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Emergence of Tick-Borne Granulocytic Anaplasmosis Associated with Habitat Type and Forest Change in Northern California

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Abstract. An important ecosystem service of intact forests is protection from some emerging infectious diseases. Tick-transmitted disease granulocytic anaplasmosis increasingly occupies second-growth forest. We hypothesized that areas of second growth would have increases in tick and rodent abundance, facilitating emergence of anaplasmosis. We predicted *Anaplasma phagocytophilum* presence as a function of biocomplexity and forest structure, including vegetation, ticks, and rodents in four sites in California. Significant risk factors for exposure included host species (woodrats with 13% seroprevalence, odds ratio [OR] = 8.3 and chipmunks with 27% seroprevalence, OR = 20.7), and park location (northern parks, OR 25.5–27.7). Exposure to *A. phagocytophilum* was more likely among chipmunks in redwood sites at one park, but with woodrats and oaks at another. Overall, transects on which small mammals showed greatest *A. phagocytophilum* exposure had high biodiversity in ticks, rodents, and vegetation, as well as intermediate-sized trees with a high mean and variance in diameter at breast height, findings which suggest that a dilution effect, where increased biodiversity reduces disease risk, does not necessarily apply in this system. Thus, enzootic and potentially emerging anaplasmosis were linked to high biodiversity and mature second-growth forest.

INTRODUCTION

Infectious diseases may emerge in areas where changes in forest canopy cover, mammal and plant species distribution, and habitat integrity have occurred, often caused by anthropogenic factors. In Europe, cases of tick-borne Lyme disease, encephalitis, and granulocytic anaplasmosis have increased in humans, pet and agricultural animals, and wildlife, expanding their geographical ranges to much of Scandinavia and eastern Europe.[1] Proposed explanations include habitat change, reduced predation, ongoing climate change, and increasing reservoir host and vector numbers. *Anaplasma phagocytophilum* is an important emerging infectious tick-transmitted pathogen of humans, horses, and dogs, which shares tick vectors and in many cases vertebrate hosts with the agent of Lyme disease.[2] Although only six human cases were confirmed as of 2006 in California,[3] this disease is common in dogs[4] and horses[5] in the western United States.

Throughout the Holarctic, the vectors of *A. phagocytophilum* are ticks in the *Ixodes ricinus* subgroup of hard ticks, including the western black-legged tick (*I. pacificus*) in western North America, the deer tick (*I. scapularis*) in eastern North America, the taiga tick (*I. persulcatus*) in Asia, and the sheep tick (*I. ricinus*) in Europe.[6] Reservoirs of granulocytic anaplasmosis are rodents, including the dusky-footed woodrat (*Neotoma fuscipes*), the western gray squirrel (*Sciurus griseus*), and the redwood chipmunk (*Tamias ochrogenys*) in California, bank voles (*Myodes* spp.) and wood mice (*Apodemus* spp.) in the Old World, and the white-footed mouse (*Peromyscus leucopus*) in the eastern United States.[7–14] The effects of habitat change on tick-borne disease in the western United States are difficult to assess accurately. Old-growth coniferous forests in the California coast range mountains are increasingly interspersed with patchworks of disturbed second-growth forest, particularly represented by oak. The increase in second growth has led to dramatic increases in humid microhabitats for ticks, which are typical in deciduous woodlands, dense brush, along ecotones, or in areas with sufficient shade and moisture. We hypothesized that areas that have experienced deforestation and second-growth succession would have an increase in tick and rodent abundance as community structure is re-assembled, facilitating disease emergence. The present study was undertaken to examine granulocytic anaplasmosis exposure and infection within the rodent guild, describe the *Ixodes* spp. tick fauna of rodents, associate high likelihood that small mammals were exposed to *A. phagocytophilum* with habitat characteristics, and predict *A. phagocytophilum* presence and potential emergence as a function of biocomplexity and forest structure, including vegetation, ticks, and rodents in four sites in northern California.

METHODS

Study site and transect establishment. Sampling was performed at four state parks with mature and peripheral redwood habitat in northern California: Humboldt Redwoods (HR) State Park (southern Humboldt County, 40°17.770, –123°59.178), Hendy Woods (HW) State Park (Mendocino County, 39°04.25; –123°28.238), Samuel P. Taylor (SPT) State Park (Marin County, 38°01.232; –122°40.774), and Big Basin (BB) State Park (Santa Cruz County, 37°10.621; –122°12.328). Within each park, twelve 50-meter × 12-meter transects were established by randomly choosing among available deer or poorly used human trails to encompass mature and peripheral redwood habitat.

Assessment of vegetation. Point intercept sampling of vegetation was performed, which consists of describing at each specific point what vegetation is present on the forest floor, canopy, and at a height of 1–3 meters at selected points. Points were taken every two meters from 0 meters to 50 meters along the length of the transect and at points 2 meters perpendicular to the transect extending 6 meters in both directions. All herbs, forbs, trees, and shrubs were identified to species; grasses and mosses were recorded only as grasses and mosses. Substrate where no vegetation was present was described as duff, sand, dirt, rock, or wood. All trees within 6 meters on each side of

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the transect were recorded and the diameter at breast height (dbh in cm) was measured if the diameter exceeded 1 cm.

Animal trapping and ectoparasite evaluation. Twice per year (spring and fall) for two years, 10 extra large Sherman live traps (HB Sherman, Tallahassee, FL) were set per transect overnight for three consecutive nights. Traps were set at locations of observed active small mammal usage or dens and baited with peanut butter and oats. Upon removal from the traps, rodents were anesthetized with ketamine (20 mg/kg) and xylazine (3 mg/kg) delivered subcutaneously, examined visually for ectoparasites, bled by retro-orbital abrasion, and the blood was anticoagulated with EDTA. Each animal was labeled with a permanent numbered metal ear tag before release at the site of capture. Because *Sorex* spp. (shrews) were often found dead in traps, their carcasses were retrieved, kept cold, and then transferred to the laboratory for sample collection. Live shrews were examined for ticks but released without further processing. All blood was kept cool or frozen at –20°C at until plasma could be separated by centrifugation. Ectoparasites were preserved in 70% ethanol. *Ixodes* spp. ticks were identified at 80–100× magnification to species using keys of Furman and Loomis[15] and Webb and others.[16] Larvae, which can be difficult to identify, were viewed under a compound microscope on a depression slide and under a dissecting microscope before identification was confirmed. Voucher specimens for each species and stage were retained in the laboratory collection. All work with animals was performed under the oversight of the University of California Davis attending veterinarian and Institutional Animal Care and Use Committee.

Serologic analysis. Plasma IgG against *A. phagocytophilum* was assayed by an indirect immunofluorescent antibody assay,[17] using *A. phagocytophilum*-infected HL-60 cells as substrate and fluorescein isothiocyanate–labeled goat anti-rat heavy and light chain IgG (Kirkegaard and Perry, Gaithersburg, MD). Samples were tested starting at dilutions of 1:20, and positive and negative control sera were included on each run. Samples were considered positive if strong fluorescence was detected at dilutions of at least 1:80, consistent with previously published cutoff values.[17]

Nucleic acid extraction and real-time TaqMan polymerase chain reaction. All sciurids, woodrats, voles, and shrews were tested by polymerase chain reaction (PCR). Deer mice (*Peromyscus* spp.) were chosen randomly for PCR testing. DNA was extracted from 200 μL of whole blood using a kit (DNeasy Tissue kit; Qiagen, Valencia, CA) according to manufacturer's instructions. Real-time PCR was performed using primers and probes as described[18] in a combined thermocycler/fluorometer (ABI Prism 7700; Applied Biosystems, Foster City, CA).

Data management and analysis. Data were maintained in Excel® (Microsoft, Redmond, WA) and analyzed with the statistical package R (R-Development Core Team, http://www.r-project.org). For all tests, the cutoff for statistical significance was $P = 0.05$. Summary statistics were calculated for each park and transect. For analysis of vegetation, percent canopy and shrub cover were calculated as the number of points where plants were present divided by all points evaluated. Species richness (S) and diversity were calculated for each park and transect, including species number, the Shannon and Wiener index H′, the Simpson's reciprocal index D ($1/\Sigma p_i$), and D/S, a measure of evenness.[19]

Small mammals were summarized as for vegetation. In addition, relative population sizes among transects were calculated using unique trap success per unit effort for deer mice, woodrats, and chipmunks. Ticks were summarized by location and host species. Exact 95% confidence intervals (CIs) for seroprevalence assuming binomial distributions were calculated in R using a proportions test. Differences in seroprevalence among small mammal species between sexes and among parks were assessed by chi-square tests. Similar calculations were performed for PCR test results. Individual risks of small mammals for exposure to and infection with *A. phagocytophilum* were assessed as a function of park, sex, and species by calculating odds ratios and 95% CIs. Multivariate logistic regression was performed to evaluate seropositivity as a function of site, host species, sex, tick load, and interactions. Potential confounding variables were evaluated based on a change of greater than 10% in the odds of other parameters after adding a confounder to the model and based on previous knowledge and biological reasoning. An optimal model was chosen to minimize Akaike's information criterion. A *t*-test was performed to evaluate differences in mean tick load between seropositive and negative (and PCR-positive and -negative) animals. We tested the hypothesis that presence of *A. phagocytophilum* differs among transects by logistic regression by using explanatory variables from the richness and diversity measure of vegetation (Table 1) and small mammals (Table 2). Initially, univariate screening of variables was performed and then all variables for which $P \leq 0.1$ included in a multivariate model.

RESULTS

Forty-eight transects were evaluated among four state parks, comprising mature and peripheral redwood ecosystems. Vegetation communities typically consisted of mixed hardwood and conifer, including redwood (*Sequoia sempervirens*), true oaks (*Quercus* spp.), tan oak (*Lithocarpus densiflorus*), Douglas fir (*Pseudotsuga menziesii*), bay laurel (*Umbellularia californica*) and others (Table 1). Fifty-eight non-tree species were recorded, most commonly blackberry (*Rubus* sp.), grasses, monkeyflowers (*Mimulus* spp.), mosses, redwood sorrel (*Oxalis oregana*), poison oak (*Toxicodendron diversilobum*), sword fern (*Polystichum munitum*), evergreen huckleberry (*Vaccinium ovatum*), rose (*Rosa* sp.), and salal (*Gautheria shallon*). Transects had on average 23.5 (range = 4–70) tree individuals, with mean species richness of 3.3 (range = 1–8) (Table 1). The dbh for the largest tree and the mean dbh of all trees were calculated and ranged from 0.34 cm to 5.25 cm with a mean of 1.47 cm for the former and ranged from 0.16 cm to 2.25 cm with a mean of 0.43 cm for the latter. The largest trees were at HR and HW. Percent canopy ranged from 0.2 to 1, and percent shrub ranged from 0.06 to 0.8. Evenness of the forest was highly variable, notably high in some of the pure redwood groves at HR and HW but much lower elsewhere (Table 1).

Eight-hundred seventy-six small mammals, including 655 individuals, were trapped at the four state parks during 2005–2007 (Table 3). The most commonly caught animals included deer mice (54% of all unique captures), woodrats (9%), and chipmunks (11%). Rarely caught species included voles (*Microtus californicus* and *Clethrionomys californicus*), shrews (*Sorex* spp.), and flying squirrels (*Glaucomys sabrinus*). The greatest number (33%) of individuals were caught at BB and fewest at HR. More animals overall were caught in the

TABLE 1
Summary of transect vegetation characteristics and diversity indices in four state parks in northern California*

Park	T	Most common non-redwood tree or shrub	No. of trees	No. of tree species	Shannon-Wiener trees	Tree mean dbh	Tree variance dbh	Largest tree dbh	Simpson's D trees	D/S trees	% Canopy cover	% Shrub
BB	1	Oak	18	4	1.05	0.26	0.07	0.95	2.08	0.41	0.846	0.33
BB	2	Oak	16	4	1.21	0.28	0.04	0.52	3.05	0.76	0.789	0.167
BB	3	Ceanothus	70	8	0.67	0.17	0.03	1.12	2.06	0.26	0.686	0.615
BB	4	Tan oak	11	2	0.59	0.71	0.14	1.54	1.66	0.83	0.987	0.353
BB	5	Tan oak	13	2	0.43	0.62	0.1	1.3	1.35	0.68	1	0.058
BB	6	Tan oak	37	5	1.06	0.29	0.05	1.1	2.43	0.49	0.865	0.282
BB	7	Oak	35	3	0.35	0.42	0.05	1.26	1.19	0.40	0.647	0.275
BB	8	Oak	22	5	1.21	0.28	0.04	0.67	2.75	0.55	0.942	0.263
BB	9	Madrone	47	2	0.57	0.26	0.02	0.54	1.61	0.81	0.891	0.314
BB	10	Tan oak	18	3	0.94	0.36	0.1	1.44	2.31	0.77	0.974	0.455
BB	11	Oak	8	3	0.97	0.36	0.13	1.12	2.46	0.82	0.301	0.744
BB	12	Oak	54	3	1.09	0.23	0.02	0.54	2.93	0.98	0.917	0.276
HR	1	Tan oak	41	4	0.54	0.24	0.02	0.69	1.35	0.34	0.981	0.321
HR	2	Tan oak, Douglas fir	61	4	0.37	0.18	0.12	0.56	1.18	0.30	1	0.654
HR	3	Bay laurel	23	6	1.5	0.3	0.06	1.02	3.78	0.63	0.949	0.494
HR	4	Madrone/Douglas fir	24	3	1.08	0.2	0.03	0.72	2.91	0.97	0.981	0.372
HR	5	None	4	4	1.39	0.32	0.06	0.42	4.00	1.00	0.237	0.321
HR	6	Bay laurel	29	6	1.27	0.42	0.1	2.48	2.56	0.43	1	0.288
HR	7	Tan oak	13	2	1.18	0.2	0.03	0.48	2.38	0.48	0.795	0.429
HR	8	Tan oak	22	2	0.47	0.45	0.17	3.39	1.41	0.71	0.853	0.75
HR	9	Tan oak	26	2	0.36	0.35	0.16	4.34	1.26	0.63	0.981	0.365
HR	10	Tan oak	17	2	0.56	0.72	0.28	3.68	1.56	0.78	0.993	0.352
HR	11	None	5	3	0.95	0.89	0.76	3.94	2.27	0.76	0.891	0.442
HR	12	Bay laurel	8	2	0.38	1.26	0.46	3.26	1.28	0.64	0.827	0.481
HW	1	Bay laurel	18	2	0.64	0.7	0.3	5.24	1.80	0.90	0.955	0.276
HW	2	Tan oak	10	2	0.33	0.95	0.23	1.79	1.22	0.61	0.654	0.321
HW	3	Tan oak	6	3	0.69	0.68	0.28	1.71	2.00	1.00	0.474	0.378
HW	4	None	16	1	0	0.59	0.18	1.88	1.00	1.00	0.859	0.308
HW	5	None	4	1	0	2.25	0.45	3.51	1.00	1.00	0.801	0.231
HW	6	Tan oak	10	3	0.94	0.17	0.03	0.34	2.38	0.79	0.756	0.429
HW	7	Bay laurel	29	4	1.21	0.21	0.02	0.68	3.01	0.75	0.955	0.404
HW	8	Tan oak	36	3	0.79	0.17	0.04	1.5	1.91	0.65	0.872	0.66
HW	9	Tan oak	26	4	0.127	0.2	0.04	1.08	3.07	0.61	0.556	0.635
HW	10	Tan oak	35	3	0.84	0.27	0.04	1.06	1.98	0.66	0.949	0.314
HW	11	Tan oak	24	4	1.11	0.26	0.05	0.86	2.64	0.66	0.885	0.487
HW	12	Tan oak	24	4	0.82	0.22	0.07	1.43	1.74	0.43	0.949	0.609
SPT	1	Tan oak	12	2	0.68	0.52	0.21	2.56	1.95	0.97	0.942	0.692
SPT	2	Tan oak	13	4	0.79	0.47	0.12	1.61	1.64	0.41	0.955	0.558
SPT	3	Bay laurel	33	4	0.92	0.23	0.03	0.63	2.25	0.56	0.942	0.628
SPT	4	Bay laurel	18	4	0.88	0.23	0.04	0.77	1.82	0.45	0.859	0.821
SPT	5	Tan oak	9	3	0.85	0.51	0.14	1.32	1.98	0.66	0.91	0.667
SPT	6	Tan oak	12	4	1.13	0.51	0.18	1.59	2.67	0.67	0.923	0.538
SPT	7	Buckeye	27	6	1.6	0.23	0.02	0.65	4.47	0.75	1	0.327
SPT	8	Bay laurel	22	4	0.71	0.34	0.06	0.13	2.30	0.58	0.917	0.692
SPT	9	Bay laurel	17	2	0.55	0.21	0.03	0.47	1.56	0.78	0.808	0.487
SPT	10	Bay laurel	46	2	0.68	0.17	0.04	0.96	1.94	0.97	0.987	0.442
SPT	11	Bay laurel	54	4	0.96	0.16	0.03	0.91	2.28	0.57	0.974	0.558
SPT	12	Douglas fir	5	3	0.95	0.43	0.12	0.87	2.27	0.76	0.929	0.494
Mean			23.5	3.3	0.8	0.42	0.12	1.47				

*T = transect; dbh = diameter in centimeters at the tree breast; D/S = measure of evenness; BB = Big Basin State Park; HR = Humboldt Redwoods State Park; HW = Hendy Woods State Park; SPT = Samuel P. Taylor State Park.

spring (629) than in the fall (249). Old recaptured animals (i.e., the animal had been caught at least six months earlier) were obtained 82 times and included 26 chipmunks, 10 woodrats, 44 deer mice, 1 flying squirrel, and 1ne vole. New recaptures (i.e., the animal had been caught in the previous week) occurred 134 times and included 16 chipmunks, 100 deer mice, 8 woodrats, and 1 vole. Transects had 1–5 mammal species and species evenness tended to be relatively low (< 30%) except at SPT (Table 2). Overall, relatively few individual small mammals were caught on any given transect with the notable exception of *Peromyscus* spp. at BB.

Ten tick species were identified on small mammals, most commonly *I. pacificus*, *I. angustus*, *I. ochotonae*, and *I. spinipalpis* (Table 4). Only immature *Dermacentor* spp. ticks and only adult *I. woodi* were recovered. Host species with high species richness were woodrats with five tick species, deer mice with eight tick species, and redwood chipmunks (*T. ochrogenys*) with six tick species. Southerly sites (BB and SPT) had primarily *I. angustus* and *I. pacificus*. In northern sites, there was greater tick diversity including seven species at HW and six species at HR. The maximum richness of ticks on any transect was 5. Tick abundance on small mammals ranged from 0 to 32 (Table 2).

The overall seroprevalence in small mammals was 7.31% (95% CI = 5.5–9.6%; Table 5). Highest values from 12% to 27% occurred in western red-backed voles, flying squirrels, dusky-footed woodrats, and redwood chipmunks (Table 5). Much lower or zero values were observed in meadow voles,

TABLE 2

Summary of mammals caught and tested for *Anaplasma phagocytophilum* on 48 transects in four state parks in northern California*

Park	T	No. of mammal species	Shannon-Wiener mammals	Simpson's D mammals	D/S mammals	No. chipmunks	No. of wood rats	No. of deer mice	No. of tick species	No. of ticks on mammals	Seroprevalence of *A. phagocytophilum*
BB	1	4	0.825	1.91	0.64	0	4	12	1	5	0
BB	2	3	0.687	3.05	0.76	0	0	8	1	1	0
BB	3	4	0.97	2.42	0.81	0	7	14	3	6	0
BB	4	3	0.649	1.58	0.53	0	0	19	2	2	0.091
BB	5	3	0.974	2.46	0.82	0	0	21	1	2	0.045
BB	6	4	0.661	1.55	0.52	0	2	15	1	1	0
BB	7	5	0.808	1.97	0.66	0	1	12	2	3	0.067
BB	8	2	0.598	1.69	0.84	0	0	12	1	1	0
BB	9	4	0.697	1.64	0.55	0	4	30	1	1	0
BB	10	3	0.659	1.61	0.54	0	1	18	3	5	0
BB	11	4	1.23	3.03	0.76	0	2	14	1	1	0
BB	12	3	1.17	2.67	0.67	2	2	20	1	1	0
HR	1	3	0.956	2.33	0.78	0	2	2	1	1	0.20
HR	2	3	0.824	1.95	0.65	0	1	1	0	0	0.09
HR	3	3	0.409	1.24	0.41	0	1	1	2	4	0.13
HR	4	2	0.451	1.38	0.69	0	0	0	3	4	0.00
HR	5	3	0.501	1.34	0.45	0	1	1	3	11	0.00
HR	6	2	0.598	1.69	0.84	0	0	0	1	2	0.33
HR	7	3	0.708	1.73	0.58	5	0	0	3	14	0.20
HR	8	1	0	1.17	0.58	0	0	0	1	2	0.20
HR	9	2	0.271	1.17	0.58	1	0	0	1	1	0.10
HR	10	3	0.463	1.29	0.43	1	0	0	1	12	0.00
HR	11	4	1.038	2.42	0.61	2	0	0	2	4	0.17
HR	12	3	1.03	2.56	0.85	4	0	0	1	3	0.45
HW	1	2	0.586	1.65	0.83	2	0	8	2	10	0
HW	2	3	0.889	2.86	0.95	6	0	5	2	3	0
HW	3	3	0.831	2.03	0.68	2	0	8	3	16	0
HW	4	2	0.637	1.80	0.90	1	0	4	1	8	0
HW	5	3	0.918	2.31	0.77	8	0	10	3	24	0
HW	6	3	0.837	2.14	0.71	8	1	8	5	17	0
HW	7	2	0.679	1.95	0.97	3	0	3	4	7	0
HW	8	5	1.354	2.86	0.57	6	4	8	4	9	0.21
HW	9	3	0.868	2.22	0.74	5	1	4	3	18	0.14
HW	10	2	0.681	1.95	0.98	4	0	7	2	4	0
HW	11	4	1.33	3.72	0.93	4	4	7	3	5	0
HW	12	4	1.34	3.65	0.91	3	4	6	3	8	0.08
SPT	1	2	0.215	1.12	0.56	0	1	13	2	4	0
SPT	2	2	0.52	1.51	0.75	0	0	8	3	5	0
SPT	3	1	0	1.32	0.66	3	0	7	1	5	0
SPT	4	1	0	1.00	1.00	0	0	2	2	4	0
SPT	5	2	0.41	1.32	0.66	0	0	9	1	1	0
SPT	6	2	0.325	1.22	0.61	0	1	6	3	6	0
SPT	7	2	0.362	1.26	0.63	0	2	13	1	5	0
SPT	8	2	0.649	1.84	0.92	0	4	9	5	15	0
SPT	9	2	0.462	1.40	0.70	0	2	18	5	23	9.5
SPT	10	2	0.41	1.32	0.606	0	1	6	1	18	0
SPT	11	2	0.223	1.12	0.56	0	0	14	1	10	0
SPT	12	3	0.63	1.54	0.77	1	3	15	4	32	0

*T = transect; D/S = measure of evenness; BB = Big Basin State Park; HR = Humboldt Redwoods State Park; HW = Hendy Woods State Park; SPT = Samuel P. Taylor State Park.

all *Peromyscus* spp., shrews, and Sonoma and Merriam's chipmunks. The PCR prevalence among small mammals tested was 5.5% (n = 175, 95% CI = 2.9–10.6), with highest values in flying squirrels, dusky-footed woodrats, and chipmunks (Table 6).

The sex ratio of all small mammals was 281 F:316 M; neither the sex ratio of seropositive small mammals (21 F:27 M) nor PCR-positive small mammals (5 F:4 M) were statistically significant ($P > 0.55$). In the spring, 6 (6.6%) of 91 animals tested were PCR positive; results in the fall were 4 (4.8%) of 84. These differences were not statistically significant ($P = 0.845$). However, for serologic analysis, seropositive small mammals were more prevalent in the fall (24 of 211 tested, 11.4%) than in the spring (24 of 448 tested, 5.4%) ($P = 0.008$). Location was an important determinant of exposure to *A. phagocytophilum*, with an order of magnitude higher seroprevalence in the two more northern parks (14–15%) than the southern parks (1–2%) (Table 6). Interaction terms between species and park were not significant ($P = 0.08$). PCR-prevalence was 0 at BB but > 0 in the three more northerly parks (Table 6). Odds ratios significantly > 1 were observed for seropositivity for the two northern parks and for woodrats and redwood chipmunks (Table 7). Exposure to *A. phagocytophilum* was more likely among chipmunks in redwood sites at one park, but more likely for woodrats and oaks at another park. The mean tick load on seropositive animals was 0.67 ticks with 0.19 ticks/seronegative animal. This difference was statistically significant ($P < 0.01$).

When we performed plotting and logistic regression for each potential variable that could account for seropositive animals on a transect, statistically significant odds ratios were detected for those variables shown in Table 7. Those variables

TABLE 3
Numbers of small mammals (and % of small mammals caught at that site) of each species caught in four state parks in northern California

Species	Big Basin	Humboldt Redwoods	Hendy Woods	Samuel P. Taylor	Total (%) of all rodents caught
Clethrionomys californicus	0 (0)	0 (0)	8 (5.1)	0 (0)	8 (1.2)
Glaucomys sabrinus	0 (0)	3 (2.2)	0 (0)	0 (0)	3 (0.5)
Microtus californicus	1 (0.5)	1 (0.7)	0 (0)	0 (0)	2 (0.3)
Neotoma fuscipes	23 (10.5)	5 (3.6)	14 (9.0)	14 (9.9)	56 (8.6)
Peromyscus boylii	2 (0.9)	0 (0)	0 (0)	0 (0)	2 (0.3)
Peromyscus californicus	125 (56.8)	0 (0)	0 (0)	0 (0)	125 (19.2)
Peromyscus maniculatus	46 (20.9)	103 (74.6)	77 (49.4)	120 (85.1)	346 (53.8)
Peromyscus truei	19 (8.6)	6 (4.4)	0 (0)	0 (0)	25 (3.8)
Sorex sp.	2 (0.9)	7 (5.1)	6 (3.9)	3 (2.1)	18 (2.8)
Tamias merriami	2 (0.9)	0 (0)	0 (0)	0 (0)	2 (0.3)
Tamias ochrogenys	0 (0)	13 (9.4)	51 (32.7)	0 (0)	64 (9.8)
Tamias sp.	0 (0)	0 (0)	0 (0)	4 (2.8)	4 (0.6)
Total	220	138	156	141	655

for which $P \leq 0.1$ were included in a multivariate model, with the following model minimizing Akaike's information criterion: seroprevalence ~ number of mammal species + park + number of *Peromyscus* spp. + number of trees + mean dbh + variance dbh + largest tree + percent shrub + vegetation D + vegetation D/S. Although the largest tree was retained in the multivariate model, a plot of the prevalence against the largest tree on a transect suggested a peak in prevalence when the dbh was intermediate (shown for the two high prevalence parks, HW and HR, in Figure 1). A purely quadratic model for the largest tree increased the R^2 to 11% ($P = 0.01$), compared with 9% ($P = 0.04$) for the linear model. Numbers of deer mice and woodrats, but not chipmunks, also were linearly related to size of the largest tree, with statistically significant R^2 of 9% ($P = 0.04$) and 12% ($P = 0.02$), respectively.

DISCUSSION

An important ecosystem service provided by intact forest ecosystems is protection from emerging infectious diseases of humans and animals. Anthropogenic change in forest integrity may modify population dynamics and interactions in reservoir hosts and arthropod vectors of disease. We show that mature redwood and peripheral forests in intermediate stages of regeneration after habitat change may have altered vertebrate host diversity and infection prevalence with the potentially fatal, tick-borne agent of human granulocytic anaplasmosis, and thus may represent a risk to humans and susceptible animals.

In multiple other regions of the world, habitat change has been associated with the emergence of amenable habitat for arthropod vectors of disease to increase in range and abundance. Examples include the change of natural grasslands to corn monocultures that contributed to the emergence of Machupo virus in Bolivia and Argentine hemorrhagic fever[20] and logging and road building in Central America, which influenced the increase in leishmaniasis by increasing suitable habitat for the sand fly vector.[21] The emergence of Lyme disease, caused by the spirochete *Borrelia burgdorferi,* is another important example. Lyme disease increased from 44 reported cases in 1977 to 19,9311 reported cases in 2006, including hundreds of cases from California.[22] The emergence in the eastern United States was caused by reforestation of abandoned farmland that led to increases of white-tailed deer (*Odocoileus virginianus*) populations and subsequent increases of the tick vector, *Ixodes scapularis*.[23] Second-growth and successional vegetation provides for increased herbivorous mammal populations (e.g., deer and rodents), particularly the eastern U.S. reservoir of Lyme disease, the white-footed mouse (*Peromyscus leucopus*). Effects driving increasing tick populations are synergistic: increased deer and rodent numbers provide for a numerical response enabling increased tick numbers, and leaf litter from shrubs increases tick survival by providing optimal habitat for diapausing and molting immature ticks.

The ecology, enzootic persistence, and probability of emergence of granulocytic anaplasmosis are intricately tied to the host-vector-pathogen relationship it shares with *Ixodes* spp. ticks and multiple small mammal hosts. The rodent reservoirs (woodrats, chipmunks, and squirrels) are differentially distributed among forest types we studied. Squirrels were observed in trees in the present study but not captured in traps. Woodrats, in particular, have been reported in association with oak and

TABLE 4
Tick diversity on rodents collected at four California state parks*

Host species (no. tested)	*Dermacentor* spp.			*Ixodes* sp.			*I. angustus*			*I. jellisoni*			*I. ochotonae*			*I. pacificus*			*I. spinipalpis*			*I. woodi*			Total		
	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L
Clethrionomys californ icus (8)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Neotoma fuscipes (56)	0	1	0	0	0	0	0	9	0	0	0	0	0	0	0	1	2	3	7	2	0	3	0	0	12	14	3
Peromyscus californicus (125)	0	0	0	2	0	1	0	3	0	0	0	0	0	0	0	0	0	1	0	0	0	3	0	0	5	3	2
Peromyscus maniculatus (346)	0	3	1	5	0	0	1	17	2	1	0	0	9	0	0	7	1	19	0	0	3	2	0	0	25	21	25
Peromyscus truei (25)	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Sorex sp. (18)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	4
Tamiaas ochrogenys (64)	0	0	1	1	0	3	1	15	3	0	0	0	7	0	0	0	6	79	0	1	0	4	0	0	13	22	86
Tamias sonomae (4)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
Total	0	4	2	8	0	4	2	26	5	1	0	0	8	0	0	9	9	107	7	3	3	12	0	0	69	60	121

*A = adult ticks; N = nymphs; L = larvae.

Table 5

Seroprevalence and polymerase chain reaction prevalence of *Anaplasma phagocytophilum* among small mammals species in four northern California state parks*

Species	*A. phagocytophilum* IFA assay		*A. phagocytophilum* major surface protein 2 PCR	
	No. seropositive	% Seroprevalence (95% CI)	No. PCR positive	% PCR prevalence (95% CI)
Clethrionomys californicus	1	12.50 (0.6–53.3)	0	0 (0–40.2)
Glaucomys sabrinus	1	25.00 (1.3–78.1)	1	25.0 (1.3–78.1)
Microtus californicus	0	0.00 (0–80.2)	0	0 (0–80.2)
Neotoma fuscipes	8	12.90 (6.1–24.4)	6	10.5 (4.4–22.2)
Peromyscus boylii	0	0.00 (0–80.2)	NT	
Peromyscus californicus	1	0.78 (0–4.9)	NT	
Peromyscus maniculatus	15	4.63 (2.7–7.7)	NT	
Peromyscus truei	1	3.45 (0.2–19.6)	NT	
Peromyscus spp.	0	0.00 (0–60.4)	NT	
All *Peromyscus*	17	3.49 (2.1–5.6)	0	0 (0–20.0)
Sorex spp.	0	0.00 (0–37.1)	0	0 (0–53.7)
Tamias merriami	0	0.00 (0–69.0)	0	0 (0–80.2)
Tamias sonomae	0	0.00 (0–60.4)	2	50.0 (15.0–85.0)
Tamias ochrogenys	21	26.92 (17.8–38.3)	1	1.4 (0–8.4)
All *Tamias*	21	24.71 (16.3–35.5)	3	3.8 (0.9–11.4)
Total	48	7.31 (5.5–9.6)	10	5.5 (2.9–10.6)

* IFA = immunofluorescent antibody; PCR = polymerase chain reaction; CI = confidence interval; NT = not tested.

are common in some second-growth coniferous forests.[24,25] Our data show that woodrats were most abundant in our older oak sites especially at HW, but also common in younger oak, Douglas fir, and redwood transects. Seroprevalence rates in woodrats in the present study were intermediate of what has been reported in other studies, ranging from relatively low positive values of 2–5% to as high as 90% in hyperenzootic locations.[26] Seropositive and PCR-positive woodrats were present in diverse transects.

At HR, chipmunks appeared to play a similar role to that of woodrats at HW (i.e., chipmunks occurred in a more specialized cluster of seropositive rodents with redwoods, and woodrats and deer mice were more generalist). Relatively few studies have evaluated the ecologies of western chipmunks. Across the parks we studied, abundant chipmunks included the Sonoma (*T. sonomae*) and redwood chipmunks. Although Merriam's chipmunk (*T. merriami*) was found at BB, it was rarely encountered except in transect 12, which was essentially early successional with chamise scrub. Merriam's chipmunk has a broad geographical and habitat range but with a preference for brush and chaparral with trees, logs, stumps, and litter.[27] Because it was infrequently observed, never found seropositive, and inhabits habitats not intended for inclusion in the present study, its role in anaplasmosis could not be determined in the present study. In contrast, the redwood chipmunk at the northern sites appeared to be a key player, often found exposed to *A. phagocytophilum*, and at high density with stable populations (e.g., with frequent recaptures of animals 1–2 years after initial capture). Redwood chipmunks are reported to inhabit humid coastal forest with extensive understory shrubby vegetation or downed woody debris.[28] At HR, there was a tendency for chipmunks to focus on more mature redwood sites although they were also common in other transects and were present at all sites in HW. Redwood chipmunks are excellent hosts for *A. phagocytophilum*, with persistent infections associated with high levels of bacteremia.[7] A study also detected a PCR-positive eastern chipmunk (*T. striatus*) from Minnesota among 28 tested.[29]

Deer mice also were common at multiple sites in all parks, but they were almost never exposed to *A. phagocytophilum*, as reported,[26,30] and our attempts in the laboratory to experimentally infect then have been unsuccessful (Foley JE, unpublished data). Deer mice function to contribute to tick persistence but not infection, potentially sharing this role with other hosts such as lizards and possibly birds, shrews, and voles, which may support juvenile *I. pacificus* but appear not to support

Table 6

Regional seroprevalence and PCR prevalence rates for exposure to *Anaplasma phagocytophilum* in small mammals in four California state parks*

Park	*A. phagocytophilum* IFA assay		*A. phagocytophilum* major surface protein 2 PCR	
	No. seropositive	% Seroprevalence (95% CI)	No. PCR positive	% PCR prevalence (95% CI)
Big Basin	4	1.7 (0.6–4.7)	0	0 (0–14.6)
Humboldt Redwoods	19	14.2 (8.9–21.5)	2	6.7 (0.9–20.5)
Hendy Woods	23	15.1 (10.0–22.1)	6	6.8 (2.8–14.8)
Samuel P. Taylor	2	1.4 (0.2–5.6)	2	8.7 (1.5–29.5)

* PCR = polymerase chain reaction; IFA = immunofluorescent antibody; CI = confidence interval.

Table 7

Risk factors for *Anaplasma phagocytophilum* seropositivity in small mammals in four state parks in northern California with statistically significant odds ratios*

Risk factor for individual serostatus	Odds ratio (95% CI)	*P*
Neotoma fuscipes	8.33 (6.14–10.53)	0.015
Tamias ochrogenys	20.7 (16.72–24.68)	3.4×10^{-8}
Humboldt Redwoods	25.53 (24.44–26.63)	6.3×10^{-5}
Hendy Woods	27.66 (26.58–28.74)	2.6×10^{-5}
Risk factor for transect serostatus		
Largest tree	4,447.07 (1.49–13,256,519.14)	0.04
No. of trees	0.85 (0.74–0.99)	0.03
Variance in dbh	1,176.15 (7.96–173,859.02)	0.01
Mean dbh	1,112.09 (0.87–1,428,306.45)	0.05
D/S vegetation	0.00 (0.00–0.67)	0.03
Percent shrub	0.00 (0.00–0.67)	0.05
No. deer mice	1.15 (1.08–1.23)	4.06×10^{-5}
No. of woodrats	0.14 (0.02–0.88)	0.03
No. of ticks	0.09 (0.02–0.57)	0.008
D/S mammals	0.05 (0.01–0.32)	0.002

* CI = confidence interval; dbh = diameter in centimeters at the tree breast; D/S = measure of evenness.

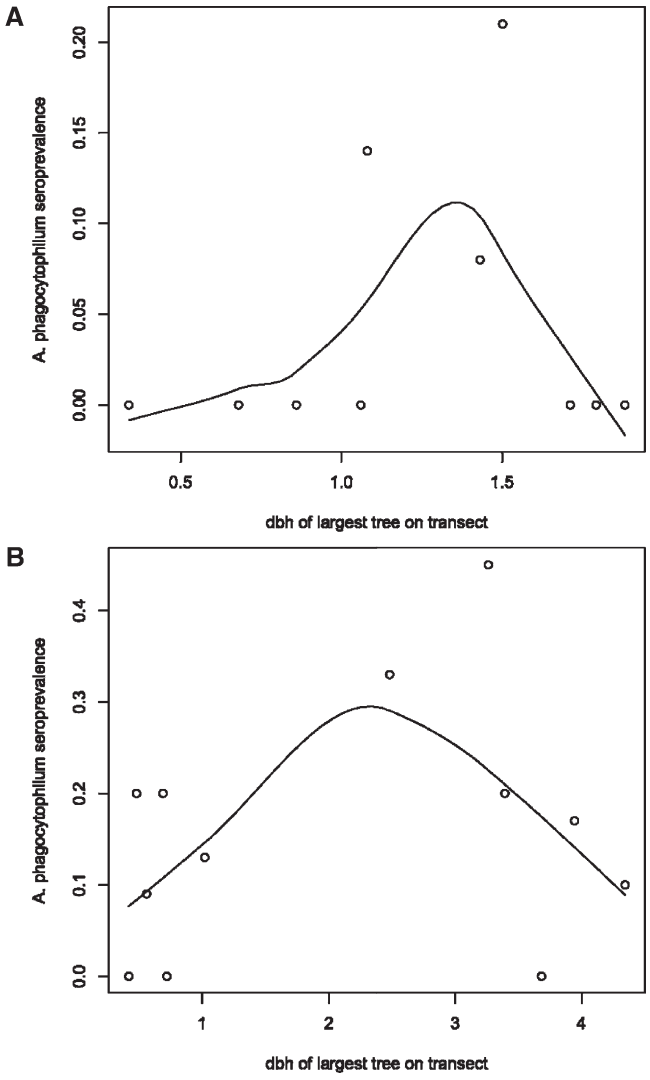

FIGURE 1. Comparison of rodents and *Anaplasma phagocytophilum* seroprevalence as a function of the diameter at breast height in meters of the largest tree individual in two state parks in northern California. **A**, Humboldt Redwoods State Park. **B**, Hendy Woods State Park.

infection. Because there is no transovarial transmission of *A. phagocytophilum* and *Ixodes* spp. ticks feed only once per stage, the key to enzootic persistence of granulocytic anaplasmosis is acquisition by juvenile ticks of infection from a small mammal reservoir, transstadial transmission through a molt, and transmission as a nymph or adult to another host. Thus, the actual contributions of the small mammals we studied to enzootic granulocytic anaplasmosis depend on the animals' abundances, habitat use, interactions with ticks, and support of vector-competent tick species. In this report and elsewhere,[7] we have found that redwood chipmunks have high mean tick burdens. Analogously, in addition to maintaining active infection, chipmunks in the eastern United States support high tick numbers, with a strong correlation between chipmunk numbers and the density of nymphal deer ticks one year later.[31]

Tick numbers and tick diversity were high at northern parks compared with southern parks, with notably high tick diversity on woodrats, deer mice, and chipmunks. The significance of this finding is not clear because it is not known whether species other than *I. pacificus* and *I. spinipalpis* could be vector competent. *Ixodes spinipalpis* was found on woodrats, deer mice, and redwood chipmunks. In Colorado where *I. pacificus* is not found, there is an *N. mexicana* and *I. spinipalpis* enzootic cycle for *A. phagocytophilum*[32] but whether it vectors this pathogen among small mammals in California is not known. *Ixodes angustus* was the second most commonly identified tick species in the present study and was also found on deer mice, woodrats, and redwood chipmunks. It was present at all sites. Not as catholic a feeder as *I. pacificus*, *I. angustus* is primarily a nidicolous tick that feeds on a variety of rodents and occasionally bites humans.[15] Its vector competence for *A. phagocytophilum* is not known.

Tick numbers have been evaluated as a function of habitat type previously.[33,34] Specifically, higher nymphal questing activity generally is associated with greater proportions of oak in an environment compared with redwood.[35] However, in addition to tick number *per se*, high tick species diversity may function with prolonged survival and questing periods for ticks to contribute to enzootic granulocytic anaplasmosis in some north coast range sites. Unfortunately, few or no datasets of which we are aware have evaluated tick species diversity across an array of habitat types in California. Interestingly, increased risk of enzootic *B. burgdorferi* was detected in sites with relatively low tick numbers but with local habitat and climate features that promoted prolonged questing seasons for nymphal ticks, with HW included in a previous study in one such area.[34]

A transect with high seroprevalence in *A. phagocytophilum* in our study had high biodiversity in ticks, rodents, and vegetation, as well as intermediate-sized trees with a high mean and variance in dbh. Particular rodent and plant species were not required for high seroprevalence. We were not able to evaluate the contributions of several other members of the community, including reptiles, large mammals, and birds in this study. However, deer and reptiles are unlikely to support enzootic anaplasmosis because the former supports primarily adult ticks and reptiles were recently shown to be reservoir incompetent for *A. phagocytophilum*.[35] Moreover, both deer and birds would likely contribute to anaplasmosis at a coarser scale than that used in the present study. Nevertheless, further evaluation of the roles of all of these vertebrates is warranted.

It is interesting to consider the generality of the present findings for other diseases in California as well, such as Lyme disease. In many respects, Lyme disease and granulocytic anaplasmosis share an ecology, with both capable of infecting woodrats and squirrels, neither infecting lizards, both vectored by the same tick, and neither apparently affecting one of the most abundant small mammals, the deer mouse.[2,6,8,35,36] One possible differences between the diseases is that, at least in the areas we studied, chipmunks play a key role as reservoirs for granulocytic anaplasmosis.[29] The pathogens share similar spatial distributions at the coarse scale including north coast range mountains and Sierra Nevada foothills. Because the present study did not systematically evaluate a range of habitat types, it was not clear how closely matched are habitat determinants of *A. phagocytophilum* and *B. burgdorferi* infection rates. This factor is an important target of ongoing research.

Old-growth redwood ecosystems are not considered as climax because of continual renewal caused by fire and other natural effects, and the age of sites in the present study could not be determined for the relatively fine scale at which data

were collected. Characteristics of old-growth redwood ecosystems include presence of individual large trees, snags, and downed logs, with multiple canopy layers, low vegetation diversity, and high variance in tree size.[37] Thus, some high prevalence transects in this study had characteristics compatible with old-growth, but the apparently oldest sites, with the largest single trees, had diminished seroprevalence compared with those sites with relatively smaller trees. Thus, the two signals associated with enzootic granulocytic anaplasmosis in this study were high biodiversity and mature second-growth forest.

These findings are not consistent with a dilution effect theory by which the force of infection for some pathogens such as *B. burgdorferi* in the eastern United States can be reduced in the presence of high biodiversity because reservoir-incompetent species buffer against the maintenance effects of reservoirs; such reservoirs could occur disproportionately in areas with low biodiversity.[38] Rather, our data suggest that heterogeneity in western communities may help support anaplasmosis, and in the present study, such conditions were present in second-growth forest. This phenomenonological pattern requires mechanistic explanation, which should be an emphasis for further study. In the end, however, it should not be surprising that a complex community would support not only diverse vegetation and vertebrates but also invertebrate vectors and disease agents.

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