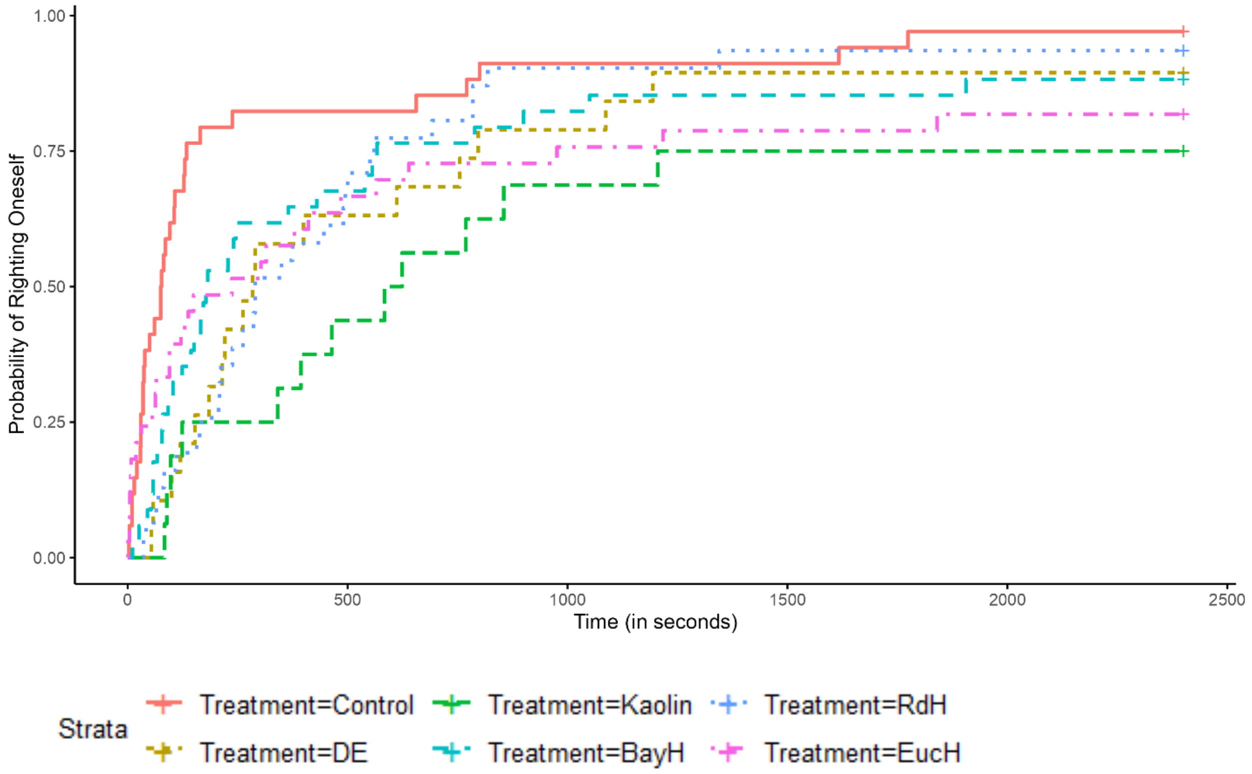


Figure 6b:

Kaplan-Meier curves depicting cumulative probability of righting oneself among *Ixodes pacificus* males in each treatment group over duration of experiment.

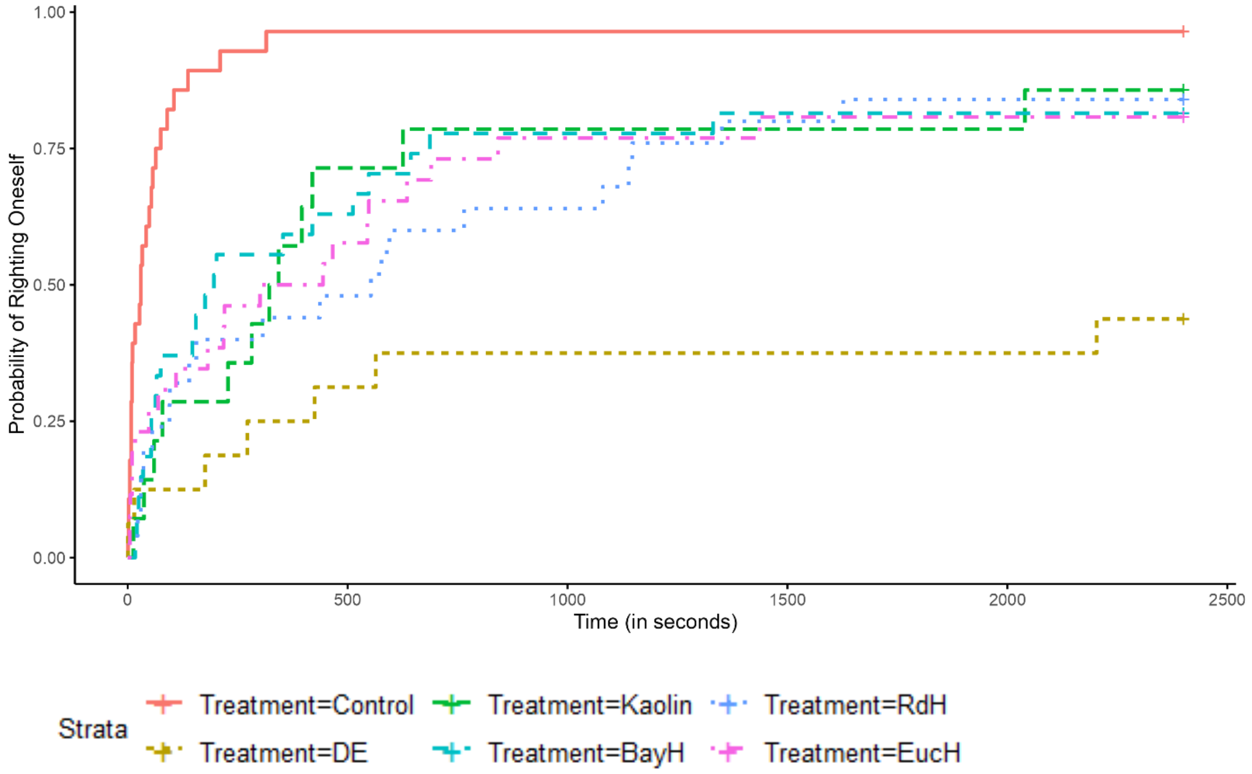


Figure 6c: Kaplan-Meier curves depicting cumulative probability of righting oneself *Dermacentor occidentalis* and *Dermacentor similis* females in each treatment group over duration of experiment.

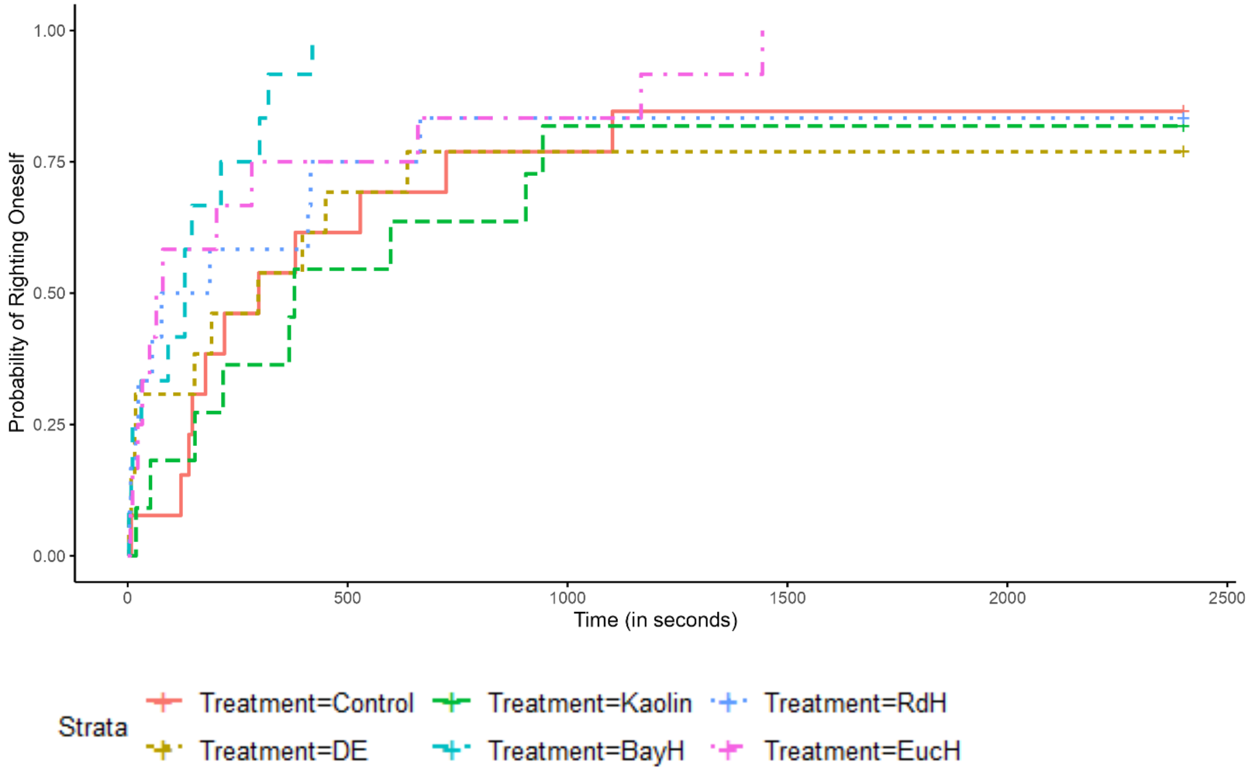
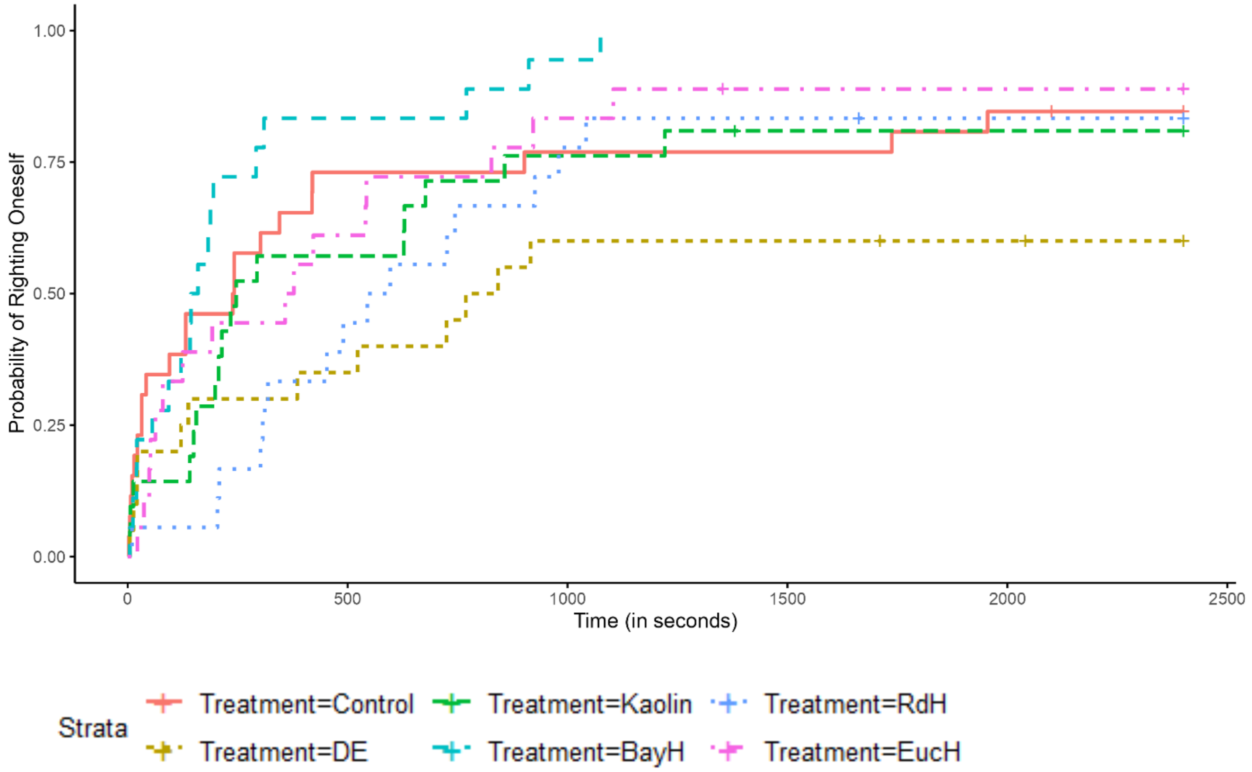
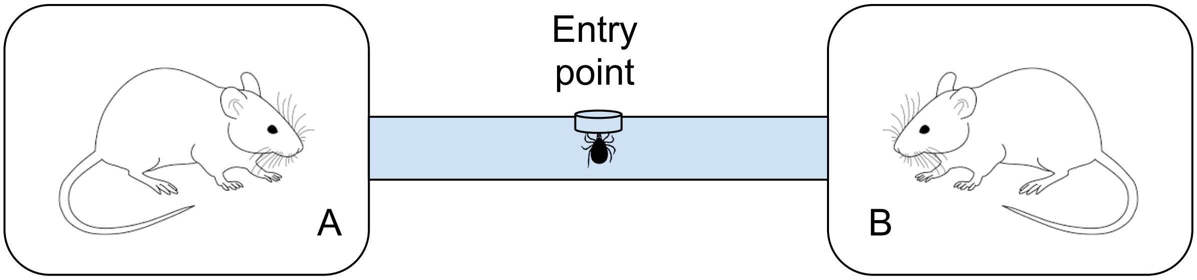


Figure 6d: Kaplan-Meier curves depicting cumulative probability of righting oneself *Dermacentor occidentalis* and *Dermacentor similis* males in each treatment group over duration of experiment.



Supplementary Figure 1: Experimental set-up for attraction trials. A control mouse (A) was placed on one side of the set-up and a mouse exposed to ash (B) was placed on the other side, both in tubes with air flow towards the tick entry point, controlled by fans on each side. Larval ticks were placed inside on the center tube at the entry point.



Supplementary Table 1: Survival of *I. pacificus* females at 24- and 48-hours compared to negative control using Fisher's Exact test (2-tailed).

Treatment	24 hours		48 hours	
	Dead/total (%)	p-value	Dead/total (%)	p-value
Negative control	1/33 (3.03)	ref	2/33 (6.06)	ref
Diatomaceous earth	11/19 (57.9)	<0.001	18/19 (94.7)	<0.001
Kaolin clay	6/16 (37.5)	0.003	14/16 (87.5)	<0.001
Bay ash	13/33 (39.4)	<0.001	28/33 (84.8)	<0.001
Redwood ash	1/32 (3.13)	1	7/32 (21.9)	0.08
Eucalyptus ash	13/33 (39.4)	<0.001	24/33 (72.7)	<0.001

Supplementary Table 2: Survival of *I. pacificus* females within each treatment and active control group at 24- and 48-hours using Fisher's Exact test (2-tailed).

Treatment	24 hours		48 hours	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Eucalyptus ash vs				
Diatomaceous earth	0.48 (0.13-1.72)	0.25	0.15 (0.00-1.28)	0.07
Kaolin clay	1.08 (0.27-4.56)	1.00	0.39 (0.04-2.28)	0.30
Bay ash vs				
Diatomaceous earth	0.48 (0.13-1.72)	0.25	0.32 (0.00-3.18)	0.40
Kaolin clay	1.08 (0.27-4.56)	1.00	0.80 (0.07-5.71)	1.00
Redwood ash vs				
Diatomaceous earth	0.02 (0.00-0.21)	<0.001	0.02 (0.00-0.14)	<0.001
Kaolin clay	0.06 (0.00-0.54)	0.003	0.04 (0.00-0.25)	<0.001

Supplementary Table 3: Pairwise comparisons of survival by *I. pacificus* females over an 11-day period using the Gehan-Breslow-Wilcoxon test with Bonferroni's correction

	Negative control	Diatomaceous earth	Kaolin clay	Bay ash	Redwood ash
Diatomaceous earth	<0.001	-	-	-	-
Kaolin clay	<0.001	1	-	-	-
Bay ash	<0.001	1	1	-	-
Redwood ash	1	<0.001	<0.001	<0.001	-
Eucalyptus ash	<0.001	1	0.39	1	<0.001

Supplementary Table 4: Male *I. pacificus* survival at 24- and 48-hours compared to negative control using Fisher's Exact test (2-tailed).

Treatment	24 hours		48 hours	
	Dead/total (%)	p-value	Dead/total (%)	p-value
Negative control	10/29 (34.5)	ref	16/29 (55.2)	ref
Diatomaceous earth	11/16 (68.8)	0.03	16/16 (100)	0.001
Kaolin clay	9/14 (64.3)	0.10	14/14 (100)	0.003
Bay ash	16/28 (57.1)	0.11	23/28 (82.1)	0.05
Redwood ash	13/25 (52.0)	0.27	17/25 (68.0)	0.41
Eucalyptus ash	16/26 (61.5)	0.06	22/26 (84.6)	0.02

Supplementary Table 5: comparison of each treatment versus each active control for male *I. pacificus* survival at 24- and 48-hours using Fisher's Exact test (2-tailed).

Treatment	24 hours		48 hours	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Eucalyptus ash vs				
Diatomaceous earth	0.73 (0.15-3.21)	0.75	0 (0.00-2.39)	0.28
Kaolin clay	0.89 (0.18-4.09)	1.00	0 (0.00-2.76)	0.28
Bay ash vs				
Diatomaceous earth	0.61 (0.13-2.59)	0.53	0 (0.00-1.82)	0.14
Kaolin clay	0.75 (0.15-3.30)	0.75	0 (0.00-2.10)	0.15
Redwood ash vs				
Diatomaceous earth	0.50 (0.10-2.16)	0.34	0 (0.00-0.76)	0.01
Kaolin clay	0.61 (0.12-2.76)	0.52	0 (0.00-0.88)	0.03

Supplementary Table 6: Pairwise comparisons of male *I. pacificus* survival over a 11-day period using the Gehan-Breslow-Wilcoxon test with Bonferroni's correction.

	Negative control	Diatomaceous earth	Kaolin clay	Bay ash	Redwood ash
Diatomaceous earth	0.02	-	-	-	-
Kaolin clay	0.04	1.00	-	-	-
Bay ash	0.15	1.00	1.00	-	-
Redwood ash	1.00	0.45	0.80	1.00	-
Eucalyptus ash	0.20	1.00	1.00	1.00	1.00

Supplementary Table 7: Pairwise comparisons of adult *D. occidentalis* and *D. similis* survival over a 11-day period using the Gehan-Breslow-Wilcoxon test with Bonferroni's correction.

	Negative control	Diatomaceous earth	Kaolin clay	Bay ash	Redwood ash
Diatomaceous earth	1.00	-	-	-	-
Kaolin clay	0.004	0.36	-	-	-
Bay ash	1.00	1.00	0.13	-	-
Redwood ash	1.00	1.00	0.01	1.00	-
Eucalyptus ash	1.00	1.00	0.01	1.00	1.00

Supplementary Table 8: *D. occidentalis* larval survival at 24- and 48-hours compared to negative control using Fisher's Exact test (2-tailed).

Treatment	24 hours		48 hours	
	Dead/total (%)	p-value	Dead/total (%)	p-value
Negative control	14/193 (7.3)	ref	28/193 (14.5)	ref
Diatomaceous earth	96/109 (88.1)	<0.001	107/109 (98.2)	<0.001
Kaolin clay	103/113 (91.2)	<0.001	108/113 (95.6)	<0.001
Bay ash	14/142 (9.9)	0.43	57/142 (40.1)	<0.001
Redwood ash	32/114 (28.1)	<0.001	42/114 (36.8)	<0.001
Eucalyptus ash	22/112 (19.6)	0.002	67/112 (59.8)	<0.001

Supplementary Table 9: larval *D. occidentalis*: comparison of each treatment versus each active control for larval *D. occidentalis* survival at 24- and 48-hours using Fisher's Exact test (2-tailed).

	24 hours		48 hours	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Eucalyptus ash vs				
Diatomaceous earth	0.03 (0.01-0.07)	<0.001	0.03 (0.00-0.11)	<0.001
Kaolin clay	0.02 (0.01-0.06)	<0.001	0.07 (0.02-0.19)	<0.001
Bay ash vs				
Diatomaceous earth	0.03 (0.01-0.07)	<0.001	0.01 (0.00-0.06)	<0.001
Kaolin clay	0.02 (0.01-0.05)	<0.001	0.03 (0.01-0.09)	<0.001
Redwood ash vs				
Diatomaceous earth	0.05 (0.02-0.11)	<0.001	0.01 (0.00-0.04)	<0.001
Kaolin clay	0.04 (0.02-0.09)	<0.001	0.03 (0.01-0.07)	<0.001

Supplementary Table 10: Pairwise comparisons of larval *D. occidentalis* survival over a 11-day period using the Gehan-Breslow-Wilcoxon test with Bonferroni's correction.

	Negative control	Diatomaceous earth	Kaolin clay	Bay ash	Redwood ash
Diatomaceous earth	<0.001	-	-	-	-
Kaolin clay	<0.001	1.00	-	-	-
Bay ash	<0.001	<0.001	<0.001	-	-
Redwood ash	<0.001	<0.001	<0.001	0.001	-
Eucalyptus ash	<0.001	<0.001	<0.001	1.00	0.001

Supplementary Table 11: *D. albipictus* larval survival at 24- and 48-hours compared to negative control using Fisher's Exact test (2-tailed).

Treatment	24 hours		48 hours	
	Dead/total (%)	p-value	Dead/total (%)	p-value
Negative control	3/54 (5.6)	ref	3/54 (5.6)	ref
Diatomaceous earth	2/63 (3.2)	0.66	19/63 (30.2)	<0.001
Kaolin clay	26/72 (36.1)	<0.001	51/72 (70.8)	<0.001
Bay ash	8/78 (10.3)	0.52	13/78 (16.7)	0.06
Redwood ash	10/65 (15.4)	0.14	27/65 (41.5)	<0.001
Eucalyptus ash	4/42 (9.5)	0.70	6/42 (14.3)	0.17

Supplementary Table 12: Comparison of each treatment versus each active control for larval *D. albipictus* survival at 24- and 48-hours using Fisher's Exact test (2-tailed).

Treatment	24 hours		48 hours	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Eucalyptus ash vs				
Diatomaceous earth	3.17 (0.43-36.71)	0.21	0.39 (0.11-1.15)	0.07
Kaolin clay	0.19 (0.04-0.61)	0.002	0.07 (0.02-0.20)	<0.001
Bay ash vs				
Diatomaceous earth	3.46 (0.66-34.66)	0.19	0.47 (0.19-1.11)	0.07
Kaolin clay	0.20 (0.07-0.51)	<0.001	0.08 (0.03-0.19)	<0.001
Redwood ash vs				
Diatomaceous earth	5.48 (1.10-53.58)	0.03	1.64 (0.75-3.65)	0.20
Kaolin clay	0.32 (0.13-0.78)	0.007	0.30 (0.14-0.63)	<0.001

Supplementary Table 13: Pairwise comparisons of larval *D. albipictus* survival over a 11-day period using the Gehan-Breslow-Wilcoxon test with Bonferroni's correction.

	Negative control	Diatomaceous earth	Kaolin clay	Bay ash	Redwood ash	
Diatomaceous earth	1.00	-	-	-	-	-
Kaolin clay	<0.001	<0.001	-	-	-	-
Bay ash	1.00	1.00	<0.001	-	-	-
Redwood ash	0.001	0.001	0.17	<0.001	-	-
Eucalyptus ash	0.23	1.00	<0.001	1.00	<0.001	0.001

Supplementary Table 13: Mixed logistic regression accounting for repeated sampling from each control analyzing the odds of larval *I. pacificus* ticks being attracted to mice post-ash exposure compared to pre-ash exposure.

Treatment	OR (95% CI)	p-value
Bay	0.99 (0.56-1.77)	0.98
Redwood	0.96 (0.54-1.72)	0.90
Eucalyptus	0.96 (0.52-1.77)	0.91

Chapter 3

Spring fever: early spring predicts lizard, tick, and Lyme disease activity in northwestern California

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Abstract.

Most Lyme disease in the far-western United States is acquired from nymphal *Ixodes pacificus* ticks. Although predicting years of elevated or early Lyme disease risk would benefit public health, predictive modeling is challenged by the tick's multiple-year life cycle and dependence on numerous vertebrate hosts. Acquiring data on hosts' population trends is expensive and labor-intensive and such data are largely lacking. We predicted nymphal abundance and questing activity with readily available climate data. We analyzed two long-term datasets of nymphal ticks from a *B. burgdorferi*-endemic site in northwestern California that ultimately experienced a prolonged drought culminating in a wildfire. The density of nymphal ticks could be predicted by temperatures in early spring of the prior year. The timing of ticks beginning to quest for hosts could be predicted by temperature and relative humidity of late spring of the prior year. When we incorporated human-caused climate change into the model, we predicted significantly earlier emergence each spring of nymphal ticks over the next century. Moreover, there may be an initial doubling in numbers of questing nymphs. Predicting tick activity from climate data provides a tool for public health and for interpreting changes in Lyme disease ecology in the far-western US.

Keywords: *Ixodes pacificus,* climate change, Lyme disease, tick-borne disease prediction

Introduction

Tick-borne disease cases, including Lyme disease (LD), the most common arthropod-borne disease in the United States, are on the rise (Paddock et al. 2016). More than 30,000 cases of LD are reported annually to the Centers for Disease Control and Prevention (CDC), and insurance claims suggest an annual disease burden of at least 476,000 cases (Kugeler et al. 2021). Untreated LD can progress to severe and prolonged cardiac, neurologic, and arthritic complications, with a financial burden exceeding $345-968 million USD per year (Centers for Disease Control and Prevention 2021, Hook et al. 2022).

Given the enormous toll of LD on human health, a model that can forecast high-risk times and places could improve surveillance and intervention, particularly with changes due to global climate change. However, such a model is challenged by the complexity of the ecology in the western US. In California, the bacterial agent of LD, *Borrelia burgdorferi* sensu lato, is transmitted to humans primarily by the nymphal stage of the western black-legged tick, *Ixodes pacificus*. This tick has a 3-yr life cycle (Marsh and Bennett 1986, Casher et al. 2002), which imposes one-year (y-1), two-year (y-2), and three-year (y-3) time lags on LD dynamics, corresponding to influences during larval, nymphal, and adult cohort years (Figure 1).

Complexity in *I. pacificus* ecology also derives from extensive variability in its hosts. This avid human-biter feeds on more than 100 species of lizards, birds, and mammals (Castro and Wright 2007). The majority of adult *I. pacificus* feed on deer, while lizards, especially the western fence lizard (*Sceloporus occidentalis*), support more larval and nymphal *I. pacificus* than all birds and mammals combined (Westrom et al. 1985, Lane and Loye 1989, Casher et al. 2002, Eisen et al. 2004a). This heavy reliance on lizards contrasts with the ecology of Lyme disease in other parts of the US, where adult *I. scapularis* similarly feed on deer but juveniles feed primarily on reservoir competent rodents such as the white-footed mouse, *Peromyscus leucopus* (Ostfeld and Keesing 2000). In principle, one might be able to model tick activity from data on host and abiotic influences. Unfortunately, there is a paucity of data on *I. pacificus* from long-term and large spatial scale studies. Worse, there are no quality long-term data on its hosts, because acquiring such data is labor- and time-intensive and thus generally cost-prohibitive.

An alternative predictive approach could be to use readily available climate data, given climate's strong influence on lizards and ticks. Specifically, fence lizards' activity is regulated strongly by temperature, even to the point of incapacitation when body temperatures fall below 10°C (Marsh and Bennett 1986). *Ixodes pacificus'* development, activity, and phenology also are heavily influenced by temperature and humidity. There are absolute temperature and humidity envelopes outside of which *I. pacificus* cannot survive (Eisen et al. 2016), their nymphal density increases with rainfall, their numbers decline once maximum air temperatures exceed 21-23°C when relative humidity is less than 83-85% (Eisen et al. 2016), and the speed of development from one life stage to the next increases with temperature (Peavey and Lane 1996, Padgett and Lane 2001). Moreover, during the annual onset of hot and dry weather in late spring, the newly fed larvae molt into nymphs and then enter behavioral diapause until the next spring in order to survive (Padgett and Lane 2001).

Here, we take advantage of long-term monitoring of nymphal *I. pacificus* at a *B. burgdorferi*-endemic site in northern California with a decade of baseline data, followed by data from a decade of drought, and ultimately wildfire in 2018. We analyze contemporaneous and time-lagged climate influences on nymphal tick densities. We aimed to: (1) characterize temporal patterns of nymphal *I. pacificus* density, (2) detect abiotic drivers that could account for the observed temporal patterns, and (3) forecast how climate change could influence tick density.

Methods

Study site

Nymphal tick data were collected at the University of California, Hopland Research and Extension Center (HREC) in southeastern Mendocino County (Lat: 39.0433, Lon: -123.0841). A map of the study region has been published previously (Timm and Vaughn 2003). HREC is a 2,168-ha agricultural research facility on the western slopes of the Mayacamas Mountains in the Russian River Valley. Average annual precipitation is 940–1,140 mm depending on altitude, with 75% of it falling during winter when mean temperatures are 7–8°C (Hopland Research and Extension Center 2024). Average summer temperatures are 21-33°C, to a maximum of 43°C. The 8-ha James II site, comprising a moderately steep upper flat and a more level lower flat, sits at about 665 m above sea level on a ridge with a western aspect and average slope of 19%. Prior to the Ranch Fire, canopy vegetation was predominantly mature *Quercus kelloggii* (California black oak) and *Arbutus menziesii* (Pacific madrone), with an understory of dense leaf litter, woody debris, and low herbaceous vegetation.

Phase I tick sampling

Phase I sampling of ticks was conducted at least twice (range 2-14 times) each spring from 1997–2007 on two established 450 m^2 grids at James II, one on each flat. Each grid was subdivided into 30 transects of 15 × 1 m^2 for a total of 900 m^2. Two people concurrently dragged a 1.0 × 1.25 m white flannel cloth with a chain hemmed into its trailing edge through leaf-litter. Ticks were identified to species with a hand lens and then released in the center of the transect from which they had been collected. Nymphal tick density was reported as mean ticks/450 m^2.

Phase II tick sampling and comparison in tick numbers across phases

In Phase II from 2014–2018, we switched to timed sampling through both leaf litter and woody debris, removing ticks for pathogen testing associated with other studies. Two people each conducted sampling for two 1-hr periods once a year in late May. In July 2018, the Ranch Fire ignited and killed some canopy trees outright, extensively reduced canopy coverage through limb destruction, and removed most of the leaf litter from the ground. After the Ranch Fire, we performed time sampling in April, May, and June in 2019 and April and May in 2020. Dragging was discontinued in June 2020 because of impenetrable understory overgrown with forbs and grasses due to succession after such extensive habitat loss (Pascoe et al. 2023).

To estimate the distances sampled and to correct for biases that can occur during timed sampling — such as incongruous walking rates — participants wore personal trackers (Charge 2 tracker, Fitbit, San Francisco, California) during the April and June 2019 sampling events. The mean distances traveled by both people combined during April was 3,910 m^2 and 2,688 m^2 in June 2019, for an overall mean of 3,299 m^2/2 people/2 hrs. To compare tick abundances across Phases I and II, an overall index for modeling the Fitbit data was calculated. This index distance then was used to convert all timed-sampling data obtained from 2014 to 2020 to temporal abundance (number nymphs collected across two hours by two subjects/3,299 m^2).

Climate data

Climate factors that could influence tick density were obtained from the Parameter-elevation Regressions on Independent Slopes Model (PRISM) dataset (PRISM Climate Group 2022). We explored daily data for precipitation, minimum temperature, mean temperature, maximum temperature, mean dew point temperature, minimum vapor-pressure deficit, and maximum vapor-pressure deficit for the 4 km region around the tick collection site for all years for which we had tick data as well as the three years prior. A version of the August-Roche-Magnus equation was used to calculate the daily relative humidity (RH): $100 \cdot \frac{\exp\left(\frac{17.625 \cdot TD}{243.04+TD}\right)}{\exp\left(\frac{17.625 \cdot T}{243.04+T}\right)}$ where *TD* is the mean daily dew point temperature and *T* is the mean daily temperature (both in °C) (US Environmental Protection Agency NA). An estimate of the daily relative humidity in leaf litter (RHleaf) was calculated using: RHleaf $= \frac{100}{1+7.2145 \cdot \exp(-0.0559 \cdot RH)}$ (Berger et al. 2014).

Statistical analysis

We used Poisson generalized linear models of nymphal density during Phase I. To control for the seasonal trend in tick density, we added a quadratic function for day of the year (i.e., $\text{day of year} + \text{day of year}^2$) to every model. Putative explanatory variables for nymphal density were the seven daily PRISM variables and estimated daily RHleaf. We averaged our outcome variable (the number of nymphs per 450m^2) across both plots and rounded up to the nearest integer. Timing and amplitude of nymphal density as a function of the seven PRISM variables and RHleaf were explored visually, incorporating 1, 2, or 3-yr time lags as appropriate based on the ecology of the tick and its hosts (Figure 1). Specifically, we plotted nymphal density against weather conditions in fall through early spring y-3 (when adults are active), year-round y-2 (eggs), spring through early summer y-1 (larval activity), and spring of the current year (nymphs). Time-lag analyses excluded 1997 because tick collections that year began mid-May, later than the following 10 years.

We defined seasons as: spring March 1–May 31, summer June 1–August 31, fall September 1–November 30, and winter December 1–February 28 or 29. We analyzed the impacts of PRISM variables, aggregated across different temporal periods, on the timing and amplitude of nymphal density. We plotted the data to identify possible linear, quadratic, or exponential functional forms for these relationships. We explored discrete numeric climatic variables that captured conditions across a given temporal period (herein called temporal-climatic windows, such as the number of days during the first 15 days of March on which conditions exceeded 12.5 °C and 80% RHleaf). Optimization of cutoffs for temperature was conducted at 0.25°C steps and for humidity by percentage points, similar to previous work (Mordecai et al. 2017). We selected cutoffs for these windows from sensitivity analysis using linear regression between the outcome variable and different cutoffs for both the period of time and the climatic predictors. We selected optimal discrete variables to be those that maximized the adjusted R^2. When we observed exponential relationships, we explored upper bounds using simulated data for the entire range of the temporal-climatic window (e.g. 0–15 if evaluating the temporal-climatic window extending through the first 15 days of March) and imposed a cap on the effect of that temporal-climatic window.

We selected the optimal model (designated “final baseline model”) from among candidate Poisson GLMs based on McFadden’s pseudo R^2’s calculated using the pscl library (Jackman

2020). Model performance was evaluated for both the overall data as well as for a subset of the data comprising only those years whose highest density was in the top 3rd quartile of all 11 years in Phase I (designated "high density years"). The final baseline model was then used to forecast Phase II tick activity; when drought occurred during Phase II, we assumed the effect of the temporal-climatic window was 0. We evaluated model performance based on the similarity between model predicted and observed nymphal tick densities using the root square mean error (RMSE).

Finally, the drought-adjusted model was used to forecast tick density into the future (Phase III) under four recommended global climate models (Pierce et al. 2018). We used HadGEM2-ES (warm/dry), CNRM-CM5 (cool/wet), CanESM2 (average), and MIROC5 (which captures more disparate possible conditions not represented in the prior three). Daily predicted maximum and minimum temperature and RH data for 2006–2095 were downloaded from Cal-Adapt (Cal-Adapt 2018) and then averaged to provide the daily means. Each model was assessed under RCP 4.5 and RCP 8.5, futures with expected statewide warming of 2–4°C and 4–7°C by the end of the century, respectively. We used the delta-change method (Fowler et al. 2007) to assess the data at finer spatial scales. When this resulted in values for relative humidities beyond feasible levels (i.e. <0 or >100), the values were converted to 0 or 100 as necessary. Similarly, if the predicted temperatures were below levels when ticks would be active and would invert coefficients from the Phase I model (i.e. negatives) producing illogical results, they were set to 0. The data were organized into decades, starting from 2006-2015 and extending to 2086-2095 with years of each decade designated year 1, year 2 etc., to year 10. The daily difference between the predicted and modeled historic values from 1996-2005 for mean temperature and relative humidity were calculated for the corresponding day and year of future decades (e.g., the first day in 1996 was compared to the first day in 2006, 2016, 2026, etc.). The projected difference was added to the observed values from the PRISM dataset from 1996-2005 (e.g., the projected difference in values calculated from comparing the 1st day in 2006 to 1996 was added to the observed values on the 1st day of 1996). We used a second strategy to incorporate drought as well, the Standardized Precipitation-Evapotranspiration Index (SPEI). A more negative SPEI value indicates drier conditions. This portion of the dataset was only available as an average of the 32 global climate models for each scenario (Thomas et al. 2018), and thus the same data was applied across all 4 simulation models for each respective scenario. Years were classified as drought years if more days were projected with a 1-month SPEI at or below -1 compared to the maximum modeled in the Phase I time period when no drought occurred. The optimal model from the Phase I analysis with the adjustments made for drought during the Phase II analysis was then used to predict tick densities under the 8 climate scenarios. All statistical analyses were performed in R v4.1.2.

Results

A total of 1,833 *I. pacificus* nymphs were collected during 73 sampling events during Phase I spanning 1997–2007. An additional 784 *I. pacificus* nymphs were collected over 10 sampling occasions in Phase II from 2014–2018. The density of nymphs ranged from 0-65 per 450 m^2 (supplementary Table 1). Each year, the “peak density” of nymphs occurred in either April or May (supplementary Figure 1). We designated those years with peak densities in the 3rd quartile of all years as “high-density years”, which in Phase I were 1998, 2002, and 2005. The only high-density year in Phase II was 2014.

Predicting peak nymphal host-seeking activity assuming no time lag

An increasingly complex set of models incorporating climate and time lags was examined to best account for the patterns observed in densities of host-seeking nymphs for each day of the year sampled during Phase I. In initial models with no time lag, climate variables poorly accounted for the fluctuations in density. A "seasonal" Poisson generalized linear model that only included influence of day of year captured the increase and subsequent decrease in nymphal density in spring and summer but failed to explain the higher amplitude in high density years (Supplementary Figure 2a, AIC: 784.82) and produced a McFadden's pseudo R^2 (R^2) of 0.41 for all years but 0.07 for high density years. The model slightly better predicted density of nymphs (AIC 652.28, R^2: 0.50) when mean daily dew point (calculated as TD= $temperature - \frac{100-\mathrm{RH}}{5}$) was added as a linear function, and slightly better again when replacing the linear function with a quadratic function of daily mean temperatures and RHleaf instead (Supplementary Figure 2b, AIC: 663.77, R^2: 0.51). However, these two models still failed to explain the amplitude in high tick density years (R^2 of 0.27 and 0.29, respectively), motivating further model candidates with time lags and focusing on the week in which densities of nymphs peaked and the amplitude of those peaks.

Harsh May conditions in y-1 capture the timing of peak nymphal host-seeking activity

The second series of candidate models examined relationships between the timing of tick activity and climate conditions during y-1, y-2, and y-3. The observed peak timing of nymphal activity was strongly negatively correlated with the number of days during the prior May (y-1) when mean temperatures $\geq$ 22.25 C and RHleaf $\leq$ 77% (adjusted R^2=0.94, Figure 2). The observed timing of nymphal peaks ranged from day 98 to 145 (median 126). For every additional day during the prior May with these climate conditions, peak activity occurred 3.42 days earlier.

This relationship between peak activity and hot, dry conditions the prior May is consistent with induced early initiation of diapause in recently molted nymphs. As a result of entering diapause early, those same nymphs a year later would have limited fat reserves and would have to terminate diapause early to quest and survive (Lees and Milne 1951, Nieto et al. 2010). In previous work, unfed nymphs in a similar habitat survived for a maximum of 325 days (Nieto et al. 2010). This region almost always has 10 or more heat events (defined as temperature ≥ 22.25 C and RHleaf ≤ 77%) per year; *I. pacificus* survives these events by entering diapause, and does so early if heat events occur earlier, i.e., by May. Supplementary Figure 3a reveals that peak density of nymphs always occurred within 325 days of the first 10 heat events of the year prior. Two other plausible candidate predictors, current temperature and photoperiod, which we included as day of year, failed to explain the observed patterns in tick activity (Supplementary Figure 3b, which shows both the moving 5-day average in y-0 mean spring temperatures and day of year). Explicitly incorporating the shift to earlier diapause into the model improved fit both to observed nymphal timing as well as the seasonal quadratic relationship in nymphal density (Supplementary Figure 4). Early diapause was incorporated into the baseline model by adjusting the “day of year” seasonal term using our “diapause-shift equation”: day of year + (coefficient of the slope, i.e., -3.42 * number of days during May the year prior with mean temperatures ≥ 22.25 C and RHleaf ≤ 77%).

Elevated March temperatures from the year prior predict years with high tick density

For each of the three Phase I high-density years, the year before had been particularly hot, specifically with temperatures during March being the hottest of the 11-year sampling period. Visual exploration revealed a strong positive exponential relationship (adjusted R-squared: 0.92, Supplementary Figure 5) between peak nymphal density and the number of days during the previous March. Based on this exploration, we detected an optimal temporal-climatic window for this relationship and defined it as the “tick-host climate optimum”, THCO. We successfully modelled the increased nymphal density in all three high-density years (R^2: 0.69, high density years R^2: 0.73, Figure 3) by adding the number of days from March–April 5th with average temperatures >10°C and maximum temperatures < 26°C, raised to 9.8 to the baseline model (AIC:429.4). Due to limitations in our data, we were unable to measure the upper limit of the effects of the THCO (assuming an infinite number of larvae and hosts). Consequently, we made the following adjustment: The model predicted tick densities exceeding 200 nymphs per 450 m^2 for THCO values greater than 2, which were not previously observed in the habitat. To address this, we imposed an upper limit on the effect of the THCO at 22 days (Figure 4). Our final baseline model was:

$$Average\ nymphal\ density = e^{(\beta_0+\beta_1 x_1+\beta_2 x_2+\beta_3 x_3+\beta_4 x_4+\beta_5 x_5+\beta_6 x_6)}$$, where

Average nymphal density = number of nymphs/450m^2,

x_1 = (optimal number of days in TCHO)$^{9.8}$

x_2 = day of year shifted to account for harsh May, y-1,

x_3 = (day of year shifted)^2,

x_4 = mean daily temperature (C□),

x_5 = (mean daily temperature)^2, and

x_6 = relative humidity in the leaf litter

Phase I nymphal density model successfully predicts Phase II data

California experienced a prolonged drought after Phase I beginning in late spring of 2007, with mean RHleaf falling below the lowest value observed throughout Phase I. RHleaf was similar or even more severely reduced in 2008, 2014, 2015, 2016, and 2021. Before correcting for drought, our final baseline model from Phase I over-predicted tick density (RMSE: 37.02), particularly in 2016 when it predicted 131 ticks would be observed at the sampling event but only 22 were recorded. We accounted for drought by setting the THCO to 0, essentially allowing dry conditions to nullify the positive effects of a hot March. This improved the model's predictive capabilities (RMSE=9.30). The model then was successfully able to predict that 2014 would experience a high tick density and that no other year during Phase II would (Figure 5). Unsurprisingly, the model predicted higher than observed densities in 2018, 2019, and 2020 which, due to the Ranch Fire, were some of the lowest on record.

Climate change will result in earlier activity of nymphs

Projections in tick activity generally trended upward with RH but did not change when more drought was projected. When we used RHleaf projections to predict drought, 7 of the 8 climate projection models suggested that the number of years when there would be high nymphal densities would trend upward. However, half of the models started to trend downwards by the end of the century (Figure 6a). The number of years per decade experiencing high nymphal densities ranged from 0-8 (more than double what was seen in Phase I). In the first round of climate change modeling, we used the same drought predictors based on RHleaf as we used in the Phase II model and our results indicated adequately high RH for ticks during much of the century. However, the climate projection models may under-predict drought and do not explicitly incorporate risk of wildfire. To better incorporate drought, we set the effect of the THCO to zero if drought was predicted using the SPEI drought index. With this adjustment, no change in high-density years was projected across all climate models. The number of years per decade experiencing high nymphal densities ranged from 0-6 (double what was seen in Phase I) but leveled off by the end of the decade (Figure 6a).

By the end of the century in all climate models, we saw nymphal emergence, peak host-seeking activity, and dormancy shifting to earlier in the year (Figure 6b). The least amount of change was seen under CNRM CM5 RCP 4.5, which projected events changing by 1–14 days. The largest impact was seen under HadGEM2 ES RCP 4.5, which projected an increase in the length of activity by 45 days, extending from the end of January until mid-August. Nymphal emergence was projected to begin as early as January, while peak activity was projected to shift to as early as the end of March under HadGEM2 ES RCP 8.5.

Discussion

Models incorporating biotic and climate drivers can forecast changing risks of disease transmitted by some *Ixodes* species (Jones et al. 1998, Schulze et al. 2009, Eisen et al. 2016, Esser et al. 2020, Rubel and Brugger 2020), but none yet predict risks of LD in the far-western US, in part due to the dearth of long-term tick data (Eisen et al. 2012), until now. We present a unique predictive analysis – relying on readily available climate data – of abundance and timing of host-seeking nymphal ticks, incorporating time lags and nonlinear influences. Our results from

18 years of data demonstrate that nymphal *I. pacificus* activity is well-predicted by climatic conditions during May and March of the preceding year, with timing of emergence explained by hotter and drier conditions in May and high densities associated with elevated March temperatures.

Earlier emergence following years when May was particularly hot and dry may occur because nymphs were forced into early diapause. In contrast with many ixodid species in which a programmed diapause is triggered by changing photoperiod, *I. pacificus* nymphs enter a behavioral diapause when triggered by harsh climatic conditions (Padgett and Lane 2001, Eisen et al. 2002, Berger et al. 2014, Gray et al. 2016). However, limited research has explored the factors that terminate *Ixodes* spp. diapause (Gray et al. 2016). Nieto et al. (2010) demonstrated that unfed ticks died within 325 days in both field sites and incubators, consistent with them having depleted some finite resource, likely fat. During a relatively mild early summer, ticks can enter diapause later with sufficient reserves to remain in diapause longer the next year. However, hot and dry conditions compel newly molted nymphs to enter diapause early. These ticks also expend energy reserves moving down into leaf litter to avoid potentially lethal hot, desiccating conditions (Lees and Milne 1951, Nieto et al. 2010). A distinct peak in activity may be anticipated the following year, as their fat reserves are depleted. This interpretation is supported by our model: the peak density of nymphs in Hopland occurred between the 98th and 145th days of the year and was strongly associated not with current temperature conditions but with May conditions of the year before.

While May had the strongest influence on timing of emergence the next year, March and early April predicted densities. During this early spring period, temperatures from 10°C-25°C provide optimal conditions both for larvae to feed (Lane et al. 1995, Eisen et al. 2016) and their lizard hosts to begin their spring activity. *S. occidentalis* begins to stir at 10°C but is limited in activity until approximately 25°C (Marsh and Bennett 1986). Notably, *S. occidentalis* acquires most *I. pacificus* ticks nocturnally while asleep in soil (Lane et al. 1995). Therefore, this temporal-climatic window provides ticks with ideal thermal conditions to locate lizards, while simultaneously restricting lizards from being too active for successful larval infestation. The fit of our model predicting nymphal activity using air temperatures was very strong even though temperatures of lizard skin and the soil and leaf litter where larval *I. pacificus* seek hosts will differ slightly (Lane et al. 1995). The strength of the relationship supports that larval tick dependence on lizards determines nymphal population density.

We also expect climate to influence the prevalence of *B. burgdorferi* in host-seeking nymphs. *Ixodes pacificus* passes *B. burgdorferi* transstadially from larvae to nymphs and from nymphs to adults, but not transovarially from adult females to their offspring (Schoeler and Lane 1993)(Z. Barrand, unpubl. data from HREC, 2021). Despite primarily feeding on lizards and to a lesser extent on more than 100 species of birds and mammals (Castro and Wright 2007), larvae and nymphs only acquire the infection from reservoir-competent hosts such as the dusky-footed woodrat (*Neotoma fuscipes)* and the western gray squirrel (*Sciurus griseus*) (Brown and Lane 1992). The primary maintenance host of *I. pacificus* adults at HREC, *Odocoileus hemionus columbianus* (Columbian black-tailed deer)*,* is reservoir-incompetent, though deer can amplify tick populations (Westrom et al. 1985, Lane et al. 1994). The predominant feeding on reservoir-incompetent lizards in the Far West tends to reduce the density of spirochete-infected nymphs because the lizards have borreliacidal proteins of the alternative complement pathway in their blood (Lane and Quistad 1998).

The ecology of LD in the eastern US, in a primarily *I. scapularis*-white footed mouse-deer cycle, is very similar to the far-western US but with the key difference of reliance on lizards for juvenile tick feeding in the west. Modeling from the eastern US revealed a higher density of nymphs two springs after acorn masting, a pulse resource to which rodent populations have a positive numerical response while cervid hosts have a behavioral response (Ostfeld et al. 1996, Jones et al. 1998, Ostfeld et al. 2006, Ostfeld et al. 2018). In contrast, we expect densities of *I. pacificus,* feeding on lizards in northwestern California, to be decoupled from acorn or similar resources and to be more closely tied to climate.

Paradoxically, a short-term lizard-removal experiment in mixed evergreen forests in Marin County in the San Francisco Bay Area suggested that *S. occidentalis* may increase LD risk by maintaining high populations of *I. pacificus* (Swei et al. 2011). After one year, many larvae had switched hosts from lizards to woodrats in the six treatment plots from which lizards were removed compared to the controls, there was a significant decrease in the density of nymphs, and a there was a decline in the density of *B. burgdorferi*-infected nymphs. However, a plausible explanation for this outcome would be the remarkable but typical spatial and temporal variability that we often see in *I. pacificus* densities, including in sites where lizards are not manipulated (e.g. (Talleklint-Eisen and Lane 1999, Tälleklint-Eisen and Lane 2000)). In our HREC dataset, we showed 5-fold differences in density of questing *I. pacificus* nymphs across years. LD risk also changes temporally: *B. burgdorferi* infection prevalence in questing nymphs varied up to 6-fold inter-annually in another study in which lizards were not removed (Eisen et al. 2004b). Moreover, there are numerous influences on LD prevalence, including climate, fire, host abundance, and influences of host blood-meal diversity on the tick microbiome and vector gene expression (Swei and Kwan 2017, Couper et al. 2020, Socarras et al. 2021, Ring et al. 2022).

Despite the inherent ecological complexity of LD ecology, we were able to predict year-to-year variation in tick abundance using climate in part because the two datasets (1997-2007, 2014-2020) had opportune features. Phase I was relatively stable climatically with no extreme drought or fire, in contrast with the profound drought culminating in a moderately severe wildfire in Phase II (Pascoe et al. 2023). To compare the two phases, we had to harmonize two different collection methods, one using set linear distance and non-removal sampling, the other using set time with removal which is further described in supplementary text (though literature indicates that results don't vary due to removal or non-removal (Salomon et al. 2020)). Timed sampling had been initiated to obtain an index of inter-annual tick abundance, to facilitate pathogen discovery, and to correct for problems that can arise with distance measurements in uneven terrain. These include collectors who walk at different rates and pausing occasionally to remove heavy tick loads from drags which requires more time to remove.

Analyzing the two phases allowed us to forecast nymphal tick abundance into the future as anthropogenic climate change continues, with the result that nymphal populations are projected to emerge earlier with the passage of time and at considerably greater densities at least in the early part of the changed climate regime. This earlier emergence is consistent with the earlier temporal shifts predicted in other studies (Monaghan et al. 2015, MacDonald et al. 2020). And, as climate data are being continually refined, our model should become increasingly accurate in predicting future tick phenology and LD risk. We emphasize though that the tight fit of our predictions with climate variables is due partly to the strong influences of temperature and RH on both ticks *and* their lizard hosts. Use of this model in other ecosystems would require training with baseline data for those systems, but the model likely would perform well for settings in certain parts of Europe and the southeastern United States where *I. ricinus* and *I. scapularis* immatures, respectively, feed on lizards as well (Majláthová et al. 2006, Ginsberg et al. 2021).

The importance of ecosystem-targeted training data also is apparent in our model's unsurprising "overprediction" of ticks after the fire at the end of Phase II. Given baseline data, the model had not been trained to manage a scenario where virtually all vegetation, leaf litter, and woody debris, including logs where we previously found abundant ticks (Lane et al. 2004), were removed. Although wildfires are expected to become more problematic as climate change progresses, they can have a uniquely devastating impact that convert firmly established habitats to completely different environments (e.g., woodlands to chaparral). Although developed for predicting the timing and peak densities of *I. pacificu*s in one endemic woodland site in northwestern California, the model is generalizable to other ecosystems, provided that several years of baseline data are available.

In the Far West's complex ecology of Lyme disease, a predictive framework that synthesizes available data and accommodates the anticipated impacts of climate change is essential for protecting public health. Understanding drivers of nymphal tick activity will also shed light on risk for exposure to other pathogens transmitted by this tick, including *Anaplasma phagocytophilum* (anaplasmosis) and *Borrelia miyamotoi* (hard tick relapsing fever). We successfully predicted the relative abundance of host-seeking *I. pacificus* with a high degree of accuracy employing readily accessible climate data. Our model is elegant in its simplicity to accomplish its intended goal given the serious challenges in assessing the diverse suite of hosts and ticks that public health workers routinely encounter while conducting surveillance in California. The strong influences of temperature and relative humidity on the interaction of larval ticks and lizards allowed us to forecast nymphal *I. pacificus* densities accurately without incorporating long-term datasets on the abundance of their primary vertebrate maintenance hosts. We posit that this novel approach could facilitate studies by other researchers who delve into the complex population dynamics of *I. pacificus* in diverse forested areas and different geographic regions during the Anthropocene.

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REFERENCES

Berger KA, Ginsberg HS, Dugas KD et al. 2014. Adverse moisture events predict seasonal abundance of Lyme disease vector ticks (*Ixodes scapularis*). Parasit Vectors. 7(1):1-8.

Brown RN, Lane RS. 1992. Lyme disease in California: a novel enzootic transmission cycle of *Borrelia burgdorferi*. Science 256(5062):1439-1442.

Cal-Adapt. 2018. LOCA Derived Data. Data derived from LOCA Downscaled CMIP5 Climate Projections. Cal-Adapt website developed by University of California at Berkeley's Geospatial Innovation Facility under contract with the California Energy Commission, https://cal-adapt.org/. Accessed October 9, 2023.

Casher L, Lane RS, Barrett R et al. 2002. Relative importance of lizards and mammals as hosts for ixodid ticks in northern California. Exp. Appl. Acarol. 26:127-143.

Castro MB, Wright SA. 2007. Vertebrate hosts of *Ixodes pacificus* (Acari: Ixodidae) in California. J. Vector Ecol. 32(1):140-149.

Centers for Disease Control and Prevention. 2021. Signs and symptoms of untreated Lyme Disease, https://www.cdc.gov/lyme/signs_symptoms/index.html, accessed 18 March 2024.

Couper LI, Yang Y, Yang XF et al. 2020. Comparative vector competence of North American Lyme disease vectors. Parasit Vectors. 13(1):1-11.

Eisen L, Eisen R, Lane R. 2002. Seasonal activity patterns of *Ixodes pacificus* nymphs in relation to climatic conditions. Med. Vet. Entomol. 16(3):235-244.

Eisen L, Eisen RJ, Lane RS. 2004a. The roles of birds, lizards, and rodents as hosts for the western black-legged tick *Ixodes pacificus*. J. Vector Ecol. 29(2):295-308.

Eisen L, Eisen RJ, Chang CC et al. 2004b. Acarologic risk of exposure to *Borrelia burgdorferi* spirochaetes: long-term evaluations in north-western California, with implications for Lyme borreliosis risk-assessment models. Med. Vet. Entomol. 18(1):38-49.

Eisen RJ, Piesman J, Zielinski-Gutierrez E et al. 2012. What do we need to know about disease ecology to prevent Lyme disease in the northeastern United States? J. Med. Entomol. 49(1):11-22.

Eisen RJ, Eisen L, Ogden NH et al. 2016. Linkages of weather and climate with *Ixodes scapularis* and *Ixodes pacificus* (Acari: Ixodidae), enzootic transmission of *Borrelia burgdorferi*, and Lyme disease in North America. J. Med. Entomol. 53(2):250-261.

Esser HJ, Liefting Y, Ibáñez-Justicia A et al. 2020. Spatial risk analysis for the introduction and circulation of six arboviruses in the Netherlands. Parasit Vectors. 13(1-20.

Fowler HJ, Blenkinsop S, Tebaldi C. 2007. Linking climate change modelling to impacts studies: recent advances in downscaling techniques for hydrological modelling. Int. J. Climatol. 27(12):1547-1578.

Ginsberg HS, Hickling GJ, Burke RL et al. 2021. Why Lyme disease is common in the northern US, but rare in the south: The roles of host choice, host-seeking behavior, and tick density. PLoS Biol. 19(1):e3001066.

Gray JS, Kahl O, Lane RS et al. 2016. Diapause in ticks of the medically important *Ixodes ricinus* species complex. Ticks Tick-borne Dis. 7(5):992-1003.

Hook SA, Jeon S, Niesobecki SA et al. 2022. Economic burden of reported lyme disease in high-incidence areas, United States, 2014–2016. Emerg. Infect. Dis. 28(6):1170.

Hopland Research and Extension Center. 2024. About the Hopland Research and Extension Center, https://hrec.ucanr.edu/.

Jackman S. 2020. Package: pscl V: 1.5.5.1. Political Science Computational Laboratory with contributions from Alex Tahk, Achim Zeileis, Christina Maimone, Jim Fearon and Zoe Meers.

Jones CG, Ostfeld RS, Richard MP et al. 1998. Chain reactions linking acorns to gypsy moth outbreaks and Lyme disease risk. Science 279(5353):1023-1026.

Kugeler KJ, Schwartz AM, Delorey MJ et al. 2021. Estimating the frequency of Lyme disease diagnoses, United States, 2010–2018. Emerg. Infect. Dis. 27(2):616.

Lane RS, Loye JE. 1989. Lyme disease in California: interrelationship of *Ixodes pacificus* (Acari: Ixodidae), the western fence lizard (*Sceloporus occidentalis*), and *Borrelia burgdorferi*. J. Med. Entomol. 26(4):272-278.

Lane RS, Quistad G. 1998. Borreliacidal factor in the blood of the western fence lizard (*Sceloporus occidentalis*). J. Parasitol. 84(29-34.

Lane RS, Kleinjan JE, Schoeler GB. 1995. Diel activity of nymphal *Dermacentor occidentalis* and *Ixodes pacificus* (Acari: Ixodidae) in relation to meteorological factors and host activity periods. J. Med. Entomol. 32(3):290-299.

Lane RS, Steinlein DB, Mun J. 2004. Human behaviors elevating exposure to *Ixodes pacificus* (Acari: Ixodidae) nymphs and their associated bacterial zoonotic agents in a hardwood forest. J. Med. Entomol. 41(2):239-248.

Lane RS, Berger DM, Casher LE et al. 1994. Experimental infection of Columbian black-tailed deer with the Lyme disease spirochete. J. Wildl. Dis. 30(1):20-28.

Lees A, Milne A. 1951. The seasonal and diurnal activities of individual sheep ticks (*Ixodes ricinus L.*). Parasitol. 41(3-4):189-208.

MacDonald AJ, McComb S, O'Neill C et al. 2020. Projected climate and land use change alter western blacklegged tick phenology, seasonal host-seeking suitability and human encounter risk in California. Global Change Biol. 26(10):5459-5474.

Majláthová V, Majláth I, Derdáková M et al. 2006. *Borrelia lusitaniae* and green lizards (*Lacerta viridis*), Karst Region, Slovakia. Emerging Infectious Disease 12(1895-1901.

Marsh RL, Bennett AF. 1986. Thermal dependence of sprint performance of the lizard *Sceloporus occidentalis*. J. Exper. Biol. 126(1):79-87.

Monaghan AJ, Moore SM, Sampson KM et al. 2015. Climate change influences on the annual onset of Lyme disease in the United States. Ticks Tick-borne Dis. 6(5):615-622.

Mordecai EA, Cohen JM, Evans MV et al. 2017. Detecting the impact of temperature on transmission of Zika, dengue, and chikungunya using mechanistic models. PLoS Neglected Trop. Dis. 11(4):e0005568.

Nieto NC, Holmes EA, Foley JE. 2010. Survival rates of immature *Ixodes pacificus* (Acari: Ixodidae) ticks estimated using field-placed enclosures. J Vector Ecol 35(1):43-49.

Ostfeld R, Keesing F. 2000. Biodiversity and Disease Risk: the case of Lyme disease. Cons. Biol. 14(3):722-728.

Ostfeld RS, Jones CG, Wolff JO. 1996. Of mice and mast. BioScience 46(5):323-330.

Ostfeld RS, Canham CD, Oggenfuss K et al. 2006. Climate, deer, rodents, and acorns as determinants of variation in Lyme-disease risk. PLoS Biol. 4(6):e145.

Ostfeld RS, Levi T, Keesing F et al. 2018. Tick-borne disease risk in a forest food web. Ecol. 99(7):1562-1573.

Changing paradigms for tick-borne diseases in the Americas. Global health impacts of vector-borne diseases: workshop summary: National Academies Press Washington, DC, USA.

Padgett KA, Lane RS. 2001. Life cycle of *Ixodes pacificus* (Acari: Ixodidae): timing of developmental processes under field and laboratory conditions. J. Med. Entomol. 38(5):684-693.

Pascoe EL, Vaughn CE, Jones MI et al. 2023. Recovery of western black-legged tick and vertebrate populations after a destructive wildfire in an intensively-studied woodland in northern California. J. Vector Ecol. 48(1):19-36.

Peavey CA, Lane RS. 1996. Field and laboratory studies on the timing of oviposition and hatching of the western black-legged tick, *Ixodes pacificus* (Acari: Ixodidae). Exp. Appl. Acarol. 20:695-711.

Pierce DW, Kalansky JF, Cayan DR. 2018. Climate, drought, and sea level rise scenarios for California's fourth climate change assessment. California Energy Commission and California Natural Resources Agency Publication Number: CNRA-CEC-2018-006.

PRISM Climate Group. 2022. Oregon State University, https://prism.oregonstate.edu, accessed 7 August 2023.

Ring K, Couper LI, Sapiro AL et al. 2022. Host blood meal identity modifies vector gene expression and competency. Molec. Ecol. 31(9):2698-2711.

Rubel F, Brugger K. 2020. Tick-borne encephalitis incidence forecasts for Austria, Germany, and Switzerland. Ticks Tick-borne Dis. 11(5):101437.

Salomon J, Hamer SA, Swei A. 2020. A beginner's guide to collecting questing hard ticks (Acari: Ixodidae): a standardized tick dragging protocol. J. Insect Sci. 20(6):11.

Schoeler GB, Lane RS. 1993. Efficiency of transovarial transmission of the Lyme disease spirochete, *Borrelia burgdorferi*, in the western blacklegged tick, *Ixodes pacificus* (Acari: Ixodidae). J. Med. Entomol. 30(1):80-86.

Schulze TL, Jordan RA, Schulze CJ et al. 2009. Precipitation and temperature as predictors of the local abundance of *Ixodes scapularis* (Acari: Ixodidae) nymphs. J. Med. Entomol. 46(5):1025-1029.

Socarras KM, Earl JP, Krol JE et al. 2021. Species-level profiling of *Ixodes pacificus* bacterial microbiomes reveals high variability across short spatial scales at different taxonomic resolutions. Genet. Test. Mol. Biomark. 25(8):551-562.

Swei A, Kwan JY. 2017. Tick microbiome and pathogen acquisition altered by host blood meal. ISME J. 11(3):813-816.

Swei A, Ostfeld RS, Lane RS et al. 2011. Impact of the experimental removal of lizards on Lyme disease risk. Proc. Royal Soc. London B: Biol. Sci. 278(1720):2970-2978.

Talleklint-Eisen L, Lane RS. 1999. Variation in the density of questing *Ixodes pacificus* (Acari:Ixodidae) nymphs infected with *Borrelia burgdorferi* at different spatial scales in California. J. Parasitol. 85(5):824-831.

Tälleklint-Eisen L, Lane RSJEE. 2000. Spatial and temporal variation in the density of *Ixodes pacificus* (Acari: Ixodidae) nymphs. Env. Entomol. 29(2):272-280.

Thomas N, Mukhtyar S, Galey B et al. 2018. Cal-Adapt: Linking Climate Science with Energy Sector Resilience and Practitioner Need. California's Fourth Climate Change Assessment, California Energy Commission. Publication Number: CCCA4-CEC-2018-015.

Timm R, Vaughn C. 2003. 'Chapter Research at Hopland: 1951-2001. An annotated bibliography. https://escholarship.org/uc/item'.

US Environmental Protection Agency. NA. HMS: Hydrologic Micro Services, https://qed.epa.gov/hms/meteorology/humidity/algorithms /, accessed 18 Mar 2024.

Westrom D, Lane R, Anderson J. 1985. *Ixodes pacificus* (Acari: Ixodidae): population dynamics and distribution on Columbian Black-tailed deer (*Odocoileus hemionus columbianus*). J. Med. Entomol. 22(507-511.

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Figure 3: Logistic relationship between peak mean abundance of *Ixodes pacificus* nymphs and number of days during March-April 5th of the prior year with mean temperatures >10 and max temperatures <26, defined as the THCO. Created with BioRender.com.

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Figure 4a. Predictions of nymphal *Ixodes pacificus* densities throughout Phase 1 shown by gray lines and observed densities by black points.

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Figure 1.

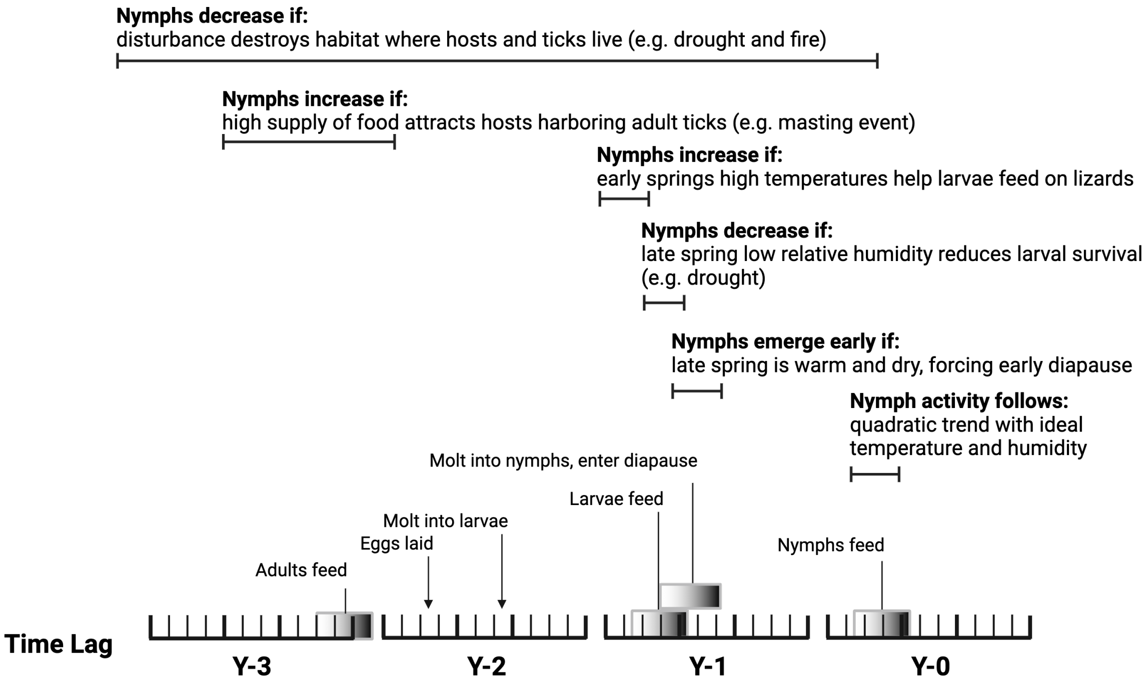
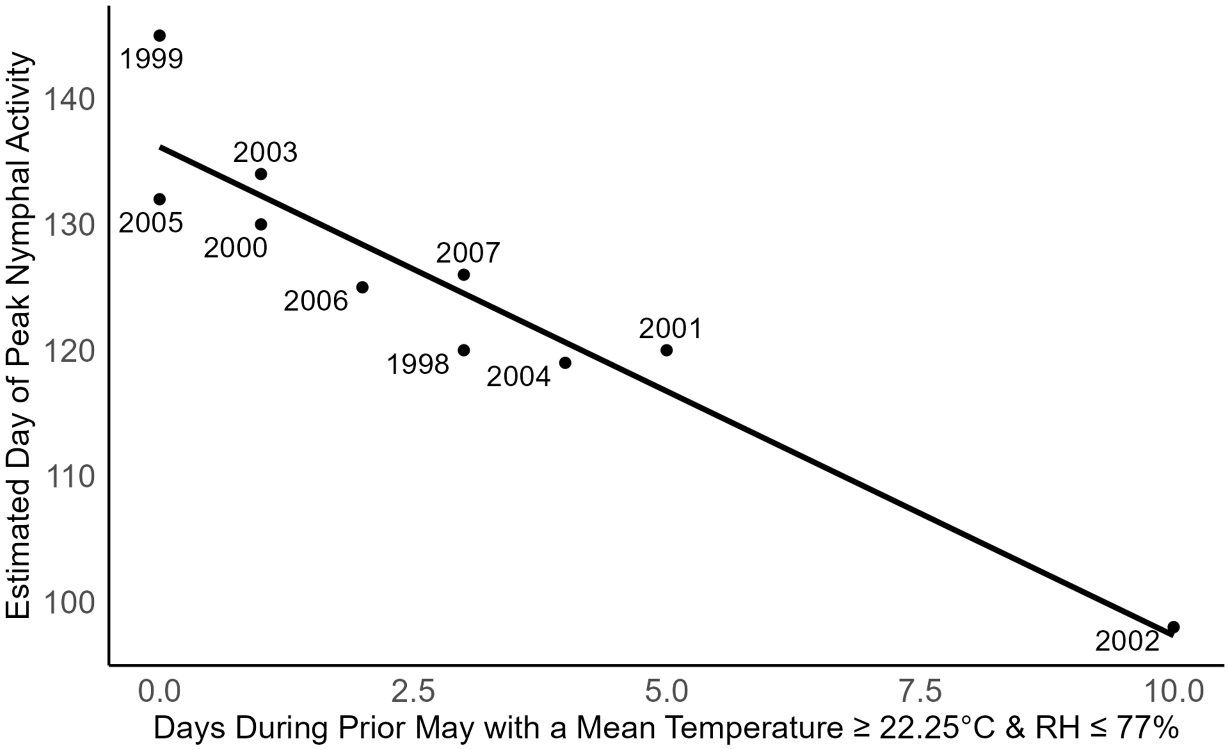


Figure 2.



1999
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110
100
0.0
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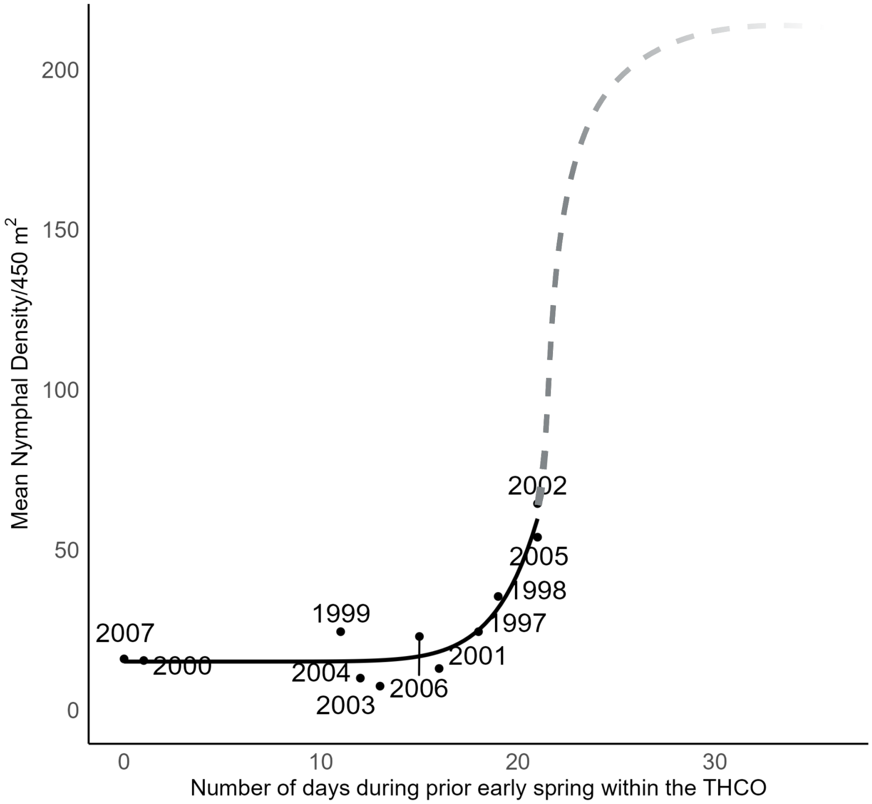
10.0
Estimated Day of Peak Nymphal Activity
Days During Prior May with a Mean Temperature ≥ 22.25°C & RH ≤ 77%

Figure 3.


Mean Nymphal Density/450 m^2
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50
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0
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20
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Number of days during prior early spring within the THCO
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Figure 4a.

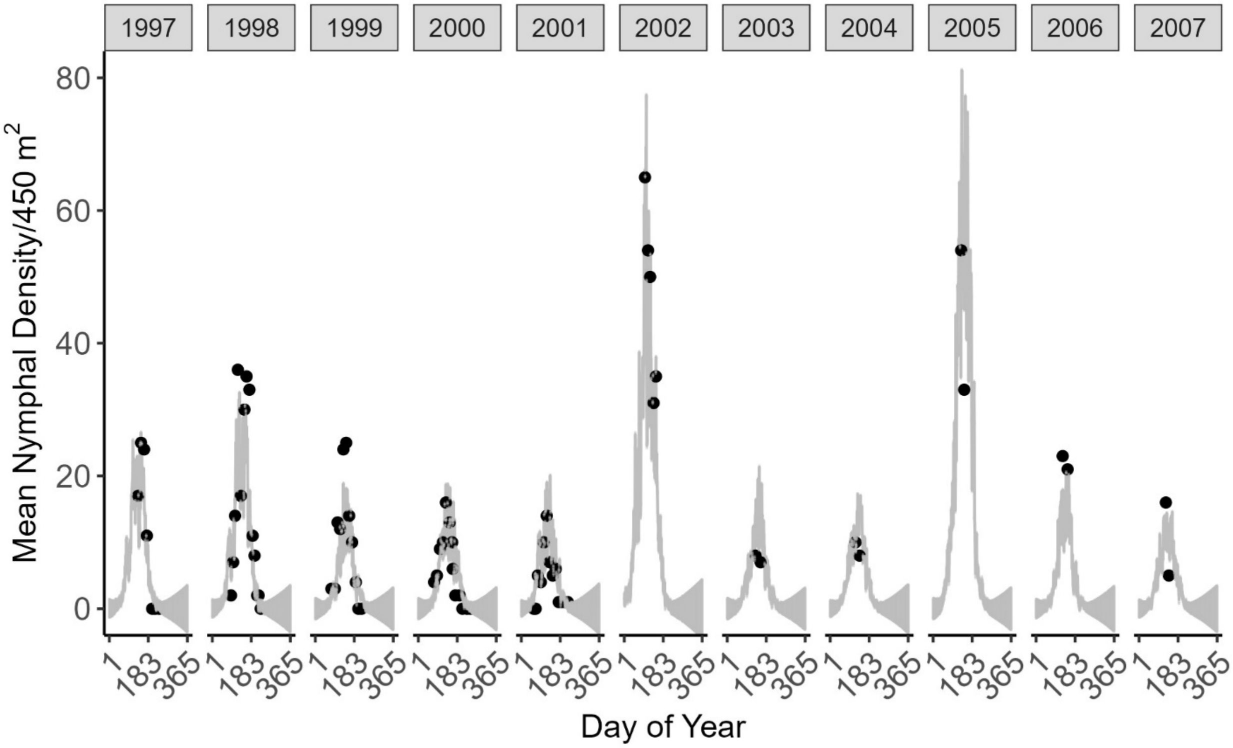


Figure 4b.

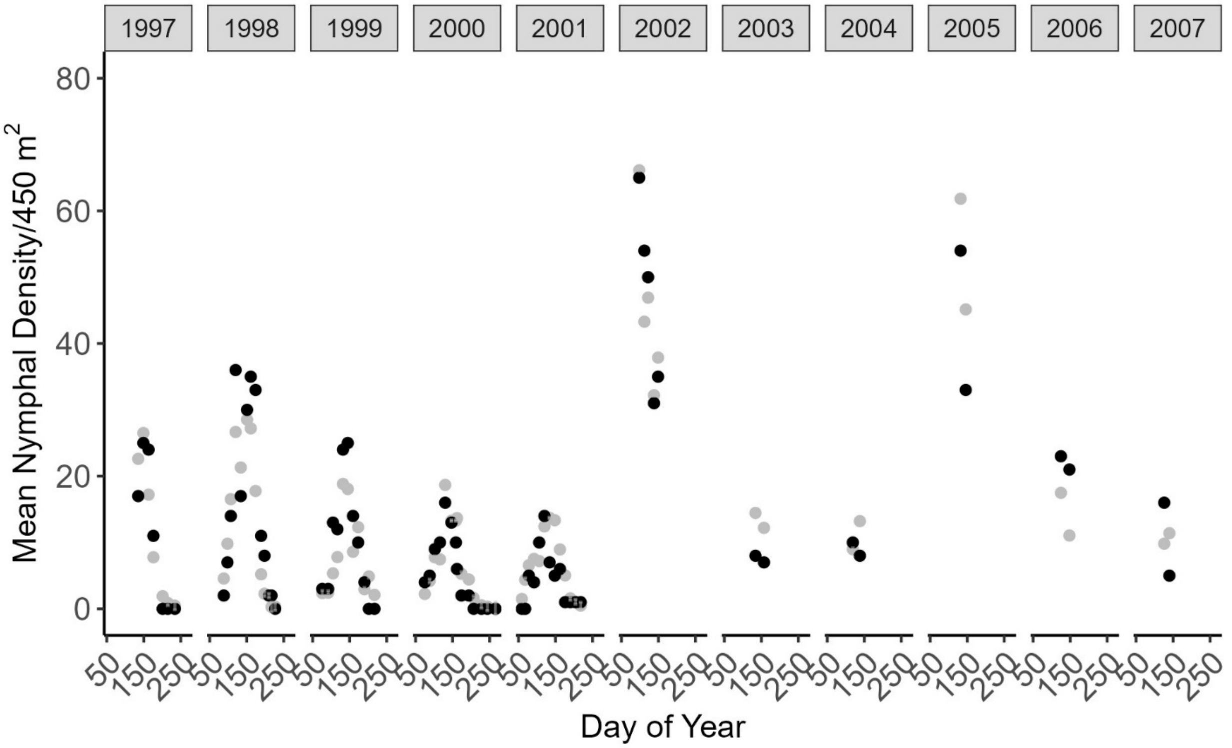


Figure 5a.

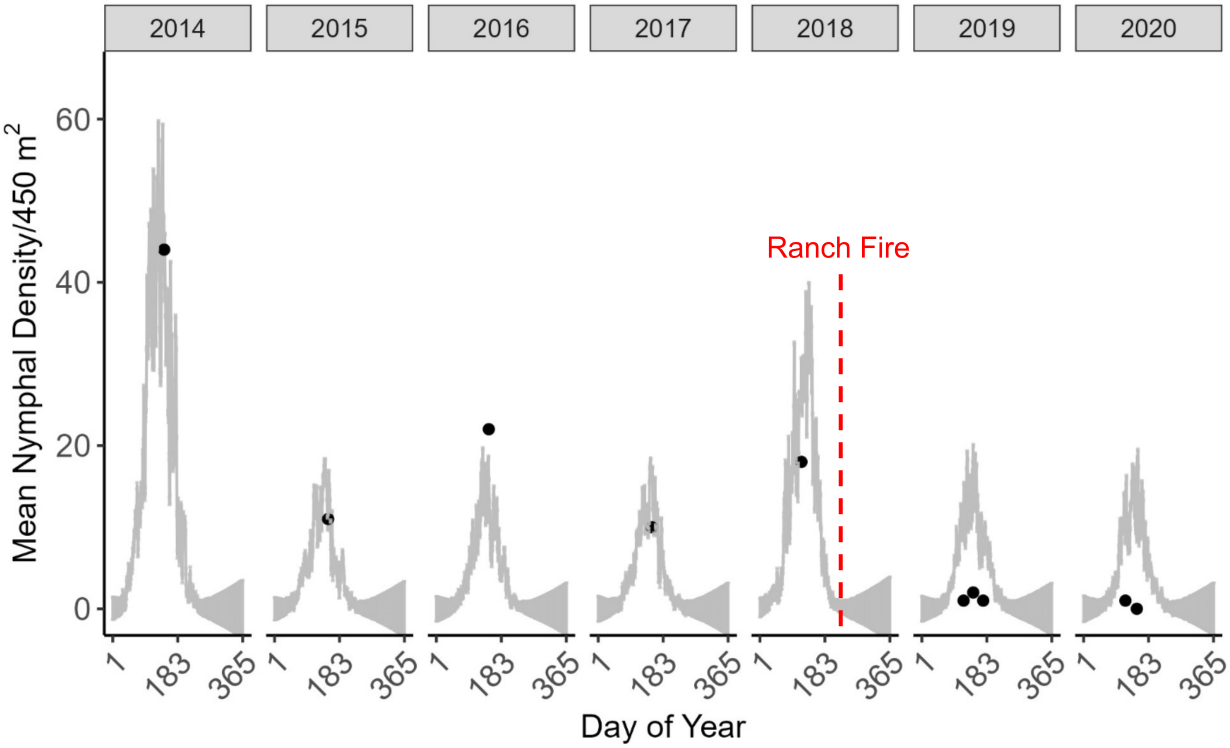

2014
2015
2016
2017
2018
2019
2020
Mean Nymphal Density/450 m^2
0
20
40
60
Ranch Fire
1
183
365
Day of Year

Figure 5b.

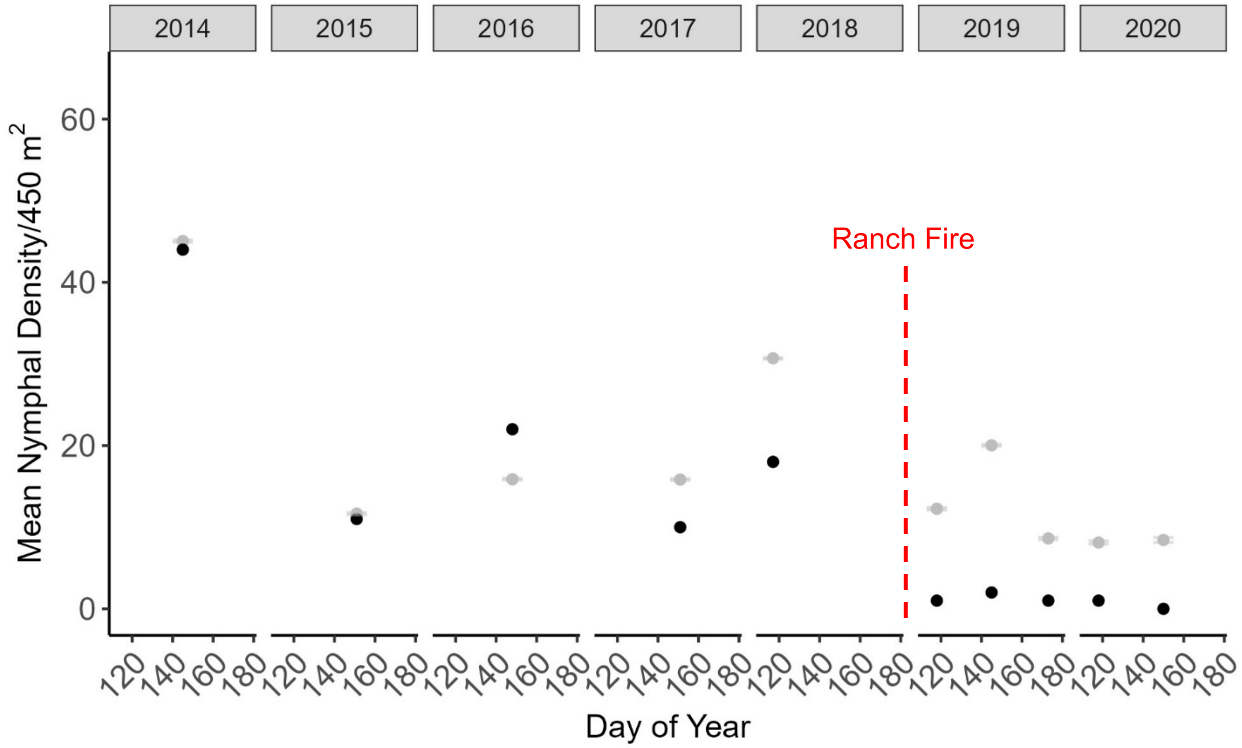

2014
2015
2016
2017
2018
2019
2020
Mean Nymphal Density/450 m^2
0
20
40
60
Ranch Fire
120
140
160
180
Day of Year

Figure 6a.

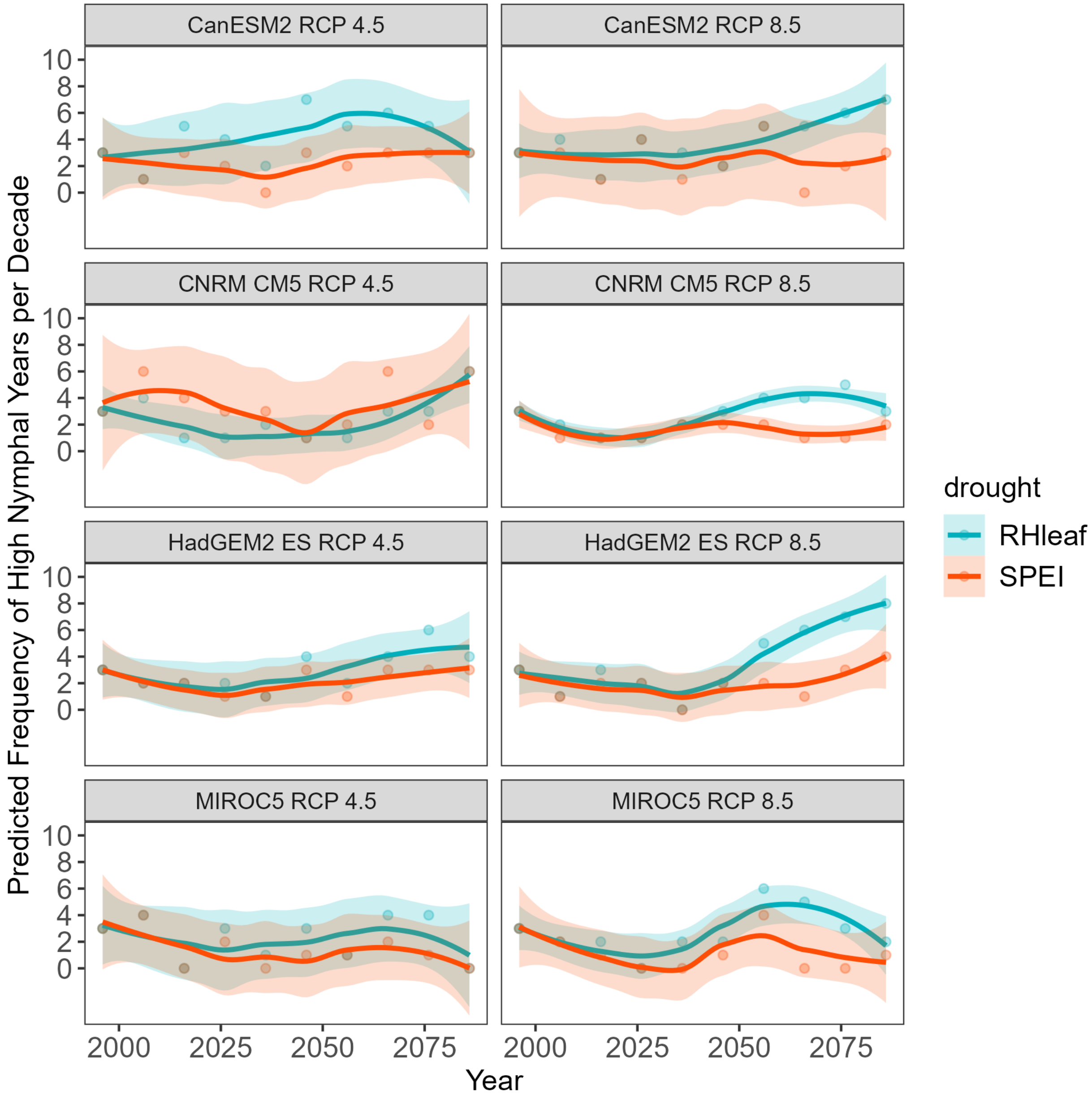
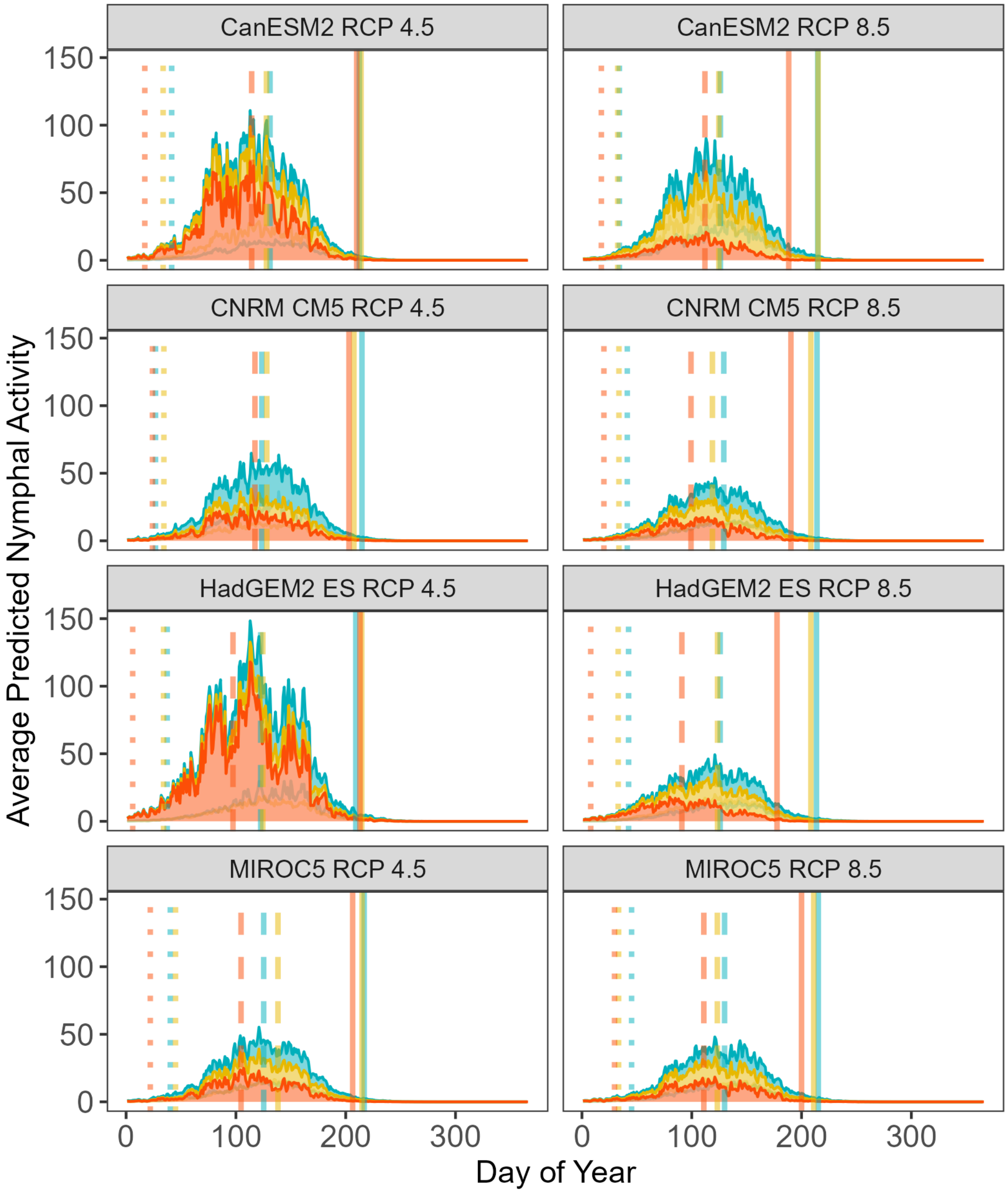

CanESM2 RCP 4.5
CanESM2 RCP 8.5
CNRM CM5 RCP 4.5
CNRM CM5 RCP 8.5
HadGEM2 ES RCP 4.5
HadGEM2 ES RCP 8.5
MIROC5 RCP 4.5
MIROC5 RCP 8.5
Predicted Frequency of High Nymphal Years per Decade
10
8
6
4
2
0
Year
2000
2025
2050
2075
drought
RHleaf
SPEI

Figure 6b.


CanESM2 RCP 4.5
CanESM2 RCP 8.5
CNRM CM5 RCP 4.5
CNRM CM5 RCP 8.5
HadGEM2 ES RCP 4.5
HadGEM2 ES RCP 8.5
MIROC5 RCP 4.5
MIROC5 RCP 8.5
Average Predicted Nymphal Activity
0
50
100
150
Day of Year
0
100
200
300

Supplementary Text:

During timed sampling, we suspected that a Fitbit worn by one of us may have recorded unrealistically lower estimates of the true distances. The units had not been calibrated against each other and a dependable measuring device before sampling was initiated, making it impossible to determine their reliability after the fact. As a result, we had to choose between having no estimate for Phase II data or using the averaged recorded distances employing both Fitbits across 8 person-hours on two dates in 2019 as a means of synchronizing timed and distance-based sampling. Even using this approximation, however, our model was extremely accurate in predicting nymphal densities during Phase II.

Supplementary Table 1: Summary of the mean density of nymphal ticks collected during Phases I and II. Tick collection during Phase I distance-based (consistently 900 m^2) non-removal sampling. Tick collection during Phase II was timed-removal collection. Fitbits were used in April and June of 2019 to measure distances traveled while sampling. The average distances between those dates were used to calculate all mean densities with an asterisk. Mean densities for all other dates were based on standardized transect-line distances.

Table 1.

Phase	**Fire**	**Year**	**Date**	**Mean Nymphal Density/450m^2**
1	Pre	1997	May 15	17
			May 29	25
			June 12	24
			June 25	11
			July 20	0
			August 3	0
			August 22	0
		1998	March 29	2
			April 8	7
			April 17	14
			April 30	36
			May 14	17
			May 31	30
			June 10	35

			June 23	33
			July 8	11
			July 17	8
			July 29	2
			August 5	2
			August 15	0
		1999	March 17	3
			April 2	3
			April 15	13
			April 27	12
			May 12	24
			May 25	25
			June 8	14
			June 22	10
			July 9	4
			July 21	0
			August 5	0
		2000	March 15	4
			March 28	5
			April 11	9
			April 25	10
			May 9	16
			May 26	13
			June 7	10
			June 10	6
			June 22	2

			July 12	2
			July 25	0
			August 15	0
			August 31	0
			September 22	0
		2001	February 28	0
			March 9	0
			March 19	5
			April 2	4
			April 16	10
			April 30	14
			May 14	7
			May 28	5
			June 11	6
			June 25	1
			July 9	1
			July 23	1
			August 6	1
		2002	April 8	65
			April 22	54
			May 2	50
			May 18	31
			May 29	35
		2003	May 14	8
			June 6	7

		2004	April 28	10
			May 17	8
		2005	May 12	54
			May 26	33
		2006	May 5	23
			May 28	21
		2007	May 6	16
			May 20	5
2		2014	May 25	44*
		2015	May 31	11*
		2016	May 27	22*
		2017	May 31	10*
		2018	April 27	18*
	Post	2019	April 28	1
			May 25	2*
			June 22	1
		2020	April 27	1*
			May 29	0*

Supplementary Figures.

Supplementary Figure 1. Density of nymphal *Ixodes pacificus* ticks collected from the Hopland Research and Extension Center (HREC) in south-eastern Mendocino County, California, between 1997-2020. See methods for collection details. The Ranch Fire, which burned from July 2018-January 2019, is denoted by the red line.

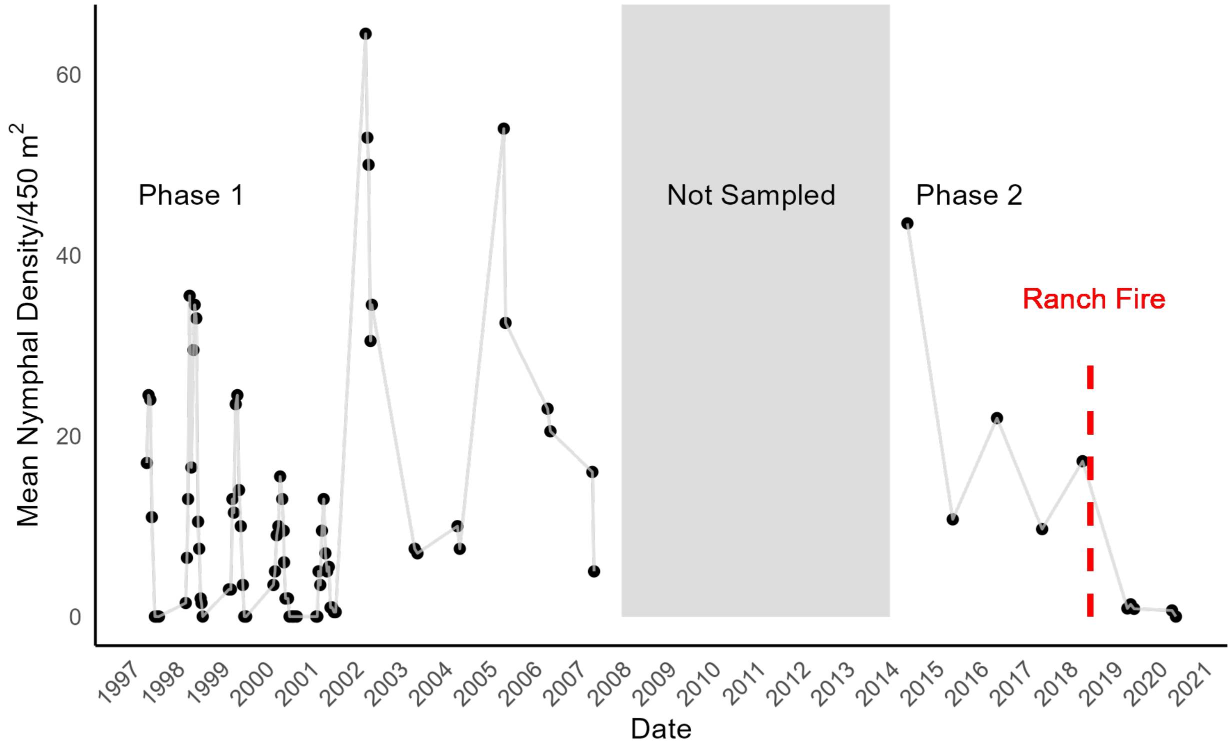
Supplementary Figure 2. Fit of predictive generalized linear models of observed nymphal *Ixodes pacificus* density at the James II woodland, HREC, between 1997-2007.
Figure 2a. Model including only Day of year +(Day of year)^2
Figure 2b. Day of year +(Day of year)^2 + (daily temperature + (daily temperature)^2)+RHleaf
* RHleaf is relative humidity in leaf litter.

Supplementary Figure 3. Possible explanations for the timing of observed peak nymphal host-seeking activity. The observed peak is shown by the solid black vertical line.
Figure 3a. Relationship between the first ten heat events during May the year prior (defined as temperature ≥ 22.25 C and RHleaf ≤ 77%) and the timing of observed peak nymphal host-seeking activity. Each additional heat event is shown in a darker shade, ranging from orange to red. The percent of larvae estimated to survive is shown on the y-axis (from [30]).
Figure 3b. Relationship between photoperiod (depicted as day of year), the moving 5-day average in mean spring temperatures, and the timing of observed peak nymphal host-seeking activity. Red points above the line were hotter and blue points below the line were colder than the 5-day average.

Supplementary Figure 4. Relationships between mean density of nymphal ticks and predictors day of year and its quadratic after adjustments using diapause-shift equation, where Day of year adjusted= Day of year × -3.42 × number of days during May the year prior with mean temperatures ≥ 22.25 C and RHleaf ≤ 77%. Observed data are represented in black; model predictions and 95% confidence intervals are shown in gray.

Supplementary Figure 5: Linear relationship between peak mean abundance of *Ixodes pacificus* nymphs and number of days during March-April 5th of the prior year with mean temperatures >10 and max temperatures <26, defined as the THCO, raised by 9.8 (adjusted R^2 = 0. 0.90).

Supplementary Figure 1.



Supplementary Figure 2.

Figure 2a.

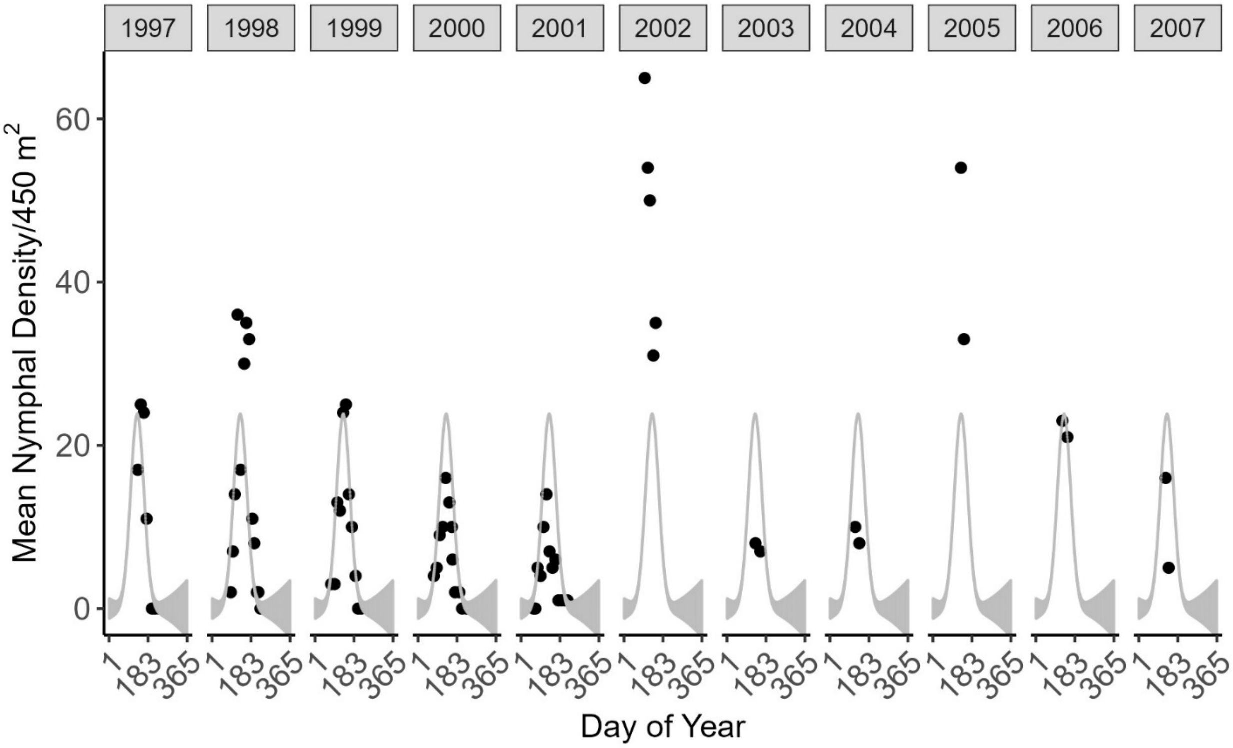
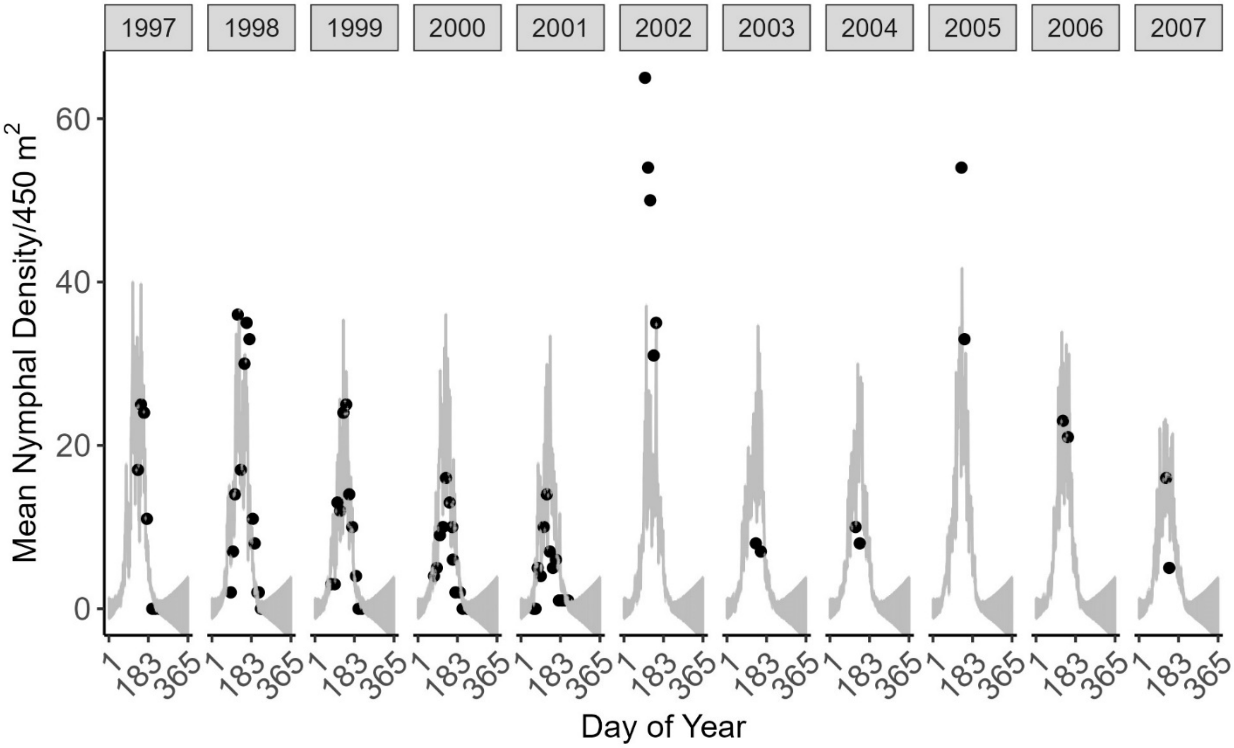


Figure 2b.


1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
Mean Nymphal Density/450 m^2
60
40
20
0
1
183
365
Day of Year

Supplementary Figure 3.

Figure 3a.

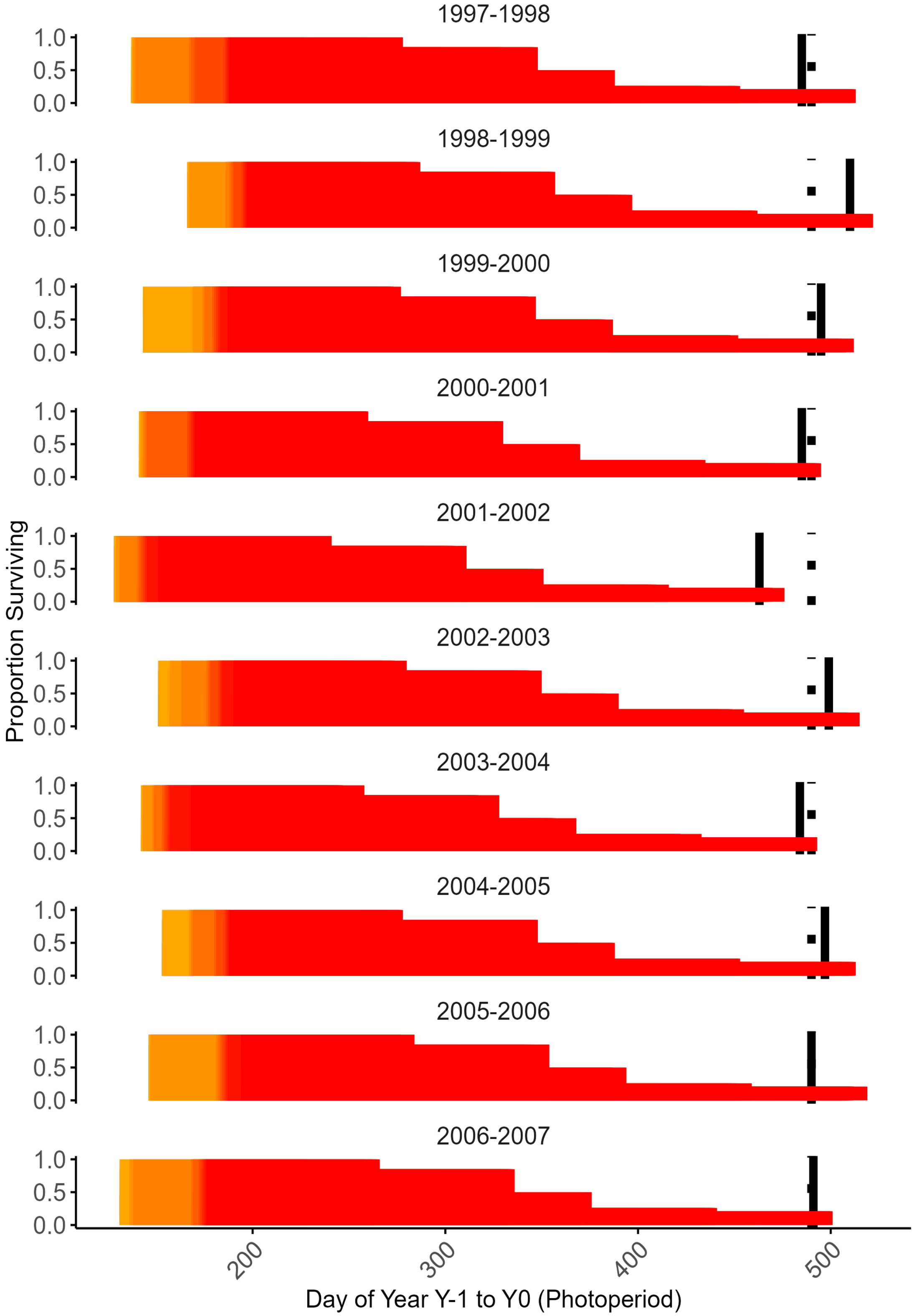
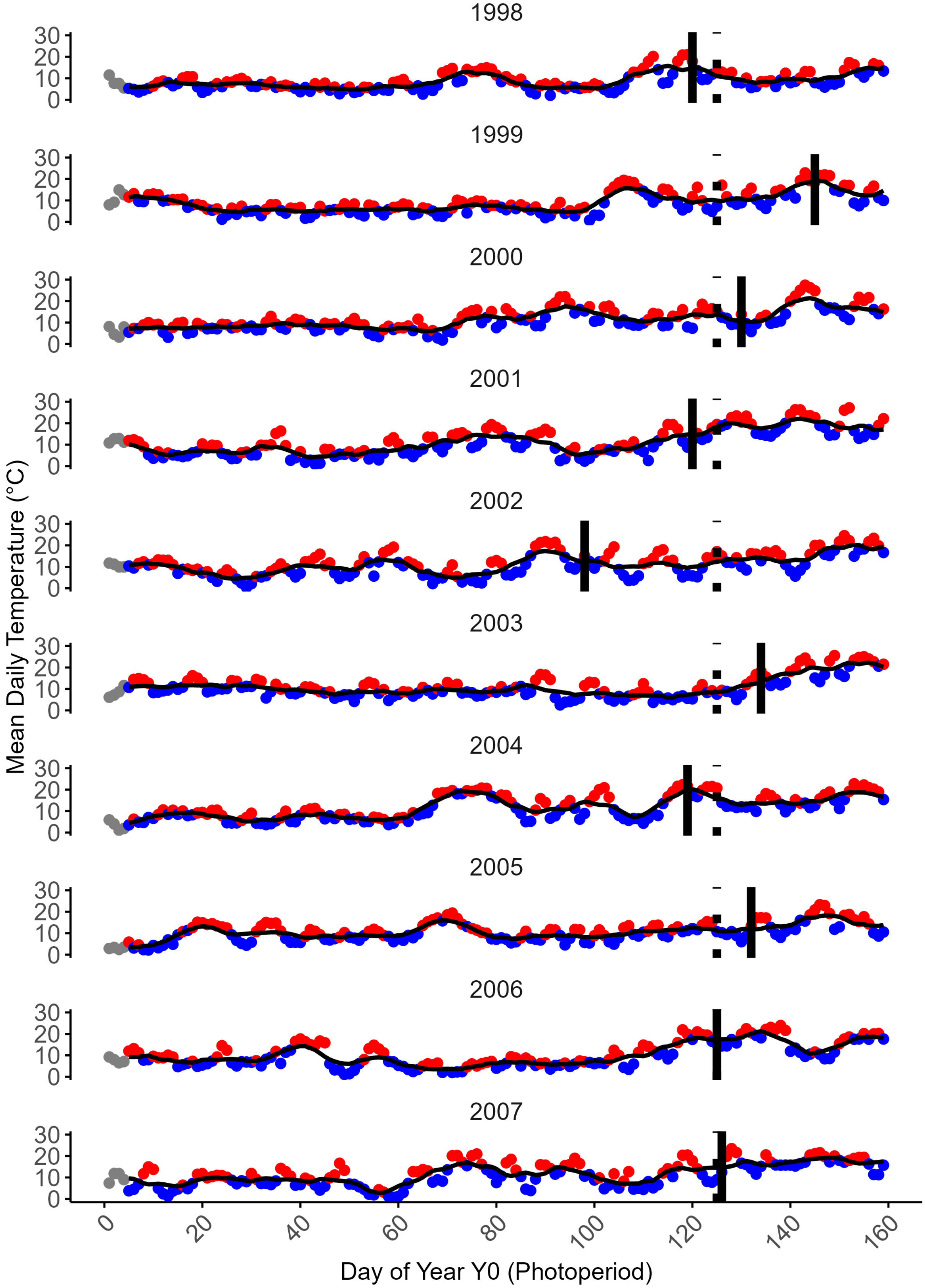
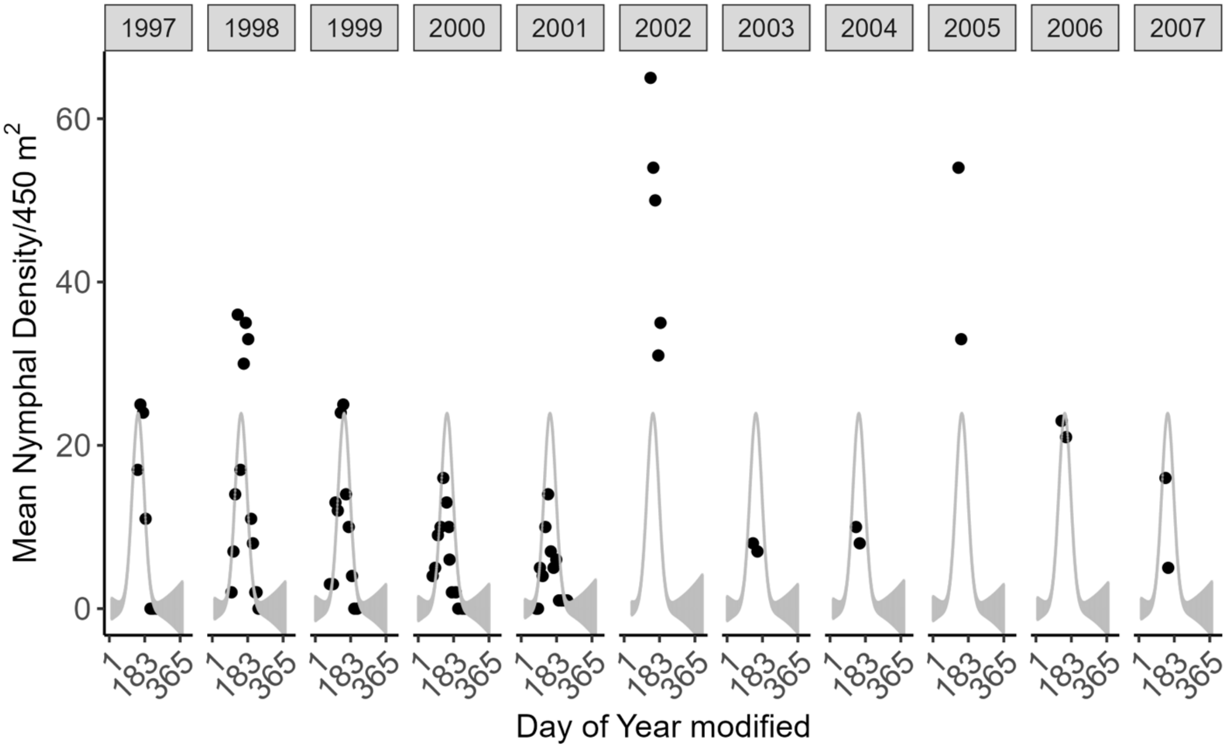


Figure 3b


1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
30
20
10
0
Mean Daily Temperature (°C)
0
20
40
60
80
100
120
140
160
Day of Year Y0 (Photoperiod)

Supplementary Figure 4. (Day of year modified by harsh May conditions year prior) + (Day of year modified by harsh May conditions year prior)^2



Supplementary Figure 5.