

Title: Microclimate conditions alter *Ixodes scapularis* (Acari: Ixodidae) overwinter survival across climate gradients in Maine, United States

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24 Abstract

25 The incidence and geographic range of vector-borne diseases have been expanding in recent
26 decades, attributed in part to global climate change. Blacklegged ticks (*Ixodes scapularis*), the
27 primary vector for multiple tick-borne pathogens in North America, are spreading rapidly
28 beyond their historic post-colonial range and are thought to be constrained mainly by winter
29 temperature at northern latitudes. Our research explored whether winter climate currently limits
30 the distribution of blacklegged ticks and the pathogens they transmit in Maine, U.S.A., by
31 contributing to overwinter mortality of nymphs. We experimentally tested tick overwinter
32 survival across large-scale temperature and snowfall gradients and assessed factors contributing
33 to winter mortality in locations where blacklegged tick populations are currently established and
34 locations where the blacklegged tick has not yet been detected. We also tested the hypothesis that
35 insulation in the tick microhabitat (i.e., by leaf litter and snowpack) can facilitate winter survival
36 of blacklegged tick nymphs despite inhospitable ambient conditions. Overwinter survival was
37 not significantly different in coastal southern compared to coastal and inland northern Maine,
38 most likely due to sufficient snowpack that protected against low ambient temperatures at high
39 latitudes. Snow cover and leaf litter contributed significantly to overwinter survival at sites in
40 both southern and northern Maine. To further assess whether the current distribution of
41 blacklegged ticks in Maine aligns with patterns of overwinter survival, we systematically
42 searched for and collected ticks at seven sites along latitudinal and coastal-inland climate
43 gradients across the state. We found higher densities of blacklegged ticks in coastal southern
44 Maine (90.2 ticks/1000 m²) than inland central Maine (17.8 ticks/1000 m²) and no blacklegged
45 ticks in inland northern Maine. Our results suggest that overwinter survival is not the sole

constraint on the blacklegged tick distribution even under extremely cold ambient conditions and additional mechanisms may limit the continued northward expansion of ticks.

Key Words: blacklegged tick; overwinter; mortality; microhabitat; snow

Introduction

Within recent decades, numerous hard-bodied tick species have spread dramatically beyond their historical ranges and increased in density within those ranges, leading to the concomitant expansion of the pathogens they transmit and the emergence and re-emergence of dozens of tick-borne diseases in humans and animals (e.g., Gubler, 1998; Randolph, 2001; Lindgren and Gustafson, 2001; Parola et al., 2005; Jaenson and Lindgren, 2011; Jaenson et al., 2012; Kilpatrick and Randolph, 2012; Mansfield et al., 2017; Nah et al., 2020). The most important disease vector ticks in northeastern North America are nymphal and adult blacklegged ticks, *Ixodes scapularis* (Eisen et al., 2017; Adams et al., 2015), a species that may transmit a diverse array of pathogens, including *Borrelia burgdorferi* sensu stricto (the primary causative agent of Lyme disease in North America), *Anaplasma phagocytophilum* (human granulocytic anaplasmosis), and *Babesia microti* (human babesiosis). The blacklegged tick first was detected in the northeastern U.S.A. in small, isolated populations in the 1920s (Larrousse et al., 1928), restricted by deforestation and availability of habitat and key vertebrate hosts, and increased in range and density both northwards and westwards across the U.S.A. substantially throughout the latter half of the 20th century (Ogden et al., 2008; Diuk-Wasser et al., 2014; Lantos et al., 2017; Bisanzio et al., 2020; Gardner et al., 2020). Since 1998, the number of U.S. counties in which the blacklegged tick reportedly is established nearly has doubled (Dennis et al., 1998; Eisen et al.,

2016), and the blacklegged tick also is spreading rapidly in southern Canada, advancing at rates of 46 km/year in Ontario (Clow et al., 2017).

Understanding the ecological mechanisms that constrain the establishment and maintenance of tick populations at the margins of their current spatial distributions may enhance our ability to predict future geographic range expansion of medically significant ticks and target public health interventions accordingly, as well as devise effective adaptive management strategies. Stimulated by change in photoperiod, blacklegged ticks enter diapause over winter as unfed or fed larvae, unfed nymphs, or unfed or fed adults (Smith and Cole, 1941; Yuval and Spielman, 1990; Rand et al., 2007; Gray et al., 2016). Diapause is terminated in response to increasing rising temperatures and increasing day-length (Belozerov, 2009; Gray et al., 2016). Because laboratory studies have shown that exposure to temperatures below -10°C for as little as two hours kills blacklegged ticks (Burks et al., 1996; VanDyk et al., 1996), it long has been hypothesized that low winter temperatures constrain their northern distribution. However, this assumption has met with mixed support in field experiments because microhabitats experienced by overwintering ticks often become decoupled from ambient conditions (Boehnke et al., 2017). Although a study conducted in Canada indicates that low temperatures can limit overwinter blacklegged tick survival severely across multiple tick life-stages (Lindsay et al., 1995), additional experiments in New York suggest that tick behavioral avoidance of adverse conditions may result in overwinter temperature having little impact on tick survival (Brunner et al., 2014), or that other microhabitat conditions, such as the presence of natural predators of ticks, may impact tick overwinter survival more strongly than cold temperatures alone (Burtis et al., 2016). At least one field experiment revealed that tick mortality during overwinter diapause is higher in early spring than during the coldest depths of winter due to tick depletion of lipid reserves as

temperatures warm (Burtis et al., 2019). Finally, multiple field studies have revealed that insulation provided by snowpack, leaf litter, or both (Lindsay et al., 1998; Lindsay et al., 1999; Brownstein et al., 2005; Dautel et al., 2016; Linske et al., 2019) can greatly enhance tick survival in the soil in an optimal microclimate despite inhospitable ambient conditions. However, all these studies took place in locations in which blacklegged ticks already occur at detectable densities. Additional field experiments north of the blacklegged tick's current range offer the opportunity to assess whether ticks can survive winter conditions in regions where populations are not established and thus provide an explicit mechanistic link between overwinter survival and tick distributions.

The state of Maine in the northeastern U.S.A., is an ideal location in which to study climate impacts on blacklegged tick overwinter survival for multiple reasons. Maine is characterized by latitudinal and coastal-inland temperature and snowfall gradients (Figure 1, Table 1) that provide a unique opportunity to conduct field experiments to study the interacting effects of broad-scale ambient climate and fine-scale microclimate conditions on blacklegged tick survival. Annual temperatures in the northeastern U.S. are increasing faster than in any other region in the country (Karmalker and Bradley, 2017). Across Maine, the average annual temperature has increased significantly since 1960, with the greatest magnitude of change occurring during the winter, accompanied by increased frequency and intensity of rainfall and snowfall (Fernandez et al., 2020). Maine also is an epidemiologically significant state, with over 2,000 human cases of Lyme disease reported in 2019 (Maine CDC, 2020), and the geographic range expansion of the blacklegged tick is ongoing. Since the first human cases of Lyme disease in Maine were reported to the CDC in 1986 (Anderson et al., 1987; Ginsberg and Ewing 1988),

the blacklegged tick and the pathogens it transmits have been spreading north and east across the state (Rand et al., 2007; Elias et al., 2021; Rounsville et al., 2021).

The goal of this study was to assess ecological pathways by which climate may currently constrain the northern distribution of blacklegged ticks. First, to determine the effects of climate on blacklegged tick overwinter survival, we tested nymphal tick overwinter survival across broad-scale temperature and snowfall gradients in Maine and assessed factors contributing to overwinter mortality over two years in locations where blacklegged tick populations are currently established and those where populations have not yet been detected. Second, to determine the effects of microclimate on blacklegged tick survival, we leveraged the experimental design and methods of Linske et al. (2019) to test nymphal tick overwinter survival in different microhabitats (i.e., snow removal, leaf litter removal, both snow and leaf litter removal, and a control in which insulation was not manipulated) spanning ambient temperature and snowfall gradients. We hypothesized that overwinter mortality decreases with increasing insulation, facilitating tick survival under harsher ambient conditions than considered by previous field experiments. Finally, to assess whether our overwinter survival findings align with the current distribution of blacklegged ticks with consequences for pathogen transmission in Maine, we conducted active surveillance of ticks during the summer when nymphs, the most epidemiologically significant life stage, are most likely to be active (Piesman et al., 1987) and screened ticks for tick-borne pathogens commonly reported in Maine (*B. burgdorferi* sensu lato, *B. microti*, and *A. phagocytophilum*).

Methods

Site selection. Enclosures containing nymphal blacklegged ticks were established at four sites across Maine in Cape Elizabeth [Cumberland County], Orono [Penobscot County], Brownville [Penobscot County], and Presque Isle [Aroostook County] (Figure 1). These sites were selected to span the state’s broad-scale temperature and snowfall gradients. Enclosures were established in mixed hardwood stands dominated by oak (*Quercus* spp.), maple (*Acer* spp.), birch (*Betula* spp.), and pine (*Pinus* spp.), which provide suitable habitat for blacklegged ticks (Ginsberg and Ewing, 1989; Adler et al., 1992; Ostfeld et al., 1995; Lindsay et al., 1999). Tree species composition and understory structure were comparable across sites, and efforts were made to maintain consistent site-level factors such as vegetation, soil type, and topography. We conducted off-host tick collections during the summer at these sites and three additional sites in Cutler, Mt. Vernon, and East Hancock (Figure 1).

Overwinter survival experiment. To assess overwinter survival as a potential mechanism to explain state-wide variation in tick densities, we conducted a two-year field experiment from 2018 to 2020. To test the hypothesis that ambient climate and microclimate affect overwinter survival, tick enclosures were constructed from one-gallon plastic buckets. Three holes were cut in the lid of each bucket and four holes were cut around the side of each bucket to allow for air flow and drainage. Each hole was covered with a small square of mesh and secured with PVC Cement (#30894, Oatey®, Cleveland, OH, USA) and all-weather tape (SI-13786BL, Gorilla®, Cincinnati, OH, USA) to prevent wildlife from interfering with the vials inside the buckets (Figure 2). At each site, buckets were inserted into ~20 cm holes dug in the ground and filled with the excavated soil to ~5 cm from the top. In compliance with the Maine Department of Inland Fisheries and Wildlife regulations, which prohibited seeding soil cores directly with ticks

(as was done in Bertrand and Wilson (1996)) to reduce the probability of the escape of ticks into the environment in areas where they are not currently established, one polypropylene vial containing 15 unfed, pathogen-free *I. scapularis* nymphs was placed on top of the soil in each enclosure. Nymphal ticks were used for this experiment because they are the most epidemiologically significant life stage (Piesman et al., 1987). Pathogen-free *I. scapularis* nymphs (three months post-molt) were obtained from the Oklahoma State University Tick Rearing Facility (Stillwater, OK, USA), which maintains a colony that originated and is refreshed from the natural population of *I. scapularis* in Stillwater, OK. Three holes were punched through the top of each vial using a tack to increase tick exposure to the temperature and humidity of the surrounding environment, and mesh was placed over the opening of each vial to prevent the accidental release of ticks. Ticks were counted before being placed in the field and again upon retrieval to ensure that no ticks escaped. At each site, the enclosures were established in an area isolated from human activities and slightly elevated relative to the surrounding area to prevent flooding in the enclosures during snowmelt.

To test the hypothesis that snow cover and leaf litter provide insulation and promote overwinter survival, four treatments were created to manipulate insulation in the enclosures based on the experimental design developed by Linske et al. (2019). The treatments were snow removal, leaf litter removal, both snow and leaf litter removal, and a control for which there was no manipulation of insulation conditions (referred to as SR, LR, SLR, and C, respectively, in figures and tables). To reflect the type and amount of leaf litter present in the surrounding area, leaf litter was obtained directly from the site and placed on top of each vial in snow removal and control enclosures before the enclosures were sealed. Leaf litter was replaced before the start of the second winter. No leaf litter was added to leaf removal and snow/leaf removal enclosures.

After each snow event which resulted in snow cover greater than 1 cm, snow was removed from the tops of snow removal and snow/leaf removal enclosures. We allowed snow to accumulate throughout the winter over leaf removal and control enclosures.

The sites in Cape Elizabeth, Orono, and Presque Isle each contained four replicates of each of the four treatments for a total of 16 enclosures at each site. The enclosures were the experimental units of the study. Due to the remote location of the site in Brownville, it was not feasible to regularly remove snow after each snow event throughout the winter. Therefore, we established four replicates of the control enclosures and no manipulated enclosures at this site. Due to flooding in the enclosures during the first winter in Orono, data from this site were excluded from analyses during the first year of the study. Prior to placement in the field, ticks were kept in a plastic bag containing a damp paper towel for humidity and stored at -4°C for storage until conditions were appropriate for placing the ticks in enclosures. The ticks were placed in enclosures in late November before significant ground frost and snow cover accumulation. The duration of the experiment was from 24 November 2018 to 26 April 2019 and from 29 November 2019 to 21 April 2020. To evaluate ticks for survival at the conclusion of the experiment, we held ticks at room temperature for at least 30 min and observed them for movement. Efforts to stimulate movement such as applying human breath or gentle prodding with forceps were applied if the ticks did not start to move after 30 min at room temperature. The ticks were considered dead if they did not respond to stimulation efforts after 30 min.

Temperature and snowfall measurements. To test the hypothesis that snow cover and leaf litter provide insulation from temperature extremes, iButton data loggers (DS1922L ThermoChron; Maxim Integrated, San Jose, CA, USA) were established inside each enclosure

next to the tick vials to record temperature (°C) once per hour. Snow depth was measured weekly and after each snow event. Because significant snow accumulation in northern sites buried ambient iButtons, ambient temperature data were obtained from the Maine Climate Office at the University of Maine Climate Change Institute (Climate Change Institute, 2020). Due to travel restrictions in 2020, weekly snowfall data in Presque Isle were obtained from the NOAA National Centers for Environmental Information (National Oceanic and Atmospheric Administration, 2020).

Off-host tick collection. To test the hypothesis that the current distribution of blacklegged ticks in Maine reflects our overwinter survival findings, we collected host-seeking blacklegged ticks at seven sites across Maine in Cape Elizabeth, Mt. Vernon, Orono, East Hancock, Brownville, Cutler, and Presque Isle (Figure 1). Collections were conducted between 10 June and 23 August in 2019, the peak activity period of nymphal *I. scapularis* in Maine (Rand et al., 2007). At each site, an 8,000 m² grid was established in an area with favorable vegetation for blacklegged ticks (i.e., mixed hardwood and significant leaf litter) to maximize the possibility of finding blacklegged ticks. Ticks were collected using a standard “dragging” method (Zimmerman and Garris, 1985), where questing ticks are collected from a 1 m² corduroy cloth attached to a dowel rod that is dragged across vegetation or leaf litter and checked for ticks every 10 m. Eight 125-m transects were dragged across the grid for a total of 1,000 m² per dragging session. Sites were dragged once every two weeks from early June to mid-August and dragging was not performed during rain or when the vegetation or leaf litter was wet enough to soak the drag cloth.

All *I. scapularis* life stages and other tick species found were collected and preserved in 70% ethanol and stored at -4°C. Larval ticks were removed from the drag cloth with a piece of

adhesive tape and stored in plastic bags. All ticks were identified to life stage and species using standard keys (Keirans and Clifford, 1978; Keirans and Litwak, 1989; Durden and Keirans, 1996; Egizi et al., 2019).

Pathogen detection. To determine nymphal infection prevalence across the state, *I. scapularis* nymphs collected during the summer of 2019 were screened for *B. burgdorferi* sensu lato (s.l.), as well as *A. phagocytophilum* and *B. microti*, two emergent pathogens transmitted by blacklegged ticks in Maine. We tested ticks collected in Cape Elizabeth, Orono, Mt. Vernon, Presque Isle, Cutler, and East Hancock. We tested all *I. scapularis* nymphs collected in Mt. Vernon (n = 15), Cutler (n = 3), and East Hancock (n = 2) and a randomly selected subset of 77 nymphs collected in both Cape Elizabeth and Orono. We also screened four *Ixodes cookei* (three nymphs and one adult) and one *Ixodes marxi* nymph collected in Presque Isle to determine whether the three pathogens of interest may be maintained in cryptic cycles in the absence of *I. scapularis* (Hamer et al., 2010).

Before DNA extraction, nymphal ticks were cut in half longitudinally and adult ticks were quartered to disrupt the exoskeleton and expose the salivary glands. DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA, USA) using a modified extraction protocol (Hojgaard et al. 2014). PCR testing was completed at the University of Maine Cooperative Extension Diagnostic and Research Laboratory. Each PCR reaction (10 μ L) contained 5 μ L of Bio-Rad iQ Multiplex Powermix (Bio-Rad, Hercules, CA, USA), 3 μ L of primers and probes (Hojgaard et al., 2014; Xu et al., 2016; Rounsville et al., 2021), and 2 μ L of extracted DNA template. PCR reactions were completed on the CFX96 Touch Real-Time PCR Detection System (Bio-Rad), and conditions were as follows: 3 min Hot-start at 95°C, followed

by 40 cycles of 95°C for 15 s and 60°C for 45 s. Each sample was run in duplicate to account for false positives; samples were determined to be positive if DNA amplified at a critical threshold ($C_t \leq 35.0$). Due to a high frequency of hybridized primers, samples that initially tested positive for *B. microti* were rerun a third time replacing the Bio-Rad iQ Powermix for PowerUp SYBR Green Master Mix to eliminate false positives.

Statistical analysis. Data analysis was conducted using the lme4 package (Bates et al., 2015) in R version 3.5.1 (R Core Team, 2013). First, we used general linear mixed models (GLMMs) to test the hypotheses that tick overwinter survival rates vary across broad-scale climate gradients in control enclosures (i.e., snow and leaf litter present) and across microhabitat treatments. Second, we used GLMMs with repeated measures to test the hypotheses that temperature and snowfall vary across study locations in control enclosures and temperature varies across manipulated microhabitat treatments. Tukey mean separation tests were carried out to detect significant differences among treatment levels ($\alpha = 0.05$) using the emmeans package in R (Lenth, 2021). Finally, we used multiple linear regression to test for associations between ambient and microclimate conditions (i.e., mean and minimum temperature, mean and maximum snow depth) and tick overwinter survival. Year was included as a random effect in all models. Tick survival data (calculated as a percentage of 15 nymphs per replicate enclosure) met assumptions for normality and homoscedasticity of variances and were not transformed.

Results

Effect of climate across large spatial scales on overwinter survival. During the first year of the study, there was no significant difference in survival of nymphal blacklegged ticks

overwinter in control conditions (i.e., snow and leaf litter present) between locations (Figure 3, Table 2). Percent survival in Cape Elizabeth, Brownville, and Presque Isle was 91%, 93%, and 71%, respectively. During the second year of the study, tick survival was highest in Cape Elizabeth (91%) and Brownville (93%), lower in Orono (69%) and lowest in Presque Isle (27%). Tick survival in control treatments was significantly lower during the second year than the first year in Presque Isle, but not in Cape Elizabeth or Brownville.

Overall, lower ambient temperatures were recorded during the first year of the study compared to the second, though neither average snow depth nor maximum snow depth was statistically different between years (Table 2, 3). Mean ambient temperature and minimum ambient temperature were highest in Cape Elizabeth and lowest in Presque Isle (Figure 4), while average snow depth and maximum snow depth were significantly higher in Brownville and Presque Isle than in Cape Elizabeth and Orono (Table 2). Multiple linear regression identified minimum and mean ambient temperature as climate variables that may explain the influence of location on tick survival in control treatments (Table 2).

Effect of microhabitat treatment on overwinter survival. Microhabitat treatment affected tick overwinter survival in both Cape Elizabeth and Presque Isle during the first year of the study but not the second (Figure 3, Table 4). At Cape Elizabeth, tick survival was highest in the control treatment followed by snow removal and leaf removal treatments, which resulted in the lowest survival (Figure 3). At Presque Isle, control, snow removal, and leaf removal treatments were similar and tick survival was lowest in the snow/leaf removal treatment (Figure 3). Similarly, microhabitat treatment affected tick survival in Orono; survival was highest in the leaf removal

treatment and lowest in the snow/leaf removal treatment. Survival was significantly higher between the first and second years in Cape Elizabeth but not in Presque Isle.

During both years of the study, treatment and location had significant effects on mean and minimum microclimate temperature (Table 4). Overall, temperatures of control and leaf removal treatments were significantly higher than snow removal and snow/leaf removal treatments, and mean temperatures were highest in Cape Elizabeth and lowest in Presque Isle (Figure 5). Multiple linear regression identified minimum and mean temperature as microclimate variables that may explain the influence of microhabitat conditions on control tick survival (Table 4).

Off-host tick collection and pathogen prevalence. We collected a total of 1,188 off-host ticks across Maine. A total of 909 *I. scapularis* were collected across the state in Cape Elizabeth, Mt. Vernon, Orono, East Hancock, and Cutler (Table 5). Additional tick species included *I. cookei* (n = 4), *I. marxi* (n = 1), *Dermacentor variabilis* (n = 43), and *Haemaphysalis leporispalustris* (n = 233) (Table 6). *Ixodes scapularis* were detected at all sites except in Brownville, where only *H. leporispalustris* was collected, and Presque Isle, where only *I. cookei* and *I. marxi* were collected. *Haemaphysalis leporispalustris* also were collected in East Hancock and Cutler, but *I. cookei* and *I. marxi* were not collected in any locations other than Presque Isle.

Overall, *B. burgdorferi* s.l. prevalence was highest in Cape Elizabeth (20%) and Orono (9.1%), and *A. phagocytophilum* and *B. microti* were only detected in ticks collected from Cape Elizabeth (Table 7). Among four *I. cookei* collected and tested from Presque Isle, one nymph was positive for *B. burgdorferi* s.l.

Discussion

Winter climate long has been hypothesized to constrain the geographic distribution and spread of vector tick species, yet no previous studies have tested this hypothesis using field experiments both in areas where ticks are abundant and in areas where ticks currently do not occur at detectable densities (Elias et al., 2020). We found that cold ambient temperature may reduce blacklegged tick overwinter survival, but ticks nonetheless can survive over winter in northern regions where reproductive populations apparently are not yet established because snow cover provides insulation from ambient conditions. Temperatures in control enclosures (i.e., leaf litter and snowpack present) remained above -10°C consistently while temperatures in northern Maine often were far lower, suggesting that ticks overwintering at the northern sites were not exposed to the lowest ambient temperatures throughout the winter because leaf litter and snow cover provided sufficient insulation to prevent freezing or desiccation. The removal of snow cover decreased tick overwinter survival more than the removal of leaf litter, demonstrating that snowpack may play an especially important role in overwinter survival in northern locations. Although we detected higher density of blacklegged ticks and prevalence of tick-borne pathogens in southern Maine compared to northern Maine, our field experiments suggest that overwinter survival of nymphs is not the primary mechanism constraining the current range of blacklegged ticks.

We found that increased insulation improved the survival of overwinter ticks in microhabitats and that insulation provides a warmer, more ideal microclimate. Snow and snow/leaf removal enclosure temperatures consistently were lower than control enclosure temperatures, suggesting that snowpack insulates the soil and thus prevented overwinter ticks from freezing despite low surface air temperatures (Decker et al., 2003; Templer et al., 2012).

342 This finding is consistent not only with other field studies of blacklegged ticks (Lindsay et al.,
343 1995; Burtis et al., 2015; Linske et al., 2019), but with several prior studies that assessed the
344 importance of snowpack and snow depth on the regulation of soil temperature and frost. Hardy et
345 al., (2001) conducted a snow removal experiment in New Hampshire, a state that, like Maine,
346 has permanent snowpack during the winter, and found that snow removal results in more
347 extensive soil frost than unmanipulated control conditions. Templer et al., (2012) tested the
348 impacts of snow removal on the abundance, richness, and diversity of soil-dwelling arthropods in
349 New Hampshire and found that a decrease in snowpack and snow depth may reduce the
350 abundance and diversity of arthropod communities. Previous tick overwinter studies conducted
351 in New York, Connecticut, and southern Maine investigating the effect of insulation on
352 overwinter tick survival found that snow removal did not decrease overwinter survival while leaf
353 litter removal did (Burtis et al., 2015; Linske et al., 2019). This research extended the latter
354 study, which was conducted in areas of Connecticut and southern Maine that support high
355 densities of blacklegged ticks, into central and northern Maine where blacklegged ticks largely
356 remain undetected. Perhaps more representative of the winter climate and low tick densities in
357 northern Maine, Lindsay et al., (1995) assessed nymphal overwinter survival in southern and
358 northern Ontario and found that nymphal survival was higher at northern sites compared to
359 southern sites due to more continuous snow cover in the north despite lower ambient
360 temperatures. We found that the effect of reduced insulation was more pronounced during the
361 first year of the study in Presque Isle, in which ambient temperatures were lower but survival
362 was higher than the second year. Thus, it is possible that the interaction between temperature and
363 snow depth is more significant in colder climates, and the combination of colder ambient
364 temperatures and greater average snow depth resulted in significant tick mortality in snow

removal treatments during the colder winter. Warmer ambient temperatures during the second year may have limited the effect of snow removal on overwinter survival because the ticks were not exposed to temperatures lower than -10°C as often as during the first year.

Tick survival under the most ecologically realistic conditions (i.e., snow and leaf litter present) decreased between the first and second years of the study in Presque Isle, which may have been caused by higher overall temperatures and reduced snowpack during the second winter. Warmer winters, which typically result in more intermittent snow cover than colder winters, can cause a greater degree of soil freezing than colder winters (Decker et al., 2003). Although ambient temperatures were higher during the second winter, control enclosure temperatures in Presque Isle were lower than during the first winter. Upon retrieval of the ticks after the second winter, we noted that several enclosures were encased in more ice in Presque Isle than the first winter, which may also have contributed to mortality. Alternatively, it is possible that the warmer spring temperatures during the second year resulted in lower survival. Warm spring temperatures induce higher energy use by ticks, which may cause increased mortality compared to low energy use during the winter (Burtis et al., 2019).

As expected, we documented a gradient of decreasing blacklegged tick density from southern to central Maine, which is consistent with previous passive surveillance studies (Rand et al., 2007; Elias et al., 2021; Rounsville et al., 2021). We detected no blacklegged ticks at our northern sites in Brownville and Presque Isle, yet other tick species including *H. leporispalustris*, *I. cookei*, and *I. marxi* were detected north of Orono. While we did not detect *I. cookei* and *I. marxi* in any locations except Presque Isle, both species are considered rare but widely distributed throughout Maine, and the distribution of these *Ixodes* species did not appear to change with the initial expansion of *I. scapularis* (Smith et al., 1992; Rand et al., 2007).

However, *I. cookei* and *I. marxi* were not detected even after intensive surveillance conducted on Mt. Desert Island, Maine, where *I. scapularis* are now well-established (A.M. Gardner unpublished data) and these species historically were endemic, suggesting that the changing distributions of tick species in endemic areas warrants additional research. Elias et al., (2021) recently reported that passively collected nymphal submission rates decreased along a longitudinal gradient in Maine where average winter temperatures were below -13°C, which is consistent with our finding of longitudinally decreasing tick density. The lower pathogen prevalence we detected in Orono compared to Cape Elizabeth is consistent with well-established correlations between nymphal tick abundance and Lyme disease cases (Rand et al., 2007; Gardner et al., 2020).

Because we found that snow cover and leaf litter insulated overwinter enclosures against low ambient temperatures in southern and northern Maine, we suggest that cold ambient temperatures do not limit blacklegged tick survival in northern areas where questing blacklegged ticks were not detected. Thus, blacklegged ticks may be limited in central and northern Maine by other biotic mechanisms such as densities of white-tailed deer (*Odocoileus virginianus*), which are crucial hosts for adult blacklegged ticks (Wilson et al., 1990). There is a correlation between deer and blacklegged tick densities in New England (Rand et al., 2004b), and several studies have suggested that lower deer densities in northern Maine may inhibit the establishment of blacklegged tick populations (Rand et al., 2004b; Telford 2007; Elias et al., 2021). Second, the greater density of coniferous forests in northern Maine compared to greater densities of deciduous forest in southern Maine (Irland 1997) may limit the size of northern tick populations, as the increased insulation caused by deciduous leaf litter compared to coniferous leaf litter creates a more favorable habitat that can increase off-host survival (Adler et al., 1992; Ostfeld et

al., 1996; Lindsay et al., 1999). Maine's climate may further constrain the distribution of ticks, as the lesser duration of time available for host-seeking over the summer may limit tick population size in northern climates (Vail and Smith 2002; Ogden et al., 2005; Diuk-Wasser et al., 2014), and colder temperatures may impact other stages of the tick life cycle (i.e, development and fecundity) (Lindsay et al., 1995; Peavey and Lane 1996; Lindsay et al., 1998; Ogden et al., 2004).

We detected higher *B. burgdorferi* s.l. prevalence in blacklegged ticks collected from southern Maine than central Maine, and *A. phagocytophilum* and *B. microti* were only detected in ticks collected in southern Maine. This is consistent with patterns in human case incidence for Lyme disease, anaplasmosis, and babesiosis; in 2019, higher incidence of Lyme disease and anaplasmosis was reported in southern Maine compared to northern Maine, and no babesiosis was reported in northern Maine (Maine CDC 2020). Although blacklegged ticks are the primary vectors of *B. burgdorferi* s.l., these pathogens have been detected in other *Ixodes* species such as *I. cookei*, *I. dentatus*, and *I. auritulus* (Levine et al., 1991; Barker et al., 1993; Morshed et al., 2005). Two other *Ixodes* species were collected in Presque Isle where no *I. scapularis* were found, and one *I. cookei* tested positive for *B. burgdorferi* s.l. Pathogen detection, however, does not imply vector competence; *Ixodes cookei* and *I. marxi* are both vectors of Powassan encephalitis virus, but experimental vector competence for *B. burgdorferi* s.l. has not been confirmed for *I. cookei* despite several studies being done and has never been evaluated for *I. marxi* (Artsob 1984; Barker et al., 1993). We tested ticks for *B. burgdorferi* s.l., which includes both pathogenic species (e.g., *B. burgdorferi* sensu stricto) and species not known to be pathogenic (e.g., *Borrelia andersonii*); thus, it is possible that the *B. burgdorferi* s.l. we detected at Presque Isle was non-pathogenic and not capable of causing Lyme disease in humans. Several

studies have found that human-pathogenic *B. burgdorferi* sensu stricto is not efficiently transmitted between *I. cookei* and their primary hosts in locations where Lyme diseases cases were reported but *I. scapularis* are not well established (Barker et al., 1992; Ryder et al., 1992). Furthermore, *I. cookei* and *I. marxi* may aggressively parasitize humans under the right conditions (Lubelczyk et al., 2010) but they are not commonly reported as feeding on humans regularly (Hall et al., 1991; Lee et al., 2019). This suggests that *I. cookei*, although capable of being infected, may not be a primary vector of *B. burgdorferi* sensu stricto in areas outside the range of the blacklegged tick.

Some limitations to this study may have contributed to our overwinter survival and distribution result. First, ticks overwintered in polypropylene vials that were placed in leaf litter or soil instead of directly in the microcosm. This may have influenced humidity and temperature that the ticks were exposed to (i.e., condensation collected in the vials and temperature in the vials may have been different from the immediate surroundings in the soil or leaf litter) (Bertrand and Wilson, 1996; Brunner et al., 2014). It has been documented that ticks that have direct access to the soil or leaf litter have a higher overwinter survival than ticks that are housed in containers due to the fact that ticks placed directly in the soil or leaf litter are able to move or relocate to a more favorable microclimate with higher relative humidity or temperature, while ticks in vials are limited in mobility (Padgett and Lane, 2001). Alternatively, although we did not measure humidity directly inside the vials, increased moisture and relative humidity inside the vials may have increased tick survival compared to ticks overwintering directly in soil or leaf litter. Second, while we made efforts to select sites with similar tree and vegetation composition, survival may also be related to site level factors such as vegetation, soil type, and topography. Third, the laboratory-reared tick colony originated in Oklahoma and belongs to the southern

clade of *I. scapularis* found in North America. Due to the population genetic and behavioral differences between northern and southern ticks (Ginsberg et al., 2014; Arsnoe et al., 2015), ticks originating from northern areas may be better adapted to surviving northeastern winter conditions, and our data thus represent an estimate of the survival rate of southern ticks exposed to a northern climate. Finally, while we conducted a comparative study between latitudinally-distinct regions, our results are not necessarily a representation of an overall overwinter survival estimate of *I. scapularis* across its entire range.

Our study revealed that manipulating microclimate insulation can reduce tick survival, which can be used to inform small-scale or local management practices to reduce leaf litter or snow cover throughout the fall and winter to potentially reduce overwinter survival. Removing leaf litter can also reduce the abundance of questing nymphs during active periods. It has been shown that active forest management can additionally alter the amount of leaf litter present (Conte et al., 2021), with potential consequences for overwinter tick survival. Thus, while labor and time intensive, removing leaf litter on a localized scale before ticks enter diapause in the late fall may reduce overwinter survival as well (Stafford and Magnarelli 1993; Schulze et al., 1995; Linske et al., 2019). In the Northeast, homeowners are most likely to encounter *I. scapularis* ticks on residential properties and peridomestic risk of tick-borne disease can be high in endemic areas (Falco and Fish 1988; Maupin et al., 1991; Feldman et al., 2015). Therefore, we suggest that small-scale management practices such as raking leaves in one's backyard may reduce individual risk of exposure to ticks (Jordan and Schulz, 2019). The findings of our study indicate that blacklegged ticks can successfully overwinter in areas north of their current range, and that snow cover plays a significant role in overwinter survival and thus the number of ticks that might emerge in the spring and summer with the potential to transmit pathogens to humans. Ultimately,

our results can be used to inform future studies assessing the relationship between climate change and the spread of blacklegged ticks, as current predictions of increasing temperatures and decreasing snowfall may affect not only overwinter survival, but tick development and reproduction. The current widening geographic range and increasing densities of blacklegged ticks portend a higher risk of tick-borne diseases to endemic and susceptible areas. As winter temperatures and snowfall are predicted to increase in the northeastern U.S.A and other areas of North America (Karmalker and Bradley, 2017; Fernandez et al., 2020), the ability to predict how climate change will impact the changing distribution of blacklegged ticks, posing a major public health concern. Understanding how climate affects blacklegged tick survival at the northern limits of their distribution can aid in predicting the future spread of ticks and tick-borne diseases.

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Competing Interests Statement

The authors declare no competing interests.

References

- Adams, D., Fullerton, K., Jajosky, R., Sharp, P., Onweh, D., Schley, A., Anderson, W., Faulker, A., Kugeler, K., 2015. Summary of notifiable infectious diseases and conditions – United States, 2013. MMWR - Morb. Mortal. Wkly. Rep. 62, 1-119.
- Amerasinghe, F.P., Breusch, N.L., Azad, A.F., Gimpel, W.F., Greco, M., Neidhardt, K., Pagac, B., Piesman, J., Sandt, J., Scott, T.W., Sweeney, K., 1992. Distribution, density, and Lyme disease spirochete infection in *Ixodes dammini* (Acari: Ixodidae) on white-tailed deer in Maryland. J. Med. Entomol. 29, 54-61.
- Anderson, J.F., Magnarelli, L.A., McAninch, J.B., 1987. *Ixodes dammini* and *Borrelia burgdorferi* in northern New England and upstate New York. J. Parasitol. 73, 419-421.
- Allan, B.F., Keesing, F., Ostfeld, R.S., 2002. Effect of forest fragmentation on Lyme disease risk. Conserv. Biol. 17, 267-272.
- Arsnoe, I.M., Hickling G.J., Ginsberg H.S., McElreath, R., Tsao, J.I., 2015. Different populations of blacklegged tick nymphs exhibit differences in questing behavior that have implications for human Lyme disease risk. PLoS ONE 10: e127450
- Artsob, H., Spence, L., Surgeoner, G., McCreadie, J., Thorsen, J., Th'ng, C., Lampotang, V., 1984. Isolation of *Francisella tularensis* and Powassan virus from ticks (Acari: Ixodidae) in Ontario, Canada. J. Med. Entomol. 2, 165-168.
- Bale, J.S., 1989. Cold hardiness and overwinter in insects. Agric. Zool. Rev. 3, 157-190.
- Barker, I.K., Lindsay, L.R., Campbell, G.D., Surgeoner, G.A., McEwen, S.A., 1993. The groundhog tick *Ixodes cookei* (Acari: Ixodidae): A poor potential vector of Lyme Borreliosis. J. Wildl. Dis. 29, 416-422.
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 97, 1-48.
- Belozеров, V.N., 2009. Diapause and quiescence as two main kinds of dormancy and their significance in life cycles of mites and ticks (Chelicerata: Arachnida: Acari). Part 2. Parasitiformes. Acarina 17, 3–32.
- Berger, K.A., Ginsberg, H.S., Gonzales, L., Mather, T.N., 2014. Relative humidity and activity patterns of *Ixodes scapularis* (Acari: Ixodidae). J. Med. Entomol. 51, 769-776.
- Bertrand, M.R. and Wilson, M.L., 1996. Microclimate-dependent survival of unfed adult *Ixodes scapularis* (Acari: Ixodidae) in nature: life cycle and study design implications. J. Med. Entomol. 33, 619-627.

- Boehnke, D., Gebhardt, R., Petney, T., Norra, S., 2017. On the complexity of measuring forests microclimate and interpreting its relevance in habitat ecology: the example of *Ixodes ricinus* ticks. *Parasit. Vectors* 10, 10-14
- Birkel, S., 2018. Winter 2017/2018 in Summary. Maine Climate Office, Climate Change Institute, University of Maine, USA.
- Brownstein, J.S., Holford, T.R., Holford, D., 2003. A climate-based model predicts the spatial distribution of the Lyme disease vector *Ixodes scapularis* in the United States. *Environ. Health. Perspect.* 111, 1152-1157.
- Brownstein, J.S., Holford, T.R., Fish, D., 2005. Effect of climate change on Lyme disease risk in North America. *EcoHealth* 2, 38-46.
- Brunner, J.L., Killilea, M., Ostfeld, R.S., 2014. Overwinter survival of nymphal *Ixodes scapularis* (Acari: Ixodidae) under natural conditions. *J. Med. Entomol.* 49, 981-987.
- Bunnell, J.E., Price, S.D., Das, A., Shields, T.M., Glass, G.E., 2003. Geographic information systems and spatial analysis of adult *Ixodes scapularis* (Acari: Ixodidae) in the Middle Atlantic region of the U.S.A. *J. Med. Entomol.* 40, 570-576.
- Burks, C.S., Stewart, R.L., Needham, G.R., Lee, R.E., 1996. The role of direct chilling injury and inoculative freezing in cold tolerance of *Amblyomma americanum*, *Dermacentor variabilis* and *Ixodes scapularis*. *Physiol. Entomol.* 21, 44-50.
- Burtis, J.C., Fahey, T.J., Yavitt, J.B., 2019. Survival and energy use of *Ixodes scapularis* nymphs throughout their overwinter period. *Parasitology* 146, 781-790.
- Campbell J.L., Ollinger S.V., Flechinger, G.N., Wicklein, H., Hayhoe, K., Bailey, A.S., 2010. Past and projected future changes in snowpack and soil frost at the Hubbard Brook Experimental Forset, New Hampshire, USA. *Hydro. Process.* 2, 2465-2480.
- Centers for Disease Control and Prevention. Lifecycle of Blacklegged Ticks. April 12, 2011; <https://www.cdc.gov/lyme/transmission/blacklegged.html>. Accessed May 6, 2020.
- Centers for Disease Control and Prevention. Lyme Disease Data and Surveillance. November 9, 2018; https://www.cdc.gov/lyme/datasurveillance/index.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Flyme%2Fstats%2Findex.html. Accessed November 27, 2018.
- Climate Change Institute, University of Maine. Maine Climate Office, Monthly Climate Data. https://mco.umaine.edu/data_monthly/. Accessed May 18, 2020.
- Clow, K.M., Leighton, P.A., Ogden N.H., Lindsay, L.R., Michel, P., Pearl, D.L., Jardine, C.M., 2017. Northward range expansion of *Ixodes scapularis* evident over a short timescale in

- Ontario, Canada. PLOS ONE 12:
e0189393. <https://doi.org/10.1371/journal.pone.0189393>
- Conte, C.E., Leahy, J.E., Gardner, A.M., 2021. Active forest management reduces blacklegged tick and tick-borne pathogen exposure risk. *EcoHealth* <https://doi.org/10.1007/s10393-021-01531-1>
- Daniels, T.J., Fish, D., Schwartz, I., 1993. Reduced abundance of *Ixodes scapularis* (Acari: Ixodidae) and Lyme disease risk by deer exclusion. *J. Med. Entomol.* 30, 1043-1049.
- Dautel, H., Kämmer, D., Kahl O., 2016. How an extreme weather spell in winter can influence vector tick abundance and tick-borne disease incidence, in: Braks et al. [eds], *Ecology and Control of Vector-borne Diseases*, pp. 335-349.
- Decker, K.L.M., Wang, D., Waite, C.E., Scherbatskoy, T., 2003. Snow removal and ambient air temperature effects on forest soil temperatures in northern Vermont. *Soil Sci. Soc. Am. J.* 67, 4.
- DeGraaf, R.M. and Rudis, D.D., 1990. Herpetofauna species composition and relative abundance among three New England forest types. *For. Ecol. Manag.* 32, 155-165.
- Diuk-Wasser, M. A., Gatewood, A. G., Cortinas, M. Roberto, Yaremych-Hamer, S., Tsao, J., Kitron, U., Hickling, G., Brownstein, J. S., Walker, E., Piesman, J., Fish, D., 2014. Spatiotemporal patterns of host seeking *Ixodes scapularis* nymphs (Acari: Ixodidae) in the United States. *J. Med. Entomol.* 43, 166-176.
- Durden, L.A. and Kierans, J.E., 1996. Nymphs of the genus *Ixodes* (Acari: Ixodidae) of the United States: taxonomy, identification key, distribution, hosts, and medical/veterinary importance. *Entomol. Soc. Am.*, Lanham, MD.
- Egizi, A.M., Robbins, R.G., Beati, L., Nava, S., 2019. A pictorial key to differentiate the recently detected exotic *Haemaphysalis longicornis* Neumann, 1901 (Acari, Ixodidae) from native congeners in North America. *ZooKeys.* 818, 117.
- Eisen, R.J., Eisen, L., Ogden, N.H., Beard, C.B., 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, 250-261.
- Eisen, R.L., Kugeler, K.J., Eisen, L., Beard, C.B., Paddock, C.D., 2017. Tick-borne zoonoses in the United States: Persistent and emerging threats to human health. *ILAR J.* 15, 319-335.
- Elias, S.P., Maasch, K.A., Anderson, N.T., Rand, P.W., Lacombe, E.H., Robich, R.M., Lubelczyk, C.B., Smith, R.P. Jr., 2020. Decoupling of blacklegged tick abundance and Lyme disease incidence in southern Maine, USA. *J. Med. Entomol.* 57, 755-765.

- Elias, S.P., Stone, B.B., Rand, P.W., Lubelczyk, C.B., Smith, R.P., 2021. History of deer herd reduction for tick control on Maine's offshore islands. *Maine Policy Review*. 30(1).
- Environmental Protection Agency. What Climate Change Means for Maine. August 2019; <https://19january2017snapshot.epa.gov/sites/production/files/2016-09/documents/climate-change-me.pdf>. Accessed April 14, 2020.
- Estrada-Peña, A., 2002. Increasing habitat suitability in the United States for the tick that transmits Lyme disease: a remote sensing approach. *Environ. Health. Perspect.* 110, 7.
- Falco, R.C., Fish, D., 1988. Prevalence of *Ixodes dammini* near the homes of Lyme disease patients in Westchester County, New York. *Am. J. Epidemiol.* 127, 826–830.
- Feldman, K.A., Connally, N.P., Hojgaard, A., Jones, E.H., White, J.L., Hinckley, A.F., 2015. Abundance and infection rates of *Ixodes scapularis* nymphs collected from residential properties in Lyme disease-endemic areas of Connecticut, Maryland, and New York. *J Vector Ecol.* 40, 198-201.
- Fernandez, I., Birken, S., Schmitt, C., Simonson, J., Lyon, B., Pershing, A., Stancioff, E., Jacobson, G., Mayewski, P., 2020. Maine's Climate Future 2020 Update. Orono, ME: University of Maine.
- Gardner, A.M., Pawlikowski, N.C., Hamer S.A., Hickling G.J., Miller J.R., Schotthoefer, A.M., Tsao, J.I., Allan B.F., 2020. Landscape features predict the current and forecast the future geographic spread of Lyme disease. *Proc. Biol. Sci. Royal. Soc.* 287, 20202278.
- Gatewood, A.G., Liebman, K.A., Vourc'h, G., Bunikis, J., Hamer, S.A., Cortinas, R.C., Melton, F., Cislo, P., Kitron, U., Tsao, J., Barbour, A.G., Fish, D., Diuk-Wasser, M.A., 2009. Climate and tick seasonality are predictors of *Borrelia burgdorferi* genotype distribution. *Appl. Environ. Microbiol.* 75, 2479-2483.
- Ginsberg, H.S., Ewing, C.P., 1988. Deer ticks, *Ixodes dammini* (Acari: Ixodidae), and Lyme disease spirochetes, *Borrelia burgdorferi*, in Maine. *J. Med. Entomol.* 25, 303-304
- Ginsberg, H.S., Rulison, E.L., Azevedo, A., Pang, G.C., Kuczaj, I.M., Tsao, J.I., LeBrun, R.A., 2014. Comparison of survival patterns of northern and southern genotypes of the North American tick *Ixodes scapularis* (Acari: Ixodidae) under northern and southern conditions. *Parasit. Vectors* 7, 394.
- Githeko, A.K., Lindsay, S.W., Confalonieri, U.E., Patz, J.A., 2007. Climate change and vector-borne diseases: a regional analysis. *Bull. W.H.O.* 78, 1136-1147.
- Gray, J.S., Kahl, O., Lane, R.S., Levin, M.L., Tsao, J.I., 2016. Diapause in ticks of the medically important *Ixodes ricinus* species complex. *Ticks Tick Borne Dis.* 7, 992-1003.
- Gubler, D.J., 1998. Dengue and dengue hemorrhagic fever. *Clin. Microbiol. Rev.* 11, 480-496.

- Hamer, S.A., Tsao, J.I., Walker, E.D., Hickling, G.J., 2010. Invasion of the Lyme disease vector *Ixodes scapularis*: implications for *Borrelia burgdorferi* endemicity. *Ecohealth* 7, 47-63.
- Hall, J.E., Amrine, J.W. Jr, Gais, R.R., Kolanko, V.P., Hagenbuch, B.E., Gerencser, V.F., Clark, S.M., 1991. Parasitization of humans in West Virginia by *Ixodes cookei* (Acari: Ixodidae), a potential vector of Lyme borreliosis. *J. Med. Entomol.* 28, 186-189.
- Hardy, J.P., Groffman, P.M., Fitzhugh, R.D., Henry, K.S., Welman A.T., Dmers, J.D., Fahey, T.J., Driscoll C.T., Tierney G.L., Nolan S., 2001. Snow depth manipulation and its influence on soil frost and water dynamics in a northern hardwood forest. *Biogeochemistry.* 56, 151-174.
- Hayhoe, K., Wake, C., Anderson, B., Liang, X.Z., Maurer, E., Zhu, J., Bradbury, J., DeGaetano, A., Stoner, A.M., Wuebbles, D., 2007. Regional climate change projections for the Northeast USA. *Mitig. Adapt. Strategies. Glob.. Chang* 13, 425-436.
- Hojgaard, A., Lukacik, G., Piesman, J., 2014. Detection of *Borrelia burgdorferi*, *Anaplasma phagocytophilum* and *Babesia microti*, with two different multiplex PCR assays. *Ticks Tick Borne Dis.* 5, 349-351.
- Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. *Biometrical J.* 50, 346-363.
- Irland, L.C., 1997. Maine's forest vegetation regions: selected maps 1858-1993. *Northeast Naturalist* 4, 421-426.
- Jaenson, T. and Lindgren, E., 2011. The range of *Ixodes ricinus* and the risk of contracting Lyme borreliosis will increase northwards when the vegetation period becomes longer. *Ticks Tick Borne Dis.* 2, 44-49.
- Jaenson, T., Jaenson, D., Eisen, L., Petersson, E., Lindgren, E., 2012. Changes in the geographical distribution and abundance of the tick *Ixodes ricinus* during the past 30 years in Sweden. *Parasit. Vectors* 5, 8
- Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L., Daszak, P., 2008. Global trends in emerging infectious diseases. *Nature* 451, 990-993.
- Jordan, R.A. and Schulz, T.L., 2019. Artificial accumulation of leaf litter in forest edges on residential properties via leaf blowing is associated with increased numbers of hosts-seeking *Ixodes scapularis* (Acari: Ixodidae) nymphs. *J. Med. Ent.*, 57, 1193-1198.
- Karmalker, A.V. and Bradley, R.S., 2017. Consequences of global warming of 1.5° C and 2° C for regional temperature and precipitation changes in the contiguous United States. *PLoS ONE*, 12, e0168697.

- Keirans, J.E. and Clifford, C.M., 1978. The genus *Ixodes* in the United States: A scanning electron microscope study and key to the adults. J. Med. Entomol. 15, 1-38.
- Keirans, J.E. and Litwak, T.R., 1989. Pictorial key to the adults of hard ticks, family Ixodidae (Ixodida: Ixodoidea), east of the Mississippi river. J. Med. Entomol. 26, 435.
- Kilpatrick, H.W., Labonte, A.M., Stafford, K.C. III., 2014. The relationship between deer density, tick abundance, and human cases of Lyme disease in a residential community. J. Med. Entomol. 51, 777-784.
- Kilpatrick, A.M., Randolph, S.E., 2012. Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. Lancet 380, 1946-1955.
- Kugeler, K.J., Farley, G.M., Forrester, J.D., Mead, P.S., 2015. Geographic distribution and expansion of human Lyme disease, United States. Emerg. Infect. Dis. 21, 1455-1457.
- Kugeler, K.J., Schwartz, A.M., Delorey, M.J., Mead, P.S., Hinckley, A.F., 2021. Estimating the frequency of Lyme disease diagnoses, United States, 2010-2018. Emerg. Infect. Dis. 27, 616-619.
- Lane, R.S., Piesman, J., Burgdorfer, W., 1991. Lyme borreliosis: Relation of its causative agent to its vector and hosts in North America and Europe. Annu. Rev. Entomol. 36, 587-609.
- Larousse, F., King A.G., Wolbach S.B., 1928. The overwintering in Massachusetts of *Ixodiphagus caucurtei*. Science 67, 351-353.
- Lee, R.E., Chen, C.P., Denlinger, D.L., 1987. A rapid cold hardening process in insects. Science 238, 1415-1417.
- Lee, X., Murphy, D.S., Johnson, D.H., Paskewitz, S.M., 2019. Passive animal surveillance to identify ticks in Wisconsin. Insects 10: e289.
- Levine, J.F., Sonenshine, D.E., Nicholson, W.L., Turner, R.T., 1991. *Borrelia burgdorferi* in ticks (Acari: Ixodidae) from coastal Virginia. J. Med. Entomol. 28, 668-674.
- Lindsay, L.R., Barker, I.K., Surgeoner, G.A., McEwen, S.A., Gillespie, T.J., Robinson, J.T., 1995. Survival and development of *Ixodes scapularis* (Acari: Ixodidae) under various climatic conditions in Ontario, Canada. J. Med. Entomol. 32, 143-152.
- Lindsay, L.R., Barker, I.K., Surgeoner, G.A., McEwen, S.A., Gillespie, T.J., Addison, E.M., 1998. Survival and development of the different life stages of *Ixodes scapularis* (Acari: Ixodidae) held within four habitats on Long Point, Ontario, Canada. J. Med. Entomol. 35, 189-199.

775 Lindsay, L.R., Mathison, S.W., Barker, I.K., McEwen, S.A., Gillespie, T.J., Surgeoner, G.A.,
776 1999. Microclimate and habitat in relation to *Ixodes scapularis* (Acari: Ixodidae)
777 populations on Long Point, Ontario, Canada. J. Med. Entomol. 36, 255-262.
778

779 Lindgren, E. and Gustafson, R., 2011. Tick-borne encephalitis in Sweden and climate change.
780 Lancet 358,16-18.
781

782 Linske, M.A., Stafford, K.C., Williams, S.C., Lubelczyk, C.B., Welch, M., Henderson, E.F.,
783 2019. Impacts of deciduous leaf litter and snow presence on nymphal *Ixodes scapularis*
784 (Acari: Ixodidae) overwinter survival in coastal New England, USA. Insects 10,
785 227 doi:10.3390/insects10080227.
786

787 Lubelczyk, C.B., Elias, S.P., Rand, P.W., Holman, M.S., Lacombe, E.H., Smith, R.P. Jr., 2004.
788 Habitat associations of *Ixodes scapularis* (Acari: Ixodidae) in Maine. Environ. Entomol.
789 33, 900-906.
790

791 Lubelczyk, C., Cahill, B. K., Hanson, T., Turmel, J., Lacombe E., Rand P.W., Elias, S.P., Smith,
792 Jr., R.P., 2010. Tick (Acari: Ixodidae) infestation at two rural, seasonal camps in Maine
793 and Vermont. J. Parasitol 96, 442-443.
794

795 MacMillan, H.A., Sinclair, B.J., 2011. The role of the gut in insect chilling injury: cold-induced
796 disruption of osmoregulation in the fall field cricket, *Gryllus pennsylvanicus*. J. Exp.
797 Biol. 214, 726-734.
798

799 Maine Center for Disease Control and Prevention, Maine Tracking Network. Tickborne disease:
800 Near real-time data. Available online: <http://data.mainepublichealth.gov/tracking/>.
801 Accessed on April 7, 2020.
802

803 Mansfield, K.L., Jizhou, L., Phipps, L.P., Johnson, N., 2017. Emerging tick-borne viruses in the
804 twenty-first century. Front. Cell. Infect. Microbiol. 7, 298.
805

806 Martens, W.J.M., Niessen, L.W., Rotmans, J., Jetten, T.H., McMichael, A.J., 1995. Potential
807 impact of global climate change on malaria risk. Environ. Health Perspect. 10, 458-464.
808

809 Maupin, G.O., Fish, D., Zultowsky, J., Campos, E.G., Piesman, J., 1991. Landscape ecology of
810 Lyme disease in a residential area of Westchester County, NY. Am. J. Epidemiol. 133,
811 1105–1113.
812

813 Maxim Integrated. DS1923 iButton Hygrochron Temperature/Humidity Logger with 8KB Data-
814 Log Memory. 19-4991; Rev 9; 10/19.
815

816 McMichael, C., Barnett, J., McMichael, A.J., 2012. An ill wind? Climate change, migration, and
817 health. Environ. Health Perspect. 120, 646-654.
818

- Moore, S., Shrestha, S., Tomlinson, K.W., Vuong, H., 2011. Predicting the effect of climate change on African trypanosomiasis: integrating epidemiology with parasite and vector biology. *J. R. Soc. Interface* 9, 817-830.
- Morshed, M.G., Scott, J.D., Fernando, K., Beati, L., Mazerolls, D.F., Geddes, G., Durden, L.A., 2005. Migratory songbirds disperse ticks across Canada, and first isolation of the Lyme disease spirochete, *Borrelia burgdorferi*, from the avian tick, *Ixodes auritulus*. *J. Parasitol.* 9, 780-790.
- Nah, K., Bede-Fazekas, Á., Trájer, A.J., Wu, J., 2020. The potential impact of climate change on the transmission risk of tick-borne encephalitis in Hungary. *BMC Infect. Dis.* 20, 34
- Neelakanta, G., Sultana, H., Fish, D., Anderson, J.F., Fikrig, E., 2010. *Anaplasma phagocytophilum* induces *Ixodes scapularis* ticks to express an antifreeze glycoprotein gene that enhances their survival in the cold. *J. Clin. Investig.* 120, 3179-3190.
- NOAA National Centers for Environmental Information, Climate at a Glance: Statewide Time Series, published July 2020, retrieved on August 4, 2020 from <https://www.ncdc.noaa.gov/cag/>
- Ogden, N.H., Bigras-Poulin, M., O'Callaghan, C.J., Barker, I.K., Lindsay, L.R., Maarouf, A., Smoyer-Tomic, K.E., Waltner-Toews, D., Charron, D., 2005. A dynamic population model to investigate effects of climate on geographic range and seasonality of the tick *Ixodes scapularis*. *Int. J. Parasitol.* 35, 375-389.
- Ogden, N.H., Lindsay, L.R., Beauchamp, G., Charron, D., Maarouf, A., O'Callaghan, C.J., Waltner-Toews, D., Barker, I.K., 2004. Investigation of relationships between temperature and developmental rates of tick *Ixodes scapularis* (Acari: Ixodidae) in the laboratory and field. *J. Med. Entomol.* 41, 622-633.
- Ogden, N.H., Lindsay, L.R., Hanicová, K., Barker, I.K., Bigras-Poulin, M., Charron, D.F., Heagy, A., Francis, C.M., O'Callaghan, C.J., Schwartz, I., Thompson, R.A., 2008. Role of migratory birds in introduction and range expansion of *Ixodes scapularis* ticks and of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in Canada. *Appl. Environ. Microbiol.* 74, 1780-1790.
- Ogden, N.H., Lindsay, L.R., 2016. Effects of climate and climate change on vectors and vector-borne diseases: ticks are different. *Trends Parasitol.* 32, 646-656.
- Ostfeld, R.S., Cebeda, O.M., Hazler, K.R., Miller, M.C., 1996. Ecology of Lyme disease: habitat associations of ticks (*Ixodes scapularis*) in a rural landscape. *Ecol. Appl.* 5, 353-361.
- Ostfeld, R.S., 2011. *Lyme Disease: The Ecology of a Complex System*. New York: Oxford University Press, Inc.

864 Padgett, K.A., Lane, R.S., 2001. Life cycle of *Ixodes pacificus* (Acari: Ixodidae): timing of
865 developmental processes under field and laboratory conditions. J. Med. Entomol. 38,
866 684-693.

867

868 Parola, P., Paddock C.D., Raoult, D., 2005. Tick-borne rickettsioses around the world: emerging
869 diseases challenging old concepts. Clin. Microbiol. Rev. 18, 719-756.

870

871 Peavey, C.A., Lane, R.S., 1996. Field and laboratory studies on the timing of oviposition and
872 hatching of the western black-legged tick, *Ixodes pacificus* (Acari: Ixodidae). Exp. Appl.
873 Acarol. 20, 695-711.

874

875 Perret, J.L., Guigoz, E., Rais, O., Gern, L., 2000. Influence of saturation deficit and temperature
876 on *Ixodes ricinus* tick questing activity in a Lyme borreliosis-endemic area (Switzerland)
877 Parasitol. Res. 86, 554-557.

878

879 Piesman, J., Mather, T.N., Dammin, G.J., Telford 3rd., S.R., Lastavica, C.C., Spielman, A. 1987.
880 Seasonal variation of transmission risk of Lyme disease and human babesiosis. Am. J.
881 Epidemiol. 126, 1187-1189.

882

883 R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for
884 Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.

885

886 Rand, P.W., Lacombe, E.H., Smith, R.P. Jr, Ficker, J., 1998. Participation of birds (Aves) in the
887 emergence of Lyme disease in southern Maine. J. Med. Entomol. 35, 270-276.

888

889 Rand, P.W., Holman, M.S., Lubelczyk, C., Lacombe, E.H., DeGaetano, A.T., Smith, R.P.,
890 2004a. Thermal accumulation and the early development of *Ixodes scapularis*. J. Vector
891 Ecol. 29, 164-176.

892

893 Rand, P.W., Lubelczyk, C., Holman, M.S., Lacombe, E.H., Smith, R.P., 2004b. Abundance of
894 *Ixodes scapularis* (Acari: Ixodidae) after the complete removal of deer from an isolated
895 offshore island, endemic for Lyme disease. J. Med. Entomol. 41, 779-784.

896

897 Rand, P.W., Lacombe, E.H., Dearborn, R., Cahill, B., Elias, S., Lubelczyk, C.B., Beckett, G.A.,
898 Smith, R.P., 2007. Passive surveillance in Maine, an area emergent for tick-borne
899 diseases. J. Med. Entomol. 44, 1118-1129.

900

901 Randolph, S.E., 2001. The shifting landscape of tick-borne zoonoses: tick-borne encephalitis and
902 Lyme borreliosis in Europe. Phil. Trans. R. Soc. Lond. B 356, 1045-1056.

903

904 Randolph, S.E., 2004. Tick ecology: processes and patterns behind the epidemiological risk
905 posed by ixodid ticks as vectors. Parasitology 129, 37-65.

906

907 Randolph, S.E., Miklisova, D., Lysy, J., Rogers, D.J., Labuda, M., 1999. Incidence from
908 coincidence: patterns of tick infestations on rodents facilitate transmission of tick-borne
909 encephalitis virus. Parasitology 118, 177-186.

- Rounsville, T.F., Dill, G.M., Bryant, A.M., Desjardins, C.C., Dill, J.F., 2021. Statewide passive surveillance of *Ixodes scapularis* and associated pathogens in Maine. Vector-Borne Zoonotic Dis. doi: 10.1089/vbz.2020.2724.
- Ryder, J.W., Pinger, R.R., Glancy, T., 1992. Inability of *Ixodes cookei* and *Amblyomma americanum* nymphs (Acari: Ixodidae) to transmit *Borrelia burgdorferi*. J. Med. Entomol. 29, 525-530.
- Schulze, T.L., Lakat, M.F., Bowen, G.S., Parkin, W.E., Shisler, J.K., 1984. *Ixodes dammini* (Acari: Ixodidae) and other ixodid ticks collected from white-tailed deer in New Jersey, USA. J. Med. Entomol. 21, 741-749.
- Schulze, T.L., Jordan, R.A., Hung, R.W., 1995. Suppression of subadult *Ixodes scapularis* (Acari: Ixodidae) following removal of leaf litter. J. Med. Entomol. 32, 730-733.
- Smith, C.N., Cole, M.M., 1941. Effect of length of day on the activity and hibernation of the American dog tick: *Dermacentor variabilis*. Ann. Ent. Soc. Am. 34, 426-431.
- Smith, R.P. Jr, Lacombe, E.H., Rand, P.W., Dearborn, R., 1992. Diversity of tick species biting humans in an emerging area for Lyme disease. Am. J. Publ. Health 82, 66-70.
- Stafford, K.C., Magnarelli, L.A., 1993. Spatial and temporal patterns of *Ixodes scapularis* (Acari: Ixodidae) in southcentral Connecticut. J. Med. Entomol. 30, 762-771.
- Telford, S.R., 2017. Deer reduction is a cornerstone of integrated deer tick management. J. Integr. Pest. Manag. 8, 25. <https://doi.org/10.1093/jipm/pmx024>.
- Templer, P.H., Schiller, A.F., Fuller, N.W., Socci, A.M., Campbell, J.L., Drake, J.E., Kunz, T.H., 2012. Impact of a reduced winter snowpack on litter arthropod abundance and diversity in a northern hardwood forest ecosystem. Biol. Fert. Soils. 48, 413-424.
- University of Maine Cooperative Extension. *Tick Surveillance Program Annual Report 2019*. <https://extension.umaine.edu/ticks/wp-content/uploads/sites/42/2020/02/UMaine-Tick-Surveillance-Program-Annual-Report-2019-web-1.pdf>. Accessed June 8, 2020.
- Vail, S.G. and Smith, G., 1998. Air temperature and relative humidity effects on behavioral activity of blacklegged tick (Acari: Ixodidae) nymphs in New Jersey. J. Med. Entomol. 35, 1025-1028.
- Vail, S.G., Smith, G., 2002. Vertical movement and posture of blacklegged tick (Acari: Ixodidae) nymphs as a function of temperature and relative humidity in laboratory experiments. J. Med. Entomol. 39, 842-846.
- Vandyk, J.K., Bartholomew, D.M., Rowley, W.A., Platt, K.B., 1996. Survival of *Ixodes scapularis* (Acari: Ixodidae) exposed to cold. J. Med. Entomol. 33, 6-10.

956
957 Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S, Fourth edition. Springer,
958 New York. ISBN 0-387-95457-0, <http://www.stats.ox.ac.uk/pub/MASS4>.
959
960 Wallace, D., Ratti, V., Kodali, A., Winter, J.M., Ayres, M.T., Chipman, J.W., Aoki, C.F.,
961 Osterberg, E.C., Silvanic, C., Partridge, T.F., Webb, M.J., 2019. Effect of rising
962 temperature on Lyme Disease: *Ixodes scapularis* population dynamics and *Borrelia*
963 *burgdorferi* transmission and prevalence. Can. J. Infect. Dis. Med. Microbiol. 2019, 1-15
964
965 Wilson, M. L., Ducey, A.M., Litwin, T.S., Gavin, T.A., Spielman, A., 1990. Microgeographic
966 distribution of immature *Ixodes dammini* ticks correlated with that of deer. Med. Vet.
967 Entomol. 4, 151–159. <https://doi.org/10.1111/j.1365-2915.1990.tb00273.x>
968
969 Xu, G., Mather, T.N., Hollingsworth, C.S., Rich, S.M., 2016. Passive surveillance of *Ixodes*
970 *scapularis* (Say), their biting activity, and associated pathogens in Massachusetts. Vector
971 Borne. Zoonotic. Dis. 16, 520-526.
972
973 Yuval, B., Spielman, A., 1990. Duration and regulation of the developmental cycle of *Ixodes*
974 *dammini* (Acari: Ixodidae). J. Med. Entomol. 27, 196-201.
975
976 Zimmerman, R.H. and Garris, G.I., 1985. Sampling efficiency of three dragging techniques for
977 the collection of nonparasitic *Boophilus microplus* (Acari: Ixodidae) larvae in Puerto
978 Rico. J. Econ. Entomol. 78, 627-631.
979

Tables

Table 1. Mean temperature and precipitation from December-February, 2021 across Northern, Central, and Coastal regions of Maine, U.S.A. Data obtained from Climate Change Institute, University of Maine.

Region (Maine, U.S.A)	Mean temperature December-February (°C)		Mean precipitation December-February (cm)	
	2018-2019	2019-2020	2018-2019	2019-2020
North	-9.6	-10.4	26.2	29.0
Central	-6.2	-6.5	32.5	30.7
Coastal	-4.4	-4.6	36.3	34.0

1000 **Table 2.** Summary of statistical tests concerning tick (*Ixodes scapularis*) survival and climate
 1001 conditions in control treatments (i.e., leaf litter and snowpack present). Asterisks indicate
 1002 statistically significant tests at $\alpha = 0.05$.

1003

Effect	df	F	P
<i>a) Tick survival in control treatments (i.e., leaf litter and snowpack present)</i>			
Location	3, 20	21.39	< 0.01*
Year	1, 20	6.08	0.02*
<i>b) Tick survival predicted by ambient conditions, December-April</i>			
Mean temperature	1, 20	11.67	< 0.01*
Mean minimum temperature	1, 20	17.63	< 0.01
Mean snow depth	1, 20	0.01	0.92
Maximum snow depth	1, 20	1.35	0.26
Year	1, 20	1.28	0.27
<i>c) Differences in ambient conditions across locations</i>			
Mean temperature	3, 20	24.14	< 0.01*
Mean minimum temperature	3, 20	20.64	< 0.01*
Mean snow depth	3, 20	86.89	< 0.01*
Maximum snow depth	3, 20	57.13	< 0.01*

1004

1005 **Table 3.** Ambient climate conditions across overwinter survival study field sites during the first
 1006 year (Winter 2019) and second year (Winter 2020) of the overwintering study.

1007

Site	Winter 2019			Winter 2020		
	Mean snow depth (cm)	Max snow depth (cm)	Mean temp (°C)	Mean snow depth (cm)	Max snow depth (cm)	Mean temp (°C)
Cape Elizabeth	3.50 ± 0.87	9.76	-0.8 ± 0.1	2.30 ± 1.45	11.20	0.1 ± 0.1
Orono	5.93 ± 1.39	15.20	-2.9 ± 0.9	3.61 ± 0.72	10.30	-2.7 ± 0.1
Brownville	35.41 ± 8.39	79.10	-4.3 ± 0.1	38.13 ± 5.90	59.00	-3.5 ± 0.4
Presque Isle	36.83 ± 5.22	81.50	-6.4 ± 0.1	44.05 ± 3.32	81.28	-2.3 ± 0.5

1008

1009 **Table 4.** Summary of statistical tests concerning tick survival and climate conditions in different
1010 microhabitat treatments. Asterisks indicate statistically significant tests at $\alpha = 0.05$.

1011

Effect	df	F	P
<i>a) Tick survival among microhabitat treatments by location</i>			
Treatment	3, 69	14.60	< 0.01*
Location	2, 69	9.58	< 0.01*
Year	1, 69	1.35	0.25
<i>b) Tick survival predicted by microclimate conditions (December-April)</i>			
Mean temperature	1, 69	31.14	< 0.01*
Mean minimum temperature	1, 69	6.39	0.01*
Year	1, 69	0.57	0.57
<i>c) Differences in mean temperature across microhabitat treatments and location</i>			
Treatment	3, 69	13.08	< 0.01*
Location	3, 69	147.15	< 0.01*
Year	1, 69	23.41	< 0.01*

1012

1013 **Table 5.** *Ixodes scapularis* densities (per 1000 m²) in Cape Elizabeth, Mt. Vernon, Orono, East
 1014 Hancock, and Cutler, in Maine, United States, June – August 2019. A total area of 1000 m² was
 1015 dragged at each site once every two weeks.

1016

Site	Life Stage	Density (per 1000 m ²)		
		June	July	August
Cape Elizabeth	Larva	5	53	63.5
	Nymph	91	84	95.5
	Adult	52	19	7
Mt. Vernon	Larva	0	0	0
	Nymph	3.5	3	8
	Adult	0.5	0	0
Orono	Larva	20	45.5	4.5
	Nymph	18.5	29	6
	Adult	1.5	0	0
East Hancock	Larva	0	0	0
	Nymph	0	1	0
	Adult	0	0	0
Cutler	Larva	1	0	0
	Nymph	0	1	1
	Adult	1	0	0

1017

1018 **Table 6.** Tick counts by species and site, June – August 2019 in Maine, United States.

1019

Site	<i>Ixodes scapularis</i>	<i>Ixodes cookei</i>	<i>Ixodes marxi</i>	<i>Dermacentor variabilis</i>	<i>Haemaphysalis. leporispalustris</i>
Cape Elizabeth	635	0	0	30	0
Cutler	6	0	0	1	8
East Hancock	2	0	0	0	187
Mt. Vernon	16	0	0	12	0
Orono	250	0	0	0	0
Presque Isle	0	4	1	0	0
Brownville	0	0	0	0	45
Species Totals	909	4	1	41	233
Total	1,188				

1020

1021 **Table 7.** Infection prevalence of questing *Ixodes scapularis* nymphs for tick-borne pathogens,
 1022 June – August 2019 in Maine, United States.

1023

Site	Number tested	Number and (%) of nymphs testing positive		
		<i>Borrelia burgdorferi</i> sensu lato (+)	<i>Anaplasma phagocytophilum</i> (+)	<i>Babesia microti</i> (+)
Cape Elizabeth	77	16 (20.1%)	11 (14.2%)	3 (3.8%)
Mt. Vernon	15	1 (6.6%)	0	0
Orono	77	7 (9.1%)	0	0
East Hancock	2	0	0	0
Cutler	3	0	0	0
Presque Isle	5	1 (2%)	0	0

1024

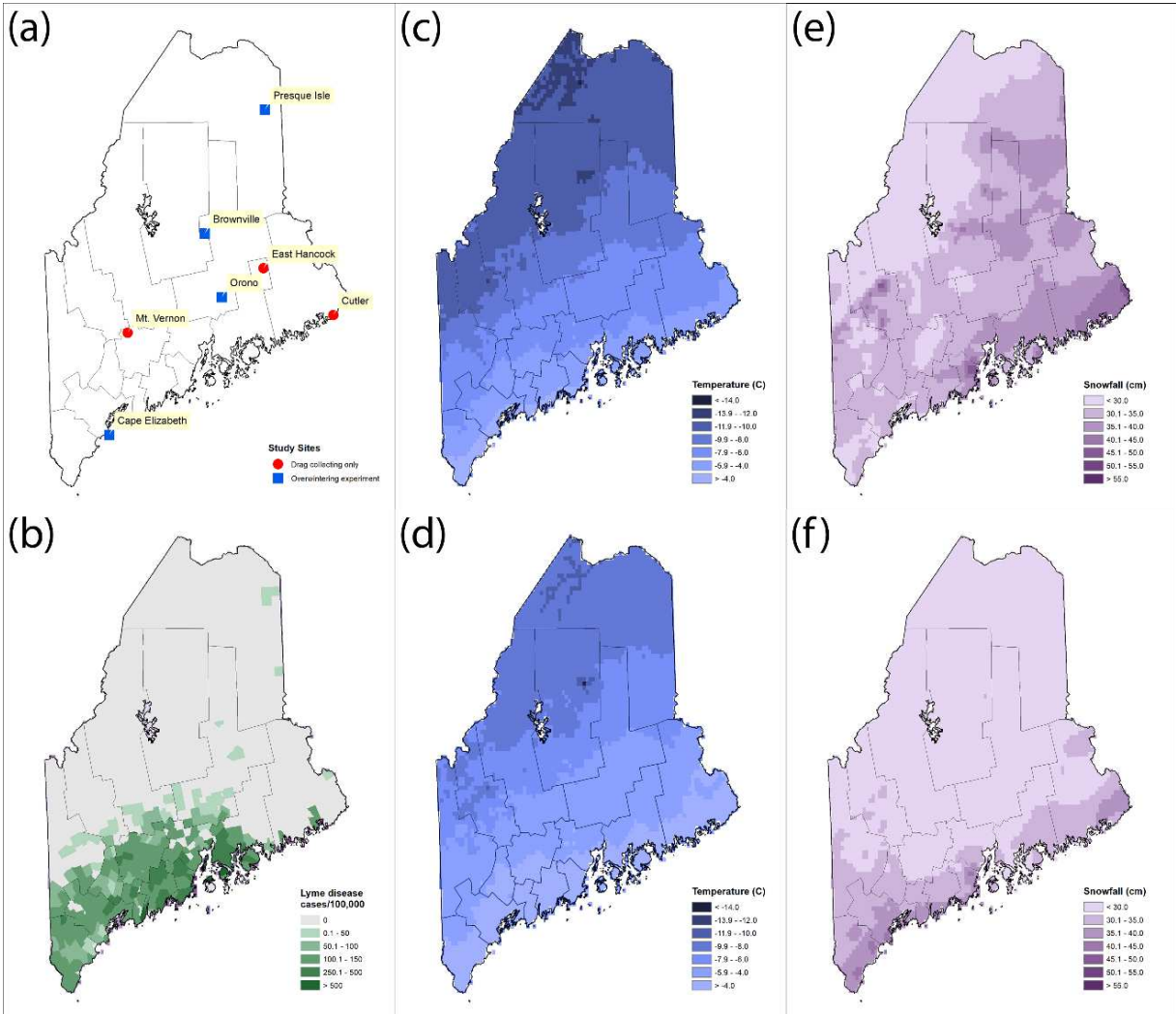


Figure 1. Maps of (A) study sites used for tick collections (red) and both tick collections and overwinter survival experiments (blue) in Maine, United States; (B) town level Lyme disease cases/100,000 people in Maine, 2019; mean temperature (°C) from December to February in (C) 2018-2019 and (D) 2019-2020; and total snowfall (cm) from December to February in (E) 2018-2019 and (F) 2019-2020. Data were obtained from PRISM (<https://prism.oregonstate.edu/>).

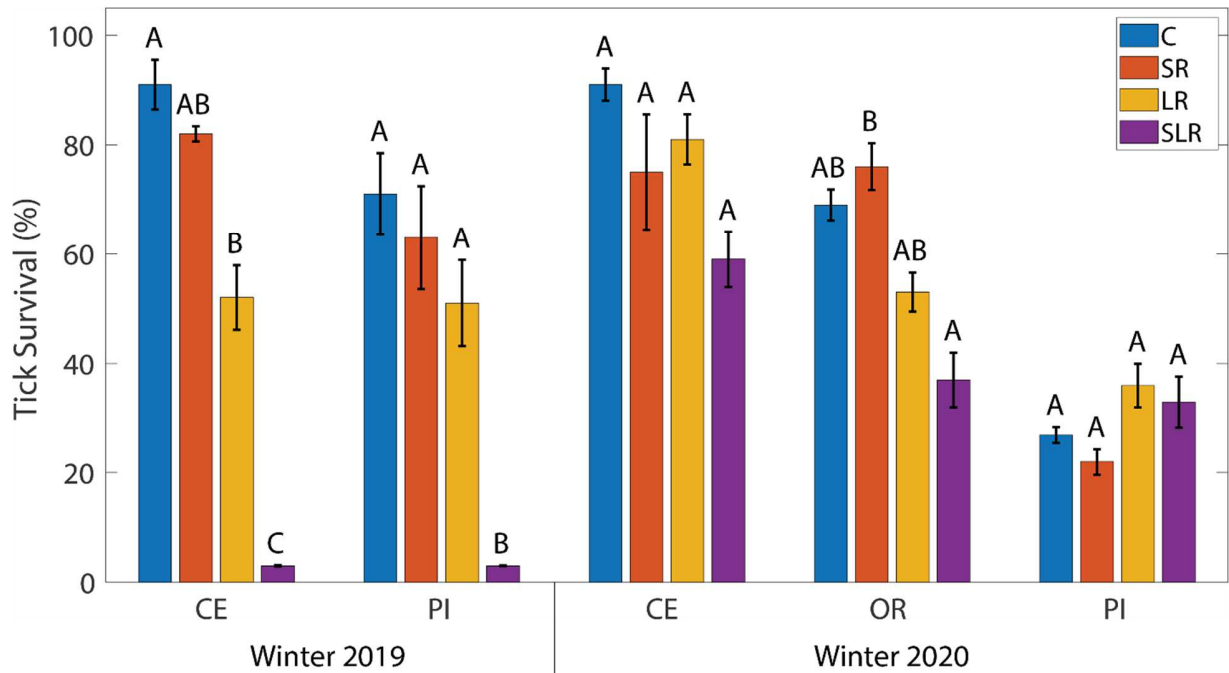


Figure 2. Percent overwinter survival of *Ixodes scapularis* nymphs in Cape Elizabeth (CE), Orono (OR), and Presque Isle (PI) across microhabitat treatments. Microhabitat treatments include a control with leaf litter and snow present (C), snow removal (SR), leaf removal (LR), and snow/leaf removal (SLR). Grouping letters indicate significant differences between means at $\alpha = 0.05$ within each year and location.

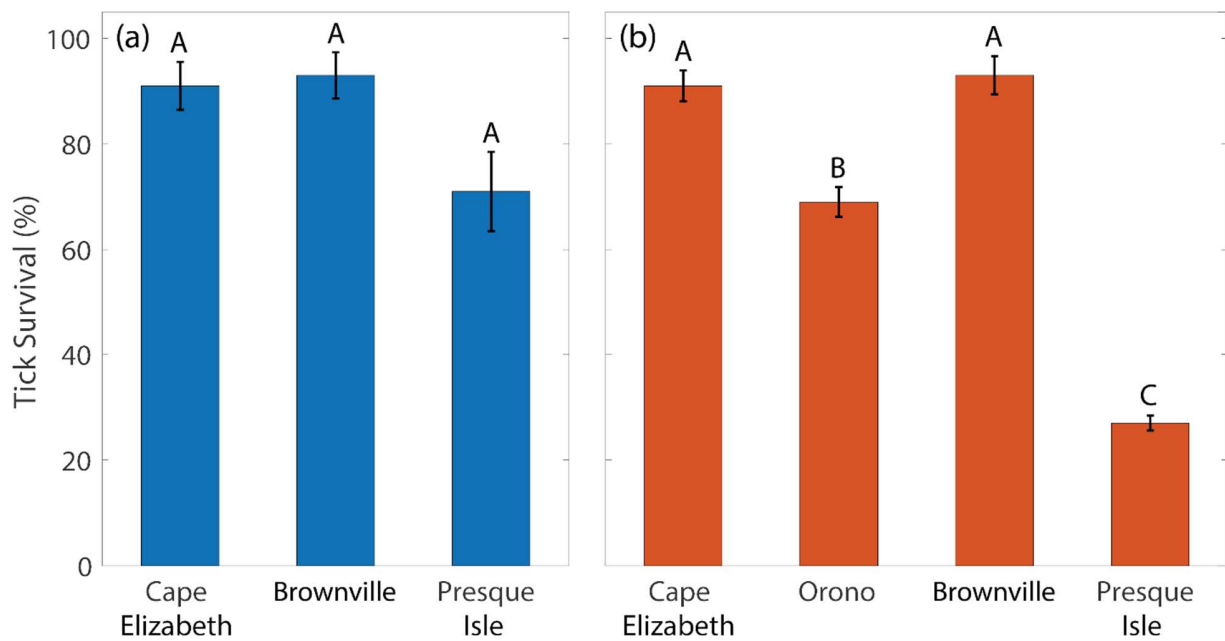
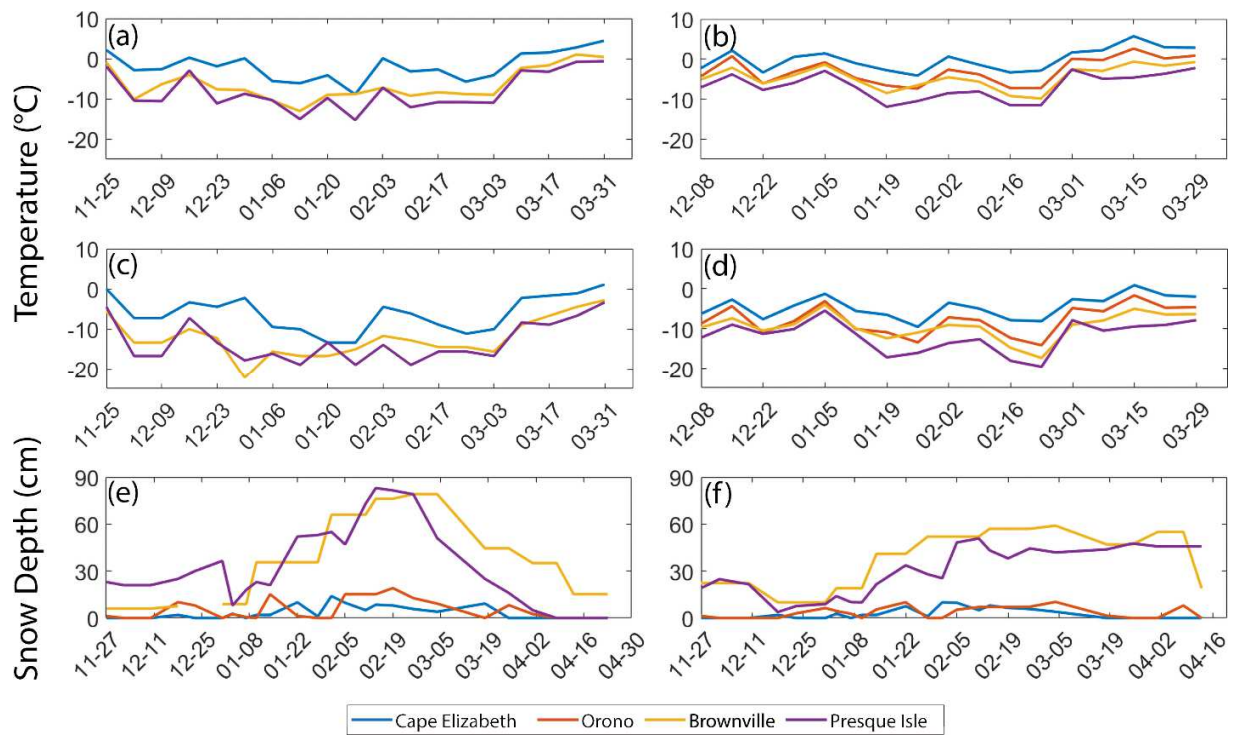


Figure 3. Percent overwinter survival of *Ixodes scapularis* nymphs exposed to control conditions (i.e., leaf litter and snowpack present) in (A) 2018-2019 and (B) 2019-2020. Grouping letters indicate significant differences between means at $\alpha = 0.05$ within each year.



1047

1048

1049 **Figure 4.** Weekly mean ambient temperature (°C) by study site in (A) 2018-2019 and (B) 2019-
 1050 2020), minimum ambient temperature in (C) 2018-2019 and (D) 2019-2020, and snow depth
 1051 (cm) in (E) 2018-2019 and (F) 2019-2020.

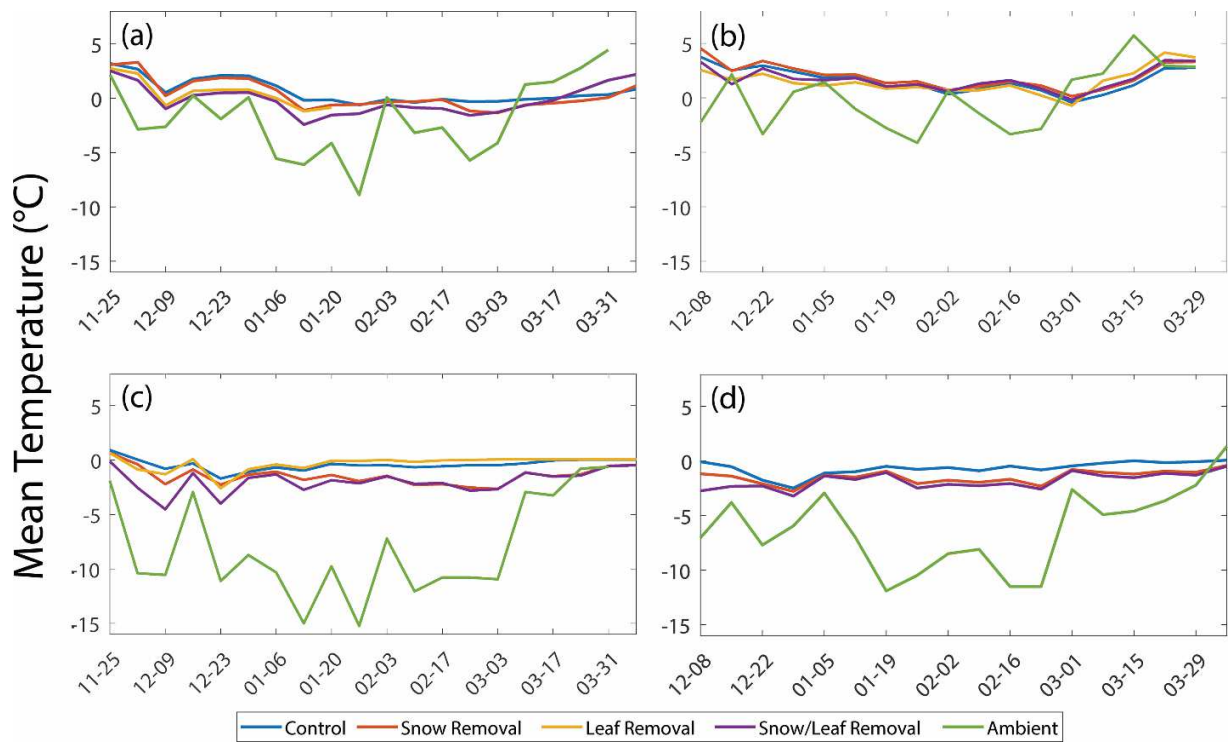


Figure 5. Weekly mean temperature (°C) across treatments in Cape Elizabeth in (A) 2018-2019 and (B) 2019-2020) and weekly mean temperature across treatments in Presque Isle in (C) 2018-2019 and (D) 2019-2020.