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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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 (*Scoloporus 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°09.50′N, 122°28′52.53″W) and 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...
For each plot, visual surveys of lizards took place along early spring, before the peak season of juvenile tick questing. S. occidentalis nymphs are shown here attached to the ear (posterior) and nuchal pocket (anterior) at various stages of engorgement. Photo by Anand Varma.

Figure 1. Western fence lizard (S. occidentalis) adult male withengorged 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.

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 (Yi = DOLi, DONi, or DINi) is Yi ~ Poisson(µi) for i = 1, . . . , n, where n = (number of plots) × (number of years) = 56; E(Yi) = var(Yi) = µi; log(µi) = α + β1 × experiment + β2 × 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 (*i*) and nymphal density, density of infected nymphs and nymphal infection prevalence results are displayed for the year following removals (*i* + 1). The baseline treatments are experiment: control, period: before, site: CCSP. The experiment × period interaction is the test of the experimental effect of lizard removal (experiment: removal × 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.)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>density of larvae (DOL)</th>
<th>density of nymphs (DON)</th>
<th>density of infected nymphs (DIN)</th>
<th>nymphal infection prevalence (NIP)</th>
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<td></td>
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<td>plot</td>
<td>$\sigma_{plot}$</td>
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<td>0.091 (0.31)</td>
<td>2.09e6 (1.5e2)</td>
</tr>
<tr>
<td>year</td>
<td>$\sigma_{year}$</td>
<td>0.01 (0.12)</td>
<td>0.023 (0.15)</td>
<td>0.52 (0.72)</td>
</tr>
<tr>
<td>fixed effects estimates ($z$-values), standard deviation and significance level</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>intercept</td>
<td>$\alpha$</td>
<td>0.36 (40.52)***</td>
<td>4.63 (27.99)***</td>
<td>1.47 (0.22)***</td>
</tr>
<tr>
<td>experiment</td>
<td>$\beta_1$</td>
<td>−0.28 (−1.73)</td>
<td>0.06 (0.36)</td>
<td>0.53 (0.04)*</td>
</tr>
<tr>
<td>period</td>
<td>$\beta_2$</td>
<td>−0.51 (−4.37)***</td>
<td>0.31 (1.76)</td>
<td>0.45 (0.16)</td>
</tr>
<tr>
<td>site</td>
<td>$\beta_3$</td>
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<td>−0.02 (0.17)</td>
<td>0.30 (0.23)</td>
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<tr>
<td>experiment × period</td>
<td>$\beta_4$</td>
<td>0.45 (17.83)***</td>
<td>−0.33 (5.77)***</td>
<td>−1.28 (0.43)***</td>
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<td>predicted delta effect size (removal plots − control plots)</td>
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<tr>
<td>no. per plot</td>
<td>171.01***</td>
<td>−14.06***</td>
<td>−4.30***</td>
<td>1.28</td>
</tr>
</tbody>
</table>

*$p < 0.05$.

**$p < 0.01$.

***$p < 0.001$.

$\beta_3 \times$ site + $\beta_4 \times$ experiment × period + $\gamma_1[i] + \phi_3[i]$. The random effect of plot is $\gamma_1[i] \sim N(0, \sigma_{plot})$ for $j = 1, \ldots, J$, where $J$ = number of plots = 14. The random effect of year is $\phi_3[i] \sim N(0, \sigma_{year})$ for $k = 1, \ldots, K$, 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 ($\phi_3$): $\log(\mu_i) = \alpha + \beta_1 \times$ experiment + $\beta_2 \times$ period + $\beta_3 \times$ site + $\beta_4 \times$ experiment × period + $\gamma_1[i] + \phi_3[i]$. The model for NIP is: $Y_i = NIP_i \sim \text{binomial}(\text{nymphs}_i, \pi_i)$ for $i = 1, \ldots, n$, where $n$ = (number of plots) = (number of years) = 56. nymphs is the number of nymphal ticks screened for B. burgdorferi on survey $i$ and $\pi_i$ is the probability of infection with *B. burgdorferi*. $E(Y_i) = \pi_i \times$ nymphs, $\text{var}(Y_i) = \text{nymphs}_i \times \pi_i \times (1 - \pi_i)$; $\logit(\pi_i) = \alpha + \beta_1 \times$ experiment + $\beta_2 \times$ period + $\beta_3 \times$ site + $\beta_4 \times$ experiment × period + $\gamma_1[i] + \phi_3[i]$, 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 (*Reithrodonotmys 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 × 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
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 ± s.e.); female: 4.05 ± 0.58 (mean ± 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 2).
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 ± 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 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 × experiment : removal’ term (β3 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.
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-lizards 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 DI N 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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