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Vector biodiversity did not associate with tick-borne pathogen prevalence in small mammal communities in northern and central California

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Abstract

Vector and host abundance affect infection transmission rates, prevalence, and persistence in communities. Biological diversity in hosts and vectors may provide "rescue" hosts which buffer against pathogen extinction and "dilution" hosts which reduce the force of infection in communities. *Anaplasma phagocytophilum* is a tick-transmitted zoonotic pathogen that circulates in small mammal and tick communities characterized by varying levels of biological diversity. We examined the prevalence of *A. phagocytophilum* in *Ixodes* spp. ticks in 11 communities in northern and central California. A total of 1020 ticks of 8 species was evaluated. Five percent of ticks (5 species) were PCR-positive, with the highest prevalence (6–7%) in *I. pacificus* and *I. ochotonae*. In most species, adults had a higher prevalence than nymphs or larvae. PCR prevalence varied between 0% and 40% across sites; the infection probability in ticks increased with infestation load and prevalence in small mammals, but not tick species richness, diversity, evenness, or small mammal species richness. No particular tick species was likely to "rescue" infection in the community; rather the risk of *A. phagocytophilum* infection is related to exposure to particular tick species and life stages, and overall tick abundance.

Keywords

Amplification effect; Dilution effect; Granulocytic anaplasmosis; Rescue effect

Introduction

The fate of a pathogen upon introduction into a community can be extinction, enzootic persistence, or emergence into an epizootic depending on biotic and abiotic factors that influence host, vector, and pathogen survival and how many individuals in each host species are susceptible, infected, or immune. Biological diversity could reduce the probability of

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particular hosts acquiring infection, increase this risk, or "rescue" infection in a community (Keesing et al., 2006). A highly biodiverse disease system provides opportunity to evaluate whether infection prevalence varies due to dilution or amplification effects. In this paper, we consider the prevalence of an obligately tick-transmitted bacterial pathogen, *Anaplasma phagocytophilum*, in communities of small mammal hosts and ixodid ticks in California. The distribution of *A. phagocytophilum* is Holarctic and the *Ixodes* spp. ticks which transmit this bacterium feed only once per stage as larvae, nymphs, and adults, transmitting the infection transstadially, but not transovarially (Munderloh and Kurtti, 1995; Foley et al., 2004). Most studies of *A. phagocytophilum* ecology have focused on bridge vectors, i.e. *Ixodes* spp. ticks such as the western black-legged tick (*I. pacificus*) in the western U.S. which has diverse feeding habits as adults, typically questing on vegetation seasonally for a variety of large mammals (Foley et al., 2004). These bridge vectors do contribute to maintenance cycles with small mammal hosts, but often host-specialist or nidicolous ticks also may be responsible for pathogen maintenance (Foley et al., 2011). California has 20 species of ticks in the *Ixodes* genus including the known vector-competent *I. pacificus* and *I. spinipalpis* and other relatively common small mammal-feeding species such as *I. woodi* and *I. angustus*. Small mammal diversity is high, and the bacteria themselves are actually a diverse set of closely related strains or genospecies (Foley et al., 2009; Rejmanek et al., 2011). Biological diversity in many areas is very high and overall highly variable.

In the present study, we collected small mammals and ticks from 11 sites where there was evidence of *A. phagocytophilum* and varying levels of tick biological diversity. We assessed ticks for the presence of *A. phagocytophilum* DNA and determined whether individual-level (tick species, stage, or capture method) or site-level factors (tick species richness, evenness, and diversity, number of ticks per host, small mammal species richness, or prevalence of *A. phagocytophilum* in small mammals) could account for the patterns of infection we obtained.

Materials and methods

Study sites and trapping

Small mammal trapping and tick collection were performed at 11 sites in northern and central California from February 2005 to January 2012 (Table 1). Sampling was performed at each site at least 6 times in that interval. At each site, transects were established along deer trails and poorly used human trails and roads. Flagging for ticks was performed over herbaceous and shrubby vegetation as well as duff and litter using a 1-m^2 white cotton flag. In order to obtain small mammals and their attached ticks, extra-large (10×10.4×38 cm) Sherman (HB Sherman, Tallahassee, FL) and Tomahawk (Tomahawk Live Trap, Tomahawk, WI) live traps were set overnight at locations of observed active rodent usage and baited with peanut butter and oats. Rodents were anesthetized with approximately 20 mg/kg ketamine and 3 mg/kg xylazine delivered SC, examined for ectoparasites, and given a permanent individually numbered metal ear tag. Blood samples were collected from the retroorbital sinus into EDTA. Ticks were removed with forceps and preserved in 70% ethanol. *Ixodes* spp. were identified to species using keys (Furman and Loomis, 1984; Webb et al., 1990). Larvae were examined under both a dissecting and a compound microscope in

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a depression slide. All work with small mammals was performed under the oversight of the UC Davis Attending Veterinarian and the Institutional Animal Care and Use Committee.

Polymerase chain reaction for *A. phagocytophilum* infection

Ticks and small mammal blood samples were assessed for *A. phagocytophilum* infection by polymerase chain reaction (PCR). DNA was extracted from mammalian blood using a kit (Qiagen Blood and Tissue Kit, Valencia, CA, USA) following manufacturer's instructions. DNA was extracted from ticks using a protocol modified from Humair et al. (2007). Ticks were surface-cleaned with 70% ethanol, the ethanol was allowed to evaporate, ticks were frozen in liquid nitrogen for 3 min, and then crushed with a pestle. The ticks were then boiled for 15 min in 100 μl of 0.7 M NH_4OH, cooled quickly for 30 s on ice, and then boiled again for 15 min in open vials to evaporate ammonia. We previously showed that ammonium hydroxide boiling did not affect DNA yield from questing ticks compared with Qiagen extraction, when we compared the cycle threshold (CT) from TaqMan PCR of the 18S rDNA gene using a purchased primer and probe set (Applied Biosystems; Cleopatra del Prado and Foley, unpubl. data). For this study, we randomly selected fed adult *I. pacificus* and subjected half to Qiagen extraction which might better remove PCR inhibitors from blood and the other half to the ammonium hydroxide method. The mean CT from Qiagen (17.4) was slightly, but significantly (p=0.003) lower than from ammonium hydroxide (20.3).

Real-time quantitative PCR was performed targeting the multiple-copy *msp2* gene of *A. phagocytophilum* as previously described (Drazenovich et al., 2006). Each 12-μl reaction contained 5 μl DNA, 1X TaqMan Universal Master Mix (Applied Biosystems), 2 nmol of each primer, and 400 pmol of probe. The amplification cycle consisted of 50°C for 2 min, 95°C for 10 min, and 40 cycles at 95°C for 15 s, followed by 60°C for 1 min. Samples were considered positive if they had a CT value <40 and characteristic amplification plots. For all reactions, 3 water negative controls and a DNA sequence-confirmed positive DNA control were included during each run.

Data 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). The cutoff for statistical significance was p=0.05.

Summary statistics were calculated for each site and overall. PCR prevalence was calculated in ticks by species, stage, and site; in hosts by genus; and in reservoir hosts (i.e. woodrats, chipmunks, and squirrels). The stage distribution of flagged ticks was compared to that for ticks removed from small mammals with a chi-square contingency test. With the non-simultaneous sampling across sites, we could not analyze for temporal trends, but acknowledge that there may have been seasonal or other dynamic influences we cannot account for.

We used a mixed logistic regression model, function glmer in the R package lme4 (Bates et al., 2011), with site as a random effect, to evaluate the dependence of PCR results in ticks on

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individual and site-level predictors. Prior to analysis, we examined potential predictor variables for collinearity and suitability for analysis. Individual-level predictors were tick stage, species, and capture method (i.e., flagging vs. rodent trapping). Due to low sample sizes, the rare species *I. auritulus* (n=1), *I. sculptus* (n=1), and *I. soricis* (n=2) were dropped from the analysis. While DNA extraction methods yielded slightly different quality and quantity DNA from flagged vs. host-fed ticks, by retaining capture method and stage in the model, we were able to detect whether there was an effect of capture method beyond the signal that derives from tick stage, since adults were more likely to be flagged than other stages.

Site-level predictors were: tick species richness (S), diversity (Simpson's reciprocal index D $[1/\Sigma(p_i)^2]$), and evenness (D/S) (Begon et al., 1996); infestation load (number of small mammal-feeding ticks found at each site divided by the total number of small mammals captured at that site); mammal species richness; prevalence of *A. phagocytophilum* DNA in small mammals; and prevalence in reservoir hosts. We included host richness in the model, but not other metrics of host diversity because we do not believe that small mammal trap success was unbiased across species. Although the same trapping bias would be expected across sites, we have confidence in the host species richness estimates, but not some other derived metrics such as population size, evenness, or diversity.

Correlation coefficients between other site-level predictors were evaluated and all but one were <0.8, and thus predictors retained in the model [small mammal and tick species richness were moderately correlated (r=0.75)]. Prevalence in reservoirs and all small mammals were correlated with r=0.85, and therefore we used only the prevalence in all small mammals in the analysis. Interactions between predictor variables were not included.

We used reverse step-wise model selection to select the model best-suited to our data (Zuur et al., 2009). We first used AIC to determine which term added the least information to the model. We then evaluated the significance of that term using a likelihood ratio (LR) test and removed the term if the LR test was not significant. This process was repeated until all terms that remained in the model were significant. Post-hoc Tukey tests were used to evaluate differences in PCR positivity among tick species and life stages utilizing the package 'multcomp' in R (Hothorn et al., 2008).

Results

A total of 1020 ticks was evaluated across 11 sites in northern and central California. There were 8 tick species including *I. angustus*, *I. auritulus*, *I. ochotonae*, *I. pacificus*, *I. sculptus*, *I. soricis*, *I. spinipalpis*, and *I. woodi. Ixodes pacificus* was found at all sites, *I. spinipalpis* at 9 sites, *I. angustus* at 8, *I. woodi* at 7, *I. ochotonae* at 5, and the other species only at one site each (Table 2). Six tick species were collected on flags, including 2 *I. angustus*, one *I. auritulus*, 324 *I. pacificus*, one *I. sculptus*, 19 *I. spinipalpis*, and 7 *I. woodi.* The total number of ticks collected on flags was 354, compared with 684 removed from small mammals. Animals from which ticks were collected included woodrats (*Neotoma* spp.), chipmunks (*Tamias* spp.), California ground squirrels (*Otospermophilus beecheyi*), tree squirrels (*Sciurus* spp.), Douglas squirrels (*Tamiasciurus douglasii*), voles (*Myodes californicus*),

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deer mice (*Peromyscus* spp.), and Trowbridge shrews (*Sorex trowbridgii*) (Table 3). The distribution of tick life stages differed between those collected on flags vs. mammals (χ^2 = 285.74, df =2, p<0.001); the majority of ticks captured by flagging were adults (adults : nymphs : larvae = 1 : 0.48 : 0.2), whereas more nymphs and larvae were removed from small mammals (adults : nymphs : larvae = 0.47 : 1 : 0.7). The distribution of tick stages is given in Table 4 (in some cases, stage and gender of ticks were not recorded – these ticks were omitted from this table).

Five percent (3.5–6.3% 95% C.I.) of all ticks were PCR-positive, varying by species, stage, and whether or not ticks were obtained from hosts (Tables 5 and 6). There were significant differences between tick species in *A. phagocytophilum* prevalence (χ^2 = 21.58, df =4, p=0.0002); *I. pacificus* had a greater prevalence (6%, 4.2–8.0% 95% C.I.) than *I. angustus* (1%, 0.2–4.2% 95% C.I.), but *I. ochotonae*, *I. woodi*, and *I. spinipalpis* were not statistically distinguishable from other species (Table 5). There were significant differences in prevalence between tick life stages as well (χ^2 = 10.18, df =2, p=0.006), with a higher prevalence in adults (5.7%; 5.5–11.6% 95% C.I.) than in larvae (2.3%; 1.1–4.7% 95% C.I.); nymphs were not significantly different from either adults or larvae (3.9%; 1.9–6.6% 95% C.I.). This pattern was driven largely by *I. pacificus*, but was reflected in *I. angustus*, *I. woodi*, and *I. spinipalpis* as well (Table 6). When tick species and life stage were accounted for, there was no evidence for a difference in *A. phagocytophilum* prevalence between ticks captured by flagging and those captured on small mammals (χ^2 =0.75, df =1, p=0.39).

Across sites, *A. phagocytophilum* PCR prevalence in ticks varied between 0 and 40% with the highest rates at Henry Cowell and Hendy Woods (Table 7). Infestation loads varied from a low of 0.053 ticks per host at Big Basin to 0.891 at Boggs Mountain, and the probability of finding *A. phagocytophilum* in ticks increased significantly with infestation load (χ^2 = 8.73, df =1, p=0.003). *Anaplasma phagocytophilum* prevalence in ticks was also a function of the *A. phagocytophilum* prevalence in small mammals (χ^2 = 14.05, df =1, p=0.0002) (Table 7). Tick species richness ranged from 2 to 4, Simpson's diversity index from 1.069 at Montgomery Woods to 4.270 at Humboldt Redwoods, and species evenness ranged from 0.31 at Hendy Woods (where very high numbers of *I. angustus* and *I. pacificus* contrasted with low numbers of *I. ochotonae*, *I. sculptus*, *I. soricis*, and *I. woodi*) to 0.82 at Henry Cowell, but none of these variables had significant associations with *A. phagocytophilum* prevalence (p>0.22 in all cases). Small mammal species richness ranged from 2 to 8 with the highest richness at Humboldt Redwoods. Small mammal and ticks species richness were moderately correlated (r=0.75). There was no evidence for an association between mammal species richness and *A. phagocytophilum* infection in ticks (χ^2 = 0.07, df =1, p=0.78).

Discussion

California, with its high biological diversity in small mammals and ticks, makes a valuable laboratory for assessing impacts of biological diversity on enzootic tick-borne pathogens. We examined the prevalence of *A. phagocytophilum* DNA in ticks from 11 sites and found infection in 5 tick species, potentially representing cryptic enzootic maintenance cycles of this pathogen. The most important determinants of tick infection were tick species and stage, tick infestation loads on hosts, and *A. phagocytophilum* prevalence in hosts.

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Across multiple species, infection prevalence increased from larvae to nymphs and was highest in adults, revealing cumulative increased risk of infection over stages. We acknowledge that for fed ticks, the extraction method was somewhat poorer than desired, but stage still accounted for risk of *A. phagocytophilum* infection across tick species, even when accounting for tick capture method in the model. We speculate that ticks extracted with a slightly better method would show an even stronger effect of stage. It was interesting that there were PCR-positive larvae although only from hosts. These are likely ticks that were feeding on infected hosts [even if the hosts test negative, the xenodiagnostic test of the tick per se is often more sensitive (Levin and Ross, 2004)], larvae that have acquired infection through skin by feeding very close to another infected tick (i.e. the host is not infected), or true transovarial transmission. It might be interesting to determine rigorously whether *I. pacificus* and nidicolous ticks tended to coinfest individual hosts: We had less than 5 host individuals coinfested with *I. pacificus* and other tick species, and this low number might serve to suggest different feeding preferences, but the number seems too small to analyze statistically. Other site predictors, i.e. measures of host and vector species richness and vector diversity and evenness, were not associated with *A. phagocytophilum* infection. Statistically, there was no particular tick species that appeared likely to "rescue" infection in the community.

In the present study, *I. pacificus*, *I. angustus*, *I. ochotonae*, *I. woodi*, and *I. spinipalpis* were found naturally infected. *Ixodes pacificus* is a broadly distributed and locally abundant vector tick that preferentially feeds on reservoir-incompetent reptiles in larval and nymphal stages, although many immature *I. pacificus* feed on small mammals or birds (Castro and Wright, 2007). However, we also observed *I. angustus*, which is vector-competent for *B. burgdorferi* and a small-mammal specialist (Furman and Loomis, 1984; Peavey et al., 2000); *I. spinipalpis*, which is vector-competent for *A. phagocytophilum* and has multiple host species including rodents and less commonly lagomorphs, birds, and humans (Furman and Loomis, 1984; Norris et al., 1997; Zeidner et al., 2000); *I. ochotonae*, a relatively infrequently encountered tick on woodrats, chipmunks, pikas (*Ochotona princeps*), and grey foxes (*Urocyon cinereoargenteus*) (Furman and Loomis, 1984); and *I. woodi* which feeds primarily on woodrats.

Infestation load on small mammals represented an important determinant of tick infection status, which is possibly an obvious finding that more ticks equate to more opportunities for pathogen transmission. However, the shape of the relationship is not known; for example if, at high loads, the response could be saturated and there would not be any increase in risk even with increasing infestation loads. This is important because loads detected in this study were moderate despite a very large capture effort and attempted removal of all *Ixodes* spp. ticks from all small mammals. Because we focus on host-associated ticks (in addition to the more wide-questing *I. pacificus*), it is possible that we underestimate tick diversity if we failed to sample some rare or trap-shy hosts, most problematically the western gray squirrel (*Sciurus griseus*), which is an important reservoir host for *A. phagocytophilum* and common at our sites but, unlike *S. carolinensis* and *S. niger*, known to be trap-shy (Lane et al., 2005; Nieto and Foley; 2008). Another host (and its specialist tick, *I. soricis*) which should be targeted in future studies is the shrew (*Sorex* spp.) because only one *I. soricis* was recovered

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and evaluated in this study and shrews appear to be rescue hosts for *B. burgdorferi* in the eastern U.S. (Logiudice and Ostfeld, 2003). In contrast however, shrews in the eastern U.S. harbored very heavy tick infestations while shrews in our study sites were very lightly infested (data not shown).

In the present study, biological diversity per se was not found to be a predictor for tick infection, possibly due to a combination of small sample size (at the site level) and small effect size. Alternatively, a dilution effect for *Anaplasma* ecology in the western U.S. may not apply for vectors. Two key requirements of a dilution effect are: (i) that the best hosts for a pathogen are also the more common hosts in a community and occur early in succession (sometimes described as “weedy” species) and (ii) density-dependent regulation of hosts such that additional host species compel the reduction in numbers of the main reservoir species. In northern California, the primary reservoirs (squirrels, chipmunks, and woodrats) do not comply with the first requirement since in almost all communities we studied, the numerically dominant species were the reservoir-incompetent *P. maniculatus* and *P. californicus* (Foley, unpubl. data). How these predictions might translate to vectors is interesting. Tick numbers are regulated by host availability, environmental factors such as humidity and entomopathogenic fungi, and predation including host grooming. Inter-species regulation could occur if increased numbers of one tick species increase host grooming of all ticks or provoke broad anti-tick immunity. However, if tick species vary in vector competencies, then a more speciose community has, on average, a greater likelihood of containing vectors.

There are multiple mechanisms by which biological diversity might be expected to alter prevalence and emergence of infectious disease (Keesing et al., 2006). For example, Lyme disease, which is caused by infection with the spirochete *Borrelia burgdorferi*, is vectored in eastern North America by the deer tick (*Ixodes scapularis*) and commonly maintained in reservoir white-footed mice (*Peromyscus leucopus*), which are the most abundant host for the tick vector and highly competent hosts for the pathogen. Hypothetically, the addition of any species into a community beyond white-footed mice reduces *I. scapularis* “nymphal infection prevalence”, i.e. dilutes the force of infection to target hosts such as humans (Ostfeld and Keesing, 2000; Logiudice and Ostfeld, 2003), but see Randolph and Dobson (2012). Adding species to a community reportedly reduces the prevalence of West Nile virus in bird communities (Swaddle and Calos, 2008), Sin Nombre virus in small mammals (Clay et al., 2009), and *Bartonella* spp. in wood mice (*Apodemus sylvaticus*) in the presence of a host of the flea that is not a host for the pathogen (bank voles, *Myodes glareolus*) (Telfer et al., 2005). In these cases, addition of species to a community can reduce contact rates of reservoir hosts and vectors, or non-reservoir species help ensure that the proportion of infected vectors is low.

In contrast, a rescue or amplification effect implies that additional species help prevent infection from going extinct. This occurs if less dominant species are particularly host-competent or even supershedders, or if less competent species help prevent infection from going extinct in the event that more competent reservoirs suffer population crashes. An example of this is the contribution of shrews (*Sorex cinereus*) to maintaining *B. burgdorferi* in the eastern U.S. (Logiudice and Ostfeld, 2003). Importantly, some “blow-out” epizootics

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can rapidly consume susceptible individuals and then become extinct. Heterogeneity in host or vector quality can keep infection at low incidence and allow enzootic infection to smolder in the community.

Diversity within and between communities in California ensures a very rich host and vector "space" for tick-borne disease, with potential for intersecting subcycles of transmission between partially host-specialist ticks and hosts. Although the present study focused on tick diversity and to a lesser degree on hosts, the bacteria are diverse as well. However, host tropism among *A. phagocytophilum* strains is poorly documented and deserves considerably greater attention in future research. It may be that infection can sequester and persist in particular ticks and hosts, a form of heterogeneity that is analogous to intersecting disease transmission core groups and spatially complex predator-prey systems, both of which have prolonged persistence time specifically because of the heterogeneity (Huffaker, 1958). For disease, the heterogeneity probably helps guard against too explosive an initial outbreak with consumption of so many susceptible individuals that there are insufficient susceptibles to maintain infection in the community afterwards.

Although most studies of biodiversity and disease risk, even for vector-borne disease, have focused on host diversity (e.g., Telfer et al., 2005; Swaddle and Calos 2008), vector diversity commonly reflects host diversity as in the present study. Thus it would be valuable to examine communities where host and vector diversity are not completely coupled, because hosts often limit infection due to immunity, while in vectors immunity is typically of lesser importance. Moreover, we wonder if our results would be different if we added *all* biotic community members, including entomopathogenic fungi, birds, lizards, deer and other diversion hosts for ticks, or any other organisms that contribute to tick population regulation. Further defining diversity beyond hosts makes for opportunities to expand insights, but also requires careful organization of data. Diversity is a characteristic of a site, but studies where site is the unit of analysis often do not allow for true replication across combinations of risk factors, and it is altogether possible that sites cannot truly be queried for driving forces of biodiversity if community assembly of each site is independent and individual. Once more details of host-pathogen-vector interactions are defined, we may still fail to find predictive patterns of risk based on diversity if the assembly history for each community of bacteria, ticks, and mammals is idiosyncratic. The data and analysis presented here may help guide surveillance and further efforts to model for disease in ecologically complex systems.

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Table 1

Characteristics of 11 study sites evaluated for *Anaplasma phagocytophilum* in ticks and small mammals from 2005 to 2012. Abbreviations for study sites are given in this table and used for subsequent tables.

Study site	Dominant vegetation	Region	County	Centroid latitude and longitude	Elevation (m)
Big Basin State Park (BB)	Redwood, chaparral live oak, tanoak, madrone,	Central coast range	Santa Cruz	37.1668; 122.2243	360
Boggs Mountain Demonstration Forest (BM)	Live oak, chaparral, Jeffrey pine	Northern interior coast range	Lake	38.8155; 123.6828	1136
Cold Canyon Preserve (CC)	Live oak, gray pine	Northern interior coast range	Yolo	38.5127; 122.0972	61
Green Diamond (GD)	Ponderosa pine, Douglas fir, tanoak, true oak	Far northwest	Del Norte, Humboldt	41.1261; 123.8134	10–690
Henry Cowell/Fall Creek State Park (HC)	Redwood, tanoak, Douglas fir	Central coast range	Santa Cruz	37.0442; 122.0725	83
Humboldt Redwoods State Park (HR)	Redwood, Douglas fir, tanoak, madrone, live oak	Northern coast range	Humboldt	41.0998; 123.9083	230
Hendy Woods State Park (HW)	Redwood, Douglas fir, live oak	Northern coast range	Mendocino	39.0691; 123.4637	168
Montgomery Woods State Park (MW)	Douglas fir, redwood	Northern coast range	Mendocino	35.3658; 123.8879	249
Quail Ridge Reserve (QR)	Chaparral, gray pine, live oak	Interior northern coast range	Napa	38.4812; 122.1035	600
Soquel Demonstration Forest (SD)	Redwood, live oak, tanoak, madrone	Central coast range	Santa Cruz	37.0478; 121.9343	450–600
Samuel P. Taylor State Park (SPT)	Redwood, live oak, tanoak, madrone	Northern coast range	Marin	38.0247; 122.7238	134

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Table 2

Numbers of ticks in each species at 11 study sites evaluated from 2005 to 2012.

Study Site	*I. angustus*	*I. auritulus*	*I. ochotonae*	*I. pacificus*, flagged	*I. pacificus from hosts*	*I. sculptus*	*I. soricis*	*I. spinipalpis*	*I. woodi*
BB	6	0	1	21	7	0	0	1	3
BM	1	0	0	8	15	0	0	25	0
CC	0	0	11	23	25	0	0	1	22
GD	61	0	0	0	1	0	0	3	12
HC	7	0	0	0	8	0	0	2	0
HR	18	1	9	21	18	0	0	8	7
HW	65	0	6	96	216	1	2	34	12
MW	1	0	0	0	29	0	0	0	0
QR	0	0	0	24	14	0	0	2	0
SD	18	0	0	32	9	0	0	0	1
SPT	17	0	1	99	16	0	0	2	4

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Table 3

Numbers of ticks in each species on various small mammal host species evaluated from 2005 to 2012.

Host species	*I. angustus*	*I. auritulus*	*I. ochotonae*	*I. pacificus*	*I. sculptus*	*I. soricis*	*I. spinipalpis*	*I. woodi*
Myodes californicus	0	0	0	1	0	0	0	0
Flag	2	1	0	324	1	0	19	7
Neotoma fuscipes	26	0	11	108	0	0	49	33
Peromyscus spp.	51	0	8	61	0	0	3	9
Sciurus spp.	2	0	0	9	0	0	3	0
Sorex trowbridgii	1	0	0	4	0	2	0	0
Otospermophilus beecheyi	0	0	0	0	0	0	0	0
Tamias spp.	80	0	6	172	0	0	4	7
Tamiasciurus douglasii	32	0	0	1	0	0	0	5

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Table 4

Number of ticks identified to species and stage found on small mammals and by flagging at 11 sites in northern and central California from 2005 to 2012. The stage of 2 flagged *I. pacificus* was not recorded, so those individuals were omitted from this table.

	Adult females	Adult males	Larvae	Nymphs
Ixodes angustus	47	0	11	132
Ixodes auritulus	1	0	0	0
Ixodes ochotonae	10	0	1	10
I. pacificus flagged	97	76	43	89
I. pacificus from host	13	1	283	43
Ixodes sculptus	1	1	0	0
Ixodes soricis	1	0	0	0
Ixodes spinipalpis	39	13	3	11
Ixodes woodi	27	1	0	26

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Table 5

Results of PCR tests for *A. phagocytophilum* among tick species either collected from vegetation or from small mammals at 11 sites in northern and central California sampled from 2005 to 2012. Cells give number of ticks testing PCR-positive (out of total numbers of ticks tested in that group) and prevalence with 95% C.I. Analyses generating the Tukey groups are described in the text (3 species were omitted from this analysis due to low sample sizes).

	Flagged ticks		**Host-associated ticks**		**Tukey group**
	PCR-positive (total tested)	**Prevalence % (95% C.I.)**	**PCR-positive (total tested)**	**Prevalence % (95% C.I.)**	
Ixodes angustus	0 (2)	0	2 (185)	1.1 (0.2–4.3)	a
Ixodes auritulus	0 (1)	0	0 (0)	0	N/A
Ixodes ochotonae	0 (0)	0	2 (28)	7.1 (1.3–25.0)	ab
Ixodes pacificus	26 (307)	8.5 (5.7–12.3)	12 (340)	3.5 (1.9–6.3)	b
Ixodes sculptus	0 (1)	0	0 (0)	0	N/A
Ixodes soricis	0 (0)	0	0 (2)	0	N/A
Ixodes spinipalpis	0 (19)	0	3 (50)	6.0 (1.6–13.8)	ab
Ixodes woodi	0 (7)	0	2 (54)	3.7 (0.6–13.8)	ab

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Table 6

Results of PCR tests for *A. phagocytophilum* among tick species at 3 developmental stages at 11 sites in northern and central California sampled from 2005 to 2012. Cells give number of ticks testing PCR-positive (out of total numbers of ticks tested in that group) and prevalence with 95% C.I.

	Larvae		Nymphs		Adults	
	PCR-positive (total tested)	**Prevalence % (95% C.I.)**	**PCR-positive (total tested)**	**Prevalence % (95% C.I.)**	**PCR-positive (total tested)**	**Prevalence % (95% C.I.)**
Ixodes angustus	0 (14)	0	1 (124)	0.8 (0.04–5.1)	1 (49)	2.0 (0.1–12.2)
Ixodes auritulus	0 (0)	0	0 (0)	0	0 (1)	0
Ixodes ochotonae	0 (1)	0	0 (10)	0	2 (17)	11.8 (2.1–37.8)
Ixodes pacificus	8 (325)	2.5 (1.2–4.9)	10 (121)	7.6 (0.04–13.9)	20 (189)	10.6 (6.7–16.1)
Ixodes sculptus	0 (0)	0	0 (0)	0	0 (1)	0
Ixodes soricis	0 (0)	0	0 (0)	0	0 (1)	0
Ixodes spinipalpis	0 (3)	0	0 (12)	0	3 (54)	5.6 (1.4–16.3)
Ixodes woodi	0 (0)	0	0 (26)	0	2 (35)	5.7 (0.9–20.5)

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Table 7

Site-level diversity, tick abundance, and *A. phagocytophilum* PCR-prevalence estimates at 11 study sites evaluated from 2005 to 2012.

Study site	Tick species richness	Tick S	Tick species evenness	Infestation load (ticks per small mammal)	Small mammal species richness	Prevalence in small mammals	Prevalence in ticks
BB	5	3.375	0.6750	0.0533	6	0.0893	0
BM	3	1.975	0.6584	0.8913	3	0	0.0222
CC	4	2.828	0.7069	0.2906	2	0	0
GD	4	1.530	0.3825	0.8506	4	0.0313	0.0649
HC	3	2.470	0.8234	0.1574	5	0.4500	0.4000
HR	6	4.270	0.7117	0.1881	8	0.0606	0.0244
HW	7	2.162	0.3088	0.4563	7	0.1292	0.0647
MW	2	1.069	0.5344	0.2913	3	0	0
QR	2	1.280	0.6400	0.2174	2	0	0.0625
SD	4	1.931	0.4828	0.1905	6	0	0.0169
SPT	5	2.827	0.7067	0.1751	4	0.0800	0.0282