Effects of environmental factors on pathogen exposure and transmission in wild rodent populations

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Dedication

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Abstract

Anthropogenic land-use change is altering ecosystems across the globe and has been implicated as a major factor increasing the spillover of zoonotic diseases from wildlife into human populations. Wild rodents are of particular importance for spillover as they host the largest diversity of zoonotic pathogens of any mammalian order. Moreover, rodent hosts of zoonotic pathogens have been found to increase in abundance in anthropogenic landscapes. In my dissertation, I investigate the effects of environmental factors related to anthropogenic land-use change on pathogen prevalence and transmission in wild rodent populations. Using an observational field study across landscape and habitat types, I broadly investigate the effects of anthropogenic development on the prevalence of zoonotic bacterial pathogens in wild *Peromyscus* mice (Chapter 1). I then turn to finer spatial scales to consider how spatial overlap can be used to approximate transmission in wildlife populations (Chapter 2). Using wild bank voles (*Clethrionomys glareolus*) as a model system, I leverage a replicated, experimental field study to quantify the effects of food supplementation and helminth macroparasite removal on vole space use and spatial overlap to approximate transmission opportunities (Chapter 3). Finally, I test how spatial overlap predicts infection of an endemic viral pathogen and examine whether the relationship between spatial overlap and infection is influenced by food abundance and macroparasite infection (Chapter 4). My research indicates that agricultural development may increase the prevalence of zoonotic bacterial pathogens in wild rodents. Further, I show that environmental factors alter the space use of wild rodents and that both environmental conditions and host traits are important to predict how spatial overlap affects transmission of an endemic pathogen. As such, my dissertation research has contributed empirical evidence that shows how environmental conditions alter zoonotic pathogen prevalence and transmission in wild rodent populations. This represents an important step forward in our ability to quantify the effects of anthropogenic land-use change on disease dynamics in wildlife, advancing our ability to understand and predict transmission dynamics and control spillover potential from wildlife into human populations.

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Introduction

Anthropogenic land-use change is affecting every ecosystem on earth and human-driven land-use change has been implicated in adverse ecological effects ranging from biodiversity declines (Caro et al., 2022; Newbold et al., 2020), to increases in regional climate extremes (Findell et al., 2017) and desertification (Burrell et al., 2020), to increased risks of zoonotic pathogen spillover from wildlife into humans (Gottdenker et al., 2014; Plowright et al., 2021). Zoonotic pathogen spillover is a complex process driven by a series of aligning ecological, epidemiology, climatic, and behavioral factors (Plowright, Parrish, et al., 2017; Schmid et al., 2015), but first and foremost, spillover requires sufficient abundance of the reservoir host and pathogen prevalence in reservoir host populations to facilitate transmission to humans (Davis et al., 2004). Pathogen prevalence and transmission in wildlife populations varies across space and time, even within a single host-pathogen system. While there are many biotic and abiotic factors that can drive these patterns (Paull et al., 2012; Tompkins et al., 2011; VanderWaal & Ezenwa, 2016), anthropogenic land-use change is particularly pervasive and relevant to current threats of zoonotic disease emergence and spillover (McMahon et al., 2018).

Anthropogenic land-use change can influence environmental factors such as resource abundance, habitat fragmentation, animal space use and movement, and interactions between wildlife and humans. These anthropogenic environmental factors can then influence pathogen transmission in wildlife both through increased pathogen exposure and increased interactions between animals. Anthropogenic environmental factors can influence wildlife exposure to pathogens: animals living near humans and livestock may be more exposed to waste, trash, and associated pathogens (Jahan et al., 2021; Murray et al., 2016); areas of high wildlife aggregation such as point-sources of food and water can accumulate pathogens shed in urine and feces or increase exposure via direct contacts (Forbes et al., 2015; Titcomb et al., 2021); and resource abundance can alter foraging behavior, exposing animals to pathogens in new environments or causing them to become more sedentary and allowing pathogens to accumulate in the environment (Hernandez et al., 2016; Murray et al., 2021).

Anthropogenic environmental factors can also influence interactions suitable for transmission by influencing the community composition of host species for pathogens

(LoGiudice et al., 2003) or altering direct and indirect interactions between animals through aggregation or sequential habitat use (Cross et al., 2007; Hirsch et al., 2016; Lewis et al., 2017). By affecting opportunities for pathogen exposure and interactions between animals, anthropogenic environmental factors can ultimately impact the transmission of pathogens and their prevalence in wildlife populations. Increased transmission among wildlife and peaks of prevalence can then increase the risk of pathogen spillover to humans (Davis et al., 2005; Khalil et al., 2019). As such, improving the understanding of how anthropogenic environmental factors ultimately drive pathogen transmission in wild populations is a priority for the effective management of disease in wildlife and in humans.

Rodents are a ubiquitous Order found on every continent (except Antarctica) and many rodents are generalist species and are highly adaptable to a large range of environmental conditions - including human-modified landscapes. As such, rodents tend to be the 'survivors' in degraded and fragmented landscapes, some even thriving in environments living alongside humans ("synanthropy"). Critically, decreases in rodent species diversity and increases in the abundance of key rodent species in human-modified landscapes is a mechanism responsible for increasing the prevalence of zoonotic disease (McCauley et al., 2015; Mills, 2006). Indeed, rodents are host to the largest number of zoonotic pathogens of any mammalian Order (Han et al., 2016), and rodent hosts of zoonotic pathogens, in particular, have been found to increase in abundance in anthropogenic landscapes (Gibb et al., 2020; Mendoza et al., 2019), making rodents a key focus of study for understanding and managing zoonotic disease in wildlife and limiting spillover potential to humans.

Moreover, rodents are reservoir hosts for many pathogens transmitted through the environment and are commonly implicated in indirect spillover pathways. Therefore, when studying rodent exposure to and transmission of these pathogens, it is critical to consider how the environment itself influences factors relevant to environmental transmission. However, our understanding of how environmental factors, particularly those associated with anthropogenic land-use change, alter pathogen exposure and transmission is still lacking. This is not only a gap in our ecological knowledge of infectious diseases, but also a detriment to effective public health interventions, limiting our ability to predict and control spillover of potentially deadly rodent-borne diseases into human populations (Abdullahi et al., 2020; Sun et al., 2019; Tian & Stenseth, 2019).

In an effort to improve our understanding of environmental anthropogenic factors on disease dynamics in wildlife, the overarching question my dissertation addresses is “How do environmental factors influence pathogen exposure and transmission in wild rodent populations?”. Specifically, I consider anthropogenic environmental factors such as agricultural development and variation in environmental food and macroparasite abundance. I address the effects of these factors through three studies which span scales: from broad landscape-level effects on populations to local habitat-level effects on individual rodents, and also investigate a range of possible transmission routes: from indirect exposure to pathogens in the environment, to direct exposure through interactions with conspecifics.

Chapter 1 investigates how large-scale environmental factors associated with anthropogenic land-use change (undeveloped vs. cropland agricultural landscape; forest vs synanthropic habitat) influence rodent exposure to environmental pathogens as evidenced by the bacterial microbiome and prevalence of zoonotic bacterial pathogens. My research addresses two specific questions: 1) How does the microbiome community of *Peromyscus* mice differ between forest and synanthropic habitat? and 2) Are zoonotic bacterial pathogens more abundant in agricultural developed landscapes? I conducted a live-trapping study of wild *Peromyscus* mice at the Cedar Creek Ecosystem Science Reserve and at the Itasca Biological Station and Laboratories and in Itasca State Park in Minnesota. DNA from fecal samples was sequenced using single-molecule, long read nanopore sequencing technology and a bioinformatic pipeline was used to taxonomically identify all bacterial reads to the species level. I show that the bacterial gut microbiome composition of *Peromyscus* varies across landscapes and habitat types. I also show that the abundance of putative pathogenic bacteria in wild *Peromyscus* may be increased by cropland agriculture. As such, this study broadly demonstrates how anthropogenic land-use change alters the bacterial gut microbiome and presence of zoonotic bacterial pathogens in wild rodents and suggests agricultural development as a potential driver of increased rodent exposure to environmental pathogens.

After investigating drivers of pathogen exposure at broad spatial scales, I then turn to finer spatial scales and focus the remaining chapters on the space use and spatial overlap of individual rodents to address how pathogen exposure and transmission in wild rodent populations are shaped by environmental factors of the ecosystems in which they reside.

Transmission events are difficult to document, particularly in wildlife, so opportunities for transmission are often approximated using relevant contacts between individuals, with the assumption that the pattern of transmission is a subset of the pattern of contact (Craft, 2015). However, if contacts are to be used to make inference about transmission, these contacts must be directly relevant to the transmission mode of the pathogen in question. Considering this, in Chapter 2 I provide an introduction to the use of networks in disease ecology with particular emphasis on the important considerations that must be made when constructing animal networks for pathogen transmission inference. We advise on best practices to ensure that how 'contact' is defined and networks are constructed aligns with biologically relevant interactions to enable meaningful inference about transmission.

With these recommendations for approximating transmission opportunities in mind, I leverage an experimental field study on wild bank voles (*Clethrionomys glareolus*, previously *Myodes glareolus*; Carleton et al., 2014; Kryštufek et al., 2020) to ask how food abundance and macroparasite infection: two environmental factors which vary naturally but can also be altered by anthropogenic forces, influence host exposure to environmentally transmitted pathogens. Here I broadly define host exposure as opportunities for transmission across a range of possible transmission routes from indirect exposure to pathogens in the environment, to direct exposure through interactions with conspecifics. Food abundance and macroparasite prevalence are environmental factors that vary naturally, but are also impacted by anthropogenic land-use change: food abundance can increase due to subsidies by agriculture or direct feeding of wildlife, but can also be decreased by habitat degradation, creating patchy patterns of food abundance across the landscape (Altizer et al., 2018). Similarly, macroparasite infection can also be altered by habitat degradation or increased wildlife use of urban areas (Arizono et al., 2012; Santicchia et al., 2015), changes in host community assemblies (Mihaljevic et al., 2018), or changes in host population density (Fichet-Calvet et al., 2003). Rodents, and bank voles especially, are a useful model system for studying spatial behavior and pathogen transmission in wildlife populations. Bank voles are abundant and are the dominant rodent species in Finland where this research was conducted, but have a range across much of central Europe and Fennoscandia. As such, a large body of research details their ecology and social and spatial behavior in a variety of habitats (Sweden, Bergstedt, 1966; Poland, Bujalska &

Grüm, 1989; United Kingdom, Crawley, 1969; Finland, Ylönen & Viitala, 1985). Previous field studies have also shown vole populations to be amenable to experimental manipulation and longitudinal capture-mark-recapture studies (Forbes et al., 2015, 2016). Finally, bank voles are host to several pathogens that can be transmitted through the environment and have the potential to infected humans (Parechovirus B; Jääskeläinen et al., 2016; e.g. Puumala hantavirus; Vaheri et al., 2013), motivating our efforts to better understand how the environment may influence transmission.

In Chapter 3, I test how food supplementation and helminth removal affect vole space use and spatial overlap with conspecifics. Space use and spatial overlap capture behaviors that can influence exposure to pathogens both indirectly in the environment and directly from conspecifics. Wild, free-ranging populations of bank voles were experimentally manipulated in a replicated, factorial experiment. Populations received one of four treatments: no manipulation, supplemental food only, helminth removal via oral anthelmintic only, or both supplemental food and helminth removal. Each treatment was replicated in three populations. Populations were monitored by capture-mark-recapture trapping grids and the locations of individual voles were recorded each month over the course of two days of live trapping. My objectives in this study were to 1) estimate vole space use by sex and reproductive status in the breeding and non-breeding season and 2) quantify spatial overlap between voles each month using network analysis. I then compared space use and spatial overlap between treatments to test the effects of food supplementation and helminth removal on vole spatial behavior. I show that food supplementation decreases vole space use and helminth removal increases space use. I also show that food supplementation increases bank vole population density. However, despite the differences in space use, I found similar spatial overlap across the experimental manipulations, suggesting that trade-offs in space use and population density may regulate spatial overlap between individual voles. The spatial overlap quantified in this chapter is most suitable to quantify transmission opportunities for zoonotic pathogens which can be transmitted through the environment, several of which are common in bank voles (e.g., Puumala hantavirus, Parechovirus B), so understanding how anthropogenic changes to their environment affects vole spatial behavior can ultimately aid in predicting the effects of environmental factors on pathogen exposure and transmission in vole populations and spillover potential to humans.

Finally, in Chapter 4, I expand on the ideas of the previous chapter to ask how environmental factors influence pathogen transmission. In this study, I test how spatial overlap in wild rodent populations predicts infection of an endemic viral pathogen, and further examine if host-level traits play a role in mediating the effects of environmental factors such as food abundance and helminth infection on the relationship between spatial overlap and infection. This chapter experimentally tests the assertions made in Chapter 3 that patterns of spatial overlap can be used to infer transmission of environmentally transmitted pathogens. I again leverage the experimental field study in bank voles (as described in Chapter 3) and additionally document individual-level infection of a viral pathogen (Puumala hantavirus) that is endemic in bank vole populations. This chapter addresses two specific questions: 1) Is the relationship between spatial overlap and infection status influenced by food abundance and helminth infection? and 2) How do vole demographic traits (sex and reproductive condition) and space use behavior moderate these effects? I show that spatial overlap does drive hantavirus infection in vole populations, but the relationship between spatial overlap and infection is altered by food supplementation and helminth removal. I also identify how differences in behavior and physiology by vole functional group (combination of sex and reproductive condition) interact with space use and spatial overlap under the different experimental manipulations to alter how spatial overlap influences hantavirus infection probability. With this study, I provide empirical evidence that manipulating food abundance and helminth infection can impact pathogen transmission and show that the interaction of host traits and spatial behavior may be important to explain different patterns of transmission under each of these manipulations.

Scientific research is a collaborative effort and the work included in my dissertation is no exception. I was the primary author of all the research presented herein, responsible for collecting the data (or contributing significantly to its collection, in the case of Chapters 3 and 4), conducting the analyses, writing the initial draft, and incorporating revisions from my collaborators. However, none of this work would have been possible without the help of my co-authors in obtaining funding, collecting data in the field, processing samples in the laboratory, lending their knowledge and expertise, and providing critical feedback to improve the written work presented herein. Each chapter therefore includes co-authors who contributed meaningfully to the work. Chapter 1 was co-authored with Evan Kipp, Sarah Weinberg, Colin Adams, Peter Larsen, and

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Chapter 1. Microbiome community composition and zoonotic bacterial pathogen prevalence in synanthropic *Peromyscus* mice

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1.1 Abstract

Rodents are key reservoirs of zoonotic pathogens and play an important role in disease transmission to humans. Importantly, anthropogenic land-use change has been found to increase the abundance of synanthropic rodents, particularly rodent reservoirs of zoonotic disease. Anthropogenic environments also affect the microbiome of synanthropic wildlife, influencing wildlife health and potentially introducing novel pathogens. Our objective was to characterize the microbiome and investigate the prevalence of zoonotic bacterial pathogens in synanthropic rodents in native and anthropogenic environments to better understand their role in pathogen maintenance and transmission. We sampled wild *Peromyscus* mice in agricultural and undeveloped landscapes and forest and synanthropic habitat in Minnesota, USA and conducted 16S amplicon sequencing using long-read Nanopore sequencing technology on fecal samples to characterize the rodent microbiome. We compared community composition and diversity between habitats and screened for the presence of putative pathogenic bacteria species. Microbiome community composition differed significantly between agricultural and undeveloped landscapes and forest and synanthropic habitat while microbiome richness, diversity, and evenness were lower in undeveloped-forest habitat compared to all other habitats. We detected overall low abundance and diversity of putative pathogenic bacteria, though the greatest number of pathogenic bacteria were detected in the agricultural-forest habitat. Our findings show that rodent microbiome community composition differs across landscapes and habitat types but suggest that landscape-level anthropogenic factors may be most important to predict zoonotic pathogen abundance. Ultimately, understanding how anthropogenic land-use change

and synanthropy affect rodent microbiomes and pathogen prevalence is important to managing transmission of rodent-borne zoonotic diseases to humans.

1.2 Introduction

Rodents are an important source of zoonotic disease spillover, accounting for a greater diversity of zoonotic pathogens than any other mammalian order (Han et al., 2016). While many factors have been proposed to contribute to this (e.g. fast-paced life history, Han et al., 2015; cyclic population fluctuations, Kallio et al., 2009) recent studies have suggested that the tendency of particular rodent species to occasionally or exclusively live in human-built environments (synanthropy) is likely a key factor (Ecke et al., 2022).

Anthropogenic land-use change, leading to habitat fragmentation and the intensification of agricultural development and urbanization, is the major driver of zoonotic pathogen spillover (Gottdenker et al., 2014). Indeed, urbanized habitat has been found to have a significant, positive effect on the abundance of rodent hosts of zoonotic pathogens compared to areas of native vegetation (Mendoza et al., 2019). Shifts in rodent biodiversity in anthropogenic landscapes could further increase zoonotic risk, as rodent hosts and non-host rodents show opposite responses to agricultural and urban habitat, with the abundance of host species increasing and non-host species decreasing compared to areas of minimally disturbed primary vegetation (Gibb et al., 2020).

However, spillover of zoonotic pathogens at the human-wildlife interface does not solely flow from wildlife into humans. Synanthropic wildlife (including rodents) also show increased prevalence of human pathogens: *Escherichia coli*, *Clostridioides difficile*, *Salmonella enterica* in Norway rats in New York City, New York (Firth et al., 2014); antimicrobial-resistant *E.coli* in racoons in Chicago, Illinois (Worsley-Tonks et al., 2021); *Salmonella* in urbanized white ibis in southern Florida (Hernandez et al., 2016), representing both a concern for wildlife health and a potential source for spillback into human populations. As such, while the relationships between land-use change, rodents, and zoonotic pathogen prevalence are still being explored, synanthropic wildlife represent both important reservoirs for zoonotic pathogens and likely drivers of pathogen maintenance and spillover in anthropogenic landscapes (Hassell et al., 2017).

Synanthropy has also been shown to impact the gut microbiome of wildlife. The gut microbiome plays a role in host health (Marchesi et al., 2016) and immune function (Schluter et al., 2020) and disruption of the normal microbiome has been linked to various health conditions in wildlife, livestock, and domestic animals (Funosas et al., 2021; Monteiro & Faciola, 2020; Suchodolski, 2022). Wildlife living in close proximity to humans often experience changes to the composition of their microbiome compared to counterparts in native habitat (e.g. rodents, Anders et al., 2022; sparrows, Berlow et al., 2021) though whether anthropogenic habitats decrease or increase microbiome diversity may vary by species (Diaz et al., 2023; Dillard et al., 2022). It is likely that changes in microbiome diversity associated with synanthropy could increase the prevalence of pathogenic bacteria in wildlife, but studies linking microbiome shifts with pathogen prevalence are limited (but see Murray et al., 2020).

Here, we characterize the microbiome and compare the abundance of zoonotic bacterial pathogens in *Peromyscus* mice in agricultural developed and undeveloped landscapes and forest and synanthropic habitat in Minnesota, USA. Our research questions were two-fold: 1) How does the microbiome community of *Peromyscus* mice differ between forest and synanthropic habitat? and 2) Are zoonotic bacterial pathogens more abundant in agricultural developed landscapes?

We expected the microbiome community of *Peromyscus* to be shaped by the surrounding landscape and specific habitat as they influence the availability of food resources and exposure to humans and their pathogens. We predicted that microbiome richness and diversity would be lower in the agricultural landscape and synanthropic habitat compared to the undeveloped landscape and forest habitat due lower diversity of food resources. We predicted that the agricultural landscape would have a higher abundance and diversity of pathogenic bacteria since the area is dominated by crop fields and human habitation and thus increased exposure to manure as fertilizer, wastewater and runoff, and trash; whereas we predicted that the undeveloped landscape would have lower pathogen abundance because the surrounding area is largely forested with little anthropogenic development. Characterizing rodent microbiomes across development gradients is important for quantifying the risk of rodent-borne zoonotic pathogen spillover and understanding how microbiome shifts associated with synanthropy may influence pathogen abundance.

1.3 Materials & Methods

Study Sites

Three major North American biomes intersect in Minnesota: the eastern deciduous forest, northern coniferous forest, and western prairie, providing diverse habitats and biological communities of hosts and pathogens. With respect to land-use, the state is dominated by agricultural cropland and forest with interspersed developed areas ranging from dense metropolitan areas to small, rural communities. Together, the biological and anthropogenic factors create a heterogeneous landscape of natural areas mixed with agricultural and urban developed landscapes where synanthropic rodents have many opportunities to overlap with humans. We focus our study on mice of the genus *Peromyscus* (i.e. *Peromyscus leucopus* and *Peromyscus maniculatis*) which are highly adaptable generalists that are common throughout Minnesota and can thrive in agricultural and urban settings as well as forests and grasslands. Importantly, *Peromysucus* mice are known reservoirs of zoonotic and foodborne pathogens (e.g. *Borrelia*, *Campylobacter* spp., *E. coli*, *Giardia* spp., hantavirus) (Jahan et al., 2021).

For our study, we focused on two landscape types: native, contiguous forest with little permanent human habitation or agriculture (hereafter “undeveloped landscape”) and a mosaic of fragmented forest interspersed with crop fields and low-density housing (hereafter “agricultural landscape”). Within each landscape, four study sites were chosen to represent two habitat types (two sites per habitat): forest habitat and synanthropic habitat around human-frequented structures (e.g. cabins, tent platforms, field station buildings, maintenance sheds and garages). Rodent sampling was conducted at two locations: the Itasca Biological Station and Laboratories at Itasca State Park (“Itasca”, undeveloped landscape) and Cedar Creek Ecosystem Science Reserve (“Cedar Creek”, agricultural landscape). Itasca is located in northern Minnesota in the northern coniferous boreal forest biome. Though the state park is frequented by hikers and visitors, the surrounding landscape is contiguous forest with no agricultural development and very sparse permanent human habitation (Figure 1.1-A). Cedar Creek is located in central Minnesota in the eastern deciduous forest and oak savanna biome. The landscape surrounding the reserve is dominated by agricultural development (e.g. pasture/hay, cultivated crops), woody and herbaceous wetlands, and low-medium intensity housing communities (Figure 1.1-B).

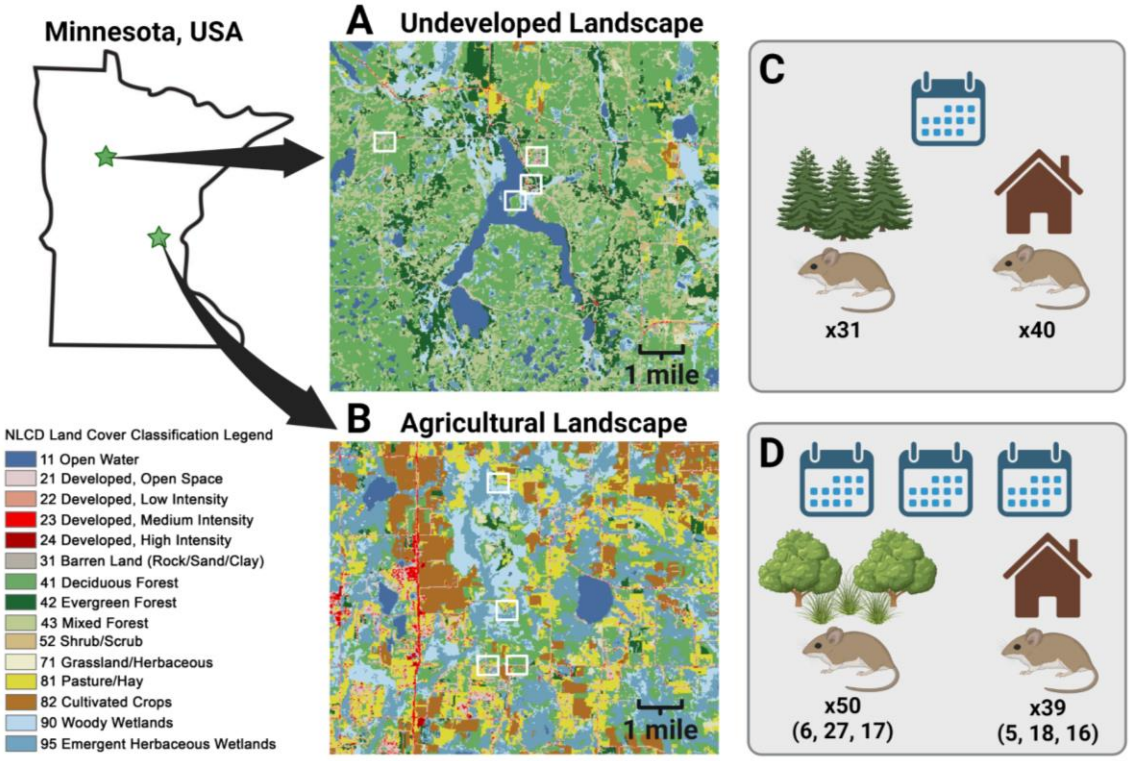

Figure 1.1. *Rodent sampling locations and sample size summary. Sampling was conducted at two locations in Minnesota, USA representing undeveloped and agricultural landscapes. Study sites are outlined with white boxes (A, B). Sample size (total number of fecal samples) in forest and synanthropic habitat is shown for each landscape (C, D). Sampling was conducted once in the undeveloped landscape and three times in the agricultural landscape. Total number of samples per landscape-habitat pairing is noted first with samples per month in parentheses below (includes multiple samples from individual mice). Maps and land cover classification legend from National Land Class Database (NLCD) 2019 (Dewitz, 2021). Figure created with BioRender.com.*

Rodent trapping

Two consecutive nights of rodent trapping were conducted at each study site (a "trapping session") using 100 Sherman live-capture traps baited with oats. Traps were opened at dusk and checked at dawn the following morning. Traps were closed during the day between trap nights at a single site and were reopened at dusk for the second night. Captured *Peromyscus* mice were sampled and then released at the point of capture. Due to the difficulty in distinguishing *P. leucopus* and *P. maniculatus* – two species found across our study landscapes in Minnesota – based solely on morphologic features, we did not attempt to identify captured *Peromyscus* mice to the species level. Captured non-target (i.e. non-*Peromyscus*) species were released immediately and were not sampled. Longitudinal trapping was conducted at the agricultural landscape sites. Each site was sampled three times throughout the summer (June, July, and August

2019) with 3-4 weeks between trapping sessions. Captured *Peromyscus* mice were marked with a metal ear tag to identify individuals at subsequent recaptures. Only one trapping session (July 2019) was conducted at the undeveloped landscape sites. For each captured *Peromyscus*, a fecal sample was collected and body mass, sex, and reproductive status were recorded (reproductive individuals identified by the presence of scrotal testes for males or any of the following traits for females: perforate vagina, enlarged nipples, palpable embryos). Individuals captured a second time within a trapping session were not resampled and were promptly released at the point of capture.

All rodent trapping and handling methods were reviewed and approved by the University of Minnesota Institutional Animal Care and Use Committee (protocol no. 1903-36892A). The objective of this study was live-capture and release but trap fatalities (3.4% [16/477] of capture events of target and non-target species) were collected with approval by the Minnesota Department of Natural Resources (MN-DNR) under Special Permit No. 28440 and were accessioned with the Bell Museum of Natural History collections.

DNA Extraction

We collected 176 fecal samples representing 153 unique individuals. Fecal samples of up to 250 mg were stored without buffer or ethanol and frozen at -80°C immediately after sampling until DNA extraction. DNA was extracted using a QIAamp PowerFecal Pro kit (Qiagen, Hilden, Germany) following manufacturer instructions both manually and using a QIAcube robotic workstation (Qiagen, Hilden, Germany). DNA extracts were quantified using a Qubit 4 fluorometer (Thermofisher Scientific, Waltham, MA, USA) using the Qubit dsDNA BR Assay Kit (Thermofisher Scientific, Waltham, MA, USA) following the manufacturer's instructions. Samples with low DNA yield (<24 ng/μL, n=16) were excluded from downstream analysis.

Library Prep & Nanopore Sequencing

For the remaining 160 samples, the Rapid 16S Barcoding Kit (SQK-16S024 [utilizing 'Kit 9' chemistry]; Oxford Nanopore Technologies [ONT], Oxford, UK) was used to prepare barcoded amplicon libraries for sequencing, largely following the manufacturer's protocol (methods described in detail in Jahan et al., 2021). First, all fecal DNA extracts were diluted in nuclease-free water to a concentration of 10-30 ng/μl. The

full-length bacterial 16S rRNA gene region (1.6kb) was amplified via PCR using specific primers and between 20-40 ng of DNA template, a long-range master mix (LongAmp Hot Start Taq, 2X; New England Biolabs, Ipswich, MA, USA), and sample-specific barcode identifier. PCR products were purified and prepared for sequencing through a series of magnetic bead wash steps (AMPure XP beads; Beckman Coulter Life Sciences, Indianapolis, IN, USA). Barcoded samples were pooled with ONT rapid sequencing adapter mixture into a final library for sequencing. Seven sequencing runs were performed with a total of 160 samples, including 24 (run 1, 2, 4), 23 (run 6), 22 (run 3, 5), and 21 (run 7) barcoded samples from individual mice. Libraries were sequenced on a FLO-MIN106 MinION flow cell utilizing R9 sequencing chemistry (Oxford Nanopore Technologies, Oxford, UK), run for 24 hours using the ONT MinKNOW GUI (v4.3.20; Oxford Nanopore Technologies, Oxford, UK).

Bioinformatic Pipeline

Raw Fast5 data from the sequencing runs were base-called using the ONT Guppy basecaller using the 'super accuracy' basecalling model (ONT configuration file: dna_r9.4.1_450bps_sup.cfg). The barcoded samples were further de-multiplexed using the Guppy barcoder to identify reads as belonging to one of the 24 unique barcodes. Reads were quality filtered (Nanopore Q score ≥ 8, corresponds to 84.15% base call accuracy) and filtered for target length (full-length bacterial 16S region approx. 1600 bp in length) using NanoFilt (v2.8.0; De Coster et al., 2018). Only reads 1200-1800 bp in length were retained for onward analysis. Summary reports were generated using Nanoq (v0.9.0; Steinig & Coin, 2022).

Taxonomic abundance profiles were generated using Emu, an expectation-maximization algorithm designed specifically to account for the increased read length and error rate often associated with long-read data such as ONT-generated sequences (v3.4.4; Curry et al., 2022). Compared to conventional taxonomic identification algorithms, Emu is able to reduce the false positive rate of identification and accurately identify long reads to species level (Curry et al., 2022). Reads were mapped using the Emu default database settings: a combination of rrnDB v5.6 (Stoddard et al., 2015) and NCBI (National Center for Biotechnology Information) 16S RefSeq downloaded on 17 September 2020 (O'Leary et al., 2016). The output of Emu is an estimated abundance (read count) of each identified species in a given sample. Because read counts are

estimated based on likelihood probabilities, outputted values are not necessarily integer counts. Values were rounded to the nearest integer for analysis.

Data Analysis

The full fecal microbiome was characterized at the sample level using measures of alpha and beta diversity (to quantify within-sample and between sample bacterial diversity, respectively). Alpha diversity indices included species richness, Shannon-Weiner diversity, Simpson diversity, and species evenness. Shannon diversity and Simpson diversity make different assumptions about species evenness and how it contributes to diversity: Shannon diversity assumes all species are present and are randomly sampled while Simpson diversity gives more weight to common species. Calculating both indices can suggest how common or rare species may affect diversity estimates for different populations. Beta diversity was quantified using the Bray-Curtis dissimilarity index to compare bacterial microbiome community composition at the species level between all pairs of samples. As a subset of the full fecal microbiome, the presence of pathogenic bacteria (foodborne and zoonotic pathogens of concern for human, livestock, and domestic animal health) was quantified at the sample level, then grouped by landscape-habitat pairing.

Rodent sampling was conducted across three months (June, July, and August) in the agricultural landscape and 18 individuals were captured and sampled in multiple months. To control for non-independence between repeat samples of the same individuals, only one sample per mouse (n=140) was included in the alpha and beta diversity analyses. We chose to include only the July sample for all recaptured mice to avoid introducing variation based on sampling month (all recaptured animals were sampled in July, but not in June or August) and to better align with the undeveloped landscape sampling (which was only conducted in July). For the analysis of pathogenic bacteria species, all samples (n=160) were used.

For the analyses of alpha and beta diversity, all samples were rarefied to the number of reads of the least abundant sample using the ‘phyloseq’ R package (v1.38.0; McMurdie & Holmes, 2013). Alpha diversity indices (richness, Shannon, Simpson, and evenness) were estimated from the rarefied data using the ‘vegan’ R package (v2.6.4; Oksanen et al., 2022). We investigated whether alpha diversity was affected by landscape or habitat type by developing a linear regression model for species richness

and Shannon diversity and a beta regression model for Simpson diversity and species evenness. In all models, the response variable was the alpha diversity index and the explanatory variables were landscape type (i.e. anthropogenic or undeveloped), habitat type (forest or synanthropic), the interaction of landscape and habitat type, mouse sex, reproductive status (reproductive or non-reproductive), body mass, and sampling month (June, July, or August). Beta diversity was visualized using non-metric multidimensional scaling (NMDS) ordination performed on the rarefied data using the Bray-Curtis dissimilarity index in the 'vegan' package. NMDS was first performed with 2-dimensions (k) and the k value was iteratively increased until the stress value was below 0.1. Non-parametric statistical analyses were performed on the rarefied distance matrices using the 'adonis2', 'anosim', 'betadisper', and 'permutest' functions also in the 'vegan' package.

For the analysis of pathogenic bacteria, species-level abundances were not rarefied and the raw estimated read counts output by the Emu pipeline were used. A list of 209 putative pathogenic bacteria species was generated using the PHI-base pathogen database (Urban et al., 2020; accessed on 13 Feb. 2023, plant-specific pathogens removed); resources from the U.S. Centers for Disease Control and Prevention on 'foodborne germs and illnesses' (CDC, 2022); and foodborne and mastitis-causing pathogens screened for by Jahan *et al.* 2021 (Jahan et al., 2021). The species-level read count abundance data from the sequenced samples was filtered for reads assigned to the pathogen species on this list. We thresholded read count per pathogen species to at least 50 reads and visualized the patterns of pathogen read count per mouse, grouped by landscape-habitat pairing. All analyses were performed in R Statistical Software (v4.1.2; R Core Team, 2021).

1.4 Results

Rodent Samples

160 fecal samples were sequenced, representing 140 unique *Peromyscus* mice. In the agricultural landscape, 50 and 39 total fecal samples were collected from forest and synanthropic habitats, respectively, across three months of rodent trapping (Figure 1.1-C). Excluding recaptures, 40 and 29 unique mouse fecal samples were collected in forest and synanthropic habitats, respectively. In the undeveloped landscape, 31 and 40

unique mouse fecal samples were collected from forest and synanthropic habitats, respectively (Figure 1.1-D).

Nanopore Sequencing Summary

After quality filtering, over 32.7 million high quality reads were retained (mean Q score 12.8 ± 0.31 s.d; Q score of 12.8 corresponds to base call accuracy of 94.75%). The mean number of reads per sample was 204,772.4, though the number of reads per sample was highly variable (standard deviation: 82,970.5; range: 74,517-517,058 reads; Table 1.1).

Table 1.1. *Summary statistics of 16S Nanopore sequencing data of mouse fecal sample DNA (after quality filtering) by landscape, habitat type, and sampling month. Mean and standard deviation are reported for number (N) of reads per sample (reported in units of thousands of reads), number of basepairs (BP) per sample (reported in units of millions of basepairs [Mb]), and read quality (Q) score. Individual sampling months in the agricultural landscape shown in italics, rows shaded in gray. Mean values across all three months shown in bold. Number of samples represents total number of fecal samples sequenced (includes repeat sampling of unique mice).*

Landscape	Habitat	Month	N samples	N reads / sample (thousands of reads)	N basepairs / sample (Mb)	Q Score
Agricultural	*Forest*	*June*	*6*	*272.18 ± 39.12*	*433.27 ± 61.68*	*13.08 ± 0.04*
Agricultural	*Forest*	*July*	*27*	*262.35 ± 62.91*	*418.69 ± 100.39*	*13.14 ± 0.06*
Agricultural	*Forest*	*August*	*17*	*248.61 ± 116.91*	*395.76 ± 186.39*	*12.84 ± 0.31*
Agricultural	**Forest**	**Summer**	**50**	**258.86 ± 82.36**	**412.64 ± 131.34**	**13.03 ± 0.23**
Agricultural	*Synanthropic*	*June*	*5*	*326.35 ± 73.84*	*517.32 ± 115.67*	*13.08 ± 0.08*
Agricultural	*Synanthropic*	*July*	*18*	*215.45 ± 21.66*	*342.53 ± 33.57*	*12.82 ± 0.04*
Agricultural	*Synanthropic*	*August*	*16*	*88.02 ± 10.85*	*139.68 ± 17.33*	*12.4 ± 0*
Agricultural	**Synanthropic**	**Summer**	**39**	**177.39 ± 88.31**	**281.72 ± 139.93**	**12.68 ± 0.25**
Undeveloped	**Forest**	**July**	**31**	**139.49 ± 22.44**	**223.04 ± 35.6**	**12.43 ± 0.22**
Undeveloped	**Synanthropic**	**July**	**40**	**214.45 ± 59.77**	**342.01 ± 95.9**	**12.88 ± 0.11**

The Emu algorithm identified 1212 unique bacterial species across the 160 fecal samples. The mean number of species per sample was 211 (standard deviation: ± 55.8; range: 82-367). Rarefaction curves were plotted for all sequenced samples (n=160). The asymptotic nature of these curves suggest reasonable sequencing depth was achieved for all samples (Figure A1). To enable direct comparisons between samples for the

alpha and beta diversity analyses, the samples used in the diversity analysis (n=140) were rarefied to the minimum number of reads per sample (74,517 reads) and species were selected without replacement to reach the desired number of reads. After rarefaction, 36 species were removed because they were no longer present in any sample after random subsampling.

Alpha Diversity

The interaction of landscape and habitat type had a moderate effect on observed species richness, Shannon diversity and Simpson diversity indices (all $p<0.05$; Table A1). The effect of the interaction of landscape and habitat type on species evenness was weaker and only marginally significant ($p=0.087$; Table A1), though there was a significant effect of landscape alone ($p=0.016$; Table A1). Mean observed species richness, Shannon diversity, Simpson diversity, and species evenness were lower in the undeveloped-forest habitat compared to all other landscape-habitat pairings (Figure 1.2; Table A2). However, contrary to our hypotheses, there was no difference in species richness, diversity, or evenness between forest and synanthropic habitats in the agricultural landscape or between agricultural-synanthropic and undeveloped-synanthropic habitat. Reproductive status (reproductive or non-reproductive individual, as noted by external morphology) had a moderate effect on Shannon and Simpson diversity and species evenness (all $p<0.01$; Table A2). None of the other parameters tested (sex, body mass, sampling month) had an effect on any alpha diversity index.

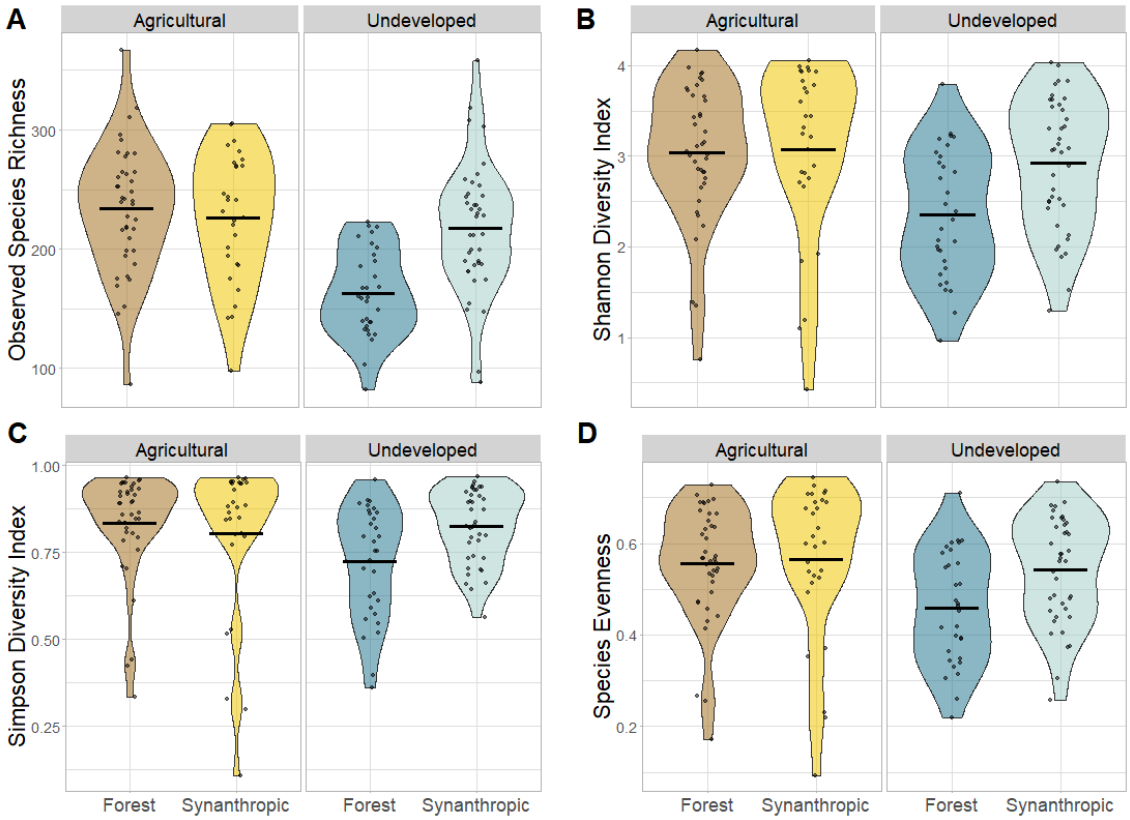

Figure 1.2. *Alpha diversity for all unique mouse fecal samples (n=140) in anthropogenic and undeveloped landscapes and in forest and synanthropic habitat according to A) observed species richness B) Shannon diversity index C) Simpson diversity index and D) species evenness.*

Beta Diversity

Across the four landscape-habitat pairings, the microbiome communities of sampled mice were dominated by three phyla: Firmicutes, Proteobacteria, and Bacteroidetes (relative abundance ≥5%) though Melainabacteria (a candidate phylum related to Cyanobacteria, Di Rienzi et al., 2013) and Deferribacteres were observed at relative abundances ≥1% in some samples (Figure 1.3). Firmicutes was the dominant phyla in most samples (relative abundance 90.1% mean ± 11.1 s.d.) followed by Proteobacteria (16.8% ± 20.0) and Bacteriodetes (8.92% ± 3.07).

Figure 1.3. *Relative abundance of bacteria phyla per sample (n=140) by landscape-habitat pairing showing phyla present at ≥1% relative abundance. Phyla observed at <1% relative abundance were grouped in a single category "Other". The microbiome of sampled mice was dominated by three phyla: Bacteroidetes, Firmicutes, and Proteobacteria.*

Bacterial microbiome community composition at the species level was compared between all pairs of samples using the Bray-Curtis dissimilarity index based on rarefied count data. A nonparametric analysis of similarities test ('anosim' function, 'vegan' R package) comparing dissimilarity indices between samples from the four landscape-habitat pairings suggested that the between-group dissimilarity in microbiome community composition was significantly greater than the within-group dissimilarity ($p=0.001$).

An NMDS ordination plot calculated based on Bray-Curtis dissimilarity indices showed a high degree of overlap between samples from the four landscape-habitat pairings (Figure 1.4). Samples from agricultural-synanthropic and undeveloped-forest habitat showed the greatest dissimilarity while samples from agricultural-forest and undeveloped-synanthropic habitat were more similar. The variability among samples was high, but an analysis of multivariate homogeneity of group dispersion ('betadisper' and 'permutest' functions, 'vegan' R package) by landscape-habitat pairing showed no significant difference in variance between the groups (permutation test, $p=0.96$), indicating that the differences in community composition were not only due to differences in sample variance.

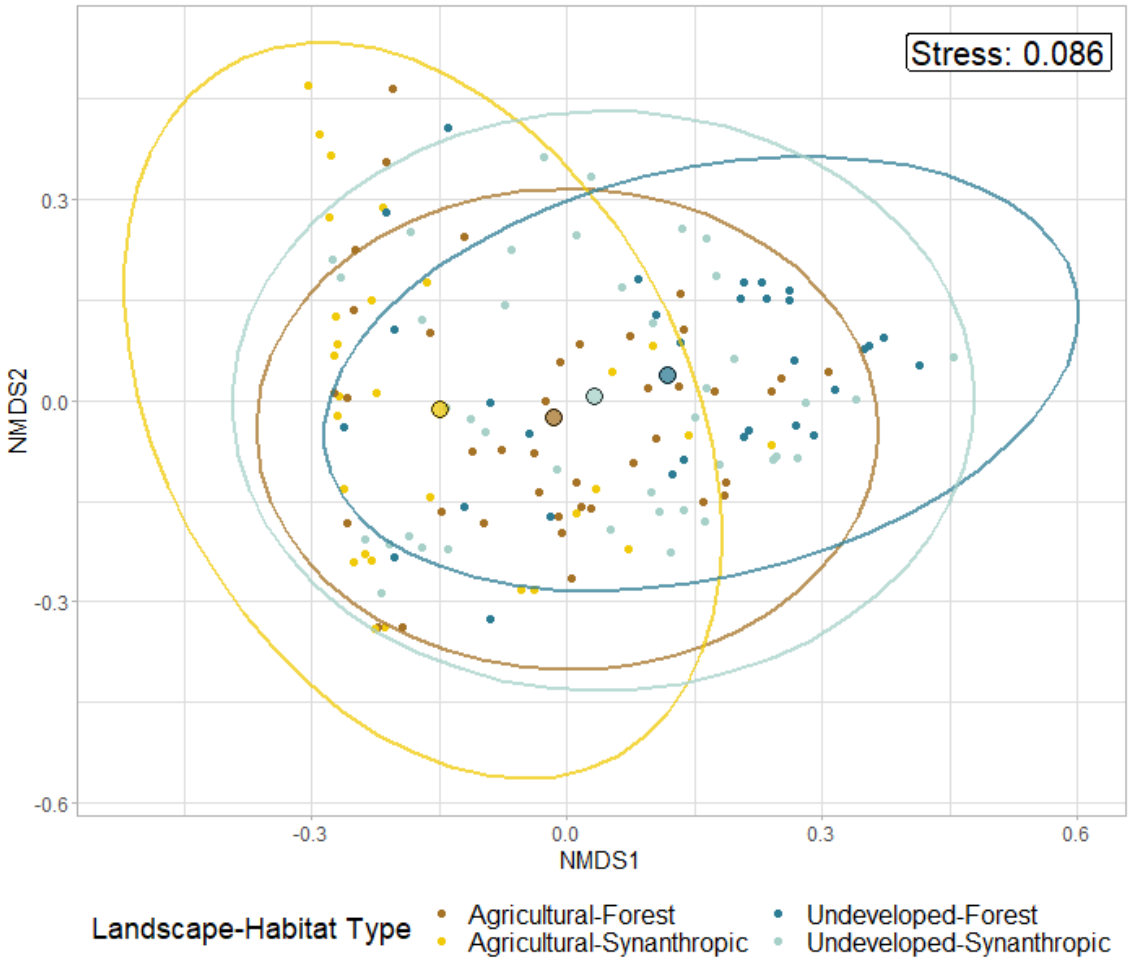

Figure 1.4. *Non-metric multidimensional scaling ordination on microbiome community composition by Bray-Curtis dissimilarity index. Non-metric multidimensional scaling ordination on microbiome community composition by Bray-Curtis dissimilarity index. Points represent individual samples, colored by landscape-habitat pairing. Ellipses denote the 95% confidence level for a multivariate t-distribution of the data points per group (centroids marked with larger points). Stress value: 0.086 (k=4).*

A nonparametric PERMANOVA analysis was used to test the effects of landscape, habitat type, mouse sex, reproductive status, body mass, and sampling month on differences in microbiome community composition using the 'adonis2' function in the 'vegan' R package with the by="margin" option to determine the marginal effect of each parameter. There was a small but significant effect of landscape and habitat, suggesting that the microbiome of sampled mice was different between agricultural and undeveloped landscapes and between forest and synanthropic habitats (PERMANOVA, $R^2_{Landscape}$=0.06, $R^2_{Habitat}$=0.04, both p=0.001; Table A3). Mouse reproductive status and body mass also had small, but significant effects (both p<0.05). However, much of the

variance in microbiome community composition was not explained by the modeled parameters (residual R^2=0.85).

Putative pathogen detection

The presence of putative pathogenic bacteria was investigated using raw read counts of all sequenced samples (n=160). Read counts from mice captured in more than one month in the agricultural landscape were pooled by bacterial species across fecal samples from a single mouse. Of the 209 putative pathogenic bacteria species screened for, 18 were identified in sampled mice (read count ≥ 50). At the population level, putative pathogen species richness was higher in agricultural-forest and undeveloped-synanthropic habitat (13 species identified; Figure 1.5) compared to agricultural-synanthropic and undeveloped-forest habitat (7 species identified). However, at the individual level, putative pathogen species richness was higher in mice in the agricultural landscape (agricultural-forest: mean putative pathogen species/mouse 1.42 ± 1.17 s.d.; agricultural-synanthropic: 1.24 ± 1.06) compared to mice in the undeveloped landscape (undeveloped-forest: 0.42 ± 0.77; undeveloped-synanthropic: 0.83 ± 0.93).

Read counts of detected putative pathogens were similar across landscape-habitat pairings with many mice having low read counts (<200 reads), though the number of mice with high read counts (>500 reads) was greatest in the agricultural-forest habitat (Figure 1.5). Across all sampled mice, *Clostridioides difficile*, *Streptococcus sanguinis*, *Enterococcus gallinarum*, *Citrobacter freundii*, and *Morganella morganii* were the most frequently detected putative pathogens (Figure 5).

Figure 1.5. *Heatmap of read counts of putative pathogenic bacteria species per mouse in each landscape-habitat pairing (count threshold >50 reads). The vertical axis represents samples from an individual mouse. Warmer colors indicate higher read abundance (natural log scale).*

1.5 Discussion

Our objective was to characterize and compare the microbiome of synanthropic rodents and the abundance of zoonotic bacterial pathogens in agricultural landscapes and synanthropic habitat in Minnesota. We found that landscape-habitat pairing affected microbiome richness and diversity but species evenness was only affected by landscape. Overall, undeveloped-forest habitat had lower mean alpha diversity (richness, Shannon and Simpson diversity, evenness) than the other three landscape-habitat pairings. Microbiome community composition at the species level was also significantly different between landscapes (agricultural versus undeveloped) and habitat types (forest vs. synanthropic). We detected reads for a number of putative pathogenic bacteria across the four habitats, mostly at low read counts. The mean number of putative pathogenic bacteria detected per mouse was higher in the agricultural landscape than the undeveloped.

Across landscape-habitat pairings, the microbiome of sampled mice was dominated by three phyla (Firmicutes, Bacteroidetes, Proteobacteria). These phyla are typical of the gut microbiome of wild *Peromyscus*, though we observed higher levels of Firmicutes and lower levels of Bacteroidetes compared to previous studies (e.g. Diaz et al., 2023; Schmidt et al., 2019). This suggests that the core fecal microbiome of the mice in our study is similar to *Peromyscus maniculatus* in other regions of North America. Only one other study has compared microbiome communities of free living *Peromyscus* in developed and undeveloped habitats (Diaz et al., 2023). We found lower richness and alpha diversity in the undeveloped-forest habitat compared to all other habitats, conversely, Diaz *et al.* found lower mean richness and Shannon diversity in urban habitats compared to undeveloped habitats. However, the directionality of alpha diversity shifts between undeveloped and developed populations is likely affected by multiple species- and system-specific factors; research in other wildlife systems has documented an increase in alpha diversity between undeveloped and developed populations (Dillard et al., 2022). Despite the differences in the direction of alpha diversity shifts, our finding that the microbiome community composition (beta diversity) between mice from undeveloped and agricultural developed landscapes was significantly different aligned with the findings of Diaz *et al.* These shifts in microbiome composition could be attributed to dietary shifts based on habitat type and food availability, particularly in synanthropic environments (Anders et al., 2022). In future studies, stable isotope

analysis similar to those conducted by Anders *et al*. could provide additional insights into the diet of synanthropic and forest mice. Such information would likely inform the microbiome composition observed in our data, as the PERMANOVA modeling approach utilized herein indicated a high degree of unexplained microbiome composition variability that was not accounted for by landscape or habitat type.

We detected 16S sequences of a number of putative pathogenic bacteria in samples from all four landscape-habitat pairings. The greatest number of mice carrying putative pathogenic bacteria and the highest mean diversity of putative pathogen species per mouse was found in the anthropogenic-forest habitat while the lowest was found in the undeveloped-forest habitat. These differences are likely explained by the landscape surrounding our sampling locations which could represent a source of infection for many of these pathogens. The forest sampling sites in the agricultural landscape were located on the periphery of a research reserve which is surrounded by crop fields, pastures, and low-density housing. By contrast, the forest sites in the undeveloped landscape were contained in a state park and the forest continues uninterrupted beyond the park boundary with little agricultural development, limiting sources of pathogen exposure. *Peromyscus* are known to forage in crop fields as well as forest habitat, so it is likely that the abundance of putative pathogens in mice in the anthropogenic-forest habitat are representative of exposure to the surrounding agricultural landscape. Indeed, *Clostridioides difficile* was the most frequently detected putative pathogenic bacteria in the agricultural landscape, aligning with literature documenting this bacteria in many species of livestock and wildlife, including antimicrobial resistant strains in urban rodents and those living on or near farms (reviewed in Weese, 2020). In agricultural settings, manure used as fertilizer may serve as a source of environmental contamination for *C. difficile* (Frentrup et al., 2021) which could provide a transmission route to rodents and other wildlife. Contrary to our predictions, the mean number of putative pathogenic bacteria per mouse was similar between forest and synanthropic habitat within a landscape, suggesting similar levels of pathogen exposure for mice between these two habitats. The synanthropic habitats sampled were all at the interface of forest and human-habitated areas. It is possible the synanthropic mice only occasionally visit the human structures where they were trapped (maintenance garages and storage areas, cabins and tent platforms, etc.) and predominantly reside in the nearby forest. Frequent movement of mice between native

vegetation and synanthropic habitat could account for similar putative pathogen exposure within a landscape type.

Accurate detection and taxonomic assignment of reads is a key assumption for community diversity and metagenomic analyses. Species richness and diversity estimates can be sensitive to the presence of rare species. The Emu algorithm has a built-in abundance threshold of 10 reads for large samples (over 1,000 reads) to control against long tails of low-abundance species which are an artifact of the probabilistic expectation-maximization model (Curry et al., 2022). As a result, Emu has a limited ability to detect rare species and thus our estimates of species richness and diversity are likely underestimations of the true community composition. However, Emu's strength is that it was specifically designed for taxonomic identification of long-read sequence data. The Emu pipeline helps to correct errors and improve the accuracy of Nanopore 16S amplicon sequencing through the expectation-maximization algorithm and has been shown to outperform algorithms designed for short-read (i.e. Illumina) data when classifying 16S Nanopore sequences (Curry et al., 2022). Because we were most interested in the species-level identification of reads for the detection of putative pathogenic bacteria, we chose to prioritize accurate taxonomic assignment over the ability to detect rare species and more accurately estimate species richness and diversity. Furthermore, Nanopore sequencing provides a key advancement over short-read microbiome sequencing in that species-level identification is possible and accurate. In future research, we see great utility for taxonomic assignment algorithms like Emu designed specifically for long-read Nanopore sequences and expect these novel methods to continue to improve the ability to accurately characterize and study species-level microbiome composition. Indeed, already the Nanopore 'Kit 12' chemistry and R10 flow cells (released in late 2021) are able to outperform Illumina sequencing with less noise and higher accuracy, specifically for species-level classification of 16S amplicon sequencing of gut microbiota (Szoboszlay et al., 2023).

It is important to clarify that, while we can be confident in accurate taxonomic assignment of the bacterial species detected in the sampled mice, their presence does not guarantee zoonotic potential. Many of these bacteria are commensal in the human and mammalian gut and may only be opportunistic pathogens or only certain serotypes possess virulence factors capable of infecting humans. Determining pathogenicity requires more in-depth genotyping or lab cultures that were outside the scope of this

research. Nonetheless, our detection of these bacteria species serves to inform the potential of *Peromyscus* mice to be reservoirs for zoonotic pathogens and can inform future studies that characterize the pathogenicity of these bacteria.

Our research supports and expands upon previous work done in Minnesota using Nanopore sequencing to identify pathogenic bacteria in synanthropic rodents. Jahan *et al.* pointed to the role that farms play in the increased abundance of putative pathogenic bacteria in synanthropic rodents (Jahan et al., 2021). However, farms are a unique anthropogenic environment with many routes of pathogen introduction, and rodents at this interface may not be representative of synanthropic rodents more broadly. Our work expands upon the foundation set by Jahan *et al.* by investigating less disturbed environments to understand the abundance and diversity of zoonotic bacterial pathogens in undeveloped and agricultural (cropland) landscapes. The diversity of putative pathogenic genera found in *Peromyscus* mice generally align between our studies: Jahan *et al.* similarly identified putative pathogenic genera including *Bacillus, Clostridium*, *Enterococcus*, and *Streptococcus* circulating in synanthropic rodents on Minnesota farms. However, we identified a higher abundance of *Clostridioides* and no pathogenic species of *Helicobacter* in our study. It is possible that these differences can be attributed to differences in how pathogen abundance was quantified: Jahan *et al*. reported abundance of reads identified at the genus level (summed across all sampled *Peromyscus*) whereas we focused on read abundance of specific pathogenic species per individual mouse. Interestingly, Jahan *et al.* found lower abundance of putative pathogenic genera in *Peromyscus* mice compared to other rodent species trapped on farms including *Mus musculus*, *Microtus pennsylvanicus*, and *Rattus norvegicus*. While our study did not include other rodent species, the limited abundance of putative pathogenic bacteria found in *Peromyscus* herein corroborates the findings of Jahan *et al.* and could indicate lower exposure for these mice compared to other synanthropic rodents.

Overall, we found that *Peromyscus* in undeveloped and agricultural landscapes in Minnesota carried low abundance and diversity of putative pathogenic bacteria (we detected, on average, 1-2 putative pathogens per mouse and zero putative pathogens in many mice). Further, many of these were opportunistic pathogens which may pose a limited risk to zoonotic transmission in the human population. Our findings suggest that agricultural landscapes play a role in increasing the abundance of zoonotic pathogens in

wild rodents; however, synanthropic habitat may be less informative of the abundance of zoonotic bacterial pathogens, particularly in environments where mice are expected to be highly mobile across interfaces between native vegetation and synanthropic areas. Taken together, our research suggests that *Peromyscus* are occasional hosts of zoonotic bacterial pathogens when sources of exposure are high (i.e. agricultural settings like crop fields and farms) but their flexibility to thrive in natural vegetation as well synanthropic habitat may act as a buffer to higher levels of zoonotic pathogen abundance.

1.6 Conclusions

The data presented herein provide a glimpse into the gut microbiome of *Peromyscus* mice in diverse landscapes of Minnesota. By sampling from populations in agricultural and undeveloped landscapes and in forest and synanthropic habitat, we find that landscape and habitat are important factors influencing microbiome community composition in wild rodents. We also identify low abundance of putative pathogenic bacteria species in these populations and suggest the role of agricultural landscapes in increasing rodent exposure to putative pathogens. Even where transmission risk seems low, infection in wildlife populations could represent sources of novel pathogenic strains, bridge hosts linking environmental contamination back to human or livestock infection, or vectors to translocate pathogens across the landscape. As such, this research underscores the importance of investigating zoonotic pathogen prevalence in synanthropic rodents and other wildlife to better characterize their potential as reservoirs and vectors for pathogen spillover at the human-wildlife interface.

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Chapter 2. Constructing animal networks for parasite transmission inference

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2.1 Abstract

For free-ranging wildlife, it is often more practical to quantify interactions between individuals rather than successful transmission events; however, defining and quantifying transmission-relevant interactions is non-trivial. Researchers have choices in the technology used to collect data on animal locations in space and time as well as the methods of analysis to define network edges from those data. These choices can significantly affect network structure and subsequent inferences drawn about transmission. We explore empirical and theoretical examples of network data collection and analysis to highlight important considerations for transmission inference. Since parasite-host behavior feedbacks have been understudied in network analyses, we discuss how to incorporate these feedbacks into network applications using existing and novel approaches.

2.1 Introduction

Transmission is the process governing how parasites (transmissible infectious agents including viruses, bacteria, protists, prions, and macroparasites such as worms and arthropods) infect new hosts and spread through populations (Thomas et al., 2001). Simple models of infectious disease dynamics, such as the compartmental frameworks pioneered in early epidemic modeling (Anderson & May, 1979), assume all individuals in a population contribute equally to transmission. However, observations of outbreak dynamics suggest that heterogeneity in transmission is the norm, not the exception, for humans (Meyers et al., 2005), livestock (Chase-Topping et al., 2008), and wildlife (VanderWaal & Ezenwa, 2016).

Dynamical disease models consider transmission as the product of two interacting components: (i) the probability of contact between individuals in a population and (ii) the conditional probability of transmission given contact (White et al., 2018a). However, transmission events are challenging to document, particularly in wildlife species. Instead, it is often more practical to estimate contacts between individuals and use contacts as a proxy for transmission (Craft, 2015). In disease ecology, 'contact' is often used very generally, even up to and including indirect contact via the environment. To acknowledge that not all opportunities for transmission involve a direct, physical association, we use the term 'interaction' to refer to opportunities for transmission inclusive of all modes of parasite transmission. These range along a continuum from direct interactions ('same place, same time') such as physical contact or close proximity to indirect interactions ('same place, different time') such as sequential exposure to a shared environment (Richardson & Gorochowski, 2015).

Networks provide a way to visualize the direction, strength, and structure of interactions within a population (Fig. 2.1). The structure or topology (the arrangement of edges between nodes) of the network can serve as a powerful tool to make inferences about parasite transmission by, for example, identifying highly connected individuals or individuals connecting otherwise segregated populations. Importantly, the transmission-relevant host interactions that determine where edges appear in the network ('edge formation') will inform both (i) the thresholds of space and time used to define edges from spatiotemporal data and (ii) the technology used to record spatiotemporal data in animal populations, and these choices can ultimately alter network topology (Fig. 2.2) (Gilbertson et al., 2020; Perkins et al., 2009). In this chapter, we discuss considerations

for defining edges and collecting data to construct networks, synthesize the application of networks in the context of studying parasite-host behavior feedback in animal systems, and identify future directions to improve network studies of parasite transmission.

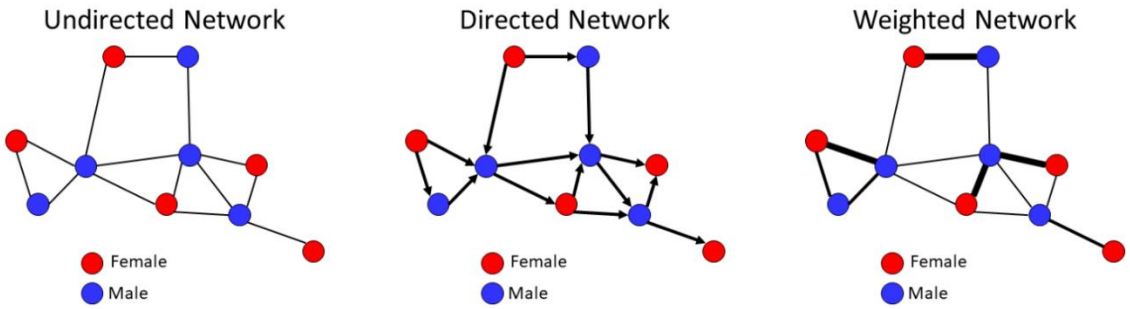

Figure 2.1 *Options for visualization of networks. In networks, individuals can be represented as points (nodes) and lines between nodes can signify interactions (edges). Attributes of individuals may be represented by node size, shape, or color. Edges in a network may be directed to show actions performed by one individual on another, and edges may also be weighted to indicate the strength of the interaction between two nodes.*

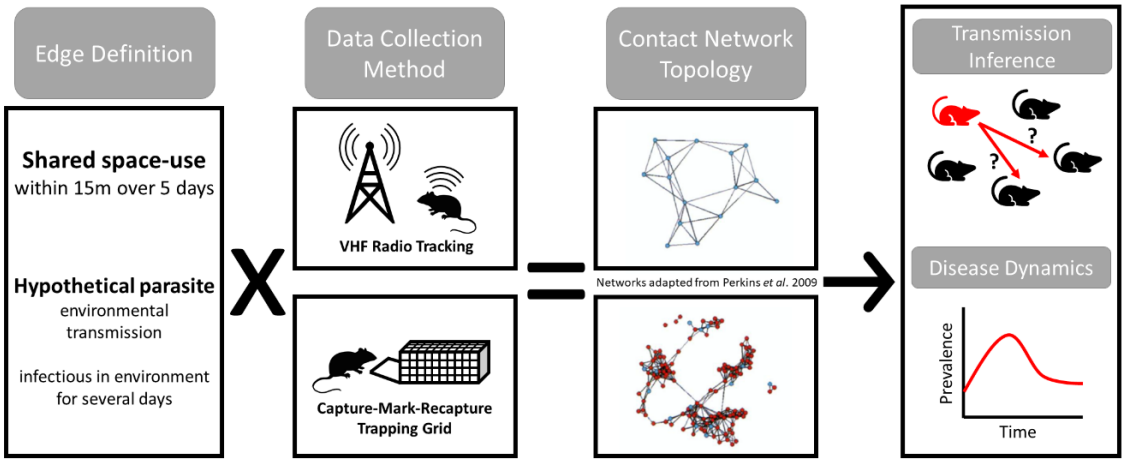

Figure 2.2 *Edge definition and data collection method can impact network topology and transmission inference. Researchers can choose how to define edges and collect network data when constructing networks. These choices can impact the resulting network topology. To illustrate, two empirical networks adapted from Perkins et al. (2009) are shown. Using a common edge definition of shared space-use, interaction data on wild mice was collected via both VHF radio tracking and capture-mark-recapture (CMR) trapping grids, and networks were constructed for each method. Significant differences in network topology arose between the two data collection methods, likely as a result of the resolution with which the method could estimate interactions. These differing network topologies would have downstream effects on transmission outcomes inferred from the networks. Networks adapted from Perkins et al., 2009. Reprinted by permission of John Wiley & Sons, Inc, © 2009.*

2.2 Constructing networks

Node identity

Networks can be built based on the interactions of individuals or groups of individuals (e.g. packs, herds) and nodes may represent a single species or individuals of different species (see section “Multilayer Networks”). The social system of a species (encompassing interactions, cohabitation, and mating between individuals) will often inform whether nodes are individuals or groups and how interactions are defined (White et al., 2017). For instance, in group-living species, membership in a herd or pack is often assumed to connect an individual to every other individual in the group (i.e. “gambit of the group”, Franks et al., 2010) and thus researchers may be most interested in how groups interact to spread parasites. In contrast, solitary or territorial animals that actively avoid conspecifics require nodes that represent individuals. It is also important to consider that missing nodes may influence network topology. As such, key goals when defining node identity should be to accurately identify individuals and repeatedly observe a representative proportion of the population.

Biological relevance

Edges in a network are only meaningful for understanding transmission if they are defined in a way that is biologically relevant to transmission of the parasite being studied (Grear et al., 2013). Consider, for example, a sexually transmitted parasite in a solitary, territorial species. Home range overlap estimated from GPS data aggregated over a year would suggest many edges between animals, but these are unlikely to represent the rare, male/female interactions necessary for transmission of the hypothetical parasite. When edges are poorly defined relative to the host-parasite biology, it can lead to problems for transmission inference (Craft, 2015). In Table 2.1, we highlight several examples of edges that are well-defined for parasites of different transmission modes.

Table 2.1 *Examples of biologically relevant edge definitions*

Study	Host-parasite pairing	Social system	Transmission mode	Edge definition	Method / Technology
Clay *et al.* 2009 (Clay et al., 2009)	Deer mouse - Sin Nombre hantavirus	Social, one mature male and several mature females (and offspring) share territory; males may fight with other males	Direct contact (likely aggressive behaviors)	**Direct interaction**: individual with powder color matching that of a marked male	Live trapping capture-mark-recapture grid & powder marking of five individuals to document their subsequent interactions upon next capture
Bull *et al*. 2012 (Bull et al., 2012)	Australian sleepy lizard - *Salmonella*	Multi-season monogamous pair bonding; some overlap of home range edges, core area with reduced overlap to same sex neighbors	Unknown, assumed environmental (fecal-oral)	**Spatiotemporal overlap** of GPS locations Synchronized GPS locations of two lizards within 2m of each other (conservatively included locations within 14m to account for varying GPS precision)	GPS loggers, locations recorded every 10 minutes
Wilber *et al*. 2019 (Wilber et al., 2019)	Domestic cattle, white-tailed deer, raccoons, opossums - Bovine tuberculosis (bTB)	Herds of cattle on farms Individual wildlife	Direct and environmental	**Strict spatiotemporal overlap**: proximity logger readings between unique animal pairs within a 3-month season (contact rates vary with season) **Shared space-use**: animal pair contacts same stationary logger within 30 days (avg. infectious period of bTB in environment)	Proximity logging collars on animals and stationary loggers on cattle-related resources Loggers detect an interaction at a mean distance of 0.88m - 60 seconds of separation before recording a new event

It can be challenging to determine how edges in a network should be defined. There may be uncertainty associated with the exact parameters for transmission of a parasite (Huyvaert et al., 2018) or parasites may have multiple modes of transmission (e.g. direct contact and environmental transmission, Wilber et al., 2019). Laboratory trials can be a useful tool to suggest a particular dose-response threshold or length of environmental persistence of an infectious agent to inform relevant interactions for transmission. However, laboratory trials are not feasible for many host-parasite pairings and a controlled lab setting is likely an imperfect approximation of what occurs in nature (Plowright et al., 2008). It may be more appropriate to "ground truth" a chosen edge definition with pilot studies in the field or using analytical approaches (this topic will receive further attention in later sections of this chapter: "Defining edges – Estimating interactions"; "Multilayer Networks"; "Choice of Method"; 2.6 Future Directions).

Defining edges - Direct observation of interactions

Network edges representing direct interactions to estimate transmission of directly transmitted parasites are best defined by the occurrence of a transmission-relevant behavior between two animals (e.g. biting or sexual intercourse). Behavioral observations, focal follows, or video monitoring represent a gold standard for defining edges as they provide information on what animals are doing during interactions and can help identify the interactions most correlated to transmission of various parasites (Drewe, 2010). For instance, Leu *et al.* (2020) constructed networks based on sexual interactions, skin contact, synchronous breathing, and social association using behavioral observations of wild bottlenose dolphins to assess the potential transmission risk of parasites spread by different routes (sexual transmission, physical contact, and aerosol). While powerful for inferring transmission, behavioral observations remain fairly uncommon for constructing networks in disease ecology as they are time intensive, require specialized training in standardized methods, and require host species that can be readily observed. In this chapter, we will focus on methods for estimating interactions between animals, assuming that host behaviors cannot always be observed directly.

Defining edges - Estimating interactions

When host behaviors cannot be easily observed, edges in a network are often defined by some degree of host overlap in space and time (spatiotemporal overlap). Spatiotemporal overlap can estimate transmission-relevant interactions for different transmission modes based

on how edges are defined, ranging from strict spatiotemporal overlap (direct interactions, e.g. sexual transmission) to asynchronous shared space-use (environmental exposure, e.g. fecal-oral transmission). Asynchronous shared space-use assumes that spatial overlap correlates with interaction frequency (Robert et al., 2012) and is often estimated via home range overlap (Fieberg & Kochanny, 2005). For edges defined by shared space-use, a transmission-relevant interaction occurs when two animals use the same space asynchronously, but within a time window relevant to the environmental persistence of the parasite. This could range from days to weeks (e.g. bovine tuberculosis, Porphyre et al., 2008) to months to years (e.g. chronic wasting disease, Schauber et al., 2015). To estimate transmission opportunities for directly transmitted parasites, edges are better defined by strict spatiotemporal overlap using narrow distance and time thresholds (e.g. fractions of a meter to several meters and seconds to hours, respectively). This can be estimated via visual observations of group membership (Sundaresan et al., 2007), proximity-logging collars (Hamede et al., 2009), or GPS collars (Schauber et al., 2015).

However, estimating edges using spatiotemporal overlap - even strict overlap - may not provide enough information to confirm an interaction has occurred or to know if the interaction was sufficient for transmission (Silbernagel et al., 2011). For instance, a direct interaction may be estimated from two animals with GPS locations within 2m of each other at the same time but this neither accounts for attraction or avoidance behavior nor distinguishes if an interaction occurs. As such, grooming may be indistinguishable from sexual intercourse but only the latter is relevant for sexual transmission. Some technologies can help address these uncertainties by approximating behavior states (e.g. accelerometers, see section “Movement ecology and disease”) or by augmenting interaction data with information about the duration of an interaction (e.g. proximity loggers).

Missing data is a persistent concern in network studies, though intentional study design (e.g. edge definition) and sensitivity analyses can help to limit and quantify the effects on study outcomes. For example, missing edges may influence network topology and could potentially lead to individuals not appearing in the network - both of which would affect transmission inference. Simulation models can be a powerful approach to incorporate sensitivity analyses into network studies to assess how robust network topology (and thus transmission inference) is to even small changes in how edges are defined (Fig. 2.3). These approaches highlight an important intersection between host social system, parasite transmission mode, and transmission inference. Some host social systems, such as highly territorial species which tend to have fewer associations between individuals, or parasite transmission modes, such as those

transmitted by direct interactions, are likely more sensitive to changes in how edges are defined as they require more strict definitions (i.e. narrow time and distance thresholds of spatiotemporal overlap) to accurately represent network topology (Gilbertson et al., 2020).

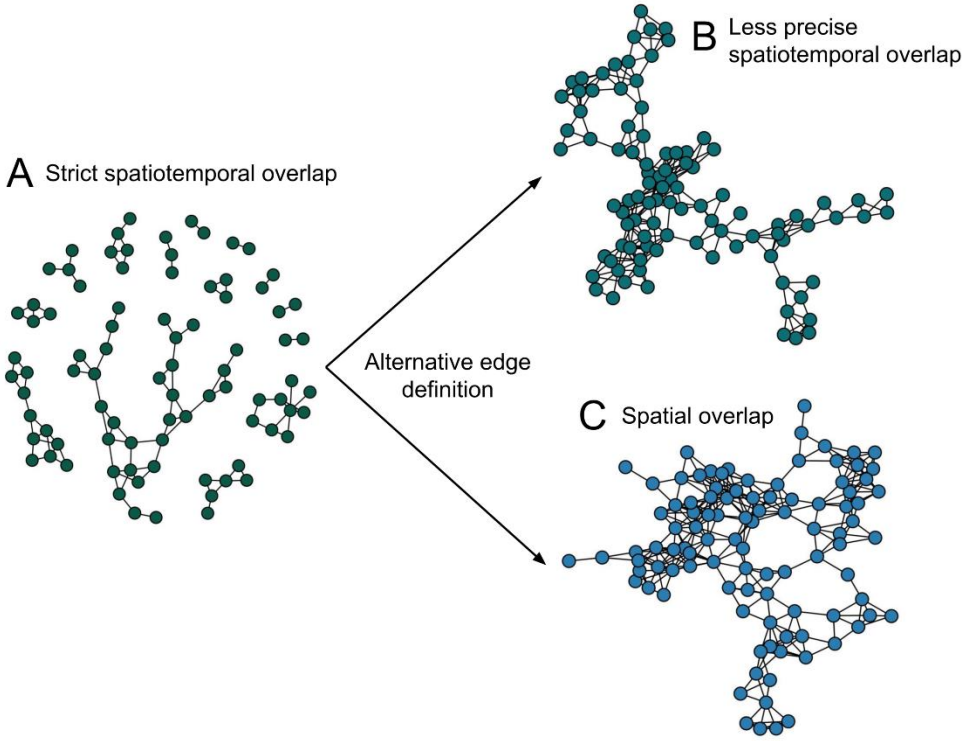

Figure 2.3 *Theoretical example of how edge definition can affect network topology. Gilbertson et al. (2020) simulated movements of a host population to generate a 'complete' network, and then generated 'sample' networks with subsets of the movement data using alternative thresholds to define edges. The 'complete' network (A) shows edges as defined by strict spatiotemporal overlap. The same data is shown in two 'sample' networks where edges are defined by (B) spatiotemporal overlap with a less precise (i.e. larger) distance threshold or (C) purely spatial home range overlap ignoring temporal elements (i.e. an infinite temporal threshold). The less precise thresholds in sample networks B and C produced more connected networks compared to the complete network (A) while the number and location of connections also led to different network topologies between networks B and C.*

Static versus dynamic networks

Temporal variation in factors such as climate, host social behavior, and host space-use can influence transmission dynamics, both for direct and environmentally transmitted parasites (Altizer et al., 2006). This variation should be considered when defining temporal thresholds for edge formation and when aggregating interactions in a population for network construction. For example, climate can seasonally alter survival of parasites transmitted via the environment (Langwig et al., 2015). This may necessitate different edge definitions over time, with longer

temporal thresholds in some parts of the year (long parasite survival) than others (shorter parasite survival). Further, temporal or seasonal variation in host social behavior or space-use (e.g. fission-fusion dynamics, host breeding season, etc.) can cause heterogeneity in interactions to vary over time (Langwig et al., 2015). For example, in a fission-fusion species like African buffalo, associations may form, dissolve, and shift from month to month (Cross et al., 2004). A static network aggregating interactions over that period would suggest that all edges are relevant to transmission and would fail to represent the importance of lasting versus ephemeral edges. Temporal dynamics can be managed by using multiple static networks which aggregate interactions over shorter periods, or by using dynamic networks which "re-wire" edges over time. However, the increased complexity of dynamic networks requires increased data requirements to collect, store, and process data—especially continuous data—and greater computational complexity to conduct statistical analyses. As such, researchers should only include the temporal complexity necessary to address their research question.

Multilayer networks

Network approaches can be extended beyond a single definition for nodes and for edge formation by constructing layered networks to compare different edge definitions or to investigate cross-species interactions in multi-host systems (Kinsley et al., 2020). Multilayer networks can represent the same individuals in a series of networks that differ based on how edges are defined. This provides a framework to test which interactions best approximate transmission opportunities (VanderWaal et al., 2014) or to investigate host-parasite systems with multiple routes of transmission (Schauber et al., 2015). Multilayer networks can also be applied to parasite transmission in multi-host systems. In this context, individuals of different species are represented in distinct layers and edges may be formed within a layer (same-species interaction) or between layers (cross-species interaction). These approaches have been used to investigate the potential for cross-species transmission via spatial overlap (Lewis et al., 2017) and via direct interactions using proximity loggers (Silk et al., 2018). Multilayer networks are a new and developing area of research, promising additional realism in the context of ecological interactions, but with the tradeoff of complexity in terms of data requirements and network construction which may not be necessary to address all research questions.

2.3 Methods of data collection

The choice of how to collect network data requires careful consideration due to the effects these decisions can have on network topology and transmission inference (Fig. 2.1). Different methods and technologies have different capabilities for capturing data on animal locations in space and time (particularly with respect to direct versus environmental transmission) and vary in the resolution of data collected. As such, the research question, host-parasite system, and edge definition together should determine the technology used to estimate interactions, with full recognition that the method of data collection may limit the use of the data for questions beyond the scope of the original study.

Choice of method

There are many options of data collection methods to record spatial and temporal data on wildlife. These can be generally classified as place-based: technology in the environment recording individuals at specific locations, or individual-based: technology attached to an animal recording their movement or interactions (Smouse et al., 2010). We present descriptions of a variety of methods that have been commonly used to collect data to construct animal networks (Table 2.2. Data collection methods), but this list is by no means exhaustive (see also Craft & Caillaud, 2011; Krause et al., 2013; Spiegel et al., 2022 and section 4.6 Future Directions). Specific considerations are highlighted in Table 2.2, but we emphasize that these methods range in cost and researcher effort to implement, and some are better suited for certain edge definitions or parasite transmission modes. Moreover, regardless of the data collection method, monitoring a representative sample is key to identifying meaningful patterns that are generalizable to the population of interest. For instance, behavioral heterogeneity may make some individuals more likely to be observed by certain methods which can bias network topology.

Table 2.2 *Data collection methods*

Place-based Monitoring **General considerations:** technology is generally less costly					
Method / Technology	**Explanation**	**Edge definitions**	**Host features**	**Examples**	**Considerations**
Trapping grid	Live-capture traps in fixed locations, regular trapping to detect animal locations; often combined with PIT tags to identify individuals	Shared space-use; less precise spatiotemporal overlap	Small-bodied and "trap-happy" animals; effective for systems where aggregation naturally occurs (e.g. around food sources)	Rodents (Perkins et al., 2009); brushtail possums (Porphyre et al., 2008)	Consider trap density relative to population size (Perkins et al., 2009); added resources may affect interaction patterns
Passive Integrated Transponder (PIT) tag	Unique identifier inserted under the skin or attached via leg band; stationary receivers record individuals at a location	Shared space-use via visits to common locations (e.g., trap, feeder); may be extended to more strict spatiotemporal overlap	Small-bodied animals *Proximity loggers on animals and stationary devices often used in a similar fashion with larger animals	Finches (Farine et al., 2015) *Cattle and deer (Wilber et al., 2019)	Assumes host congregation at stationary receivers is congruent with other interaction behavior
Visual observations (including gambit of the group, GoG)	Identify individuals by unique markings or VHF/GPS collar; record spatial location, individual associations, or group members	Direct interactions & associated behaviors; strict spatiotemporal overlap; group membership	Uniquely identifiable individuals; most effective with group-living species (GoG can be used to track fission-fusion dynamics)	Meerkats (Drewe, 2010); chimpanzees (Rushmore et al., 2013); GoG: African buffalo (Cross et al., 2004)	Time-intensive to collect; difficult to monitor many individuals at once; direct observations may not be feasible for many species

Table 2.2 *(continued) Data collection methods*

Individual-based Monitoring **General considerations:** requires entire population to be tagged for optimal resolution; difficulty extrapolating locations between fixes: technology is generally more costly							
Method / Technology	**Explanation**	**Edge definitions**	**Host features**		**Examples**	**Considerations**	
Proximity loggers	Devices attached to animals record other devices within a researcher-defined distance	Strict spatiotemporal overlap	Generally larger-bodied hosts *logger size/weight may be prohibitive for small-bodied animals		Tasmanian devils (Hamede et al., 2009); badgers and cows (Silk et al., 2018)	Interaction distances set at discrete thresholds (e.g. 0.5m, 2m, etc.)	
VHF monitoring	Radio transmitter emits a signal which can be picked up by a manual device (or stationary tower)	Shared space-use; strict spatiotemporal overlap	Range of host body sizes	Works well for solitary individuals or long-distance movement	Rodents (Perkins et al., 2009); bobcats and puma (Lewis et al., 2017)	Requires very high frequency of data collection for high temporal resolution	Must consider localization error (error in exact location estimation), can be biased by habitat type, animal behavior
GPS monitoring	GPS device broadcasts animal location at specified time points; can provide high-resolution information on animal location and movement	Shared space-use; strict spatiotemporal overlap	Generally, larger-bodied hosts *collar size/weight may be prohibitive for some smaller-bodied animals		Deer (Schauber et al., 2015); lizards (Bull et al., 2012)	Trade-off temporal resolution vs. longevity of sampling	

Choosing the data collection method that best estimates transmission-relevant interactions may require conducting pilot studies with different technologies or pairing studies of free-ranging wildlife with observations of captive populations (e.g. enclosures with controlled populations). For example, Lavelle *et al.* (2014) fitted a population of white-tailed deer with collars equipped with cameras, proximity loggers, and GPS devices to compare interaction rates as measured by the three devices. They found that cameras and GPS underrepresented interactions among deer and only proximity loggers provided interaction rate estimates that were representative of actual rates. Thus, using only one method of data collection and failing to investigate other options or consider potential limitations could result in over- or under-estimation of transmission-relevant interactions.

Implementation of the technology

Once the data collection method is chosen, there are additional considerations about how the technology will be implemented: specifically, the temporal resolution (how frequently data are collected) and longevity of sampling (the length of time over which data are collected). For individual-based monitoring in particular, there are trade-offs to balance data resolution and longevity; frequently recording data points provides high spatiotemporal resolution, but often comes at the cost of a shorter battery life. However, if the sampling effort is inadequate (e.g. too coarse such that data points are too far apart in time), this can result in failure to detect interactions and, consequently, yield inaccurate inference about transmission (Gilbertson et al., 2020). We recommend that decisions about implementation of data collection methods be motivated by (i) the host species, including the frequency of transmission-relevant interactions and how seasonality may impact these; (ii) the parasite of interest, including the infectious period or environmental persistence of infectious agents; and (iii) the technology itself, including the lifetime of monitoring equipment or the resolution at which individual data points can be recorded. Considering all these factors together will help align the appropriate choice of method and the degree of sampling effort necessary to observe transmission-relevant interactions.

2.4 Network analysis and application

Methods for analyzing networks can be grouped into three classes: social network analysis, statistical modeling, and simulation modeling. However, these designations are not mutually exclusive and a network analysis pipeline may include several approaches used in parallel. We will briefly discuss network analysis under each of these three classes and illustrate how networks can be used to identify key individuals or potential pathways of transmission, concluding with applications of network data for management of parasite transmission in animal populations. For a deeper dive into the varied uses of network data to explore parasite transmission in wildlife populations, we recommend the review by White *et al.* (2017).

Social network analysis

Social network analysis (SNA) is used to quantify aspects of network structure at both the node- (individual) and network- (population) levels. For those new to SNA, several 'how-to' guides provide a general introduction (Croft et al., 2008; Farine & Whitehead, 2015). A vast number of network metrics (also called network statistics) have been developed to examine both heterogeneity and patterns in node interactions as well as network structure. Sosa *et al.* (2021) provide an overview of the most commonly used social network measures including their uses and interpretations. When SNA is applied to parasite transmission, the goal is often to compare node position in a network to node-level characteristics or disease status (Godfrey, 2013). Studies may seek to correlate characteristics of highly connected individuals to form generalized conclusions about host factors influencing transmission potential. For example, individuals with high betweenness (i.e. frequently lying on the shortest path between other nodes) play an important role in connecting others in their network and may act as superspreaders. In a study comparing social networks and *E. coli* transmission networks in giraffes, individuals that were highly connected or occupied 'bottleneck' positions in the social network tended to occupy the same positions in the transmission network, suggesting that an individual's social association patterns could be used to inform their transmission potential (VanderWaal et al., 2014).

Statistical modeling

Statistical modeling can be applied to networks to conduct hypothesis tests or compare networks, however these analyses require careful consideration due to the non-independence of network data (Croft, Madden, et al., 2011; James et al., 2009). Statistical modeling approaches such as exponential random graph models (ERMGs) (Silk & Fisher, 2017) or stochastic actor-oriented models (Fisher et al., 2017) enable researchers to investigate factors influencing patterns of node interactions in the network. These approaches can help test hypotheses about what factors drive observed network topology; for example, do edges tend to be formed between same-aged individuals. Statistical network models have been widely used in human social and disease research (Goodreau et al., 2009), but tools like ERGMs or probabilistic network modeling (Yang et al., 2021) are relatively new to animal disease ecology. We suggest the review by Silk *et al*. (2017) as a starting point to learn more about these approaches.

Simulation modeling

Simulation models ultimately link networks to epidemiology to investigate how parasite transmission might play out in a network of a given structure. Generally, simulation modeling also involves social network analysis and statistical modeling to (i) take empirical network data and identify important factors governing edge formation and network structure, (ii) generate new iterations of the network that reflect these same patterns, and (iii) simulate outbreaks on the new networks to understand how network structure affects epidemiological processes. Simulation modeling can be used retrospectively to investigate the network connections that may explain observed transmission dynamics. For instance, simulation modeling of Serengeti lion networks during the 1994 canine distemper outbreak was used to suggest the critical role of spillover events from other carnivore hosts in maintaining the epidemic in the lion population (Craft et al., 2009). Simulation models can also be used proactively to predict transmission dynamics or investigate questions of how seasonal or sickness-induced host behavior may affect outbreak dynamics (Reynolds et al., 2015).

Network applications for disease management

If animals in a population exhibit heterogeneity in the number of transmission-relevant interactions, networks can be important for decision-making in disease

management. Network data suggesting heterogeneity in interactions can be used to inform the scope or timing of management efforts (via vaccination, culling, or isolation) by identifying highly connected individuals or seasonal variation in network connections (Rushmore et al., 2013). Simulations on empirically derived networks can also be used to compare the outcomes of different management strategies in ways that are not feasible or ethical in empirical contexts. Robinson *et al.* (2018) used network simulations based on empirical networks of interactions to compare two candidate vaccination campaigns in endangered Hawaiian monk seals: (i) target the most connected individuals or (ii) vaccinate any available animal. They found that vaccinating any available animal more quickly led to protective levels of immunity than waiting to vaccinate highly connected seals but required more vaccines to achieve the same decrease in outbreak size. As such, social network analysis and simulation modeling approaches can be powerful tools to combine empirical data, hypothetical scenarios, and epidemiological modeling to devise effective management actions to control infectious disease outbreaks in animal populations.

2.5 Synthesis: Networks in the context of parasite-host behavior feedback

Thus far, we have emphasized the use of animal networks for understanding how an individual's position in a network or the overall network topology affects transmission inference. Less often is the reverse relationship considered: how parasite infection influences host behavior and thus network position or overall network topology. Assuming that interaction behavior and parasite infection are independent ignores the possibility of parasite-host behavior feedback and limits our understanding of disease dynamics. As such, incorporating feedback loops into disease ecology, and particularly the study of animal networks, has been highlighted as an important gap in order to more fully understand transmission dynamics (Ezenwa et al., 2016; Godfrey, 2013; Hawley & Altizer, 2011; Hawley & Ezenwa, 2022).

The ways in which data are collected and networks are constructed can often make it difficult to identify and effectively study this feedback in free-ranging wildlife systems. First, networks have primarily been constructed using the interactions of uninfected animals (Craft & Caillaud, 2011). However, empirical studies indicate that sickness behavior can have important—and system-specific—consequences for network connections and parasite transmission, further emphasizing the importance of recording

observations on infected animals. For example, some infected animals may interact less or be avoided by uninfected conspecifics, decreasing their network connections (Croft, Edenbrow, et al., 2011; Lopes et al., 2022) while in other cases, the reverse is true and sickness-induced lethargy can result in an increase in host interactions (Franz et al., 2018). Second, studies of wildlife disease often operate with a cross-sectional view of infection status and interactions, requiring researchers to extrapolate the dynamic processes that created these patterns (Heisey et al., 2006). Cross-sectional studies are able to quantify how infected hosts are connected in the network but lack the ability to disentangle correlation and causation.

Addressing these limitations to robustly study parasite-host behavior feedback requires longitudinal monitoring to observe individuals that become infected and to document any associated changes in interaction patterns. Collecting such data via observational studies of wildlife populations is no easy task. Instead, field experiments manipulating infection status (e.g. deworming via anthelmintic) can be a powerful means to explicitly examine how parasite infection affects host interactions. The number of studies explicitly manipulating parasite infection in wildlife to investigate the effects on network structure remains limited (White et al., 2017), but anthelmintics have been used to study the influence of helminth infection on host population cycles (Pedersen & Fenton, 2015) and viral infection dynamics (Ezenwa & Jolles, 2015), providing informative precedents for parasite removal experiments in wildlife.

Even when experiments can be implemented in wildlife systems, persistent issues of small sample sizes, confounding factors, and limited replication may hamper the ability to robustly test the impact of parasite infection on host interactions. In these situations, simulation approaches informed by empirical data can be used to examine the effects of parasite-induced host behavioral changes on transmission dynamics at a higher resolution (Box 2.1 Parasite-host behavior feedback) (Hawley & Altizer, 2011; White et al., 2017). Building these models requires knowledge about how infection is likely to alter behavior. In wildlife-parasite systems that are difficult to observe, adequate information to inform models may be limited. Nonetheless, even if a range of possible scenarios can be tested, simulations are a powerful approach to model the population-level effects of infection-induced behavioral changes and test the implications of varying behavioral changes via sensitivity analyses.

Box 2.1 *Parasite-host behavior feedback*

Reynolds *et al.* (2015) used a simulation approach to model the feedback effects of parasite-induced host behavioral changes on rabies transmission in wild raccoons. Different rabies-induced behaviors ('furious' or 'dumb') have been observed in wild raccoons which may differentially affect transmission (Rosatte et al., 2006). Reynolds *et al.* (2015) used interaction data from proximity loggers in a healthy population of wild raccoons to simulate a population with a comparable number and durations of edges. They then investigated a series of outbreak scenarios with rabies-induced behavioral change (Fig 2.4A-D) where infected racoons displaying 'dumb' behavior were simulated via a decrease in number of interactions while 'furious' behavior corresponded to an increase.

In general, furious behavior led to a faster spread of rabies and increased final outbreak size while dumb behavior had the opposite effect (Fig. 2.4). When a combination of dumb, furious, and normal behavior was simulated, there was little difference in speed of spread or final outbreak size compared to simulations with no behavioral changes (Reynolds et al., 2015). This study illustrates how simulations can help identify circumstances under which parasite-induced host behavioral change is likely to be important to transmission outcomes. We recommend researchers conduct such sensitivity analyses, as the insights provided can be helpful in developing new hypotheses and/or identifying the most important data to collect to reduce uncertainty about the role parasite-host behavior feedback plays in shaping transmission dynamics.

"Raccoon" by dave and rose is licensed with CC BY 2.0.

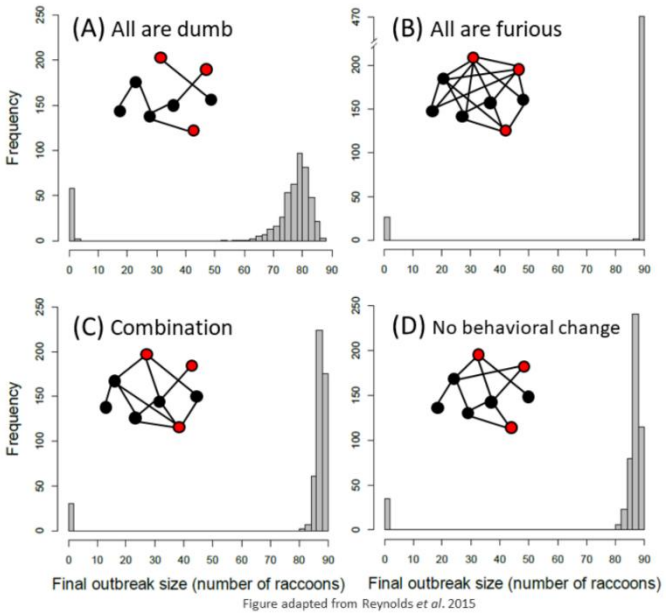

Figure adapted from Reynolds *et al.* 2015

Figure 2.4. *Final rabies outbreak sizes under simulation conditions for a population of suburban racoons. Panel (A) all infected animals are dumb (B) all are furious *note y-axis break (C) one third are furious, dumb, normal (D) all are normal. Toy networks illustrate the modeled changes to the number of interactions made by infected individuals (represented by red nodes). Photo reproduced under Creative Commons Attribution 2.0 International (CC BY 2.0) license. Figure adapted from Reynolds et al., 2015. Reprinted by permission of John Wiley & Sons, Inc, © 2015.*

2.6 Future directions

This section introduces some alternative approaches to investigate animal networks and parasite transmission. It is worth noting that pursuing future research in these directions does not preclude research using the methods already introduced (see 2.3 Methods of data collection). We encourage additional field and experimental studies, for example, using anthelmintics to study parasite-host behavior feedback. Nonetheless, the following are promising avenues to more precisely track transmission, improving our ability to study parasite-host behavior feedback in animal populations.

Transmission networks from parasite genomic sequencing

One potential approach to circumvent the challenges of estimating interactions is to use transmission networks based on parasite genomes to better understand social or interaction-based networks and transmission (Gilbertson et al., 2018). For example, several studies have found that genetic relationships among commensal bacterial and viral agents can illuminate social relationships between hosts (Bull et al., 2012; VanderWaal et al., 2014). Such findings, however, have not been consistent across host species and infectious agents (e.g. Blyton et al., 2013), so further work is necessary to determine under what conditions infectious agents—including commensal agents—may successfully act as proxies of social or transmission-relevant interactions.

As genomic sequencing technology becomes cheaper and more accessible, advances in software tools can now allow researchers to infer transmission networks directly from high resolution parasite genomes (Hall et al., 2016). Some transmission network inference approaches can even provide an estimate of the time of infection (Didelot et al., 2017). This is likely to be a coarse or uncertain estimate, and would require parasites with rapid mutation rates, but could provide a temporal window before and after which to look for evidence of behavioral change in studies of parasite-host behavior feedback. Though promising, these transmission network approaches require intensive population sampling, and large genome sizes can introduce computational challenges (but see Didelot et al., 2017). Nonetheless, the integration of molecular epidemiology and network science is a rapidly developing topic in disease ecology and promises exciting insights into the dynamics of transmission.

Intensive non-invasive sampling

A key challenge in studies of parasite-host behavior feedback is determining the time point of infection to better understand the causal relationships between infection and behavior. Diagnostic field sampling can be challenging in wildlife, especially in cryptic or secretive species, species living in inhospitable habitats, or species of conservation concern where field capture and sampling is particularly risky (Krause et al., 2013). Advances in non-invasive sampling (e.g. feces, Cristescu et al., 2019; saliva, Evans et al., 2016; carrion- and feces-consuming flies, Hoffmann et al., 2016) may help address some of these issues by allowing detection of infection without invasive handling. Longitudinal, repeated non-invasive sampling would increase the probability of detecting infection state conversion and a temporal window for the time of infection. If coupled with direct observations or a single capture event to deploy individual-based monitoring technology, researchers could then analyze behavioral, movement, or interaction data with this known time of infection information, and thereby test for evidence of infection-induced behavioral change in studies of parasite-host behavior feedback.

Movement ecology and disease

Increasingly, researchers are calling for integration of methods and theories from movement and disease ecology (Dougherty et al., 2018; Spiegel et al., 2022; White et al., 2018b), and these ideas are relevant to studying parasite-host behavior feedback processes. For example, linking movement and disease may help determine if movement patterns are associated with a diseased or infectious state. However, such research questions may require intensive and/or invasive methods in at least a subset of the population either to detect the onset of infection; for example, body temperature monitoring to detect febrile states as an indicator of infection (Timsit et al., 2011), or to acutely characterize animal activity; for example, biological sensor tags to detect locomotion and energetic expenditure or physiological responses like heart rate or ventilation (Wilson et al., 2015). Intensive non-invasive sampling may be particularly relevant for addressing such issues (see section “Intensive non-invasive sampling”). In addition, improved statistical approaches or approximations may be particularly relevant for detecting a relationship between disease and changes in movement behavior (e.g. continuous time movement models, Hooten & Johnson, 2017; step selection functions,

Thurfjell et al., 2014). Advances in technology can also provide increased resolution of individual-based movement monitoring; for example, applying accelerometers, GPS collars, and proximity logging devices together may be able to better interrogate the links between interactions, movement, and behavioral states, albeit with increased financial and data processing costs to implement. Linking movement and disease ecology is likely to be a fruitful avenue for better understanding transmission dynamics and parasite-host behavior feedback.

2.7 Conclusions

How edges are defined and interactions estimated from spatiotemporal data has the power to affect network topology. Therefore, the process of collecting data and constructing networks must be rooted in an understanding of the biology of the host-parasite system and align with the relevant mode of parasite transmission and the host interactions necessary to facilitate it. Informed choices will best enable meaningful inference about parasite transmission, though it is important to acknowledge that networks of interactions are often, at best, approximations of true transmission networks. And yet, improved technology and modeling frameworks are closing the gap between inferring transmission and directly documenting transmission. Continued advancements in network approaches, transmission inference, infection detection, and movement analyses will open doors to more fully understand transmission processes as a product of feedback between animal behavior and parasite infection, providing powerful insight into generalizable processes driving transmission at an individual and a population level.

2.8 Acknowledgements

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Chapter 3. Effects of food supplementation and helminth removal on space use and spatial overlap in wild rodent populations

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3.1 Abstract

Animal space use and spatial overlap can have important consequences for population-level processes such as social interactions and pathogen transmission. Identifying how environmental variability and inter-individual variation affect spatial patterns and in turn influence interactions in animal populations is a priority for the study of animal behavior and disease ecology. Environmental food availability and macroparasite infection are common drivers of variation, but there are few experimental studies investigating how they affect spatial patterns of wildlife. Bank voles (*Clethrionomys glareolus*) are a tractable study system to investigate spatial patterns of wildlife and are amenable to experimental manipulations. We conducted a replicated, factorial field experiment in which we provided supplementary food and removed helminths in vole populations in natural forest habitat and monitored vole space use and spatial overlap using capture-mark-recapture methods. Using network analysis, we quantified vole space use and spatial overlap. We compared the effects of food supplementation and helminth removal and investigated the impact of season, sex, and reproductive status on space use and spatial overlap. We found that food supplementation decreased vole space use while helminth removal increased space use. Space use also varied by sex, reproductive status, and season. Spatial overlap was similar between treatments despite up to three-fold differences in population size. By quantifying the spatial effects of food availability and macroparasite infection on wildlife populations, we demonstrate the potential for space use and population density to moderate spatial overlap in wildlife populations. This has important implications for spatial processes in wildlife including pathogen transmission.

3.2 Introduction

How animals use space in their environment and interact with conspecifics is fundamental to understanding proximity- and contact-driven processes such as social interactions (Kusch & Lane, 2021) and pathogen transmission (Silk et al., 2018). Interactions with conspecifics are frequently heterogeneous, reflecting individual variation due to animal sex and reproductive status and associated social behavior or, alternatively, can be driven by environmental variability in habitat quality or population density (Craft, 2015; Tompkins et al., 2011). Since animal space use and conspecific interactions are crucial determinants of pathogen exposure, identifying how these are shaped by individual-level variation and environmental variability has important implications for mitigating effects of novel and zoonotic pathogens on wildlife, domestic animals and people (Silk, Croft, Delahay, Hodgson, Boots, et al., 2017). However, studies that seek to disentangle the relative contributions of environmental and individual variation to interactions between animals are rare.

Food availability is a common source of environmental variability that can influence pathogen transmission by altering host space use and spatial overlap (Becker et al., 2015). At local scales, low food availability may increase animal space use through increased foraging, while areas of high food availability may decrease movement through increased site fidelity; heterogeneity in food availability across the landscape may promote movement through increased attraction and dispersal between patches (Becker, Snedden, et al., 2018). Wildlife aggregation around point food sources (e.g., bird feeders, landfills, crop fields) can increase pathogen transmission by facilitating both direct interactions between infected and susceptible animals (Altizer et al., 2018; Forbes et al., 2015) and indirect interactions through environmentally transmitted microparasites accumulated at the site (Cowie et al., 2016; Cross et al., 2007).

Individual variation among animals can also affect space use and spatial overlap with potential consequences for pathogen transmission (VanderWaal & Ezenwa, 2016) and superspreading of infection (Lloyd-Smith et al., 2005). For example, in rodents, testosterone-mediated behavior in mature males can increase their contacts with conspecifics and transmission potential (Grear et al., 2009; Perkins et al., 2008) and is hypothesized to be a key driver of commonly observed male bias in infection prevalence (Krasnov et al., 2012). Individual variation may also arise through differing burdens of

macroparasites such as helminths, with potentially opposing effects on space use and spatial overlap. Helminth infection could increase feeding behavior, requiring infected hosts to expand their foraging area (Brown et al., 1994). Conversely, helminths may cause sickness-induced lethargy in infected animals and decrease their activity, thus decreasing their space use and interactions with conspecifics (Ghai et al., 2015). Further, since space use and spatial overlap can influence macroparasite exposure, positive feedbacks between macroparasite exposure and space use could exacerbate or regulate within-population variation in macroparasite burden and movement (Hawley et al., 2021).

Much of our knowledge of how food availability and macroparasite infection impact microparasite transmission in wildlife comes from studies of laboratory animals under highly controlled conditions (Croft, Edenbrow, et al., 2011) or observational studies in wildlife (Jolles et al., 2008). However, in natural settings, food availability and macroparasite infection frequently co-vary. Experienced together, macroparasite infection and low food availability can have synergistic effects on host immune condition (susceptibility, shedding rate), resulting in more extreme effects on microparasite transmission than either has in isolation (Budischak et al., 2015; Forbes et al., 2016). Ultimately, explicit investigation through rigorous field experiments on wildlife are necessary to test the independent and synergistic effects of environmental and individual variation on transmission in their ecological context (e.g., Sweeny et al., 2021). Such research is pertinent and timely because urbanization and other land-use change could alter patterns of food availability and macroparasite burdens that exacerbate pathogen emergence and transmission at the human-wildlife interface (Plowright et al., 2021).

Our goal was to determine the effects of food supplementation and macroparasite removal on space use and spatial overlap in wildlife populations. We used bank voles (*Clethrionomys glareolus*, previously *Myodes glareolus*; Carleton et al., 2014; Kryštufek et al., 2020) as our study species as they are amenable to experimental manipulation, are a common host for macroparasite infection (e.g., helminths like *Heligmosomoides glareoli* can have a population-level prevalence of up to 80%; Haukisalmi & Henttonen, 2000), and have well-documented natural history, particularly with respect to space use behavior (Tamarin et al., 1990). Reproductive males hold territories that are larger than those of females and that overlap with territories of males and females. Reproductive females are territorial toward other reproductive females but

may overlap more with non-reproductive females in the non-breeding season (Bujalska, 1990). Home range sizes of reproductive males are approximately double those of reproductive females (reproductive males average: 1800 m^2; females: 900 m^2; Bujalska & Grüm, 1989). In both sexes, space use of non-reproductive voles is less than that of reproductive voles (non-reproductive females, males average: 700 and 800 m^2, respectively; Bujalska & Grüm, 1989). Both food availability and population density are thought to regulate vole territory size (Bondrup-Nielsen & Karlsson, 1985).

Here we experimentally manipulated wild bank vole populations via food supplementation and removal of intestinal macroparasites (helminths) and monitored individual- and population-level responses. Specifically, we 1) quantified space use by sex and reproductive status in the breeding and non-breeding seasons and 2) quantified spatial overlap between voles each month and visualized these "overlaps" using network approaches. Space use and spatial overlap were compared between treatments to test the effects of food supplementation and helminth removal on vole spatial patterns. As abundant food often increases small mammal population density through immigration (Prevedello et al., 2017), we hypothesized that food supplementation would decrease vole space use and increase spatial overlap. We hypothesized that helminth infection would increase host movement (due to increased feeding behavior), and thus expected that helminth removal would decrease space use and spatial overlap.

3.3 Materials & Methods

Study site and experimental design

We conducted a two-factor field experiment in the boreal forests of southern Finland (61.0775°N, 25.0110°E) where bank voles are the dominant rodent species. These forests receive snowfall from approximately November-April, and absence of snow cover makes monitoring and experimental manipulation of voles most feasible from May-October. Twelve study sites were established in old-growth spruce forest patches. All sites were at least two kilometers apart to prevent dispersal between replicate populations (maximum dispersal distance estimated to be 500 m in heterogeneous habitat, Gliwicz & Ims, 2000). At each site, a standardized trapping grid (100 m x 100 m, 1 hectare) was established of 61 uniquely identified traps spaced 10 meters between grid rows and columns to monitor the vole population (Figure 3.1).

Sites were randomly assigned to one of four treatment pairings (“treatments”): no manipulation (“unfed-control”; “U-C”); helminth removal but no food supplementation (“unfed-deworm”; “U-D”); food supplementation but no helminth removal (“fed-control”, “F-C”); and both food supplementation and helminth removal (“fed-deworm”; “F-D”). Each treatment was replicated at three sites (Figure 3.1). Sites assigned to food supplementation received a feed mix of mouse chow pellets and sunflower seeds evenly distributed on the trapping grid every two weeks from May through November (if snowfall had not yet accumulated). The supplemental feed mix was dosed at 7.54 kcal/m^2 (following Sweeny et al., 2021) consisting of 7.875 kg of mouse chow pellets (Altromin 1324 10 mm pellets maintenance diet for rats and mice [3227 kcal/kg]; Altromin Spezialfutter GmbH & Co. KG, Lage, Germany) and 7.875 kg sunflower seeds (as fed, 6350 kcal/kg). Voles at deworm sites were given an oral dose of 10 mg/kg Ivermectin and 100 mg/kg Pyrantel on their first capture in a trapping occasion; this combination of medication is effective at treating larval and adult helminth infections (Clerc et al., 2019). Voles at control sites received a matching weight-based control dose *(*17.5% sucrose solution) on their first capture in a trapping occasion.

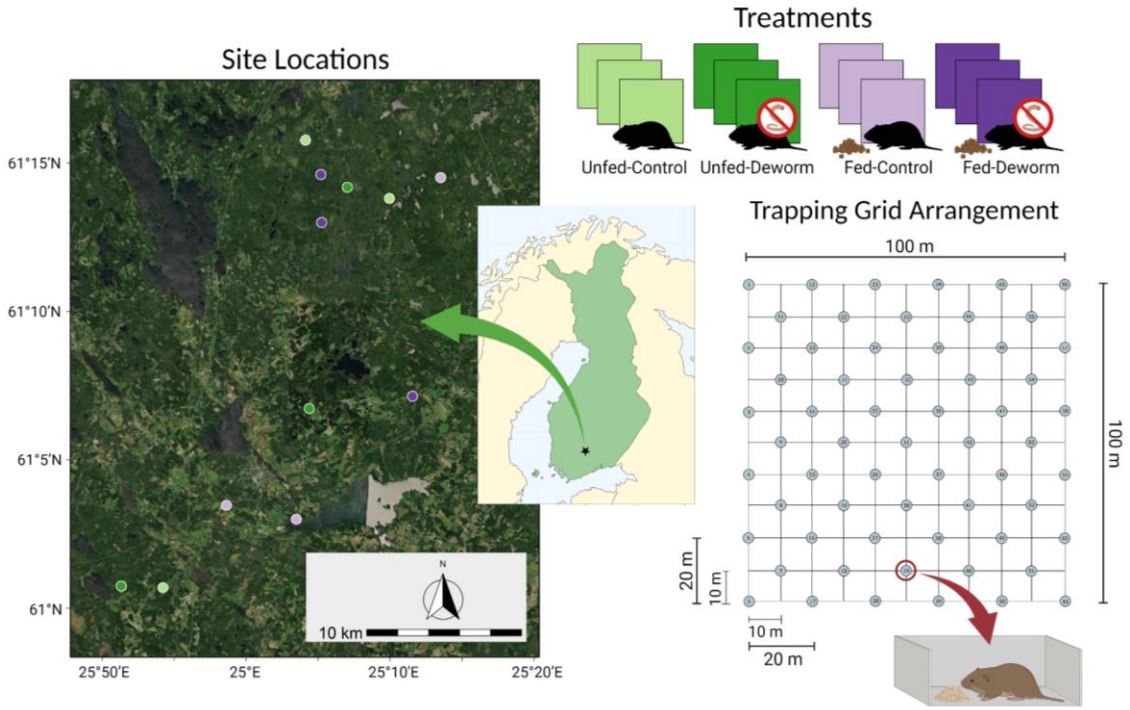

Figure 3.1. *Field study design and experimental set-up. ("Site Locations") Twelve field sites were established in southern Finland. ("Treatments") Sites were assigned one of four treatment pairings: unfed-control, unfed-deworm, fed-control, and fed-deworm and each treatment was replicated at three sites each. ("Trapping Grid Arrangement") The trapping grid included 61 traps in 11 rows and 11 columns. Rows and columns were spaced 10 meters apart and traps (dots) were placed in an offset arrangement with 20 meters to the next trap in the same row and column and 14.14 meters to the next trap on the diagonal.*

Data collection

Vole populations were longitudinally monitored via capture-mark-recapture (CMR) methods. Every four weeks from May-October 2021, Ugglan multi-capture live traps (Grahnab, Sweden) were baited with whole oats and set for 48 hours (a "trapping occasion"). Traps were checked each morning and evening at approximately 0600 and 1600 (four checks per trapping occasion) and captured animals were processed and then released at the point of capture. Six trapping occasions were conducted at each site during the study period (total trap events: 17,568). Upon first capture, all voles were injected with a Passive Integrated Transponder ("PIT tag"; "Skinny" PIT Tag, Oregon RFID, USA) for unique identification. The trap number, PIT tag number, sex, and reproductive status were recorded and a fecal sample was collected (to quantify helminth infection intensity and prevalence via fecal egg counts) for each vole on their first capture each trapping occasion. If a vole was recaptured in a given trapping

occasion, only the trap number and PIT tag number were recorded, no samples were collected, and the vole was released at the capture location.

At each capture, voles were categorized as reproductive (perforate vagina, lactating, or pregnant females; males with scrotal testes) or non-reproductive. Reproductive status per vole was summarized within the summer breeding season (June-August) and the autumn non-breeding season (September-October in southern Finland; Kaikusalo, 1972) to account for seasonal differences in vole behavior. If a vole was ever recorded as reproductive during a trapping occasion within the season, it was recorded as reproductive in that season. In autumn, voles that were reproductive in summer were also considered reproductive in autumn, even if external traits were not observed in September or October. Voles were further classified into four functional groups: reproductive males, reproductive females, non-reproductive males, and non-reproductive females. Functional groups are population subgroups that are relatively uniform in their behavior, physiology, and immunology (Haukisalmi et al., 1995; Myllymäki, 1977a, 1977b). Conducting epidemiological analysis at this level pools individuals into biologically meaningful groups and enables more realistic conclusions to be drawn (Henttonen, 2022).

All trapping, handling, and sampling of wild bank voles was conducted under approval of the University of Arkansas Institutional Animal Care and Use Committee (IACUC #19105) and the Finnish Animal Ethics Board (ESAVI-17810-2019). Access to forest sites was provided by private landowners and by Metsähallitus Metsätalous Oy (MH 6302/2019).

Data analysis

To understand general patterns of vole space use and how they were influenced by food supplementation and helminth removal, the mean space use of voles in each functional group was characterized in both the breeding and non-breeding seasons for each treatment. Within a single study site, overlapping space use between pairs of voles (“pairwise space-use overlap”) was used to approximate opportunities for direct and indirect interactions (Robert et al., 2012). The total pairwise space-use overlap of a focal vole with all its neighbors (“individual spatial overlap”) was quantified to investigate how opportunities for interactions varied among individuals within a population. When interactions like spatial overlap are heterogeneous among individuals, network analysis

is a useful method to visualize their frequency and distribution within a population (Croft et al., 2008; Krause et al., 2007). We constructed spatial overlap networks to visualize all the pairwise space-use overlap between voles at each study site to investigate how the experimental manipulations affected opportunities for direct and indirect interactions at the population level.

Vole capture numbers in May were very low (zero to five animals per site), prohibiting network construction for all sites, therefore May capture data were excluded from downstream analysis. Additionally, since space use was estimated by functional group (the combination of sex and reproductive status), only animals with both sex and reproductive status data recorded were included in the analysis.

Modeling factors that impact seasonal space use

Vole space use was approximated by aggregating capture location data among individuals by population subgroups to characterize space use for an average individual in each group (Wanelik & Farine, 2022). This approach allows space use and spatial overlap to be estimated for individuals, even if they are caught only once or only in one trap, and offers a robust way to detect biological effects of shared space use when using sparse CMR data where repeat observations per individual are limited or heterogeneous (Wanelik & Farine, 2022).

Using this approach, seasonal vole space use was quantified by aggregating vole capture locations from each trapping occasion June-October (hereafter “month”) into a summer breeding season (June-August) and an autumn non-breeding season (September-October). We defined a vole’s seasonal “centroid” as the weighted average of all locations where it was trapped that season, allowing multiple captures in the same trap to more strongly influence the centroid than a single capture in a trap. For each vole, the distance from this centroid to every trap in the trapping grid (n=61) was calculated. Then, distances for all voles (across the entire study) were pooled by season.

We used generalized linear models (GLMs) to characterize the probability of capturing a vole in a trap a given distance from its seasonal centroid and investigate factors influencing space use (i.e., sex, reproductive status, treatment), separately in the summer and autumn. In each model, the response variable was whether a vole was caught in a given trap (where 1 indicated it was caught, 0 indicated it was not) and the

explanatory variables were: the natural log distance of the trap from the vole's seasonal centroid, vole sex, reproductive status (reproductive/non-reproductive), site-level food treatment (unfed/fed), helminth treatment (control/deworm), and interaction effects between distance and each of: sex, reproductive status, food treatment, and helminth treatment, and a three-way interaction between distance, food treatment, and helminth treatment. The interaction terms were examined to identify parameters that significantly affected the relationship between distance from the seasonal centroid and capture probability, indicating differences in space use. Model interactions were visualized using the 'visreg' R package (Breheny & Burchett, 2017).

Characterizing seasonal space use

We then used the significant predictors of capture probability as identified by the seasonal space use GLMs to divide voles into population subgroups and characterize space use separately for each group. The distances from a vole's seasonal centroid to every trap in the trapping grid were pooled across voles, grouped by the combination of season*sex*reproductive status*food treatment*helminth treatment (n groups = 32). For each group, we fitted a negative sigmoidal curve (Equation 3.1; fitted using a Bernoulli GLM, where 1 indicated an animal was caught in a given trap, 0 indicated it was not; as developed by Wanelik & Farine, 2022) to characterize the average space use of a vole in that group:

$$P(d) = \frac{1}{1 + e^{-a-bd}} \quad (3.1)$$

Where space use was defined as the declining probability *P* of capturing a vole an increasing distance (*d*) from its seasonal centroid as determined by: *a* (describing the size of the space use radius), *b* (describing how steeply the capture probability declines as distance increases), and *d* (the logarithmic distance from the centroid). Thus, space use was separately characterized in the summer and autumn for each treatment, for voles of each functional group.

Estimating pairwise space-use overlap and constructing spatial overlap networks

Space-use overlap was estimated between all pairs of voles captured at a study site in a given month. These pairwise interactions were then used to create spatial overlap networks to visualize all the interactions among individuals in the observed

population at each site, in each month. First, the monthly centroid for every vole was calculated as the weighted average of all trap locations where it was captured that month (1-4 captures/month possible). The appropriate seasonal space use for a given vole's sex, reproductive status, the site-level food and helminth treatments, and the season (as calculated above using Eq. 3.1) was centered at this monthly centroid.

Overlap between all pairs of voles observed in a month at a site was then estimated based on the overlap in the two voles' space use (as developed by Wanelik & Farine, 2022). Specifically, we calculated the amount of pairwise space-use overlap by first predicting the (independent) probabilities of capturing vole 1 and vole 2 (using Eq. 3.1) at each grid point (x, y) in a grid of coordinates X and Y overlapping both of their space use. The amount of pairwise space-use overlap between the two voles ($E_{1,2}$) was estimated by taking the lower of the two voles' capture probabilities at each grid point (x, y) and summing them across all grid points and similarly summing the higher of the two capture probabilities across all grid points. The summed minimums were then divided by the summed maximums to get a ratio indicating how similar the two voles' capture probabilities were across space (Equation 3.2, developed by Wanelik & Farine, 2022):

$$E_{1,2} = \frac{\Sigma_X \Sigma_Y \, min(P_1(\sqrt{(x_1-x)^2+(y_1-y)^2}), P_2(\sqrt{(x_2-x)^2+(y_2-y)^2})}{\Sigma_X \Sigma_Y \, max(P_1(\sqrt{(x_1-x)^2+(y_1-y)^2}), P_2(\sqrt{(x_2-x)^2+(y_2-y)^2})} \quad (3.2)$$

The more similar the space use of the two voles, the ratio ($E_{1,2}$) approaches 1 (the two voles have similar capture probabilities at each grid point, indicating more complete overlap), whereas the more different their space use, the ratio approaches 0 (where one vole has high capture probability, the other has low capture probability, indicating very little overlap).

We then constructed spatial overlap networks visualizing the pairwise space-use overlap between all observed voles at each study site using the 'igraph' R package (Csardi & Nepusz, 2006). Separate networks were constructed for each month June-October to capture the temporal dynamics of network structure and account for animal interactions that vary through time due to changing population size, demographics, or seasonal behaviors (Pinter-Wollman et al., 2014). In the networks, voles were represented as nodes and only voles captured in a given month were included in the network. Connections between nodes (edges) were undirected and were weighted by

the values of space-use overlap between pairs of voles (the edge weight between voles 1 and 2, $E_{1,2}$) with no thresholding of edge weights.

Quantifying individual vole spatial overlap

We quantified individual spatial overlap within the vole populations using weighted degree (Table 3.1). Values of weighted degree were unfiltered, allowing for very small measures of weighted degree at the extreme edges of two voles' space use, indicating a very low probability of overlap (e.g., weighted degree <0.01). Two additional measures of spatial overlap: unweighted degree and normalized unweighted degree, are presented in Appendix B.1 Individual spatial overlap - Additional metrics. All network metrics were calculated using the 'igraph' R package (Csardi & Nepusz, 2006).

Table 3.1. *Description of network metrics and their biological relevance.*

Network Metric		Network Definition	Biological Relevance
NODE LEVEL	Weighted degree	Number of edges connected to a focal node by the weight of each edge; weights represent amount of spatial overlap	Sum of all pairwise space-use overlap a focal vole shared with its neighbors ("individual spatial overlap")
NETWORK LEVEL	Degree distribution	Frequency distribution of degree counts in a network (weighted or unweighted)	Quantifies the heterogeneity in individual spatial overlap in the population
	Network Size	Total number of nodes in the network	Number of voles observed during a trapping occasion at a given site

Network data are inherently non-independent and degree is influenced by network size, limiting the ability to make direct comparisons between networks of varying size (Farine & Whitehead, 2015). For an analysis of the effects of treatment on network size, see Appendix B.2 Network size. The degree measures of all observed voles per treatment and month were visualized as degree distributions using density plots. In these, the x-axis indicates the value of interest, and the height of the curve suggests the

frequency of occurrence of that value in the population. Narrow distributions suggest low variation in the population values while wide distributions indicate greater variation.

Data analysis was conducted in program R version 4.1.2 (R Core Team, 2021).

3.4 Results

We captured and recorded sex and reproductive status data for 742 unique voles during our study period (June-October 2021). Of these, 445 voles (60%) were captured at least twice (mean and standard deviation captures per vole: 2.75 ± 2.33 [range: 1-17 captures]). We documented 905 captures across the 12 sites in summer (June-August) and 1107 captures in autumn (September-October). These capture data were used to inform our seasonal space use models.

At first capture (i.e., pre-treatment) there was no difference in helminth infection status or helminth infection intensity (measured in eggs per gram of feces) between voles in the control versus deworm treatments (deworming treatment parameters in generalized linear model, linear model [respectively] both $p>0.58$). At subsequent captures (i.e., post-treatment) deworming treatment strongly decreased helminth infection intensity in treated voles compared to control voles receiving sugar water (linear mixed-effects model deworming treatment parameter $p<0.02$) while deworming moderately decreased infection status in treated compared to control voles (GLMM deworming parameter $p<0.08$).

Seasonal space use

In summer, the seasonal space use model indicated that the effect of distance on capture probability differed by vole sex, reproductive status, and site-level food supplementation treatment (GLM interaction terms between each parameter and distance from centroid: all $p<0.001$; Table B3; Figure B3A-C). No effect of helminth removal was detected (GLM interaction term between helminth treatment and distance from centroid: $p=0.18$, Table B3; Figure B3D).

When we estimated summer space use (using Equation 1) for each functional group in each treatment, we found evidence for male voles having larger space use than females and reproductive voles having greater space use than non-reproductive voles (Figure 3.2). Food supplementation generally decreased vole space use, compared to unfed treatments (Figure 3.2).

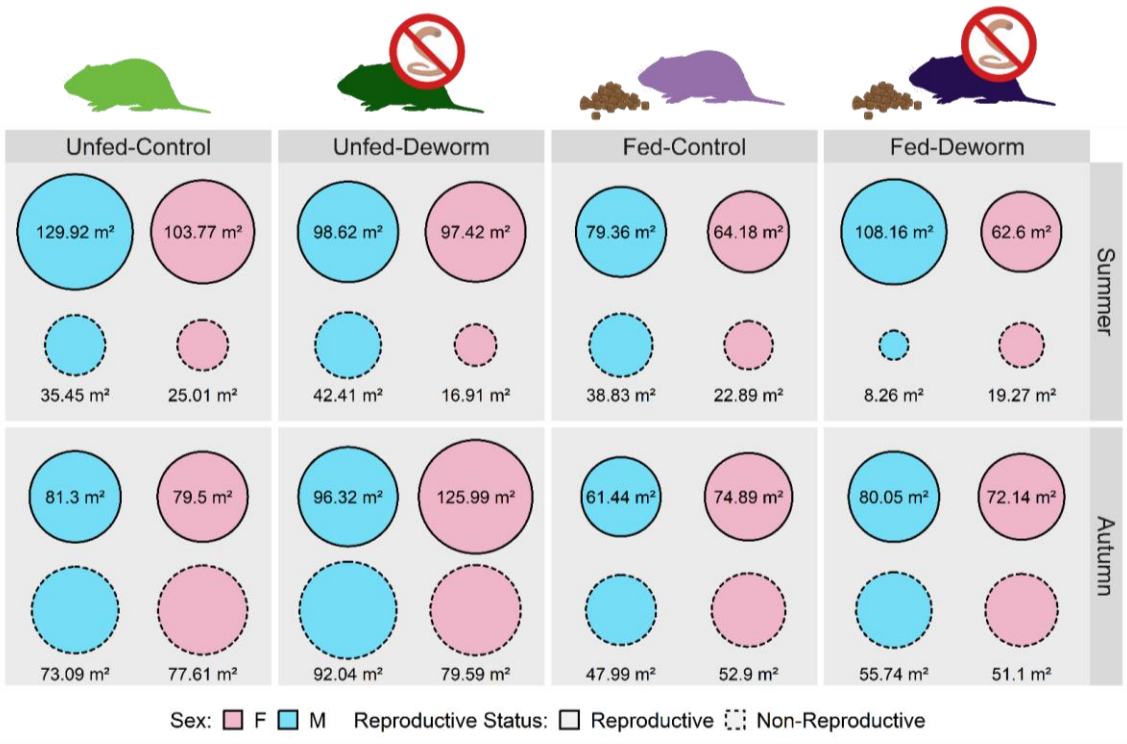

Figure 3.2. *Space use of bank voles by sex and reproductive status across treatments in the summer breeding season (June-August) and autumn nonbreeding season (September-October). Circles represent the mean space use area (areas reported in m^2) where the probability of capture is greater than 0.01.*

In autumn, the seasonal space use model indicated that the effect of distance on capture probability differed by vole reproductive status, site-level food treatment, and helminth treatment (GLM interaction terms between each parameter and distance from centroid: all $p<0.012$, Table B4; Figure B4B-D). No effect of vole sex was detected in autumn (GLM interaction term between sex and distance from centroid $p=0.68$, Table B4; Figure B4A).

When estimating space use, we found that reproductive voles had greater space use than non-reproductive voles, though space use of reproductive and non-reproductive voles was more similar in autumn than in summer (Figure 3.2). The space use of reproductive voles generally decreased or remained similar from summer to autumn. The space use of non-reproductive voles increased from summer to autumn. Food supplementation decreased vole space use compared to unfed treatments (Figure 3.2). Helminth removal had the opposite effect: voles in deworm treatments had increased space use, compared to voles in control treatments (Figure 3.2).

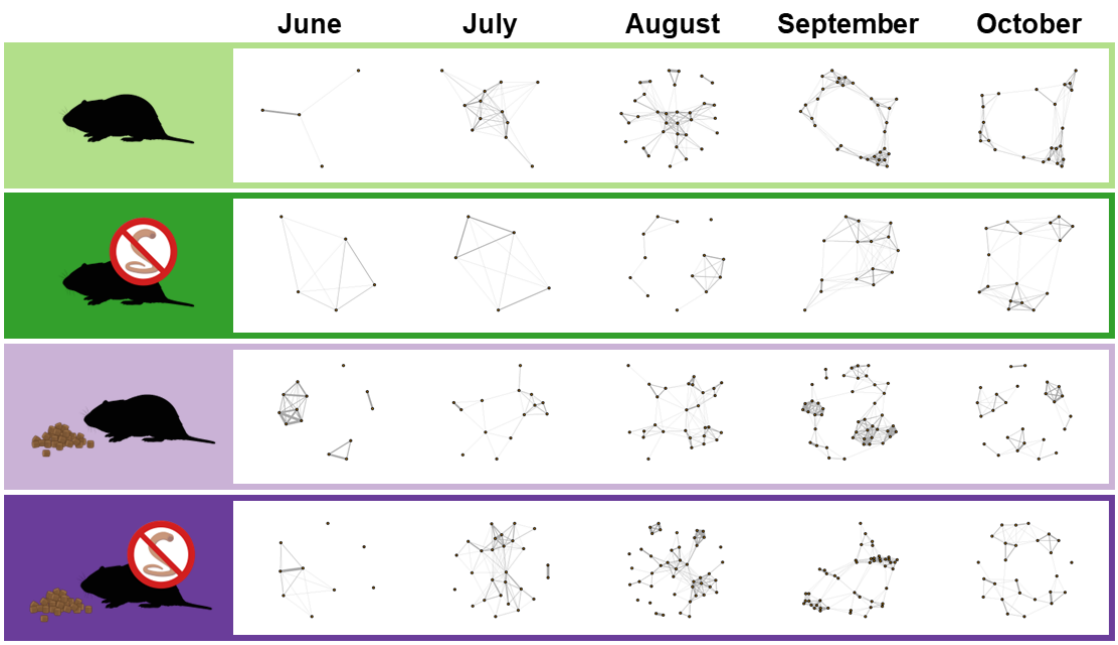

Figure 3.3. *Spatial overlap networks of vole pairwise space-use overlap. Nodes represent individual voles, edges represent pairwise space-use overlap, thicker edges indicate greater space-use overlap between voles. For each treatment, networks from one study site are shown. Edge weights are thresholded to a minimum edge weight of 0.05 for visualization purposes only.*

Spatial overlap networks

In every month, all voles overlapped with at least one other vole and populations appeared densely and homogeneously connected if all edges were included in the network (i.e., no edge weight thresholding). However, edges of low weight (<0.05) were common and more heterogeneous network structure appeared with minimal edge weight thresholding (Figure 3.3). Network size was highly variable across months and treatments (range: 2-52 voles). Networks were smallest in June, peaked in size in August (unfed treatments) or September (fed treatments), and then decreased slightly though October (Figure 3.4A; see also Appendix B.3 Network Size).

Distributions of weighted degree, indicating individual vole spatial overlap (total spatial overlap of a focal vole with its neighbors, weighted by the amount of pairwise space-use overlap with each neighbor) were similar between the unfed-control, fed-control, and fed-deworm treatments in a given month in both summer and autumn. In these treatments, weighted degree distributions were widest in September when the most voles with high degree (weighted degree >3) were observed (Figure 3.4B). In the unfed-deworm treatment, weighted degree distributions were more narrow in June-

September, reaching a smaller maximum. Overall, the mean weighted degree was similar across all four treatments in a given month.

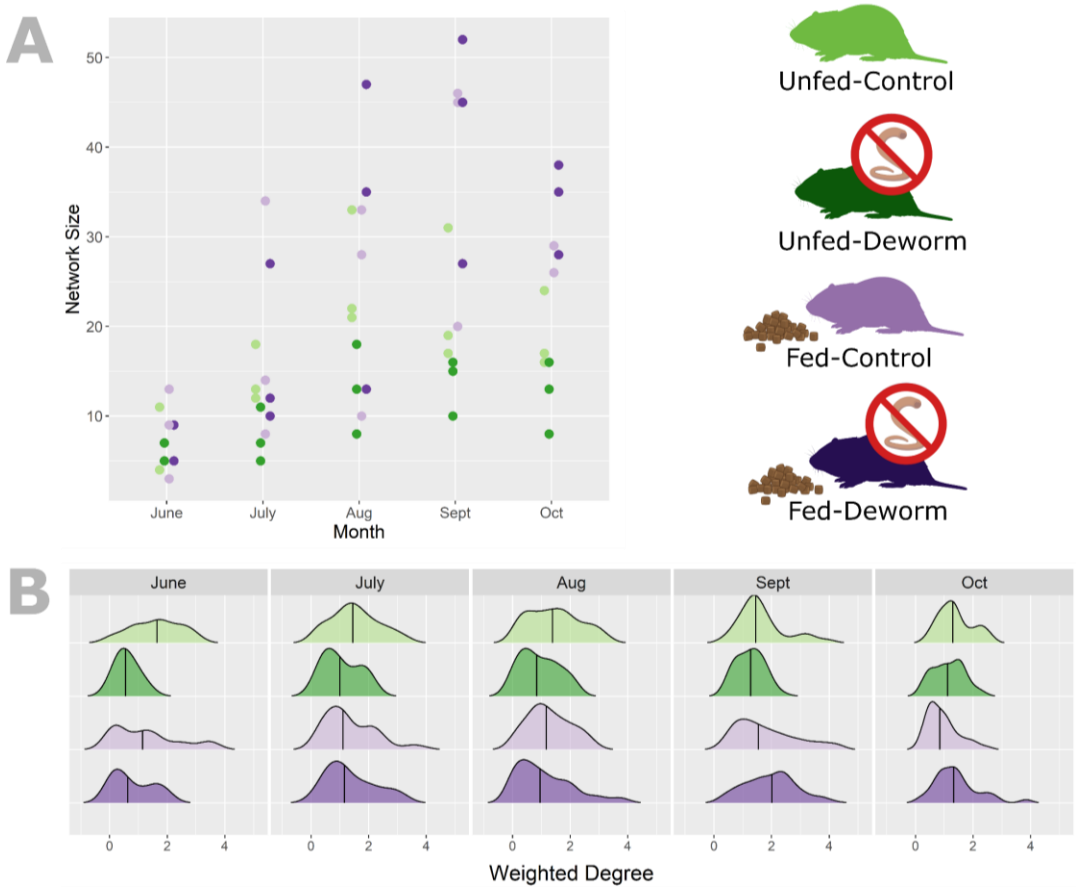

Figure 3.4. *Network size and weighted degree distributions of vole populations. (A) Network size (number of voles) by month. One data point shown per replicate study site. (B) Density distributions of weighted degree (total spatial overlap per vole, weighted by the amount of overlap with each of its neighbors) by month and treatment.*

3.5 Discussion

Variation in food availability and macroparasite infection can alter individual space use and intraspecific interactions in ways that influence spatial processes in animal populations. Through experimental food supplementation and helminth removal in wild bank vole populations, we quantified the effects of environmental and individual variation on space use and spatial overlap. Food supplementation decreased vole space use, while helminth removal increased vole space use but only in autumn. Space use was also influenced by functional group (combination of sex and reproductive status) and season. Mean individual vole spatial overlap was similar between treatments in a

given month, despite up to three-fold differences in population density. Our findings show that both environmental variability and individual variation influence animal space use, but that interactions between space use and population density can maintain consistent spatial overlap in wildlife populations under different environmental conditions.

Significant effects of both food supplementation and deworming treatment on vole seasonal space use were detected, though the effect sizes were generally small. The experimental and replicated design of our study and the large seasonal sample size of captured animals likely enables the detection of subtle and potentially important differences in space use. Food supplementation decreased vole space use in both summer and autumn. These findings align with the general understanding that supplemental food decreases bank vole home range size (Bondrup-Nielsen & Karlsson, 1985; Mazurkiewicz, 1983). However, food supplementation also increased population density, introducing a confounding factor which cannot be fully disentangled from the observed effects on space use. Increased population density can increase territorial behavior, resulting in smaller, more easily defended space use - potentially independent of food availability (Davis et al., 2015). Decreased space use under food supplementation is likely a complex response driven by interacting effects of food availability and population density.

In autumn, deworming treatment increased vole space use as compared to voles in populations with unmanipulated helminth infection. This effect was not observed in the summer, suggesting a time-lagged effect of deworming treatment. This may be due to factors at the individual level: e.g., it takes time for the medication to start working and for behavioral effects of helminth infection to be impacted, or at the population level: several months of trapping may be necessary to treat a sufficient proportion of individuals and observe population-level effects (Pedersen & Fenton, 2015) or repeated treatments per individual may be necessary to decrease the environmental burden and limit infection (Knowles et al., 2013). Increased space use by voles in the deworm treatments lends support to the hypothesis that animals with high helminth burdens exhibit sickness behavior and decrease their movements (Ghai et al., 2015). One potential confounding factor is that population density was very low across the unfed-deworm treatment, thus the increased space use may be a result of minimal territorial constraints and not a direct result of helminth removal. However, population sizes were

more similar between the fed treatments (and, in some cases, populations were larger in fed-deworm than fed-control sites) and space use was still greater with deworming treatment, supporting helminth removal being a biologically meaningful factor influencing vole space use.

Space use interacted with vole sex and reproductive status as expected based on vole biology. We found that male space use was greater than that of females during the breeding season and space use of reproductive voles was greater than that of non-reproductive voles which is consistent with the established literature (Bondrup-Nielsen & Karlsson, 1985; Bujalska, 1990). However, while we are confident in our space use estimates for reproductive voles, the space use of non-reproductive voles may be more difficult to estimate, particularly in the summer. On average in the summer months, non-reproductive voles were captured less frequently and in fewer traps than reproductive voles (whereas capture frequency and trap usage were similar in autumn). We also observed very small space use by non-reproductive voles in summer. This could be representative of their true space use (e.g., a 'sit and wait' strategy, Bujalska, 1990), but may also be the result of many young voles who are captured once and then disperse off the trapping grid and are not captured again, artificially 'shrinking' the observed space use of non-reproductive voles. While we can not disentangle the relative impacts of these two factors, our observations still provide valuable comparisons of reproductive vole space use across seasons and environmental and individual variation by food supplementation and helminth removal.

It was surprising to us that, despite the differences in space use and population density, we did not observe clear differences in individual spatial overlap by treatment, even when population sizes differed. We found that food supplementation increased population density, consistent with previous manipulations in wild vole populations (Bujalska & Janion, 1981). It has been hypothesized that territorial behavior among reproductive females should be weakest when food is abundant and evenly distributed and population density is high (Ostfeld, 1985). Previous studies in *Clethrionomys* voles have supported this hypothesis, demonstrating that food addition decreases reproductive female space use, but increases spatial overlap between females (Andrzejewski & Mazurkiewicz, 1976; Ims, 1987). While we did observe the decreases in both male and female space use with food supplementation, we did not observe an increase in spatial overlap in fed treatments compared to unfed. Similar spatial overlap

despite differences in population density could be explained by a non-linear interaction of space use with population density. Individuals may move more freely and have larger space use at lower densities (i.e., unfed treatments), but constrain their space use and only contact their nearest neighbors at high densities (fed treatments) (Davis et al., 2015). Thus, it is possible the effects of food supplementation on space use and population density balance out: resulting in similar patterns of spatial overlap between fed and unfed populations, despite differences in population density.

Our network construction methods were chosen specifically to minimize the effect of low or missing observations of individuals on network structure. Networks constructed from trapping data often assign edges between individuals caught in the same or adjacent traps (e.g., Grear et al., 2009; Perkins et al., 2009), but this can bias degree measures if capture frequency is heterogeneous. The methods used herein combine capture data across individuals to define an average space use that is applied to every individual of a functional group. In this way, the behavior of animals captured many times fills in the 'missing' data for animals captured only once or twice and minimizes the effect of capture frequency on network measures (Wanelik & Farine, 2022). One drawback to this approach is that aggregating behavior across functional groups eliminates individual variation within the group. While the behavior of small, highly connected subsets of the population undoubtedly impacts transmission dynamics (e.g., "superspreaders"; Lloyd-Smith et al., 2005), our objective was to quantify and compare space use between treatments and populations. By grouping voles by functional group, we allow behavior to vary across biologically meaningful axes such as sex and reproductive status (Henttonen, 2022), while capturing the average behavior of each population subclass which can be more readily generalized to other animal systems.

Spatial overlap can be linked to many direct and indirect interactions between animals including social interactions and pathogen transmission. When direct observation of direct interactions in wildlife populations is limited or impossible, thoughtfully aligned measures of spatial overlap can be used to approximate and draw inference about these interactions (Craft & Caillaud, 2011; Mistrick et al., 2022). For example, spatial overlap in a window of time consistent with the environmental persistence of a virus can be used to approximate indirect transmission opportunities where direct contact between animals is not required (Godfrey et al., 2010). In this

context, our findings that spatial overlap was similar across treatments could suggest that opportunities for environmental transmission may remain relatively constant even when population size increases such as under high resource availability. However, differences in space use by sex and reproductive status could bias functional groups such as mature males toward greater exposure to environmental pathogens or greater potential to shed infectious material over larger areas (Krasnov et al., 2012). As such, the utilities of space use and spatial overlap data can be extended to various fields, providing insight into animal behavior and its ecological consequences, even when it cannot be measured directly.

3.6 Conclusions

This study provides necessary, empirical evidence of the effects of food supplementation and helminth removal on space use and spatial overlap in wild rodent populations. Field studies investigating the effects of environmental and individual variation on animal spatial patterns are limited, thus this work addresses a gap in our understanding of factors influencing opportunities for direct or indirect interactions in wildlife populations. We demonstrate that environmental variability due to supplemental food and individual variation in macroparasite infection drive rodent space use. We have also identified the potential for space use and population size to trade off, allowing individuals in a population to maintain similar levels of spatial overlap despite variation in their environment. As such, our research demonstrates that environmental variability and individual variation drive animal spatial behavior and interact with population dynamics in wildlife populations and therefore represent important drivers of population-level processes such as social interactions and pathogen transmission.

3.7 Acknowledgements

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Chapter 4. Environmental conditions alter how spatial overlap predicts viral infection status in wild rodent populations

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4.1 Abstract

Spatial overlap between animals in wildlife populations can be used to examine how the interactions between individuals influence pathogen transmission. Environmental conditions and individual host traits and behavior can influence animal space use and spatial overlap, but it is unclear how these factors interact with animal spatial patterns to drive pathogen transmission. Our objective was to investigate if the effect of spatial overlap on pathogen infection varied based on food abundance and macroparasite infection. We also explored factors such as animal sex and reproductive condition that could moderate the effect of food and macroparasites on space use behavior and infection. We experimentally manipulated wild bank vole populations via food supplementation and helminth removal and quantified vole space use, spatial overlap, and infection of an endemic hantavirus. We investigated the relationship between spatial overlap and infection probability under each manipulation to determine if the environmental conditions influenced how spatial overlap drove transmission. Previous spatial overlap drove current hantavirus infection, but food supplementation and helminth removal altered the effects of spatial overlap on infection. Vole sex and reproductive condition were important factors dictating whether spatial overlap increased or decreased infection probability and the traits of individuals most important for transmission varied by experimental manipulation. Our research provides rare empirical evidence linking viral infection to space use in populations of a wildlife species. As such, we highlight the importance of incorporating and investigating biologically meaningful axes of environmental and inter-individual variation when studying disease dynamics in wildlife systems to capture factors driving heterogeneity in transmission.

4.2 Introduction

Networks are a powerful tool for investigating how heterogeneity at the individual level scales up to population-level effects. In disease ecology, networks have been used in a wide range of wildlife-pathogen systems to examine how the behaviors and traits of individual animals influence transmission dynamics at the population level (Godfrey, 2013). Animal behavior, particularly how individuals use and overlap in space, creates opportunities for direct and indirect contact that can facilitate pathogen transmission (Davis et al., 2015; Robert et al., 2012). Network approaches can identify behaviors that are linked to transmission by testing for correlation between network connections and infection prevalence (Corner et al., 2003; Leu et al., 2010). Combining host traits and infection prevalence with network data can identify the relative contributions of different demographics in the population to transmission (Fenner et al., 2011; VanderWaal et al., 2013). In these ways, network approaches have provided major insights into the behaviors and traits driving transmission in wildlife populations and are often used to generalize more broadly to other host-pathogen systems (White et al., 2018b).

However, hosts and their pathogens, and thus population disease dynamics, are inextricably linked to the environmental context in which they occur. Therefore, it is not enough to examine the relationship between host behaviors and traits and pathogen transmission, the environmental conditions which influence them must also be considered. Environmental conditions such as food abundance (Becker et al., 2015) and seasonality (Altizer et al., 2006) influence animal space use and space sharing behavior and can facilitate pathogen transmission (VanderWaal & Ezenwa, 2016). Food abundance can impact host space use by increasing space use through increased foraging when food is scarce and decreasing space use through greater site fidelity when food is abundant (Becker, Hall, et al., 2018). Increased spatial overlap in areas of high food abundance can increase the prevalence of directly and indirectly transmitted pathogens (Cross et al., 2007; Forbes et al., 2015). Spatial overlap among conspecifics and between species can also change seasonally based on aggregation behavior driven by climate or resources and can similarly drive transmission in wildlife populations (Hirsch et al., 2016; VanderWaal et al., 2017).

Environmental conditions can also influence animal traits at the population- and individual-levels. Seasonality and food abundance influence host demography at the population level by driving birth pulses (Ostfeld & Keesing, 2000; Touzot et al., 2020)

and potentially influencing sex ratios of offspring (Geffroy & Douhard, 2019; Navara, 2018). Population density, which is itself influenced by seasonality and food abundance, can alter individual-level host traits by altering the timing of sexual maturation (Prévot-Julliard et al., 1999). In addition, demographic traits (e.g., sex, reproductive condition) influence spatial behavior in many species, further linking animal behavior, demographic traits, and environmental conditions in ways that may influence pathogen transmission.

Although macroparasites have well-established effects on animal behavior (Ezenwa et al., 2016), the effects of macroparasite infection on animal space use, thus pathogen transmission potential, has been minimally investigated in wildlife. Macroparasite infection may induce sickness behavior leading to decreased energetic activities such as movement and mating and thus decreased host space use (Ghai et al., 2015). Uninfected animals may also be able to detect infection in conspecifics and avoid interactions, decreasing space use sharing (Croft, Edenbrow, et al., 2011; Kavaliers et al., 2014). Alternatively, removal of macroparasites may have negligible effects on host space use (Bloomer et al., 1995). Despite these findings, much is still unknown about the effects of parasite infection on behavioral changes in wildlife including how individual parasite-induced behaviors scale up to influence transmission at the population level (Hawley & Altizer, 2011). Further experimental studies in wildlife are necessary to robustly measure the effects of parasitism on host spatial behavior (Hawley & Ezenwa, 2022).

While many network studies have investigated relationships between animal behavior and traits and transmission, most are observational in nature or conducted with minimal replication. As such, it is difficult to determine whether host behaviors and traits always correlate with infection as observed, or if their relationship with infection is dependent upon specific environmental conditions. To advance our understanding of transmission in wildlife systems, we must incorporate and investigate the effects of realistic natural heterogeneity in the environment when studying the links between animal spatial behavior, demographic traits, and pathogen prevalence (Tompkins et al., 2011; VanderWaal & Ezenwa, 2016). Ultimately, addressing questions of how relationships between networks of animal interactions and infection prevalence vary under different environmental conditions requires employing rigorous experimental designs.

Rodents and their endemic pathogens provide a tractable model system for field experiments investigating the effects of environmental conditions on infection prevalence (Forbes et al., 2016; Sweeny et al., 2021). For this study, we leveraged a well-characterized rodent-hantavirus system: bank voles (*Clethrionomys glareolus*, previously *Myodes glareolus*; Carleton et al., 2014; Kryštufek et al., 2020) and Puumala hantavirus. Bank vole populations are amenable to experimental manipulation and capture-mark-recapture studies. They are common hosts for macroparasite infection (e.g., helminths such as *Heligomosomoides glareoli*, Haukisalmi & Henttonen, 2000) and are the primary wildlife host of Puumala hantavirus, which is endemic in their populations at approximately 50% prevalence (Voutilainen et al., 2016). Animal space use behavior is essential to inform modeling approaches and guide model interpretation. Bank vole space use behavior is well-documented, including how it varies by sex, reproductive condition, and season (Tamarin et al., 1990). Sexually mature male space use is greater than that of mature females and mature males will share space with other mature males and with multiple mature females (Mazurkiewicz, 1971). Mature females are territorial toward other mature females (Koskela et al., 1997), but may tolerate overlap with sexually immature subadults, particularly in the non-breeding season (Bujalska, 1970). Subadult space use is much smaller than that of mature voles and space use sharing is variable: some subadults may disperse to find new territory while others may employ a 'sit and wait' strategy where overlap on the periphery of mature voles' territories is tolerated (Bujalska, 1990). Both food abundance and population density are thought to regulate the size of vole space use (Bondrup-Nielsen & Karlsson, 1985; Jonsson et al., 2002).

We experimentally manipulated two environmental factors: food abundance and helminth macroparasite infection, and longitudinally measured vole space use and hantavirus infection to address two questions: 1) Is the relationship between spatial overlap and infection status influenced by environmental conditions? and 2) How do vole demographic traits (sex and reproductive condition) and space use behavior moderate these effects? We expected space use to be influenced by food supplementation and helminth removal (Chapter 3 of this dissertation). We hypothesized that the relationship between spatial overlap and infection status would vary by treatment, based on how the treatment affected population density and space use. We also predicted that the relationships been spatial overlap and infection status would be moderated by

demographic traits: where demographic groups less tolerant of overlap (i.e., mature females) would experience limited effects of treatment altering the relationship between spatial overlap and infection status compared to those more tolerant of overlap (i.e., subadults, mature males).

4.3 Materials & Methods

Study site and experimental design

A replicated two-factor field experiment was conducted in the boreal forests of southern Finland (61.0775°N, 25.0110°E) where bank voles are the dominant rodent species. Twelve study sites - spaced at least two kilometers apart to prevent dispersal between replicate populations - were established in old-growth spruce forest. A standardized trapping grid (100 m x 100 m, 1 hectare) consisting of 61 uniquely identified traps spaced 10 meters between grid rows and columns was established at each site to monitor the vole population (Figure 4.1A). Sites were assigned one of four treatment pairings ("treatments"): no manipulation ("unfed-control"; "U-C"); helminth removal but no food supplementation ("unfed-deworm"; "U-D"); food supplementation but no helminth removal ("fed-control", "F-C"); and both food supplementation and helminth removal ("fed-deworm"; "F-D") with each treatment replicated at three sites (Figure 1). Food supplementation sites received a feed mix of mouse chow pellets and sunflower seeds evenly distributed on the trapping grid every two weeks from May through November. The feed mix consisted of equal parts by weight (7.875kg each) of mouse chow (Altromin 1324 pellets [3227 kcal/kg]; Altromin Spezialfutter GmbH & Co. KG, Germany) and sunflower seeds (as fed, 6350 kcal/kg) dosed at 7.54 kcal/m^2 (following Sweeny et al., 2021). At deworm sites, voles received an oral dose of 10 mg/kg Ivermectin and 100 mg/kg Pyrantel (effective at treating infection with larval and adult helminths, Clerc et al., 2019) at their first capture each trapping occasion. At control sites, voles received a matching weight-based dose of sugar water *(*17.5% sucrose solution) at their first capture each trapping occasion.

Rodent sampling

Vole populations were longitudinally monitored via capture-mark-recapture methods over the course of two years (2021 and 2022). Each year, every four weeks from May-October, Ugglan multi-capture live traps (Grahnab, Sweden) were baited and

set for a 48 hour “trapping occasion”. Over the trapping occasion, traps were checked four times: twice daily at approximately 0600 and 1600, and captured animals were sampled and then released at the capture location. In each year of the study, six trapping occasions were conducted at each site (total trap events: 17,568 per year in both 2021 and 2022). At their first capture, all voles were injected with a Passive Integrated Transponder (“PIT tag”; Oregon RFID, USA) for unique identification. At their first capture in a trapping occasion, the trap number, PIT tag number, sex, and reproductive condition of each vole were recorded. Vole reproductive condition was categorized as either a sexually mature adult (“reproductive”) or sexually immature subadult (“non-reproductive”). Voles were considered reproductive if they had a perforate vagina, visible nipples, or were pregnant (females) or if they had scrotal testes (males). A fecal sample was collected from each vole and fecal egg counts were performed to quantify helminth infection prevalence and intensity. A blood sample was also collected from the retro-orbital sinus of each vole to screen for hantavirus infection (see ‘Serological assay for hantavirus infection’). If a vole was captured more than once in a trapping occasion, only the trap number and PIT tag number were recorded at subsequent captures, no additional samples were collected, and the vole was released at the capture location.

Demographic traits (e.g., sex, reproductive condition) can be used to categorize a population into functional groups: population subgroups that are uniform in their behavior, physiology, and immunology (Haukisalmi et al., 1988; Myllymäki, 1977a, 1977b). Conducting epidemiological analysis at the level of functional groups provides the biological context to better investigate differences in contacts and infection prevalence within the population, enabling more in-depth analyses and realistic conclusions to be drawn (Henttonen, 2022). Bank voles were classified into four functional groups: reproductive males, reproductive females, non-reproductive males, and non-reproductive females. Space use of reproductive voles differs seasonally between the summer breeding season (May-August in southern Finland) and the autumn non-breeding season (September-November in southern Finland, Kaikusalo, 1972) and the composition of each functional group changes through time as voles are born and disperse or die. For this reason, we assigned functional groups separately in summer and autumn. Reproductive condition was summarized across the entire season, such that an individual with sexual traits in any month June-August was classified as

reproductive in the summer season. In autumn, voles were classified as reproductive if sexual traits were observed in September or October or if the vole was classified as reproductive in the summer (even if no traits were observed in autumn).

All trapping and handling of wild bank voles was conducted under approval of the University of Arkansas Institutional Animal Care and Use Committee (IACUC #19105) and the Finnish Animal Ethics Board (ESAVI-17810-2019). Access to forest sites was provided by private landowners and by Metsähallitus Metsätalous Oy (MH 6302/2019).

Serological assay for hantavirus infection

Once per trapping occasion, a blood sample was collected from the retro-orbital sinus of all captured voles >10g (sample volume in µL = vole body mass*10, up to 150µL) using 75µL capillary tubes (micro-hematocrit sodium heparinized, Hirschmann, Germany). Whole blood samples were maintained in a cooler on ice until they were centrifuged and the serum separated, and then frozen at -20°C until samples were tested. Infection status (infected or uninfected) was determined by detecting PUUV-specific IgG antibodies in blood serum via validated immunofluorescence assays (IFA), as described previously (Kallio-Kokko et al., 2006). Hantavirus infection in bank voles is chronic, thus the presence of IgG antibodies indicates current infection (Forbes et al., 2018; Meyer & Schmaljohn, 2000). A subset of voles found to have inconsistent IFA results between subsequent samples (e.g., positive test followed by one or more negative tests, alternating positive and negative tests) were removed from the dataset and excluded from downstream analysis. Maternal antibodies can persist in young voles for up to 80 days (Kallio et al., 2006) and are indistinguishable from true infection via IFA, which is likely to be the source of inconsistent IFA results in individuals that were first captured at a young age.

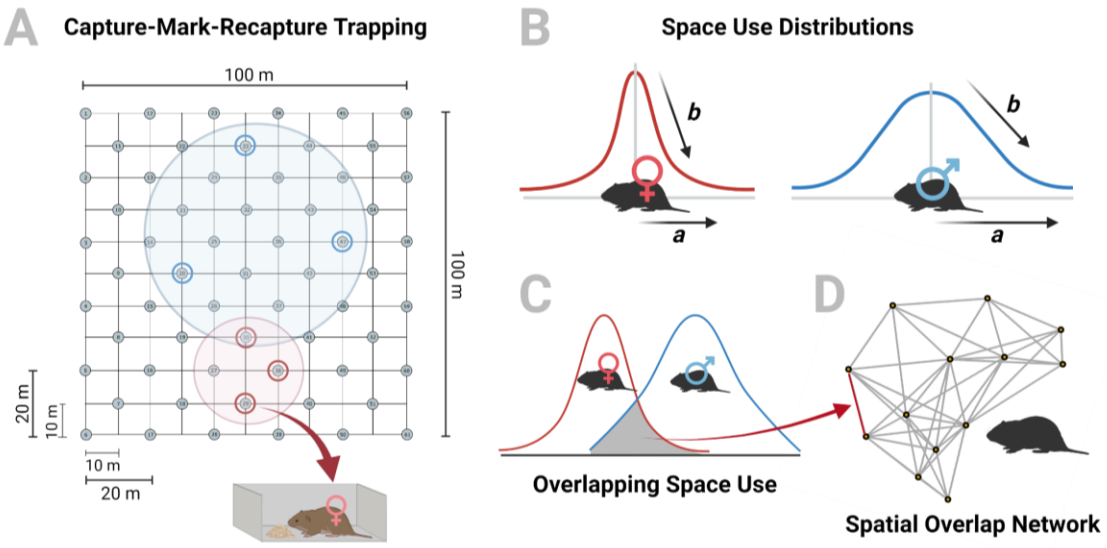

Figure 4.1. *Conceptual figure of data collection and analysis methods. A) Voles were monitored via capture-mark-recapture methods and the trapped locations of individuals were recorded. B) Trapped locations were pooled across individuals by functional group (combination of sex and reproductive condition) in the summer and autumn. A negative sigmoidal curve was used to characterize the declining probability of capturing a vole with increasing distance from the centroid of their trapped locations to estimate the average space use for a vole of each functional group. C) Space use overlap between pairs of voles was estimated each month based on the amount of overlap between their space use and D) the amount of pairwise space use overlap was used to inform the edge weights of spatial overlap networks of each population of voles. Figure created with BioRender.com.*

Data Analysis

The capture-mark-recapture data was used to estimate vole space use by season and visualize spatial overlap (defined here as mutual use of habitat, either concurrently or sequentially) between voles within a trapping occasion using networks. Vole captures in May, after the winter period, were very low (0-5 voles per site) so May data were excluded from analyses. Space use was estimated across the summer breeding season (June-August) and autumn non-breeding season (September, October) to align with seasonal changes in vole behavior (Bondrup-Nielsen & Karlsson, 1985). Space use sharing was visualized in a shorter time frame (within a single, 48-hour trapping occasion) to approximate opportunities for environmental transmission of Puumala hantavirus, which is readily shed in urine and feces and can remain infectious in the environment for up to two weeks (Kallio, Klingström, et al., 2006).

Methods utilized herein were developed by Wanelik & Farine (Wanelik & Farine, 2022) and have been proposed as a method to detect biological effects of spatial

overlap even from sparse trapping data where observations per individual may be limited or heterogeneous. Briefly, observations of individuals and their space use were pooled by treatment and season and the space use behavior of each functional group (e.g., reproductive females, non-reproductive males) was modeled separately (Figure 4.1B). Previous research in our populations has established that vole space use varies by sex, reproductive condition, and treatment between the summer breeding and autumn non-breeding seasons (Chapter 3 of this dissertation). Using this aggregate spatial behavior, a space use distribution could be fit to all individuals of that functional group to infer behavior for individuals where trapping data were limited. The weighted average trapped location for each individual vole was then used to inform a center point of their activity area each month, and the appropriate seasonal space use distribution (see below) was drawn around this point. This was repeated for all animals observed in a given month. Space use overlap was then estimated between all pairs of individuals each month based on these overlapping distributions (Figure 4.1C) and these values of overlap were used to construct spatial overlap networks (Figure 4.1D).

Estimating space use distributions

The seasonal activity center for each vole was defined as the weighted average of all trapped locations that season (such that multiple captures in the same trap would have a stronger influence than a single capture in a trap). Then, the distance from this activity center to each trap in the trapping grid (n=61) was calculated for each vole. The calculated distances per vole were pooled by grouping variables and a negative sigmoidal curve (Equation 4.1 [developed by Wanelik & Farine, 2022]; fitted using a Bernoulli GLM, where 1 indicated an animal was caught in a given trap, 0 indicated it was not) was used to define the space use distribution of a vole in that group.

$$P(d) = \frac{1}{1 + e^{-a-bd}} \quad (4.1)$$

The space use distribution represents the probability (*P*) of capturing a vole a distance (*d*) from its seasonal activity center as determined by the parameters: *a* (describing the size of the space use area), *b* (describing how rapidly capture probability declines away from the activity center) and *d* (the logarithmic distance from the center; Figure 4.1B).

To calculate the space use distributions used to construct the spatial overlap networks, voles were grouped by year, season, treatment, and functional group (combination of sex and reproductive condition) and a separate space use distribution was estimated for each group (n groups = 64; 2 years * 2 seasons per year * 4 treatments * 2 sexes * 2 reproductive conditions).

Constructing space use overlap networks

Networks were constructed for each site, one network for each of the five trapping occasions (trapping occasions: June-October, hereafter called “month”). In each month, for each vole, the weighted average of all locations where the vole was trapped that month (1-4 captures/month possible) was used as its monthly activity center, and the appropriate space use distribution (as described above; estimated by functional group for a given year, season, and treatment) was centered at that point. In this way, a vole captured in July and August would have the same ‘summer’ space use distribution in each month, but that distribution could be centered at different locations on the trapping grid, based on where the vole was captured in July versus August.

Space use overlap was estimated between all pairs of voles at a site in a given month based on their space use distributions by overlaying the two distributions and calculating the area of overlap (as developed by Wanelik & Farine, 2022) (Figure 4.1C). In detail: the amount of space use overlap was calculated by first predicting the probability of detecting each vole (using Eq. 4.1) in a grid of coordinates X and Y overlapping both of their space use distributions. The amount of overlap between voles 1 and 2 was calculated by dividing the sum of the lowest values at each point on the grid (x, y) by the sum of the largest values at each point (x, y) using Equation 4.2 (developed by Wanelik & Farine, 2022):

$$E_{1,2} = \frac{\Sigma_X \Sigma_Y min(P_1(\sqrt{(x_1-x)^2+(y_1-y)^2}), P_2(\sqrt{(x_2-x)^2+(y_2-y)^2})}{\Sigma_X \Sigma_Y max(P_1(\sqrt{(x_1-x)^2+(y_1-y)^2}), P_2(\sqrt{(x_2-x)^2+(y_2-y)^2})} \quad (4.2)$$

Where $P_n\left(\sqrt{(x_n - x)^2 + (y_n - y)^2}\right)$ is the probability of observing individual n, with a seasonal activity center of (x_n, y_n) at point (x, y) from Eq. 4.1.

Values of space use overlap between two individuals range from 0 (no overlap) to 1 (complete overlap). The measures of space use overlap were used to construct

monthly, undirected, weighted networks using the 'igraph' R package (Csardi & Nepusz, 2006) where voles were represented as nodes and edges were assigned a value corresponding to the amount of space use overlap between the two voles ($E_{1,2}$; Figure 4.1D). All calculated edge weight values were used with no thresholding, which is generally preferable to picking an arbitrary weight threshold and removing edges of lower weight or using unweighted edges (Farine & Whitehead, 2015). Only voles captured in a given month were included in the network.

Measures of weighted degree were used to quantify the amount of overlap between a focal vole and its neighbors. Weighted degree is the sum of the space-use overlap associated with all edges connected to a node, representing the total space-use a focal vole shared with all its neighbors. We partitioned weighted degree based on sex and reproductive condition to calculate the amount of overlap a focal vole had with males versus females (male- or female degree) and reproductive vs. non-reproductive voles (reproductive- or non-reproductive-degree). We also partitioned weighted degree by functional group to quantify a focal vole's overlap with reproductive males, reproductive females, non-reproductive males, and non-reproductive females. These degree measures were calculated for every vole observed at a study site in a given month.

Modeling infection status

We wanted to investigate how spatial overlap between bank voles affects Puumala hantavirus infection. Seroconversion of Puumala-infected bank voles is detectable as early as 7-14 days post-exposure (Hardestam et al., 2008), however, because each trapping occasion only spanned 48 hours and subsequent trapping occasions were spaced one month apart, transmission that occurred during one month would not be detected until the following month. We therefore investigated the effect of a vole's network position in the previous month on its infection status in the current month. It was rare to capture a vole in two months that were not consecutive (e.g., June and August but not July) but in the cases where that did occur (25/713 [3.5%] of recaptures), we used the previous capture to inform current infection. Previous capture was restricted to within a sampling year, such that July was the first month when infection status (as informed by June network position) was considered.

In addition to spatial overlap, we quantified the tendency for voles to visit different traps as a measure of exploratory behavior (VanderWaal et al., 2013). Exploratory behavior can be an important trait influencing environmental exposure and direct interactions that may not be fully captured in spatial overlap. Exploratory behavior was measured as the number of different traps a vole was caught in, relative to the expected number of traps for that number of captures. A power regression model ($y = 0.98x^{0.74}$) was fitted to the observed data of the number of unique traps and the number of captures per vole across a sampling year, one point for each vole in our dataset. A vole's measure of exploratory behavior was the residual of its observed number of unique traps minus the expected number of traps for a vole that was captured that many times, based on the model. Positive values indicate an individual used more unique traps than expected whereas negative values indicate an animal used fewer unique traps than expected (VanderWaal et al., 2013).

We used mixed-effects logistic regression models fitted using the 'lme4' R package (Bates et al., 2015) to investigate the relationship between hantavirus infection status and spatial overlap network degree measures. Explanatory variables used in all models included individual-level traits: sex, reproductive condition, exploratory behavior, and population-level traits: treatment, previous month, previous network size, sampling year. Numeric variables were scaled and centered to improve model convergence. Previous month was coded as a numeric variable in the models for the unfed-control, unfed-deworm, and fed-deworm treatments. However, singularity issues in model fitting for the fed-control treatment resulted in previous month being coded as a categorical variable with levels ordered chronologically. Site was included as a random effect to help account for inherent variation among populations.

Each degree measure (weighted degree, weighted degree by sex [male/female]; weighted degree by reproductive condition [reproductive/non-reproductive]; weighted degree by functional group [reproductive male/reproductive female/non-reproductive male/non-reproductive female]) was used as the explanatory variable in separate models. We also explored interaction effects of sex, reproductive condition, and both sex and reproductive condition for each of these degree measures, each in separate models. All candidate models were assessed by AIC and the most parsimonious was selected using Akaike Information Criterion (AIC). Data analysis was conducted in program R version 4.1.2 (R Core Team, 2021).

4.4 Results

We obtained sex and reproductive condition data and hantavirus serology results for 1,029 captures in 2021 and 1,062 captures in 2022. These datasets with sex, reproductive condition, and hantavirus infection status were used to construct space use overlap networks for all sites in June-October 2021 and 2022. To model hantavirus infection, only animals with captures in at least two separate months were used, resulting in datasets of 346 captures in 2021 and 367 captures in 2022.

Space use distributions & spatial overlap networks

In the summer breeding season (June-August), seasonal estimated space use was greater for males than females (mean ± standard deviation area of 99% probability of capture; reproductive male: 206.1 m^2 ± 34.0; reproductive female: 167.1 m^2 ± 37.2; Figure 4.2A June-Aug) and greater for reproductive voles compared to non-reproductive voles (non-reproductive male: 70.0 m^2 ± 30.7; non-reproductive female: 44.5 m^2 ± 13.3; Figure 4.2A Aug). In the autumn non-breeding season (Sept-Oct), space use was more similar for males and females and reproductive and non-reproductive voles, though reproductive vole space use was still slightly greater than that of non-reproductive voles (mean ± standard deviation, reproductive voles: 158.0 m^2 ± 31.9 ; non-reproductive: 124.3 m^2 ± 25.9; Figure 4.2A Sept-Oct). Reproductive vole space use decreased from summer to autumn while non-reproductive vole space use increased. These trends were consistent across treatments. By treatment, food supplementation decreased space use in both seasons in 2021 (GLM, $p<0.001$), but no effect of food supplementation was detected in 2022 ($p<0.5$). In both years, helminth removal increased space use in autumn ($p<0.01$), but had no effect in summer ($p<0.1$).

Spatial overlap networks were constructed for all months June-October at all sites (n=12) in 2021 and all months June-October at 11 of 12 sites in 2022 (one site had no animals in June 2022 so networks were only constructed for July-October). Without edge weight thresholding, the observed spatial overlap networks generally showed high connectivity (nearly all possible edges between individuals were realized; Figure 4.2B) but many of these edges were of very low weight (less than 0.05; Figure 4.2C), indicating low probability of overlap between a given pair of voles. Weighted degree (and weighted degree partitioned by sex, reproductive condition, and functional group) were included without thresholding in all models. Weighted degree (sum of all edge weights

per node) distributions were generally similar across treatments, though distributions tended to be more right skewed (indicating more voles with higher weighted degree) in the fed treatments compared to unfed and in 2022 compared to 2021. Mean degree was similar between treatments, ranging from 1.0 to 1.85.

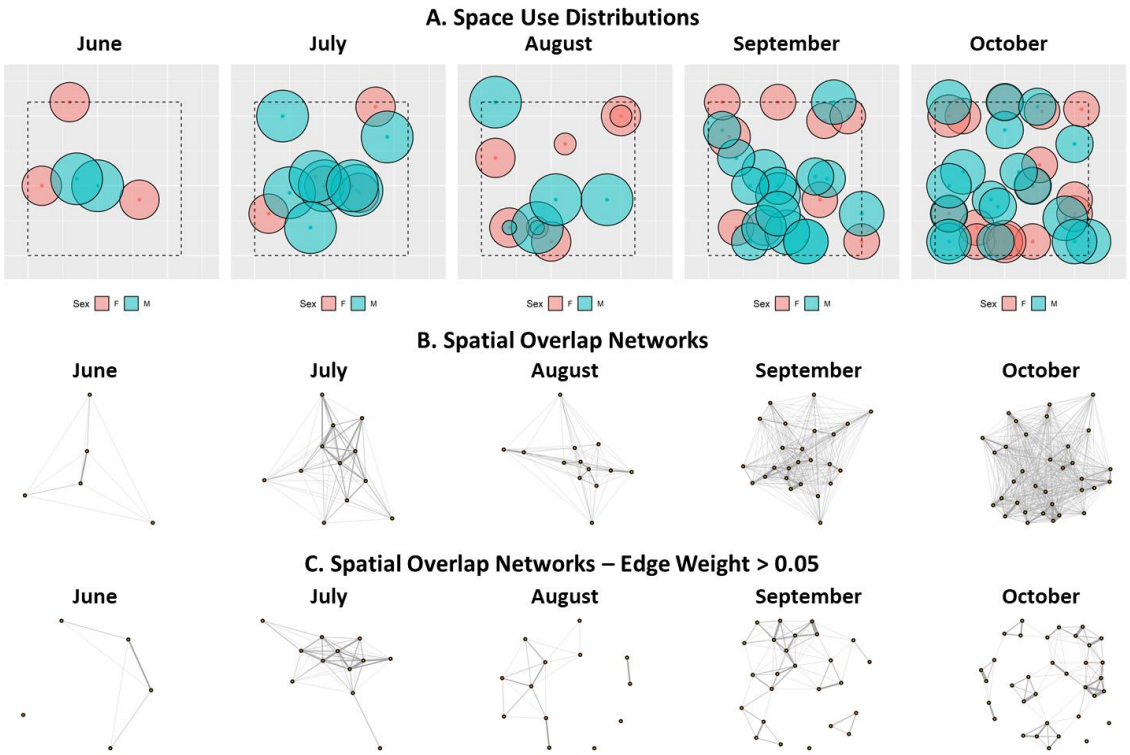

Figure 4.2. *Space use diagrams and spatial overlap networks for a vole population (fed-deworm treatment, site "Puro") in 2021. A) Colored circles indicate space use of the voles captured at the site each month. Space use was characterized for each functional group by season, therefore the space use of e.g. reproductive females was the same in June, July, and August and was recalculated for September/October. Large circles in June/July show only reproductive voles. Non-reproductive males and females (smaller circles) first appear in August. Males (blue) had larger space use than females (pink) in both summer and autumn. Reproductive and non-reproductive voles had more similar space use in autumn compared to summer. B) Spatial overlap networks were constructed for June-October from the monthly space use based on the amount of space use overlap between pairs of voles. With no edge weight threshold, spatial overlap networks in all months appear densely and homogeneously connected. C) Minimal edge weight thresholding (pairwise space-use overlap <0.05 removed) of the spatial overlap networks shows more heterogeneous network structure where clusters of more closely interacting nodes (voles) show similar patterns of overlap to the space use diagrams.*

Models fit by treatment

A series of candidate models predicting infection status from previous degree, individual-level traits, and population-level traits were fit to data from each treatment

separately. Overall sample size was lower in the unfed-deworm treatment (N observations=110) due to low vole capture numbers as compared to the other three treatments (N observations U-C=183; F-C=189; F-D=231). Low sample size of hantavirus-positive non-reproductive voles in three of the treatments (unfed-control, unfed-deworm, and fed-control) prevented us from fitting the full suite of candidate models to these datasets (for full list of candidate models fit by treatment see Table C1). In the unfed-control treatment, no hantavirus-positive non-reproductive voles were present in the dataset so candidate models were fit without the 'reproductive condition' parameter.

The best-fit model for the unfed-control and unfed-deworm treatments was the reproductive condition degree:sex model while the best-fit model for the fed-control and fed-deworm treatments was the functional group degree:sex:reproductive condition model.

In the unfed-control treatment, previous spatial overlap affected the probability of current hantavirus infection for male voles. Space use sharing with non-reproductive voles decreased the probability of infection in males while spatial overlap with reproductive voles increased the probability of infection (non-reproductive degree: Odds Ratio (OR)=0.05, 95% Confidence Interval (CI) (0.00-1.41), p=0.079; reproductive degree: OR=1.73, CI (1.07, 2.81), p=0.026; Figure 4.3; Table C2).

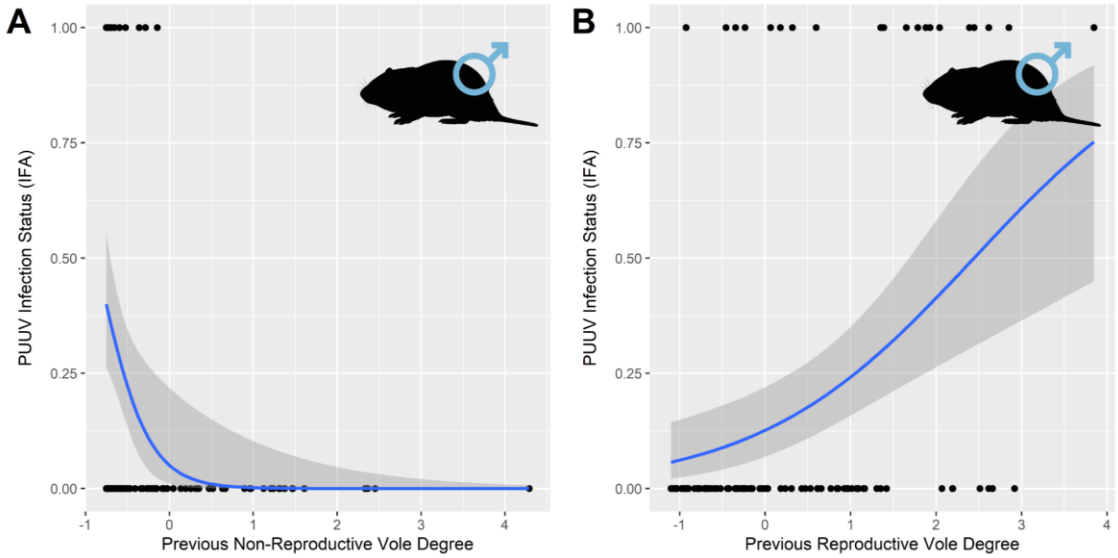

Figure 4.3. *Plots of correlation between current Puumala hantavirus (PUUV) infection status and previous overlap network degree for male voles in the unfed-control treatments. Previous degree measured as A) spatial overlap with non-reproductive voles and B) spatial overlap with reproductive voles. Degree measures were scaled and centered.*

In the unfed-deworm treatment, previous spatial overlap with non-reproductive voles increased the probability of current infection in female voles (OR=20.5, CI (1.70-248), p=0.017; Figure C1; Table C3).

In the fed-control treatment, previous spatial overlap with non-reproductive male voles increased the probability of current infection for reproductive females (OR=2.85, CI (1.14-7.15), p=0.025**;** Table C4). For a discussion of possible effects of spatial overlap with reproductive females on infection status in non-reproductive voles, see Appendix C.1 Results and Discussion.

In the fed-deworm treatment, previous spatial overlap affected the probability of current infection for reproductive females. Spatial overlap with reproductive males decreased the probability of infection for reproductive females while spatial overlap with reproductive females increased the probability of infection for reproductive females (reproductive male degree: OR=0.29, CI (0.11-0.79), p=0.015; reproductive female degree: OR=4.26, CI (1.55-11.7), p=0.005; Figure 4.4; Table C5).

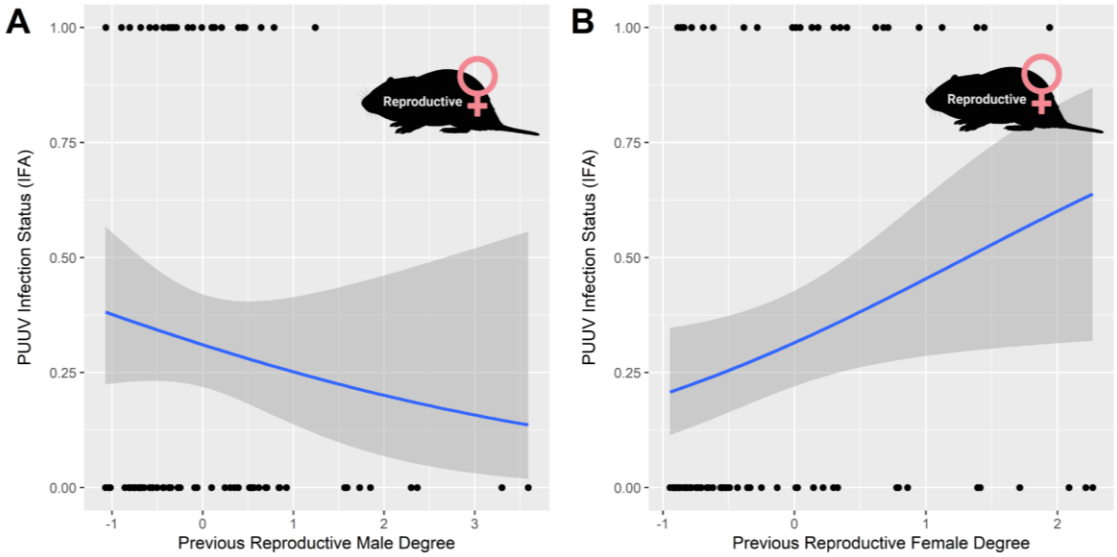

Figure 4.4. *Plots of correlation between current Puumala hantavirus (PUUV) infection status and previous overlap network degree for reproductive female voles in the fed-deworm treatments. Previous degree measured as A) spatial overlap with reproductive male voles and B) spatial overlap with reproductive female voles. Degree measures were scaled and centered.*

Exploratory behavior was negatively correlated with current infection in both the fed-control and fed-deworm treatments, indicating less exploratory animals were more likely to be infected (F-C: OR=0.58, CI (0.37-0.91), p=0.017; F-D: OR=0.63, CI (0.42-0.96), p=0.030). Exploratory behavior was not correlated with current infection in either of the unfed treatments (p>0.35).

4.5 Discussion

Environmental conditions can alter animal space use and spatial overlap in ways that influence pathogen transmission. We experimentally manipulated food abundance and helminth infection in wild bank vole populations and investigated the effects on the relationship between spatial overlap and infection status. Spatial overlap with other voles drove hantavirus infection probability in subsequent months. However, this was only the case for certain groups of focal voles and their overlapping neighbors and food supplementation and helminth removal dictated how spatial overlap drove infection probability. Thus, we show that spatial overlap can predict transmission opportunities for environmentally transmitted pathogens, and we suggest that patterns of spatial overlap by sex and reproductive condition may change as food abundance, helminth infection,

and population density change, resulting in different effects of spatial overlap on pathogen transmission.

The unfed-control treatment can serve as a baseline to establish the relationship between spatial overlap and infection status under unmanipulated conditions. For males in this treatment, space sharing with reproductive voles increased hantavirus infection probability, while space sharing with non-reproductive voles decreased infection probability. This aligns with what we know about Puumala hantavirus in bank vole populations: reproductive animals are more likely to be infected than non-reproductive animals because they have had longer cumulative exposure, are actively interacting with the local population during breeding, and are no longer protected by maternal antibodies (Bernshtein et al., 1999; Voutilainen et al., 2016). Male-biased infection is commonly observed in host-pathogen systems across taxa and is often attributed to greater space use and interactions by males due to mate searching. As such, our study corroborates established epidemiological and ecological knowledge and suggests the importance of adult reproductive individuals as a key demographic driving transmission of pathogens with chronic infection patterns (Clay et al., 2009; Plowright, Manlove, et al., 2017).

In both the unfed-deworm and the fed-control treatments, spatial overlap with non-reproductive (male) voles increased infection probability in (reproductive) female voles (parenthetical traits only significant in the fed-control treatment). Small sample size, particularly of infected animals, in the unfed-deworm treatment may have impacted the estimated magnitude of increase and associated confidence interval, but the model nonetheless indicated significant, positive effects. Our finding that overlap with non-reproductive males increases infection probability challenges the common assumption that reproductive males are key individuals for transmission. While several network studies have pointed to the key role that adult males play in pathogen transmission (e.g., Ferrari et al., 2004; Grear et al., 2009; Perkins et al., 2008), preemptively limiting studies to focus on reproductive animals could result in missing or misidentifying major players in transmission. Our findings indicate that non-reproductive individuals (particularly non-reproductive males) can play an important role in transmission, especially if they are highly mobile via more exploratory behavior or highly social with conspecifics (VanderWaal et al., 2013).

It was surprising that different environmental manipulations: helminth removal alone and food supplementation alone, produced similar relationships between previous

spatial overlap and increased infection probability. We propose that these patterns may be driven by different mechanisms: Food supplementation increased population sizes and the number of young non-reproductive voles. Conversely, helminth removal increased vole space use. Nonetheless, these two treatments had similar effects on the spatial overlap of females and non-reproductive voles, leading to similar transmission outcomes. Even in species like bank voles where reproductive animals are territorial, reproductive individuals may perceive non-reproductive individuals as 'sex-less', making them more tolerant of greater spatial overlap (Henttonen, 2022). When population size or space use increases, this could create more overlap between non-reproductive and reproductive conspecifics (e.g., non-reproductive animals sharing their mother's territory before dispersing or establishing at the edge of her territory, Bujalska, 1990) and could increase transmission. This could, in part, explain the shift in non-reproductive voles becoming more important for transmission, at least to females, under more favorable environmental conditions such as more abundant food and decreased parasitism.

In the fed-deworm treatment, spatial overlap between reproductive females increased infection probability in reproductive females. Food supplementation in rodent populations has been found to increase the number of reproductive females (Mazurkiewicz, 1994) while also decreasing home range size of reproductive females and increasing space use sharing (Desy et al., 1990; Ims, 1987; e.g., Ostfeld, 1986). Together, these effects may increase the frequency of female-female transmission. In territorial species, increased overlap between females could increase the frequency of direct, antagonistic interactions between reproductive females, which may increase transmission. Alternatively, increased density of females under abundant resources may lead to more amicable behavior and space use sharing, potentially as an energetic tradeoff or result of kin structure among females (Johnsen et al., 2019). In either scenario, transmission between females is increased under increased population density, regardless if females behave territorially or amicably toward other females.

Contrary to spatial overlap between reproductive females, spatial overlap with reproductive males decreased the probability of infection in reproductive females. This finding was surprising as male bank voles are commonly found to have higher hantavirus infection prevalence than females (Olsson et al., 2002) and their larger space use observed in our study would suggest greater exposure to pathogens shed in the environment. One potential explanation is that female voles can detect and avoid

infection in conspecifics and minimize their overlap with infected individuals. In mate choice studies, female rodents show aversive responses and avoid odors of males parasitized with ectoparasites (Kavaliers et al., 2003), helminths (Kavaliers et al., 2014), and microparasites (Kavaliers & Colwell, 1995). Alternatively, the different effects of spatial overlap with males and females on infection probability in female voles could be driven by spatial overlap equating to different levels of pathogen exposure. Spatial overlap between males and females may provide opportunities for indirect exposure but limited direct contact, whereas female-female overlap provides more consistent and direct contact between voles (i.e., mating occurs once, but antagonistic interactions between females defending territory are more frequent).

When considering spatial overlap for transmission, it is critical to consider the identities of the two overlapping individuals and how this may shape their behavior - particularly with respect to attraction or avoidance (White et al., 2017). As we observed, overlap with reproductive voles increased the probability of infection in males (unfed-control) but overlap with reproductive males decreased the probability of infection in reproductive females (fed-deworm). When behavior and immunology differ between population subgroups, analyses at the subgroup level will yield conclusions that are biologically meaningful (Henttonen, 2022). Spatial overlap may be informative, but it is essential to consider host biology and its effect on behavior to understand what animals are doing when they overlap, how much they may be interacting, and therefore what the potential transmission routes are.

4.6 Conclusions

Our research provides rare empirical evidence linking viral infection to space use in populations of a wildlife species. We demonstrate the utility of network connections informed by spatial overlap to draw inference about transmission in wild rodent populations. Moreover, we test the relationship between space use and infection status across biologically meaningful variation in food abundance and helminth infection and identify environmental abundance of food and macroparasites as key factors influencing how spatial overlap drives infection status. We find that spatial overlap has different effects on infection probability based on the sex and reproductive condition of overlapping individuals and based on environmental conditions. This is important because it shows that subgroups of the population become more or less important for

transmission when population density or space use change. As such, our research highlights the importance of incorporating and investigating individual-level and environmental heterogeneity into studies of pathogen transmission in wildlife to explain variation in transmission across space and time.

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Conclusions

Environmental factors vary naturally across habitat- and landscape-level scales and are also influenced by human-driven land-use change. Importantly, variation in environmental factors can drive patterns of pathogen spread and persistence in wildlife populations. As key hosts of zoonotic pathogens and a highly adaptable Order that are capable of thriving in degraded, fragmented, and anthropogenic environments, rodents have a great potential to play a major role in maintaining, spreading, and transmitting zoonotic pathogens to humans. Thus, the research presented here helps to explain how environmental factors - including those related to anthropogenic land-use change - affect individual rodent exposure to pathogens, and ultimately the transmission of pathogens within rodent populations. Moreover, this research is timely and important for the improved understanding of the transmission dynamics of infectious diseases in wildlife populations and managing spillover risk from wildlife to human populations.

In this dissertation, I have examined the effects of anthropogenic environmental factors on spatial patterns of pathogen exposure and transmission using wild rodent populations as a model system. In Chapter 1, I identified broad landscape- and habitat-level effects of anthropogenic land-use change that alter the microbiome and increase the prevalence of zoonotic bacterial pathogens in wild rodents. In Chapter 2, I reviewed the use of contact networks for studying transmission in animal populations and outlined important recommendations to ensure that interactions documented in animal populations are biologically relevant to infer transmission of parasites and pathogens. Using these recommendations, in Chapter 3 I quantified the effects of environmental variability in food abundance and helminth infection on patterns of space use and spatial overlap in wild rodent populations, finding that space use is affected but spatial overlap may be more robust to changes. The results of this chapter suggest the role that animal social behavior plays in moderating changes in opportunities for transmission under varying food and macroparasite burdens, a finding that is generalizable to many other wildlife species. Finally, in Chapter 4, I test the ability of spatial overlap to predict transmission of an environmental pathogen, showing that both food abundance and macroparasite infection interact with host traits to influence how spatial overlap predicts infection status. These findings have major implications as they provide empirical evidence for the role of environmental heterogeneity in shaping transmission in wildlife populations. Beyond advancing our theoretical knowledge, this research also contributes

to future management of infectious disease in wildlife populations by showing that the most important population subgroups for limiting transmission are dependent on specific environmental factors. In this way, targeted control efforts must incorporate an understanding of host contact behavior and how the abundance of food or macroparasite infection may alter these interactions.

Together, these chapters have contributed much-needed empirical evidence that shows how environmental factors such as food and macroparasite abundance alter zoonotic pathogen prevalence and transmission in wildlife populations and suggests the role that anthropogenic land development may play in wildlife disease dynamics. My work has also demonstrated that spatial overlap defined from capture-mark-recapture data, carefully aligned with relevant biological interactions, can provide valuable insight into transmission dynamics in wildlife populations. This research therefore represents an important step forward in our ability to quantify the effects of environmental factors (such as those influenced by anthropogenic forces) on disease dynamics across wildlife-pathogen systems, advancing our ability to understand, predict, and control transmission and spillover potential from wildlife into human populations.

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Appendix A. Supplemental Information for Chapter 1

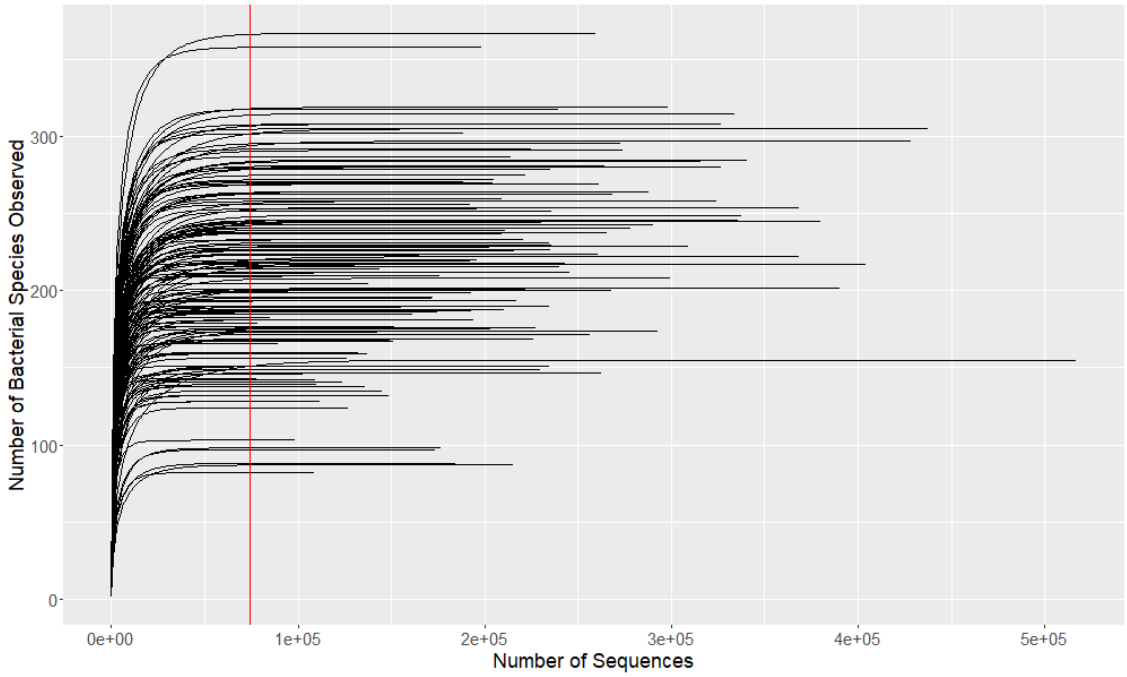

Figure A1. *Rarefaction curves for all mouse fecal samples (n=160). The red vertical line indicates minimum sample size (74,517 reads) to which all samples (n=140) were rarefied for alpha and beta diversity analyses. The asymptotic nature of the curves suggests reasonable sequencing depth was achieved at the rarefied sample size.*

Table A1. *Linear regression (left column) and beta regression models (right column) comparing alpha diversity indices between landscape-habitat pairings. Bolded values indicate significance.*

Linear Regression Model

Richness

Variable	b	SE	p
(Intercept)	207.301	20.848	<0.001
Landscape (Undeveloped)	-73.44	12.659	**<0.001**
Habitat (Synanthropic)	-9.724	12.231	0.428
Sex (Male)	7.457	10.159	0.464
Reproductive (Breeding)	-18.312	10.397	0.081
Body Mass	1.724	0.859	**0.047**
Month (June)	6.430	20.201	0.751
Month (August)	-21.534	14.229	0.133
Landscape:Habitat Type (Undeveloped:Synanthropic)	66.396	16.944	**<0.001**

Shannon

Variable	b	SE	p
(Intercept)	2.866	0.328	<0.001
Landscape (Undeveloped)	-0.628	0.199	**0.002**
Habitat (Synanthropic)	0.019	0.192	0.921
Sex (Male)	-0.151	0.160	0.346
Reproductive (Breeding)	-0.449	0.163	**0.007**
Body Mass	0.022	0.013	0.098
Month (June)	-0.045	0.317	0.887
Month (August)	0.170	0.224	0.448
Landscape:Habitat Type (Undeveloped:Synanthropic)	0.545	0.266	**0.043**

Beta Regression Model

Simpson

Variable	b	SE	p
(Intercept)	1.464	0.339	<0.001
Landscape (Undeveloped)	-0.539	0.201	**0.007**
Habitat (Synanthropic)	-0.092	0.201	0.647
Sex (Male)	-0.218	0.162	0.177
Reproductive (Breeding)	-0.437	0.167	**0.009**
Body Mass	0.020	0.014	0.160
Month (June)	-0.067	0.326	0.838
Month (August)	0.166	0.238	0.484
Landscape:Habitat Type (Undeveloped:Synanthropic)	0.565	0.272	**0.038**

Evenness

Variable	b	SE	p
(Intercept)	0.142	0.216	0.513
Landscape (Undeveloped)	-0.317	0.131	**0.016**
Habitat (Synanthropic)	0.011	0.127	0.930
Sex (Male)	-0.160	0.105	0.129
Reproductive (Breeding)	-0.336	0.108	**0.002**
Body Mass	0.015	0.009	0.100
Month (June)	-0.072	0.209	0.731
Month (August)	0.200	0.149	0.179
Landscape:Habitat Type (Undeveloped:Synanthropic)	0.301	0.176	0.087

Table A2. *Alpha diversity (observed species richness, Shannon diversity, Simpson diversity, species evenness) for all unique mouse fecal samples (n=140) in the anthropogenic and undeveloped landscapes and in forest and synanthropic habitat. Indices were calculated per sample and the values reported represent the mean and standard deviation for a given landscape-habitat pairing. Individual sampling months in the anthropogenic landscape are shaded in gray, mean values across all three months are shown in bold.*

Landscape	Habitat	Month	N	Observed Richness [a]	Shannon Diversity [b]	Simpson Diversity [c]	Evenness [d]
Agricultural	*Forest*	*August*	*10*	*237.3 ± 54.25*	*3.26 ± 0.63*	*0.87 ± 0.11*	*0.6 ± 0.1*
Agricultural	*Forest*	*July*	*27*	*237.89 ± 45.88*	*3.07 ± 0.61*	*0.85 ± 0.11*	*0.56 ± 0.1*
Agricultural	*Forest*	*June*	*3*	*183.67 ± 102.84*	*2.02 ± 1.68*	*0.57 ± 0.33*	*0.38 ± 0.28*
Agricultural	**Forest**	**Summer**	**40**	**233.68 ± 53.23**	**3.04 ± 0.76**	**0.83 ± 0.15**	**0.56 ± 0.13**
Agricultural	*Synanthropic*	*August*	*7*	*175.57 ± 26.18*	*3.06 ± 0.71*	*0.82 ± 0.15*	*0.59 ± 0.12*
Agricultural	*Synanthropic*	*July*	*18*	*234.56 ± 52.76*	*2.96 ± 1.11*	*0.77 ± 0.27*	*0.54 ± 0.19*
Agricultural	*Synanthropic*	*June*	*4*	*274.75 ± 25.08*	*3.62 ± 0.45*	*0.91 ± 0.06*	*0.64 ± 0.08*
Agricultural	**Synanthropic**	**Summer**	**29**	**225.86 ± 54.1**	**3.08 ± 0.97**	**0.8 ± 0.23**	**0.57 ± 0.17**
Undeveloped	**Forest**	**July**	**31**	**162.61 ± 36.35**	**2.35 ± 0.71**	**0.72 ± 0.16**	**0.46 ± 0.12**
Undeveloped	**Synanthropic**	**July**	**40**	**217.52 ± 54.49**	**2.92 ± 0.72**	**0.82 ± 0.1**	**0.54 ± 0.12**

[a] Estimated number of observed species as identified by the expectation-maximization algorithm (Emu)

[b] Shannon diversity index gives equal weight to common and rare species

[c] Simpson diversity index gives higher weight to common species when calculating diversity

[d] Evenness measures the relative abundance of different taxa (Evenness = Shannon / Richness)

Table A3. *PERMANOVA statistical results for Bray-Curtis dissimilarity indices of microbiome community composition analyzed by landscape, habitat type, mouse sex, reproductive status, body mass, and sampling month using the 'adonis2' function in the 'vegan' R package.*

Variable	Df	SumOfSqs	R2	F	Pr(>F)
Landscape	1	1.1449	0.0450	6.9904	0.001
Habitat	1	0.9127	0.0359	5.5731	0.001
Sex	1	0.2261	0.0089	1.3804	0.184
Reproductive Status	1	0.3810	0.0150	2.3265	0.035
Body Mass	1	0.5527	0.0217	3.3749	0.006
Sampling Month	2	0.5611	0.0221	1.7129	0.055
Residual	132	21.6183	0.8500	NA	NA
Total	139	25.4344	1.0000	NA	NA

Appendix B. Supplemental Information for Chapter 3

B.1 Individual spatial overlap - Additional metrics

Methods

We explored individual spatial overlap within the vole populations using measures of weighted degree, unweighted degree, and normalized unweighted degree, calculated for each vole (**Table B1**).

Table B1. *Description of additional network metrics and their biological relevance*

Network Metric		Network Definition	Biological Relevance
NODE LEVEL	Unweighted degree	Number of edges connected to a focal node	Number of unique neighbor voles with whom a focal vole overlapped
	Normalized unweighted degree	Number of unweighted edges connected to a focal node divided by the number of nodes in the network less one; for comparison between networks of different sizes	Proportion of the voles in the observed population with whom a focal vole overlapped

Weighted degree (presented in the main text) was chosen to quantify the amount of overlap a focal vole had with its neighbors as a measure of cumulative opportunities for direct interactions or indirect exposure to a shared environment.

However, weighted degree does not capture the number of unique individuals a focal vole overlaps with, which could be important for transmission. We therefore also calculated unweighted degree (**Table B1**). For unweighted degree, thresholds of weighted degree (below which voles were considered not to meaningfully overlap) of 0.05, 0.01, 0.005, and 0.001 were investigated and the least restrictive threshold that resulted in voles with unweighted degree values of 0 was used to calculate unweighted degree (**Figure B1**).

Population density was highly variable between treatments which would increase the maximum possible unweighted degree for an individual in a large population compared to a small population. To better compare the number of unique overlaps across populations of varying size, we also calculated unweighted degree, normalized

by the population size. This measure therefore represents the proportion of the population with whom a focal vole overlapped in space.

Results

Thresholds of weighted degree (weighted degree >0.05, >0.01, >0.005, >0.001) were investigated to choose an appropriate minimum value to constitute an overlap for unweighted degree. The threshold at 0.01 was chosen as a moderately restrictive threshold that resulted in some of the weakest overlaps having an unweighted degree of zero while still maintaining heterogeneity in the distribution of unweighted degree values (**Figure B1**).

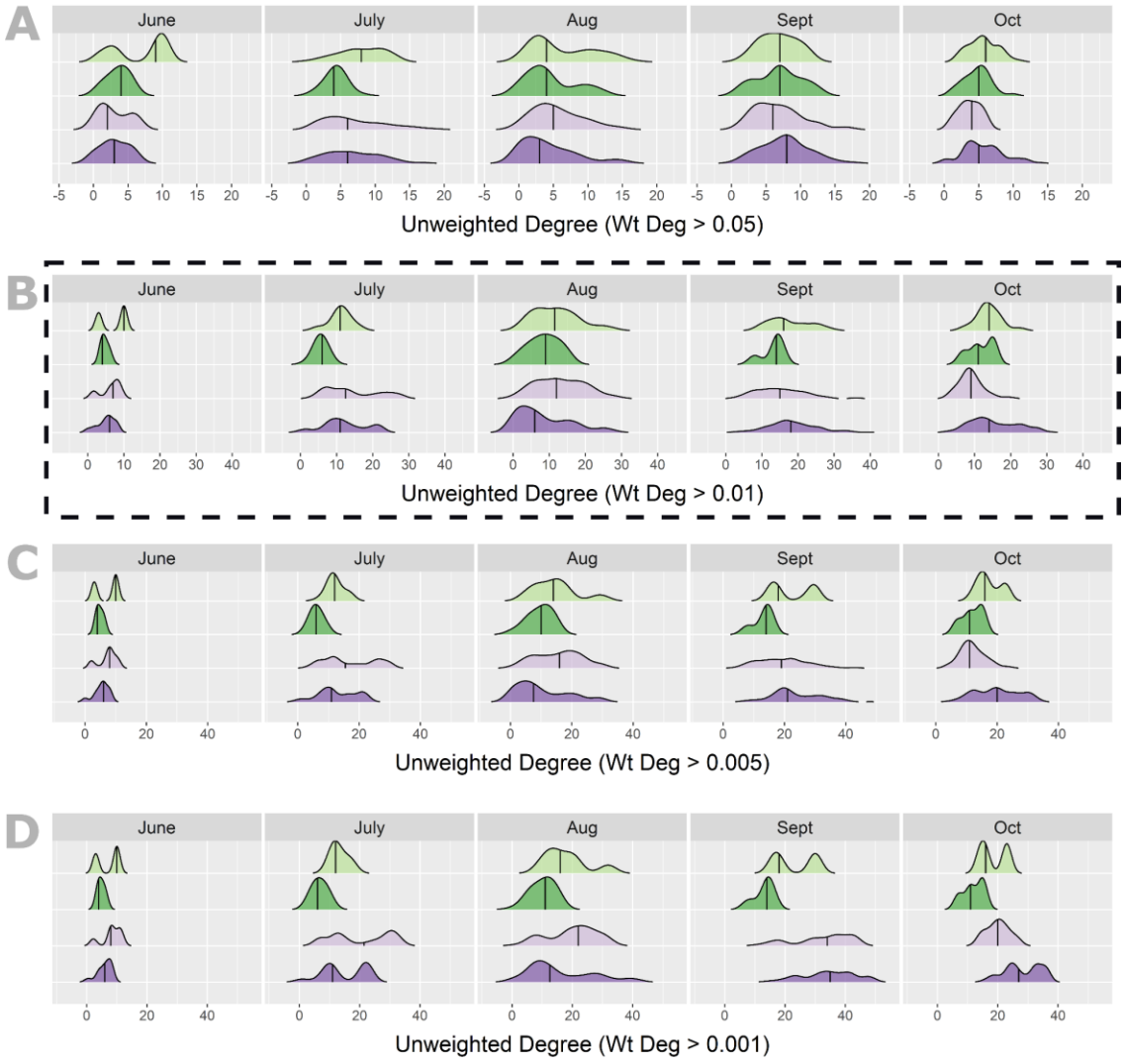

Figure B1. *Sensitivity analysis of unweighted degree. Density distributions of unweighted degree with varying thresholds of weighted degree to constitute an overlap (A) weighted degree >0.05, (B) weighted degree >0.01, (C) weighted degree >0.005, and (D) weighted degree >0.001. Dotted line indicates the threshold used for analysis in the main text (weighted degree >0.01).*

Distributions of unweighted degree (the number of unique neighbor voles with whom a focal vole overlapped, using a threshold of >0.01 to define an overlap) were generally wide and fairly similar between the unfed-control, fed-control, and fed-deworm treatments in a given month in summer and autumn (**Figure B2C**). Unweighted degree in the unfed-deworm tended to have a more narrow distribution in most months. Like weighted degree, mean unweighted degree was similar across all four treatments in a given month.

Unweighted degree normalized by network size (i.e., the proportion of the population with whom a focal vole overlapped) was similar across treatments in June-August and showed wide distributions where voles overlapped with anywhere from 0-100% of the observed population. However, normalized unweighted degree was most different between treatments in autumn (**Figure B2D**). In September and October, on average, voles in the unfed treatments overlapped with a majority to nearly all of observed population at their site (U-C: mean normalized unweighted degree 0.765 ± standard deviation 0.154; U-D: 0.990 ± 0.032) while voles in the fed treatments overlapped with less than half of the observed voles at their site (FC 0.381 ± 0.140; FD 0.470 ± 0.176).

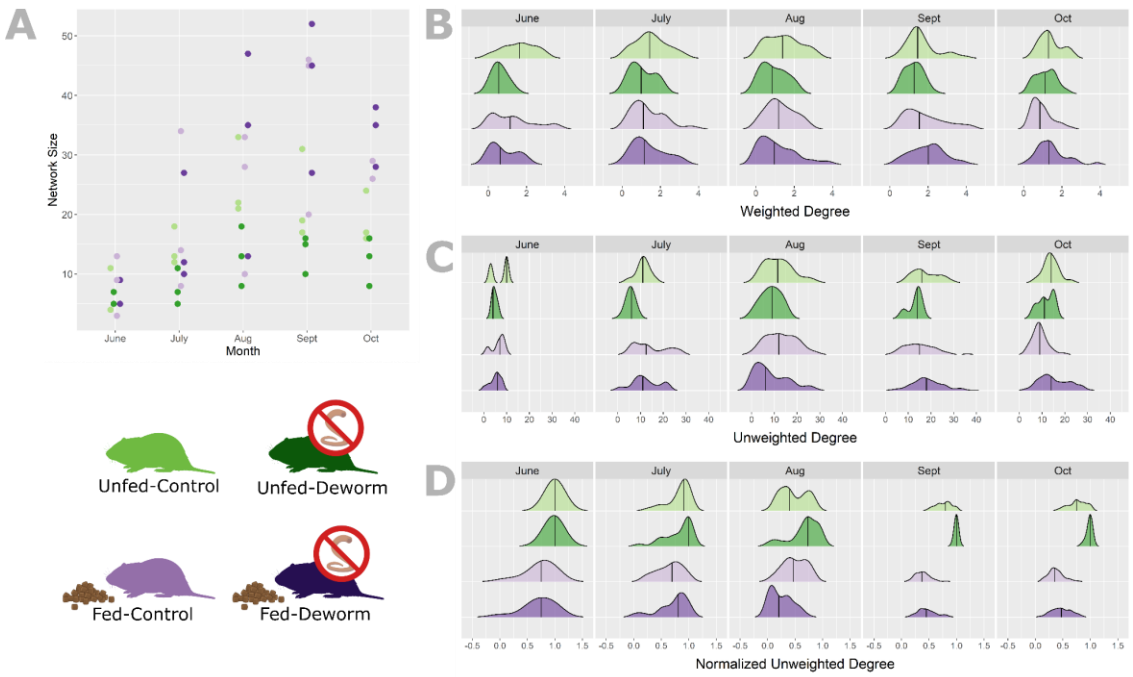

Figure B2. *Network size and distributions of individual vole spatial overlap. (A) Network size (number of voles) by month. One data point shown per replicate study site (as shown in main text). Density distributions by month and treatment of three metrics quantifying individual spatial overlap (B) weighted degree (spatial overlap per vole, weighted by the amount of overlap with each of its neighbors; as shown in main text), (C) unweighted degree (number of unique neighbors each focal vole overlapped with where weighted degree ≥0.01), and (D) unweighted degree normalized by network size.*

Discussion

The number of voles a focal vole overlapped with was highly variable among individuals, even within a treatment. Counterintuitively, in September when populations were largest, fed treatments had more voles that overlapped with very few neighbors (0-5 other voles) whereas this was not observed in the unfed treatments. High resource abundance clearly increased vole population density, increasing the potential maximum number of overlaps, but abundant resources may also enable voles to maintain sufficiently small territories and limit their overlap to only a few other animals (Ims, 1987). Overall, this created more heterogeneity in the number of voles a focal vole overlapped with in the larger populations of fed treatments. This increased heterogeneity, along with differences in population size between treatments, influenced the proportion of the observed population voles overlapped with. In unfed treatments in autumn, voles on average overlapped with 75-99% of the observed population, compared to 38-47% of the population in fed treatments where populations were much larger. This would further suggest that voles change their space use to maintain a similar absolute amount of spatial overlap, and not a similar proportion of neighbors overlapped with, when population density increases. While contact rates in populations are assumed to be either density-dependent or frequency dependent, population size and interactions between animals may not always scale, particularly if there are social behaviors limiting contacts (Cross et al., 2009). Our findings align with those of previous research in vole systems which indicate that contact-based transmission rates are a saturating function of host density (Smith et al., 2009), potentially indicating that contact rates are not as dichotomous as they seem. This is an exciting area of research which could benefit from future experimental studies to empirically test relationships between host density and contact patterns.

B.2 Network size

Methods

To understand how network size was affected by treatment and to contextualize potential confounding factors influencing degree measures, we investigated the factors affecting network size using a linear mixed-effects model fit by restricted maximum likelihood (REML) using the ‘lme4’ package (Bates et al., 2015). We modeled the main effects of food supplementation (unfed/fed), helminth removal (control/deworm), and

month. Site was included as a random effect to account for multiple measures at each site and variation between replicate sites within a treatment. Network size was log-transformed to normalize distribution of the data.

Results

Network size was moderately affected by food treatment (β=0.48, p=0.022) and by month (β=0.30, p<0.001; **Table B2**) with fed treatments and months later in the year having larger networks. Deworming treatment had no effect on network size (β=-0.17, p=0.40; **Table B2**).

Table B2. *Summary of linear mixed-effects model predicting log-transformed values of network size by food supplementation, helminth removal, and month. Site is included as a random effect in all models (n = 12 sites, total observations = 60).*

	log(Network Size)			
Predictors	*Estimate*	*Std. Error*	*CI*	*p*
(Intercept)	1.96	0.19	1.58-2.35	**<0.001**
Food Addition	0.48	0.20	0.07-0.89	**0.022**
Helminth Removal	-0.17	0.20	-0.59-0.24	0.398
Month	0.30	0.04	0.22-0.38	**<0.001**
Random Effects				
σ^2	0.18			
$\tau_{00\ Site}$	0.09			
ICC_{Site}	0.34			
N_{Site}	12			
Observations	60			
Marginal R^2 / Conditional R^2	0.482 / 0.659			

ICC = Intra-class correlation coefficient.

B.3 Space use

Table B3. *Model summary for the generalized linear model describing how the probability of capturing a vole varies with the natural logarithm of distance from its seasonal centroid, indicating vole space use, in the summer breeding season (June-August).*

Parameter	OR †	95% CI ‡	p-value
Log Distance	0	0.00, 0.00	**<0.001**
Sex			
Female			
Male	0.21	0.12, 0.37	**<0.001**
Reproductive Status (seasonal)			
Reproductive			
Non-Reproductive	0	0.00, 0.00	**<0.001**
Food Treatment			
Unfed			
Fed	1.84	0.91, 3.80	0.093
Helminth Treatment			
Control			
Deworm	1.24	0.58, 2.68	0.58
Food Trt * Helm Trt			
*Fed * Deworm*	0.57	0.20, 1.65	0.3
Log Dist. * Sex			
*Log Dist. * Male*	4.59	2.70, 8.03	**<0.001**
Log Dist. * Reproductive Status			
*Log Dist. * Non-Reproductive*	13,447	1,922, 121,475	**<0.001**
Log Dist. * Food Treatment			
*Log Dist. * Fed*	0.31	0.16, 0.58	**<0.001**
Log Dist. * Helminth Treatment			
*Log Dist. * Deworm*	0.65	0.33, 1.22	0.18
Log Dist. * Food Trt * Helm Trt			
*Log Dist. * Fed * Deworm*	2.57	1.00, 6.74	0.053

† OR - Odds Ratio ‡ CI - Confidence Interval

In summer, females were more likely to be captured within one trap of their centroid while males were more likely to be captured at greater distances (**Figure B3A**). Non-reproductive voles were very likely to be captured only in one trap while reproductive voles were captured over a greater distance (**Figure B3B**). Voles in unfed treatments were more likely to be captured 1-2 traps away from their centroid compared to voles in fed treatments (**Figure B3C**). Voles in deworm and control helminth treatments were equally likely to be captured at all distances from their centroid (**Figure B3D**)

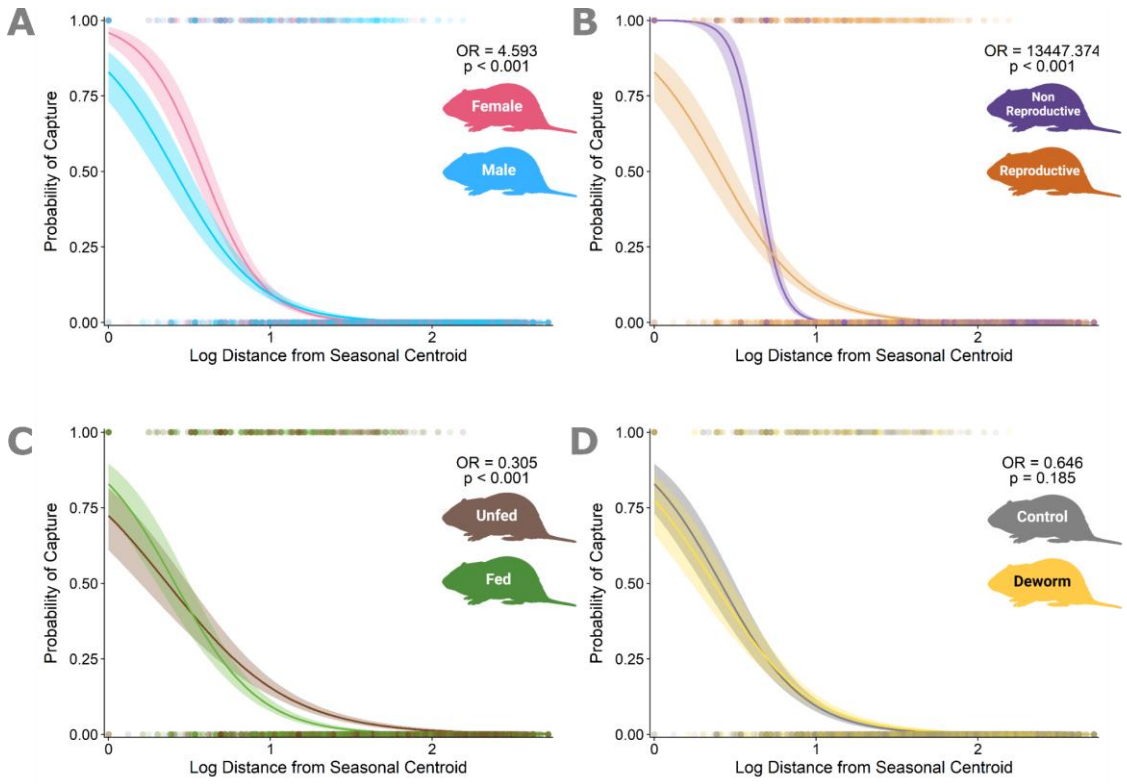

Figure B3. *Subgroup-specific curves describing the change in capture probability with increasing distance from the seasonal centroid in the summer breeding season (June-August). Lines represent the fitted generalized linear model (GLM) for summer space use partitioned by each of the interaction terms in the model to show how the effect of distance on capture probability varies by (A) Sex: male vs. female bank voles, (B) Reproductive status: reproductive vs. non-reproductive bank voles, (C) Food treatment: voles in fed vs. unfed treatments, and (D) Helminth treatment: voles in deworm vs. control helminth infection treatments. Points show the raw data (whether a vole was captured at a given distance, 1, or not, 0). Distances are measured in trapping grid cells (1 grid cell=10 m). Odds ratios (OR) and p-values are reported for the interaction effect of each variable with the natural log of distance as modeled by the summer space use GLM (see* ***Table B3****).*

Table B4. *Model summary for the generalized linear model describing how probability of capturing a vole varies with the natural logarithm of distance from its seasonal centroid, indicating vole space use, in the autumn non-breeding season (September-October).*

Parameter	OR †	95% CI ‡	p-value
Log Distance	0	0.00, 0.01	**<0.001**
Sex			
Female			
Male	0.75	0.42, 1.34	0.34
Reproductive Status (seasonal)			
Reproductive			
Non-Reproductive	0.61	0.33, 1.11	0.1
Food Treatment			
Unfed			
Fed	2.15	0.91, 5.11	0.080
Helminth Treatment			
Control			
Deworm	0.43	0.19, 0.97	**0.043**
Food Trt * Helm Trt			
*Fed * Deworm*	1.91	0.58, 6.20	0.28
Log Dist. * Sex			
*Log Dist. * Male*	1.13	0.64, 2.02	0.68
Log Dist. * Reproductive Status			
*Log Dist. * Reproductive*	2.46	1.37, 4.38	**0.002**
Log Dist. * Food Treatment			
*Log Dist. * Fed*	0.24	0.10, 0.57	**0.001**
Log Dist. * Helminth Treatment			
*Log Dist. * Deworm*	2.61	1.23, 5.54	**0.012**
Log Dist. * Food Trt * Helm Trt			
*Log Dist. * Fed * Deworm*	0.55	0.17, 1.81	0.33

† OR - Odds Ratio ‡ CI - Confidence Interval

In autumn, males and females were equally likely to be captured at a given distance from their seasonal centroid (**Figure B4A**). Non-reproductive and reproductive voles were equally likely to be captured within a trap distance from their centroid but reproductive voles were more likely to be captured further than one trap (**Figure B4B**). Voles in the fed and unfed treatments were also equally likely to be captured less than one trap from their centroid, but voles in the unfed treatments were more likely than fed treatment voles to be captured at least one trap from their centroid (**Figure B4C**). The difference was subtle between voles in the control and deworm treatments, but dewormed voles were more likely to be captured more than one trap from their seasonal centroid, compared to voles with unmanipulated helminth infections (**Figure B4D**).

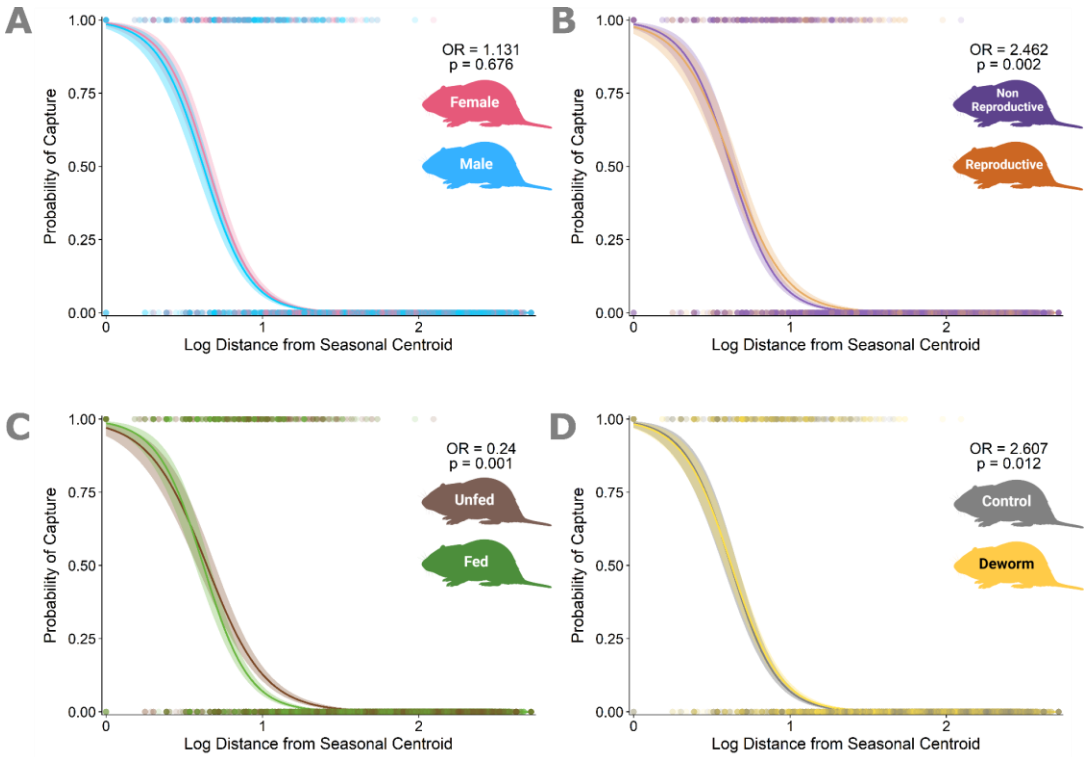

Figure B4. *Subgroup-specific curves describing the change in capture probability with increasing distance from the seasonal centroid in the autumn non-breeding season (September-October). Lines represent the fitted generalized linear model (GLM) for autumn space use partitioned by each of the interaction terms in the model to show how the effect of distance on capture probability varies by (A) Sex: male vs. female bank voles, (B) Reproductive status: reproductive vs. non-reproductive bank voles, (C) Food treatment: voles in fed vs. unfed treatments, and D) Helminth treatment: voles in deworm vs. control helminth infection treatments. Points show the raw data (whether a vole was captured at a given distance, 1, or not, 0). Distances are measured in trapping grid cells (1 grid cell=10 m). Odds ratios (OR) and p-values are reported for the interaction effect of each variable with the natural log of distance as modeled by the autumn space use GLM (see* ***Table B4****).*

Appendix C. Supplemental Information for Chapter 4

Table C1. *Candidate models fit by treatment. Green boxes marked with an 'X' indicate the models (columns) that were fitted for each treatment (rows). Twelve candidate models were considered: Four degree measures: weighted degree, weighted degree by sex; weighted degree by reproductive condition ("repro"); weighted degree by functional group ("fxnl grp"; combination of sex, reproductive condition) were used as explanatory variables in separate models. For each degree measure, three separate models were fitted with interaction effects by sex, reproductive condition, and both sex and reproductive condition, respectively.*

	Weighted Degree			Wt. Degree by Sex			Wt. Degree by Repro			Wt. Degree by Fxnl Grp		
	:Sex	:Repro	:Sex:Repro	:Sex	:Repro	:Sex:Repro	:Sex	:Repro	:Sex:Repro	:Sex	:Repro	:Sex:Repro
Unfed-Control	X			X			X			X		
Unfed-Deworm	X	X	X	X	X	X	X	X	X	X	X	
Fed-Control	X	X	X	X	X	X	X		X	X		X
Fed-Deworm	X	X	X	X	X	X	X	X	X	X	X	X

Table C2. *Model summary for generalized linear mixed-effects model predicting hantavirus infection status for voles in the unfed-control treatment.*

Variable	OR	95% CI	p-value
Sex			
Female			
Male	0.4	0.04, 4.02	0.43
Exploratoriness	1.26	0.75, 2.14	0.38
Previous Month	0.67	0.31, 1.47	0.32
Previous Network Size	1.51	0.32, 7.00	0.6
Year			
2021			
2022	1.07	0.39, 2.95	0.9
Repro Degree * Sex			
*Repro Degree * Female*	0.85	0.29, 2.51	0.77
*Repro Degree * Male*	1.73	1.07, 2.81	**0.026**
Non-Repro Degree * Sex			
*Non-Repro Degree * Female*	0.43	0.05, 3.56	0.43
*Non-Repro Degree * Male*	0.05	0.00, 1.41	**0.079**

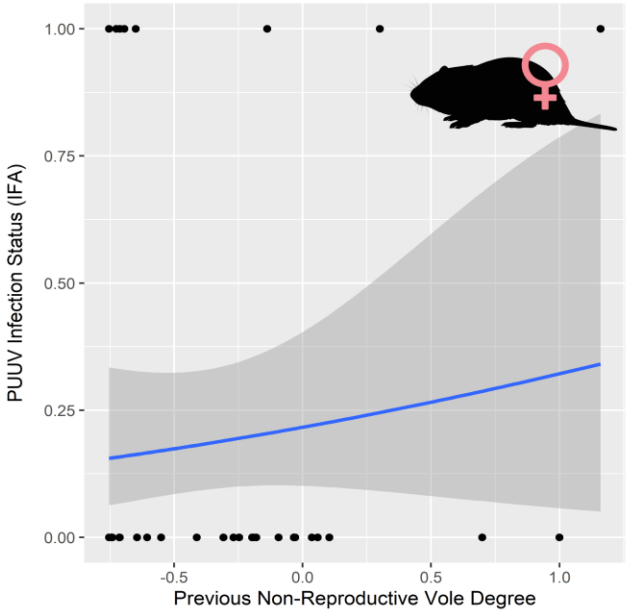

Figure C1. *Plot of correlation between current Puumala hantavirus (PUUV) infection status and previous overlap network degree for female voles in the unfed-deworm treatments. Previous degree measured as spatial overlap with non-reproductive voles. Degree measures were scaled and centered.*

Table C3. *Model summary for generalized linear mixed-effects model predicting hantavirus infection status for voles in the unfed-deworm treatment.*

Variable	OR	95% CI	p-value
Sex			
Female			
Male	1.06	0.17, 6.79	0.95
Reproductive Condition (seasonal)			
Reproductive			
Non-Reproductive	0.69	0.12, 4.07	0.68
Exploratoriness	1.04	0.53, 2.04	0.9
Previous Month	0.31	0.13, 0.78	**0.012**
Previous Network Size	0.41	0.02, 8.87	0.57
Year			
2021			
2022	0.3	0.08, 1.11	0.071
Repro Degree * Sex			
*Repro Degree * Female*	0.82	0.12, 5.72	0.84
*Repro Degree * Male*	1.6	0.38, 6.79	0.52
Non-Repro Degree * Sex			
*Non-Repro Degree * Female*	20.5	1.70, 248	**0.017**
*Non-Repro Degree * Male*	1.89	0.37, 9.64	0.44

Table C4. *Model summary for generalized linear mixed-effects model predicting hantavirus infection status for voles in the fed-control treatment.*

Variable	OR	95% CI	p-value
Sex			
Female			
Male	2.06	0.59, 7.20	0.26
Reproductive Condition (seasonal)			
Reproductive			
Non-Reproductive	0	0.00, 0.20	**0.009**
Exploratoriness	0.58	0.37, 0.91	**0.017**
Previous Month			
June			
July	1.31	0.20, 8.58	0.78
August	0.9	0.12, 6.54	0.92
September	0.18	0.02, 2.01	0.16
Previous Network Size	1.26	0.53, 2.97	0.6
Year			
2021			
2022	0.04	0.01, 0.15	**<0.001**
Repro Male Degree * ReproCond * Sex			
*R M Degree * Repro * Female*	0.72	0.25, 2.09	0.55
*R M Degree * Repro * Male*	1.65	0.86, 3.16	0.13
*R M Degree * Non-Repro * Female*	4.24	0.42, 42.8	0.22
*R M Degree * Non-Repro * Male*	0.02	0.00, 5.95	0.18
Non-Repro Male Degree * ReproCond * Sex			
*N-R M Degree * Repro * Female*	2.85	1.14, 7.15	**0.025**
*N-R M Degree * Repro * Male*	0.93	0.27, 3.17	0.9
*N-R M Degree * Non-Repro * Female*	2.96	0.39, 22.4	0.29
*N-R M Degree * Non-Repro * Male*	5,005	0.61, 40,943,420	0.064
Repro Female Degree * ReproCond * Sex			
R F Degree * Repro * Female	0.73	0.32, 1.65	0.45
R F Degree * Repro * Male	1.22	0.63, 2.36	0.55
R F Degree * Non-Repro * Female	0	0.00, 0.10	**0.010**
R F Degree * Non-Repro * Male	0	0.00, 0.25	**0.019**
Non-Repro Female Degree * ReproCond * Sex			
*N-R F Degree * Repro * Female*	0.85	0.41, 1.79	0.68
*N-R F Degree * Repro * Male*	0.55	0.19, 1.60	0.27
*N-R F Degree * Non-Repro * Female*	0.36	0.08, 1.58	0.18
*N-R F Degree * Non-Repro * Male*	7.49	0.56, 101	0.13

C.1 Results and Discussion

Effects of spatial overlap with reproductive females on infection status in non-reproductive voles

In the fed-control treatment, non-reproductive male and female voles with previous high spatial overlap with female breeders were less likely to be currently infected (Males: OR=0.0, CI (0.0-0.01), p=0.01; Females: OR=0.0, CI (0-0.25), p=0.019; Figure C2). However, the sample size of infected non-reproductive voles was very low (males n=2; females n=4) limiting our ability to draw robust conclusions from these findings.

Young bank voles may remain in their mother's territory before dispersing to establish their own territory (Bujalska, 1990). Our finding that increased overlap with reproductive females may decrease infection probability in non-reproductive individuals could indicate a protective effect of remaining in natal territory. Non-reproductive voles with high spatial overlap with reproductive females may not explore widely themselves, limiting their exposure to environmental pathogens.

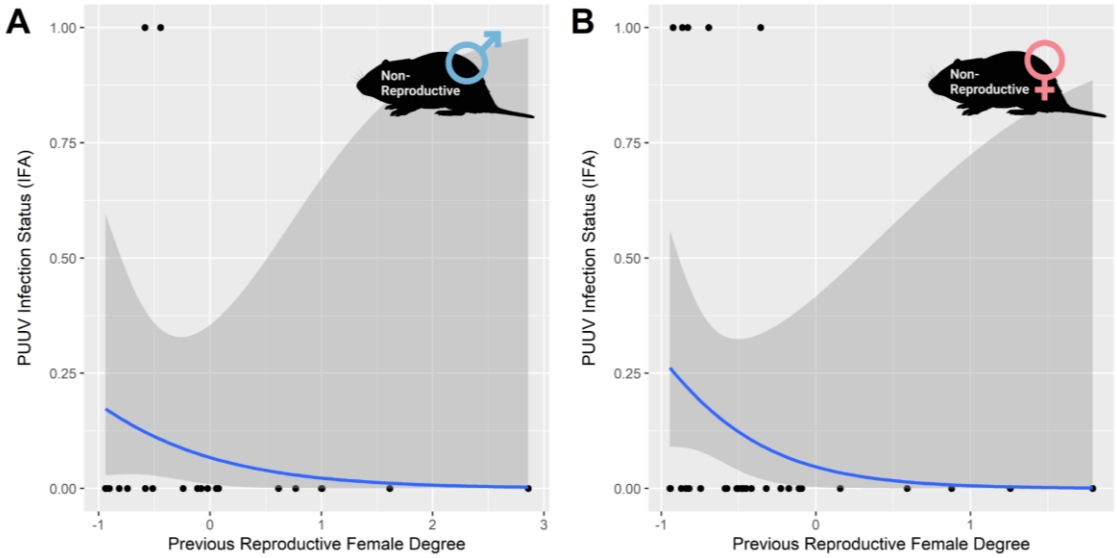

Figure C2. *Effect of previous reproductive female degree on current Puumala hantavirus (PUUV) infection status in non-reproductive bank voles in the unfed-control treatment. A) Effect of overlap with reproductive females on infection status in non-reproductive male voles. B) Effect of overlap with reproductive females on infection status in non-reproductive female voles. Degree measures were scaled and centered.*

Table C5. *Model summary for generalized linear mixed-effects model predicting hantavirus infection status for voles in the fed-deworm treatment.*

Characteristic	OR	95% CI	p-value
Sex			
Female			
Male	3.97	1.48, 10.6	**0.006**
Reproductive Condition (seasonal)			
Reproductive			
Non-Reproductive	0.01	0.00, 0.73	**0.036**
Exploratoriness	0.63	0.42, 0.96	**0.030**
Previous Month	1.59	0.79, 3.19	0.2
Previous Network Size	0.43	0.17, 1.11	0.082
Year			
2021			
2022	3.22	1.04, 9.93	**0.042**
Repro Male Degree * ReproCond * Sex			
*R M Degree * Repro * Female*	0.29	0.11, 0.79	**0.015**
*R M Degree * Repro * Male*	1.63	0.89, 3.00	0.11
*R M Degree * Non-Repro * Female*	4.49	0.09, 213	0.45
*R M Degree * Non-Repro * Male*	0.03	0.00, 3.83	0.16
Non-Repro Male Degree * ReproCond * Sex			
*N-R M Degree * Repro * Female*	1.78	0.76, 4.14	0.18
*N-R M Degree * Repro * Male*	0.76	0.40, 1.45	0.41
*N-R M Degree * Non-Repro * Female*	0.09	0.00, 39.8	0.44
*N-R M Degree * Non-Repro * Male*	1.62	0.73, 3.60	0.24
Repro Female Degree * ReproCond * Sex			
R F Degree * Repro * Female	4.26	1.55, 11.7	**0.005**
R F Degree * Repro * Male	0.78	0.45, 1.34	0.37
R F Degree * Non-Repro * Female	5.16	0.03, 956	0.54
R F Degree * Non-Repro * Male	1.56	0.37, 6.62	0.54
Non-Repro Female Degree * ReproCond * Sex			
*N-R F Degree * Repro * Female*	1.03	0.30, 3.58	0.96
*N-R F Degree * Repro * Male*	0.74	0.33, 1.65	0.46
*N-R F Degree * Non-Repro * Female*	0	0.00, 57.8	0.18
*N-R F Degree * Non-Repro * Male*	1.07	0.19, 5.94	0.94