

## THE ROLE OF LIZARDS IN THE ECOLOGY OF LYME DISEASE IN TWO ENDEMIC ZONES OF THE NORTHEASTERN UNITED STATES

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**ABSTRACT:** We examined the role of lizards in the ecology of Lyme disease in New York and Maryland. We collected data on vector tick infestations, measured lizard “realized” reservoir competence for the Lyme disease spirochete *Borrelia burgdorferi*, and estimated lizard population density. These data were incorporated into a model that predicts a host’s ability to influence the prevalence of *B. burgdorferi* in the tick population, a primary risk factor in the epidemiology of Lyme disease. Published data on other northeastern hosts were included in the model to provide a reference for interpreting the importance of lizards as hosts. The model results indicate that 5-lined skinks (*Eumeces fasciatus*) are dilution hosts, capable of reducing infection prevalence in the tick population by 10.7–51.5 percentage points, whereas eastern fence lizards (*Sceloporus undulatus*) are not dilution hosts in the areas studied. Owing to moderate burdens of larval ticks, relatively high population densities, and reservoir incompetence, *E. fasciatus* may play an important role in the ecology of Lyme disease by reducing vector infection prevalence and associated human risk of infection.

Lyme disease was first described in southern Connecticut, United States, in the 1970s, and it is now a major public health problem in several parts of the country (Centers for Disease Control and Prevention, 2004). Most U.S. cases originate from 2 geographic hyperendemic regions (the northeastern and upper midwestern United States), but scattered cases also occur in the southern and western United States (Centers for Disease Control and Prevention, 2004). Globally, Lyme disease also has been reported from eastern Canada, Europe, Asia, and northern Africa, exhibiting a Holarctic distribution (Sonenshine, 1994). The causative agent, *Borrelia burgdorferi*, is maintained in an enzootic cycle, and it is exclusively transmitted by species of *Ixodes* (Ixodidae) with vertebrate hosts serving as the exclusive source, or reservoir, of infection for ticks. Tick population density and *B. burgdorferi* infection prevalence, particularly within the nymphal stage, are critical risk factors influencing rates of human exposure to Lyme disease (Barbour and Fish, 1993; Ostfeld and Keesing, 2000).

The primary vector in eastern North America is the black-legged tick (*Ixodes scapularis*), which is known to feed on many species of mammals, birds, and lizards (Keirans et al., 1996). These hosts vary strongly in their contribution to the abundance and infection prevalence of the tick population. In the northeastern United States, the white-footed mouse (*Peromyscus leucopus*) and eastern chipmunk (*Tamias striatus*) are the most important reservoir hosts due to the large numbers of immature *I. scapularis* that they feed and the large proportion of those that they infect with *B. burgdorferi* (Lane et al., 1991; Piesman and Gray, 1994; Ostfeld et al., 2006). Both nymphal infection prevalence and the density of infected nymphs in any given year are best predicted by either abundance of these rodent hosts or by their food resources (acorns) (Ostfeld et al., 2006). However, understanding the impacts of alternative hosts seems equally important. Using field studies combined with an empirically based simulation model, LoGiudice et al. (2003) and Brisson and Dykhuizen (2004) determined that gray squirrels (*Sciurus carolinensis*) were an important “dilution host” in the northeastern United States because they are abundant,

feed many larval ticks, and are incompetent reservoirs for *B. burgdorferi*. LoGiudice et al. (2003) and Ostfeld and LoGiudice (2003) argued that the composition of the host community, particularly the relative abundance of reservoir and dilution hosts, should influence local Lyme disease risk. In particular, high relative abundance of dilution hosts in the host community is expected to reduce the proportion of nymphal ticks that are infected with *B. burgdorferi*, and hence risk of human exposure to this pathogen.

In parts of Asia, Europe, Africa, and the southern and western United States, lizards are frequently parasitized by species of *Ixodes* (Bauwens et al., 1983; Hayashi and Hasegawa, 1984; Manweiler et al., 1990, 1992; Matuschka et al., 1991; Apperson et al., 1993; Oliver et al., 1993; Levine et al., 1997; Wright et al., 1998; Kollars et al., 1999; Talleklint-Eisen and Eisen, 1999; Schall et al., 1999; Eisen et al., 2001; Casher et al., 2002; Durden et al., 2002), and with a few exceptions (Levin et al., 1996; Clark et al., 2005; Dsouli et al., 2006; Richter and Matuschka, 2006) seem to be incompetent reservoirs for *Borrelia* (Lane, 1990; Manweiler et al., 1992; Matuschka et al., 1992; Lane and Quistad, 1998; Wright et al., 1998; Kuo et al., 2000; Lane et al., 2006). To assess the potential role of lizards in the ecology of Lyme disease in areas within the northeastern United States, we sampled lizard and tick populations in New York and Maryland. The lizard species in the study region are small (<50 g), diurnal, terrestrial to semi-arboreal, and are among the most geographically and ecologically widespread lizard species in the eastern United States (Conant and Collins, 1991). For all lizard species present, we estimated 3 primary variables that influence species-specific contributions to infection prevalence in host-seeking nymphal ticks: (1) infestation levels by larval ticks, (2) host population density, and (3) reservoir competence. Our goal was to integrate the potential role of lizards with those of better studied mammalian and avian hosts in affecting nymphal infection prevalence in the northeastern United States.

### MATERIALS AND METHODS

#### Study area

Our study area was located in parts of the northeastern United States experiencing high prevalence of Lyme disease. Three sites were located in the Atlantic coastal plain of Maryland, i.e., Jug Bay Wetlands Sanctuary (Jug Bay), the Smithsonian Environmental Research Center (SERC), and Cove Point, and 2 sites in the Hudson Highlands region of New York, i.e., Hudson Highlands State Park (Bull Hill) and the

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West Point Military Reservation (Cragston Lakes). Sites were mostly forested and dominated by assemblages of hardwood (*Quercus prinus*, *Q. rubra*, *Q. velutina*, *Q. coccinea*, *Q. alba*, *Fagus grandifolia*, *Carya ovata*, *Acer rubrum*, *A. saccharum*, *Liquidambar styraciflua*, *Nyssa sylvatica*, and *Liriodendron tulipifera*) and softwood tree species (*Pinus rigida*, *P. strobus*, *P. virginianus*, *P. echinata*, and *Tsuga canadensis*).

#### Data collection

At each of the 5 sites, 2 sampling plots were established. Except for 1 plot located at Bull Hill (60 × 100 m), plots measured 100 × 100 m (1 ha), and they were placed at least 100 m apart. Tick burden, host population density, and questing tick abundance were sampled in the field, whereas nymphal infection prevalence and host reservoir competence were measured in the laboratory using wild-caught ticks and wild-caught hosts.

Lizards were sampled monthly at each site from March to October 2003, by actively searching vegetation and cover objects within and immediately adjacent to the study plots. Lizards were captured by hand or noose. Once captured, snout-to-vent length (SVL), total length (TL), and mass of each individual were measured to the nearest 0.1 mm, 0.1 mm, and 0.1 g, respectively. Adult five-lined skink (*Eumeces fasciatus*) and broadhead skink (*E. laticeps*) were sexed using external morphology (Vitt and Cooper, 1986). Adult six-lined racerunner (*Aspidoscelis sexlineatus*) (formerly *Cnemidophorus sexlineatus*; Reeder et al., 2002) also were sexed using external morphology (Stebbins, 1954). *Sceloporus undulatus* were sexed by the presence of enlarged postanal scales, which are apparent in all ages. External sexual dimorphism in *A. sexlineatus* and *E. fasciatus* is not apparent until the advent of sexual maturity; thus, sex of immature individuals of these species was not assigned. Lizards were surveyed for tick infestations with a ×10 lens. Genus, number (burden), stage, and attachment site of feeding ticks were recorded for each infestation. Specific identifications were made on all ticks that dropped off hosts in the lab during the period before xenodiagnostic studies (see below). Before release, lizards were individually marked by toe-clipping for permanent identification.

Host-seeking ticks were sampled monthly in each plot by dragging a 1.0-m<sup>2</sup> piece of corduroy cloth over the substrate (Falco and Fish, 1992). Each sample consisted of 5 30-m transects, totaling 150 m. At the end of each transect, the cloth was lifted from the substrate and visually inspected for ticks. Attached ticks were identified, counted, and removed from the cloth. Nymphs collected during drag sampling were collected to estimate infection prevalence, and larvae were collected for use in lizard xenodiagnosis. Prevalence of *B. burgdorferi* infection in the nymphal populations was estimated using direct immunofluorescence assay (DFA; Ostfeld et al., 2006). Ticks were washed once in 70% ethanol and twice in deionized water and ground in phosphate-buffered saline (PBS). Three 5-ml aliquots of tick suspension were placed in separate wells in a multiwell slide, air-dried, and fixed in cold acetone for 10 min. Fluorescein rabbit anti-*B. burgdorferi* conjugate was incubated in wells at 37 C for 45 min, after which slides were washed in PBS, dried, and mounted with a coverslip. Slides were examined systematically (3 wells/tick) to categorize each tick as either infected or uninfected. DFA potentially can produce positive assays in ticks infected with *Borrelia* species other than *B. burgdorferi*, including *B. miyamotoi*, leading to the potential for false positives. In addition, the potential exists for DFA to fail to detect very small populations of spirochetes in ticks, and therefore to lack sensitivity. However, our estimates of *B. burgdorferi* infection prevalence in ticks using DFA have been corroborated within 1.6 percentage points by independent polymerase chain reaction (PCR) assessments of infection prevalence in the same cohorts of ticks (Qiu et al., 2002; Brisson and Dykhuizen, 2004). The PCR assays are specific to outer surface protein C are highly sensitive, and they are diagnostic of *B. burgdorferi* sensu lato. These comparisons indicate that both false positives and false negatives from our tick samples using DFA should be rare.

We sought to determine "realized reservoir competence" of lizards by testing field-caught lizards for their ability to infect feeding ticks with *B. burgdorferi*. Realized reservoir competence (Schauber and Ostfeld, 2002) is defined as the probability that uninfected ticks feeding from a specific host will acquire infection from that blood meal. This measure differs from true reservoir competence in that the latter is an attribute of hosts known to be infected at the time of tick feeding. Therefore, estimates of realized reservoir competence are typically con-

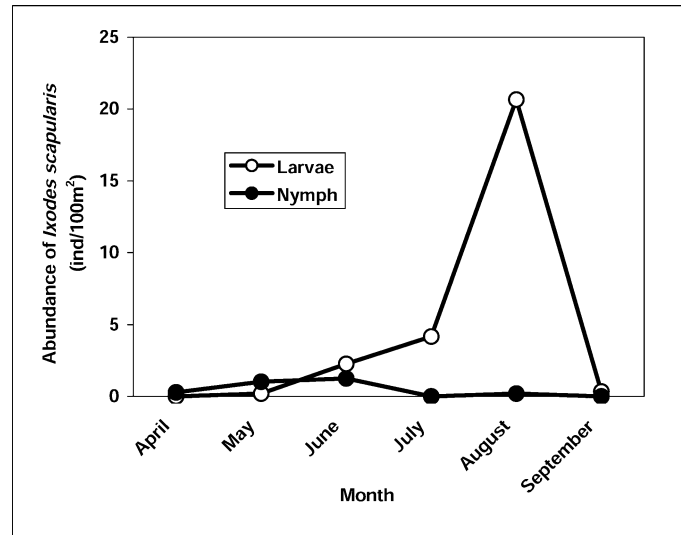


FIGURE 1. Monthly mean abundance of host-seeking immature *Ixodes scapularis* by drag sampling at five sites in New York and Maryland in 2003.

servative, given that lack of transmission from the host to the feeding tick can result from the host not being infected rather than from the host not transmitting an existing infection. Although realized reservoir competence provides less information on host physiology and host-pathogen interactions, it provides more information on the roles played by hosts in infecting vectors under field conditions. Realized reservoir competence was determined via xenodiagnosis using wild-caught *I. scapularis* larvae and lizards (Sonenshine, 1994). After capture, lizards were brought to the lab and housed individually in 0.64-cm (0.25-in.) wire mesh cages (30 cm in length × 13 cm in width × 13 cm in height), suspended over water-filled bins. Food and water were provided ad libitum. Each lizard was provided with a 60-W incandescent bulb at one end of the cage for radiant heat and moistened paper towels at the other to provide a humid shelter. The ambient temperature within the laboratory was approximately 20 C. Larvae matching the origin of each lizard were allowed to attach, feed, and drop off of the host into the water below. Replete larvae were recovered and placed in glass vials partially filled with water-saturated plaster and incubated at 27 C until they molted to nymphs. Resulting nymphs were tested for *B. burgdorferi* using DFA. Each lizard was infested with 2 consecutive groups of 15 wild-caught larval *I. scapularis*, beginning no longer than 4 days after capture. Lizards were exposed to the second group once feeding by the first group concluded. After tick feeding, all lizards were returned to their respective capture locations no longer than 21 days from the date of capture.

Because we did not inoculate lizards with *B. burgdorferi*, our xenodiagnosis method relies on presumed natural inoculation to determine the host's ability to maintain and transmit *B. burgdorferi* to feeding ticks. To determine the probability that an individual lizard was exposed to at least 1 infected nymph ( $P_i$ ) in the month preceding our xenodiagnosis trial, we relied on the binomial probability distribution (Ginsberg, 1993):

$$P_i = 1 - (1 - k)^n$$

where  $n$  is the mean number of nymphs hosted in the preceding nymphal season (April–June), and  $k_i$  is the proportion of nymphs infected in the population (NIP).

Based on the duration of larval attachment observed during the xenodiagnostic portion of this study (5–8 days), mean nymphal burdens were multiplied by the approximate number of weeks in the nymphal season ( $n = 12$ ; Fig. 1) to estimate  $n$ . The binomial probability distribution also allowed us to estimate the probability that at least 1 individual in a sample was exposed to an infected nymph ( $P_i$ ), where  $n_s$  is the number of individuals in the sample, and  $k_s$  is the proportion of exposed individuals in the sample ( $P_i$ ; Ginsberg, 1993).

TABLE I. Mammal and bird data used in the dilution potential model, taken from LoGiudice et al. (2003).

Host species		Model parameter		
		Mean larval burden, $B_i$	Population density, $N_i$	Reservoir competence, $C_i$
Eastern chipmunk	<i>Tamias striatus</i>	36	10	55
Raccoon	<i>Procyon lotor</i>	127	0.2	1.3
Short-tailed shrew	<i>Blarina brevicauda</i>	62.9	25	41.8
Songbirds	*	1.7	31.6	11.7
Shrews	<i>Sorex</i> spp.	55.5	25	51.2
Squirrels	†	142	8.1	14.7
Striped skunk	<i>Mephitis mephitis</i>	66.8	0.05	9.7
Virginia opossum	<i>Didelphis virginiana</i>	254	1	2.6
White-footed mouse	<i>Peromyscus leucopus</i>	27.8	20	92.1
White-tailed deer	<i>Odocoileus virginianus</i>	239	0.25	4.6

\* American robin (*Turdus migratorius*), wood thrush (*Hylocichla mustelina*), veery (*Catharus fuscescens*), and ovenbird (*Seiurus aurocapillus*).

† Gray squirrel (*Sciurus carolinensis*) and red squirrel (*Tamiasciurus hudsonicus*).

### Data analysis

Infestations are described as infestation prevalence and mean burden. Infestation prevalence is the proportion of the sample infested with 1, or more, ticks. Mean burden is the total number of ticks observed infesting lizards divided by the total number of lizards in the sample. Data used to describe infestations were restricted to numbers collected during stage-specific peak abundances of immature *I. scapularis* (nymph: April–June; larvae: July–August; Fig. 1). Infestation statistics and population density estimates are restricted to individuals born before lizard sampling, which includes adult and subadult lizards. Individuals captured within and immediately adjacent to the study plots were used to describe infestations. A Mann–Whitney *U*-test was used to assess differences in infestation. Relationships between tick burdens and questing tick abundance were grouped by month and analyzed with Spearman rank correlation. Tick abundance data used in correlations were restricted to sites in which that species was sampled. The slope of the relationship between tick burden and tick abundance was then determined using linear regression. Our estimates of host population density within plots were generated using both Jolly–Seber and Schnabel methods for estimation of abundance from mark-and-recapture studies (Krebs, 1989). Because 1 of the lizard species (*E. fasciatus*) was not observed to move more than a few meters between observations, populations of this species were assumed to be largely closed to significant immigration and emigration from the study plots. Thus, Schnabel's method was used to estimate density. Two other species, *S. undulatus* and *A. sexlineatus*, were often observed to move distances greater than the width of study plots (100 m). Therefore, populations of these 2 species were considered open, and the Jolly–Seber method used. When multiple estimates of host population density were attained, the mean (individuals/hectare) was used in the model.

### Model approach

To compare the potential for lizards to influence Lyme disease risk with those of other hosts for blacklegged ticks, we integrated lizard hosts into the model of LoGiudice et al. (2003). This model makes

quantitative predictions about the ability of a host species to change NIP when that host is added to a simulated host community. The initial host community consists solely of white-footed mice (*P. leucopus*), with succeeding communities consisting of *P. leucopus*, plus the focal host(s). The percentage difference in NIP between the initial and succeeding host communities is the dilution potential of the focal host. The model in LoGiudice et al. (2003) uses 4 parameters to determine dilution potential:  $N_i$ , population density (individuals/hectare);  $B_i$ , tick burden (mean number of larval ticks/host);  $S_i$ , molting percentage (percentage of larvae that transstadially molt); and  $C_i$ , reservoir competence (mean proportion of larval ticks infection by the host).  $S_i$ , the factor with the weakest influence, was omitted from the model in this study. We used the remaining 3 parameters to determine the contribution of the focal host,  $i$ , to NIP. Because we did not collect data on the 3 parameters for nonlizard hosts at the sites where we studied lizard populations, we relied on the data collected in similar habitat types in southeastern New York for mammals and songbirds ( $N_i$ ,  $B_i$ ,  $C_i$  from LoGiudice et al. [2003]; Table I). Although we have not established that the same community of mammals and songbirds exists in the sites where we sampled lizards, all of the host species included are widespread in forested habitats of New York and Maryland.

## RESULTS

### Infestations

Among the 5 study sites, a total of 306 lizards (432 observations), representing 5 species (*A. sexlineatus*, *E. fasciatus*, *E. laticeps*, *Sceloporus undulatus*, and *S. lateralis*) were sampled. *Aspidoscelis sexlineatus*, *S. lateralis*, and *E. laticeps* were restricted to Maryland sites. *Eumeces fasciatus* and *S. undulatus* were found in New York and Maryland sites (Table II).

Infestations were observed throughout the study period. Immature *I. scapularis* were the only ticks observed infesting liz-

TABLE II. Distribution of adult and subadult lizard captures/recaptures among Maryland and New York study sites from March to October 2003.

Species	Bull Hill	Cove Point	Cragston Lakes	Jug Bay	SERC*
<i>Aspidoscelis sexlineatus</i>		10/2		35/14	
<i>Eumeces laticeps</i>		7/2			
<i>Eumeces fasciatus</i>	14/2†	17/1	59/18	37/3	12/2
<i>Sceloporus undulatus</i>	55/19	41/19		17/10	
<i>Scincella lateralis</i>		3/0			

\* Smithsonian Environmental Research Center.

† Number of captured individuals/total number of recaptures.

TABLE III. Infestation prevalence, mean burden (SE), and infestation range of adult and subadult lizards infested by immature *Ixodes* ticks from study sites in New York and Maryland during stage-specific peaks in tick abundance (larvae: July and August; nymphs: April–June).

Species	Larvae				Nymphs			
	n	Prevalence	Mean	Range	n	Prevalence	Mean	Range
<i>Aspidoscelis sexlineatus</i>	12	0 (0/12)	0	0	26	0 (0/26)	0	0
<i>Eumeces fasciatus</i>	30	46.7 (14/30)	2.0 (0.969)	0–29	88	29.5 (26/88)	0.364 (0.065)	0–2
<i>Eumeces laticeps</i>	1	0 (0/1)	0	0	1	100 (1/1)	4	4
<i>Sceloporus undulatus</i>	81	6.5 (4/46)	0.086 (0.052)	0–4	28	7.1 (4/28)	0.17 (0.12)	0–3
<i>Scincella lateralis</i>	0	0 (0/0)			3	0 (0/3)	0	0

ards, despite detection of sympatric lone star ticks (*Amblyomma americanum*), dog ticks (*Dermacentor variabilis*), and adult *I. scapularis* in field sampling. Immature *I. scapularis* infestations were observed on *E. laticeps*, *E. fasciatus*, and *S. undulatus*, but not on *A. sexlineatus* or *S. lateralis* (Table III). Coincident attachment (cofeeding) of larvae and nymphs was infrequent, observed only once on *E. laticeps*, and *S. undulatus*, making up only 3.2% of the observed infestations ( $n = 62$ ). Mean burdens were significantly larger for *E. fasciatus* than *S. undulatus* (nymphs:  $U = 4465.0$ ,  $P < 0.001$ ; larvae:  $U = 576.5$ ,  $P < 0.01$ ; Table III). Infestation prevalence of *E. fasciatus* was also significantly larger than the infestation prevalence of *S. undulatus* (nymph:  $\chi^2 = 19.814$ ,  $P < 0.001$ ; larvae:  $\chi^2 = 7.095$ ,  $P < 0.01$ ; Table III). Because of our small *E. laticeps* ( $n = 7$ ) and *Scincella lateralis* ( $n = 3$ ) samples, comparative analyses of infestations were not performed for these species.

### Host population density

Population densities were estimated for *E. fasciatus*, *S. undulatus*, and *A. sexlineatus* within plots that yielded adequate captures and recaptures, but estimation of *S. lateralis* and *E. laticeps* population density was precluded by our small sample of each species (Table II). Cragston Lakes (2 plots) and Bull Hill (1 plot) yielded our estimates of *E. fasciatus* population density (21.2–46.3 individuals/ha), and a single plot from Bull Hill yielded our estimate of *S. undulatus* population density (6.25 individuals/ha). *Aspidoscelis sexlineatus* density was estimated from a single plot at Jug Bay (15.0 individuals/ha; Table IV).

### Realized reservoir competence

Ten *S. undulatus* collected from Jug Bay ( $n = 3$ ) and Cove Point ( $n = 7$ ) were used in the xenodiagnosis trials. Twenty *E. fasciatus* collected from Jug Bay ( $n = 9$ ), SERC ( $n = 2$ ), Crag-

ston Lakes ( $n = 4$ ), Bull Hill ( $n = 2$ ), and Cove Point ( $n = 3$ ) were used in the trials. Of the larval ticks ( $n = 164$ ) that successfully molted after feeding on *E. fasciatus* none (0%) were infected, and of those ( $n = 51$ ) that fed on *S. undulatus*, only 1 (2%) was infected (Table V).

Our estimates of exposure probability show a 50.9% probability of exposure for individual *E. fasciatus* and a 99.9% exposure probability for the entire *E. fasciatus* xenodiagnosis sample. Exposure probabilities were much lower for *S. undulatus*, with a 12.9% probability that an individual was exposed to 1, or more, infected ticks, and a 75.0% probability that at least 1 of the *S. undulatus* in the xenodiagnosis sample was exposed to infected nymphs during the preceding nymphal season.

### Tick abundance

Immature *I. scapularis* were collected in drag samples in all months sampled (April–October; Fig. 1), and they were observed at all sites and study plots. Larvae were detected in drag samples from April to September, and nymphs were detected from April to July (Fig. 1). Larval abundance increased during July and August, reaching a peak in August (22.8 larvae/100 m<sup>2</sup>; Fig. 1). Mean nymphal abundance also showed monthly variation, but of considerably lower magnitude, resulting in a less pronounced peak that spanned April to June, with the highest abundance observed in June (1.6 nymphs/100 m<sup>2</sup>; Fig. 1).

Questing larval densities at our sites were substantially lower than the sites where LoGiudice et al. (2003) determined larval burdens of mammalian and avian hosts. We expected that lizards occupying sites with higher densities of questing larvae would have higher larval burdens than observed at our sites, and we see this relationship with *E. fasciatus*, which shows a significant positive relationship between monthly mean larval density and monthly mean tick burden ( $r_s = 0.943$ ,  $P < .05$ ,  $n$

TABLE IV. Population density of lizards within study plots. *Eumeces fasciatus* estimates were generated using Schnabel's method and *Sceloporus undulatus* and *Aspidoscelis sexlineatus* estimates were generated using the Jolly–Seber method.

Species	Site	Plot	n/plot	95% CI*	Plot area (ha)	Density (n/ha)
<i>E. fasciatus</i>	Cragston	1	21.2	12.9–36.7	1.0	21.2
<i>E. fasciatus</i>	Cragston	2	46.3	17.2–169.9	1.0	46.3
<i>E. fasciatus</i>	Bull Hill	1	18.5	5.5–104.2	0.6	30.8
<i>S. undulatus</i>	Bull Hill	1	3.8	2.1–10.2	0.6	6.3
<i>A. sexlineatus</i>	Jug Bay	1	15	2.6–172.4	1.0	15

\* Confidence interval.



TABLE V. Realized reservoir competence of wild-caught *Eumeces fasciatus* and *Sceloporus undulatus* for *Borrelia burgdorferi* infection shown by xenodiagnosis using *Ixodes scapularis* larvae.

Species	No. lizards	Mean larvae/lizard	Range larvae/host	No. positive/n tested (%)
<i>E. fasciatus</i>	20	8.2	(1–18)	0/164 (0)
<i>S. undulatus</i>	10	5.1	(1–11)	1/51 (1.96)

= 6). Therefore, estimates of the dilution potential of lizards can be expected to be conservative compared with the estimates from LoGiudice et al. (2003) from the other host species. To provide an estimate of the maximum dilution potential of the lizards and to create an expected range, we adjusted larval tick burden data to reflect habitats more typical of the northeastern hyperendemic zone. To generate a scaling coefficient, we used the slope of the regression line of monthly means of questing larvae abundance and monthly means of *E. fasciatus* larval burden ( $m = 0.158$ ) and then multiplied this coefficient ( $m$ ) by the larval density measure (114 larvae/100 m<sup>2</sup>) of LoGiudice et al. (2003), to generate a new value for the mean larval burden of *E. fasciatus* ( $B_{i, scaled} = 18.0$ ). This scaled estimate of larval burden falls within the range of *E. fasciatus* larval infestations observed in this study (Table III).

### Nymphal infection prevalence

Host-seeking nymphs were collected at 4 sites and tested for *B. burgdorferi*. Nymphal infection prevalence grouped by study site was Cove Point, 0% ( $n_{nymphs} = 9$ ); Cragston Lakes, 17.2% ( $n_{nymphs} = 29$ ); SERC, 25.8% ( $n_{nymphs} = 31$ ); and Jug Bay, 9.3% ( $n_{nymphs} = 43$ ). A Pearson's chi-square test showed no significant heterogeneity among sites in NIP ( $\chi^2 = 4.16$ ,  $P = 0.24$ ).

### Model results

The model results show *E. fasciatus* to be an effective dilution host, whereas *S. undulatus* is not. Change in NIP predicted from incorporation of *E. fasciatus* into the initial species-poor community consisting only of white-footed mice was a 10.7% reduction, from 92.1 to 82.6% of nymphs infected (Fig. 2). Incorporation of *S. undulatus* into the initial species poor community reduced NIP from 92.1 to 92%, a 0.1% reduction (Fig. 2). When we incorporate our scaled estimate of larval burden into the model to estimate the maximum dilution potential, the presence of *E. fasciatus* reduced NIP by 51.5%, from 92.1 to 44.7%.

## DISCUSSION

### Tick burdens and reservoir competence of lizards

*Ixodes scapularis* infestations were commonly observed on lizards at all study sites and in 9 of the 10 study plots. *Eumeces fasciatus* were the most frequently and heavily parasitized of the 5 species studied followed by *S. undulatus*. The high importance of *E. fasciatus* at our study sites is in agreement with the majority of published studies, as is the lesser importance of *S. undulatus* and *A. sexlineatus* (Apperson et al., 1993; Oliver et al., 1993; Levine et al., 1997; Kollars et al., 1999; Durden et al., 2002). Inadequate sample sizes for *S. lateralis* and *E.*

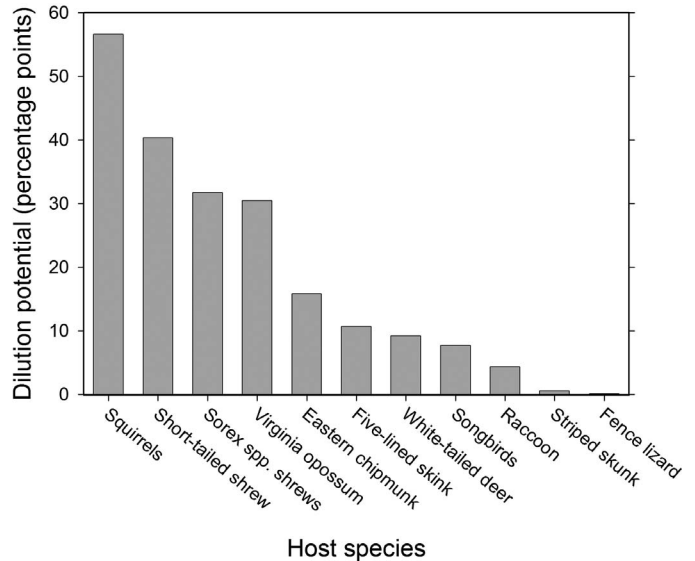


FIGURE 2. Dilution potential of host species in a host community containing white-footed mice and the focal host. Dilution potential is the percent change in nymphal infection prevalence (NIP) due to the addition of the focal species to the host community, as determined by the model of LoGiudice et al. (2003) and described in Materials and Methods.

*laticeps* prevented us from determining their role as tick hosts. In addition, despite the presence of questing adult *I. scapularis*, this life stage was not seen on any of the lizards. Although other species of tick (*A. americanum* and *D. variabilis*) were present within the study sites, these species were not observed infesting lizards.

The results of our xenodiagnostic trials showed low reservoir competence for both wild-caught *E. fasciatus* (0%) and *S. undulatus* (2%; Table V). Because we neither inoculated lizards with *B. burgdorferi* nor confirmed their infection status before conducting xenodiagnosis, our measure of reservoir competence, termed “realized reservoir competence” by Schaubert et al. (2002), must be interpreted with caution. In particular, low realized reservoir competence could reflect low proportion of lizards infected rather than their infectiousness to feeding ticks. However, the observed burdens of nymphal ticks on lizards combined with moderate to high infection prevalence in the nymphal population suggest that exposure rates at least for *E. fasciatus* are relatively high and that their low (0) realized reservoir competence is unlikely to have resulted from low exposure to *B. burgdorferi*. In contrast, low nymphal infestation (Table III) and low infection prevalence of nymphs at sites from which the *S. undulatus* originated did not allow us to distinguish between low reservoir competence per se and low exposure probability as the cause of low transmission to ticks feeding on this species.

These results provide evidence for the existence of a barrier to acquisition, maintenance, and/or transmission of *B. burgdorferi* in *E. fasciatus*. Such barriers have been documented in the western fence lizard (*Sceloporus occidentalis*; Lane, 1990; Manweiler et al., 1992; Lane and Quistad, 1998; Kuo et al., 2000; Lane et al., 2006) and southern alligator lizard (*Elgaria multicarinata*; Wright et al., 1998) in the western United States, and they are likely in the sand lizard (*Lacerta agilis*) of Europe

(Matuschka, 1992; but see Richter and Matuschka, 2006). The inability of lizards to act as reservoirs has been suggested as contributing to low Lyme disease prevalence in the western United States (Lane and Loye, 1989; Lane, 1990; Manweiler et al., 1992; Kuo et al., 2000; Casher et al., 2002) and Europe (Matuschka et al., 1991, 1992). However, other studies show that the reservoir incompetence of lizards is not universal. Levin et al. (1996) found *Eumeces inexpectatus* to infect 23.6% (n = 424) of feeding larvae with *B. burgdorferi*, and Clark et al. (2005) found evidence of *B. burgdorferi* infection in 8 of 9 lizard species (*E. laticeps*, *E. inexpectatus*, *S. lateralis*, *Ophisaurus ventralis*, *A. sexlineatus*, *Anolis sagrei*, *Anolis carolinensis*, *S. undulatus*, and *Sceloporus woodi*) from South Carolina and Florida as well as evidence of transmission to feeding larvae in *E. laticeps* at least 5 mo after infection. Lizards also have been shown to be competent reservoirs in a zoonotic cycle in the Mediterranean region of Africa (*Psammotromus algirus*; Dsouli et al., 2006), and Europe (*Lacerta agilis* and *Podarcis muralis*; Richter and Matuschka, 2006) involving *Borrelia lusitaniae* and *Ixodes ricinus*.

### Dilution potential

Our modeling approach suggests that *E. fasciatus* can be an effective dilution host, but that *S. undulatus* is not likely to play a significant role in the reduction of nymphal infection prevalence and is, therefore, a poor dilution host in the northeast. Given the comparable reservoir competence values and similar population densities of *E. fasciatus* and *S. undulatus*, it is clear from the model that infrequent and relatively small tick burdens prevent *S. undulatus* from having a measurable impact on nymphal infection prevalence. Under the conditions of the model, *E. fasciatus* was a more effective dilution host than white-tailed deer, songbirds, raccoons, skunks, and fence lizards (Fig. 2).

Larval infestation of lizards in our study was lower than has been described in prior studies of the same hosts (Apperson et al., 1993; Oliver et al., 1993; Levine et al., 1997; Kollars et al., 1999; Durden et al., 2002) and perhaps was a function of relatively low densities of questing larvae at our study sites. When we reassessed the role of *E. fasciatus* in diluting nymphal infection prevalence based on larval burdens expected in more heavily tick-infested areas of the northeast, we found a dramatic increase in the dilution potential of *E. fasciatus* from 10.7 to 51.5 percentage points. We suspect that the true dilution potential of *E. fasciatus* falls somewhere between the measured (low) and scaled (high) value.

In the northeastern United States, most lizard populations are limited to discrete, isolated, and relatively xeric habitat patches. Yet, the apparent ubiquity of lizard parasitism by immature *I. scapularis* observed in this study, combined with the wide sympatry of lizard and blacklegged tick geographic ranges, suggests the tick–host–*B. burgdorferi* relationships described in this study exist wherever *I. scapularis* and these lizards co-occur. Fully gauging the importance of lizards in the ecology of Lyme disease in the northeast and reconciling differing reservoir competence results will require further study of host communities that contain lizard populations, but this study clearly shows the potential for lizards, especially *E. fasciatus*, to play an important role in the ecology of Lyme disease as a dilution host.

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