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ARTICLE

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Off-host survival of blacklegged ticks in eastern North America: A multistage, multiyear, multisite study

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Abstract

Climatic conditions are widely thought to govern the distribution and abundance of ectoparasites, such as the blacklegged tick (*Ixodes scapularis*), vector of the agents of Lyme disease and other emerging human pathogens. However, translating physiological tolerances to distributional limits or mortality is challenging. Ticks may be able to avoid or tolerate unsuitable conditions, and what is lethal to one life history stage may not extend to others. Thus, even after decades of research, there are clear gaps in our knowledge about how climatic conditions determine tick distributions or patterns of abundance. We present results from a 3-year study combining daily hazard models and data from field experiments at three sites spanning much of *I. scapularis*' current latitudinal distribution. We examine three predominant hypotheses regarding how temperature and vapor pressure deficits affect stage-specific survival and transition success and consider how these results influence population growth and distribution. We found that larvae are sensitive to temperature and vapor pressure deficits, whereas mortality of nymphs and adults is consistent with depletion of energy reserves. Consistent with prior work, we found that overwinter survival was high and successful stage transitions (e.g., fed nymphs molting to adults) were sensitive to temperature. Collectively, results from this comprehensive, multiyear, multistage field study suggest that population growth of *I. scapularis* is less limited by restrictive climatic conditions than has been broadly assumed, although influences on larval survival may slow tick population growth and establishment in some desiccating conditions. Further studies should integrate climate effects on stage-specific survival to better understand these effects on population dynamics and range expansion in a changing climate.

KEYWORDS
arthropod vector, climatic conditions, survivorship, temperature-dependent development

[Correction added on 4 May 2023, after first online publication: Author name Mary Killilea has been corrected in this version.]

INTRODUCTION

Anthropogenic climate change is affecting the distribution and abundance of species involved in the transmission of pathogens that infect wildlife, livestock, and humans (Altizer et al., 2013; Harvell et al., 2002). In particular, there is a great deal of concern over how the demography and behavior of arthropod vectors of zoonotic pathogens will change as the climate continues to warm (Caminade et al., 2019). To predict future changes in the distribution and intensity of the diseases caused by these pathogens, it is important to understand the specific relationships between climatic variables (e.g., temperature, humidity) and demographic variables (e.g., survival, reproduction) of the vectors. This study addresses these relationships in the blacklegged tick, one of the most important zoonotic pathogen vectors worldwide, in the eastern United States.

Hard ticks (Ixodidae) spend a majority of their life free-living in the environment, where they are exposed to highly variable environmental conditions (Sonenshine, 1993). As ectotherms, key processes such as rates of development (e.g., eclosion, molting) vary with temperature (Lindsay et al., 1995; Ogden et al., 2004) and so ticks are thought to be unable to complete their lifecycle in areas that are too cold (Ogden et al., 2005). Within these developmental constraints, climatic conditions are thought to limit tick distributions and abundances more directly, though mortality (e.g., Gaff et al., 2020; Ginsberg et al., 2014; Johnson et al., 2018; Ogden et al., 2021). Laboratory studies show that both high and low temperature extremes, as well as low humidity, are lethal to several species in the genus *Ixodes* (reviewed by Ostfeld & Brunner, 2015). The range of conditions permissive for behaviors such as questing for hosts is likely even more restrictive (Gilbert et al., 2014; Perret et al., 2003). Despite the clear sensitivity of tick survival to temperature and humidity in the laboratory, these apparent lethal thresholds do not translate to obvious limits in the field. For instance, while ticks are exquisitely sensitive to freezing and desiccation in the laboratory, they are able to avoid or tolerate such conditions in nature (e.g., Ginsberg et al., 2017; Gray et al., 2016), and we observe tick populations established in locations that routinely experience what would seem to be lethal conditions. Thus, while environmental conditions are widely thought to restrict the range and abundance of tick populations (e.g., Brownstein et al., 2003, 2005; Estrada-Peña, 2002; Killilea et al., 2008; Leal et al., 2020; Leighton et al., 2022; Ogden et al., 2021; Tufts et al., 2021), the lessons from laboratory studies are not always coherent with field observations (Ostfeld & Brunner, 2015). Climate-based predictions of habitat suitability and potential tick distributions, under current or future climatic conditions, thus remain challenging.

Translating ticks' physiological tolerances, as determined in laboratory studies, into distributional limits or population dynamics has been difficult for a number of reasons. First, it has been difficult to study ticks, especially juvenile stages, under ecologically realistic conditions in a way that allows researchers to infer causal relationships. For instance, many studies have attempted to link densities of a particular life stage of ticks to conditions experienced by earlier stages (e.g., predicting densities of questing nymphs based on average humidity when larvae were questing the prior summer). However, observed tick densities are a product of numerous confounding processes, such as historical presence or dispersal limitation (Diuk-Wasser et al., 2006; Hahn et al., 2016; Hamer et al., 2010), tick densities in prior years (i.e., autocorrelation in population sizes), and the rates at which ticks find vertebrate hosts (Lord, 1992; Ostfeld et al., 2018; Randolph, 2004; Randolph & Steele, 1985).

Tracking survival in tick-housing enclosures, first used by Lindsay et al. (1995), addresses many of these confounding influences, but the enclosures themselves may alter microclimatic conditions or restrict normal behaviors (e.g., questing) that could influence metabolism and exposure to hazardous conditions. For instance, Bertrand and Wilson (1996) found that providing adult ticks "free-range" enclosures increased survival over short periods of time because they had access to microhabitats in the soil and leaf litter. More recent enclosure designs have overcome these challenges to a large extent with largely permeable mesh containers and structures provided to allow questing. As a consequence, survival estimates are increasingly representative of natural conditions. Indeed one study by Nieto et al. (2010) found that survival of nymphal *I. pacificus* was similar in the incubator and field, and survival of larvae was higher in field enclosures than in a laboratory incubator with similar temperatures but lower humidity.

Second, many studies document mortality over coarse time intervals (e.g., over the several months to a year between one life stage and the next). This makes it difficult to discern which conditions most strongly affected overall survival (e.g., a slow, continual loss with desiccating conditions as opposed to a single event during a cold-snap; Burks et al., 1996). An alternative study design that surmounts these issues is to take sequential samples at short time steps and analyze the pattern of survivorship as a function of climate-related hazard during those shorter intervals. The idea is that the probability of surviving to some point in time is simply the product of the probability of having survived all of the prior time steps (e.g., days), each of which is the complement of the (daily) hazard of mortality. This hazard can be linked to microclimatic conditions with any reasonable function (e.g., a logistic curve).

With sufficient serial sampling it is possible to estimate the relationship between particular conditions and hazard, which provides a means of predicting expected survival over time under realistically varying conditions. However, the only published attempt to use this method to understand tick survival suggested that at least overwintering survival of *I. scapularis* nymphs was largely unaffected by low temperatures or relative humidity and instead reflected a more-or-less constant hazard through time (Brunner et al., 2012).

The third difficulty in identifying predictive climate-tick survival relationships is that the spatial and temporal replication of most studies has been insufficient to quantify consistent relationships. Studies have generally been conducted at one or two sites, usually well within the established distribution of the focal tick species, which at least raises the possibility that the studies do not include restrictive conditions. Most studies are also fairly short-term relative to the *Ixodes* life cycle, often just one transition and life stage or a series of transitions within a year (but see Lindsay et al., 1995). It has thus been difficult to differentiate idiosyncrasies of survivorship in one place and time from the general patterns of survival with prevailing environmental conditions.

This problem is made worse by the fact that the life cycle and phenology of ticks vary geographically. For instance, *I. scapularis* in the northeastern United States has a 2-year life cycle with distinct activity peaks for each life stage during particular times of year (Levi et al., 2015), whereas in the southeastern United States, they may complete their life cycle in 1 year (Lavender & Oliver Jr., 1996). Moreover, in the Southeast ticks exhibit greater plasticity in their activity levels throughout the year, presumably in response to climatic conditions (Clark et al., 1998; Durden et al., 2002; Lavender & Oliver Jr., 1996). Thus, not only do conditions during a particular season vary considerably in space (e.g., latitudinally), so too do the life stages that are active during that time.

Given these challenges, we set out to quantify how temperature and moisture conditions influence stage-specific survival and key developmental transitions of *I. scapularis* in semi-natural enclosures deployed across three field sites spanning much of the latitudinal range of the species over a period of 3 years. We were particularly interested in understanding the extent to which microclimatic conditions cause direct mortality of flat (unfed), host-seeking tick stages, in addition to the overwintering survival of nymphs. Our first hypothesis, in accordance with the prior literature, was that the hazard of mortality would strongly increase as microclimatic conditions became more desiccating and temperatures more extreme (i.e., warmer in the summer and colder in the winter), although overwintering survival was expected to be high relative to survival in active seasons (Brunner et al., 2012; Burtis et al., 2016; Lindsay et al., 1995; Volk et al., 2022). However, we expected these relationships with conditions would not be linear, but would instead reflect thresholds beyond which hazard increases rapidly. Our second hypothesis was that adult ticks, because of their lower surface area:volume ratios and greater biomass energy reserves (Needham & Teel, 1991), would be less sensitive to desiccation than nymphs and nymphs would be less sensitive than larvae. To evaluate these first two hypotheses, we used a hazard-based model to assess survival as a function of temperature and vapor pressure deficit and, more broadly, identify conditions that might regulate each free-living stage in turn.

Our third hypothesis was that key developmental transitions—fed larvae molting into nymphs, fed nymphs molting into adults, and fed adults laying eggs that hatch larvae—would occur sooner under warmer conditions in a manner predicted by a growing-degree day model. The underlying assumption is that rates of enzymatic reactions, and thus development (e.g., towards molting), increase exponentially with temperature until the point where high temperatures begin denaturing these enzymes (Higley et al., 1986; Moore & Remais, 2014). However, across a fairly wide range of temperatures rates of development increase approximately linearly with temperature, and so researchers often assume a linear relationship between temperature and development above some threshold temperature where development becomes possible (e.g., Ogden et al., 2004; Rand et al., 2004, but see Moore & Remais, 2014 for discussion of this assumption). Thus, doubling the temperature (above this minimum) should roughly halve the time to molting or larval eclosion. Knowing how each life stage responds to prevailing temperature and moisture conditions should generate more informed expectations for the changing distribution of ticks and risk of tick-borne disease.

METHODS

Sites and plots

These studies were conducted at three field sites along a latitudinal climatic gradient in eastern North America with extant tick populations, each a Department of Defense installation: Fort Drum (FD) in northern New York, West Point (WP) in southeastern New York and in the heart of the species' current range, and Camp Lejeune (CL) in coastal North Carolina and south of the zone of "high incidence" of Lyme disease (https://www.cdc.gov/lyme/datasurveillance/lyme-disease-maps.html; Figure 1). The life cycle in all three sites appears to be 2 years, although this is not, to our knowledge,

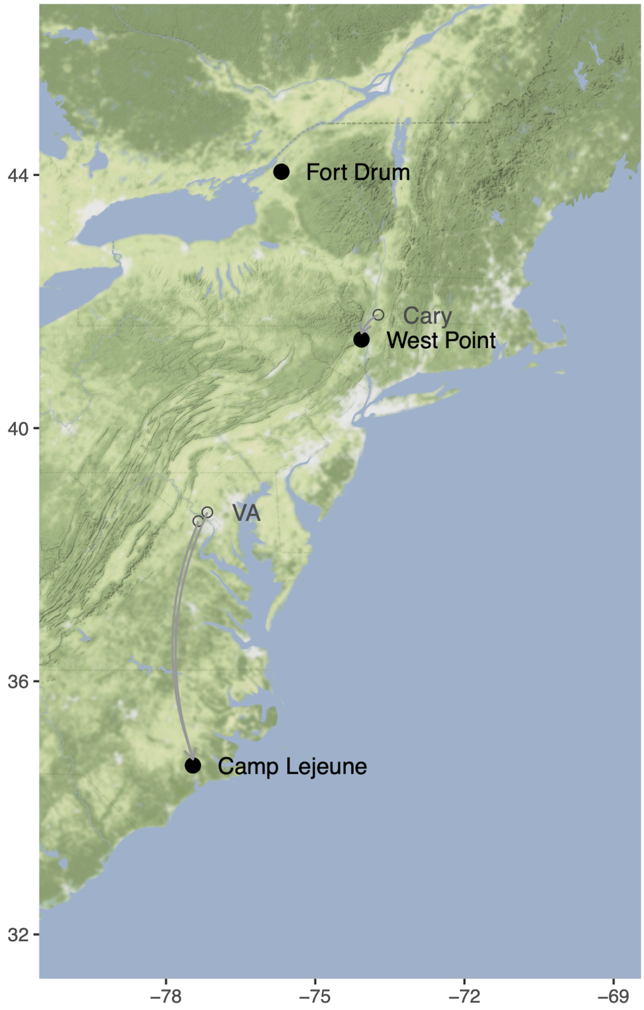

FIGURE 1 Map of the three field sites used in this study (black points) along the eastern United States. Ticks used at Fort Drum and West Point came from these sites, but West Point also received some ticks from the Cary Institute ("Cary" on the map). Ticks used at Camp Lejeune were collected from two sites in northeastern Virginia ("VA" on map). See text for details.

well studied in the areas around CL. All sites were forested, although FD and CL had more mid- and understory vegetation and less rocky terrain and soil than WP. The primary overstory trees were white oak, loblolly pine, mockernut hickory, and tulip poplar at CL; sugar maple, red oak, red maple, and American beech at WP; and red oak, red maple, and white oak at FD. See Appendix S1: Table S1 for additional details of canopy cover and soil characteristics. Collectively, these sites span a wide range of abiotic conditions and with which to evaluate the roles that climate may play in the off-host survival and development of *I. scapularis* within the current known geographic range. In particular, CL was consistently several degrees warmer than the other two sites and often relatively more desiccating (Appendix S1: Table S2 and Figure S2), so we predicted greater climate-related mortality, but faster developmental transitions within this site.

Within each field site, two 20 × 20 m plots, each enclosed within a 25 × 25 m fenced area, were selected in closed canopy forests of mixed hardwoods and conifers, the main habitat of *I. scapularis* (Killilea et al., 2008). Soil core enclosures (SCEs) were positioned on the intersections of a 20 × 20 grid, ensuring ≥1 m between SCEs, for a maximum of 400 SCEs per grid. Each position was used only once. A total of 1495 SCEs with ticks were deployed over the three field seasons across the three sites (Appendix S1: Table S2). One of the two plots per site was used for SCEs deployed in 2017 and 2018 and the second plot was used for SCEs deployed in 2018 and 2019.

Enclosures

Ticks were housed in semi-natural soil core enclosures that consisted of a core of soil (10 cm diameter by 5 cm deep) with intact leaf litter on top, extracted from the ground using a golf-hole corer, placed in a PVC ring with four 15 mm diameter holes along its circumference to ensure drainage, with three wooden dowels inserted into the soil (18–23 cm above the soil surface) to allow questing, and enclosed in two organza fabric bags (Appendix S1: Figure S1; Brunner et al., 2012; Burtis, 2017). Approximately half of the SCEs also included two data loggers to record microclimate conditions every hour. Hygrochron DS1923 iButtons (Maxim Integrated, San Jose, CA) were used to measure air temperature and percent relative humidity inside the organza fabric bags just above the surface of the soil core while Thermochron DS1922L iButtons (Maxim Integrated, San Jose, CA) were used to measure soil temperature 5 cm below ground, under the PVC soil core. The Hygrochron loggers are not waterproof and so are not recommend for use below ground. While soil moisture meters were deployed (see Appendix S1: Section S1) to better describe conditions below ground, multiple failures prevented us from using these data in analyses, so only above-ground humidity measurements are available. See the Appendix S1: Section S1 for additional details of SCE construction.

Tick deployments

Tick deployments in SCEs were similar whether they included flat, host-seeking, or overwintering nymphs or fed ticks expected to undergo developmental transitions (i.e., molting or egg laying and eclosion); where they deviate we note the differences. A literature review was conducted to determine the phenology of each life stage in each site's region. Deployments and recovery of tick SCEs were then scheduled to span the relevant period when host-seeking life stages were active and when molting, oviposition, and hatching would be expected to occur at each field site (see Appendix S1: Table S2 for details of deployment periods).

Ticks used for all deployments were collected from the wild using drag cloths as close to the field sites as possible, but those deployed at WP were supplemented with ticks from at the Cary Institute of Ecosystem Studies (Cary) in Millbrook, New York (~45 km NNE) when necessary and those deployed at CL came from NE Virginia (~445 km N; see Appendix S1: Section S1) and Cary. Ticks designated for fed tick deployments were first fed on mammalian hosts (see Appendix S1: Section S1) and then placed in programmable growth chambers to acclimate them to the climatic conditions of their destination site for 3–5 days prior to field deployment. Ticks designated for newly-molted nymph deployments were first fed on mammalian hosts, allowed to molt, and then acclimated for 2–3 days before deployment (see Appendix S1: Section S1).

Deployments involved placing live ticks on the soil core surface within each SCE. The organza bags were then twisted and tied shut to prevent ticks from escaping. The number of ticks placed in a SCE varied with the life stage (e.g., 15 flat larvae per SCE, but just one fed adult female) as well as with the availability of live ticks (see Appendix S1: Table S2 for details). Also, we ensured that when multiple ticks were deployed in an SCE they came from at least two holding vials to avoid potential vial-specific effects. Similarly, SCEs holding fed juvenile ticks received ticks fed on at least two mice to minimize potential host effects influencing survival estimates. The use of vertebrate animals for tick feeding was approved by Cary's IACUC (permit number 2017-04).

Survival and molting assessments

All three sites were visited every 2 weeks during deployment periods. During each visit a predetermined number of randomly selected SCEs from each deployment date were removed from the plot—generally alternating between one and three SCEs for flat tick deployments and, for fed tick deployments, two SCEs prior to when molting was expected based on prior literature and three to four SCEs afterwards—and transported, unopened in a Ziploc bag and held in a cooler with ice, to Cary to assess tick survival (flat tick deployments) or molting success or larval hatching (fed tick deployments). If SCEs were saturated due to recent rain, paper towels were added to each Ziploc bag to absorb excess moisture that could affect tick survival during transport and holding.

At Cary, SCEs were allowed to acclimate to room temperature for 1–5 days and were then destructively sampled by disassembling, piece by piece, and examining each component thoroughly for ticks. Additional specific search methods were employed for finding flat versus newly-molted ticks and engorged ticks. Because flat ticks exhibit questing behavior, these stages were lured into sight using the searcher's breath and gentle handling of the soil core and leaf litter, for up to 30 min. per SCE depending on the amount of leaf litter. Engorged and newly-molted tick SCEs were searched visually for up to 40 min., after which time the soil core and leaf litter were placed in a Berlese Funnel for 3 days (light intensity 6 Watts, 13 Watts, and 25 Watts over the 3 days) and the ethanol catch vial searched meticulously. Counts of recovered engorged ticks were not used in our analyses. When SCEs were deployed with a fed adult, searchers examined material until (1) the adult was located, (2) an egg mass was located, or (3) at least one living larvae was found, with the search target appropriate to the laying and eclosion phenology determined from prior literature. Organza fabric bags were also assessed for holes and the presence and size of holes noted. Those SCEs where damage to the bags may have allowed ticks to escape were excluded from analyses (see Appendix S1: Section S2).

Microclimate data

Plot-level microclimatic conditions were averaged by first finding the mean temperature or vapor pressure deficit (VPD; calculated with the ASCE standardized reference evapotranspiration equation; Allen et al., 2005) for each iButton data logger and then by taking the mean across the iButtons deployed at a plot within a site in a given day. Only data from above-ground iButtons were used for the tick survival and molt analyses presented here, since they provided both the temperature and relative humidity data necessary for calculating VPD. When microclimate data were not available due to logger failure, data from associated SCEs were excluded from analyses.

MODEL STRUCTURE

Analyses of flat, host-seeking, and overwintering tick deployments

To address our first hypothesis, that extreme microclimatic conditions (i.e., high and/or low temperature and high VPD) influence mortality of flat ticks, we modeled the daily hazard of mortality, λ_i. In general the probability of survival to day *t* is:

$$S_t = \prod_{i=1}^{i=t} (1-\lambda_i) = \exp\left(\sum_{i=1}^{i=t} \log(1-\lambda_i)\right). \quad (1)$$

We assumed there was some *baseline* hazard that was modeled as either a constant (λ_0) or as a site-year-specific baseline hazard (λ_{SY}). In full models, we added additional hazard due to daily conditions (λ_{C_i}), such that $\lambda_i = \lambda_0 + \lambda_{C_i}$ or $\lambda_i = \lambda_{SY} + \lambda_{C_i}$. We modeled the relationship between recorded microclimate conditions (e.g., temperature) and this additional hazard as a flexible logistic function (Figure 2),

$$\lambda_{C_i} = \frac{\kappa}{1+\exp(-\beta[C_i - \alpha])}, \quad (2)$$

where β is the slope with measured condition, C_i, α is the half-maximum, and κ is the maximum additional hazard. Thus, the minimum daily hazard is $\lambda_i = \lambda_0$ or $\lambda_i = \lambda_{SY}$ and the maximum daily hazard is $\lambda_i = \lambda_0 + \kappa$ or $\lambda_i = \lambda_{SY} + \kappa$. The model is flexible in that it allows flat relationships (no effect of conditions, $|\beta| \approx 0$), graded slopes ($|\beta|$ intermediate), and thresholds ($|\beta|$ large). Thus, the hazard model allowed us to test the corollary of our first hypothesis, that relationships between specific temperature and desiccation conditions and tick mortality would involve thresholds. Moreover, the overall effect of conditions can vary from negligible ($\kappa \approx 0$) to quite important ($\kappa > \lambda_0$ or λ_{SY}; Figure 2). Note that we included the conditions in the pre-deployment period (e.g., when ticks were in the laboratory or being transferred to the site) in our estimation of cumulative hazard.

Four versions of this model were fit to data: (1) a null model where daily hazard was a function of the site-year-specific baseline hazards ($\lambda_i = \lambda_{SY}$); (2) a full model with temperature hazards added to the site-year-specific baseline hazards ($\lambda_i = \lambda_{SY} + \lambda_{T_i}$); (3) a model with a constant baseline hazard across all sites and years plus the site-specific daily temperature hazards ($\lambda_i = \lambda_0 + \lambda_{T_i}$); and (4) a full model with site-year-specific baseline hazards and added hazards from vapor pressure deficit ($\lambda_i = \lambda_{SY} + \lambda_{VPD_i}$). The third model ($\lambda_0 + \lambda_{T_i}$) was designed to test whether latitudinal differences in survival could be primarily attributed to their differences in temperature, as opposed to some unmeasured site- or year-specific factor(s) contained in the first and second models (λ_{SY} and $\lambda_{SY} + \lambda_{T_i}$). That is, whether after explaining temperature-related hazards, site-specific factors would be of negligible importance. Note that we did *not* fit a model with only the vapor pressure deficit predicting hazard, $\lambda_i = \lambda_0 + \lambda_{VPD_i}$, because we had no expectation that vapor pressure deficits would follow a predictable pattern with latitude. For the rest of this report, we refer to the four models by their definitions of daily hazard (i.e., the right-hand sides of the equations in the descriptions above).

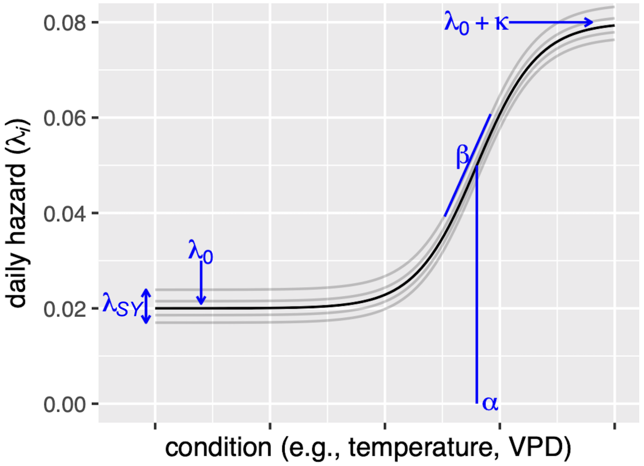

FIGURE 2 Schematic depicting how the daily hazard of mortality might relate to microclimatic conditions according to a logistic function. The role of each parameter (Greek letters in blue) is described in the text.

Finally, we fit a fifth, post hoc, phenomenological logistic model in which tick survival was allowed to decrease sigmoidally with time from an arbitrary starting point prior to when ticks would be active (1 April for the larvae and nymphs and 1 October for the adults). This was spurred in part by a consideration of the nymph survival data, in which we observed high survivorship for long periods before a rapid decline, whereas the daily hazard-based models, above, assume survival declines exponentially, with the rate determined by conditions. We refer to this as the phenomenological "time" model. (See Appendix S1: Section S1 for details of this and the hazard models.)

These five models were fit to each flat life stage (host-seeking larvae, nymphs, and adult; overwintering nymphs) separately. Survival data from flat adult female and male SCEs were combined and analyzed together as "flat adult" SCEs. CL spring larva deployments were combined with the flat larva deployed later in each field season and analyzed together as "flat larva" SCEs. Overwintering nymph SCEs, however, were analyzed separately from flat (host-seeking) nymph SCEs. Use of these five models for each flat life stage allowed us to test our second hypothesis, which was that sensitivity to desiccation would be lowest in adults and highest in larvae.

Analyses of developmental transitions in fed tick deployments

For the fed larvae to newly molted nymph and fed nymph to newly molted adult transitions, we modeled the number of newly-molted ticks, out of the number of fed ticks deployed per SCE, as an increasing, logistic function of the cumulative degree days, DD_i up to day i when the SCE was destructively sampled. While degree-day models typically treat the number of degree days required to reach a developmental milestone (e.g., molting) as a sharp threshold, below which there is no chance of molting and above which molting is a certainty (e.g., Ogden et al., 2005; Rand et al., 2004), the logistic function allows for the probability of molting to increase more slowly with the accumulation of degree days (DD). This also allows for greater flexibility in fitting a linear temperature-development rate model to data that is derived from a developmental process that is certainly more complex than this model assumes (Moore & Remais, 2014). We let the logistic model saturate at a probability less than 1 to allow for mortality or other reasons ticks might not be observed. Note that we do not incorporate our observations of whether engorged ticks were found alive or dead to separately estimate the probabilities of surviving and molting because these designations were considered less reliable. Our model thus describes the joint probability of surviving and molting.

We fit two versions of the model to each fed-tick deployment (see Appendix S1: Section S1 for details). In the first, we assumed a fixed threshold temperature for the DD calculations using estimates of development rates derived from Ogden et al. (2004), who measured development times in fed ticks held at a range of constant temperatures. Linear regressions were fit to the inverse of the estimated times to molt for larvae and nymphs in the Ogden et al. (2004) study, excluding temperature ≥30°C where all ticks died or development slowed, to estimate development rates. Then the x-intercept was inferred from the regression equation, which corresponds to the threshold temperature. This threshold was 3.7°C for nymphs and 8.1°C for larvae, and the overall number of degree days above these thresholds were 699 and 477 for nymphs and larvae, respectively. (Note that this was not the method employed by Ogden et al., 2004, although they later used DD >0°C as a threshold for the entire tick life cycle as detailed in Ogden et al., 2005). Our logistic model was then conditioned on the accumulated DD above these thresholds experienced by each larval or nymphal SCE. In the second version of the model, we estimated these thresholds directly from the data. CL spring larva deployments were combined with the fed larva deployed later in each field season and analyzed together as "fed larva" SCEs. Again, we included the temperature in the period prior to deployment in the field in our calculations of DD in all models.

We used the second version of this model, where the threshold was estimated from the data, to describe the probability of observing larvae in SCEs deployed with a single fed (and presumably mated) female as a function of degree days. Note that this model describes the joint probability of fed females surviving and laying eggs, and those egg masses hatching larvae. These approaches allowed us to evaluate our third hypothesis, that transitions between life stages would occur sooner under warmer conditions, according to growing-degree day models.

Model fitting and priors

All of the above models were coded in the Stan language (Stan Development Team, 2021) and run using the Rstan package (Stan Development Team, 2020) or with the ulam function in the rethinking package (McElreath, 2020) as an intermediary. Regularizing, but otherwise uninformative priors were chosen through prior predictive checks to avoid nonsensical outcomes (e.g., ticks surviving on average for less than a day or more than several years). Model results were qualitatively robust to choice of priors, as long as prior distributions restricted numerical errors (e.g., divide-by-zero errors). See Appendix S1: Section S1 for additional details, including the specific priors.

To explore and compare the predictive performance of our models we used leave-one-group-out cross validation, with each site-year group left out in turn. Performance was measured with expected log-predictive density (elpd) and model performance was compared by differences in elpd along with the standard errors (among left-out groups) in these differences (Vehtari, 2020). This model scoring method assesses how well new data are accounted for by the posterior predictive distribution of candidate models. Models with the highest elpd generate predictions closest to the left-out data.

RESULTS

Climatic conditions

Microclimatic conditions were similar, at a course scale at FD and WP, and generally warmer at CL (Appendix S1: Figure S2 and Table S2). For instance, the average mean daily temperature from July through August in 2017–2019 was 19.7, 22.1, and 21.0°C at FD, 20.0, 21.7, and 21.8°C at WP, but 25.2, 25.6, and 26.7°C at CL. Similarly, winters were colder at FD and WP—average mean daily temperatures in November through February were 0.0, −0.9, and −1.2°C at FD and 0.6, 0.4, and 1.0°C at WP—and much milder at CL: 10.3, 10.9, and 11.5°C. Desiccating conditions were more idiosyncratic. In the summer months (July and August), average mean daily VPD varied among sites in 2017 from 0.12 to 0.20 kPa, whereas in 2018 FD had twice the average VPD as the other sites—0.43 kPa compared to 0.22 kPa at CL and 0.17 kPa at WP—and in 2019 CL had the high average of 0.54 kPa compared with 0.35 kPa in FD and 0.31 kPa at WP. That said, none of these sites experienced prolonged periods with conditions shown to cause rapid mortality to a closely related *Ixodes* species in the laboratory (e.g., >2 kPa in Herrmann & Gern, 2010; Appendix S1: Figure S2).

Survival of flat, host-seeking ticks (hypotheses 1 and 2)

We deployed 8691 flat ticks in 786 SCEs, of which 7624 ticks were in the 697 SCEs considered usable (e.g., lacked holes in organza coverings that might facilitate tick escape)—259 SCEs at CL, 219 at FD, and 219 at WP (full data are available in Brunner et al., 2023a). Among these useable SCEs we observed a striking amount of variability in mortality even between replicate SCEs deployed at the same time and in the same plot, especially in flat larva and flat nymph SCEs (Appendix S1: Figure S2). Indeed, no ticks were recovered alive from 50% of the 230 flat larva SCEs that were not overwintered and 53% of the 201 flat nymph SCEs not overwintered, whereas none of the 50 overwintering nymph SCEs included in analyses had zero recovery and just 9% of the 165 flat adult SCEs had no live ticks recovered. Although overwintering nymph SCEs had high survival through the winter at all sites, nearly all flat larva SCEs had zero ticks survive through winter. The most northern site, FD, had the lowest flat larva mortality across the field season and was the only site from which surviving larvae were recovered after winter (Figure 3). We now consider the hazard relationships separately for each stage.

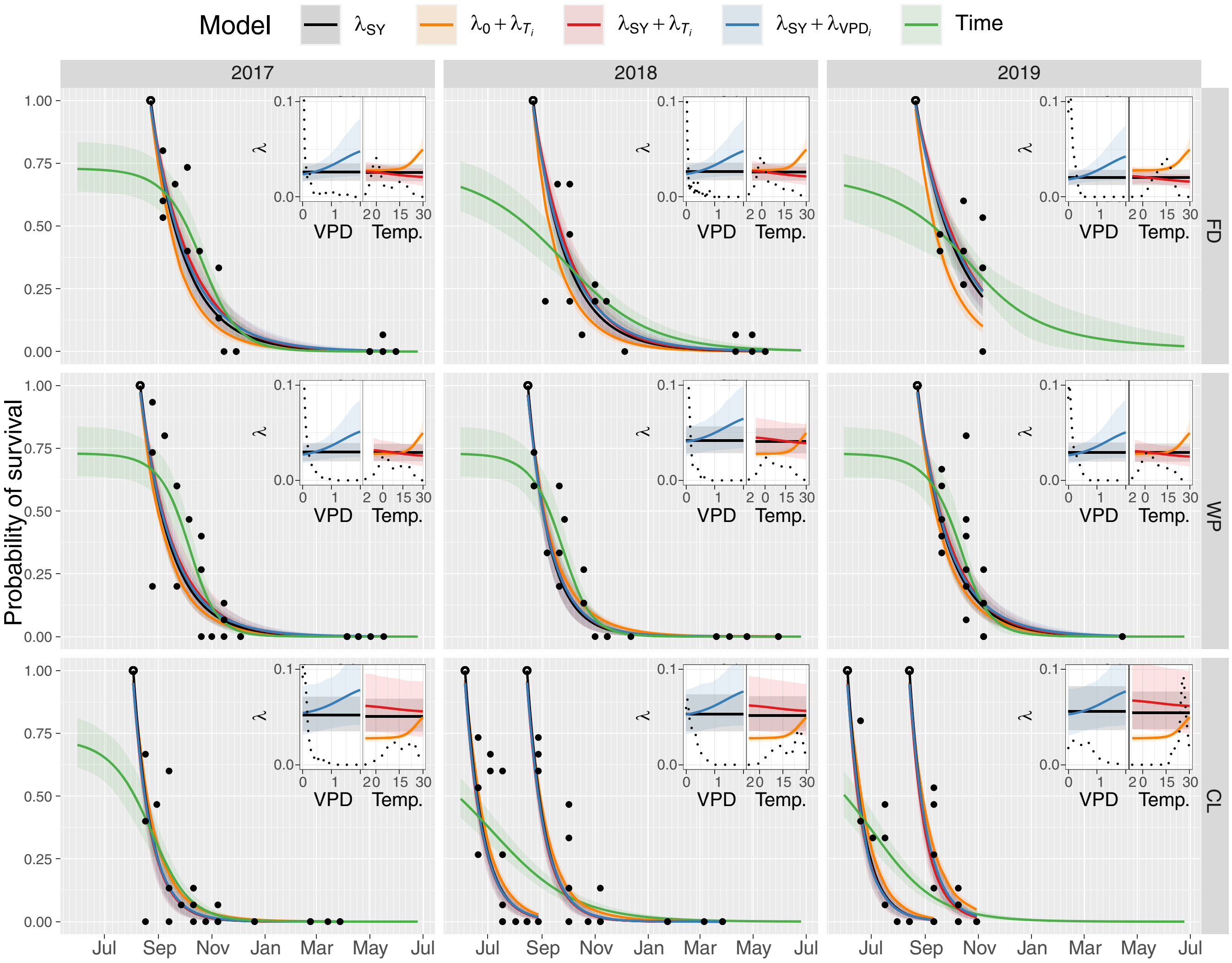

FIGURE 3 Posterior predictions of flat larvae survival over time from the four a priori daily hazard models and the post hoc phenomenological "time" model. Insets within each panel illustrate the estimated relationships between mean daily vapor pressure deficit (VPD; kPa) and temperature (Temp.; °C) and daily hazard for each site-year. See caption of Figure 4 for more details.

Flat host-seeking nymphs, not overwintered

Comparing the four a priori hazard models fit to the not overwintered flat nymph SCE data, we found that all of the models where the baseline hazard varied by site-year were much better at out-of-sample prediction (e.g., higher elpd) than the model with a constant baseline hazard (Table 1). The elpd for the $\lambda_{SY} + \lambda_{T_i}$ model with temperature was higher than the same model with VPD—a difference of 17.4— although high standard errors for these estimated differences suggest these differences in out-of-sample predictive accuracy vary a great deal between site-years (Table 1). Qualitatively, even in the $\lambda_{SY} + \lambda_{T_i}$ model there was little evidence of a strong effect of temperature on hazard, and thus survival rates; despite the improved predictive fit, the estimated relationship between hazard and temperature was quite flat, only deviating noticeably from the constant hazard model (λ_{SY}) at the upper temperatures, such as those experienced at CL in 2018 and 2019 (Figure 4). Similarly, while the estimated hazard in the $\lambda_{SY} + \lambda_{VPD_i}$ model tended to increase at high VPD (above ~1 kPa), such conditions were rarely observed and there was little confidence in this elevated risk (Figure 4).

The phenomenological "time" model, where survival was a sigmoidal function of date, seems to capture the general pattern in the data much better than the daily hazard-based models (Figure 4). In particular, this model was able to describe the high survival we observed early in the deployment period before survivorship dropped more-or-less exponentially in July and August, regardless of when the ticks were deployed. The hazard-based models, on the other hand, all necessarily predict essentially exponentially declining survival beginning at deployment. This phenomenological model was clearly superior at out-of-sample prediction (Table 1).

Overwintered nymphs

With just 50 useable observations of overwintering nymph survival (see Appendix S1: Section S2) combined with the fairly high survivorship overall (Figure 5), there was little capacity to estimate the relationship between temperature or VPD and hazard with precision (Figure 5), and all models were virtually equivalent at out-of-sample prediction (Table 2). Both models with hazard increasing with temperature ($\lambda_{SY} + \lambda_{T_i}$ and $\lambda_0 + \lambda_{T_i}$) suggest a moderate, if poorly estimated, increase in daily hazard that ramps up slowly with temperature, accelerating above about 15–20°C. This trend seems to highlight the distinction between the period of fall/winter inactivity and therefore low hazard, and the period of spring host-seeking activity and therefore higher mortality hazard. Hazard also seems to steadily increase with VPD, diverging from the constant hazard model (λ_{SY}) between ~0.5 to 1 kPA (Figure 5). The overwintering nymphs, however, rarely experienced these more desiccating conditions and the uncertainty around these relationships is quite large. The phenomenological time model was as poor at out-of-sample prediction as the rest (Table 2). It seems that there was simply a fraction of nymphs in our study that survived over winter (70% at FD, 76% at WP, and 77% at CL), and it did not vary over time in a notable or predictable way.

TABLE 1 Differences in the expected log predictive density (Δ elpd) for flat nymph models for a new site-year dataset between each model and the model with the largest elpd, the standard error of these differences (SE Δ), and the estimates and standard errors of the elpd estimates.

Model	Δ elpd	SE Δ	elpd	SE elpd
Time	0.0	0.0	−349.3	69.9
$\lambda_{SY} + \lambda_{T_i}$	−70.3	31.9	−419.6	72.8
λ_{SY}	−82.8	30.9	−432.0	84.9
$\lambda_{SY} + \lambda_{VPD_i}$	−87.8	32.6	−437.0	84.7
$\lambda_0 + \lambda_{T_i}$	−134.1	44.6	−483.4	101.6

Flat, host-seeking larvae

Unlike with the flat nymphs, where survival was initially high and then sharply decreased, survivorship of larvae appeared to decline rapidly after each deployment (Figure 3). Moreover, the out-of-sample predictive accuracy of the hazard-based models was substantially better than the phenomenological, "time" model (Table 3). Among these a priori models, the one where hazard increased with VPD had the highest elpd, suggesting it performed best at out-of-sample prediction, but its performance was not clearly distinguishable from the two temperature-dependent models due to large standard errors around the differences in elpd (Table 3).

In the VPD model ($\lambda_{SY} + \lambda_{VPD_i}$), there was a moderately large increase in daily hazard from the lowest to highest VPD (Figure 3). The effect is well within the bounds of our uncertainty, however, and only notably diverges from the constant hazard model (λ_{SY}) in VPD ranges that were rarely observed (Figure 3). CL was generally the driest site during larval deployments, particularly in 2019, which may explain why CL was estimated to have a higher baseline hazard. Conversely, the generally less desiccating conditions observed at FD and WP might account for the lower baseline hazards predicted

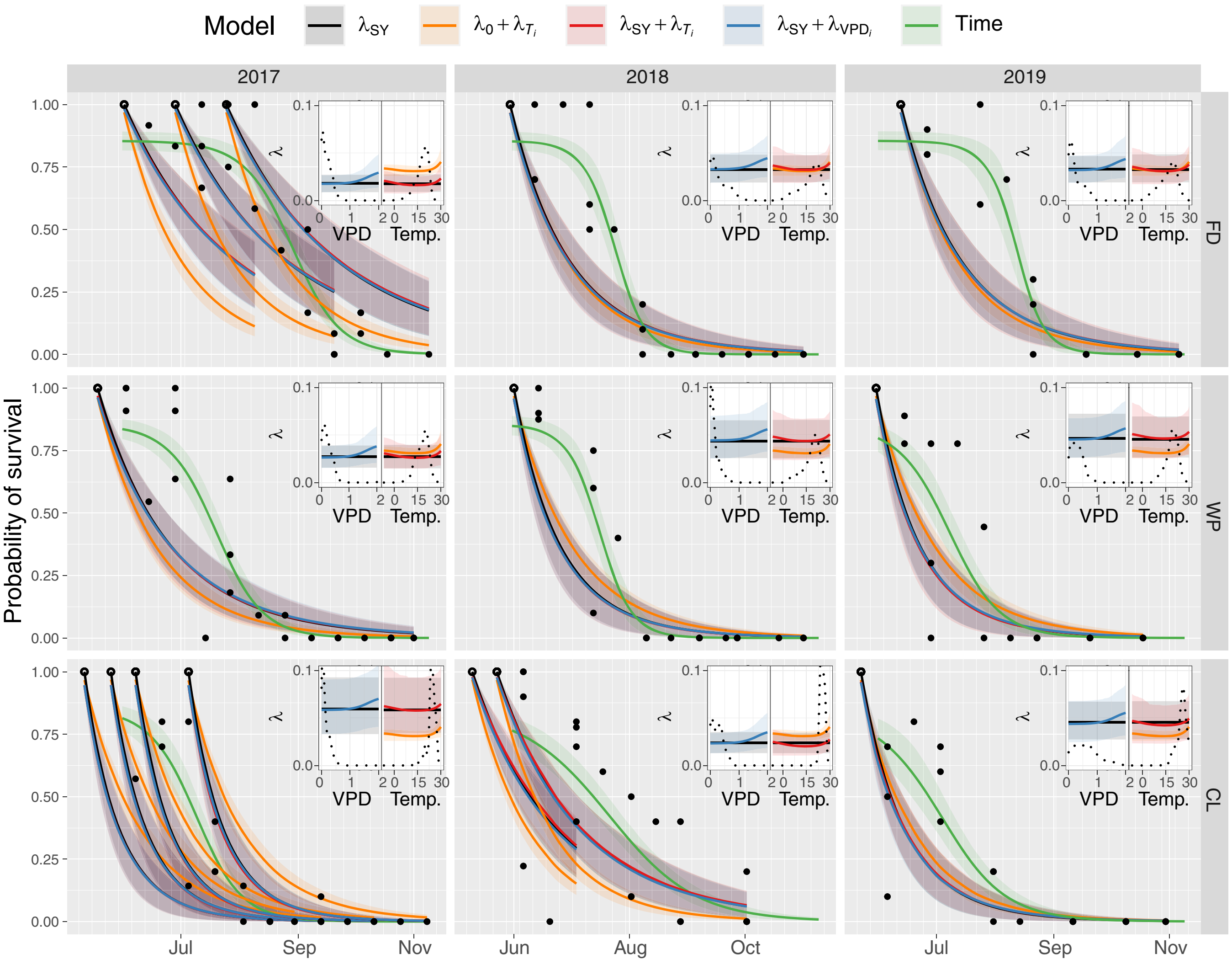

FIGURE 4 Posterior predictions of flat nymph survival over time from the four a priori daily hazard models and the post hoc phenomenological "time" model. Points represent the start of the deployment period (open circles) and then observed proportions of flat nymphs surviving in SCEs (filled circles). Lines are means of the predictions of survival from deployment, for the four a priori models, or from 1 April, for the phenomenological time model, and the envelopes their 80% CIs. Note that these posterior predictions account for the mean survival among SCEs within a site-year, but do not include the variation among SCEs that is part of the four a priori models. Insets within each panel illustrate the estimated relationships between mean daily vapor pressure deficit (VPD; kPa) and temperature (Temp.; °C) and daily hazard for each site-year (lines and envelopes are the mean expectation and 80% CI), while the dashed lines are proportional to the frequency of VPDs and temperatures experienced during the deployments in each site-year.

for these sites. Taken together, evidently some of the site-to-site variation is attributable to site-level differences in VPD.

Flat, host-seeking adults

Estimated daily hazards were generally low for flat adults, reflecting their relatively high survival, with the exception of CL in 2019 when there was a rapid decline in survivorship by May relative to the same time in 2018 (Figure 6). This year at CL was somewhat hotter and dryer than in the past, which might have contributed to the increased mortality rate. Indeed, the estimated hazard increased dramatically in the temperature models ($\lambda_0+\lambda_{T_i}$ and $\lambda_{SY}+\lambda_{T_i}$) at temperatures above 20°C, which was only experienced at CL in 2019 (Figure 6). Similarly, the $\lambda_{SY}+\lambda_{VPD_i}$ model predicted a noticeable increase in daily hazard when VPD increases above ~1 kPA, conditions rarely experienced by deployed adults, except at CL in 2019, though the uncertainty around these relationships is quite large (Figure 6). Among the daily hazard-based models, those basing hazard on temperature were dramatically better at out-of-sample prediction performance than either the constant site-year hazard or VPD-based hazard model (Table 4).

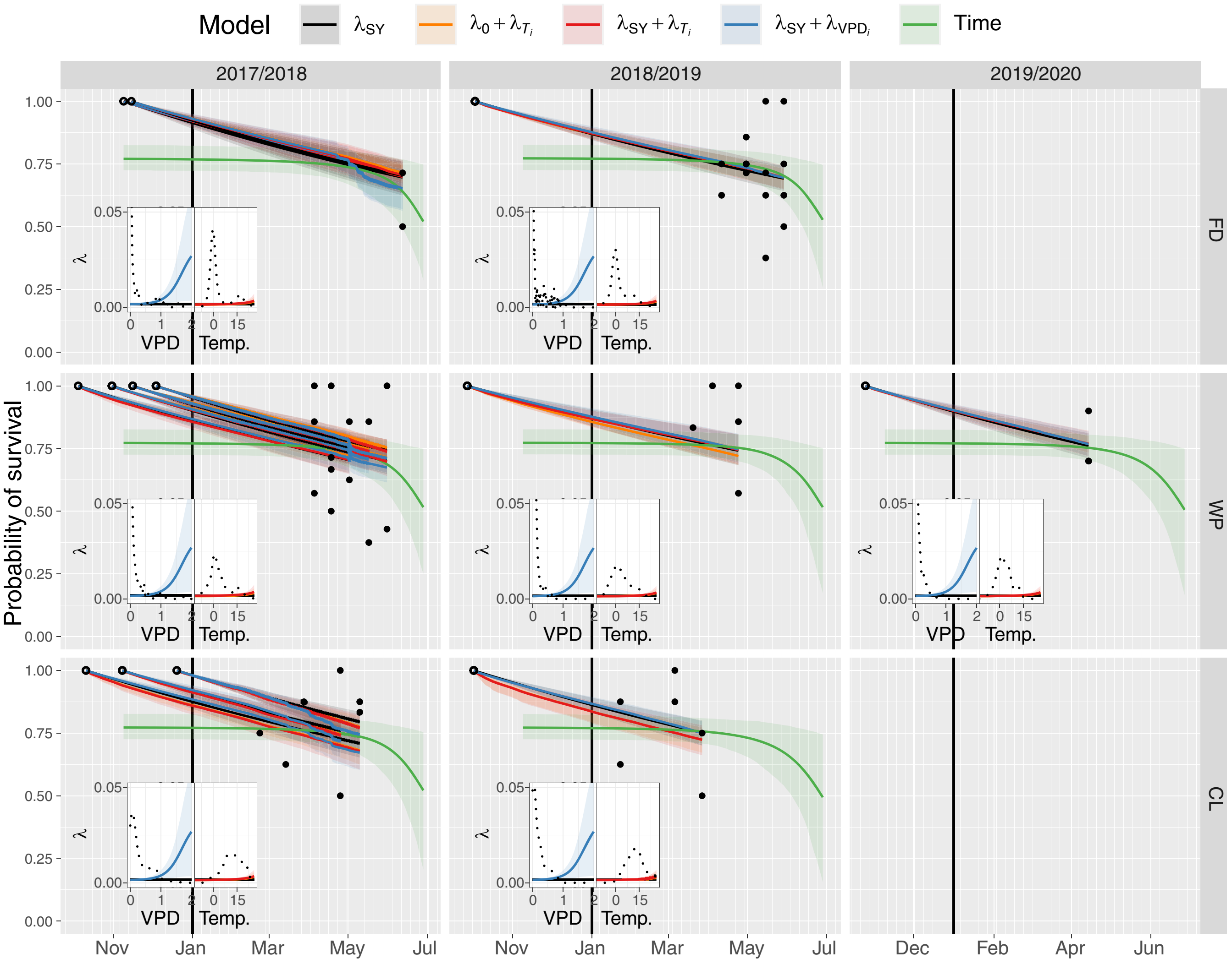

FIGURE 5 Posterior predictions of overwintering nymph survival over time from the four a priori daily hazard-based models and the post hoc phenomenological "time" model. Insets within each panel illustrate the estimated relationships between mean daily vapor pressure deficit (VPD; kPa) and temperature (Temp.; °C) and daily hazard for each site-year. See caption of Figure 4 for more details.

TABLE 2 Differences in the expected log predictive density (Δ elpd) for overwintering nymph models for a new site-year dataset between each model and the model with the largest elpd, the standard error of these differences (SE Δ), and the estimates and standard errors of the elpd estimates.

Model	Δ elpd	SE Δ	elpd	SE elpd
Time	0.0	0.0	−83.4	22.5
$\lambda_{SY} + \lambda_{VPD_i}$	−0.4	0.8	−83.8	22.2
λ_{SY}	−0.4	0.7	−83.8	22.2
$\lambda_0 + \lambda_{T_i}$	−0.7	0.5	−84.2	22.5
$\lambda_{SY} + \lambda_{T_i}$	−0.8	0.5	−84.2	22.4

TABLE 3 Differences in the expected log predictive density (Δ elpd) for flat larva models for a new site-year dataset between each model and the model with the largest elpd, the standard error of these differences (SE Δ), and the estimates and standard errors of the elpd estimates.

Model	Δ elpd	SE Δ	Elpd	SE elpd
$\lambda_{SY} + \lambda_{VPD_i}$	0.0	0.0	−436.1	40.9
$\lambda_0 + \lambda_{T_i}$	−7.6	10.5	−443.7	37.9
$\lambda_{SY} + \lambda_{T_i}$	−8.0	11.0	−444.1	43.9
λ_{SY}	−11.2	4.7	−447.3	40.4
Time	−165.4	108.1	−601.5	139.7

The phenomenological time model was, on average, substantially better at out-of-sample prediction than the temperature-based models, although again, there was a great deal of uncertainty in these differences (Table 4). This model predicted fairly constant survivorship for many months (from October 1) prior to a rapid decline in

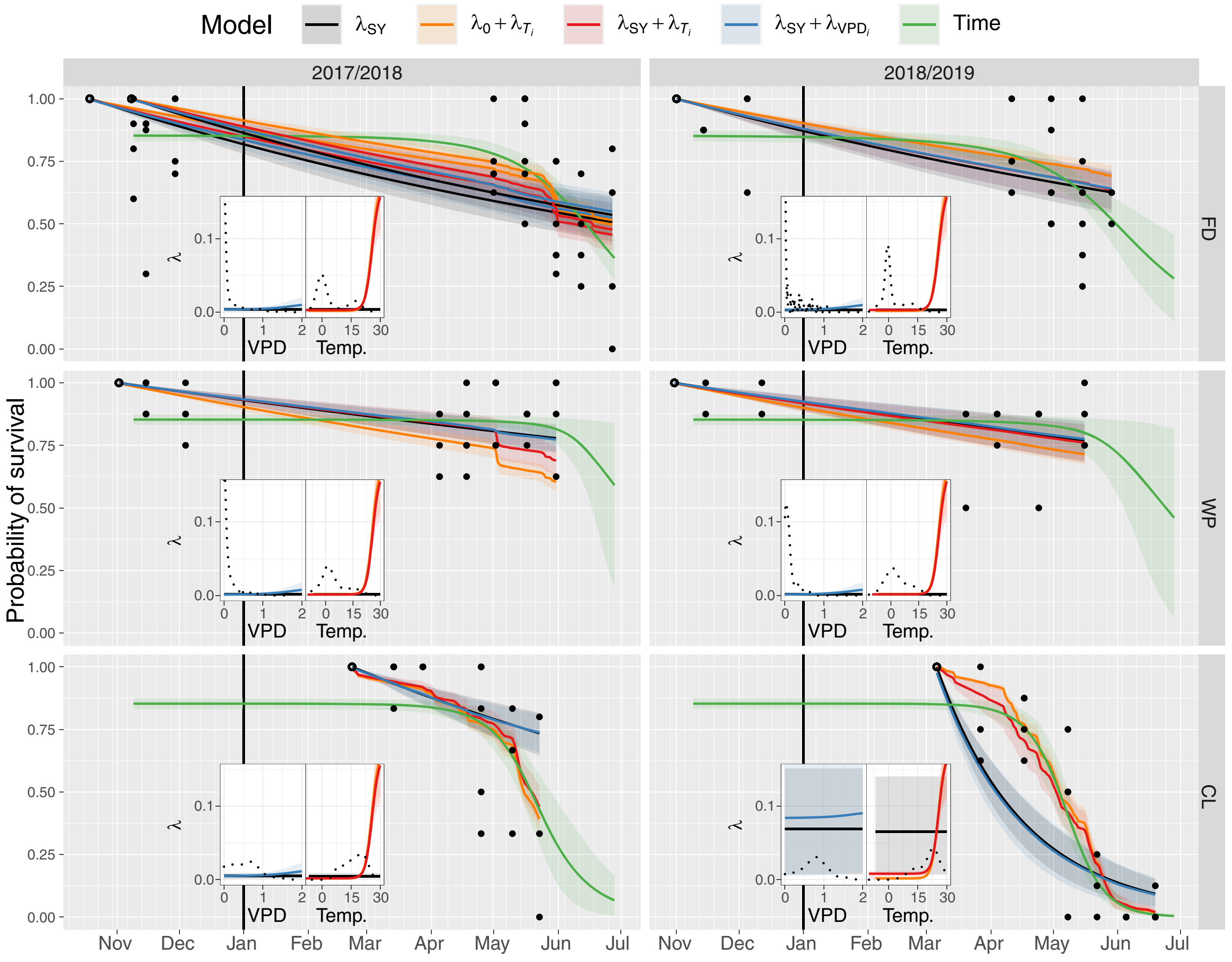

FIGURE 6 Posterior predictions of flat adult survival over time from the four a priori daily hazard models and the post hoc phenomenological "time" model. Insets within each panel illustrate the estimated relationships between mean daily vapor pressure deficit (VPD; kPa) and temperature (Temp.; °C) and daily hazard for each site-year. See caption of Figure 4 for more details.

May or June (Figure 6), although this decline was not always observed in the data (e.g., see WP in Figure 6). This was a reasonable description of the flat adult survival data overall, but there is a great deal of variability in outcomes among SCEs retrieved on a given date, especially at FD. The time model tended to predict lower survival than the hazard-based models early in the deployment period and higher survival in spring for the two northern sites (Figure 6).

Developmental transitions in fed ticks (hypothesis 3)

We deployed 3313 fed immature ticks in 359 SCEs in total, of which 3183 were in the 345 SCEs considered usable in the analyses: 167 fed nymph and 178 fed larva SCEs. There was a great deal of variation in the number of newly-molted ticks observed among the SCEs recovered at the same time (Figures 7 and 8). However, after

TABLE 4 Differences in the expected log predictive density (Δ elpd) for flat adult models for a new site-year dataset between each model and the model with the largest elpd, the standard error of these differences (SE Δ), and the estimates and standard errors of the elpd estimates.

Model	Δ elpd	SE Δ	elpd	SE elpd
Time	0.0	0.0	−329.1	59.0
$\lambda_{SY} + \lambda_{T_i}$	−10.9	13.1	−339.9	69.4
$\lambda_0 + \lambda_{T_i}$	−18.8	19.1	−347.8	74.2
$\lambda_{SY} + \lambda_{VPD_i}$	−109.6	56.1	−438.6	104.2
λ_{SY}	−139.9	79.3	−468.9	125.0

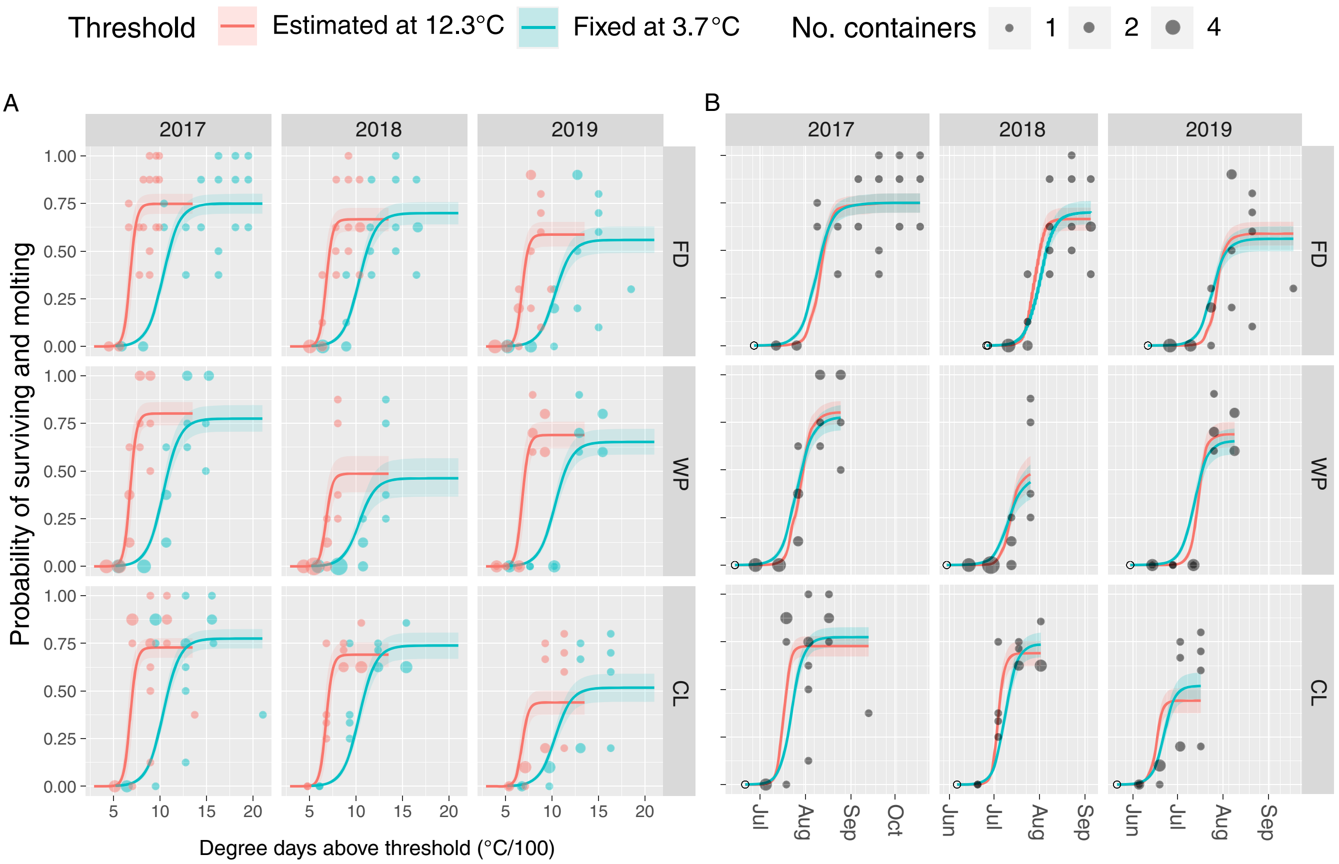

FIGURE 7 (A) Estimated relationships between the accumulated degree days above the fixed or estimated threshold and the probability of molting for fed nymphs for each site and year. (B) Posterior predictions of the probability of molting over time based on degree-day models. Lines represent the mean expectation and the shaded areas are the 80% CI. Points represent the proportion of fed nymphs found as newly-molted adults when ticks were recovered.

molting began, few SCEs had complete failures; just 17 (13%) of the 128 fed larvae SCEs retrieved after molting was first observed had no newly-molted ticks recovered and none of the 78 fed nymph SCEs (Figures 7 and 8). There were 263 SCEs each containing a single fed and (presumably) mated adult female, all but 8 of which were usable in the analyses.

Fed nymph to newly molted adult transition

The growing degree day model with the assumed threshold of 3.7°C above which development occurs captured the general pattern of molting probability (Figure 7A). When we allowed the threshold temperature at which development begins to vary (with a prior centered on 3.7°C) we saw strong support for higher threshold temperatures, with a mean around 12.3°C (95% CI = 11.0–13.6°C). The slope is rather higher in this case, too, indicating a distinct threshold number of degree days to achieve molt (Figure 7A). The posterior estimates of the threshold temperature and the total number of degree days required to achieve a 50% chance of molting were highly, negatively correlated ($r = -0.969$), but the number of degree days was still reasonably well estimated at ~679 DD (95% CI = 630–735). The posterior predictions of this more flexible model deviated from the fixed model in a few places, perhaps most notably at site CL, producing a more "threshold-like" curve (Figure 7B). Overall, however, both models described the general pattern in the data.

The maximum expected probability of molting and surviving varied a great deal among years within a site and, to a comparable degree, among sites within a year (see the asymptotes in Figure 7A). To some degree, this may reflect incomplete sampling (e.g., SCEs in WP in 2018 were not collected after July at which point they were only just attaining the degree days needed to ensure molting), but more generally it seems to be caused by differential pre- or post-molting mortality among years and sites. There was no evidence that the overall probability of molting and surviving declined with longer expected times to molt (Appendix S1: Figure S4).

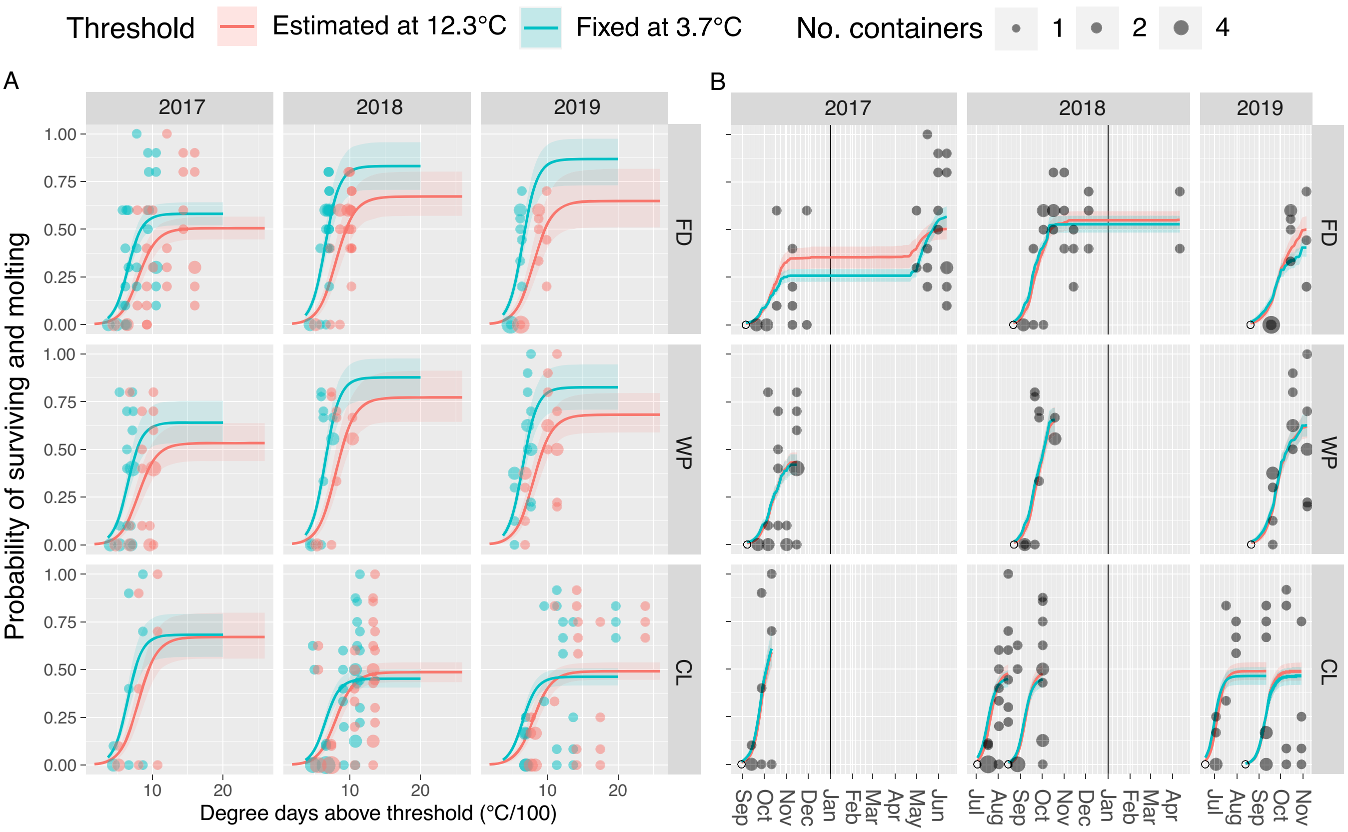

FIGURE 8 (A) Estimated relationships between the accumulated degree days above the fixed or estimated threshold and the probability of molting for fed larvae for each site and year. (B) Posterior predictions of the probability of molting over time based on degree-day models. Lines represent the mean expectation and the shaded areas are the 80% CI. Points represent the proportion of fed larvae found as newly-molted nymphs when ticks were recovered.

Fed larva to newly molted nymph transition

When we used the assumed threshold of 8.1°C for when degree day accumulation is initiated, the model broadly captured the increasing nature of molting probabilities. However, the fit model suggested larvae require many more degree days than the prior expectation from our re-analysis of Ogden et al. (2004): 652 versus 477 on average. When the model was allowed to fit the threshold temperature from the data, the estimated threshold was somewhat lower, around 3.2°C (95% CI = 0.5–5.8°C), and a larger number of degree days were needed to complete molting (~816 DD; 95% CI = 720–917 DD).

The predictions of the two models were quite similar, except for site FD where the lower estimated threshold led to an earlier accumulation of DD and thus an earlier expectation of molting (Figure 8). Both models predict that only a fraction of ticks deployed in FD in the fall of 2017 accumulated sufficient DD for molting prior to the cold of winter, although additional DD the following spring further increased the probability of molting (Figure 8). However, even at this site, there was sufficient DD to molt before winter temperatures preclude further development.

It is worth noting that there was considerable noise in the observed number of newly molted nymphs at any given time, even well after the threshold number of DD had been achieved (Figure 8). In all cases, there were a substantial number of SCEs with few or zero surviving newly-molted nymphs, suggesting mortality is an important process in this transition. These models are clearly missing some process (e.g., variable mortality) that influenced the observed survival + molt outcomes. Again, there was no evidence that the overall probability of molting and surviving declined with the expected times to molt in these field sites (Appendix S1: Figure S4).

Fed adult female to newly hatching larvae transition

We estimated that the transition from fed adult female to hatching larvae required roughly 751 (95% CI = 493–1021) degree days above 9.6°C (95% CI = 6.7–12.7°C). All sites accumulated sufficient DD to complete the transition. The model predicts that on average 72.7% of females would produce clutches yielding larvae (and so nearly 30% failed), though there was considerable variation among sites (Figure 9). Females deployed at CL had much greater success than those at WP and FD. This higher success at CL was at least coarsely associated with an expected time to complete the developmental transition that was less than half that required at WP and FD (Appendix S1: Figure S4), perhaps in part because these females were deployed in the spring rather than prior fall.

DISCUSSION

Blacklegged ticks spend a majority of their life off-host, subject to the hazards of freezing, overheating, and desiccation. At the same time, ticks are capable of avoiding suboptimal conditions (e.g., by staying under leaf litter or in the soil) and maintaining homeostasis in the face of these hazards (e.g., by rehydrating). The extent to which climatic conditions restrict tick populations has therefore been the subject of a great deal of debate. Our study investigating the off-host survival and probability of completing key developmental transitions of all life stages of *I. scapularis* in three sites distributed across the species' current geographical range and over several years was uniquely comprehensive. It was especially well-suited to test an overarching hypothesis that climatic conditions restrict tick geographic distributions. However, rather than finding simple, consistent, or strong effects of abiotic variables, our results highlight the complexity of off-host survival.

One of the key, but unexpected, observations was the large degree of variability in survival among otherwise identically treated groups of ticks. That is, while tick survival in some field-deployed soil core enclosures (SCEs) was quite high (80% or greater), demonstrating that it was possible for ticks to survive under the prevailing conditions, adjacent SCEs often had zero survivors. This was especially pronounced in flat larvae and flat nymphs (Figures 3 and 4), but flat adult survival was also quite variable at some timepoints (Figure 6). We are uncertain about the cause(s) of this variability, but likely explanations include micro-scale variability in the occurrence or density of predatory arthropod communities or entomopathogenic fungi (reviewed in Burtis, Yavitt, et al., 2019). Whatever the cause(s), the commonness of SCEs with zero ticks

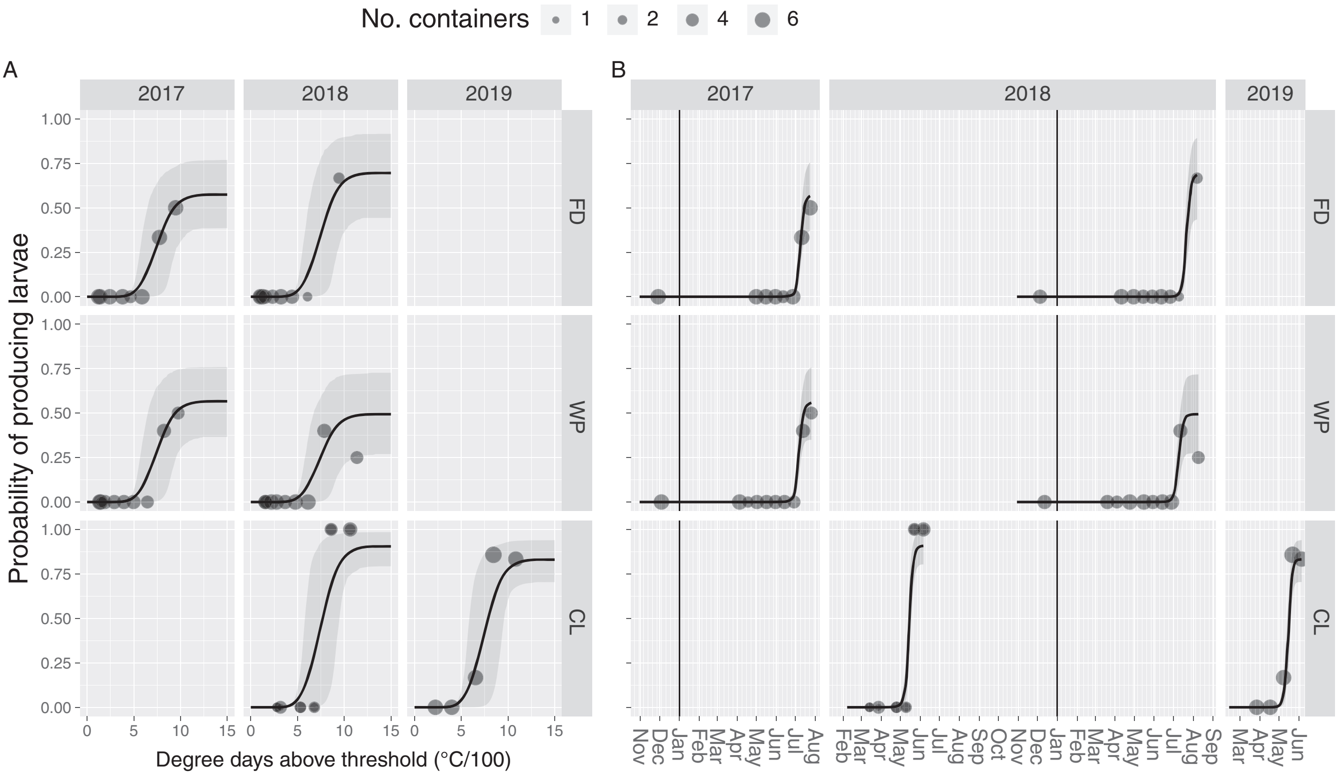

FIGURE 9 (A) Estimated relationships between the accumulated degree days above the estimated threshold temperature and the probability of offspring larvae being produced for each site and year. (B) Posterior predictions of the probability of offspring larvae being produced over time based on the degree-day model. Lines represent the mean expectation and the shaded areas are the 80% CI. Points represent the proportion of soil core enclosures deployed with a fed female in which larval ticks were recovered.

recovered—roughly half—suggests that unmeasured variables might play an important role in site-level, average survival of ticks off hosts.

In spite of this variability, we explored our hypotheses regarding how microclimatic conditions would shape patterns of tick survival, and whether even short periods of especially desiccating, warm, or cold conditions can lead to notable tick mortality. Our hazard-based models of tick survival imply a constant mortality rate, all else equal (i.e., type II survivorship), but this rate is modified by microclimatic conditions. The overall pattern of survivorship was thus expected to be one of exponential decline, with faster rates of decline under desiccating conditions or especially high or low temperature. Larval ticks largely fit this pattern (Figure 3) and, as expected a priori (e.g., Ginsberg et al., 2017; Stafford, 1994), the daily hazard rate increased under desiccating conditions. This is consistent with the hypothesis that larval ticks are very sensitive to desiccation (Yoder & Spielman, 1992). It also suggests regional patterns in humidity will translate to differences in survival times (e.g., Ginsberg et al., 2017). Indeed, median survival times were less than 15 days at CL, where there were more frequent periods of high vapor pressure deficit, as compared to 25–35 days at FD.

While we observed a fairly strong effect of microclimate, specifically vapor pressure deficit, on larval survival, there was little support for similar effects in flat nymphs or adults. As noted above, these hazard models generally predict an exponential decline in survivorship as soon as ticks are deployed, but questing nymphs and adults maintained moderately high survivorship for several weeks or months, respectively, before experiencing substantial mortality (Figures 4 and 6). Instead, the post hoc model that treats survivorship as a sigmoidal function of time better described the observed pattern of nymph and adult survival (Figures 4 and 6). Our empirical and statistical modeling results make it clear that these stages are much less vulnerable to climate-related hazards than larvae, as one might expect from their lower surface area:volume ratios and larger sizes and energy reserves, both of which likely buffer them from desiccation risk (reviewed in Needham & Teel, 1991). Moreover, this suggests that the basic assumption of our a priori hazard models, that the daily risk of mortality is constant and independent of time, is flawed or at least incomplete. Instead, we suggest that a nymph's or adult's hazard of mortality may not stem as much from the direct risk of drying out, freezing, or overheating, but indirectly, from exhausting their energy supply (i.e., lipid reserves; Burtis, Fahey, & Yavitt, 2019; Nieto et al., 2010; Steele & Randolph, 1985). That is, barring finding a blood meal host, which we experimentally excluded in this study, most nymphal and adult ticks die of starvation rather than environmental stress or injury. Size and energy reserves are also important for larval survival (Ginsberg et al., 2017), but perhaps less so than for the following life stages.

Climatic conditions should still play a critical role in survival even if ticks starve before desiccating or succumbing to extreme temperatures. Temperatures, for instance, influence the rate of energy consumption directly, as tick metabolism is temperature-dependent (Alasmari & Wall, 2021), and both temperature and vapor pressure deficit likely affect this rate indirectly, as ticks spend energy questing under favorable conditions or rehydrating under desiccating conditions (Kahl & Knülle, 1988; Needham & Teel, 1991; Stafford, 1994). Indeed, a condition-dependent energy budget might provide an explanation for differences in median survival times between years and sites, although more definitive tests await further analyses.

It is worth noting that overwintering nymphs tended to have quite high survival into spring, as in prior studies (e.g., Brunner et al., 2012; Burtis, Fahey, & Yavitt, 2019; Lindsay et al., 1995; Volk et al., 2022), across sites. In contrast to early suggestions that vulnerability to freezing would restrict blacklegged ticks to places with relatively mild conditions (McEnroe, 1977, 1979), the collective evidence suggests that the quiescent period during winters when ticks are presumably insulated by leaf litter and snow, exposes ticks to little risk of mortality (Burtis, Fahey, & Yavitt, 2019; Volk et al., 2022). The observation that overwintering survival was similar in our southern and northern sites is also consistent with a behavioral diapause or quiescence (reviewed in Belozerov, 2009 and Gray et al., 2016), slowing the rate at which ticks consume their energy reserves (e.g., Burtis, Fahey, & Yavitt, 2019). Indeed, to the extent we could estimate the hazard relationships, hazard tended to increase with temperature and, especially, VPD, suggesting nymphs became more vulnerable to mortality under conditions that would cause them to emerge from quiescence, as in Burtis, Fahey, and Yavitt (2019), although we have no direct evidence of quiescence in this study. In summary, our data are most consistent with the hypothesis that at least nymphal and adult blacklegged ticks die from starvation, which is presumably hastened by activity, higher temperatures, and the need to rehydrate. We suggest future models of tick abundance and distributions more explicitly consider this energetic perspective, as well as the behavioral and physiological responses to conserving energy, such as diapause and quiescence.

The short survival times of larvae in the environment (2–5 weeks) suggest a narrow window of time in which to find a bloodmeal host before dying. This, in turn, suggests contacts with potential hosts might be rate-limiting in the transition of larvae to (possibly *Borrelia*-, *Anaplasma*-, or *Babesia*-infected) nymphs, which is important both for tick population dynamics as well as the risk of tick-borne infections (Barbour & Fish, 1993). This is consistent with an analysis of a time series of (infected) nymph densities in New York that found the best predictors of nymph density in a given year was the density of small mammals in the prior year or their food resources (i.e., acorns) 2 years prior (Ostfeld et al., 2006, 2018). The much longer survival times of nymphs and adults in the field—median survival times of 4–11 weeks post deployment for nymphs and 2–8 months for adults—suggest host contacts are probably less limiting at these stages than they are for larvae. Over such periods of time, even very low contact rates are likely to accumulate to ensure a tick finds a host, and we may expect relatively few nymphs or adults to go unfed. These surprisingly short survival times and their consequences merit further study.

Collectively, our results suggest that climatic conditions have the most influence on the transition from free-living larvae to newly molted nymphs. While we did not observe clear thresholds in conditions beyond which larvae could not survive, daily hazard was elevated with elevated vapor pressure deficits, which hastened their demise. Likewise, shorter larval survival times are also likely to translate into reduced host-finding success, thus magnifying the impact of desiccating conditions. Models of ixodid tick population growth often suggest the larva-to-nymph transition is quite influential (e.g., Dunn et al., 2013; Estrada-Pena & Estrada-Sanchez, 2013), so even moderate effects of desiccating conditions are likely to translate into important effects on tick population growth rates. While all three of our sites appear suitable for persistence of black legged tick populations currently, our results suggest that in drier areas, or places that become more desiccating in the future, survival through the larvae-to-nymph transition may be limiting. Indeed, even nymphal black legged ticks in the Southeast have an apparently genetic predisposition to quest for hosts in the humid leaf litter rather than under more desiccating conditions higher off the ground, suggesting desiccation risk is already an important source of mortality in this region (Arsnoe et al., 2015, 2019).

Black legged ticks are ectotherms; hence, the rates at which they transition through key developmental processes, including molting, egg laying, and eclosion, are temperature sensitive (e.g., Ogden et al., 2005). Previously published models of climatic influences on ixodid tick populations and distributions have emphasized the role temperature plays in development rates and shown that duration of the life cycle might be extended in colder regions to the degree that even low levels of hazard might reduce survival to reproduction below replacement levels (e.g., Estrada-Pena & Estrada-Sanchez, 2013; Ogden et al., 2005). We found that a degree-day model, similar to that in prior studies (e.g., Ogden et al., 2005), worked well at predicting molting times, especially for fed nymphs molting into adults (Figures 7 and 8), and the timing of larvae hatching from egg masses laid by fed females (Figure 9). While we did not find any evidence that prevailing temperatures at any of our distributed sites might prove insufficient for ticks to complete these transitions (Figures 7–9), our results are consistent with the hypothesis that this temperature-dependent development might place hard limits on tick distributions in cooler, northern habitats.

We observed a great deal of variability among SCEs in terms of the number of newly-molted ticks observed, but it was not possible to discern whether this was due to differences in molting success or in survival before or after molting. However, the observation that there was much more variation in survival from fed larvae to newly-molted nymphs than from fed nymphs to newly-molted adults is at least consistent with the latter. Similarly, at least at a coarse scale, there was no clear relationship between longer transitions being associated with fewer newly-molted ticks recovered (Appendix S1: Figure S4), as is often assumed when these models of temperature-dependent development are extended to distributional predictions, although we caution that our study was not designed to distinguish how or when molting ticks died.

Collectively, there was much less of a clear signal of microclimatic conditions leading to mortality than we expected given the prior literature on tick population dynamics and recent rates and directionality of *Ixodes* range expansions (e.g., Bertrand & Wilson, 1996; Ginsberg et al., 2017; Gray et al., 2016; Leal et al., 2020; Nieto et al., 2010; Ogden et al., 2021; Stafford, 1994; Tufts et al., 2021). Beyond the well-established temperature-dependence of development in ectothermic organisms leading to fairly clear relationships between molting or larval hatching probability and accumulated degree-days (Figures 7–9; Lindsay et al., 1995; Ogden et al., 2004, 2005), only larvae appear particularly sensitive to climatic conditions. Nymphs and adults, on the other hand, appear constrained by energetic reserves (the consumption of which may also be influenced by climatic conditions). Our findings thus suggest that most life stages of ticks are much better at avoiding or compensating for challenging environmental conditions than previously assumed. One caveat is our finding that some, as-of-yet undetermined variable, did result in catastrophic mortality that was spatially heterogenous at within-plot scales. Beyond this unknown mortality source, we suspect, desiccation-related mortality of larvae may already be sufficient in some areas to restrict population establishment and growth.

AUTHOR CONTRIBUTIONS

Jesse L. Brunner, Shannon L. LaDeau, Mary Killilea, and Richard S. Ostfeld designed the studies and procured funding. Elizabeth Valentine and Megan Schierer led the research teams with oversight from Richard S. Ostfeld. Jesse L. Brunner led the analyses and produced the first draft the manuscript. All authors contributed to and edited the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Brunner et al., 2023a) are available in Dryad at https://doi.org/10.5061/dryad.931zcrjnz. Code (Brunner et al., 2023b) is available in Zenodo at https://doi.org/10.5281/zenodo.6803051. Code output (Brunner et al., 2023c) is available in Zenodo at https://doi.org/10.5281/zenodo.6803053.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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