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Impact of the experimental removal of lizards on Lyme disease risk

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The distribution of vector meals in the host community is an important element of understanding and predicting vector-borne disease risk. Lizards (such as the western fence lizard; *Sceloporus occidentalis*) play a unique role in Lyme disease ecology in the far-western United States. Lizards rather than mammals serve as the blood meal hosts for a large fraction of larval and nymphal western black-legged ticks (*Ixodes pacificus*—the vector for Lyme disease in that region) but are not competent reservoirs for the pathogen, *Borrelia burgdorferi*. Prior studies have suggested that the net effect of lizards is to reduce risk of human exposure to Lyme disease, a hypothesis that we tested experimentally. Following experimental removal of lizards, we documented incomplete host switching by larval ticks (5.19%) from lizards to other hosts. Larval tick burdens increased on woodrats, a competent reservoir, but not on deer mice, a less competent pathogen reservoir. However, most larvae failed to find an alternate host. This resulted in significantly lower densities of nymphal ticks the following year. Unexpectedly, the removal of reservoir-incompetent lizards did not cause an increase in nymphal tick infection prevalence. The net result of lizard removal was a decrease in the density of infected nymphal ticks, and therefore a decreased risk to humans of Lyme disease. Our results indicate that an incompetent reservoir for a pathogen may, in fact, increase disease risk through the maintenance of higher vector density and therefore, higher density of infected vectors.

Keywords: disease ecology; host composition; *Borrelia burgdorferi*; tick burden; host preference

1. INTRODUCTION

The emergence of human diseases caused by zoonotic pathogens transmitted by arthropod vectors presents one of the biggest threats to public health [1]. Human risk of exposure to such diseases generally increases with increasing population density of vectors infected with the zoonotic pathogen [2] and is the product of vector density and vector infection prevalence. Because different vertebrate hosts for the vector vary in their ability to support vector survival (host competence) and to infect the vector (reservoir competence) [3], the density of infected vectors often depends on the suite of host species available for blood meals. Consequently, the species composition and relative abundance of the host community are crucial in determining risk of exposure to vector-borne zoonotic pathogens [3,4].

Lyme disease is the most common vector-borne disease in the United States [5] and one of the most well-studied examples of how host assemblage affects disease risk [4,6,7]. *Ixodes* ticks, the genus of ixodid ticks responsible for transmitting the Lyme disease pathogen



(*Borrelia burgdorferi*) worldwide, have three post-egg life stages: larva, nymph and adult, that each take one blood meal from a vertebrate host. Larvae and nymphal ticks are both active in the spring and early summer, with larval densities peaking slightly earlier in the season than nymphs [8]. Adult *I. pacificus* ticks are active in the winter months. Nymphal ticks are primarily responsible for transmitting *B. burgdorferi* infections to people [9]. Because *B. burgdorferi* is not vertically transmitted [10,11], the tick vector must acquire the pathogen from one of its three blood meals. Consequently, the distribution of larval blood meals determines the prevalence of infection in nymphs and therefore disease risk to humans.

In the far-western United States, Lyme disease is vectored by the western black-legged tick, *I. pacificus* [12]. Mammals such as the dusky-footed woodrat (*Neotoma fuscipes*), western grey squirrel (*Sciurus griseus*), California kangaroo rat (*Dipodomys californicus*), and deer mouse (*Peromyscus maniculatus*) maintain *B. burgdorferi* as reservoir hosts [13–15]. However, not all species are equal in their reservoir competency. *Neotoma fuscipes* and *S. griseus* are the most competent reservoirs whereas *P. maniculatus* has generally low competence and contributes little to the total risk of Lyme disease [13–16]. By contrast, lizards, rather than mammals, host the largest proportion, up to 90 per cent, of the juvenile (larval

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Table 1. Table of predicted responses of *I. pacificus* to lizard removal given scenarios of complete host switching and no host switching. (Summary of the observed results from this study are shown in the last column.)

	predictions		observed results
	with host switching by ticks 	without host switching by ticks 	
questing larval ticks (year t)	no effect	increase	increase
larval burdens on other hosts (year t)	increase	no effect	increased on female <i>N. fuscipes</i> , no effects on male <i>N. fuscipes</i> , no effect on <i>P. maniculatus</i>
density of nymphs (year $t + 1$)	no effect	decrease	decreased
nymphal infection prevalence (year $t + 1$)	increase	increase	no detectable effect
density of infected nymphs (year $t + 1$)	increase	decrease if above change in DIN > change in NIP, otherwise increase	decreased

and nymphal) *I. pacificus* population [17]. In addition to providing a large majority of larval blood meals, the immune system of lizards such as the western fence lizard (*Sceloporus occidentalis*) and the southern alligator lizard (*Elgaria multicarinata*) actively kills the Lyme disease bacterium during tick feeding [18,19]. As ticks imbibe host blood, the lizards' alternative complement system kills the spirochete in the midgut diverticula of infected *I. pacificus* ticks [20]. The presence of lizards may be partly responsible for the significantly lower rates of infection found in nymphal *I. pacificus* in California compared with the highly endemic region of Lyme disease in the northeastern United States [4,15,21].

Although the impact these lizards have on *B. burgdorferi* in ticks has been described [19,20], the ecological role of lizards in Lyme disease ecology has not been examined experimentally. Lizard abundance can vary dramatically as can both vertebrate tick burdens and infection prevalence in the far-western United States [22,23], but the effect of this variation on the abundance of infected ticks is unknown. If ticks switch to other hosts when lizards are scarce, and feed with equal success, then tick abundance might not decline and infection prevalence would increase (table 1). Alternatively, reduced lizard abundance might lower tick abundance if ticks generally fail to find alternative, high-quality hosts (table 1). Some recent studies suggest that questing *I. pacificus* larvae may be able to identify lizards as preferred hosts for blood meals and that natural distributions of larvae are non-random [24]. If a strong preference for lizards as hosts does exist, then ticks may not choose to switch to an alternate host in the absence of *S. occidentalis*.

The degree to which pathogen vectors redistribute their blood meals in the absence of a particular host species can have important consequences on disease risk [3]. The most direct way to assess the impact of particular host species on the distribution of larval blood meals in the host community and consequent disease risk is through direct experimental manipulation of host species abundance.

We assessed the impacts of experimentally reduced lizard density on abundance and infection prevalence of *I. pacificus* and on tick distributions on the remaining

hosts, which were not manipulated. This study focused on the removal of *S. occidentalis* because this species is abundant on our study plots, diurnal, easy to capture and feeds a high proportion of juvenile ticks in oak woodland sites (figure 1). We chose not to manipulate another local lizard host, *E. multicarinata*, because it is highly cryptic and difficult to capture, though its abundance is unclear owing to the aforementioned points. Understanding the redistribution of larval *I. pacificus* following lizard removal would contribute significantly to understanding the maintenance of Lyme disease in California and help guide predictions on the relationship between host species assemblage and vector-borne diseases in general.

2. MATERIAL AND METHODS

Fieldwork for this study took place in Marin County, CA, north of San Francisco. Plots were established in two sites, China Camp State Park (CCSP) (38°0'9.50" N, 122°28'2.53" W) in San Rafael, CA, and Marin Municipal Water District Sky Oaks headquarters (37°58'5.39" N, 122°36'15.20" W). All plots were established in mixed evergreen forests composed of coast live oak (*Quercus agrifolia*), California bay laurel (*Umbellularia californica*), Pacific madrone (*Arbutus menziesii*), California black oak (*Quercus kelloggii*), Douglas fir (*Pseudotsuga menziesii*), and occasionally coastal redwood (*Sequoia sempervirens*).

There is a high degree of spatial and temporal variability in both vertebrate tick burdens and infection prevalence in the far-western United States [22,23]. To help overcome these inherent challenges, this study used plots that had been studied extensively for 2 years, leading up to our manipulation with detailed baseline data on vertebrate population density, tick burdens, vertebrate infection prevalence, and tick density and infection prevalence [8,25]. This study incorporated temporal and spatial variability in replicate experimental plots, invoking a replicated before-after-control-impact (BACI) design [26–28].

Of 14 long-term 1 ha plots, six were selected as experimental removal plots and eight as control plots. These experimental plots were evenly distributed at our two sites. Western fence lizard populations were estimated using mark–recapture techniques at all plots prior to removal in



Figure 1. Western fence lizard (*S. occidentalis*) adult male with engorged and feeding *I. pacificus* ticks. Larvae and nymphs are shown here attached to the ear (posterior) and nuchal pocket (anterior) at various stages of engorgement. Photo by Anand Varma.

early spring, before the peak season of juvenile tick questing [29]. For each plot, visual surveys of lizards took place along five evenly spaced transect lines, 22 m apart. Sighted lizards were sprayed on their dorsum with a diluted latex paint mixture using an Idico hand tree-marking gun (Idico Products Co., Miami, FL, USA; [22]). Use of the spray gun permitted the marking of lizards from 5 to 10 m away, and the marking is temporary, lasting only until the lizard's next moult [22]. Lizard surveys took place over three consecutive days with a different paint colour used each day to determine a lizard's encounter history. Each plot was surveyed once for three consecutive days in February 2008 prior to lizard removals. Population abundance was estimated using the Huggins closed-capture model in program MARK [30].

Lizard removals took place between 13 March and 10 April 2008. Lizards were captured using slip nooses. Lizard removals were conducted at each site until search time for a single lizard exceeded 30 min. Removal effort therefore varied depending on the size of the local lizard population. Captured lizards were sexed, measured and batch marked with liquid paper to identify them as a removed individual. The parietal eye was also painted over to minimize homing behaviour [31–33]. Lizards were temporarily placed in a cloth bag and then transported and released into a suitable habitat 1–3 km from the original point of capture.

Baseline data on vertebrate tick burdens were collected in 2006 and 2007 [8]. Tick burden data on small mammals following lizard removal were collected from 22 April to 8 May 2008. Small mammals were trapped using extra-large Sherman live traps (7.62 × 9.53 × 30.48 cm; H.B. Sherman Traps, Tallahassee, FL, USA) for three consecutive nights. Each plot was arrayed with 10 rows of 10 trapping stations. Each row was 11 m apart and each trapping station had two traps for a total of 200 traps per plot per night. Following lizard removal, there was a total of 8400 trap nights in this study. All captured animals were given an individually numbered fingerling eartag (National Band and Tag Co.,

Newport, KY, USA), identified by species and sex and weighed. All ticks were removed from the ears and head of each animal before being released at the point of capture.

The effect of lizard removals on the density and infection prevalence of questing ticks was evaluated by sampling larval ticks in the year of removals (time t) and nymphal ticks the year after the experimental manipulation to follow the larval cohort from time t to time $t + 1$. Each plot was sampled for ticks by dragging a 1 m² white flannel cloth through the forest understorey. At each plot, 500 m² was sampled twice during the peak nymphal questing period from 22 April to 8 May in 2008 and from 18 March to 19 April in 2009. All collected ticks were stored in 70 per cent EtOH for laboratory identification of species and molecular testing of infection with *B. burgdorferi* sensu stricto. All ticks were extracted using a DNeasy tissue extraction kit (QIAGEN, Valencia, CA, USA), and tested for infection by real-time PCR [8]. All positively screened ticks were then tested by nested PCR [14] and sequenced to positively identify the infection strain.

(a) Statistical analysis

We performed two main analyses in our study. First, we examined the impact of lizard removal on larval tick burdens on other tick hosts and second, we looked at the impact on questing larval and nymphal ticks. The impact of lizard removals on small mammal tick burdens was evaluated using generalized linear mixed-effect models (GLMMs) [34] with Poisson errors for count data [35]. GLMMs can incorporate repeated measures on plots and account for temporal or spatial trends. The impact of lizard removal was assessed on the population density of host-seeking ('questing') larval ticks (density of larvae (DOL)), nymphal ticks (density of nymphs (DON)), nymphal infection prevalence (NIP) and density of infected nymphs (DIN). Because of the inherent variability in tick density and infection data, a GLMM analysis granted us greater power of detection especially given the availability of baseline data on our plots and the replication of our large-scale experiment. For both types of analyses, independent class variables were defined as follows: (i) *year*, allowed for the structure of 2 years of 'before' data and 1 year of 'after' data; (ii) *plot*, corresponds to the long-term monitoring plots where the experiment was carried out; (iii) *site* was included to allow for generality of the results across two study sites; (iv) *period*, indicated before versus after the experimental manipulation and corresponds to a temporal control and includes both control and manipulated plots; and (v) *experiment*, plots were designated as 'removal' or 'control' in the period before and after the removals. The fixed factors tested in the GLMM models included period, experiment, period × experiment and site. With the exception of period × experiment, the other fixed effects tested in the model were additive. The factor of interest in all GLMM analyses is the period × experiment interaction (i.e. the impact of the removals on plots in period: after and experiment: removal). Plot was included as a random factor to account for repeated measures among years. Year was used as the unit of temporal variation within the random-effects portion of the model. Maximum likelihood was used to fit the model and the best model was determined using the Akaike information criterion [34,35]. The model for tick density ($Y_i = \text{DOL}_i, \text{DON}_i$ or DIN_i) is $Y_i \sim \text{Poisson}(\mu_i)$ for $i = 1, \dots, n$, where $n = (\text{number of plots}) \times (\text{number of years}) = 56$; $E(Y_i) = \text{var}(Y_i) = \mu_i$; $\log(\mu_i) = \alpha + \beta_1 \times \text{experiment} + \beta_2 \times \text{period} +$

Table 2. Results of the impact of lizard removals on *I. pacificus* ticks. (Data from generalized linear mixed-effect models (GLMMs) are shown for larval *I. pacificus* in the year of removals (t) and nymphal density, density of infected nymphs and nymphal infection prevalence results are displayed for the year following removals ($t+1$). The baseline treatments are experiment: control, period: before, site: CCSP. The experiment \times period interaction is the test of the experimental effect of lizard removal (experiment: removal \times period: after) and is indicated in bold. The z -value is the Wald statistic. The GLMM model-predicted difference in effect size between the removal and control plots is given as delta effect size in the indicated units.)

parameter		density of larvae (DOL)	density of nymphs (DON)	density of infected nymphs (DIN)	nymphal infection prevalence (NIP)
<i>random effect variance and standard deviation</i>					
plot	σ_{plot}	0.09 (0.30)	0.091 (0.31)	2.09e6 (1.5e2)	0.11 (0.33)
year	σ_{year}	0.01 (0.12)	0.023 (0.15)	0.52 (0.72)	0.10 (0.31)
<i>fixed effects estimates (z-values), standard deviation and significance level</i>					
intercept	α	6.36 (40.52)***	4.63 (27.99)***	1.47(0.22)***	-2.73 (-11.71)***
experiment	β_1	-0.28 (-1.73)	0.06 (0.36)	0.53 (0.04)*	0.37 (1.72)
period	β_2	-0.51 (-4.37)***	0.31 (1.76)	0.45 (0.16)	-0.87 (-2.08)*
site	β_3	0.02 (0.16)	-0.02 (0.17)	0.30 (0.23)	n.a.
experiment \times period	β_4	0.45 (17.83)***	-0.33 (5.77)***	-1.28(0.43)**	0.11 (0.38)
<i>predicted delta effect size (removal plots - control plots)</i>					
δ effect size		no. per plot 171.01***	no. per plot -14.06***	no. per plot -4.30***	per cent infection 1.28

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

$\beta_3 \times \text{site} + \beta_4 \times \text{experiment} \times \text{period} + \gamma_j[i] + \psi_k[i]$. The random effect of plot is $\gamma_j[i] \sim N(0, \sigma_{\text{plot}})$ for $j = 1, \dots, \mathcal{J}$, where \mathcal{J} = number of plots = 14. The random effect of year is $\psi_k[i] \sim N(0, \sigma_{\text{year}})$ for $k = 1, \dots, K$, where K = number of years = 4. The model for DIN had a random effect structure with an intercept and a slope term for the random effect of year (ψ_k): $\log(\mu_i) = \alpha + \beta_1 \times \text{experiment} + \beta_2 \times \text{period} + \beta_3 \times \text{site} + \beta_4 \times \text{experiment} \times \text{period} + \gamma_j[i] + b_{k1} + \psi_k[i]$. The model for NIP is: $Y_i = \text{NIP}_i \sim \text{binomial}(\text{nymphs}_i, \pi_i)$ for $i = 1, \dots, n$, where $n = (\text{number of plots}) \times (\text{number of years}) = 56$, nymphs_i is the number of nymphal ticks screened for *B. burgdorferi* on survey i and π_i is the probability of infection with *B. burgdorferi*. $E(Y_i) = \pi_i \times \text{nymphs}_i$, $\text{var}(Y_i) = \text{nymphs}_i \times \pi_i \times (1 - \pi_i)$; $\text{logit}(\pi_i) = \alpha + \beta_1 \times \text{experiment} + \beta_2 \times \text{period} + \beta_3 \times \text{experiment} \times \text{period} + \gamma_j[i] + \psi_k[i]$, where the random effects are as described above. Burden data were analysed for each species (*N. fuscipes* and *P. maniculatus*). For *N. fuscipes*, males and females were evaluated separately.

Tick density data and infection prevalence data were also analysed using the same BACI design described for tick burden data with Poisson errors for the density of ticks and density of infected ticks (count data) and a binomial error distribution for NIP models (presence/absence data). All GLMM models were fit using the lmer routine in the lme4 package in R [36].

3. RESULTS

In 2008, a total of 447 *S. occidentalis* were captured and relocated from six, 1 ha plots (electronic supplementary material, table S1). Estimates of lizard populations prior to removal were conducted early in the season for lizards, which explains why more lizards were removed from each experimental plot than was estimated to be present (electronic supplementary material, table S1). Because *S. occidentalis* is highly territorial and unlikely to expand its home range during the breeding season [37], influx

of lizards onto removal plots was minimal in our subsequent visits to the plots, but no formal counts were conducted. No relocated lizards were found on any of the plots after removal.

Lizard removals had an immediate effect on the local pool of questing larval ticks. We found a significant increase in questing larval *I. pacificus* density on experimental removal plots (table 2 and electronic supplementary material, table S2). These additional *I. pacificus* larvae on removal plots probably reflected the portion of the larval population left stranded by the absent lizards and apparently were not able to immediately find an alternate blood meal host following lizard removal.

From 2006 to 2008, we found only two mammal species that consistently maintained substantial tick burdens, *N. fuscipes* ($n = 544$) and *P. maniculatus* ($n = 995$). We encountered other species as well (e.g. California vole (*Microtus californicus*), western harvest mouse (*Reithrodontomys megalotis*) and *Sorex* spp. shrews), but in such low numbers and tick burdens [25] that they were not included in our analyses of tick burden. The western grey squirrel (*S. griseus*) is a potentially important reservoir but was not encountered in our study and has been difficult to trap by others on our sites [16]. Therefore, the remainder of this study focuses on *N. fuscipes* and *P. maniculatus* as unmanipulated tick hosts.

The impact of lizard removal on *I. pacificus* larval burdens on small mammals was species-specific, and in the case of woodrats, gender-specific. *Neotoma fuscipes* had significantly higher burdens post-removal (period \times experiment) than before (table 3 and figure 2), whereas no difference in tick burden was found for *P. maniculatus* (table 3 and figures 2 and 3). Gender of *N. fuscipes* was an important factor in larval burden response. Female *N. fuscipes* had higher larval burdens on lizard-removal plots but male *N. fuscipes* had lower larval burdens following lizard removal (table 3). Thus, the experimental effects on female *N. fuscipes* dominate the full model

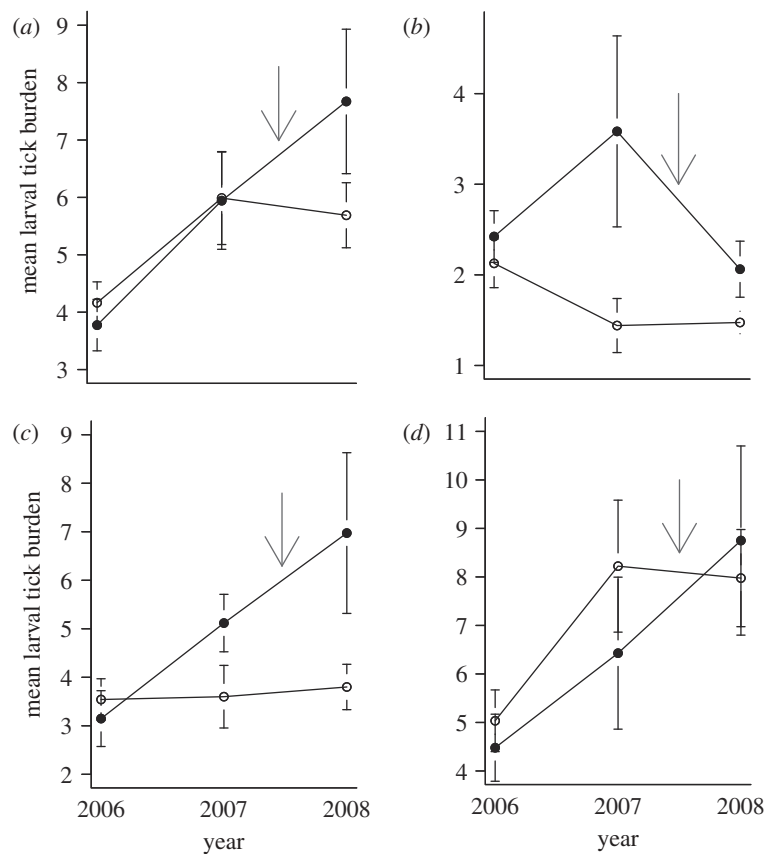


Figure 2. Larval burdens of *I. pacificus* on small mammals from 2006 to 2008. (a) *Neotoma fuscipes* burden is shown by mean burdens on control and experimental plots by year. (b) *Peromyscus maniculatus* burden is shown by mean burdens on control and experimental plots by year. *Neotoma fuscipes* burden is shown for both (c) females and (d) males. Experimental removal plots are indicated by the closed black circles and the controls are indicated with open circles. The experimental removal of *S. occidentalis* lizards took place on experimental plots before the 2008 season and is indicated by the arrows. Error bars indicate 1 s.e.

Table 3. Results of larval *I. pacificus* burdens on mammal species. (GLMM results are shown for dusky-footed woodrat (*Neotoma fuscipes*), male woodrats, female woodrats and deer mice (*P. maniculatus*). Model results are presented or the experimental effect of lizard removals on tick burdens in the year they were conducted—in 2008 (*t*). GLMM model estimates represent the slope of the interaction term representing the main experimental effect, shown in bold. The baseline treatments are experiment: control, period: before, site: CCSP. The experiment \times period interaction is the test of the experimental effect of lizard removal (experiment: removal \times period: after) and is indicated in bold. The *z*-value is the Wald statistic. The GLMM model-predicted difference in effect size between the removal and control plots is given as delta effect size of larvae burden per animal.)

	parameter	<i>N. fuscipes</i>	<i>N. fuscipes</i> male	<i>N. fuscipes</i> female	<i>P. maniculatus</i>
sample size		<i>n</i> = 544	<i>n</i> = 257	<i>n</i> = 287	<i>n</i> = 995
random effect variance and standard deviation					
plot	σ_{plot}	0.059 (0.24)	8.88e5 (9.4e2)	0.05 (0.23)	4.28e - 10 (2.07e - 5)
year	σ_{year}	0.03 (0.17)	0.22 (0.46)	0.04 (0.19)	4.49e - 8 (2.12e - 4)
fixed effects estimates (<i>z</i> -values) and significance level					
intercept	α	1.61 (9.33)***	1.99 (9.28)***	1.38 (6.63)***	0.77 (4.86)***
experiment	β_1	-0.07 (-0.43)	0.46 (1.27)	0.28 (1.22)	0.26 (1.09)
period	β_2	0.09 (0.44)	0.02 (0.17)	-0.18 (-0.67)	-0.35 (-4.77)***
site	β_3	-0.36 (0.17)*	0.08 (0.27)	n.a.	n.a.
experiment \times period	β_4	0.36 (4.67)***	-0.54 (-2.26)*	0.81 (6.46)***	0.18 (1.92)
predicted delta effect size (removal plots - control plots)					
		larvae per animal	larvae per animal	larvae per animal	larvae per animal
δ effect size		1.54***	-0.45*	4.89***	0.42

**p* < 0.05.

***p* < 0.01.

****p* < 0.001.

results (table 3). On control plots, we observed that larval burdens were higher on male compared with female *N. fuscipes* (male: 7.74 ± 1.08 (mean \pm s.e.); female: 4.05 ± 0.58 (mean \pm s.e.)). Therefore, lizard removal

disproportionately elevated larval tick burden on female woodrats (table 3). Our GLMM model predicted that female *N. fuscipes* larval burden was higher on experimental removal plots by 4.89 larvae per animal (table 3 and

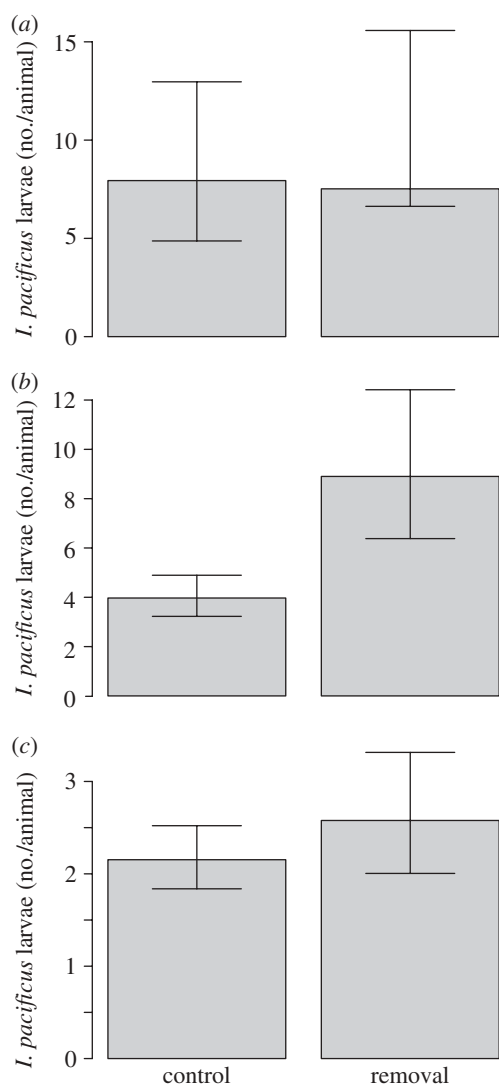


Figure 3. Generalized linear mixed-effect model (GLMM) predictions of larval *I. pacificus* burdens on (a) *N. fuscipes* males on control and removal plots, (b) *N. fuscipes* females on control and removal plots, and (c) *P. maniculatus* on control and removal plots. Error bars indicate 1 s.e.

figure 3). For both sexes combined, the model-predicted difference in the number of larvae per animal was 1.54 (table 2). Based on mean abundance of *N. fuscipes* over 3 years (17.11 woodrats·per plot; [8]), this translated to an additional 26.35 larvae on woodrats per plot over a 3 day trapping period. Lizard larval burden on the control plot in 2008 was 14.13 ± 2.10 (mean \pm s.e.), and with a mean abundance of lizards of 39.09 per plot over 3 years [8], this left an average of 507.52 larvae without a host on each lizard-removal plot. So the increased larval burden on *N. fuscipes* was not enough to absorb 94.81 per cent of larvae that would have fed on lizards, at least on the hosts we monitored. We therefore found incomplete (5.19%), species and gender-specific host switching of larval *I. pacificus* following removal of *S. occidentalis*.

The year following lizard removal (2009), we examined the density and infection prevalence of nymphs from the original larval cohort. We found that lizard removal differentially affected different metrics of disease risk. The total abundance of nymphal ticks was significantly lower on lizard-removal plots with a model-predicted

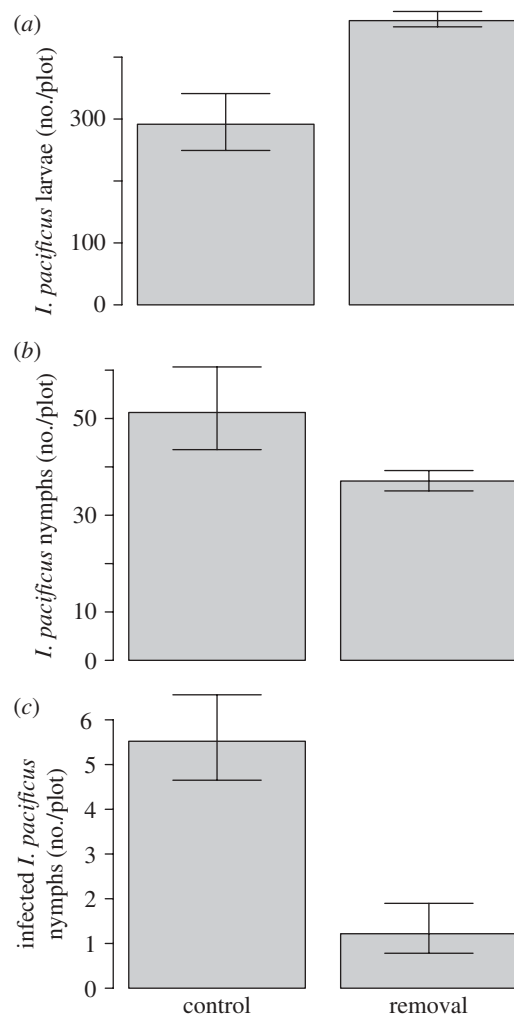


Figure 4. GLMM predictions of (a) *I. pacificus* larval density per plot in the year of lizard removals on control and lizard-removal plots, (b) *I. pacificus* nymphal density on control and lizard-removal plots, and (c) density of infected *I. pacificus* nymphs the year following lizard removals on control and lizard-removal plots. Error bars indicate 1 s.e.

delta effect size (difference between removal and control plots) of -14.06 nymphs per plot (table 2 and figure 4). The GLMM estimates represent the slope of each parameter. The main experimental effect of lizard removal is in the 'period : after \times experiment : removal' term (β_3 in table 2). We found no detectable effect of lizard removal on NIP in year $t + 1$ (table 2). Because lizard removal reduced total nymph density without changing their infection prevalence, we found that the DIN was significantly lower on lizard-removal plots relative to control plots by -4.3 ticks per plot (table 2 and figure 4).

4. DISCUSSION

The important role played by lizards such as *S. occidentalis* in the ecology of Lyme disease in the far-western United States has been described previously [13,19] but the ecological impact of *S. occidentalis* on the distribution of larval meals, and therefore disease risk, is not well understood. We found that lizard removal resulted in higher density of questing larval ticks in the year of removals

and lower DON the following year, indicating that many questing larvae were unable to find a host after lizards were removed. Following lizard removal, a small portion (5.19%) of larval *I. pacificus* did switch to a competent reservoir host (*N. fuscipes*), but this did not result in a detectable difference in infection prevalence of nymphs the following year. The total DIN is an important metric of Lyme disease risk and is the product of the DON and the NIP. Given the negative impact of lizard removal on nymph density but not infection prevalence, our GLMM analysis indicated that lizard removal resulted in lower DIN. Thus, we show that despite the cleansing effect of lizards on ticks infected with *B. burgdorferi*, the net impact of lizard removals was a reduction in the DIN and therefore risk of human exposure to Lyme disease.

These results were unexpected. What is currently known about the role of *S. occidentalis* in Lyme disease suggests that the removal of an antibacterial host in the community should increase Lyme disease risk [38]. But this expectation does not take into account the important impacts lizards have on tick density, which in the absence of a significant change in tick infection prevalence can determine overall disease risk. In addition, the expectation that lizard removal would increase Lyme disease risk assumes that the loss of lizard hosts will cause many of the larval ticks that would have fed on lizards to switch to other hosts, which are likely to be more competent reservoirs for *B. burgdorferi*. However, although we detected a small increase in larval burdens on a reservoir-competent host, female woodrats, when lizards were removed, this increase was inadequate to increase infection prevalence in the subsequent generation of nymphs. The total number of additional larval blood meals on lizard-removal plots was 1.54 larvae per woodrat, or approximately 26.35 per plot, which was not enough to significantly affect infection rates in nymphs the following year, particularly when woodrat infection prevalence and transmission rates are considered as well [8]. It is also possible that some of the larvae on lizard-removal plots that would have fed on *S. occidentalis* switched to alligator lizards. Switching from one reservoir-incompetent host to another would lessen the potential for an increase in infection prevalence. Unfortunately, we could not assess tick burdens on this cryptic host.

Apparently, higher larval burden on a reservoir-competent species does not necessarily translate to higher nymphal infection. Perhaps transmission is a negative density-dependent function of tick load, or survival from the larval to nymphal stage is compromised at higher burden levels. While it is not entirely clear why NIP was unaffected by lizard removal, the loss of lizards from removal plots can, by itself, explain the reduction in nymph density. Only a huge increase in NIP following lizard removal could compensate for the reduced tick density we observed, potentially resulting in a higher DIN. Our experiment rejected this scenario. However, as a one-time, pulsed experiment, these results only apply to a single cohort of ticks in one coastal county in California. The effect of long-term removal of lizards would depend on whether *I. pacificus* is ultimately limited by hosts for its juvenile stages, hosts for the adult stage or something else.

We found that increased larval burdens primarily affected female *N. fuscipes*. This was unexpected because sexual dimorphism in ectoparasite burden usually results in higher burdens on males [39]. Male-biased parasite loads probably arise from immunosuppression from elevated androgen levels [40] and larger home-range sizes. In fact, on control plots, larval burdens were higher on males. But the effect of lizard removal disproportionately increased burdens on female woodrats. While surprising, sampling for larval tick burdens on small mammals took place during their reproductive season, so females may have been immunocompromised because they were pregnant or lactating [41].

Although some larvae found an alternate host on female *N. fuscipes* following lizard removals, most were unable to successfully feed on an alternate host, leading to an overall reduction in nymph density the following year. This may be because encounter rates between larval ticks and non-lizard hosts are low. Alternatively, it is possible that tick preference for *S. occidentalis* is sufficiently strong that in the absence of lizards, the majority of *I. pacificus* larvae chose to not infest non-lizard hosts upon encounter. Under laboratory conditions, *I. pacificus* will preferentially feed on *S. occidentalis* when given a choice between four hosts: *S. occidentalis*, *P. maniculatus*, California kangaroo rat (*D. californicus*) and California towhee (*Pipilo crissalis*) [24]. Together, these results suggest that *I. pacificus* may exhibit some degree of host choice in the field that strongly affects their local density and infection prevalence. It is also possible that host grooming behaviour may limit tick burdens above a maximum threshold on other host species in this system.

Incomplete host switching following lizard removals can substantially impact predictions regarding the relationship between community disassembly and Lyme disease risk [3,7]. In the northeastern United States, Keesing *et al.* [3] found that the removal of eastern grey squirrels (*Sciurus carolinensis*) could either lower or raise the DIN depending on the extent of tick redistribution on the remaining host community. Our study provides experimental evidence that cautions against the assumption that the absence or removal of a tick host would result in complete host switching. Factors such as encounter rate, host preference and aversion to alternate hosts may play a critical role in the degree of host switching. The nature of the redistribution of larval meals following the removal of a species or community disassembly can strongly affect predictions of Lyme disease risk.

Theoretical and empirical research on Lyme disease, as well as other vector-borne and non-vector-borne diseases, supports a negative association between host diversity and disease risk or pathogen transmission [42–46]. This relationship, termed the dilution effect [46], occurs when the species most vulnerable to biodiversity loss tend to reduce transmission, whereas those that persist as biodiversity is lost tend to amplify risk. In the Lyme disease system of the eastern United States, small rodents such as the white-footed mouse (*Peromyscus leucopus*) and eastern chipmunk (*Tamias striatus*) are highly competent reservoirs for *B. burgdorferi* and competent hosts for *Ixodes scapularis* ticks [3]. These species persist, and even thrive, in low-vertebrate-diversity communities that occupy fragmented landscapes [47]. By contrast, other

species such as Virginia opossums (*Didelphis virginiana*), which are incompetent reservoirs for *B. burgdorferi* and poor hosts for black-legged ticks, occur in diverse vertebrate communities but disappear when forests are fragmented [3,47]. The current study suggests that the California Lyme disease system might behave differently. In contrast to the eastern USA, where poor reservoirs are also poor tick hosts, California's western fence lizard is an incompetent reservoir but amplifies tick populations. Our study shows that the amplifying effect of *S. occidentalis* on immature ticks dominates the diluting effect on tick infection prevalence, as evidenced by the net decrease in the DIN after lizards were removed (see also [48]).

To test the effect of changes in host community composition on disease risk, we chose an experimental approach of removing a host species. Experimental host removal provides advantages over comparative studies of disease risk among sites in which host community composition varies naturally. In particular, an experiment reduces the likelihood that unmeasured independent variables will produce spurious correlations. However, our experimental approach has limitations as well. For example, if lizards are highly insensitive to natural or anthropogenic forces that reduce vertebrate diversity in California, then our experiment might not mimic natural sequences by which communities are disassembled. Therefore, our manipulation is not a straightforward test of the dilution effect. Future studies should combine the assessment of host-species-specific effects on Lyme disease risk with that of species-specific responses to forces causing biodiversity loss in diverse habitats.

All protocols were approved by China Camp State Park, Marin Municipal Water District, California Department of Fish and Game and the University of California, Berkeley Animal Care and use Committee (protocol R092-B).

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