Patterns of diversity along a habitat size gradient in a biodiversity hotspot

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Abstract. Habitat destruction and loss are the gravest threats to wildlife worldwide. We examined the correlation between habitat patch size and species richness and abundance of mammals and reptiles in a biodiversity hotspot in California. Within nine forest fragments standardized for age, topography, climate, and vegetation cover, we tested the effects of patch size and isolation on biodiversity. To measure species richness and abundance, we used wildlife cameras for meso-and-large vertebrates, mark-and-recapture analyses for small mammals, mark-and-resight analysis for reptiles, and standard dragging techniques for tick collections because they are frequent ectoparasites on vertebrates in oak woodland habitats. Our results show that meso-and-large vertebrate richness and abundance increase with patch area as does tick density. Surprisingly, small mammal species richness and abundance peak in intermediate-sized fragments. Resource limitation and competition at the smallest habitats and predation at the largest patches may be responsible for this pattern. Further, there is a significant decrease in invasive species richness with habitat patch size. We found that habitat destruction and fragmentation are acting upon species and communities in context-dependent ways that is critical to conservation planning, land use design, and ecosystem function.

Key words: biodiversity; California oak woodland; disease ecology; fragmentation; habitat loss; intermediate disturbance hypothesis; invasive species; island biogeography theory.

INTRODUCTION

Habitat loss and fragmentation are the most dire threats to global biodiversity (Fahrig 2003, Millenium Ecosystem Assessment 2005) and a root cause for species extinctions (Baillie et al. 2004). Accordingly, the impact of habitat loss and fragmentation on biodiversity and ecosystem function has been a central research question in conservation biology for decades (Kruess and Tscharntke 1994, Meffe and Carroll 1994). It is important to differentiate between habitat loss and habitat fragmentation. Habitat fragmentation is defined as a landscape-scale process in which a large habitat area is converted into a number of smaller habitat patches. These patches are smaller in their total area compared to the original habitat and are isolated from each other by a non-habitat matrix (Wilcove et al. 1986). Habitat loss describes the measurable amount of habitat lost. It can operate on different scales and is regarded as an effect of habitat fragmentation. While habitat loss has consistently negative effects on biodiversity, the effects of habitat fragmentation on biodiversity can be both positive and negative (Fahrig 2003). Human land use development leads to habitat loss and habitat fragmentation, resulting in the division of formerly large continuous habitat into small and isolated habitat patches (Ranta et al. 1998,
The study and management of habitat loss and fragmentation has its roots in island biogeography theory (IBT; MacArthur and Wilson 1967, Ewers and Didham 2006). This theory—developed to predict patterns of species richness on oceanic islands (Watling and Donnelly 2006)—predicts decreasing levels of species richness in smaller, more isolated habitat patches (Preston 1962, MacArthur and Wilson 1967). Both scientists and conservationists have applied IBT to terrestrial ecosystems to explain patterns of biodiversity in fragmented terrestrial ecosystems (Allan et al. 2003, Prugh et al. 2008, Tulloch et al. 2016). Yet evidence for IBT in terrestrial systems is limited (Debinski and Holt 2000, Cook et al. 2002, Paciencia and Prado 2005).

If consequences of habitat loss, such as increased human activity and greater edge effects, are acting as disturbances, the intermediate disturbance hypothesis may be able to explain species richness patterns. This hypothesis predicts that the greatest species diversity occurs in habitats that experience an intermediate degree of disturbance since too high or too low levels of disturbance favor disturbance-adapted species and climax communities, respectively (Connell 1978).

In recent years, the number of studies addressing the impacts of habitat loss and fragmentation has steadily increased, making the lack of empirical evidence on the effects of habitat loss in California, a biodiversity hotspot, even more glaring. The California Floristic Province is among the world’s 25 most biologically rich and endangered terrestrial ecoregions with 44% endemism in plant and vertebrate species (Myers et al. 2000). It is also considered one of the most threatened ecoregions on the planet (Brooks et al. 2006) largely due to rapid expansion of human development and agriculture. In this study, we empirically evaluate the response of different animal taxa to varying degrees of habitat loss and test community disassembly rules in a hardwood forest ecosystem. The patterns we find in this research can function as a predictive framework for terrestrial ecosystems beyond California oak woodlands.

To address species-specific responses to habitat loss, we established a research design which can be easily replicated and applied beyond specific regions or species. We analyzed the effects of habitat loss of woodlands surrounded by human settlement and/or agriculture in Northern California. We selected nine forested patches along a habitat size gradient, ranging from 2.5 to 2700 ha, to measure the impact on diversity. By rigorously standardizing for time since fragmentation, topography, temperature, precipitation, exposure, elevation, and vegetation cover, we were able to focus on the effects of fragment size (measured in hectares [ha]) and isolation (measured by proximity index as described in Giladi et al. 2011, Gustafson and Parker 1994). We employed wildlife cameras, small mammal live-trapping, and reptile noosing in mark-and-resight studies as well as tick sampling to investigate the effects of habitat loss on (1) species richness, (2) abundance, and (3) diversity. Further, we examined community composition and community disassembly—the ongoing process of nonrandom species losses and declines (Zavaleta et al. 2009) across taxonomic groups and trophic levels. We expected species richness, abundance, and diversity of native species to increase with increasing fragment size independent of broader taxonomic group. We also separately analyzed the relationship between habitat loss and invasive species richness. According to the diversity resistance hypothesis, which argues that diverse species compositions are highly competitive and therefore less susceptible to invasion (Elton 1958), we hypothesized that there is better ecosystem health and resiliency against biological invasion in larger, more diverse habitat fragments.

**Materials and Methods**

**Site selection**

To determine the relationship between habitat loss and vertebrate diversity, nine distinct forest fragments delineated by agriculture, major roads, and/or urban space were chosen around the San Francisco Bay Area (Fig. 1). The habitat patches ranged in size from 2.5 to 2775 ha (Table 1). Sites were selected using a combination of Esri world imagery (Esri 2015) and a land use/land cover data set veg06 compiled by the California Department of Forestry (CDF) and Fire Protection’s Fire and Resource Assessment Program (FRAP) in cooperation with the USDA Forest Service Region 5 Remote Sensing Laboratory (RLS). This land use/land cover layer utilizes data spanning from 1997 to 2002 and distinguishes 41 land cover categories.
Fig. 1. Sample sites. The nine circles represent the nine sample sites in the Bay Area within a radius of 78 km. Circle size indicates relative site area along a habitat size gradient, ranging from 2.5 to 2700 ha.
based on two common classification schemes: the California Wildlife Habitat Relationships system and Anderson Land use/Land cover classification scheme. Housing density information is provided from the 2000 U.S. Census. Although the land use/land cover data were last updated in 2006 and have a resolution of only 30 m, it has proven to be largely accurate, admitting some minor changes in urban land cover expansion in the intervening decade. These discrepancies were located and modified using the corresponding Esri world imagery basemap for 2015 with a resolution of 0.3 m. In one case—Tiburon Uplands Preserve—the percentage of oak woodland was determined visually based on Esri world imagery to compensate for its clear misclassification of forest as urban space in fveg06 (Appendix S1: Fig. S1). Within the nine forest patches, we determined time since fragmentation of each patch by averaging the ages of seven properties bordering the focal patch. Those properties included freeways, highways, and randomly chosen buildings bordering the focal patch. We then calculated the average time since fragmentation of these bordering properties ranging from 40 to 58 yr. Temperature and precipitation were assessed for the month of April, the main sampling season. Average temperature ranged from 13° to 15°C, and average precipitation ranged from 37 to 56 mm. Both values were derived from PRISM Climate Group, Oregon State University 2015.

Percentage of oak woodland vegetation cover was determined based on the percentage of pixels classified as oak woodland compared to all pixels comprising each forest patch. All sites comprise 57% oak cover or more (Appendix S1: Fig. S2e). Isolation was measured using the proximity index (Gustafson and Parker 1994, Giladi et al. 2011) within a 1000 m buffer around each of the nine focal patches. The proximity index sums the ratios of patch area ($a_i$) to the square distance of all habitat patches to the focal patch ($h_{ij}$).

$$
PI_i = \sum_{j=1}^{n} \frac{a_i}{h_{ij}^2}
$$

Seven of the nine patches had at least one small, vegetated corridor leading to another vegetated patch not covered by houses or pavement. Those corridors never exceeded 1% of the patch perimeter (Esri world imagery, Esri 2015). None of these corridors led to a patch that exceeded half the size of the original patch except for the biggest forest patch. Since forest patch size is analyzed categorically and not absolutely, the fact that the largest forest fragment opens into another bigger forest patch should not bias the analysis. Abiotic and biotic parameters including isolation, temperature, precipitation, elevation, aspect of sampling grid, and vegetation cover were standardized for all nine sites (Appendix S1: Fig. S2).

**Vertebrate sampling and tick collection**

Standardized sampling grids (0.5 ha) were established at each site based on the following criteria: (1) Grids were located at least 20 m away from the forest edge (determined by spatial restrictions of the smallest site), (2) the grids were located under oak cover, and (3) direct north facing slopes were avoided due to microclimatic biases.

To measure species richness, relative abundance, and diversity of large- and medium-sized vertebrates, we employed one motion-sensored wildlife camera per site (Reconyx PC800 in spring 2016 and Bushnell Trophy Cam HD in fall/winter 2016/2017). The cameras were

| Site abbr. | Site full name | County   | Latitude | Longitude | Area (ha) | Perimeter/area |
|------------|----------------|----------|----------|-----------|-----------|----------------|
| SMI        | St. Margarita Island | Marin   | 38.0095  | –122.5242 | 2.5       | 233.6          |
| WP         | Worcester Park | Santa Clara | 37.2221 | –121.9665 | 11        | 531.5455       |
| JSCP       | Junipero Serra County Park | San Mateo | 37.6099 | –122.4229 | 57        | 103.3158       |
| WDLP       | Water Dog Lake Park | San Mateo | 37.5061 | –122.3026 | 130       | 97.18462       |
| HOS        | Heintz Open Space | Santa Clara | 37.2326 | –121.9294 | 188       | 91.15426       |
| TUP        | Tiburon Upland Preserve | Marin   | 37.8893  | –122.4523 | 293       | 86.13652       |
| LFY        | Lafayette Reservoir | Contra Costa | 37.8790 | –122.1424 | 499       | 63.67335       |
| CCSP       | China Camp State Park | Marin   | 38.0010  | –122.4893 | 1193      | 40.10813       |
| SLRP       | Spring Lake Regional Park | Sonoma | 38.4281  | –122.6212 | 2775      | 24.16757       |
installed on trees at a height of 20–50 cm facing an obvious game trail. The cameras were within the 0.5 ha sampling grid at each site. Public trails were avoided to protect visitors’ privacy within public parks. Camera traps were set to medium trigger sensitivity and programmed to take three photographs within a 1-s interval with a 30-s delay before a subsequent trigger. Camera traps were active 24 h/d using an infrared flash for night photographs. We did not use baits or lures. Cameras recorded a total of 40 camera days in spring from April to May 2016 and 60 d in fall/winter from November 2016 to January 2017.

Small mammals were trapped within the 0.5-ha sampling grid using a 7 × 7 trapping array with 11.8 m spacing between sampling stations. Two extra-large Sherman live traps (7.6 × 9.5 × 30.5 cm; H.B. Sherman Traps, Tallahassee, Florida, USA) were positioned at each of 49 trapping stations facing opposite directions. Sherman traps were baited with oatmeal and peanut butter for three consecutive nights at each site during peak tick season (beginning of April–mid-May). All captured animals were identified by species, sexed, weighed, measured, marked with ear tags, and searched for ticks; ticks were removed; and 2-mm tissue samples were taken using standard ear punch methods. For tissue collection, animals were anesthetized with isoflurane (Sigma) solution. The animals were placed in the bag for 5–10 s with a jar containing cotton with 5 mL of 5% isoflurane/propanediol solution and monitored until the animal went limp at which point the animal was removed from the bag and processed as described above. Each captured small mammal was uniquely marked with ear tags for mark-and-recapture analyses, allowing abundance and diversity assessments for small mammals. All animals were released at the point of capture. Small mammal species included deer mouse (Peromyscus maniculatus), california mouse (Peromyscus californicus), pinion mouse (Peromyscus truei), dusky-footed woodrat (Neotoma fuscipes), Californian vole (Microtus californicus), brown rat (Rattus norvegicus), black rat (Rattus rattus), and western harvest mouse (Reithrodontomys megalotis).

Lizards were located by visual surveys along transect lines within the 0.5-ha sampling grid totaling 495 m at each site. Encounters included western-fence lizards (Sceloporus occidentalis), southern alligator lizards (Elgaria multicarinata), western skink (Eumeces skiltonianus), gopher snake (Pituophis catenifer), and western rattlesnake (Crotalus oreganus).

Slip-noosing, a standard herpetological procedure, was used to collect western-fence lizards. All captured animals were sexed, weighed, measured, and marked, and attached ticks were counted. Each animal was individually marked allowing for abundance assessment among western-fence lizards. Western-fence lizards were released at the point of capture.

Ticks were collected using standard dragging and flagging methods in which a white cotton cloth was dragged or flagged within the 0.5-ha sampling grid totaling 495 m at each sampling site. Drag cloths were checked every 30 m, and ticks were transferred to vials containing 70% ethanol, before transported to laboratory facilities. Tick species were identified using binominal keys using a dissecting microscope (Furman and Loomis 1984, Kleinjan and Lane 2008). All protocols were approved by institutional animal care and use protocol (# AU16-05).

**Data analysis.**

Small mammal and western-fence lizard abundance estimates.—Statistical analyses were performed in R v. 3.2.3 (R Core Team 2015) with RStudio v. 0.99.902 (RStudio Team 2016). Small mammal and western-fence lizard abundances were calculated using mark-recapture statistical analysis implemented in R using the package RCapture (Rivest and Baillargeon 2015). Each species was analyzed separately for all nine forest patches (Appendix S1: Table S1). Abundance estimates were selected based on Akaike information criterion (AIC).

Large vertebrate abundance and diversity estimates.—Photographs from camera traps were date and time stamped, allowing for species-sighting records within 30-s intervals. Consecutive photographs of the same species were considered independent if taken ≥30 min apart (O’Brien et al. 2003). If photographs contained several individuals of the same species within one image, all individuals in the picture were counted. If consecutive photographs within 30 min showed individuals of the same species, but the individuals were clearly distinguishable,
for example, adult vs. juvenile or antlered vs. antlerless, we counted the individuals separately. Finally, we created a matrix of encounter rates (number of independent photographs per species/trap day) from the independent photographs for all nine sites. Using this matrix, we were able to calculate relative abundance (numbers of independent photographs per species/trap-days). By placing one single camera trap at each of our nine forest fragments, we sought to standardize our sampling effort at all nine sites. This may have underestimated diversity at the larger sites. Further, one camera trap per site cannot account for the different movement patterns and home range sizes of species encountered. Deer, for example, were the most abundant species captured in our camera trap data (see Appendix S1: Fig. S3a for large vertebrate abundance excluding deer). To minimize these effects, we operated each camera for long periods of time—40 and 60 d—and collected data during two different seasons—spring, and fall/winter. From our observations, this technique captured more species and recorded reencounters of species with large home ranges and frequent movement such as bobcats and foxes.

For species richness and relative abundance, we analyzed both seasons spring and fall/winter for a total of 100 camera trap-days. For relative abundance estimates, we excluded western gray and fox squirrel data from fall/winter due to seasonal behavior shifts among squirrels caching food, which would have resulted in an overinflated representation of western gray and fox squirrels. Since deer comprised a large proportion of the observed large vertebrates, we analyzed the relationship between site area and deer abundance separately to ensure that large vertebrate abundance data were not purely driven by deer abundance. For analyses between *Ixodes pacificus* ticks and their primary reproductive host, mule deer, we only used data collected in the same season (spring 2016).

Invasive species richness estimates.—We calculated invasive species richness based on our small mammal as well as our camera trap data for each of the nine patches. In addition to domestic house cats (*Felis catus*), we encountered Virginia opossums (*Didelphis virginiana*), Brown rats (*R. norvegicus*), and one Black rat (*R. rattus*), all of which are invasive to California (Jameson and Peeters 1988).

Habitat size and diversity analyses.—We tested frequency distribution of our data with a combination of the Shapiro-Wilk test of normality and the goodness-of-fit test derived from the package *vcd* (Meyer et al. 2016). To assess the effect of habitat patch size on small mammal, lizard, and large vertebrate species richness and abundance, we conducted simple linear regression analyses with the *lm* function in R. If no linear regression was determined, we tested for polynomial relationships. To describe nonlinear trends between site area and small mammal richness as well as small mammal abundance, we fitted a second-order polynomial regression with site area and used the *predict* function in RStudio. If neither linear nor polynomial relationships could be detected, we concluded that there are no trends depending on habitat patch size.

Multiple regression analysis.—To analyze more complex, species-specific interactions responsible for structuring invasive species richness and tick density, we conducted generalized linear model analyses (GLM) using the *glm* function. For invasive species richness, our fixed effects were site area, native small mammal species richness, native small mammal abundance, native large vertebrate species richness, and native large vertebrate abundance. For nymphal *I. pacificus* density, our fixed effects were site area, small mammal richness, small mammal abundance, large vertebrate richness, large vertebrate abundance, lizard species richness, and western-fence lizard abundance. Native, large vertebrate species richness was cosine transformed, and site area, nymphal tick density, and small mammal richness were log transformed. Stepwise, backward selection was used to select the most parsimonious model based on the AIC score.

Pathogen analyses.—Tick was identified to life stage and species under a dissecting microscope. After a surface sterilization with 70% ethanol and hydrogen peroxide, mammal ear biopsy tissue, nymphs, and adult *I. pacificus* ticks were extracted using the DNeasy blood and tissue kit (Qiagen, Valencia, California, USA). We tested for two bacterial pathogens transmitted by ticks in California, *Borrelia burgdorferi*, the causative agent of Lyme disease and *Borrelia miyamotoi* a relapsing fever pathogen. For ticks, we used a nested PCR targeting the 16S rRNA gene using primers that can amplify *B. burgdorferi* and

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B. miyamotoi simultaneously (Bunikis et al. 2004). For mammal tissue, we used a nested PCR targeting the 5S–23S rRNA intergenic spacer region with forward and reverse primers that can amplify B. burgdorferi (Lane et al. 2004). After PCR, samples were purified using SeraPure magnetic beads and then sequenced on an ABI 3730 using forward and reverse internal primers (Life Technologies, Grand Island, New York, USA). Sequences were aligned and edited in Geneious v7 and aligned to reference sequences using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS

Overall, we sampled 1138 large vertebrates from 40 d of camera trapping between the beginning of April to mid-May 2016 and 60 d of camera trapping from November 2016 to January 2017. Between April and May of 2016, a total of 202 small mammals, 153 lizards, and 1859 questing ticks were collected (Appendix S1: Table S2).

Patterns in species richness as well as abundance across habitat fragments differed tremendously between large vertebrates, small mammals, and lizards. Overall, lizard species richness and western-fence lizard abundance did not follow a clear linear or polynomial trend based on habitat patch size. Large vertebrate species richness was positively, though non-significantly correlated with patch size ($R^2 = 0.186$, $P = 0.247$). Small mammal richness and abundance showed no linear trend with habitat patch size. Instead, small mammal richness displayed a monotonic polynomial trend with the function: richness ~ site area (log) + [site area (log)]^2. This polynomial trend was not significant ($R^2 = 0.356$, $P = 0.268$; Fig 2a). Species richness patterns were generally in alignment with patterns of species abundance. Western-fence lizard (Sceloporus occidentalis) abundance showed no clear trend with patch size. Large vertebrate abundance increased significantly as a linear function of site area ($R^2 = 0.488$, $P = 0.036$). When disentangling the relationship between large vertebrate abundance, deer abundance, and site area, we found that large vertebrate abundance, excluding deer, increased significantly with site area ($P = 0.013$, $R^2 = 0.488$; Appendix S1: Fig. S3a). Deer abundance alone showed a positive but non-significant trend with site area ($P = 0.144$, $R^2 = 0.279$; Appendix S1: Fig. S3b). Therefore, we conclude that large vertebrate abundance is not significantly driven by deer alone. Small mammal abundance significantly peaked at intermediate-sized (100–300 ha) forest fragments and was best fit with a second-order polynomial function of the form: site area (log) + [site area (log)]^2 ($R^2 = 0.709, P = 0.024$; Fig 2b).

We encountered the greatest species richness and abundance of invasive species at the smallest habitat patches (<100 ha; Fig. 3a). Invasive species richness ranged from zero to four at each site and included F. catus, D. virginiensis, Rattus norvegicus, and Rattus rattus. The best fit model for predicting invasive species richness included site area and native large vertebrate species richness (Table 2).

Tick drags for questing ticks yielded 1859 ticks representing five hard tick species Ixodes pacificus, Dermacentor occidentalis, Dermacentor variabilis, Dermacentor albipictus, and Ixodes spinipalpus. The majority of these were various life stages of I. pacificus (95.75%). The best fit model for predicting density of I. pacificus nymphs included site area, large vertebrate species richness, small mammal abundance, western-fence lizard abundance, lizard species richness, and small mammal species richness (Table 2). The relationship between density of I. pacificus nymphal ticks and site area is shown in Fig 3b. Site area, large vertebrate species richness, and small mammal abundance were all statistically significant predictors in the model (Table 2). Since deer are the primary reproductive host of I. pacificus, we singled out relative abundance of deer from large vertebrate relative abundance and by using a simple linear regression found a strong, positive correlation between relative abundance of deer and the density of nymphs (DON; $R^2 = 0.526$, $P = 0.027$; Fig 3c). Larval I. pacificus abundance had a non-significant, positive trend with relative abundance of deer ($R^2 = 0.427$, $P = 0.0567$). Among all I. pacificus ticks, we encountered one I. pacificus nymphal tick infected with Borrelia miyamotoi, a relapsing fever pathogen (Mun et al. 2006), at one of our large sites (1193 ha). At two intermediate-sized forest fragments (11 and 200 ha), we detected one Neotoma fuscipes infected with Borrelia burgdorferi and one N. fuscipes infected with Borrelia bissettii, a closely related genomospecies.
in the *B. burgdorferi* sensu lato complex (Postic et al. 1998).

**DISCUSSION**

We found evidence for taxonomically driven patterns of species diversity along a gradient of habitat loss. Our data show a strong pattern of small mammal abundance peaking at intermediate-sized study areas (100–300 ha), while large vertebrate abundance increased linearly with habitat patch size. We found that invasive species richness and abundance are highest in the smallest and most species-poor habitat patches (<100 ha) and are highly associated with habitat disturbance. Our results also found a significant increase in *Ixodes pacificus* nymphal density with increasing patch size. Patterns of small mammal abundance and large vertebrate abundance are mirrored in the species richness data trends. For instance, we found large vertebrate species richness (e.g., deer, raccoons, top predators) increased with habitat patch size, conforming to predictions of IBT (MacArthur and Wilson 1967). In contrast, our results show that small mammal species richness also peaks in intermediate-sized forest fragments.

The peak in small mammal abundance in intermediate-sized fragments may be explained by examining the factors regulating small mammal populations at the smallest and largest patches. We posit that disturbance and resource limitation is regulating small mammals at the smallest habitat patches. In contrast, greater predation pressure may be exerting top-down regulation on small mammals at the largest, most intact sites.

(Fig. 2. Continued)

Fig. 2. Relationship of (a) species richness and (b) abundance as a function of habitat fragment area. Vertebrate responses are separated into large vertebrates, estimated by camera trap data, small mammals, estimated by mark–recapture analysis, and reptiles, estimated by mark–resight methods. Dashed lines represent non-significant relationships (*P* > 0.05). Large vertebrate species richness (narrow dashed line) increases linearly with site area (*P* = 0.247). Small mammal species richness (thick dashed line) follows a non-significant polynomial function that peaks at intermediate-sized fragments. Lizard species richness follows no clear pattern with site area, and a best fit line is not displayed. In the abundance graph, solid lines represent significant relationships (*P* ≤ 0.05). Large vertebrate abundance (narrow solid line) increases linearly with site area (*R*² = 0.488, *P* = 0.036). Small mammal abundance (thick solid line) follows a significant polynomial function (*R*² = 0.709, *P* = 0.025) with the highest abundance at intermediate-sized fragments. Western-fence lizard abundance follows no clear trend dependent on site area, and a best fit line is not displayed.
Our data showing an increase in large vertebrate richness and abundance, which include small mammal predators, support this theory. However, our data on small mammal predators alone were too limited to test this hypothesis directly. Still, if predation pressure was the main limiting factor for small mammal abundance, we would expect an inverse relationship between habitat patch size and isolation with more small mammals in smaller forest fragments as has been observed with Peromyscus leucopus in the eastern United States (Nupp and Swihart 1996, Krohne and Hoch 1999). Yet we found lower abundances of small mammals as habitat patch size decreases from 57 to 2.5 ha. One possible explanation is that resource quality is lower and levels of disturbance are higher at smaller sites. In addition, competition for those limited resources can play an important role on community structure. Peromyscus mice were absent from habitats 11 ha or smaller and the smallest forest patch (2.5 ha) contained no native small mammals at all. This nonrandom loss of species indicates differences in competitive ability between species.

Table 2. Multivariate GLM analysis.

| Variable                                | Estimate | SE  | t-value | P-value |
|-----------------------------------------|----------|-----|---------|---------|
| Invasive species richness (AIC 26.344)  |          |     |         |         |
| Intercept                               | 3.154    | 0.71| 4.41    | 0.005** |
| Site area (log)                          | -0.92    | 0.30| -3.03   | 0.023*  |
| Native large vertebrate species richness (cos) | 1.17     | 0.59| -1.97   | 0.097   |
| Density of nymphal Ixodes pacificus ticks (AIC – 0.598) |         |     |         |         |
| Intercept                               | 1.01     | 0.5 | 2.05    | 0.178   |
| Site area (log)                          | 0.94     | 0.1 | 9.42    | 0.011*  |
| Small mammal abundance                  | -0.03    | 0.01| -4.93   | 0.039*  |
| Small mammal species richness (log)     | 0.32     | 0.15| 2.08    | 0.174   |
| Large vertebrate species richness       | -0.35    | 0.05| -6.38   | 0.024*  |
| Lizard species richness                 | 0.41     | 0.12| 3.33    | 0.079   |
| Western-fence lizard abundance          | -0.03    | 0.01| -3.89   | 0.060   |

Notes: AIC, Akaike information criterion; GLM, generalized linear model. Significance of model parameters are denoted with a * for \( P < 0.05 \) and ** \( P < 0.001 \).
with *Neotoma fuscipes* likely the stronger competitor compared to *Peromyscus* spp (Swei et al. 2012). Our findings suggest that small mammal populations are not only driven by habitat patch size but also are influenced by resource limitation and biotic interactions with other species, including predators. The influence of both bottom-up and top-down regulation on small mammals resulted in the highest small mammal abundance in intermediate-sized fragments between 50 and 300 ha.

Our small mammal richness results do not conform to the predictions of IBT, and instead, they more closely resemble patterns predicted by the intermediate disturbance hypothesis. This hypothesis predicts that the greatest species diversity occurs in habitats that experience an intermediate degree of disturbance since too high or too low levels of disturbance favor disturbance-adapted species and climax communities, respectively (Connell 1978). Our smallest habitat patches experience more human activity and greater edge effect relative to habitat area, both of which can be considered disturbances. In addition, harsher conditions at the edge of a habitat patch, such as wind patterns, changes in foliage density, humidity, and temperature, have also been described as disturbances (Harper et al. 2005). In this study, the ratio of perimeter to interior area (edge) closely tracks with the total habitat area (see Appendix S1: Fig. S2b), and thus, patterns in small mammal richness and abundance might be equally explained by edge effect as by area.

The nonlinear trend of mammal abundance as well as species richness peaking at intermediate-sized forest fragments could only be revealed by assessing a large range of fragment sizes (2.5–2700 ha), greater than other comparable studies. However, covering such a large range of fragment sizes comes at the cost of having little replication within the patch sizes. Due to the time and resource limitations of this study, our study was designed to span a large range of habitat fragments over increased replication to increase the statistical power of our analyses. Given this, we were able to detect nonlinear trends, such as a significant polynomial pattern of small mammal abundance. Covering such a large range of fragment sizes has the potential to uncover otherwise undetected ecological patterns. For example, population dynamics appear to be shaped by both predation pressure and resource limitation. In addition, assessing such a large range of fragment sizes is necessary for understanding geographically complex ecological systems such as Lyme disease ecology which depends on a variety of vertebrate hosts, all exhibiting differences in habitat behavior.

These findings have implications for biological conservation, whether the focus is on overall biodiversity or the protection of target species. Moreover, small mammal populations play an important role in disease ecology as reservoirs of viruses and bacteria such as hantavirus, salmonellosis, and *Yersinia pestis*, the causative agent of plague (CDC, 2017a). In addition to these directly transmitted diseases, rodents act as primary reservoirs for many vector-borne diseases including cutaneous leishmaniasis, Lyme disease, relapsing fever, and Rocky Mountain spotted fever (CDC, 2017b). This implies that our results are relevant not only for species conservation but also for improved ecosystem management and zoonotic disease ecology. With this in mind, we sampled western blacklegged ticks (*I. pacificus*), the main vector for Lyme disease in California (Burgdorfer et al. 1985). Our results indicate a significant increase in *I. pacificus* nymphal density with increasing patch size. These findings, however, are contrary to previous studies conducted in the eastern United States that found significant declines in nymphal tick density as habitat patch size increased (Allan et al. 2003, Brownstein et al. 2005). Since Lyme disease is mainly transmitted to humans by nymphal ticks as vectors, the calculation of DON is commonly used to assess disease risk for specific areas (LoGiudice et al. 2003). For the western United States, our findings indicate a higher risk of encountering ticks that transmit disease in larger, more intact habitats. Often these are the same habitats that tend to be well used for outdoor recreation and for extended periods of time. For example, at our nine study sites, campgrounds for overnight stays only existed in the largest two forest patches. Studies on the eastern United States predicted that the highest risk of infection would be associated with the smallest sites which are more regularly frequented by humans, though usually for shorter intervals (Allan et al. 2003). However, compared to our study which looked at a habitat patch size gradient ranging from 2.5 to 2700 ha, Allan et al.
(2003) included study sites spanning a scale of 0.7–7.6 ha. This more restricted scale may have limited the ability to determine the larger scale or nonlinear impacts of habitat patch size on nymphal tick density and infection prevalence. Larger tick hosts like deer as well as other small mammal predators may not be present or highly abundant in smaller habitat patches. In addition, there are considerable differences in the enzootic cycle and pathogen ecologies between Lyme disease transmission in the western and eastern United States that may also explain the differences we observed.

Studies from the eastern United States found increased DON in smaller habitats was correlated with increased *P. leucopus* abundance in those smaller forest fragments (Nupp and Swihart 1996, Krohne and Hoch 1999, Allan et al. 2003). In our study, we observed a negative correlation between small mammal abundance and DON. While juvenile *I. pacificus* mainly feed on lizards and small mammals, deer are the reproductive host for *I. pacificus* and thus highly important for the tick life cycle (Telford et al. 1988, Jaenson and Talleklint 1992, Lane et al. 1994). Previous studies found much lower overall tick abundance in habitats with very few or no deer compared to areas with medium or high deer densities (Stafford et al. 2003). However, the precise relationship between juvenile tick abundance at medium or high deer densities remains unresolved (Kilpatrick et al. 2017). In this study, we demonstrated that nymphal *I. pacificus* density is significantly and positively correlated with deer abundance. In addition, larval *I. pacificus* density displayed a positive, though non-significant trend with deer abundance. Larval tick abundance can be interpreted as being highly correlated with adult tick abundance. Hence, our results strongly suggest that the *I. pacificus* life cycle, distribution, and abundance are highly dependent on deer population dynamics. Density of nymphs does not assess disease risk per se since it does not take tick infection prevalence in account. Therefore, to determine whether habitat loss, host diversity, and the relative abundance of hosts play a significant role in predicting disease risk, it is necessary to calculate the density of infected nymphs (DIN) which can serve as a proxy for human risk of Lyme disease (LoGiudice et al. 2003). We found one *N. fuscipes* infected with *Borrelia burgdorferi* and one *N. fuscipes* infected with *Borrelia bissettii*, another closely related Lyme disease agent in the *B. burgdorferi* sensu lato complex (Postic et al. 1998). Both infected *N. fuscipes* came from intermediate-sized forest fragments (11 and 200 ha). Further, we encountered one *I. pacificus* nymphal tick infected with *Borrelia miyamotoi*, a relapsing fever pathogen (Mun et al. 2006), at one of our larger sites (1193 ha). The prevalence of *B. burgdorferi* was much lower than previous studies in the region (Swei et al. 2011, 2012, Salkeld et al. 2015) and may have been driven by California’s five-year long drought that lasted from 2012 to 2016. Further data collection is necessary to analyze the effects of habitat loss and host diversity on DIN under non-drought conditions.

Our study provides empirical support for foundational ecological theories related to species diversity and abundance and demonstrates that habitat loss has multipronged and species-specific impacts. There is, however, one exemption in our findings: invasive species. Invasive species were only encountered in the smallest forest patches regardless of the taxonomic group of the invader. While there is some theoretical framework and much speculation predicting that small habitat fragments are more vulnerable to invasion (Collinge 2000, With 2004, Harper et al. 2005), little empirical evidence has yet been found demonstrating these patterns beyond specific target species. Our results suggest that habitat protection and biodiversity act as buffers against biological invasion.

Human settlement, infrastructure, and agriculture are rapidly expanding worldwide. These activities fragment natural habitats and affect ecosystems globally. Understanding the impact of habitat loss on biodiversity is an important theoretical and conservation priority, but there are surprisingly few empirical studies that evaluate the general impact of habitat loss and fragmentation across a broad taxonomic spectrum. This is particularly true in California, a biodiversity hotspot where it appears that multiple regulating forces act on diverse species assemblages. While some species display standard species-area curves such as large vertebrates, small mammal communities may be structured by resource limitation at the smallest sites and top-down predation pressures at larger sites. Our results suggest that natural communities respond in
taxonomically specific, but predictable ways to inhabit loss and provide a path to help predict and guide efforts in biological conservation, ecosystem management, and public health.

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