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Macro-parasites and micro-parasites co-exist in rodent communities but are associated with different community-level parameters

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ABSTRACT

Wildlife species are often heavily parasitized by multiple infections simultaneously. Yet research on sylvatic transmission cycles, tend to focus on host interactions with a single parasite and neglects the influence of co-infections by other pathogens and parasites. Co-infections between macro-parasites and micro-parasites can alter mechanisms that regulate pathogenesis and are important for understanding disease emergence and dynamics. Wildlife rodent hosts in the Lyme disease system are infected with macro-parasites (i.e., ticks and helminths) and micro-parasites (i.e., *Borrelia* spp.), however, there has not been a study that investigates the interaction of all three parasites (i.e., *I. pacificus*, *Borrelia* spp., and helminths) and how these co-infections impact prevalence of micro-parasites. We live-trapped rodents in ten sites in northern California to collect feces, blood, ear tissue, and attached ticks. These samples were used to test for infection status of *Borrelia* species (i.e., micro-parasite), and describe the burden of ticks and helminths (i.e., macro-parasites). We found that some rodent hosts were co-infected with all three parasites, however, the burden or presence of concurrent macro-parasites were not associated with *Borrelia* infections. For macro-parasites, we found that tick burdens were positively associated with rodent Shannon diversity while negatively associated with predator diversity, whereas helminth burdens were not significantly associated with any host community metric. Ticks and tick-borne pathogens are associated with rodent host diversity, predator diversity, and abiotic factors. However, it is still unknown what factors helminths are associated with on the community level. Understanding the mechanisms that influence co-infections of multiple types of parasites within and across hosts is an increasingly critical component of characterizing zoonotic disease transmission and maintenance.

1. Introduction

Wildlife species can be infected with a broad range of macro-parasites and micro-parasites, often at the same time. Yet most zoonotic disease research focuses on a single parasite and neglects the interactions between multiple parasites within the host, leading to an incomplete understanding of pathogen impacts (Vaumourin et al., 2015). The increased pace of disease emergence necessitates consideration of multiple parasitic infections in wildlife hosts to better predict and prevent transmission (Friggens and Beier, 2010; Hahn et al., 2021; Jones et al., 2008; Messina et al., n.d.; Millette et al., 2020; Patz et al., 2000, 2008; Rizzoli et al., 2019; Swei et al., 2020; Tidman et al., 2021; Webster et al., 2016; Wolfe et al., 2005).

Parasites can be classified as micro-parasites (e.g., bacteria and viruses), endo-macroparasites (e.g., helminth worms), or ecto-macroparasites (e.g., ticks and mites). These various parasite types have distinct life history traits such as mechanisms of host infection and tissue tropism. At the same time, different parasite types can interact directly within a single host by competing for space or nutrition (Behnke et al., 1999; Bell et al., 2006; Graham, 2008; Mideo, 2009; Rynkiewicz et al., 2015). Parasites can also interact indirectly through the hosts' immune system. Pressures on hosts such as chronic stress, low resource availability, sex hormone response, and interfering immune responses from multi-parasitic infections; can suppress the immune response in

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wildlife, leading to the increase of disease morbidity (Cizauskas et al., 2014, 2015; Ezenwa et al., 2012; Ezenwa and Jolles, 2011; Jolles et al., 2008a; Petney and Andrews, 1998). For example, mammalian hosts deploy T-helper 1 cells to combat micro-parasitic infections and deploy T-helper 2 cells when combating a macro-parasite infection. The pathways which initiate the deployment of either of these cells are cross-regulating. Therefore, only one type of infection can be fought by the host's immune system at a time (Morel and Oriss, 1998; van Riet et al., 2007), leading to immune suppression in co-infected hosts. Furthermore, T-helper 2 responses are positively correlated with macro-parasite burdens (Maaz et al., 2016) and as parasites are aggregated across host populations (Shaw et al., 1998) the relative differences in individuals macro-parasite burdens have an impact on the individuals overall health (Jolles et al., 2008b). Research aiming to understand this immune interaction traditionally focuses on the interaction between internal macro-parasites and micro-parasites (Budischak et al., 2012; Ezenwa and Jolles, 2011; Jolles et al., 2008a) but a multi-parasite approach involving ecto-parasites is warranted.

In the United States, ticks are the most common vector of disease and are responsible for transmitting the greatest number of zoonotic pathogens (Swei et al., 2020). They transmit a variety of micro-parasites, most notably, the spirochete bacterium(s) that cause Lyme disease and hard tick relapsing fever (i.e., *Borrelia* species) (Eisen et al., 2017; Paddock et al., 2016; Rosenberg et al., 2018; Swei et al., 2020). *Borrelia burgdorferi* sensu lato (i.e., *Borrelia burgdorferi* and *Borrelia bissettiae)* and relapsing fever Borreliae (i.e., *Borrelia miyamotoi*) are naturally maintained in a sylvatic cycle involving *Ixodes* species tick vectors and wildlife hosts, particularly rodents. These rodent hosts are also commonly parasitized with helminths, thus providing an ideal model system to investigate multi-parasitic interactions within a host. A study in the UK investigated how mice experimentally infected with a commonly isolated helminth from wild rodents impacted the feeding success of ticks or the pathogenicity success of *Borrelia afzellii*, and found no influence helminth infections on the transmission of *Borrelia afzellii* (Maaz et al., 2016). Field surveys of wild host populations and their most common co-infections are necessary to document prior to lab experimentation of the molecular mechanisms defining these multi-parasite interactions.

Macro-parasites, particularly ticks, spend a large portion of their life cycle off host in the environment. As ectotherms, ticks are sensitive to abiotic changes and will modify their behavior (e.g., host-seeking questing) to avoid desiccation, while also trying to successfully find a blood meal (Perret et al., 2003; Thomas et al., 2020). Similarly, many species of helminths are environmentally transmitted and spend a great portion of their life cycle exposed to environmental conditions before a host ingests them. Therefore, abiotic conditions are likely to influence the distribution of macro-parasites in general. This study seeks to understand patterns of co-occurrence between these three types of parasites, micro-parasites and two types of macro-parasites, to better understand how they may facilitate or inhibit one another.

We investigated the community dynamics between three parasites: a microparasite (i.e., *Borrelia* spp.), an ecto-macroparasite (i.e., ticks), and an endo-macroparasite (i.e., helminths) within rodent hosts in a Lyme disease endemic area in the western United States. The goal of this study is to 1) characterize co-infections and burden intensity of micro- and macro-parasites within individual hosts; 2) understand how macro-parasites are associated with host community dynamics and environmental inputs (temperature, precipitation); as well as 3) analyze how macro-parasites are associated with micro-parasite infection within individual hosts.

2. Materials and methods

2.1. Field collection

Field collections were conducted in ten oak woodland fragmented forest patches that were selected in the San Francisco Bay Area. These forest patches were standardized by landscape and vegetation composition but varied in their habitat patch size and fell along a gradient from high fragmented (2.5 ha) to intact (>4000 ha) (Lawrence et al., 2018; Salomon et al., 2021; Sambado et al., 2020). Within each habitat patch, a half hectare sampling grid was established at least 20 m from the edge of the patch, under oak canopy cover, while avoiding north facing slopes (Talleklint-Eisen and Lane, 1999; Tälleklint-Eisen and Lane, 2000).

We conducted three consecutive days of rodent live-trapping at each site between the months of April and May in 2018 to target juvenile *Ixodes pacificus* activity (MacDonald, 2018). At each site, a total of 49 trapping stations were established in a 7 × 7 grid with each trap station located 11.8 m apart. Two extra-large Sherman Live Traps (7.6 × 9.5 × 30.5 cm; H.B. Sherman Traps, Tallahassee, FL, USA) were placed at each trapping station for a total of 98 traps per grid (Machtinger and Williams, 2020). At five of the sites, 15 tomahawk traps (16L x 5W × 5H in; Tomahawk Live Traps, Hazelhurst, WI) were set at every other trapping station to target larger rodents. Total trapping events totaled to 3165 events across all sites. Traps were baited with a combination of oats and peanut butter, and set out from dusk until dawn. Captured rodents were marked with unique ear tags (National Band & Tag Company Co., KY), weighed, aged, sexed, and identified to species (Machtinger and Williams, 2020). The following samples were collected from each rodent: 2-mm ear biopsies, whole blood from the retro-orbital vein, any and all attached ticks, and feces. Feces were collected directly from the animal's rectum during handling procedures, into a 1.7 mL Eppendorf tube. Animals were anesthetized with a 50% isoflurane and propanediol 3–4 solution prior to retro-orbital bleeding. After processing and recovery from anesthesia, all animals were released at the point of capture. All protocols were approved by institutional animal care and use protocol (#AU16-05). Ear biopsies and attached ticks were each stored in 70% ethanol at 4 °C until further processing. Whole blood was collected in EDTA tubes, held on ice in the field, flash frozen with liquid nitrogen, and then stored at −80 °C until further processing. Rodent feces were brought back to the lab and stored at −20 °C until further lab processing.

To assess the association between questing ticks and ticks attached to hosts, questing ticks were collected using standard dragging methods where a white cotton cloth was dragged within the 0.5 ha sampling grid, totaling 495 m^2 at each sampling site (Eisen et al., 2018; Salomon et al., 2020). Drag cloths were checked for ticks every ~15 m and ticks were transferred to vials containing 70% ethanol. Questing ticks were collected during two different time intervals; first during the three days of rodent trapping in April and early May and then again in the first week of June 2018 (Barbour et al., 1985; MacDonald, 2018) to capture seasonal dynamics of *I. pacificus* larvae and nymphs.

Two Bushnell Trophy Cam HD (Overland Park, KS) were set up at each site to capture terrestrial vertebrate densities and richness. Cameras were strapped to a tree roughly 60 cm from the forest floor, facing opposite directions along a game trail. Camera motion detection settings were set to 'normal sensitivity' to take a series of three photos at a time, with each photo being 1 s apart, and pausing 30 s before another capture (Lawrence et al., 2018). Cameras were active 24 h a day for 40 days of analysis between the months of April 1 through May 10 of 2018.

2.2. Molecular analysis

Tissue and blood samples were extracted using the DNeasy Tissue Extraction kit according to the manufacturer's instructions (Qiagen, Valencia, California, USA). A few adjustments were made to the protocol; for tissue samples, an additional 70% ethanol wash step was added to ear tissue extractions and the final product of both blood and tissue samples were eluted in 100 μL of AE buffer to concentrate the eluate. Once DNA was extracted, microparasite infection was determined using two separate nested PCR protocols. Within hosts there is niche partitioning of *Borrelia* species where *B. burgdorferi* sensu lato is found in endothelium, and relapsing fever *Borrelia* species, including

B. miyamotoi, circulate in the hosts' blood (Barbour et al., 2009; Barbour and Hayes, 1986; Sambado et al., 2020). Therefore, one PCR protocol was used on ear tissue samples targeting the 5S–23S rRNA intergenic spacer region to detect *Borrelia burgdorferi* sensu lato which includes the Lyme disease bacterium, *B. burgdorferi,* as well as other related genospecies such as *B. bissettiae*. Another PCR protocol was used on blood samples and targets the 16S–23S rDNA intergenic spacer region for detecting relapsing fevers *Borrelia* species, such as *B. miyamotoi* and related bacteria (Barbour et al., 2009; Bunikis et al., 2004; Postic et al., 1994; Sambado et al., 2020). All nested PCR products were tested in triplicate and visualized by gel electrophoresis on a 1.8% agarose gel. Positive samples were then purified using the SeraPure magnetic beads prior to sequencing on an ABI 3730. All positive sequences were edited and aligned using Geneious v 11.15 software and identified to species by aligning to reference sequences on NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.3. Tick species identification and abundance estimates

Collected ticks were identified under a dissecting microscope to species and life stage using taxonomic keys (Furman and Loomis, 1984; Kleinjan and Lane, 2008). Questing tick abundances were quantified by species, life stage, and site. Attached tick abundances (Busht et al., 1997) per species and life stage, were quantified for each animal, which we refer to as 'tick burden' throughout the text.

2.4. Helminth detection

Rodent helminth infection was determined by processing rodent feces using a standard protocol to detect helminth eggs. For each sample, 0.1 g of feces was processed from each animal by homogenizing rodent feces in a Sugar-Med solution (Bechtel et al., 2015; Benbrook and Sloss, 1955; Foreyt, 2001; Parkinson et al., 2011). Samples were then centrifuged at 500 RCF for 5 min in a centrifuge with a 161 mm rotor in 15 mL test tubes. After centrifugation, more sugar solution was added to the 15 mL test tube to create a reverse meniscus and coverslips were pressed on top of the solution. After 15 min, coverslips were transferred to glass slides and examined at 40× total magnification under a standard compound microscope. Eggs were identified to genera (Benbrook and Sloss, 1955; Foreyt, 2001) and quantified for each rodent. Total egg abundance (Busht et al., 1997) was quantified per animal, which we refer to as 'helminth burden' throughout the text since fecal egg abundance are positively correlated to adult worm burden (Bryan and Kerr, 1989; Kim et al., 2011; Sithithaworn et al., 1991).

2.5. Remotely sensed climate data

When assessing macro-parasite burdens across rodent hosts at the community level (i.e., site), we included abiotic metrics such as temperature, precipitation, and maximum vapor pressure deficit to account for climate impacts on tick populations outside of our host community analysis (i.e., Shannon diversity of vertebrate hosts and predators). Climate data for each site was used from the Oregon State Parameter-elevation Regression on Independent Slopes Model (PRISM) Climate Group (downloaded on 2022-05-01) which provides estimates of primary climate elements such as mean temperature (tmean, °C), precipitation (ppt, mm), and maximum vapor pressure deficit (vpdmax, kPA) ("PRISM Climate Group," 2014). Data from April 2018 was selected for analysis because peak sampling of all ten sites occurred throughout the month of April (Supplemental Fig. 2). We chose to select the average over a period of one month because ticks are more sensitive to shifts in monthly climate characteristics than yearly averages and their peak activity season (i.e., phenology) occurs between April and May. Climate data for mean temperature is derived as the average of maximum temperature and average minimum temperature that is averaged over all days in the month. To represent precipitation, we selected monthly total precipitation (ppt). Maximum vapor pressure deficit is the daily maximum vapor pressure deficit averaged over all days in the month. Vapor pressure deficit (vpd) is a variable related to humidity and temperature and is calculated as the difference (in units of pressure) between actual vapor pressure and the saturation vapor pressure (e.g., the amount of water air can hold before it becomes liquid). A high vpd can be thought of as an environment with higher temperature and lower humidity levels. Maximum vpd has been shown to be a significant predictor of tick abundance and questing activity, which is why it is incorporated in these analyses (Bacon et al., 2022; Diuk-Wasser et al., 2010; Hacker et al., 2021; Hahn et al., 2016). Single point climate estimates were taken at a 4 km resolution at given site GPS points from Salomon et al., 2021); Lawrence et al. (2018); Salomon et al. (2021); Sambado et al. (2020).

2.6. Community vertebrate metrics

Community vertebrate metrics (i.e., Shannon diversity and abundance estimates) were calculated as done in Lawrence et al., (2018); Salomon et al., (2021); Lawrence et al. (2018); Salomon et al. (2021). Briefly, all captured rodents were marked with unique ear tag numbers in order to calculate mark recapture estimates for site abundances in R (v. 0.99.902) using the '*Rcapture*' package (Baillargeon and Rivest, 2007). Predator abundance estimates were calculated from camera traps, each captured photo within 30 min of the same species was considered as one individual. From these photographs of activity for each species, we divided by the number of days the camera trap was active (40 days). Using these activity estimates as proxy's for abundance, we were able to calculate a relative Shannon diversity index with the *'vegan'* package in R for each site (Oksanen, 2016). Similarly the rodent Shannon diversity index was calculated with the '*Rcapture*' abundance estimates for each site.

2.7. Statistical analysis

Statistical analysis was broken into three parts: 1) characterizing the distribution of macro- and micro-parasites across individual hosts, 2) understanding how macro-parasites are associated with host community dynamics as well as abiotic metrics (negative binomial models), and 3) analyzing how macro-parasites are associated with micro-parasites within individual hosts (binomial models and negative binomial models). Specific model formulation for each part can be found in the statistical analysis subsections. Terminology for all statistical analysis is as follows: micro-parasites examined included a single *Borrelia* spp. or multiple *Borrelia* species (i.e., *B. burgdorferi*, *B. bissettiae, B. miyamotoi*, or an uncharacterized relapsing fever *Borrelia*) and the type of micro-parasite included in analyses is stated explicitly in generalized linear mixed effects model interpretation. Macro-parasites included ticks (*Ixodes* sp. and *Dermacentor* sp.) and helminths (*Trichuris* sp., *Capillaria* sp., *Aspiculuris* sp., and *Hymenolepis* sp.) and were evaluated together (all tick spp. and all helminth spp.) or individually as separate groups of ticks or helminths. A complete list of each combination of micro- and macro-parasites evaluated in the analyses can be found in the Supplement (Table 3). All analyses were done in RStudio version 1.4.1717 ("RStudio: Integrated Development Environment for R," 2021).

2.8. Characterizing the distribution and host community associations of macro- and micro-parasites

We first calculated the observed infection prevalence (Busht et al., 1997) of each infection type (*Borrelia* spp., ticks, and helminths) by dividing the number of individuals to be positive for each parasite type by the number of individuals tested for each infection type. We calculated 95% Confidence Intervals using the Agresti-Coull method. Wilcoxon rank sum tests were used to test for significant differences between the two genera of rodent hosts (i.e., *Neotoma* and *Peromyscus*

spp.) of both the median tick burdens and the median helminth burdens. Additionally, Wilcoxon rank sum tests were used to test for significance between uninfected and *Borrelia* spp. infected rodents and both their median tick burdens, and their median helminth burdens.

To understand how macro-parasites (ticks and helminths) are associated by individual host demographics, community structure (i.e., vertebrate diversity), and abiotic metrics (i.e., temperature, precipitation, vapor pressure deficit); we fitted a generalized linear mixed effects model (GLMM) with a negative binomial distribution to account for over-dispersed count data of tick and helminth burdens. All models were built and ran separately for ticks and helminths. For each model, the outcome of interest was ticks and helminths burdens on individual hosts. For the host demographic analysis, fixed effects were genera, sex, and age (i.e., adult or juvenile) with the random effect of site. Rodent weight was removed from the analysis due to its high collinearity with age and genera. For the site-level community structure analysis, the fixed effects were rodent Shannon diversity, and predator Shannon diversity, with the random effect of genera. Lastly, for the abiotic metrics in April 2018, negative binomial models were run separately for the fixed effects of mean temperature (°C), total monthly precipitation (mm), and max vapor pressure deficit (kPA) due to their high collinearity with each other, with host genera as the random effect. Additional details of how abiotic metrics were collected and applied can be found in Supplemental Table 2 and Supplemental Fig. 2.

2.9. Macro-parasites and micro-parasites interactions within individual hosts

To understand how micro-parasite infections (*Borrelia* spp.) are associated with macro-parasites (ticks and helminths), a binomial logit model was fitted with the presence/absence of a *Borrelia* spp. infection as the response variable with predictors of tick and helminth burden, presence/absence of tick and helminths, and genera of host. Additionally, we ran separate models for only *Neotoma* spp. as they had higher infection prevalence's of macro-parasites compared to *Peromyscus* species. For models that just looked at *Neotoma* spp., we removed genera as a fixed effect. Separate binomial logit models were run for all *Borrelia* spp., *Borrelia burgdorferi* sensu lato, relapsing fever *Borrelia* spp., and individual *Borrelia* spp. (e.g., *B. burgdorferi* sensu stricto, *B. bissettiae*, *B. miyamotoi*, and an unnamed tick-borne relapsing fever-like organism described in Sambado et al., 2020; Sambado et al., 2020)). Some predictor variables were explored for interactions as well for these models, each model structure is presented in Supplemental Table 2.

For each set of these analyses, the best fit model was selected by the lowest Akaike Information Criterion (AIC) score, and candidate models were compared with and without each predictor variable using one-sided likelihood-ratio tests with the 'anova' function in R. A list of all model types and structures can be found in Supplemental Table 2.

3. Results

3.1. System characterization

A total of 2812 questing ticks were collected from these ten collection sites, with the majority being *I. pacificus* larvae (76%, n = 2125). Other life stages of *I. pacificus* collected consisted of 336 nymphs and 84 adults. We also collected *Dermacentor occidentalis*, *Dermacentor similis* (previously known as *Dermacentor variabilis*) (Lado et al., 2021), *Haemaphysalis leporispalustris*, and *Ixodes spinipalpus*, but in much lower numbers.

Rodent sampling totaled 3165 trapping events across 10 field sites and resulted in 313 individual rodents captured. Six rodent species were encountered, included 98 *N. fuscipes*, 7 *Microtus californicus*, 30 *P. californicus*, 35 *P. maniculatus*, 141 *P. truei,* and two *Reithrodontomys megalotis*. Camera trap analysis can be reviewed in depth within Lawrence et al., (2018) and Salomon et al., 2021 (Lawrence et al., 2018; Salomon et al., 2021). But briefly here, eight different predator species were identified across the 10 sites including: *Puma concolor* (mountain lion), *Canis latrans* (coyote), *Lynx rufus* (bobcat), *Urocyon cinereoargenteus* (gray fox), *Procyon lotor* (raccoon), *Mephitis mephitis* (striped skunk), *Didelphis virginiana* (Virginia opossum), and *Felis catus* (domestic cat).

3.2. Characterizing the distribution of macro- and micro-parasites across individual hosts

Due to resource constraints and animal safety we could not collect blood, tissue, and feces from all 313 rodents. We examined 184 rodent blood samples and 307 rodent ear tissue samples for pathogen infection with microparasites. A total of 56 samples (hosts) were positive for *Borrelia* spp. based on 5S–23S IGS and 16S–23S IGS rDNA nested PCR analysis. All but two samples produced sequences that were identified to species and submitted to GenBank (Accession numbers in Supplemental Table 3). We submitted 33 sequences to GenBank, but two samples we determined positive via base pair length on gel electrophoreses (one *B. bissettiae* and another *B.* miyamotoi) produced poor quality sequences. Furthermore, 21 samples were identified as an uncharacterized tick-borne relapsing fever *Borrelia* species (Supplemental Table 3). For statistical analyses, we considered 53 *Borrelia* positives. We detected 13% of the rodent's blood (n = 24) were infected with *Borrelia* species that cause relapsing fever, and 10% of rodents were infected with *B. burgdorferi* sensu lato in ear tissue samples (n = 31). Of the relapsing fever causing species that we isolated, 3 were *B. miyamotoi,* and 21 were infected with an uncharacterized relapsing fever *Borrelia* species (Sambado et al., 2020). Of the *B. burgdorferi* sensu lato group we isolated, 19 were identified as *B. burgdorferi* sensu stricto and 19 *B. bissettiae* positive samples. There were two *Peromyscus* spp. that were co-infected with *B. burgdorferi* sensu stricto and the uncharacterized relapsing fever *Borrelia* species (Sambado et al., 2020). *Neotoma fuscipes* were found to be infected with *B. burgdorferi* sensu lato at a greater proportion than *Peromyscus* spp. (Supplemental Table 3). All positive samples are summarized in Supplemental Table 3, with their corresponding Genbank accession numbers. Sequences of the novel uncharacterized relapsing fever *Borrelia* species isolated from rodent hosts do not have accession numbers, but are first reported in Sambado et al., (2020); Sambado et al. (2020).

From the 312 rodents checked for ticks, we collected 538 attached ticks that consisted mainly of *I. pacificus* larvae (80%, n = 431) (Supplementary Table 4). Additionally, we collected other life stages of *I. pacificus* (nymphs = 3, adults = 3), *I. angustus* (larvae = 7, nymphs = 22, adults = 8), *I. spinipalpis* (larvae = 28, nymph = 1, adult = 2), *Ixodes woodi* (nymphs = 6), *D. occidentalis* (larvae = 20, nymphs = 7) attached to the various rodents. All of these tick species were removed from *Neotoma fuscipes*, but two individual *Peromyscus* species (*P. maniculatus* and *P. truei*) had tick burdens of 5 and 4 composed of three different species at Windy Hill Open Space Preserve (*I. angustus, I. pacificus,* and *I. spinipalpis*). From 99 *N. fuscipes* we removed 337 *I. pacificus,* five *I. angustus*, 27 *I. spinipalpis*, three *D. occidentalis*, and 6 *I. woodi* ticks. While from 207 *Peromyscus* spp., we removed only *97 I. pacificus, 32 I. angustus, 4 I. spinipalpis*, and *20 D. occidentalis.*

A total of 107 rodent fecal samples were collected and tested, yielding an overall helminth infection prevalence of 31% (n = 33, Supplemental Table 1). Four different macro-parasite genera, belonging to two different phyla (Nematoda and Platyhelminthes), were identified. The most common helminth infection identified was *Trichuris* (Nematoda: Trichocephalida) species (n = 32). In addition, co-infections of *Trichuris* and *Aspiculuris tetraptera* (Nematoda: Oxyurida) within a *P. californicus* and *Capillaria* (Nematoda: Enoplia) within two different *Neotoma fuscipes.* Lastly, we identified a single occurrence of *Hymenolepis* (Platyhelminthes: Cyclophyllidea) infection within a *Peromyscus californicus*.

Of 103 rodents that were tested for all three types of parasites (i.e.,

ticks, helminths, and *Borrelia* species), 7% were concurrently infected with them all (n = 7, Table 1 and Fig. 2). Meanwhile, 15% (n = 15) of hosts were not parasitized by any of the infections we examined in this study (Table 1). The most common host co-infection was a combination of ticks and helminths at 18% (n = 19). The second most common co-infection observed was with ticks and *Borrelia* species, at 11% (n = 32). The least common co-infection was between helminths and *Borrelia* species, with nine rodents (8.74%) infected with both. Rodent species that most commonly hosted the parasitic infections we examined were *N. fuscipes* and *P. truei* (Supplemental Table 1 and Supplemental Fig. 1). However, due to low numbers of certain species (i.e., *Peromyscus californicus* and *Peromyscus maniculatus*), hosts were grouped into two genera (i.e., *Peromyscus* spp. and *N. fuscipes*) for regression models.

The Wilcoxon rank sum test found that tick and helminth median burdens were significantly different between *N. fuscipes* (W = 12806, p-value <0.001) and *Peromyscus* spp. (W = 1984.5, p-value <0.001), with *N. fuscipes* hosting higher burdens of these macro-parasites (Fig. 1). Another Wilcoxon rank sum test found that both tick (W = 5263, p-value <0.05) and helminth (W = 1515, p-value <0.05) median burdens were significantly different for rodents that were uninfected versus *Borrelia* infected. The mean tick burden was higher in *Borrelia* infected rodents (infected = 2.4 mean tick burden, uninfected = 1.6 mean tick burden) whereas the helminth burden was higher in *Borrelia* uninfected rodents (uninfected = 4.6, infected = 1.5).

Neotoma fuscipes were more likely to be infected with *B. burgdorferi* sensu lato and have higher burdens of both ticks and helminths compared to *Peromyscus* species (Table 2). When looking at tick burdens, significant predictors such as *Peromyscus* spp. (estimate = −1.45 ± 0.21, p-value <0.001), and juvenile status (estimate = −0.79 ± 0.33, p-value = 0.02) were negatively associated with tick burdens, while male rodents had a positive association with tick burdens (estimate = 0.47 ± 0.19, p-value = 0.01). For helminth burdens, significant predictors included a negative association of *Peromyscus* spp. with helminths counts (estimate = −3.24 ± 0.56, p-value <0.001) and juvenile status (estimate = −2.85 ± 0.94, p-value <0.01).

3.3. Host community dynamics and abiotic metrics association with parasites

Tick burdens are associated with different host community dynamics such as rodent diversity, predator Shannon diversity, and abiotic metrics (i.e., temperature and vapor pressure deficit), whereas helminths did not have a significant relationship with any of those predictors (Table 3). Tick burdens had a positive association with rodent Shannon diversity (estimate = 0.80 ± 0.18, p-value <0.001) but a negative association with site predator Shannon diversity (estimate = −0.48 ± 0.16, p-value <0.01). Abiotic metrics such as mean temperature (°C; estimate = 0.65 ± 0.19, p-value <0.01) and vapor pressure deficit max (kPA; estimate = 0.11 ± 0.05, p-value = 0.02) were significant predictors of tick burdens

Table 1
Prevalence of co-infections for all hosts between *Borrelia*, ticks, and helminths with a 95% Confidence Interval (95% CI).

	Positives	Tested	Observed Infection Prevalence (%)	95% CI
No infections	15	103	NA	8.9–23
Only *Borrelia* spp.	20	306	6.54	4.2–9.9
Only Ticks	17	309	5.50	3.4–8.7
Only Helminths	13	103	12.62	7.4–21
Ticks + Helminths	19	107	17.76	12–26
Borrelia spp. + Helminths	9	103	8.74	4.5–16
Borrelia spp. + Ticks	32	306	10.46	7.5–14
Borrelia spp. + Ticks + Helminths	7	103	6.80	3.1–14

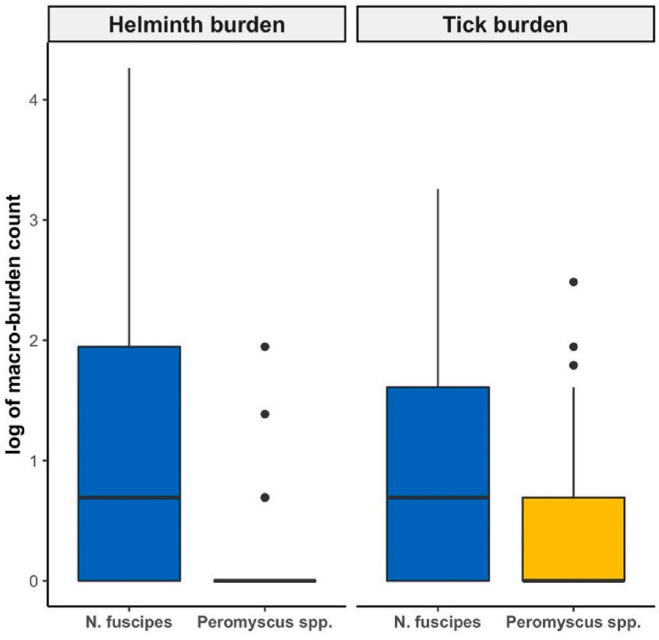

Fig. 1. A boxplot of the mean macro-parasite burden across *N. fuscipes* and *Peromyscus* species. The log of helminth (left panel) and tick (right panel) counts on individual rodents were used for visualization purposes.

while precipitation was not. All *Borrelia* spp. were investigated separately to identify community level impacts (i.e., Shannon diversity of rodents and predators) on pathogen presence in *N. fuscipes*. The most fit model included both predator and rodent Shannon diversity, where rodent Shannon diversity has a significant positive association with *Borrelia burgdorferi* sensu lato (Table 2).

3.4. Macro- and micro-parasite associations within individual hosts

We did not find a significant relationship between micro- and macro-parasite infection in shared rodent hosts. We investigated different combinations of models with macro- or micro-parasite presence/absence as a response to the counterpart parasite burden or presence, but none of the models were significant (Supplemental Table 2).

4. Discussion

Rodents in a Lyme disease endemic region of California experience simultaneous infections of helminths, ticks and *Borrelia* species (Table 1 and Supplemental Table 1). However, when testing how these three separate infections are associated with each other, there was no significant association observed. This survey supports the findings of Maaz et al., (2016), that helminth infections do not alter *Borrelia* spp. transmission (Maaz et al., 2016). Patterns of macro-parasite distribution within hosts and across host communities varied for both ticks and helminths. Ticks were the only macro-parasite significantly associated with various community diversity metrics such as rodent Shannon diversity (positive), predator Shannon diversity (negative), and climate variables (i.e., mean temperature and max vapor pressure deficit) (Table 3). We found that adult *N. fuscipes* were more likely to harbor ticks than sympatric juvenile *Peromyscus* species, and described higher loads of helminth infections within *N. fuscipes* than sympatric *Peromyscus* species (Table 3). The variation in tick and helminth distribution across hosts, along with the different associations within host community structure and abiotic metrics; suggests that ticks are more sensitive to extrinsic host community level factors (i.e., host demographics, host predation, and host diversity) (Tables 2 and 3), than by direct parasite interactions within the host (e.g., immune cross reactivity or resource

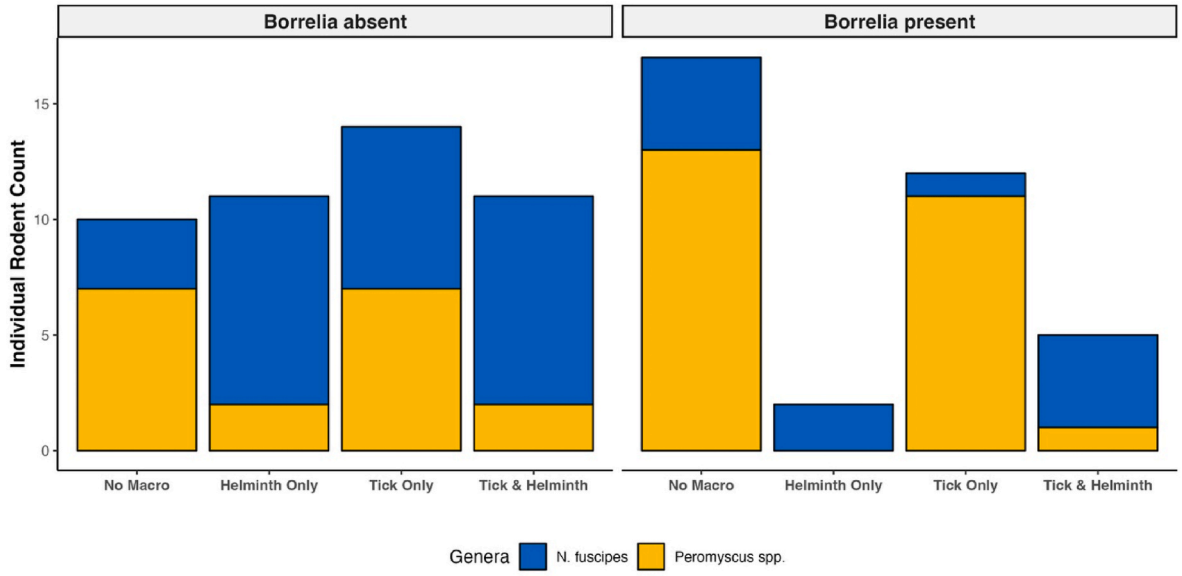

Fig. 2. A histogram of the counts of individual rodents that were uninfected or infected with *B. burgdorferi* sensu lato with certain macro-parasite infections. The types of infection were: no tick or helminth burden on an individual rodent (No macro-parasite), helminth burden only (Helminth Only), tick burden only (Tick Only), or both ticks and helminths were found on individual rodent (Tick & Helminth). The colors denote for *N. fuscipes* (blue) and *Peromyscus* species (yellow). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Significant regression models for part 1 of the analyses: characterize the distribution of macro- and micro-parasites across individual hosts. Levels of significance are indicated with * (*** p-value <0.001; ** p-value <0.01; * p-value <0.05).

Response	Explanatory	Estimate	Std. Error	z-value	p-value
Rodent tick burden (negative binomial model) with site as random effect					
	Genus-Peromyscus	−1.45	0.206	−7.03	<0.001**
	Sex-Male	0.474	0.193	2.45	0.0143 *
	Age-Juvenile	−0.786	0.330	−2.38	0.0173 *
Rodent helminth burden (negative binomial model) with site as random effect					
	Genus-Peromyscus	−3.06	0.575	−5.32	<0.001***
	Sex-Male	−0.675	0.524	−1.29	0.198
	Age-Juvenile	−2.51	0.963	−2.60	0.00922 **
Rodent *B. burgdorferi* sensu lato infection (binomial model)					
	Genus-Peromyscus	−2.12	0.601	−3.53	<0.001***
	Sex-Male	−0.145	0.551	−0.264	0.792
	Age-Juvenile	−0.829	1.07	−0.768	0.443

Table 3
Significant regression models for part 2 of the analyses: understand how macro-parasites are influenced by associated with host community dynamics. Levels of significance are indicated with * (*** p-value <0.001; ** p-value <0.01; * p-value <0.05).

Response	Explanatory	Estimate	Std. Error	z-value	p-value
Tick burden (negative binomial model) with genera as random effect					
	Rodent Shannon diversity index	0.796	0.177	4.51	<0.001***
	Predator Shannon diversity index	−0.483	0.163	−2.96	0.00308 **
Tick burden (negative binomial model) with genera as random effect					
	Mean temperature	0.645	0.190	3.41	<0.001***
Tick burden (negative binomial model) with genera as random effect					
	Max vapor pressure deficit	0.111	0.0490	2.26	0.0238 *
***Borrelia* spp. infection (binomial model) for *N. fuscipes* only**					
	Rodent Shannon diversity index	−1.157	0.600	−2.07	0.0388 *
	Predator Shannon diversity index	0.530	0.485	1.09	0.275

competition).

We found the distribution of helminths and ticks on individual hosts were significantly different between *Neotoma fuscipes* and *Peromyscus* species (Fig. 1). Concordant with other tick findings (Brown and Lane, 1996; Salomon et al., 2021; Swei et al., 2012), *N. fuscipes* had larger tick burdens than *Peromyscus* species, and our results identified higher helminths burdens on *N. fuscipes*, which could be consistent with larger bodied rodents having higher helminth burdens (Froeschke and Matthee, 2014; Mohd Zain et al., 2012; Nunn et al., 2003; Poulin, 1996). Behavioral differences between *N. fuscipes* and *Peromyscus* spp. may lead to increased encounter rates with ticks. Examples of behavioral differences between species include a lack of grooming behaviors, larger territories, or use of constructed middens which create favorable microclimates for ecto-parasites (Cranford, 1977; Eisenberg, 1962; Kinsey, 1976; Wallen, 1982; Whitford and Steinberger, 2010). Majority of the helminth infections were of the *Trichuris* species. Based on lab studies, this helminth has been shown to only reach full development in the presence of certain gut flora (Hayes et al., 2010). The high helminth burdens found in *N. fuscipes* compared to *Peromyscus* spp. could be highlighting differences in gut flora between the two hosts or simply different exposure to microenvironments favorable to helminths. While it was not possible to identify the helminths to species, all of the helminth genera identified in this study have zoonotic disease potential and suggests that helminth infections in rodent populations of close proximity to urban spaces require examination in tandem with tick-borne diseases.

Our research identified difference in macro-parasite distribution across an ecological community, with tick burdens responding significantly to extrinsic factors like Shannon diversity of rodents and predators as well as climatic variables, whereas helminth burdens were not significantly associated with any of those variables (Table 2). Looking at the relationships between tick burden and host community structure, we see opposing associations for predator Shannon diversity (negative) and rodent Shannon diversity (positive). The negative relationship between predator Shannon diversity and rodent tick burdens is consistent with

earlier work reported in Salomon et al., (2021); Salomon et al. (2021). We hypothesize that this relationship is due to an increase of diverse predator activities that causes rodents to leave their nests less frequently, resulting in a decrease in encounter rates between rodents and questing ticks (Calabrese et al., 2011; Hofmeester et al., 2017; Hudson et al., 1992). With increased predator diversity there is an increase in diversity of behaviors and chemical cues that rodents have to interpret, increasing their fear response and decreasing their behaviors of leaving their nests (Moll et al., 2017, 2020; Suraci et al., 2019a, 2019b). Surprisingly, predator diversity did not impact the helminth burden of these rodent hosts and may represent the different modes of transmission for helminths. For instance, the majority of attached ticks were *I. pacificus*, therefore since this species is known to display questing behavior, these attached ticks were most likely encountered while the rodent was outside of its nest. Conversely, *Trichuris* eggs are excreted by hosts in feces and then ingested orally. *Neotoma fuscipes* are known to create middens (Moore et al., 2020; Whitford and Steinberger, 2010) that are typically within close range of their nests. Therefore, greater predation pressure may not deter *N. fuscipes* from their midden use nearby their nest, resulting in a higher likelihood of encounter with helminths. Teasing apart the predation of these rodents and reduction of movement due to predator activity by analyzing movement data, would be useful to better understand the negative impact predators have on parasitism patterns of rodent populations. Whereas the positive relationship between tick burdens and rodent Shannon diversity (Table 2) is hypothesized to be an indication of overall habitat quality and environmental conditions that are favorable to rodent populations and tick survivorship (MacDonald, 2018; Macdonald et al., 2017; Williams and Ward, 2010).

As ectoparasites are free-living organisms, ticks spend most of their lives in the environment rather than on hosts. This trait makes them sensitive to climatic parameters impacting their ability to survive off-host and during questing. Considering our last community level metric, climate, we found that tick burdens were positively associated with site level climate variables such as mean temperature and maximum vapor pressure deficit (Table 2 and Supplemental Table 2). In temperate San Francisco Bay Area, higher maximum vapor pressure deficit leads to an increased potential for tick-host encounters due to increased tick habitat suitability, increased tick survivorship, and increase period of host-seeking (Bacon et al., 2022; Diuk-Wasser et al., 2010; Hacker et al., 2021; Hahn et al., 2021). Our analysis shows that higher maximum vapor pressure deficit also increases tick burdens on hosts, providing more evidence that this parameter increases epidemiological risk of tick-borne pathogens.

Extending beyond the macro-parasite relationship with these community level metrics (i.e., Shannon diversity, climate), we found the presence of *B. burgdorferi* sensu lato infections in *N. fuscipes* significantly associated with only rodent Shannon diversity (Table 3). Essentially, in less diverse rodent communities there is a higher probability for a *N. fuscipes* to be infected with *B. burgdorferi* sensu lato (Table 3). Host diversity has been routinely connected to decreasing the prevalence of *B. burgdorferi* sensu lato by the literature and is referred to as the dilution effect hypothesis (Schmidt and Ostfeld, 2001). However, we are providing evidence that the host community diversity's impact on pathogen transmission may not be so clear. Our evidence suggests that the higher burden of *Ixodes* spp. increases probability of *B. burgdorferi* sensu lato presence in a host (Figs. 1 and 2) (Ostfeld et al., 2018; Salomon et al., 2021). However, our regression results show that tick burdens increase on hosts as rodent host diversity increases, while *B. burgdorferi* sensu lato decreases as rodent host diversity increases (Table 3). These discordant results suggest that this relationship is surprisingly nonlinear and highlights the need for more investigation surrounding the interactions of host community diversity, macro-parasites, and microparasites.

We sought to detect interactions between *Borrelia* spp., ticks, and helminths and found simultaneous infections of all three at a higher rate than expected (Table 1) but did not detect any evidence of interaction between them (Supplemental Table 2). However, when we compare uninfected and *B. burgdorferi* sensu stricto infected *N. fuscipes* with the Wilcoxon rank sum test, there was a significant difference in burdens of ticks and helminths but in opposite directions. Where *B. burgdorferi* sensu stricto infected *N. fuscipes* had a higher burden of ticks but a lower burden of helminths compared to uninfected *Neotoma fuscipes*. However, when we investigated these differences further with the binomial regression model, the analyses were not powerful enough to detect potential associations of macro-parasites on micro-parasites (Fig. 2). We found that *N. fuscipes* were more likely to be infected with *B. burgdorferi* sensu lato than *Peromyscus* spp. (Table 3), which may reflect the lower tick burdens mice had in comparison to woodrats ($W = 12806$, p-value <0.001). Since *B. burgdorferi* sensu lato is a tick-borne pathogen, these results are not surprising (Ostfeld et al., 2018; Salomon et al., 2021). What was unexpected, is that despite finding co-occurrences for all three types of infections, there was no significant associations between all three infections within individual hosts. Future studies with a larger sample size of *Borrelia* and helminth infected *N. fuscipes* would give more power to address these questions.

The mechanisms behind these coinfections are indirectly driven by community factors such as rodent diversity and predator diversity. Our results suggest that habitats with dynamic wildlife populations simultaneously facilitate diversity of parasites such as helminths and ticks. Counterintuitively, higher rodent diversity inhibits *B. burgdorferi* sensu lato infection within *N. fuscipes*, a rodent host which has been found to be a highly competent reservoir for *B. burgdorferi* sensu lato (Brown and Lane, 1994; Swei et al., 2012). This study has large implications on disease dynamics while stressing the need for more research so we can better understand how multi-parasitic infections are distributed amongst wildlife reservoirs and are driven by community diversity with consequences for system-wide disease transmission and risk. Changing environmental conditions may alter community interactions between hosts and their parasites and pathogens, it is therefore vital to understand these dynamics to prevent and manage the transmission of human infectious diseases.

Ethics approval and consent to participate

All procedures performed in this study involving animals were in accordance with the ethical standards of the California Department of Fish and Wildlife permit (ID # SC-8407) and the San Francisco State Animal Care and Use Committee (IACUC AU15-06).

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. Code for regression models and figures can be found at https://github.com/sbsambado/MultiParasite.

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Authors' contributions

JS, AC, SBS collectively conducted field work. JS, AC, SBS, and SS processed all field samples in the lab. JS and SBS analyzed data. JS prepared the initial manuscript. All authors wrote and edited the final manuscript.

Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2023.08.006.

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