The Effect of Wildfires on Local Tick Populations and Human Disease Risk Along Popular Hiking Trails in Northern California

By

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Integrative Pathobiology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

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2024

ABSTRACT

Although wildfires are increasingly common in California, information regarding their effect on tick populations and pathogen prevalence is limited. This study explores the questing tick population and potential human disease risk in two hiking areas, Stebbins Cold Canyon (SCC) and Skyline Wilderness Park (SKY), after varying wildfire exposure. Stebbins Cold Canyon experienced two wildfires over the past ten years, the Wragg Fire in 2015 and the LNU lightning complex fire in 2020, whereas SKY was exposed solely to the Atlas Fire in 2017. Questing ticks were collected monthly over three seasons at each location. Tick species were identified and screened for known and emerging human pathogens using quantitative PCR. Human foot traffic, provided by park directors, was recorded for each location based on trailhead counters (SCC) and admission fees (SKY). Foot traffic (mean $\pm$ SD) at SCC for spring, summer, and fall was 3,236 $\pm$ 1,238, 1,743 $\pm$ 522, and 2,557 $\pm$ 449, respectively. Foot traffic (mean $\pm$ SD) at SKY for spring, summer, and fall was 5,861 $\pm$ 834, 5,030 $\pm$ 70, and 4,435 $\pm$ 263, respectively. Negative binomial regression was used to compare tick count data, with location and season as interaction terms. In total, 459 ticks were collected: 54 adult ticks from SCC and 405 from SKY, including 279 (68.9%) larvae, 2 (0.5%) nymphs, and 124 (30.6%) adults. At SCC, *Ixodes pacificus* accounted for 92.6% (50/54) of collected ticks. The remaining 7.4% consisted of *Dermacentor occidentalis* (4/54). At SKY, *I. pacificus* (16.8%, 68/405), *D. occidentalis* (12.3%, 50/405), *D. similis* (0.7%, 3/405), and *D. albipictus* (70.4%, 285/405) were collected. No statistical difference was seen in tick counts across locations when removing the large amount of *D. albipictus* larvae from the model ($p = 0.352$). These findings suggest that SCC's plant, host, and tick communities are recovering after the most recent wildfire, likely expedited by increased rainfall. However, SKY had significantly more ticks than SCC ($p < 0.001$) when including

larvae, likely due to the lack of leaf litter at SCC compared to SKY. *Borrelia burgdorferi* SL and *Rickettsia rhipicephali* were only found at SKY with a prevalence of 0.8% (1/121) and 0.7% (2/121), respectively. *Rickettsia tillamookensis* and *Babesia duncani* were found at both locations. Prevalence for *R. tillamookensis* at SCC and SKY was 3.7% (2/54) and 1.7% (2/121), respectively. Lastly, *B. duncani* was identified in 3.7% (2/54) and 1.2% (5/405) of ticks at both SCC and SKY, respectively.

Keywords: ticks, questing, wildfire, tick-borne, ecology, drought

ACKNOWLEDGEMENTS

Funding for this project was provided by the UC Davis Natural Reserve System and the Maurer-Timm Endowment. We thank the following parks and their directors, including Stebbins Cold Canyon and Skyline Wilderness Park, for their support and assistance in this project. Similarly, thank you to the UC Davis Real-Time PCR Research and Diagnostics Core Facility for assistance in logistics, protocol development, and sample analysis.

1. INTRODUCTION

The prevalence and propagation of vector-borne disease is influenced by factors that affect the biology and behavior of vectors, reservoirs, and hosts (Rosenburg et al., 2018). Examples include environmental changes in precipitation, temperature, and available shelter, among others. A majority of vector-borne diseases, including tick-borne diseases (TBDs), are maintained by small mammal or bird reservoirs, with humans as incidental hosts. For example, the white-footed mouse is a reservoir for *Borrelia burgdorferi*, the causative agent of Lyme disease, and *Ixodes* ticks vector the disease, bridging the gap between animals and humans. Due to this multifactorial relationship, environmental extremes, like wildfires, drought, and varying temperatures, can significantly affect animal reservoir and tick populations, altering disease prevalence and subsequent human risk.

From 2004 to 2019, TBD cases in the United States more than doubled, reaching 50,865 reported cases in 2019 (Tickborne Disease Surveillance Data Summary, cdc.gov). Similarly, California had the highest number of reported TBD cases on the West Coast (Rosenberg et al., 2018). TBDs comprise 77% of all vector-borne disease cases, with Lyme, anaplasmosis, and spotted fever rickettsiosis accounting for most cases in the United States (Rosenberg et al. 2018). This increase in TBD is likely a result of globally increasing temperatures, which have led to the northward expansion of tick habitats and ticks' improved survival, faster maturation, and shorter life cycles (Ogden et al. 2016). Because of these environmental changes, ticks are questing for hosts earlier and longer each year, increasing human risk (Ogden et al. 2016).

With climate change comes drier conditions, stronger winds, and an increased risk for wildfire frequency, size, and severity (Goss et al., 2020). California has endured multiple years of extreme wildfires. In 2018, 7,948 individual wildfires burned 1.6 million acres; in 2020, 4

million acres burned, making it the largest wildfire season in CA history (Cal Fire 2018 and 2020 incident archives, fire.ca.gov). Frequent and extreme wildfires affect the local vegetation and destroy available shelter and food for wildlife, therefore altering local tick populations. Prior studies have demonstrated changes in tick populations, both in quantity and species type, in the short-term post-wildfire (Pascoe et al., 2020). Nevertheless, the time it takes for tick populations to return to burned areas is unknown and likely varies based on multiple factors. Wildfires' effect on tick abundance in the short and long term, and human TBD risk is an area of ongoing research.

This study describes the host-seeking tick population over a 9-month period along popular hiking trails in Solano and Napa counties after exposure to varying frequencies of wildfires over the last 10 years. The specific aim was to describe and compare the medium to long-term effects of wildfire exposure on tick populations and subsequent human disease risk, with the hypothesis being that there will be a significant decrease in the local tick population and overall disease prevalence in regions exposed to more frequent wildfires over the last 10 years. This study will provide further data on the effects of weather extremes and climate change on tick populations. Lastly, this data can be used to educate the public regarding the current disease risk along these popular hiking trails.

2. MATERIALS & METHODS

2.1. Study locations- wildfire history

This study was performed at Skyline Wilderness Park (SKY) in Napa County and Stebbins Cold Canyon (SCC), a University of California natural reserve located between Napa and Solano County (Figure 1). Both locations were picked based on their proximity to each other, similarity

in vegetation, popularity, and wildfire exposure. Here, wildfire exposure is defined by the frequency of extreme wildfires over a 10-year period.

Skyline Wilderness Park encompasses 850 acres along the Napa County foothills, with elevations ranging from 200 to 1,630 feet. The park includes Lake Marie, a man-made pond along the southern end, and Marie Creek, which runs throughout the length of the park. Multiple vegetation types exist, including mostly grasslands, blue and live oak, dense riparian woodlands, savannas, and chaparrals at higher elevations. Trails are lined with dense areas of leaf litter throughout. In 2017, the Atlas fire burned 80% of the park, focusing along the northeast portion, according to park directors. The fire burned 122 days from October 9, 2017, to February 9, 2018, damaging 51,624 acres along Napa and Solano counties (Cal Fire 2018 incident archives, fire.ca.gov).

Stebbins Cold Canyon is a 638-acre reserve located along the California Inner Coast Range, with elevations ranging from 300 to 2,500 feet. The reserve sits along a slope flanking the southeast end of Lake Berryessa, a man-made reservoir. The canyon vegetation consists of grasslands, savannas, chaparrals, blue and live oak, and riparian woodlands, similar to SKY. Along the lower elevations, Cold Creek runs parallel to the main hiking trail. This creek contains water throughout the end of winter and spring and is commonly dry from summer onward. Before the 2015 Wragg fire, the reserve consisted mainly of blue oak and riparian woodlands with dense leaf litter along lower elevations and chaparral along higher elevations (Pascoe et al., 2020). The Wragg fire burned 8,051 acres and lasted for 14 days from July 22, 2015, to August 5, 2015. Due to its location, SCC was the first region to be burned in this fire, including most of the reserves' chaparral, oak woodlands, leaf litter, and vegetation (Pascoe et al., 2020).

In 2020, the LNU lightning complex fires burned 363,200 acres in 46 days from August 17th to October 2nd, making it the 4th largest wildfire in California history (Cal Fire 2020 incident archives, fire.ca.gov). The fires spanned five counties, including Napa, Solano, Lake, Sonoma, and Yolo counties. Dry lightning led to the three fires that made up the LNU complex, with the Hennessey Fire surrounding Lake Berryessa being the largest fire, consuming SCC.

2.2.Study locations- drought history

Prior to 2023, California experienced varying severities of drought. The US Drought Monitor grades drought on a D0-D4 scale, describing regions as either abnormally dry (D0), or in drought, including moderate (D1), severe (D2), extreme (D3), and exceptional (D4) drought. From 2012-2017, drought ranged from D0-D4, with up to 60% of the state experiencing D4 level drought between 2014-2017 (California Drought data, https://www.drought.gov/states/california). In 2020-2022, drought levels ranged from D2-D4, with 50% of the state graded as D4 from 2021-2022. Throughout both timeframes, drought in Napa (Figure 2A) and Solano (Figure 2B) counties encompassed 100% of both counties (U.S. Drought Monitor Data, https://droughtmonitor.unl.edu).

Winter and spring of 2023 brought heavy rain and snow, relieving a majority of the state's drought. As of spring 2023, only 19.1% of the state is considered abnormally dry, and 6.5% is experiencing moderate drought (D1). Both Napa and Solano counties are considered drought-free (U.S. Drought Monitor Data, https://droughtmonitor.unl.edu). According to the National Integrated Drought Information System (NIDIS), in 2023, California experienced the 15th wettest January-June since 1895, with a total of 20.79 inches in precipitation and an increase of 6.61 inches from normal (California Drought data, https://www.drought.gov/states/california). Due to

the heavy rain, multiple landslides occurred at SCC between February and March, limiting hiking access to the area and sample collection.

2.3.Collection procedure

Ticks were collected monthly from April to December along popular hiking trails at SCC and SKY. At SCC, collection was conducted along 1 mile of the Homestead Trail for 4 hours each time. At SKY, the collection was conducted for a similar distance and time, along the River to Ridge Trail. All collection was performed using flags made of white cloth, as previously described (Newman et al. 2019). To evaluate human risk, flags were brushed along leaf litter, grasses, and foliage directly on trail edges, where foot traffic from hikers and pets was considered most likely. Flags were checked for ticks every 5 feet to ensure ticks did not fall off prior to collection. Larvae were considered part of the same nest if they were characteristically clumped close together on the flag, in which case they were stored and processed as pooled batches. Larvae that could not be confirmed to be part of the same nest or were removed from the flag at different times were processed individually. Ticks were preserved in 70% ethanol at room temperature and identified by the same individual to species, stage, and sex using dichotomous keys and published literature (Dubie et al., 2017; Furman & Loomis, 1984).

Human foot traffic was recorded for each location for the entirety of the study period. For SCC, hiking traffic was based on trailhead counters at the start of the Homestead trail. At SKY, hikers, equestrians, and mountain bikers accessing the trailheads were included based on admission fees. For both locations, use data was provided by park directors.

2.4. *DNA extraction*

As all collected ticks were unengorged, DNA was extracted using the alkaline lysis method with ammonium hydroxide (NH_4OH), as previously described (Humair et al. 2007). In brief,

ticks were dissected into quarters, incubated at 100°C with 0.7 M NH_4OH for 15 minutes, cooled rapidly on ice, and then incubated at 100°C for a second time to evaporate any remaining ammonia. All remaining liquid, containing extracted DNA, was removed and stored at 4°C until further processing. Tick larvae determined to be from the same nest were extracted together in pools of 5.

2.5. Pathogen analysis

All ticks were screened for the presence of human TBDs previously confirmed in the area and TBDs of potential emerging concern. For tick species known or suspected to vector a specific pathogen, DNA was tested individually. For those species that were not suspected or known primary vectors of the pathogen, DNA from 5 individual samples was pooled, and only positive pools were tested individually. *Dermacentor albipictus* ticks were only tested for *B. duncani* because they are one host ticks, feeding mainly on mule deer in California during each stage, and rarely on people (Swei et al., 2019). All other specimens were screened for *Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu lato (Bbsl), *B. miyamotoi, Francisella tularensis, Babesia duncani,* and *Bartonella* spp. DNA was initially tested for rickettsial pathogens within the spotted fever group using a Pan-*Rickettsia* qPCR protocol targeting a conserved region of the 23S rRNA (Kato et al. 2013). Positive samples were further screened for *R. rickettsii*, *R. philipii* (364D), *R. massiliae, R. rhipicephali,* and *R. tillamookensis*. All primers, probes, genes, and sources are detailed in Table 1.

Extracted DNA was screened using Applied Biosystems StepOnePlus and QuantStudio 7 Real-Time PCR systems (Applied Biosystems, MA). All samples were run in duplicate, with 1 positive control and 3 non-template negative controls per set of reactions. All reactions were 12 µL total, including 6 µL master mix, 0.6 µL primer and probe mix, 0.4 µL water, and 5 µL DNA.

In each reaction, 5 and 2.5 picomoles of both primers and probe were used, respectively. TaqMan Universal PCR Master Mix and Maxima Probe/ROX qPCR Master Mix (2X) (Thermo Scientific, MA) were used, with ROX as the passive reference dye. The reporter dye for all probes was 6-carboxyfluorescein (6-FAM), except for *Borrelia miyamotoi* and *R. rhipicephali,* in which 2'-chloro-7'-phenyl-1,4-dichloro-6-carboxyfluorescein (VIC) was used. The non-fluorescent minor groove binder, Black Hole Quencher 1 (BHQ-1), was used as the quencher for all probes. Thermocycler conditions were 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. Samples were considered positive based on cycle threshold (Ct) values <40 with characteristic amplification curves.

Synthetic positive controls comprising a single oligonucleotide strand containing both forward and reverse primers and probe sequences were used for pathogens when genomic DNA was unavailable. For *B. duncani,* the following sequence was used: 5'-TGG GCG GTA GCC TCG C**GC TTC CTA ACC CGA GAC CAA** CTT TGC GGT TCG CCG TAC GGC CCC TGA TGT CG GGG CGT **TAC TTT TCA CCC CGC CAG TG**-3' (Wilson et al. 2015). For *R. tillamookensis,* the following sequence was used: 5'-CAT GAG ATC ACC **GCT TGC TGA TTT AAA GGA AAT GCA** CAG TTA GGC ATC CAT GCG GCG GTG CTC CAA ACT CCG ATT **CCC CAA ATC TCT TAT CAA CTT CCT** GTA CGT AA-3' (Paddock et al. 2022). Portions related to primers and probes are bolded and underlined, respectively.

2.7 Statistical analyses

All data analysis was performed using R (v4.3.2, R Core Team, 2023) and JMP Pro (v17.0.0, JMP Statistical Discovery, 2022). A p-value <0.05 was used to infer statistical significance. As ticks were collected at each location every month, a seasonal categorical variable was created as follows: spring (April-June), summer (July-September), and fall (October-December). Temporal

mean abundance was reported as the mean number of ticks collected per hour ± SD for each location per season. Initial univariate analysis was performed to identify predictors of interest and define further analyses. Generalized linear models (GLMs) were used to analyze count data based on season and location, as well as the interaction between the two. Due to over-dispersion of data, negative binomial regression was used, using the location "SKY" and season "spring" as reference terms. For categorical variables, particularly sex, chi-square was performed to assess differences between locations, with post-hoc adjusted standardized residual analysis. Absolute infection prevalence was reported as a percentage and calculated as the number of positive samples over the total number tested. As larvae from the same nest were pooled in groups of 5, a positive pool was assumed conservatively to contain only one infected tick. Therefore, prevalence estimates were reported as the minimum infection prevalence (MIP) in these cases. Due to the low level of disease prevalence, further statistical analysis was not performed.

3. RESULTS

In total, 459 questing ticks were collected throughout the April to December study period (Table 2). Fifty-four ticks were collected from SCC, and 405 ticks were collected from SKY over 9 months (Fig. 3). At SCC, only questing adult ticks were collected at all time points. However, at SKY, larval (68.9%, 279/405), nymphal (0.5%, 2/405), and adult (30.6%, 124/405) ticks were collected, with all larvae and nymphs collected in the fall from areas of dense leaf litter. Of the questing adults, 37.0% (20/54) and 54.8% (68/124) were females at SCC and SKY, respectively, with a significant difference in the distribution between the two locations (p = 0.029). Post-hoc standardized residual analysis indicated a larger deviation between the observed and expected counts for females (-1.30) and males (1.28) at SCC compared to females (0.86) and males (-0.85) at SKY. Four tick species were identified, including *Dermacentor albipictus, D.*

occidentalis, D. similis, and *Ixodes pacificus*. At SKY, *I. pacificus* (16.8%, 68/405), *D. occidentalis* (12.3%, 50/405), *D. similis* (0.7%, 3/405), and *D. albipictus* (70.4%, 285/405) were collected. At SCC, *I. pacificus* accounted for 92.6% (50/54) of ticks collected. The remaining 7.4% consisted of *D. occidentalis* (4/54) (Fig. 4). Human traffic was high at both locations throughout the study period (Fig. 3). Foot traffic (mean ± SD), at SCC for spring, summer, and fall was 3,236 ± 1,238, 1,743 ± 522, and 2,557 ± 449, respectively. Foot traffic (mean ± SD) at SKY for spring, summer, and fall was 5,861 ± 834, 5,030 ± 70, and 4,435 ± 263, respectively.

Using location and season as predictors, tick count as the outcome, and SKY and spring as reference terms, initial univariate analysis confirmed statistical correlation for SCC ($p = 0.004$), fall ($p = 0.024$), and summer ($p = 0.007$). When initially comparing SCC to SKY, without the effect of season, a significant difference was seen between locations (IRR=0.15, 95% CI= 0.06-0.37, $p<0.001$), indicating an 85% decrease in questing tick exposure in SCC (Table 3a). However, after accounting for the effect of season on location, location was not a statistically significant predictor of tick count ($p = 0.251$), except for in the fall ($p = 0.024$) (Table 3b). When looking at seasons, overall tick abundance was highest in the fall (IRR=6.43, 95% CI= 2.15-19.22, $p <0.001$) and lowest in the summer (IRR= 0.21, 95% CI= 0.06-0.72, $p = 0.013$) at SKY, compared to spring. However, the seasonal effect varied between locations, with fall in SCC experiencing a reduced tick density compared to SKY (IRR= 0.16, 95% CI 0.03-0.78, $p = 0.024$).

As *D. albipictus* larvae made up a large portion of count data at SKY in the fall, larval count data was removed in the second GLM, focusing solely on questing adults and nymphs. Univariate analysis found no statistical correlation for SCC ($p=0.17$) or fall ($p=0.86$). Negative binomial regression was used with the same predictors, reference terms, and variables as before.

Unlike the initial model, when comparing SCC to SKY, no significant difference was seen between locations in any season (Table 3c).

Overall, pathogen prevalence was low at both locations for all time points (Table 4). None of the collected ticks tested positive for *A. phagocytophilum, B. miyamotoi, Bartonella* spp., and *F. tularensis*. In total, 63.4% (111/175) of adult ticks were positive on initial pan-*Rickettsia* screening, of which 3.6% (4/111) were *Dermacentor* spp. and 96.4% (107/111) were *I. pacificus*. All ticks positive on pan-*Rickettsia* were negative for *R. rickettsii*, *R. philipii*, and *R. massiliae*. However, 1.7% (2/121) of the ticks at SKY and 3.7% (2/54) at SCC, all of which were *I. pacificus,* tested positive for *R. tillamookensis*. Similarly, 1.7% (2/121) of the ticks at SKY, both of which were *D. occidentalis,* carried *R. rhipicephali*. As human pathogens were the focus of this study, further analysis was not performed to determine Rickettsia spp. in the remaining samples. *Babesia duncani* was detected at both locations. At SCC, 2 *I. pacificus* adults tested positive for *B. duncani* (3.7% (2/54)) in the spring, and at SKY, 1.2% (5/405) of ticks tested positive in the fall, including one *I. pacificus* adult and four *D. albipictus* larvae. Lastly, 0.8% (1/121) of ticks tested positive for *B. burgdorferi* SL in the fall at SKY.

4. DISCUSSION

This study describes the questing tick population and associated human disease risk at two hiking areas, SCC and SKY, in Northern California, following differing wildfire exposure. Stebbins Cold Canyon was exposed to two extreme wildfires, including the Wragg Fire in 2015 and the LNU Fire in 2020. However, SKY was only exposed to a single wildfire, the Atlas Fire, in 2017. Despite observing seasonal variations in questing tick abundance between these locations, no significant difference was found in the adult and nymphal tick counts between SCC and SKY. An important finding in this study was the large amount of *D. albipictus* larvae present

at SKY. When including *D. albipictus* larvae counts in the GLM model, a substantial difference in tick abundance was seen between the two locations (p<0.001). Specifically, SCC exhibited an 85% lower risk of tick exposure compared to SKY. Overall, SKY displayed greater species diversity, including *D. occidentalis*, *D. similis, D. albipictus*, and *I. pacificus*, while only *D. occidentalis* and *I. pacificus* were collected at SCC. Despite the varied species, both locations displayed low pathogen prevalence. Potential human pathogens detected include *B. duncani* and *R. tillamookensis* at both SCC and SKY and *B. burgdorferi* sl at SKY.

4.1. Pathogen prevalence

The first case of human babesiosis in North America was diagnosed in California in 1966 due to *B. duncani* (Scott et al., 2021). Since then, 14 cases of *B. duncani*-associated babesiosis have been reported along the Pacific coast, with nine cases having potential exposure in California (Swei et al., 2018). Canada has recently reported an increase in *Babesia* associated human cases, including *B. duncani* and *B. odocoilei* (Scott et al., 2017 and 2021). The first confirmed case of locally acquired *B. duncani* in Ontario was reported in 2017 (Scott et al., 2017). Prior to this, cases diagnosed in Canada usually had prior tick exposure along the west coast. In this case, a man was confirmed to have symptoms caused by *B. duncani* after a bite from an *I. scapularis* nymph. Likewise, in 2021, two clinically ill individuals in Ontario tested positive for *B. odocoilei*, one bitten by an *I. scapularis* tick (Scott et al., 2021). Most importantly, disease caused by *B. duncani*, and likely *B. odocoilei*, are reportedly more difficult to treat than *B. microti*, the main cause of babesiosis on the east coast (Scott et al., 2021). Considering these factors, further surveillance in the region for *B. duncani* and investigations into alternative reservoirs and vectors are needed.

The primary tick vector and enzootic cycle for *B. duncani* is unknown. Initially, *I. pacificus* and *I. scapularis* were assumed vectors, mostly due to their ability to vector *B. microti*. However, recent evidence suggests that *D. albipictus* and the mule deer (*Odocoileus hemionus*) may be the primary vectors and reservoir species for this disease, respectively (Swei et al., 2019). As *D. albipictus* is a one-host tick, most human exposure would likely occur from questing larvae or rare incidental adult ticks that have fallen off large ungulates.

Two strains of *B. duncani* have been sequenced in California, WA-1 and BH3, with WA-1 being the predominant strain identified in ticks along the coast (Swei et al., 2019). Prior studies in Napa County have demonstrated *B. duncani* (strain WA-1) in 3.0% of *D. albipictus* larvae tested (Swei et al., 2019). Napa was reported to have the lowest prevalence compared to other nearby counties, including Sonoma (4.8%), Marin (20.7%), and Contra Costa (4.7%) (Swei et al., 2019). Most importantly, this finding supports transovarial transmission of the pathogen in *D. albipictus* ticks, an important aspect of vector competency for some species. Similarly, 86.8% of mule deer captured in Mono County were reported to be seropositive, although with the BH3 strain (Swei et al., 2019). Importantly, Mono County is located along the eastern border of central California, about 250 miles from Napa County. The seroprevalence of *B. duncani* in mule deer within Napa and surrounding counties has not yet been reported. Although these findings suggest that *D. albipictus* and mule deer may play a role in the enzootic cycle, further studies regarding *D. albipictus* vector competency and demonstrating the presence of the WA-1 strain in mule deer in the region are required.

A surprising finding in this study was the presence of *B. duncani* in 2 adult *I. pacificus* ticks at SCC. Prior to this, no studies have reported the presence of *B. duncani* in Solano County or *I. pacificus* in California. Unfortunately, due to the high Ct values (36-39) further sequencing and

strain identification was not pursued. Interestingly, a survey of *I. pacificus* in California identified *B. odocoilei* in 3% of total ticks tested, coming from Alameda, Humboldt, Napa, Placer, and San Bernardino counties (Eshoo et al., 2015). Given the diverse feeding preferences of *I. pacificus*, further studies assessing other potential reservoirs and the vector capacity of these ticks are warranted. *I. pacificus* have a wide range of hosts that they feed on, including small and large mammals, birds, and reptiles (McVicar et al., 2022). At SCC, prior studies have found western fence lizards *(Sceloporus occidentalis*), southern alligator lizards (*Elgaria multicarinata*), dusky-footed woodrats (*Neotoma fuscipes*), and mice of the *Peromyscus* spp., all of which are recorded hosts of *I. pacificus* (McVicar et al., 2022, Pascoe et al., 2020). Similarly, all three stages of *I. pacificus* are known to parasitize deer (Westrom et al., 1985). Although mule deer were not seen by the main investigator (CMK) during collections, camera data at SCC throughout the study timeframe confirms the presence of mule deer, particularly along the Homestead Trail by Cold Creek (UC Davis Natural Reserves Camera Trap Program, youtube.com/@universityofcaliforniadavi890). Relatedly, an increase in *I. pacificus* habitats in California has been predicted within this century, with areas along the northwest coast projected to have some of the largest increases in area (Hahn et al., 2021). Determining this species' role in both *B. duncani* and *B. odocoilei* is critical for human health. Unfortunately, *B. odocoilei* was not tested for in our current study. However, this does not diminish the important role that this pathogen may play in the region in the future.

Rickettsia tillamookensis was initially isolated from *I. pacificus* in 1967 in Oregon (Paddock et al., 2022). In 2021, whole genome sequencing of the isolated strain identified the pathogen as a transitional *Rickettsia* species termed *R. tillamookensis*. The prevalence of *R. tillamookensis* among *I. pacificus* ticks collected via flagging throughout CA was reported to be 1.9% and 2.9%

in adults and nymphs, respectively (Paddock et al., 2022). For Napa and Solano counties, prior studies have cited 2.7% and 2.1%, respectively (Paddock et al., 2022). Our study found a similarly low prevalence in SKY (1.7%) and a higher prevalence at SCC (3.7%).

As *I. pacificus* is the most frequently identified human-biting tick along the west coast, accounting for 60-70% of bites, concerns about the pathogenicity of *R. tillamookensis* in people are reasonable (Furman & Loomis, 1984, Paddock et al., 2022). Virulence studies have demonstrated the pathogen's ability to cause a low-grade febrile illness in intraperitoneally infected guinea pigs (Hughes et al., 1976). However, no confirmed cases of human infections have been reported. Regardless, many reports have predicted the expansion of *I. pacificus* populations within CA due to changing climate. It is predicted that by the middle of this century (2026-2045), regions suitable for *I. pacificus* will have expanded by 23% (Hahn et al., 2021). Similarly, by the end of the century (2086-2099), the suitable area will have grown by 86%, including over 1/3 of open access land, like SKY and SCC (Hahn et al., 2021). Due to this potential increase in human exposure, further studies evaluating the pathogenicity of *R. tillamookensis* and correlations between tick exposure and disease are essential.

A majority of *I. pacificus* in our study tested positive for *Rickettsia* spp. (90.7% (107/118)). These positives are most likely due to the endosymbiont G021. This endosymbiont is widespread in *I. pacificus* ticks in northern CA, with up to100% transovarial and transstadial transmission (Alowaysi et al., 2019). G021 contributes a functional enzyme needed for folate synthesis, which cannot be synthesized by ticks on their own and is generally acquired from the diet (Alowaysi et al., 2019, Bodnar et al., 2018).

In our study, 2 ticks tested positive for *R. rhipicephali* (1.14%, 2/175). To date, this pathogen has not been associated with any human infection. However, similar to *R. tillamookensis*, this

pathogen has been shown to cause disease in guinea pigs (Labruna et al. 2007). Regardless of its pathogenicity, *R. rhipicephali* may play an important role in preventing multiple rickettsial infections in a single tick. This theory came about as no co-infection with spotted fever rickettsial pathogens has been identified in ticks carrying *R. rhipicephali* (Ortega-Morales et al., 2019). Studies have demonstrated the ability of primary infecting rickettsial pathogens, like *R. rhipicephali*, to exclude or inhibit co-infection of a tick vector with a second rickettsia (Macaluso et al. 2002). This protection has likely led to the low prevalence of spotted fever rickettsia and disease transmission in certain parts of the country (Merino et al. 2020, Zeringota et al. 2016, Niebylski et al. 1997).

4.2.Controlled burns and wildfires

Prescribed or controlled burning has been shown to have multiple benefits, including reduction of wildfire risk, management of diseased and invasive plants, and control of insect populations, particularly ticks. In addition, prescribed burns can help restore the surrounding ecosystem. Ash from burned vegetation provides nutrients to the soil more rapidly than natural decomposition. In dense forests, the removal of canopy cover allows for more sunlight to penetrate, helping younger trees and plants grow. For some pine trees, fire is a required element for reproduction as the fire helps melt resin on pinecones, releasing their seeds and facilitating germination (Encyclopedic entry on Controlled Burning, education.nationalgeopgraphic.org). Although these fires remove canopy cover, the trees themselves still survive, growing leaves in the following year.

Along with the ecological benefits, controlled burns have been used for years to control tick abundance and subsequent disease risk (Gleim et al. 2019) with mixed results, likely due to their lack of intensity, regularity, and homogeneity. Although fire will kill ticks directly, heterogenous

burn areas leave portions of less damaged land that can continue to harbor small mammals in the remaining understory and leaf litter, leading to only a transient decline in ticks (Pascoe et al., 2020). In these regions, vegetation grows rapidly, allowing for a quicker return of animal and tick populations (Pascoe et al., 2020). Because of this, prescribed burns are most effective for tick control if done regularly over a long time period to more permanently alter the area's vegetation, remove canopy cover, and allow for more sunlight penetration to the understory (Gleim et al., 2019). In a recent study, regions exposed to over ten years of prescribed burning, with burns every 2 to 4 years, demonstrated a significant reduction in tick abundance compared to immediately adjacent unburned regions (Gleim et al., 2019). However, another study on the short-term effects of prescribed burns found increased tick abundance two years after a controlled burn in the Missouri Ozarks; larval tick densities for *Amblyomma americanum* were over 6 times higher in burned areas compared to non-burned controls (Brian Allan, 2009). This was reportedly due to the immediate use of burned sites by white-tailed deer, an important host for this tick species.

Much of the difference in the effect that prescribed burns and wildfires have on the ecosystem has to do with the severity of the burn and the burn pattern (Eisenburg et al., 2019). With extreme wildfires, high severity can be more easily achieved due to the overabundance of "fuel," leading to the development of large homogenous burn patches (Eisenberg et al.). Here, wildfire fuel consists of live shrubs and grasses as well as thick layers of dead foliage like pine needles, leaf litter, and twigs. Underneath dead foliage, dry soil can further add to the fuel, leading to increased severity. In extreme cases, this can cause the complete removal of trees, leaf litter, and vegetation in large regions, encouraging open habitats and direct sun, as was seen with the Wragg and LNU fires at SCC. Because controlled burns do not have as much fuel and are

often created to minimize potential fuel sources, they rarely reach the same severity, causing heterogeneous burn patches. Similarly, controlled burns are often performed during particular times of the year, like early spring, when less fuel is available to minimize risk (Eisenberg et al., 2019).

4.3.Tick abundance and wildfires

Wildfires can affect tick abundance due to direct tick death, loss of habitat, particularly leaf litter and canopy cover, and the displacement of hosts (Pascoe et al., 2020 and 2023). Leaf litter plays a critical role in the tick life cycle, providing a moist, cool, and shaded environment for ticks. This is important, especially for gravid females laying eggs, as seen by the large amount of *D. albipictus* larvae collected in leaf litter at SKY. With extreme wildfires, the underlying leaf litter burns completely, leaving the exposed floor. Mature trees also burn, removing shade, protection, and the leaf litter source. These burned areas will initially be covered with various grasses and shrubs well before the return of canopy cover and dense leaf litter, which can take well over two years (Pascoe et al., 2023). Tree roots burned below the exposed soil will kill the tree and prolong the time for canopy cover and wildlife habitats to return, sometimes taking over 100 years (Meghan Snow, 2022). Wildfire studies have demonstrated a decrease in questing nymph and larvae count post-wildfire due to this loss of leaf litter (Pascoe et al., 2023). Likewise, a study evaluating SCC before and after the 2015 Wragg fire found a 16-fold decrease in vegetation a year after the fire (Pascoe et al., 2020). Although the LNU fire occurred 3 years ago, the amount of leaf litter and canopy cover seen at SCC, compared to SKY, during our current study was minimal. No larvae were collected at SCC, unlike SKY. Similarly, many regions that once contained leaf litter were now covered in varying densities of grasses. This lack

of leaf litter is likely to continue to affect the local tick population by minimizing shade, moisture, and protected areas for gravid females.

When comparing the sex of adult ticks between locations, a statistical difference was seen between locations ($p = 0.029$), with 54.8% (68/124) and 37.0% (20/54) of ticks being females at SKY and SCC, respectively. Based on calculated standardized residuals, this difference was mainly driven by the counts at SCC, compared to SKY. The biological blood-feeding requirements differ between female and male hard ticks. Female ticks require a blood meal at all life stages (larvae, nymph, adult) and will feed to repletion as adults prior to egg-laying. However, males of some species do not need to feed as adults, only as larvae and nymphs. Adult males quest to find a female, mating on the host while females feed. There is no data regarding changes in the proportions of questing male and female ticks post-wildfire. Hypothetically, with the removal of leaf litter and transient declines in host abundance, environmental changes may inadvertently favor male over female ticks in the immediate 2-3 years post-fire.

With habitat destruction, changes in host abundance, including lizards and various small and large mammals, may alter the tick population. Multiple studies have documented the immediate return of deer to severely burned regions (Pascoe et al., 2020, Kreling et al., 2021). At SCC, deer were noted within a few weeks after the 2015 Wragg fire (Pascoe et al., 2020). Similarly, a study evaluating the 2018 Mendocino Complex Fire noted deer returning to burned regions within hours after the fire, ignoring nearby unaffected regions with better covering and food (Kreling et al., 2021). For two years after the Mendocino Fire, deer continued to be the most commonly seen species using camera trapping data (Pascoe et al., 2023). As adult ticks are the most common life stage to feed on deer, ticks could be re-introduced to burn areas this way. However, with the lack of leaf litter and shade, it is unlikely that these ticks would survive, especially long enough for

egg laying, which can take multiple weeks, unlike what was previously discussed with controlled burns and *A. americanum* larval abundance (Brian Allan, 2009).

In the first two years after the 2015 Wragg Fire, camera traps and small mammal trapping data at SCC documented a 5.5 times increase in rodents, including deer mice (*Peromyscus* spp.), house mice (*Mus musculus*), and voles (*Microtus californicus*) compared to 2010 pre-fire data (Pascoe et al., 2020). They also reported minimal change in the proportion of infested hosts pre and post-fire at SCC, with 12.5% and 13.7% of small mammals infested, respectively (Pascoe et al., 2020). After the Mendocino Fire, a significant increase in rodents was seen between 2019 and 2020, with only 1 deer mouse captured in 2019, followed by 37 in 2020 (Pascoe et al., 2023). Many of these vertebrate animals are considered fast life-history species, meaning they have short lifespans, can increase populations quickly, and adapt quickly to changes in environment and climates, as is seen post-wildfire. Along the East Coast, these species reportedly lead to the return of tick populations and pathogen prevalence in controlled burned areas if burns are not performed regularly, suggesting their importance in returning tick populations post-wildfire (Pascoe et al., 2020). Although these fast-life history species are unlikely to be reservoir hosts for TBDs on the West Coast, compared to the East Coast, they can easily transport larvae and nymphs to burned locations and provide a bloodmeal source for survival.

Interestingly, in the MCF study, of the *I. pacificus* ticks collected from captured vertebrates, 95.3% (164/172) came from western fence lizards (*Sceloporus occidentalis*) in 2020, while only 4.7% (8/172) came from mice (Pascoe et al., 2023). Unlike small rodents, these lizards are not considered fast-life history species. However, they are an important host for larval and nymphal

I. pacificus ticks in California and likely play a role in the survival and eventual repopulation of this tick species post-fire (Lane et al., 1989).

4.4.Drought and wildfires

Drought, similar to habitat and host populations, can directly affect tick abundance. The effect of drought on tick populations post-wildfire is complex as drought not only plays a crucial role in wildfire development but also tick survival, questing, and host presence. This relationship makes it difficult to decipher which event, drought or wildfire, mostly affects tick populations. Although wildfires can occur regardless of drought, particularly during times of strong wind and warmer temperatures, drought does increase the risk of wildfires. Looking back at drought severity data and wildfire burn areas from 1984-2018, drought severity significantly predicts the size of burn areas in California (Angela Chen, 2021). Drought further amplifies wildfires by drying out live and dead fuels, increasing the probability of a fire starting and the rate that the fire will spread (Littell et al., 2016).

Dermacentor spp. are known to be more resistant to warmer temperatures and drier conditions compared to *Ixodes* spp. (Pascoe et al., 2020). Similarly, because of their ability to manage drier and warmer weather, *Dermacentor* spp. are more tolerant to sun exposure and can survive and quest in more open habitats, whereas *Ixodes* spp. prefer cooler, humid, and covered regions (MacDonald et al., 2018). Regardless of wildfire, in cases of severe drought, as seen in both Napa and Solano County from 2011 to 2023, the environment will likely favor *Dermacentor* spp. actively searching for hosts.

Studies at SCC regarding the tick population in 2010 reported identifying 3 tick species, including *I. pacificus, I. spinipalpis,* and *I. woodi*, which made up 59.52%, 2.38%, and 38.1% of ticks, respectively (Pascoe et al., 2020). After the Wragg fire in 2015, species collected and their

proportions changed, including the introduction of *D. occidentalis*, making up 44.19%, and a decrease in *I pacificus* (41.86%) and *I. woodi* (6.98%) (Pascoe et al., 2020). These changes in species were most likely due to a combination of changes in drought severity and habitat pre and post-fire. In 2010, Solano County drought was graded as D0 and D1, 3 grades below 2015 data (U.S. Drought Monitor Data, https://droughtmonitor.unl.edu). There is no published data regarding the tick population and vegetation after the LNU fire that affected SCC in 2020. However, in our current study, *I. pacificus* and *D. occidentalis* accounted for 92.6% and 7.4% of questing ticks, respectively. Despite SCC experiencing a second wildfire, the large portion of *Ixodes* spp. collected in this study, compared to *Dermacentor* spp., indicates a return to SCC's pre-fire conditions, likely expedited by a large amount of rain at the beginning of 2023, relieving the region of drought and allowing for an environment more suitable for questing *I. pacificus*. This is further demonstrated by the lack of statistical difference seen in the adult and nymphal tick counts between SKY and SCC. Most importantly, these changes in questing species lead to changes in human disease risk, as certain species vector certain pathogens and are more prevalent during certain times of the year.

Like ticks, certain vertebrate hosts adapt to changes in climate better than others. Fast-life history species, like house mice, adapt quickly, whereas other animals, like woodrats (*Neotoma fuscipes*), are less tolerant of drought, preferring to be near water sources and undercover (National Wildlife Federation, nwf.org). Prior to the Wragg Fire, SCC had a large population of woodrats (Pascoe et al., 2020). However, post-fire, small mammal capture data showed a decline in woodrats, with 85 captured pre-fire and pre-drought in 2010 and only 5 at SCC post-fire (Pascoe et al., 2020).

Changes in species diversity and proportions between SKY and SCC are likely due to a combination of factors, including the effect of wildfire, precipitation differences, and other ecological factors that differ between the two locations. In SCC, the Homestead Trail runs parallel to Cold Creek, which overflowed from rain, flooding portions of the trail throughout spring 2023 during collection, as observed by one of us (CMK). Similarly, minor variations in the overall drought grading in Napa compared to Solano County in the years prior to our study could explain a portion of this variation (Fig. 2). Both *Ixodes* spp. and *Dermacentor* spp. have life spans ranging from 2-3 years. Therefore, factors from years back can still affect the current questing adult population.

We did not encounter any *I. spinipalpis* or *I. woodi* in our study, compared to other studies in the region. This is most likely due to differences in sampling methods; flagging vs trapping small mammals. Flagging mainly collects questing ticks on grasses or leaf litter. Ticks can also be collected by trapping the vertebrates they are infesting. Both *I. woodi* and *I. spinipalpis* prefer small mammal burrows where they can feed on animals that inhabit the area, making them more likely to be caught via small mammal trapping.

4.5. Wildfire and pathogen prevalence

Studies evaluating the effect of wildfires on pathogen prevalence have shown mixed results, likely due to differences in burn severity and extent, drought, and the effects of various tick host species (Pascoe et al., 2023; MacDonald et al., 2018). In controlled burns, burn frequency and severity have been found to be consistently associated with a decrease in tick abundance for a short time (Stafford et al., 1998). However, comparisons in pathogen prevalence pre and post-wildfire are difficult to find as most controlled burns are not of the same severity and uniformity as wildfires, and many factors can affect pathogen prevalence. Few studies have

been able to demonstrate consistent differences pre and post-wildfire. After the 2018 Mendocino complex fire, populations of questing ticks declined within the first year as ticks feeding on small mammal and lizard hosts increased, suggesting a potential transient decrease in human risk post-fire (Pascoe et al., 2023). Similarly, with the loss of microhabitats in leaf litter, repopulation of tick density in a burned region may be hampered and could take multiple years. However, as demonstrated, factors like rainfall can help expedite an area's return to pre-fire tick populations, likely due to increased deer and small mammal traffic in areas with water.

Despite a substantial increase in rainfall at SCC and a similarity in adult tick counts between SCC and SKY, pathogen prevalence remained low. Considering the 2-3 year lifespan of the ticks collected and that hard ticks only feed once per life stage, it is possible that pathogen prevalence will increase in the coming years as the mammal and tick populations continue to regrow post-fire. Further studies covering both the short-term and the long-term effects of wildfire on tick abundance and pathogen prevalence in a single area are required.

4.6. Study limitations

The main limitation of this descriptive study is the small sample size at each location for each time point. A seasonal categorical variable was used to increase power, combining 3 months of collection for each season (spring, winter, fall). Similarly, further statistical comparisons between locations and seasons could not be performed due to the low pathogen prevalence among samples. Unfortunately, due to weather extremes, SCC was not accessible during January, February, and March of 2023, limiting our ability to provide data for a full 12-month period. Lastly, although this was mainly a descriptive study, the addition of a third nearby location, unexposed to wildfires, could have served as a control, facilitating further comparisons.

Due to public health concerns, brief surveys during sample collection focused on hikers' tick knowledge would have been an interesting addition to this study. As seen by the human traffic data, at both parks, both regions are well traversed. A better understanding of people's knowledge gaps regarding ticks and associated disease risks could help minimize future risks in an ever-changing climate.

CONCLUSION

This study describes the questing tick population and human pathogen prevalence at SCC and SKY after differing wildfire exposure. Stebbins Cold Canyon has been exposed to two intense wildfires in recent years, including the 2015 Wragg Fire and the 2020 LNU fire. Skyline Wilderness Park has experienced one wildfire, the 2017 Atlas fire. Overall, tick counts between locations were not significantly different except for in the fall, where a significant increase in ticks was present at SKY. This increase was due to the overabundance of *D. albipictus* larvae found within leaf litter on trails. Three years after the LNU fire, SCC continues to have minimal areas of leaf litter and canopy cover compared to SKY. However, the lack of difference in adult tick counts between locations and the increased proportion of *I. pacificus* seen at SCC would suggest that SCC is recovering post-wildfire and returning to pre-fire tick populations. This recovery was likely expedited by the heavy rainfall at the beginning of this year. *Babesia duncani* was found in *I. pacificus* ticks at SCC in the spring and *D. albipictus* larvae at SKY in the fall, with a prevalence of 3.7% and 1.2%, respectively. Further studies surrounding vector competency and the rate of transovarial transmission are required for *B. duncani*, especially given the large amount of *D. albipictus* larvae found at SKY. Based on hiking data provided by park directors, increases in trail use follow seasonal trends for questing ticks at SCC, with

increases seen in the spring and fall, further emphasizing the need for studies regarding vector competency for this pathogen.

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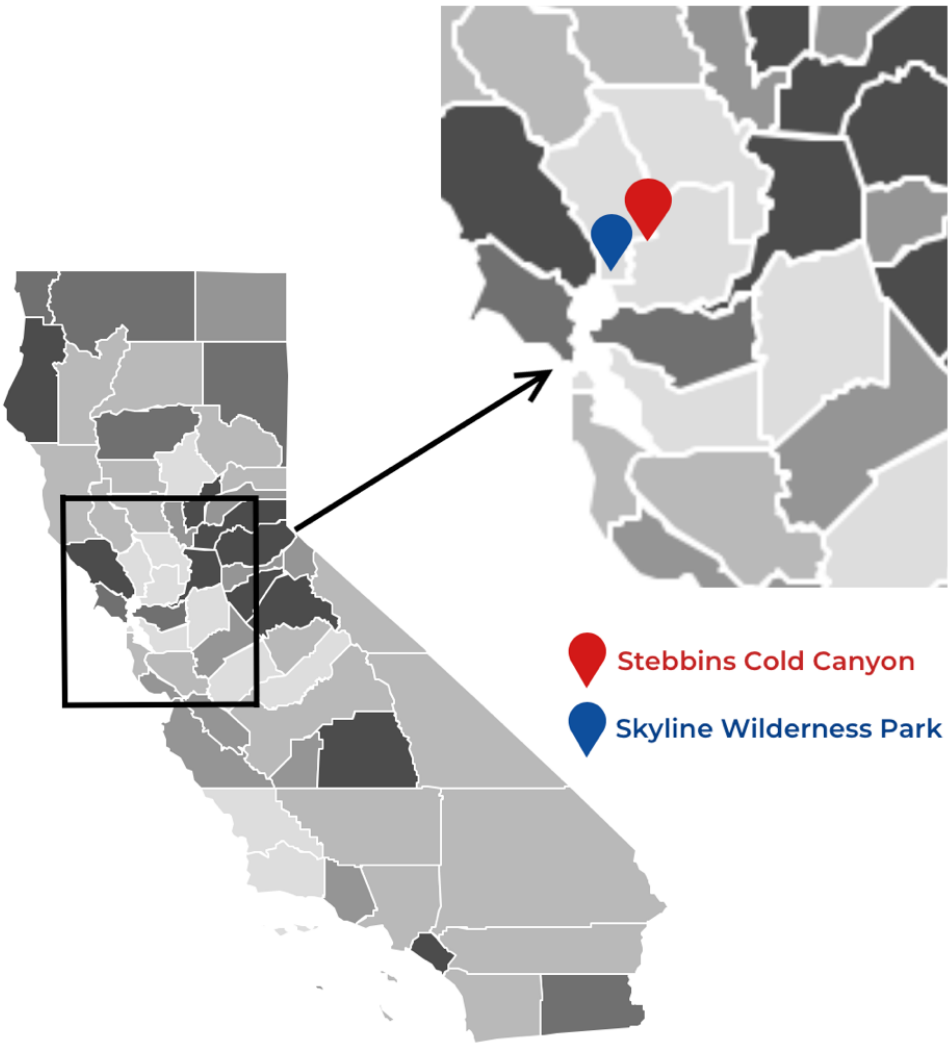
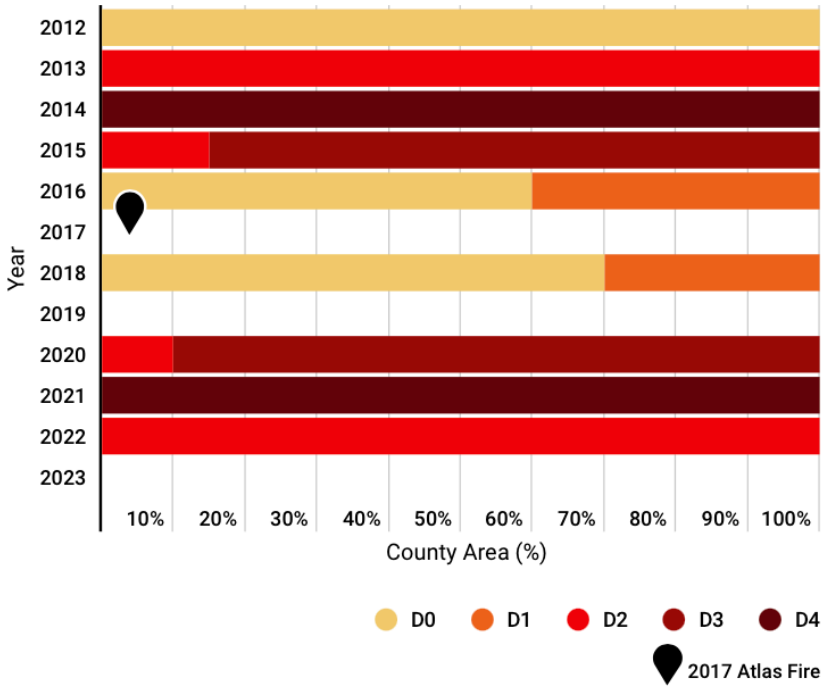


Fig. 1. Zoomed in map of California by county. The two study sites Skyline Wilderness Park (SKY), and Stebbins Cold Canyon (SCC), were in Napa and Solano County.

A)



B)

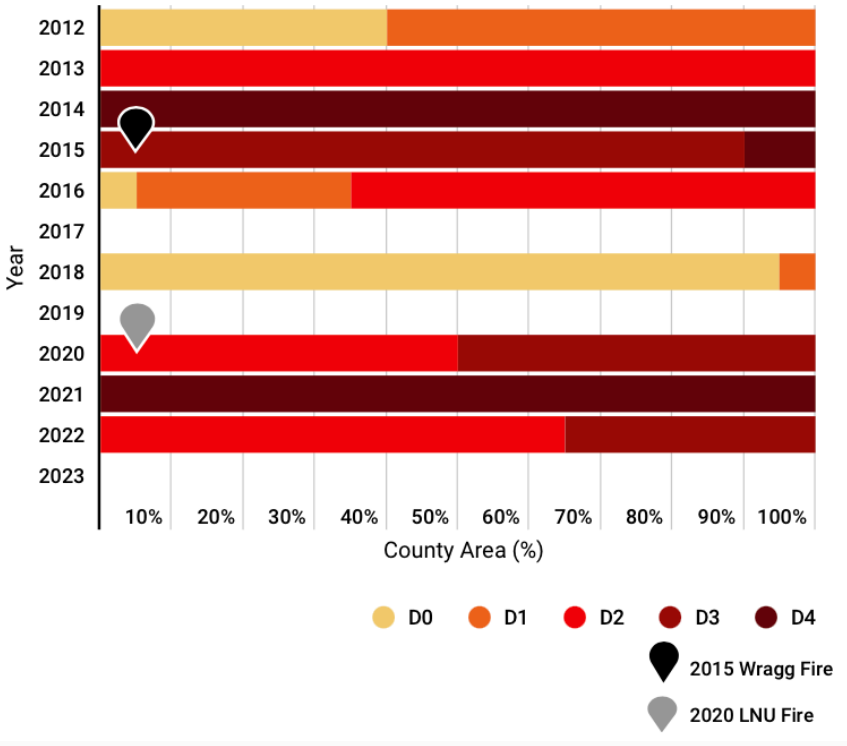


Fig. 2. Chart depicting yearly drought severity and the percent of county involvement in A) Napa and B) Solano counties from January 2000- January 2023. D0- abnormally dry, D1- moderate drought, D2- severe drought, D3- extreme drought, and D4- exceptional drought. Years that are white indicate no drought within the entire county. Data was adapted from the U.S. Drought Monitor (https://droughtmonitor.unl.edu).

Table 1: Primers and probes used for qPCR detection of various bacteria known to cause human disease in *Ixodes* spp. and *Dermacentor* spp., including *A. phagocytophilum*, *Borrelia* spp., *Babesia* spp., *F. tularensis*, *Bartonella* spp., and various *Rickettsia* spp.

Organism	Target	Primers (5'-3')	Probe (5'-3')	Resource
A. phagocytophilum	*msp* 2	F: AGTTTGACTGGAACACACCTGATC R: CTCGTAACCAATCTCAAGCTCAAC	TTAAGGACA ACATGCTTG TAGCTATGG AAGGCA	Drazenovich et al. 2006
Borrelia burgdorferi sl	16S rDNA	F: GCTGTAAACGATGCACACTTGGT R: GGCGGCACACTTAACACGTTAG	TTCGGTACT AACTTTTAG TTAA	Barbour et al. 2009
*Borrelia miyamotoi**	16S rDNA	F: GCTGTAAACGATGCACACTTGGT R: GGCGGCACACTTAACACGTTAG	CGGTACTAA CCTTTCGATT A	Barbour et al. 2009
Rickettsia spp.	23S rRNA	F: AGCTTGCTTTTGGATCATTTGG R: TTCCTTGCCTTTTCATACATCTAGT	CCTGCTTCTA TTTGTCTTGC AGTAACACG CCA	Kato et al. 2013
R. rickettsii	gltA	F: AAATCAACGGAAGAGCAAAAC R: CCCTCCACTACCTGCATCAT	TCCTCTCCA ATCAGCGAT TC	Kato et al. 2013
R. philipii	nusG	F: AGGATGTCATTGTTGCGGGA R: TCTCCTAGATTGCCACGCAG	ATGCGTCGT CATAGCCGT AG	Karpathy et al. 2019
R. massiliae	Genomic DNA	F: CTYCCCTCCCATCTGAACT R: CCTTTGGAGTTAGCAATACTTATAC	TCTAAATCA TGACCTGTTT CTGAATA	Publication in progress
*R. rhipicephali**	9666 intergenic sequence	F: CCAACCTTTTGTTGTTGCAC R: TTGGATCAGTGTGACGGACT	CACGTGCTG CTTATACCA GCAAACA	Renvoise et al. 2012
R. tillamookensis	aspS	F: GCTTGCTGATTTAAAGGAAATGCA R: AGGAAGTTGATAAGAGATTTGGGG	CCATGCGGC GGTGCTCCA AACT	Paddock et al. 2022
Francisella tularensis	Tul4	F: ATTACAATGGCAGGCTCCAGA R: TGCCCAAGTTTTATCGTTCTTCT	TTCTAAGTG CCATGATAC AAGCTTCCC AATTACTAA G	Versage et al. 2003
Babesia duncani	ITS-1	F: GCTTCCTAACCCGAGACCAA R: CACTGGCGGGGTGAAAAGTA	TACGGCCCC TGATGTCGG GG	Wilson et al. 2015
Bartonella spp.	ssrA	F: GCTATGGTAATAAATGGACAATGAA ATAA R: GCTTCTGTTGCCAGGTG	ACCCCGCTT AAACCTGCG ACG	Diaz et al. 2012

*Reporter dye: VIC

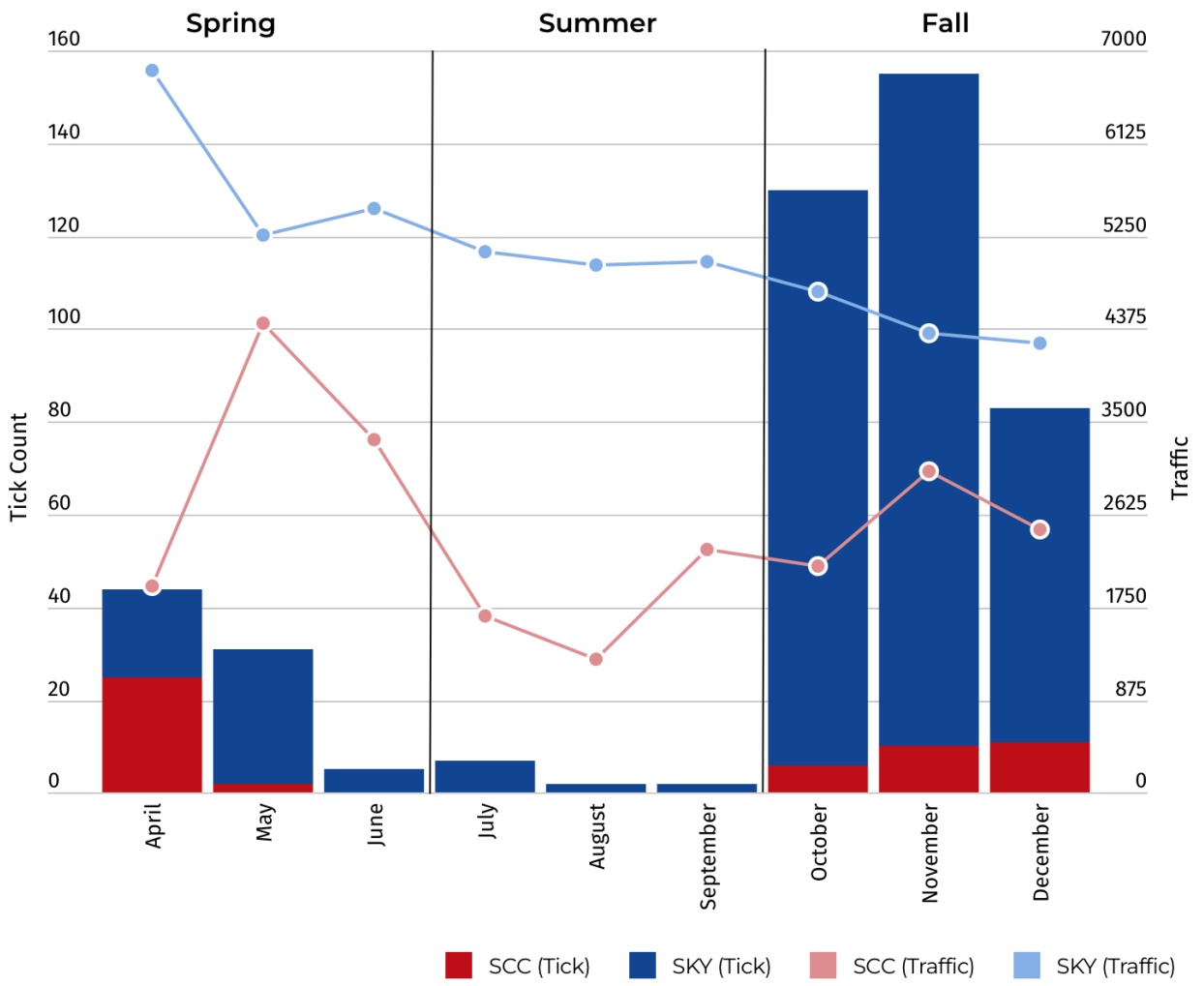


Fig.3. Chart depicting total number of ticks collected per month (bar chart) and foot traffic (lines) at both SCC and SKY. Foot traffic is measured as the number of people entering the trailhead per month based on counters at SCC. At SKY, use traffic includes hikers, equestrians, and mountain bikers utilizing the trails. All use traffic data was provided by park directors at both locations. Ticks were collected via flagging along popular hiking trails for a total of 1 mile during a 4 hour duration. No ticks were collected over 4 hours at SCC during the end of spring (June) and summer (July-September).

Table 2: Total number and species of questing ticks collected along hiking trails within Stebbins Cold Canyon and Skyline Wilderness Park, per season. Temporal mean abundance is depicted as mean number of ticks collected per hour ± SD for each season and location.

Season	Location	Total	Mean ± SD	*D. occidentalis*	*D. similis*	*D. albipictus*	*I. pacificus*
Spring	SCC	27	2.3 ± 3.5	14.8% (4/27)	0	0	85.2% (23/27)
	SKY	53	4.4 ± 3.0	75.5% (40/53)	3.8% (2/53)	0	20.8% (11/53)
	Total	**80**	-	**55.0% (44/80)**	**2.5% (2/80)**	**0**	**42.5% (34/80)**
Summer	SCC	0	0	0	0	0	0
	SKY	11	0.9 ± 0.7	81.8% (9/11)	9.1% (1/11)	9.1% (1/11)	0
	Total	**11**	-	**81.8% (9/11)**	**9.1% (1/11)**	**9.1% (1/11)**	**0**
Fall	SCC	27	2.3 ± 0.7	0	0	0	100.0% (27/27)
	SKY	341	28.4 ± 9.4	0.3% (1/341)	0	83.3% (284/341)	16.7% (57/341)
	Total	**368**	-	**0.3% (1/368)**	**0**	**77.2% (284/368)**	**22.8% (84/368)**
Total		**459**	**-**	**11.8% (54/459)**	**0.7% (3/459)**	**62.1% (285/459)**	**25.7% (118/459)**

Spring- April, May, June, Summer- July, August, September, Fall- October, November, December, SCC- Stebbins Cold Canyon, SKY- Skyline Wilderness Park, L-larvae, N-nymph, A-adult

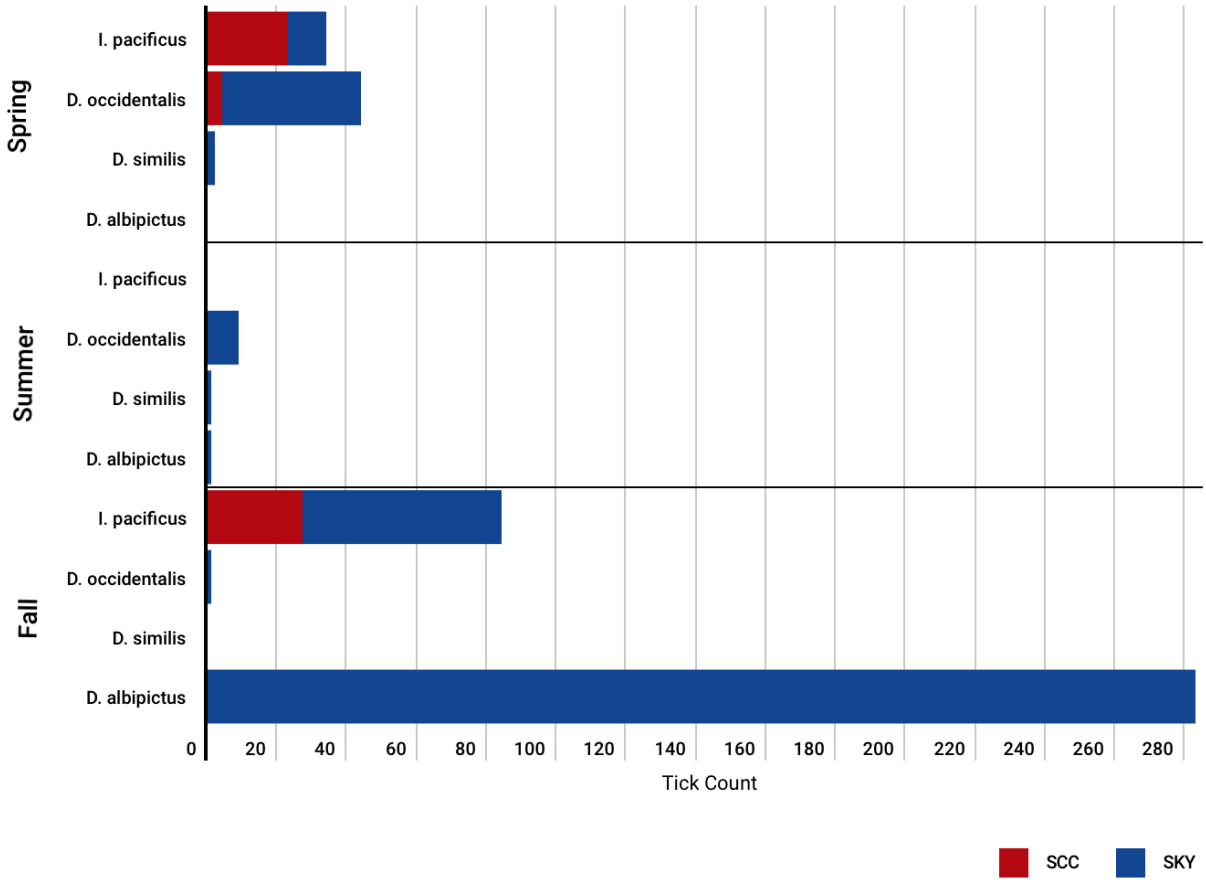


Fig.4. Bar chart depicting species count per season and location throughout April to December 2023. No ticks were collected at SCC in the summer. Mainly *I. pacificus* and *D. occidentalis* were collected at SCC. At SKY, *D. similis* and *D. albipictus* were also collected. Ticks were collected via flagging along popular hiking trails for a total of 1 mile during a 4 hour duration. No ticks were collected over 4 hours at SCC during the end of spring (June) and summer (July-September). Spring- April, May, June, Summer- July, August, September, Fall- October, November, December, SCC- Stebbins Cold Canyon, SKY- Skyline Wilderness Park.

Table 3: Results of negative binomial regression for tick count data at SCC and SKY. For the GLM, predictor variables were location and season, reference terms were SKY and Spring, and the response variable was total tick count. A) GLM results, including larvae, looking at the individual effects of location and season. B) GLM results, including larvae, looking at the interaction of location and season; C) GLM results, excluding larvae. Interactions between variables are indicated by variable 1: variable 2.

A)

Variable/Interaction	Coefficient	SE	IRR	95% CI		*p*
				Lower	Upper	
Intercept	3.61	0.41	36.89	16.37	83.11	**2.00×10^{-16}**
Location- SCC	-1.90	0.46	0.15	0.06	0.37	**3.51×10^{-5}**
Season- Fall	0.87	0.50	2.39	0.9	6.35	0.08
Season- Summer	-2.60	0.63	0.07	0.02	0.26	**3.78×10^{-5}**

SE= estimated standard error. IRR= incident rate ratio. CI= confidence interval.

B)

Variable/Interaction	Coefficient	SE	IRR	95% CI		*p*
				Lower	Upper	
Intercept	2.87	0.40	17.67	7.99	39.06	**1.30×10^{-12}**
Location- SCC	-0.67	0.59	0.51	0.16	1.61	0.25
Season- Fall	1.86	0.56	6.43	2.15	19.22	**8.56×10^{-4}**
Season- Summer	-1.57	0.63	0.21	0.06	0.72	**0.01**
Location-SCC: Season-Fall	-1.86	0.82	0.16	0.03	0.78	**0.02**
Location- SCC: Season-Summer	-19.93	5442.46	0	0	-*	1.00

SE= estimated standard error. IRR= incident rate ratio. CI= confidence interval.
*Upper 95% CI too large to be calculated

C)

Variable/Interaction	Coefficient	SE	IRR	95% CI		*p*
				Lower	Upper	
Intercept	2.87	0.50	17.67	6.66	46.9	**1.18×10^{-8}**
Location- SCC	-0.68	0.72	0.51	0.12	2.08	0.35
Season- Fall	-0.68	0.81	0.51	0.11	2.47	0.83
Season- Summer	-1.57	0.75	0.21	0.05	0.91	**0.04**
Location-SCC: Season-Fall	0.56	1.15	1.74	0.18	46.9	0.88
Location- SCC: Season-Summer	-19.92	5442.46	0	0	-*	1.00

SE= estimated standard error. IRR= incident rate ratio. CI= confidence interval.
*Upper 95% CI too large to be calculated

Table 4: Quantitative PCR results from questing ticks along SCC and SKY. A) Prevalence for all pathogens except *Rickettsia* spp. B) Prevalence of various *Rickettsia* spp. Samples were considered positive with Ct < 40 and characteristic amplification curves. Absolute prevalence is reported as the percent of positive ticks (number of qPCR positive/total number tested per location).

A)

		Percent positive ticks (no. qPCR positive/no. tested)					
		A. phagocytophilum	***B. duncani***	***Bartonella* spp.**	***Borrelia* spp.**		***F. tularensis***
					Miyamotoi	***Bbsl***	
Spring	SCC	0%	**7.4% (2/27)**	0%	0%	0%	0%
	SKY	0%	0%	0%	0%	0%	0%
Summer[a]	SCC[b]	-	-	-	-	-	-
	SKY	0%	0%	0%	0%	0%	0%
Fall	SCC	0%	0%	0%	0%	0%	0%
	SKY	0%	**1.5% (5/341)**	0%	0%	**1.7% (1/58)**	0%
Total	SCC	0%	**3.7% (2/54)**	0%	0%	0%	0%
	SKY	**0%**	**1.2% (5/405)**	**0%**	**0%**	**0.8% (1/121)**	**0%**
	Total	**0%**	**1.5% (7/459)**	**0%**	**0%**	**0.6% (1/175)**	**0%**

[a] In summer, no *Ixodes* spp. were collected at either SCC or SKY, and therefore, none were tested.
[b] In summer, no ticks were collected at SCC.

B)

		Percent positive ticks (no. qPCR positive/no. tested)					
		Pan-*Rickettsia*	***Rickettsia* spp.**				
			Rhipicephali	**364D**	***Massiliae***	***Tillamookensis***	***Rickettsii***
Spring	SCC	**81.5% (22/27)**	0%	0%	0%	0%	0%
	SKY	**28.3% (15/53)**	3.8% (2/53)	0%	0%	**1.9% (1/53)**	0%
Summer[a]	SCC[b]	-	-	-	-	-	-
	SKY	0%	0%	0%	0%	0%	0%
Fall	SCC	**92.6% (25/27)**	0%	0%	0%	7.4% (2/27)	0%
	SKY	**84.5% (49/58)**	0%	0%	0%	**1.7% (1/58)**	0%
Total	SCC	**87.0% (47/54)**	0%	0%	0%	3.7% (2/54)	0%
	SKY	**52.9% (64/121)**	**1.7% (2/121)**	**0%**	**0%**	**1.7% (2/121)**	**0%**
	Total	**63.4% (111/175)**	**1.1% (2/175)**	**0%**	**0%**	**2.3% (4/175)**	**0%**

[a] In summer, no *Ixodes* spp. were collected at either SCC or SKY, and therefore, none were tested.
[b] In summer, no ticks were collected at SCC