Quantifying dilution and amplification in a community of hosts for tick-borne pathogens

Taal Levi[1,2,3], Felicia Keesing[4], Robert D. Holt[3], Michael Barfield[3], Richard S. Ostfeld[2]

Appendix S1

Supporting Methods

Field data methods

We used mark-recapture techniques to estimate mouse and chipmunk densities on three permanent trapping grids, each consisting of 242 Sherman traps arranged in pairs in an 11-by-11 grid covering approximately 2.25 ha. Each grid was trapped for two consecutive nights every 3-4 weeks between April or May and October or November, depending upon the year, from 1994 through 2012. Upon first capture, white-footed mice and eastern chipmunks were given numbered metal ear-tags for individual identification. While holding the animals by the scruff of the neck, we exhaustively counted the number of larval and nymphal *I. scapularis* on their heads (entire head including under the chin, cheeks, and top of head and ears).

To estimate the density of questing larvae and nymphs in each plot and year, we dragged 1 m^2 white corduroy drag cloths along 450 m of transect approximately every 3 weeks from April through November each year. We counted and removed all ticks in 30 m intervals. For each plot and year, we estimated larval and nymphal abundance as the peak density (ticks encountered per 100 m^2 during the drag session with highest abundance).

Parameterization with field data

Emergence

The timing of larval emergence, which is governed by b in eqn (2), was determined based on 19 years of data from a small-mammal trapping program at the Cary Institute of Ecosystem Studies in Dutchess County, southeastern New York (see above; Ostfeld *et al.* 2006). We ignored the modest early (spring) larval activity corresponding to overwintering larvae from the previous cohort. Based on our trapping data, we determined that the larval peak occurs approximately 20 days after the trough of larval activity in mid-summer, leading to a value of $b = 1/20$ in eqn (2), which characterized both the increase and tail of larval activity (Fig. 2B). The quantity H influences the total density of larvae, which in turn influences *DIN*, but its value does not influence the fractional change in *DIN* when a host species is removed. As such, its value is irrelevant to our inference about whether hosts are dilution or amplification hosts, which is measured as a fractional change in the density of infected nymphs. The estimation of the parameter H and the predicted values of *DIN* are presented in the Supporting Information (Fig. S1).

Host specific encounter and mortality rates

We determined host-specific encounter and mortality rates using field data, experiments, and the literature. B_i is the number of larvae successfully feeding on an individual of host species i during the larval peak, S_i is the proportion of attached larvae that survive to successfully feed, and D_i is the population density (ha^{-1}) of species i (Table 1). B_i has been determined for most hosts by bringing wild-caught animals into the lab during the larval peak and counting engorged larvae that fall off the animal (see

LoGiudice *et al.* 2003, Table 1). S_i has been determined experimentally by placing 100 larvae on each host and counting the number that successfully feed to repletion (see Keesing *et al.* 2009, Table 1). D_i is taken from the literature and field data (Table 1). The probability of larval survival while feeding has not been determined for all hosts. For hosts lacking this data, we used both higher and lower values of larval survival. We assumed that short-tailed shrews (*Blarina brevicauda*) and masked shrews (*Sorex* spp.) might groom similarly to mice (high survival) or chipmunks (moderate survival), that raccoons and skunks would groom similarly to chipmunks (moderate) or opossums (very low survival), and that larvae would have high survival on deer similar to that on mice. Larval survival on deer may be higher if deer are especially poor groomers, but increasing the survival probability to $S_i = 0.8$ did not change our results (Table 1). We used two values of questing larvae mortality ($\mu_Q = 0.01, 0.1$; see below).

We convert the survival probability, S_i, into the mortality rate μ_i, using

$$e^{-\mu_i \tau} = S_i \rightarrow \mu_i = \frac{-ln(S_i)}{\tau}. \qquad \text{(eqn S1)}$$

In our model, the body burden of each host species (the number of attached ticks per host individual) is a dynamic outcome of the model. We solved for the encounter rate a_i by considering the steady state peak body burden A_i^* / D_i (total density of larvae attached to host species *i* divided by the population density of species *i*) when the density of questing larvae is assumed to be constant, $Q(t)=Q^*$. Setting eqn (3) to zero and solving for a_i yields

$$a_i = \frac{A_i^*}{D_i}\left(\frac{\mu_i}{Q^*(1-e^{-\mu_i \tau})}\right). \qquad \text{(eqn S2)}$$

The peak body burden A_i^* / D_i is equivalent to the post-grooming body burden divided by probability of surviving, B_i / S_i, assuming that Q^* is near the peak questing larval density (since B_i was measured on animals captured near this peak). Substituting our observed parameters into eqn (S2) yields the formula for a_i,

$$a_i = \frac{-B_i ln(S_i)}{Q^* S_i (1-S_i)\tau}. \quad \text{(eqn S3)}$$

Previous research using removal sampling found mean density of questing larvae during the peak, Q^*, of 115,000 ha^{-1} (Daniels, Falco & Fish 2000). Q^* is an important parameter because it influences the calculated value of the encounter rate a_i, which is a key parameter determining whether ticks that would have fed on host A instead redistribute to feed on host B when host A is removed. We therefore ran our model for values of Q^* from 10,000 to 200,000 (equivalent to a range of densities of 1-20 larvae per m^2) in intervals of 5000.

Mortality rate of questing larvae

The relationship between host diversity and Lyme disease risk is influenced by the mortality rate of questing larvae. If mortality while questing is high, then the probability of encountering any host before death becomes low and removing even poor hosts leads to more tick deaths before feeding (when assuming additivity). If mortality while questing is low, then larvae are increasingly likely to encounter a competent host should a poor host be removed from the community. In a field experiment, Lindsay *et al.* (1998) found no noticeable decline in the abundance of unfed larvae held in the field from mid-July through mid-November, suggesting low mortality. The fraction of unfed larvae surviving winter was dependent on the year and habitat type, but overwintering larvae were frequently active beginning in April of the subsequent year; however, nearly

all unfed overwintering larvae died by July (Lindsay *et al.* 1998). We assumed that on average larvae survive a season ~100 days long, from mid-July to late October, leading to a mortality rate of $\mu_Q = 0.01$ day^{-1}. We also ran the model at the extreme value of $\mu_Q = 0.1$ day^{-1}, corresponding to an average life span of 10 days, to illustrate how the results change if larvae are unlikely to encounter a host before death.

Parameterizing *H*

H in eqn (2) is unknown, so we ran the model using values of *H* from 300 to 4000, which correspond to an emergence rate of 2207 to 29430 larvae per day during the peak (i.e. when $t=1/b$ in eqn 2). We determined the value of *H* that best fit the model output of both the peak body burden (i.e. the maximum of $A_i(t) / D_i$) and the density of questing larvae during the peak, which Daniels *et al.* (2000) estimated to be 115000 ha^{-1} with removal sampling. The value of *H* does not influence our inference about whether hosts are dilution or amplification hosts, which is measured as a percent change in the density of infected nymphs, but *H* does impact the absolute densities of questing larvae, attached larvae, and infected nymphs in our numerical output.We obtained a value of $H = 2200$ by fitting this unknown parameter to maximum body burdens output from the model (Fig. S1 shows the body burdens are a function of *H* and Q^*; the dashed lines are the estimated values of *H* and Q^* and their crossing is at the peak body burden for each host).

Analytical solution to differential equations

These differential equations can easily be solved numerically, but with a little work they can also lead to analytical solutions that we derive below.

$$\frac{dQ}{dt} = Hte^{-bt} - \sum_{i=1}^{N} a_i D_i Q - \mu_Q Q. \quad \text{(eqn S4)}$$

$$\frac{dA_i}{dt} = a_i D_i Q - \mu_i A_i - a_i D_i e^{-\mu_i \tau} Q(t-\tau). \quad \text{(eqn S5)}$$

$$\frac{dF_i}{dt} = a_i D_i e^{-\mu_i \tau} Q(t-\tau). \quad \text{(eqn S6)}$$

First, solve for $Q(t)$ using the method of undetermined coefficients.

Rearrange eqn (S4) into the inhomogeneous differential equation

$$\frac{dQ}{dt} + m_Q Q - Hte^{-bt} = 0 \quad \text{(eqn S7)}$$

where

$$m_Q = \sum_{i=1}^{N} a_i D_i + \mu_Q \quad \text{(eqn S8)}$$

Recall that the general solution is the sum of the complementary solution and the particular solution. The complementary solution to this differential equation is the solution to $dQ_c / dt + m_Q Q_c = 0$, which is simply the exponential

$$Q_c(t) = c_1 e^{-m_Q t}. \quad \text{(eqn S9)}$$

The form of the particular solution for Hte^{-bt} is

$$Q_p(t) = (\alpha + \gamma t)e^{-bt}. \quad \text{(eqn S10)}$$

Plugging $Q = Q_c + Q_p$ into eqn (S7) and solving for the coefficients leads to the general solution

$$Q(t) = Q_c(t) + Q_p(t) = c_1 e^{-m_Q t} + \frac{H}{m_Q - b}\left(t - \frac{1}{m_Q - b}\right)e^{-bt}. \quad \text{(eqn S11)}$$

We solve for c_1 using the initial value $Q(0)=0$, leads to

$$Q(t) = \frac{H}{(m_Q - b)^2}(e^{-m_Q t} - e^{-bt}) + \frac{Hte^{-bt}}{m_Q - b} \quad \text{(eqn S12)}$$

for $t > 0$. $Q(t)$ can now be plugged into eqns (S5) and (S6) (noting that $Q(t)$ is 0 for $t < 0$) to solve for $A_i(t)$ and $F_i(t)$. Fed larvae begin to be produced after τ time units (i.e. after a full blood meal), leading to the integral solution for $F_i(t)$

$$F_i(t) = \int_\tau^t \frac{dF_i}{dt} dt = a_i D_i e^{-\mu_i \tau} \int_\tau^t Q(t-\tau) dt. \quad \text{(eqn S13)}$$

This integral is tedious, but not difficult, to solve, leading to

$$F_i(t) = K\{m_Q[b(b-m_Q)(t-\tau) + 2b - m_Q]e^{-b(t-\tau)} - b^2 e^{-m_Q(t-\tau)} + (m_Q - b)^2\} \quad \text{(eqn S14)}$$

where $K = a_i D_i H e^{-\mu_i \tau} / [b^2 (m_Q - b)^2 m_Q]$ and $t > \tau$ (before that F_i is 0). Because questing larvae fall off to zero, this can be simplified by solving at $t = \infty$, ($t = \infty$ and $t > 150$ yield nearly identical results; Fig. 2), which leaves only the last term in eqn (S11),

$$F_i(\infty) = K(b - m_Q)^2 = \frac{a_i D_i H e^{-\mu_i \tau}}{b^2 m_Q}. \quad \text{(eqn S15)}$$

By substituting eqn (S8) for m_Q, eqn (S15) can be written as

$$F_i(\infty) = \frac{H}{b^2} \frac{a_i D_i e^{-\mu_i \tau}}{\sum_{i=1}^N a_i D_i + \mu_Q} \quad . \quad \text{(eqn S16)}$$

This is a very intuitive result. H/b^2 is the total abundance of larvae, which can be obtained by integrating the emergence function, $\frac{a_i D_i}{\sum_{i=1}^N a_i D_i + \mu_Q}$ is the probability that host i is encountered, and $e^{-\mu_i \tau}$ is the probability of surviving on host i to reach the fed class.

$A_i(t)$ can be solved using a similar approach. First, note that the delay differential equation for $A_i(t)$ is linear. Therefore, its solution is the sum of the solution to $dA_i/dt + \mu_i A_i = a_i D_i Q$ and $dA_i/dt + \mu_i A_i = a_i D_i e^{-\mu_i \tau} Q(t-\tau)$. However, the right sides of these equations are the same except for the constant term and a time delay. Therefore, the solution to the second is just a scaled, delayed version of the solution to the first. The first equation can be solved in the same way as $Q(t)$ was found above, except that the form of

the particular solution will also include an exp{-$m_Q t$} term. The resulting equation for larvae attached to host *i* is

$$\begin{aligned} A_i(t) &= C_1 C_2(t) && \text{for } t < \tau \\ &= C_1[C_2(t) - e^{-\mu_i \tau} C_2(t-\tau)] && \text{for } t > \tau \end{aligned} \qquad \text{(eqn S17)}$$

where

$$C_1 = \frac{Ha_i D_i}{(\mu_i - b)^2 (m_Q - b)^2 (\mu_i - m_Q)}$$

and

$$C_2(t) = (\mu_i - b)^2 e^{-m_Q t} - (m_Q - b)^2 e^{-\mu_i t} + (\mu_i - m_Q)\{[b^2 - b(m_Q + \mu_i) + \mu_i m_Q]t + 2b - m_Q - \mu_i\} e^{-bt}.$$

Table S1. Data, variables and parameters used in the model

Data	**Interpretation**
D_i	Population density of host species *i*
R_i	Realized reservoir competence of species *i*: Proportion of nymphs that were infected after feeding as larvae on host *i*
S_i	Probability a tick survives on host species *i*: Proportion of 100 larvae that were experimentally placed on hosts that successfully engorged and detached
B_i	Post-grooming body burden: Cumulative number of larvae dropped off of species *i* when brought into laboratory.
Parameter	**Interpretation**
a_i	Per-capita encounter rate with host species *i*
μ_i	Per-capita death rate when attached to host species *i*
τ	Days of attachment
Q^*	Mean density of questing larvae during the peak
H	Initial slope of the function defining emergence of larvae through time, $E(t) = Hte^{-bt}$, the total size of the larval cohort is H/b^2
b	1/*b* determines the timing of the peak of larval through time $E(t) = Hte^{-bt}$
Variable	**Interpretation**
$Q(t)$	Density of questing larvae at time *t*
$A_i(t)$	Density of larvae attached to host species *i* at time *t*
$F_i(t)$	Density of larvae that have successfully completed feeding on host species *i* at time *t*

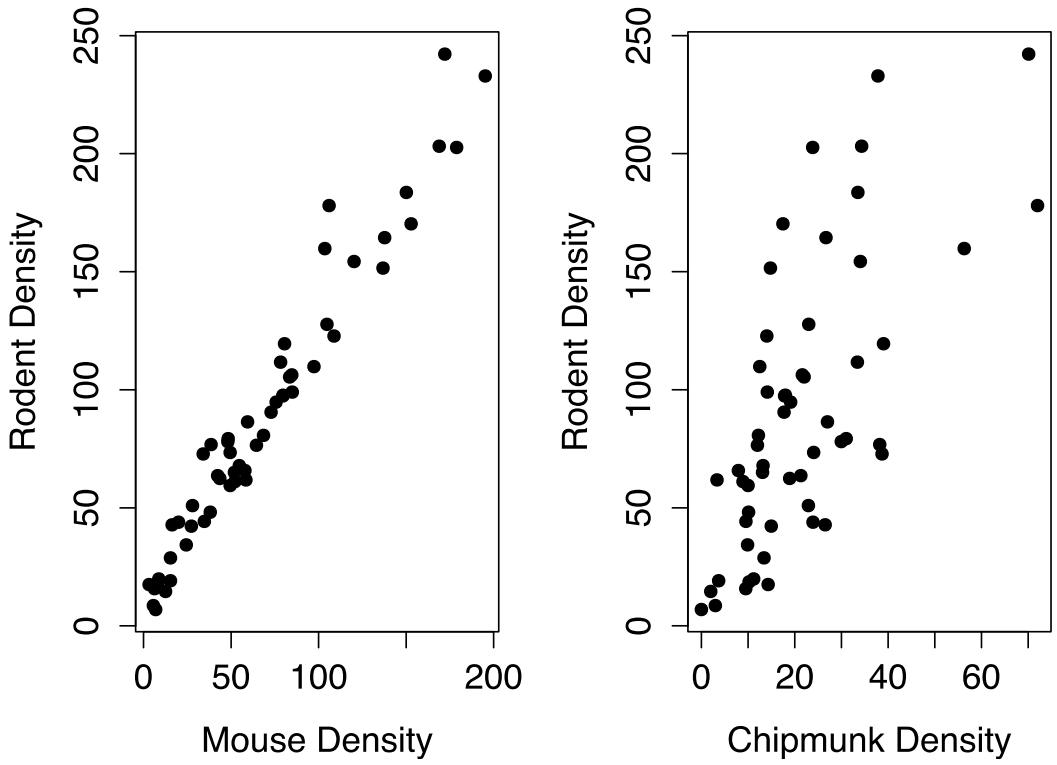
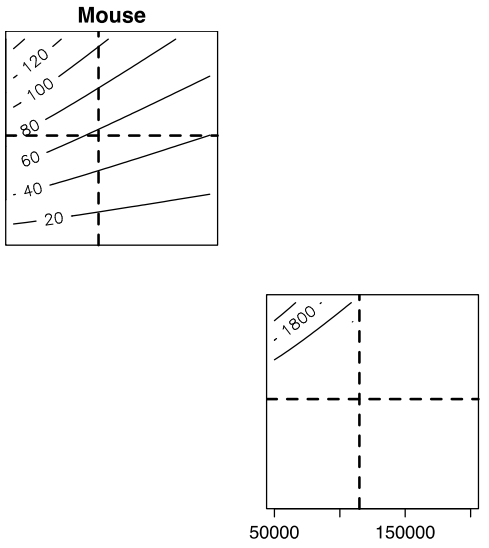


Figure S1. Scatterplots relating mouse density and chipmunk density to total rodent density. Because mice are much more abundant than chipmunks, and are highly correlated with chipmunks (Pearson's test $p < 0.0001$, $r = 0.52$), mouse density is nearly perfectly correlated with total rodent (mouse + chipmunk) density ($r = 0.98$, $p < 10^{-16}$).

A



B

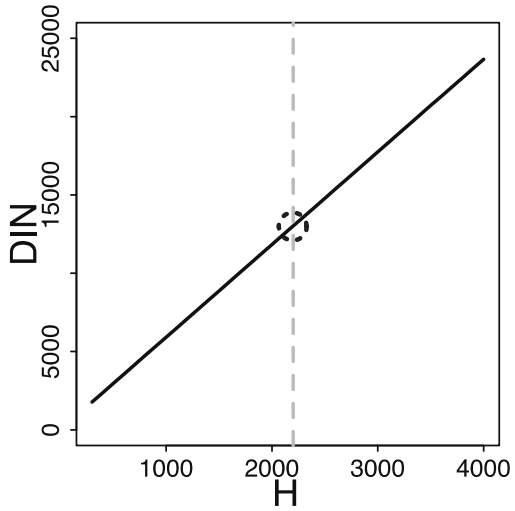


Figure S2. (A) Parameterizing the emergence function using model output. The parameter *H* in eqn (2) is unknown. While *H* does not influence the percent change in *DIN*, it does influence the maximum body burden of hosts and the total resulting density of infected nymphs. We fit *H* so that the maximum number of attached larvae per host derived from model output ($\max(A_i) / D_i$) equaled the maximum number of attached larvae per host derived from data (B_i / S_i) at our estimate of the density of questing larvae at the peak, Q^* (115,000). The solid lines are the peak body burdens; the dashed lines are the estimated Q^* and *H*, which intersect at the peak body

burdens determined from the data. (B) Prediction of the total density of infected nymphs by the complete vertebrate community as a function of *H*.