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Research

Ixodes scapularis (Acari: Ixodidae) Nymphal Survival and Host-Finding Success in the Eastern United States

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Abstract

The blacklegged tick (*Ixodes scapularis* Say) is the primary vector of *Borrelia burgdorferi* sensu stricto (Spirochaetales: Spirochaetaceae), the Lyme disease agent in North America. The basic reproduction number (R_0) for *B. burgdorferi* in *I. scapularis* in the Northeast is highly sensitive to the probability that engorged larvae survive the winter, molt into nymphs, and find a host. These processes are dependent on local environmental variables, including climate, host population size and movement, and tick behavior. A simple model is presented for estimating host-finding success from the ratio of tick abundance in two subsequent years, accounting for overwinter survival and possible differences in host associations between nymphs and larvae. This model was parameterized using data from two sites in mainland Connecticut and two on Block Island, RI. Host abundance and tick burdens were estimated via mark–recapture trapping of the primary host, *Peromyscus leucopus* Rafinesque. Overwintering survival was estimated using engorged larvae placed in field enclosures at each site. Only nymphs were recovered alive, and no significant differences in model parameters were observed between Connecticut and Block Island. Host-finding success was predicted to be high across a wide range of host association patterns at three of four sites. Assuming equivalent host association between larvae and nymphs, R_0 was also estimated to be greater than one at three of four sites, suggesting these conditions allow for the persistence of *B. burgdorferi*. The model output was highly sensitive to differences between nymphal and larval host associations.

Key words: Lyme disease, *Peromyscus leucopus*, *Borrelia burgdorferi*, blacklegged tick, white-footed mouse

The ability of a tick to molt to the next life stage, survive, and find a new host is key to the life cycle of a tick and any pathogens it may vector. Although multiple studies have investigated various aspects of some tick life cycles, key life-history traits and life-stage transitions have yet to be measured empirically for most tick species. Identifying which parameters in the complex life cycle of tick-borne pathogens are most influential for tick and pathogen persistence and spread is especially critical for *Ixodes scapularis* Say (blacklegged tick), the primary vector for the Lyme disease bacterium *Borrelia burgdorferi* sensu stricto in eastern North America, as well as for other human pathogens such as *Borrelia miyamotoi* (Spirochaetales: Spirochaetaceae) (relapsing fever), *Borrelia mayonii* (Spirochaetales: Spirochaetaceae) (Lyme disease), *Babesia microti* França, 1912 (Piroplasmida: Piroplasmorida) (human babesiosis), *Anaplasma phagocytophilum* (Rickettsiales: Ehrlichiaceae) (human granulocytic anaplasmosis), *Ehrlichia* spp. (Rickettsiales: Anaplasmataceae) (human ehrlichiosis), and Powassan virus (Powassan encephalitis) (Tokarz et al. 2014, 2018; Nelder et al. 2016). Furthermore, limited empirical information is available on the influence of abiotic conditions (e.g., temperature) and biotic resources (e.g., host availability) on key life-history parameter traits, such as overwintering survival and the ability to successfully find and feed on a host (Vail and Smith 2002, Rodgers et al. 2007, Linske et al. 2019).

To identify key parameters for pathogen persistence and spread, a previous study by Dunn et al. (2013) developed a model for the basic reproduction number (R_0) for horizontally transmitted *I. scapularis*-borne infections, such as Lyme disease, based on field measurements from the Northeast United States. R_0 is an index of the likelihood that a particular pathogen would persist or spread in the population ($R_0 \geq 1$) or eventually disappear ($R_0 < 1$; Dunn et al. 2013). Dunn et al. (2013) found the value of R_0 was most sensitive to the combined probability that a nymph survives the winter and that it finds a reservoir competent host. Although other studies have assessed nymphal survival (Yuval and Spielman 1990, Brunner et al. 2014, Burtis et al. 2019), the probability that a nymph encounters a host has not been empirically estimated in field settings.

The main objective of this study was to model host-finding success, parameterized with data on tick overwinter survival, tick burdens on hosts, and host population sizes at two eastern United States field sites corresponding to island and mainland regions, and to

investigate the model's sensitivity both to these measured parameters and to the unmeasured role of differential host associations between nymphs and larvae. To estimate tick overwinter survival, engorged larvae were placed in enclosed tubes on a grid in different field sites and monitored monthly for 1 yr. Host and vector abundance and tick burden on hosts were calculated from the same field sites during a 3-mo host-trapping season in the summers of 2015 and 2016. For host and tick burden estimates, *Peromyscus leucopus* Rafinesque (white-footed mouse), the primary host species of *I. scapularis* immature tick life stages in the eastern United States, was the focal species. *Peromyscus leucopus* is a semiarboreal, primarily nocturnal New World mouse with a wide distribution throughout North America and is a highly competent reservoir host of *B. burgdorferi* (Mather et al. 1989, Schmidt and Ostfeld 2001). The abundances and tick burdens of other hosts were not directly measured, and their potential influence on the model was assessed using a host association parameter. Incorporating regional variation in nymphal survival and host-finding success into models of tick-borne pathogen transmission and persistence can allow for more accurate predictions of the potential for Lyme disease emergence and can help identify targets for control.

Methods and Materials

Study Sites and Design

The field portion of the study was designed to simultaneously estimate overwinter tick survival and the tick burdens on *P. leucopus* hosts in the same trapping site.

Study sampling grids were established at four sites for this experiment: two in mainland Connecticut (Connecticut 1 [CT-1]: 41°21′49.5″N, 72°46′35.8″W; Connecticut 2 [CT-2]: 41°22′27.0″N, 72°46′40.6″W) and two on Block Island, RI (Block Island 1 [BI-1]: 41°09′25.2″N, 71°35′22.9″W; Block Island 2 [BI-2]: 41°09′47.6″N, 71°33′58.1″W). Flags were placed every 10 m at each grid 'node', with the grids varying in size based on habitat availability; a subset of flags were randomly chosen and survivorship tubes were placed around them. Size of the grids was as follows: CT-1 had 12 × 12 nodes, 18 random flags, 198 tubes; CT-2 had 12 × 11 nodes, 17 random flags, 187 tubes; BI-1 had 12 × 10 nodes, 13 random flags and 143 tubes; BI-2 had 10 × 6 nodes, 8 flags, 88 tubes. Sampling from each trapping site occurred for 11 mo, from October 2015 through the immature tick active seasons in August 2016.

Survivorship Tube Construction and Tick Molting

The round base of a plastic disposable culture tube (12 × 75 mm; USA Scientific, Ocala, FL) was cutoff using an electric blade. A 7.5 × 5 cm piece of tan plastic mesh (300 µm) was glued to the top of the tube and sealed in a cylindrical fashion using a hot glue gun; additionally, a strip of duct tape was wrapped around this area for added protection and to prevent the tick from escaping. Tubes were thoroughly checked for holes before being used in the field and after collection. During the last week of August 2015, one pathogen-free fully engorged larval stage *I. scapularis* (Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: engorged *I. scapularis* larvae, NR-4115) was placed inside each survivorship tube using a fine tip paintbrush. Ticks were acclimated to environmental conditions for approximately 8 h. The sturdy test tube portion was plunged approximately 3 cm into the ground, creating a dirt plug that prevented escape, and allowed the tick access to the soil and leaf litter, while the mesh top allowed the tick access to ambient conditions (Fig. 1A). This design allowed the ticks to choose between remaining in the leaf litter or to perform questing behaviors. Eleven survivorship tubes were inserted into the soil in a circle approximately 1 m around each randomly chosen flag at each study site (Fig. 1B).

At the end of each month from October 2015 to August 2016, one survivorship tube was removed from each flag at each site and taken back to the lab for processing. The mesh top was cut in half and thoroughly investigated to locate the tick. If the tick was not found in the upper mesh area, the soil collected in the plastic tube area was removed and sifted through by hand. If the tick could still not be located, enough water was added to the dirt to potentially allow the ticks to float to the surface. If the tick was still not located after 30 min of searching, the sample was labeled as 'dead'.

Small Mammal Sampling and Tick Burdens

Small mammals were trapped every other week for three consecutive nights for seven trapping sessions each from May to August 2015 and 2016. Sherman live traps (7.62 cm × 8.89 cm × 22.86 cm; H.B. Sherman Traps, Inc. Tallahassee, FL) were placed at each of the grid nodes and baited with peanut butter, oats, and sunflower seeds. Traps were opened at dusk and closed at dawn to target nocturnal animals only. Attached *I. scapularis* larval and nymphal ticks were carefully removed with fine forceps and the number of ticks collected was recorded from each animal. Mice were immobilized via

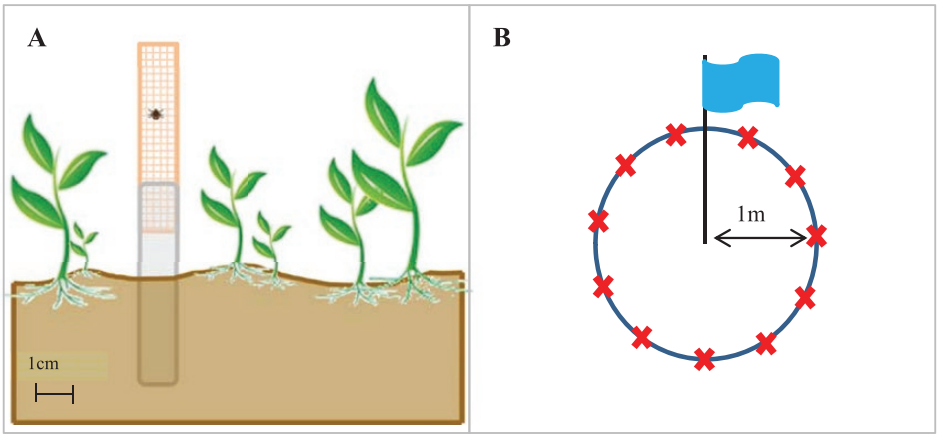

Fig. 1. Survivorship tube construction and placement. (A) A single engorged larva was placed into the upper mesh area of the tube, the hard plastic base was then pushed into the ground. (B) Eleven tubes were placed in a circle approximately 1 m from flags randomly located in the trapping grids.

scruffing technique and the entire body was checked for ticks for at least 20 min; however, there is a possibility some ticks may have been missed. Host-seeking *I. scapularis* nymphs were also collected by dragging a 1 m × 1 m corduroy along the trap lines, stopping every 10 m to collect the attached ticks.

Statistical Analyses

Tick Survivorship Estimation

Nymph survival times were analyzed as interval-censored or 'current status' data (i.e., ticks found dead were assumed to have died at an undefined time between tube placement and collection and ticks found alive were classified as dying at an undefined time between collection and time infinity). Missing ticks were omitted if the associated tube was damaged or if a dirt plug was not present when the tube was removed from the ground. Otherwise, missing ticks were assumed to have decomposed and were treated as dead. The interval and icenReg libraries in R to fit nonparametric and parametric survival curves to interval-censored data were used (Fay and Shaw 2010, Anderson-Bergman 2017). Nonparametric maximum likelihood estimation was used to generate nonparametric survival curves for individual sites, individual regions, and all sites combined. Survival curves were compared between sites and regions using log-rank tests. Because of high agreement between all survival curves, parametric curves were ultimately only fit to the data from all sites combined. The Weibull distribution was chosen for goodness of fit (by plotting its cumulative incidence function against that of the nonparametric curve) and relative ease of interpretation.

Host Abundance Estimation

A mark–recapture study was conducted to assess the abundance of white-footed mice, incorporating data from the seven trapping sessions for each year (2015 and 2016). To account for recruitment, host abundance was modeled in R using loglinear robust design models (as implemented by Rcapture R package; Baillargeon and Rivest 2007). Closed population analyses were first performed within each trapping period to identify appropriate models (constant capture variability [M0], temporal variation [Mt], individual heterogeneity [Mh] as approximated by a Chao, Poisson, Darroch, or gamma model, respectively, or temporal variation and individual heterogeneity [Mht]). Of the resulting models, a fitted robust design model was then selected based on Akaike information criterion. Temporary emigration was tested by conducting a likelihood ratio test of the fitted model and the model with temporary emigration. For one of the Connecticut sites in 2015, no robust design model converged, as a result of several trapping sessions without captures. In this case, a full-likelihood Jolly-Seber model was used and parameterized in terms of time-specific population size (as implemented by opencr R package (Efford 2019). This model structure allowed for estimation of session-by-session host abundances in an open population under the assumption that capture probabilities were equal for tagged and nontagged individuals. The differences in *P. leucopus* density between sites and trapping seasons, as estimated by the number of unique individuals/grid area at each site, was estimated using Welch's unpaired *t*-test.

A parametric model of mouse abundance at each site was then modeled with a function that accounted for the two summer breeding periods previously observed in northeastern US *P. leucopus* populations (Jackson 1952, Jacquot and Vessey 1998, Dunn 2014):

$$\frac{dN}{dt} = b(t)N - \left(\mu_M + \frac{N}{K}\right)N$$

where N represents mouse abundance, μ_M is the baseline death rate, and K is the carrying capacity (to account for density-dependent mortality). The birth rate at time t, $b(t)$, is defined as follows:

$$b(t) = \phi(H(t-\tau_1) - H(t-\tau_2) + H(t-\tau_3) - H(t-\tau_4))$$

where ϕ is the per capita birth rate, H represents the Heaviside step function, and τ_n define the timing of the birth peaks. The mouse abundance equation was integrated with respect to N to generate $N(t)$ using an additional parameter N_0, or the mouse abundance at the start of the season. This model was chosen a priori to allow easy interpretation of the effects of individual parameters on host-finding success.

Resulting curves were fit to estimated abundances using maximum likelihood estimation assuming negative binomial error distribution. Initial values for the optimization procedure were selected based on values from the literature (Dunn et al. 2013; Table 1), with τ_n adjusted to match observed abundance peaks.

Tick Burden Estimation

At each site, the tallied 2015 larval and 2016 nymphal burdens of mice were recorded on their first per-session capture (excluding recaptured mice that had already undergone tick removal during a session). After Brunner and Ostfeld (2008), nymphal burden was modeled as a right-shifted lognormal curve, and larval burden as a right-shifted normal curve with a later lognormal curve:

$$\overline{Z}_N(t) = \begin{cases} H_N e^{-\frac{1}{2}\left[\frac{\ln\left(\frac{(t-\tau_N)}{\mu_N}\right)}{\sigma_N}\right]^2} & \text{if } t \geq \tau_N \\ 0 & \text{otherwise} \end{cases}$$

$$\overline{Z}_L(t) = \begin{cases} H_N e^{\frac{1}{2}\left[\ln\left(\frac{(t-\tau_E)}{\mu_E}\right)\right]^2} & \text{if } t \leq \tau_L \\ H_L e^{\frac{1}{2}\left[\frac{\ln\left(\frac{(t-\tau_L)}{\mu_L}\right)}{\sigma_L}\right]^2} + H_E e^{-\frac{1}{2}\left[\ln\left(\frac{(t-\tau_E)}{\mu_E}\right)\right]^2} & \text{otherwise} \end{cases}$$

where $\overline{Z}_N(t)$ is the mean on-host nymphal burden at time t; $\overline{Z}_L(t)$ is the mean on-host larval burden at time t; H_N, H_E, and H_L define burden heights; τ_N, τ_E, and τ_L define timing of burden curves; μ_N, μ_L, and μ_E define shift to peak burden; and σ_N and σ_L define shape parameters of the burden peaks (Table 1). Curves were fit to measure burdens using the maximum likelihood function for negative binomial count data. Initial values for the optimization procedure were selected based on data from prior years.

Host-Finding Success Estimation

The following formula was used to estimate host-finding success at each site, incorporating the fitted parametric equations described above:

$$c(t) = \frac{\sum_{t_{0,\ 2016}}^{t} \frac{\overline{Z}_N(t)N(t)}{s_N(t)d_N} h_N}{\sum_{t_{0,2015}}^{\tau} \frac{\overline{Z}_L(t)N(t)}{d_L}}$$

where $c(t)$ is the cumulative host-finding success of questing nymphs at time t, $\overline{Z}_N(t)$ is the mean on-host nymphal burden at t, $N(t)$ is mouse abundance at t, $s_N(t)$ is the proportion of nymphs surviving at t, $\overline{Z}_L(t)$ is the mean on-host larval burden at time t, d_N is the mean time nymphs spend attached to hosts, and d_L is the mean time larvae spend attached to hosts (values which are required when comparing populations of on-host nymph to on-host larvae). The numerator is summed from 1 April 2016 mouse trapping season $t_{0,2016}$ to time

Table 1. Parameters used in the formulas and the model including the range of values and the outcomes in the partial rank correlation coefficients (PRCC) and lower and upper confidence intervals (LoCI and UpCI, respectively)

Parameter	Definition	Values			PRCC		
		Minimum	Maximum	Source	Est	LoCI	UpCI
On-host nymphal burden $Z^N(t)$							
H^N	Height of nymphal burden on hosts	1.73	4.96	Current study	0.345	0.206	0.455
τ^N	Timing of nymph burden (d)	124	139	Current study	0.107	−0.012	0.246
μ^N	Shift to peak nymph burden (d)	13.90	28.80	Current study	0.333	0.181	0.497
σ^N	Shape parameter for nymph burden	0.493	0.792	Current study	0.265	0.129	0.372
On-host larval burden $Z^L(t)$							
H^E	Height of first larvae peak	0.477	1.53	Current study	−0.109	−0.247	−0.003
H^L	Height of second larvae peak	1.04	3.74	Current study	−0.468	−0.582	−0.339
τ^E	Timing of early larval peak (d)	151	172	Current study	0.041	−0.054	0.147
τ^L	Timing of late larval peak (d)	192	203	Current study	−0.005	−0.147	0.149
μ^E	Shift to early larval peak (d)	16.50	21.90	Current study	−0.109	−0.253	0.025
μ^L	Shift to late larval peak (d)	13.70	21.60	Current study	−0.056	−0.195	0.090
σ^L	Shape parameter for larval burden	0.551	3.96	Current study	−0.379	−0.531	−0.248
Mouse abundance 2015 $N(t)$							
$N_{0,15}$	Initial mouse abundance	40.40	101	Current study	0.012	−0.099	0.157
$\mu^M_{,15}$	Baseline mouse death rate	0.017	0.046	Current study	0.879	0.845	0.912
ϕ_{15}	Per capita mouse birth rate	0.039	0.079	Current study	−0.862	−0.903	−0.825
K_{15}	Carrying capacity	9,900	10,100	Dunn et al. (2013)	0.025	−0.137	0.176
$\tau_{1,15}$	Start of first mouse birth peak	70	109	Current study	0.467	0.363	0.578
$\tau_{2,15}$	End of first mouse birth peak	160	188	Current study	−0.461	−0.574	−0.360
$\tau_{3,15}$	Start of second mouse birth peak	170	200	Current study	0.495	0.375	0.592
$\tau_{4,15}$	End of second mouse birth peak	207	300	Current study	−0.569	−0.653	−0.466
Mouse abundance 2016 $N(t)$							
$N_{0,16}$	Initial mouse abundance	99.90	101	Current study	0.122	−0.021	0.265
$\mu^M_{,16}$	Baseline mouse death rate	0.011	0.026	Current study	−0.697	−0.772	−0.621
ϕ_{16}	Per capita mouse birth rate	0.026	0.051	Current study	0.716	0.644	0.769
K_{16}	Carrying capacity	9,900	10,100	Dunn et al. (2013)	−0.050	−0.239	0.102
$\tau_{1,16}$	Start of first mouse birth peak	51	60.40	Current study	−0.157	−0.344	0.052
$\tau_{2,16}$	End of first mouse birth peak	160	180	Current study	0.042	−0.131	0.175
$\tau_{3,16}$	Start of second mouse birth peak	170	201	Current study	−0.082	−0.226	0.055
$\tau_{4,16}$	End of second mouse birth peak	196	250	Current study	−0.033	−0.152	0.123
Survivorship sN							
β_0	Initial proportion of surviving ticks	1.71	1.78	Current study	−0.063	−0.226	0.077
β_μ	Relative rate of change in proportion of surviving ticks	−0.013	−0.008	Current study	−0.455	−0.561	−0.351
Host-finding success $c(t)$							
d^N	Host attachment time of nymphs (d)	3	5	Dunn et al. (2013)	−0.155	−0.323	−0.001
d^L	Host attachment time of larvae (d)	4	6	Dunn et al. (2013)	0.274	0.127	0.398
h^N	Host association ratio	0.10	10	Current study	0.904	0.873	0.934

t, whereas the denominator is summed from 1 April 2015 season ($t_{0,2015}$) to 30 November 2015 (τ; notation adapted from Dunn et al. 2013), a span designed to include potentially unobserved early- and late-season questing while excluding months during which ticks are prohibitively unlikely to seek hosts. This assessment of parameter c assumes an effectively closed tick population from 2015 to 2016 with the exception of mortality accounted for by the survivorship curve $s_N(t)$.

$\overline{Z}_N(t)$, $\overline{Z}_L(t)$, $N(t)$, and $s_N(t)$ were defined by the parametric equations fit to each data set as described above. Values for d_L and d_N were set to 4 and 5 d, respectively, after the parameterization of Davis and Bent (2011). The remaining parameter, h_N, is the 'host association ratio' or the proportion of nymphs that parasitize non-mouse hosts divided by the proportion of larvae that parasitize non-mouse hosts, included to account for the uncertainty in the relative abundance of larvae and nymphs in non-mouse hosts. Values less than 1 signify that larvae are more likely to parasitize non-mouse hosts compared to nymphs, whereas values greater than 1 indicate that nymphs are more likely to parasitize non-mouse hosts compared to larvae. This parameter is functionally indistinguishable from a parameter accounting for differences between on-host detection rates of larvae and nymphs. Because parameter c cannot take on a value greater than 1, the upper limit of h_N is constrained:

$$h_N \leq \frac{\sum_{t_{0,2015}}^{\tau} \frac{\overline{Z}_L(t)N(t)}{d_L}}{\sum_{t_{0,\ 2016}}^{\tau} \frac{\overline{Z}_N(t)N(t)}{s_N(t)d_N}}$$

Note that since the parameter expresses differential nymphal host associations relative to larval host associations, it does not provide any information on the absolute proportions of nymphs or larvae that parasitize non-mouse hosts.

Sensitivity Analysis and Basic Reproduction Number (R_0) Estimation

To assess drivers of host-finding success calculated at each study site, a sensitivity analysis was conducted for the end-of-season cumulative host-finding success. Parameter ranges were defined as uniform distributions between the minimum and maximum values for each parameter calculated across the four sites (Table 1). Because the sensitivity analysis was intended to analyze differences between sites, survival curves and tick attachment times (which were modeled as identical between sites) were held constant. h_N was evaluated between 0.1 and 10 to capture a range of plausible values—in order to preserve the independence of the tested variables, the value was not constrained relative to other parameters. Sensitivity analysis was conducted in R using Latin hypercube sampling as formulated in the pse package (Chalom and Prado 2017). The model was run using 250 parameter combinations with 100 bootstrap replicates. Outputs as a function of each parameter were plotted to ensure that associations between parameter and model output were monotonic. Partial rank correlation coefficients (PRCC) were then calculated to assess the strength of the linear associations between each parameter and model output, holding all other parameters constant. Symmetric Blest Measure of Agreement (Coolen-Maturi and Elsayigh 2010) was calculated between the PRCC of this model and an identical model run over 300 samples of parameter combinations to ensure adequate sample size.

To assess whether the estimated values of $s_N(t)$ and $c(t)$ derived from field measurements would allow for persistence of *B. burgdorferi* ($R_0 > 1$) at the study sites, R_0 values were estimated using fixed point estimates for other parameters from a previous publication (Dunn et al. 2013; Supp Materials [online only]). Because these fixed point estimates include modeling transmission efficiencies between ticks and *P. leucopus* specifically and accounts only for disease persistence in the *P. leucopus* population (no non-mouse hosts), R_0 was only assessed at $h_N = 1$.

Results

Tick Survival Estimates ($s_N(t)$)

Over the 11-mo study, 39.2–59.4% of the ticks placed were recovered per site from the survivorship tubes (Table 2). Only nymphs were recovered alive; dead engorged larvae were recovered in 2.5% of tick recoveries from October to February at the Connecticut sites ($n = 12$) and from October to December from the Block Island sites ($n = 4$). In 5.2% of all tubes ($n = 33/629$) where no tick was found, the dirt plug was also missing, suggesting that these ticks could have escaped from the bottom part of the tube during removal from the field. Higher tick recovery rates were observed at the Connecticut sites (CT-1 = 49.5%, CT-2 = 59.9%) compared with the Block Island sites (BI-1 = 39.2%, BI-2 = 45.5%). There were no significant differences in survival between sites or regions by log-rank test (Supp Fig. 1 [online only]). Median time to death across all sites as estimated by Weibull regression was 211 d. Mortality was 100% by the final day of the experiment (Fig. 2).

Host Abundance ($N(t)$) and Tick Burdens ($(\overline{Z}_L(t), \overline{Z}_N(t)$)

From the robust design models for mouse abundance data in the 2015 trapping season, there was evidence of heterogeneity for the CT-2 and BI-2 sites (Mh Chao model); however, the simpler homogeneous model was favored at the BI-1 site (M0 model). As noted in the methods section, CT-1 was modeled by an open-population Jolly–Seber model. From the robust design models for the mouse abundance data in the 2016 trapping season, the heterogeneity model was favored at the BI-1 site, whereas the homogeneous model was favored at the CT-1, CT-2, and BI-2 sites. Inclusion of temporary emigration did not significantly affect the fit of any model during either season (Fig. 3). The dual-peaked model described in the Methods section was fit to each set of abundance estimates, with the highest calculated RMSE (for site BI-1 in 2016). Mouse density was calculated to account for the differences in grid area between each of the sites, *P. leucopus* population density was significantly higher in 2016 compared with 2015 at the

Table 2. Monthly survivorship of engorged larvae expressed as a percent (number of recovered ticks)

Site	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Average
CT-1 (18)	50.0 (9)	83.3 (15)	50.0 (9)	61.1 (11)	83.3 (15)	72.2 (13)	55.5 (10)	55.5 (10)	16.7 (3)	11.1 (2)	5.5 (1)	59.4
CT-2 (17)	88.2 (15)	76.5 (13)	76.5 (13)	76.5 (13)	76.5 (13)	70.6 (12)	52.9 (9)	47.0 (8)	35.3 (6)	41.2 (7)	17.6 (3)	49.0
BI-1 (13)	46.1 (6)	61.5 (8)	53.8 (7)	46.1 (6)	53.8 (7)	38.5 (5)	61.5 (8)	23.1 (3)	30.8 (4)	15.4 (2)	0.0 (0)	39.2
BI-2 (8)	37.5 (3)	75.0 (6)	37.5 (3)	50.0 (4)	62.5 (5)	75.0 (6)	37.5 (3)	62.5 (5)	25.0 (2)	12.5 (1)	25.0 (2)	45.5

The total number of tubes at each site is written in parentheses. CT-1 = Connecticut 1; CT-2 = Connecticut 2; BI-1 = Block Island 1; BI-2 = Block Island 2.

Connecticut sites ($P < 0.0001$, df = 26, Welch's $t = -9.0158$) and Block Island sites ($P < 0.0001$, df = 26, $t = -6.1329$; Fig. 3). Additionally, the density of *P. leucopus* mice on Block Island was significantly higher than in Connecticut in 2015 ($P < 0.0001$, df = 26, $t = -7.1685$) and 2016 ($P < 0.0001$, df = 26, $t = -7.5114$; Fig. 3). No significant differences in mouse density were observed between sites at the same location except that CT-2 was significantly higher compared with CT-1 in 2015 ($P = 0.0212$, df = 12, $t = -2.6492$).

Mean larval burdens on mice throughout the sampling period at both Block Island sites (BI-1 mean burden 19 ticks per mouse, ± SD 18; BI-2 19 ± 19) were significantly higher than those at CT-2 (5.8 ± 6.7; $P < 0.01$; Fig. 4A). There were no significant differences within regions or between CT-1 (13 ± 13) and other sites. Overall, nymphal burdens were significantly higher at BI-1 (1.9 ± 3.0) compared with CT-1 (0.7 ± 1.5; $P < 0.01$) and CT-2 (0.7 ± 1.3; $P = 0.020$) and higher at BI-2 (1.5 ± 2.7) compared with CT-1 ($P = 0.033$; Fig. 4B), and there were no significant differences within regions (Fig. 5). Other host species were also collected in Sherman traps; however, only 0.26% of immature ticks (larvae and nymphs) were found on these hosts (Supp Table 1 [online only]). Other host species present in the study area but not captured by the sampling protocol are described in Supp Materials (online only).

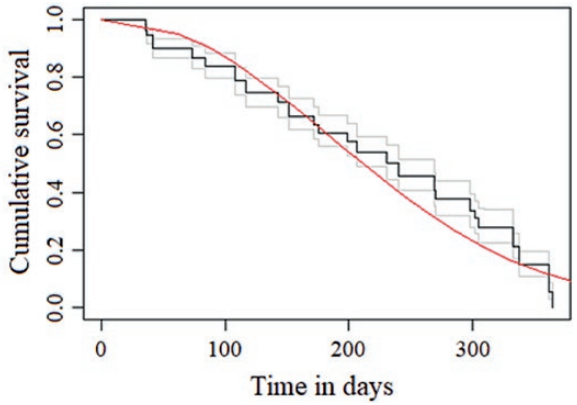

Fig. 2. Nonparametric maximum likelihood estimator (NPMLE) for cumulative survival of *Ixodes scapularis* engorged larvae across all sites. Time is measured in days since placement of engorged larvae in field survivorship tubes. Light gray lines represent 95% confidence intervals. The curved line indicates the parametric survival curve estimated with Weibull regression.

Host-Finding Success ($c(t)$)

For $h_N = 1$, cumulative host-finding success accounting for tick survival (parameter c) was similar at all sites (CT-1: 0.808; BI-1: 0.713; BI-2: 0.832) with the exception of CT-2 (0.154; Fig. 5). The value of h_N corresponding to a c of 1 by the end of the season (i.e., 100% host-finding success by the end of the season) was 1.24 for CT-1, 6.51 for CT-2, 1.40 for BI-1, and 1.21 for BI-2. Increased values of h_N here indicate that a larger number of nymphs successfully parasitizing non-mouse hosts would be required to result in a 100% host-finding success rate.

Sensitivity Analysis, Partial Rank Correlation, and Basic Reproduction Number (R_0) Estimates

Symmetric Blest Measure of Agreement between the 250- and 300-sample model runs was 0.85, indicating a high degree of agreement between the two and suggesting sufficient sample size. The

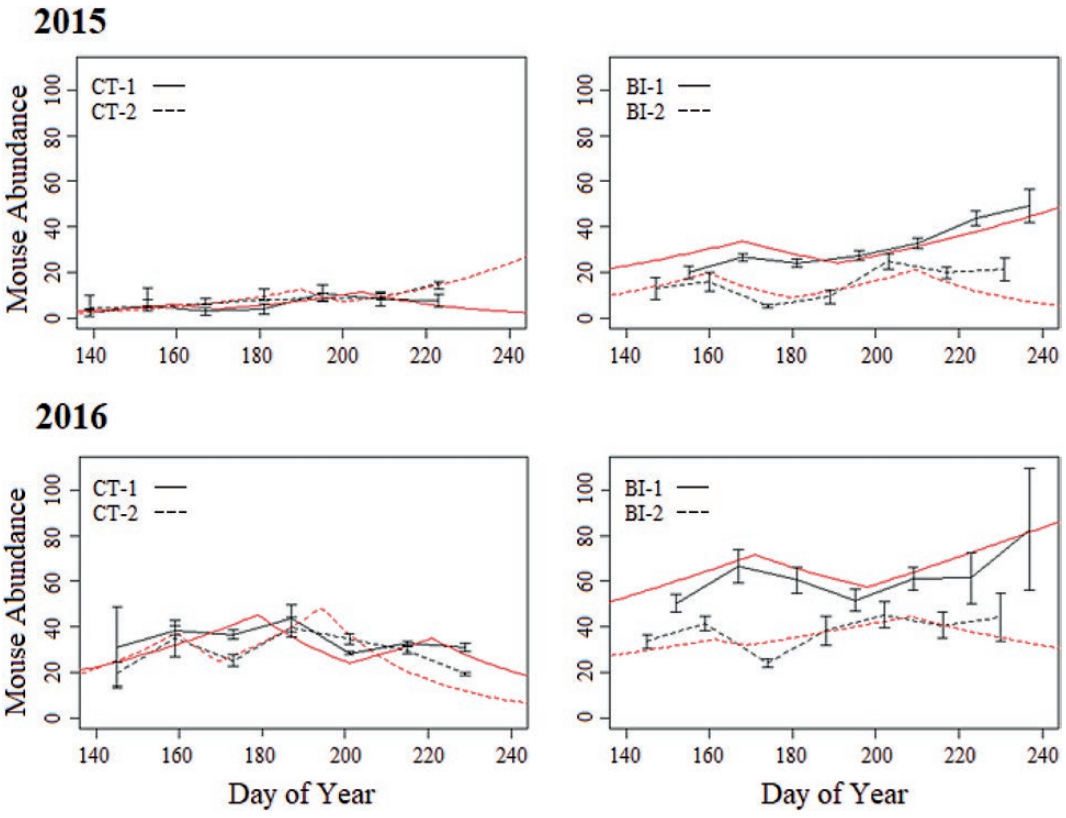

Fig. 3. Mouse abundance estimates per grid (CT-1: 120 × 120 m; CT-2: 120 × 110 m; BI-1: 120 × 100 m; BI-2: 60 × 100 m) were calculated by site for summer trapping seasons (sessions 1–7 depicted by data points with corresponding months along *x*-axis) in 2015 and 2016. Error bars represent SE. Curves are predicted abundances based on the two-peaked abundance function described in Materials and Methods.

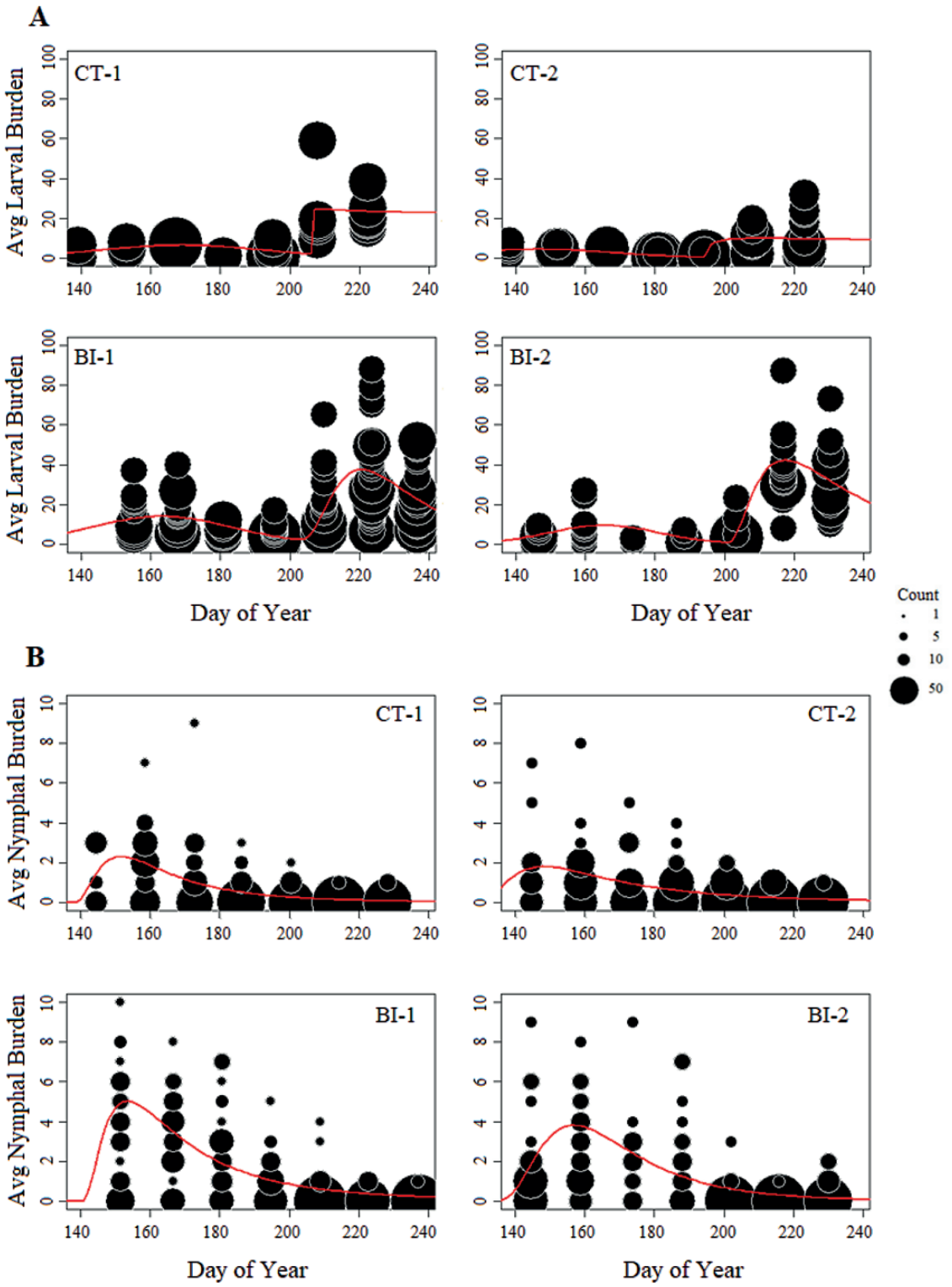

Fig. 4. Larval burden in 2015 (A) and nymphal burden in 2016 (B) of *Ixodes scapularis* found on *Peromyscus leucopus* mice in Connecticut (CT-1 and CT-2) and Block Island (BI-1 and BI-2) for each of seven trapping sessions (May–August). The size of each circle represents the frequency of mice trapped on that date with the corresponding tick burden (see legend). The curved line represents the fitted burden curve as described in Materials and Methods.

distribution of model outputs derived from each of these model runs, as represented by the empirical cumulative distribution function, showed that 64.8% of the generated values for parameter c were less than 1, and therefore mathematically possible, given that c is a probability that exists between 0 and 1 (Supp Fig. 2 [online only]). The remaining parameter combinations generated impossibly high values of c, which is a consequence of not constraining the upper limit h_N to preserve the independence of parameters from one another. No parameters showed significantly non-monotonic relationships with model output by scatterplot analysis. PRCC for the model

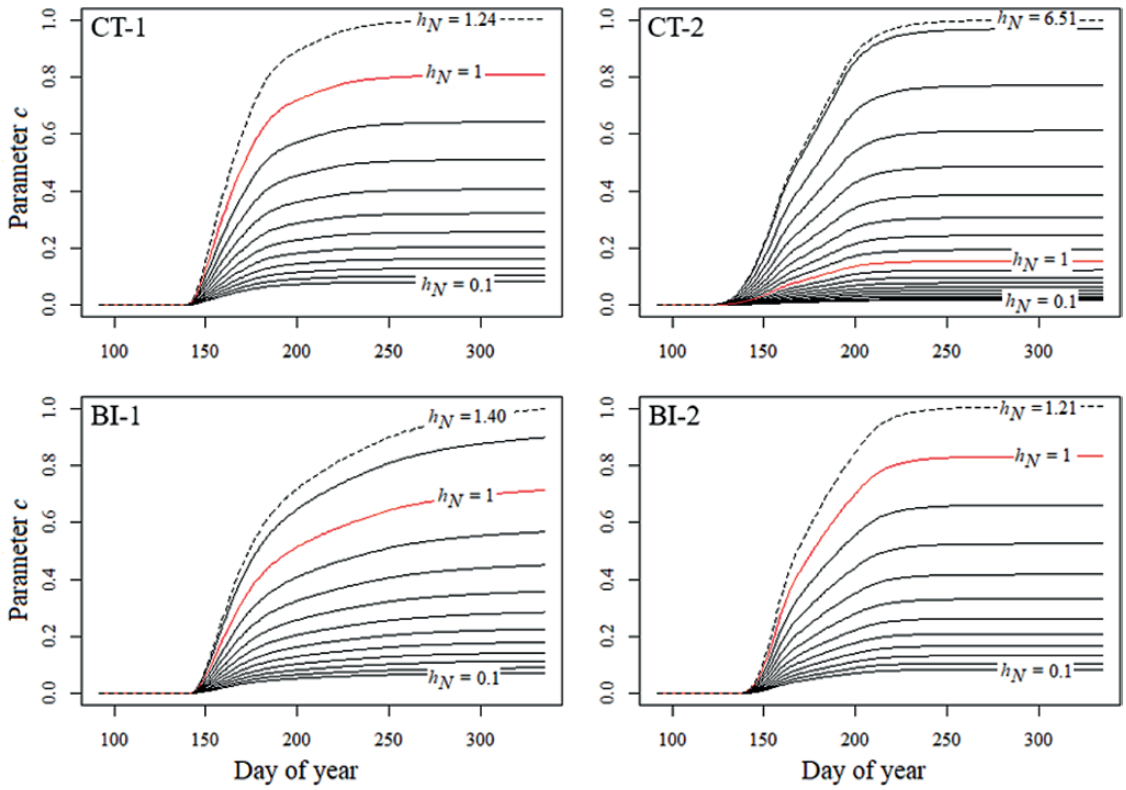

Fig. 5. Cumulative mean probability a surviving nymph will find a host by time t (parameter c), calculated for each study site in 2016. Solid lines represent estimates of $c(t)$ using increasing values of the host association ratio h_N on a logarithmic scale. Lowest line corresponds to $h_N = 0.1$ (indicating that larvae are 10 times more likely to infest mice than are nymphs), while the uppermost dashed line corresponds to the upper bound of h_N (for which $c = 1$).

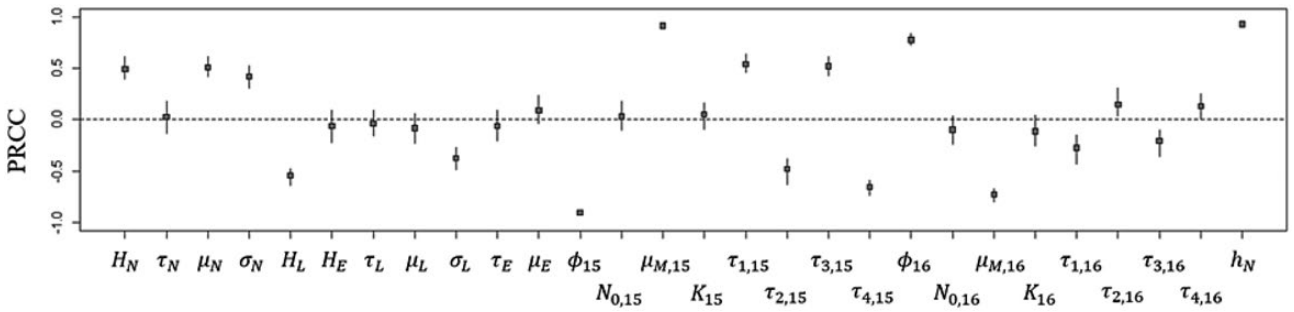

Fig. 6. Partial rank correlation coefficient (PRCC) for the model showing the linear associations between parameter and model output when other coefficients are held constant. Whiskers indicate 95% confidence intervals as calculated from 100 bootstrap replicates.

estimated the highest ranked parameters in the sensitivity analysis with positive effects were h_N, $\mu_{M,15}$, ϕ_{16}, $\tau_{1,15}$, μ_N, $\tau_{3,15}$, H_N, σ_N, and τ_N. Parameters with significant negative effects were ϕ_{15}, $\tau_{4,15}$, H_L, $\mu_{M,16}$, σ_L, and $\tau_{2,15}$ (Fig. 6).

Using the R_0 formulation and fixed point estimates of other relevant parameters derived from Dunn et al. (2013), the mean values of $s_N(t)$ and $c(t)$ at all sites yielded R_0 mean estimates for *B. burgdorferi* greater than 1, assuming $h_N = 1$, at all sites except CT-2 (CT-1: 1.25, CT-2: 0.546, BI-1: 1.09, BI-2 1.25).

Discussion

This study modeled nymphal host-finding success ($c(t)$) using parameters estimated from field data—an endeavor that involves a great deal of uncertainty in both those variables measured in the field and in the unmeasured variables pertaining to non-mouse hosts. In choosing to fit a set of parametric curves to the collected data, sacrifice of some precision for the ability to conduct an interpretable sensitivity analysis was necessary. Nevertheless, this approach does allow general conclusions to be drawn about the system and plausible ranges for host-finding success. In particular, accounting for a wide range of possible differences in host associations between nymphs and larvae caused host-finding success to be highly sensitive to the host association parameter.

Using additional parameter estimates from modeling studies informed with field data collected at the same sites (Dunn et al. 2013, 2014), and assuming no host association bias between nymphs and larvae, the parametric equations for nymphal survival, larval and nymphal burdens, and mouse abundances estimated in this study resulted in R_0 values greater than 1, implying persistence by the end of the field season at all sites with the exception of CT-2. Assuming equal host association ratios (h_N) across sites, parameter $c(t)$ (i.e., the

likelihood that a tick surviving to time t would successfully find a host) is again similar across all sites except CT-2. The outlier status at CT-2 is likely driven at least in part by the tails of the fitted mouse abundance curves, which appear to overestimate and underestimate late-season abundances in 2015 and 2016 relative to other sites, respectively, and therefore may be artifactual. The relatively high values of parameter c predicted at the other sites, though associated with plausible values for R_0, are somewhat surprising. This result could be explained by a value of h_N less than 1, although as discussed below, this explanation is unlikely. Alternately, the results may reflect one or more potential sources of bias, including higher rates of mouse capture in 2016, which would artificially inflate on-host nymph populations (note that the abundance of *P. leucopus* mice was significantly higher in 2016 than in 2015 at both Block Island and mainland Connecticut sites) compared with 2015; unrepresentatively low survival rates as a result of the use of survivorship tubes; significant changes in tick or mouse populations outside the sampling period not in accordance with the fitted curves; or a tick population that was not truly closed between 2015 and 2016 and was instead fed by either an immigrant population or a subset of newly molted nymphs that overwintered twice before questing.

Model output was most sensitive to the parameter accounting for the unmeasured host association ratio (h_N), which includes differences in host association between larvae and nymphs as well as potential differential detection bias during field collection. Large differences between the proportion of larvae feeding on non-mouse hosts and that of nymphs feeding on non-mouse hosts could therefore skew any measure of host-finding success (or R_0) based primarily on mouse capture (large differences in detection rates between on-host larvae and on-host nymphs would be mathematically equivalent). Other mammalian host species collected during this study showed minimal contributions to feeding immature stage ticks compared to *P. leucopus*; however, larger mammals and birds, not sampled in this study, play a role in feeding ticks (LoGiudice et al. 2003) even on Block Island, which is characterized by a depauperate mammalian community where nonrodent larger hosts would only be white-tailed deer (Huang et al. 2019). Prior studies in the northeastern United States suggest that nymphs are more likely to attach to non-mouse hosts than are larvae (Giardina et al. 2000). This would lead to h_N values greater than 1, increasing the expected value of parameter c.

Additional parameters with positive impacts on c were those associated either with higher nymphal populations in 2016, as with H_N (nymphal peak height), σ_N (related to nymphal peak width), and μ_N (with higher values resulting in a later nymphal peak, coinciding with the second mouse population peak); with higher mouse populations in 2016, as with ϕ_{16} (mouse birth rate); or with lower mouse populations in 2015, as with $\mu_{M,15}$ (mouse death rate) and $\tau_{1,15}$, and $\tau_{3,15}$ (with higher values resulting in a later start to the season's first and second mouse population peaks, respectively). Parameters with negative effects on c were those associated either with larger larval populations in 2015, as with σ_L (related to the width of the second larval peak) or H_L (heights of the larval peaks); higher mouse populations in 2015, as with $\tau_{2,15}$ and $\tau_{4,15}$ (with higher values resulting in a later end to the season's first and second mouse population peaks, respectively) or ϕ_{15} (mouse birth rate); or with lower mouse populations in 2016, as with $\mu_{M,16}$ (mouse death rate).

The direction in which these parameters influence parameter c is unsurprising, although it is important to note that the sensitivity of the model to these parameters still reaches significance despite limited variability between sites with respect to the measured variables.

No significant relationship between location or weather and tick survivorship was observed, in part because temperature and relative humidity differences between sites were small (data not shown). The limited role of climate in survivorship is supported by previous studies with larger climatic differences across sites (Stafford 1994, Bertrand and Wilson 1996, Brunner et al. 2014). Nymph lipid content was analyzed as a biological factor that may contribute to tick overwinter survival and was found to decrease significantly from all sites over time, but was not significantly different between regions (data not shown). Total tick recovery from all survivorship tubes regardless of site or region was 50% on average, and it is unclear whether dead ticks decomposed or if they were missed during processing. Only molted nymphs were recovered alive; engorged larvae were never recovered alive and no engorged larvae were recovered after February, suggesting that larvae that did not molt died. It is unclear why some larvae molted and others did not as all larvae were from the same cohort, fed at the same time, and were placed in the field on the same day.

Other drivers of tick survival, including differences in tick behavioral patterns in response to the local density of tick or host populations, were not directly investigated. Survival and host-seeking behavior, and therefore possibly host-finding success, are also likely not independent, as ticks may engage in riskier behaviors as the chance of mortality increases (McClure and Diuk-Wasser 2019).

Similarly, no significant differences in larval and nymphal burdens on mice were observed between Block Island and mainland Connecticut as regions; although in both years mean larval and nymphal burdens were significantly higher at both the Block Island sites compared with one of the Connecticut sites. The density of mice at the Block Island sites was significantly higher than at the Connecticut sites in 2015 and 2016.

The parameter $c(t)$ is critical in the population dynamics of ticks and pathogens, but difficult to measure. Parameters not directly measured in this study, most notably differences in host association between larvae and nymphs, may significantly affect estimates of $c(t)$. Acknowledging these potential sources of bias, the model described here helps identify other significant and measurable variables that can guide future empirical studies, allowing for more realistic predictions of Lyme disease risk in new regions or regions undergoing ecological change.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

Acknowledgments

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