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Relative Roles of Host Exposure and Parasite Establishment in Producing Patterns of Infection Using Eptesicus Fuscus (Chiroptera: Vespertilionidae) and Its Helminths as a Model System

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RELATIVE ROLES OF HOST EXPOSURE AND PARASITE ESTABLISHMENT IN PRODUCING PATTERNS OF INFECTION USING *EPTESICUS FUSCUS* (CHIROPTERA:VESPERTILIONIDAE) AND ITS HELMINTHS AS A MODEL SYSTEM

by

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Parasite abundance and community structure could vary depending on conditions that are both extrinsic and intrinsic to the host. Variation in parasite burden, resulting from heterogeneous exposure to parasites or heterogeneous establishment of parasites in the host, often drives transmission dynamics. Changes in parasite community structure influence parasite species diversity and thus, diversity of infection within a single host or entire host population. I sought to quantify relative roles of exposure and establishment in producing patterns of infection using bats, *Eptesicus fuscus*, and their helminths. I utilized subsets of data from bats captured in 16 colonies from three states to answer: 1) Are differences in helminth communities among host populations influenced by variation in environmental characteristics or by geographical distance alone? 2) Do male and female bats use different strategies to resist or tolerate helminths? and 3) Is variation in parasite burden among hosts best explained by ecological factors that determine exposure to infective stages or by intrinsic factors that promote parasite establishment within hosts? To accomplish this we recorded sex, age, date, location, body condition, and helminth burden for each bat. I performed three immune assays to determine immunocompetence and assessed host genetic heterozygosity at 11 neutral microsatellite

markers. Using a variance partitioning approach, I found that helminth communities did not differ based on geographical distance; rather community composition was significantly associated with anthropogenic disturbance. Thus, human land use could drive significant patterns of parasite community dissimilarity, most likely by changing the presence or abundance of intermediate hosts. Using generalized linear models, I found that individual bats invest in different facets of immunity based on body condition and relative costs of parasite resistance. Thus, host condition regulates trade-offs between self-maintenance and immunity, not sex. Using structural equation modeling, I found that both exposure and establishment play significant roles in creating heterogeneous helminth burdens. By uncovering associations between extrinsic conditions, intrinsic variation, helminth abundance, and parasite community structure I discovered that host-parasite relationships are highly contextual. Thus, we cannot take one-size-fits-all approaches to transmission dynamics and must carefully consider host and worm when predicting helminth burdens or diversity of infection.

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CHAPTER I

ENVIRONMENTAL CONDITIONS SIGNIFICANTLY IMPACT PATTERNS OF PARASITE COMMUNITY DISSIMILARITY IN BIG BROWN BATS (*EPTESICUS FUSCUS*)

Introduction

Species composition, diversity, and richness of ecological communities vary though time and space and reflect the combined influences of ecological and evolutionary processes (Buckley and Jetz 2008). One of the most pervasive macroecological patterns is distance decay of dissimilarity in species composition among ecological communities, where spatially close communities are often more similar to one another than to communities that are spatially distant (Nekola and White 1999). This pattern has been described for a variety of organisms, from microbes to vertebrates, across numerous habitats and ecosystems (see review in Soininen et al. 2007). However, the rate at which community dissimilarity changes across space and time may vary substantially among taxa, environments, or both, and describing and understanding the underlying causes of such variation is key to our understanding of the mechanisms driving patterns of biodiversity.

Distance decay may be the outcome of three non-exclusive mechanisms (Nekola and White 1999, Tuomisto et al. 2003, Soininen et al. 2007): 1) increasing environmental dissimilarity with distance, with distance decay resulting from differential performance of species under different environmental conditions; 2) landscape resistance to dispersal, with distance decay resulting from the presence of dispersal barriers limiting the movement of organisms in heterogeneous environments; or 3) intrinsic limits on dispersal

ability of organisms even in homogenous environments. These processes produce the same pattern, and both niche-based (environmental and dispersal barriers) and neutral processes (dispersal limitation) may act simultaneously to produce a distance-decay relationship. However, it is possible to disentangle the relative influence of various mechanisms through the simultaneous examination of multiple potential drivers. For example, inclusion of environmental characteristics that may influence the distribution and abundance of the taxa in question into distance-decay analyses has revealed an important role for niche-based mechanisms in contributing to rates of species turnover, as environmental effects often explain a greater proportion of the variation in community dissimilarity than geographic distance across a wide range of taxa (e.g. Thieltges et al. 2009, 2010, Siefert et al. 2013, Wang et al. 2013). Similarly, the rate of distance decay may vary as a function of species dispersal ability, which is often related to species traits such as body size or dispersal mode (Fellis and Esch 2005, Soininen et al. 2007), habitat or host specificity (Krasnov et al. 2010a, Timi et al. 2010), and by both spatial scale and latitude (Soininen et al. 2007, Locke et al. 2012).

Patterns of distance decay in community dissimilarity have been demonstrated not only for free-living organisms, but also for parasitic organisms (typically of vertebrates; see Poulin 2003 for a review). Parasites are a unique group with which to study distance decay, as the completion of their life cycle typically requires direct and intimate interactions with other species, and rate of distance decay may therefore depend not only on the environmental requirements and dispersal capability of the parasites, but also those of their definitive and intermediate (if necessary) hosts. Distance-decay relationships have been observed among parasitic communities of a wide variety of vertebrate hosts,

including mammals (Poulin 2003, Krasnov et al. 2005, Vinarski et al. 2007, Krasnov et al. 2010a,b,), birds (Svensson-Coelho and Ricklefs 2011, Locke et al. 2012), amphibians (Campião et al. 2012), and both freshwater (Poulin and Morand 1999, Poulin 2003, Karvonen and Valtonen 2004, Fellis and Esch 2005, Poulin et al. 2011, Locke et al. 2013) and marine (Pérez-del-Olmo et al. 2009, Thieltges et al. 2010) fish, but our understanding of the relative roles of niche- and dispersal-based processes in determining rates of distance decay is limited. When comparing parasite communities at the level of the host species or host community, geographic distance is a poor predictor of dissimilarity, while environmental distance and/or phylogenetic relatedness among host species are important predictors of community dissimilarity for ectoparasitic mites and fleas parasitizing rodents (Vinarski et al. 2007, Krasnov et al. 2010a,b). At the level of individual hosts (infracommunities) or host populations (component communities), habitat use and environmental factors such as salinity are important predictors of helminth parasite community dissimilarity in fish (Thieltges et al. 2010, Poulin et al. 2011, Locke et al. 2013), and environmental characteristics, such as climatic variables, rather than geographic distance explained variation in component community dissimilarity in gamasid mites parasitizing rodents (Vinarski et al. 2007). More evidence from a greater number of host-parasite systems is required to resolve the relative influence of environmental factors on spatial variation of community dissimilarity.

Environmental factors should have strong effects on parasite transmission and population dynamics, and hence parasite community structure and dissimilarity, either through direct effects on free-living stages characterized by narrow ecological requirements (Pietrock and Marcogliese 2003, Thieltges et al. 2009) or indirect effects on

intermediate and definitive hosts. In particular, parasite communities reflect the local diversity of free-living intermediate and definitive hosts (Hechinger and Lafferty 2005, Hechinger et al. 2007, Krasnov et al. 2010a), and the distribution and abundance of free-living hosts may be determined by the distribution and quality of suitable habitats and associated environmental conditions (e.g. Karvonen and Valtonen 2004, Krasnov et al. 2005, Poulin et al. 2011, Campião et al. 2012, Míguez-Lozano et al. 2012). For parasites with complex life cycles, environmental heterogeneity reduces the probability that all necessary hosts and environmental conditions necessary to complete the life cycle will coincide, and I would expect to observe limited community dissimilarity over large spatial scales as a function of environmental variation (Thieltges et al. 2009).

Here I examine the relative roles of spatial distance and environmental conditions on component- and infra-community dissimilarity of intestinal helminths parasitizing big brown bats (*Eptesicus fuscus*) in the midwestern region of the United States. Big brown bats are relatively common and widely distributed throughout most of North America. They form aggregations in buildings or trees during the summer consisting primarily of reproductive females and their offspring, whereas males may be found in the same roosts as females or may roost solitarily (Kurta and Baker 1990). Females tend to be philopatric to roosts or roosting areas and may return to the same site year after year (e.g. Lausen and Barclay 2006). Colony members typically forage for insects within several kilometers of their roost site (Kurta and Baker 1990, Wilkinson and Barclay 1997); however, males have larger foraging ranges than females, and bats have been observed foraging up to 11-13 km from their roost site (Arbuthnott and Brigham 2007, Wilkinson and Barclay 1997). Foraging takes place in a variety of habitats, including over water, and in or along the

edge of openings such as agricultural fields, meadows, roads, and forest gaps. Over both small and large spatial scales, big brown bats may encounter a variety of habitats, and individuals may encounter multiple habitat types in a single foraging bout. Intestinal parasite communities of big brown bats tend to be dominated by digenean trematodes that use aquatic snails as first intermediate hosta and insects with aquatic stages as second intermediate hosts (Etges 1960). Aquatic insect communities may vary substantially in relation to habitat characteristics (e.g. Mykra et al. 2007, Heino et al. 2013), and because big brown bats forage in heterogeneous habitats across large spatial scales, they may encounter highly variable communities of intermediate hosts. These scenarios could account for the variability in *E. fuscus* parasite communities from different areas of the United States (Nickel and Hansen 1967, Blankenspoor and Ulmer 1970, Lotz and Font 1985). Relative to the freshwater and marine systems often examined for distance decay of parasite communities, our host-parasite system provides an ideal opportunity to examine the relative roles of niche- and dispersal-based mechanisms in shaping patterns of parasite community dissimilarity in a highly mobile terrestrial host species that has the opportunity to encounter multiple habitats over short timescales.

Our specific objectives were to determine: 1) the independent contribution of geographic and environmental distances on the dissimilarity of intestinal helminth component communities between populations of big brown bats; 2) which environmental variables best explained variation in community dissimilarity, assuming that environmental distance was a significant predictor; and 3) whether similar patterns of decay of dissimilarity with geographic or environmental distance were observed for parasite communities within individual hosts (infracommunity) and within host

populations (component community). I used both compositional (based on species identities and relative abundance) and phylogenetic (based on the phylogenetic distance between species in different communities) measures of community dissimilarity to provide a robust examination of the ways in which parasite communities change with distance and changing environmental conditions. By comparing infra- and component communities I may gain insight into processes occurring over different timescales. Infracommunities may be responsive to local environmental conditions as they are assembled over ecological time scales through demographic processes, the dynamics on transmission and establishment, and interspecific interactions, whereas component communities are assembled over evolutionary time scales through the interplay of speciation, extirpation, and colonization (Poulin 2007, Timi et al. 2010). Importantly, the observed richness of component communities is determined by sampling effort, which, in turn, determines the probability of encountering rare species (Guegan and Kennedy 1996), whereas the richness of infracommunities can be completely quantified. This, combined with increased statistical power associated with a higher sample size of infracommunities (determined by the number of hosts rather than the smaller number of host population sampled), allows for robust statistical testing, and allows us to test which specific host characteristics, such as age and sex, influence community dissimilarity. I provide valuable data on the processes influencing the biodiversity of parasites in a poorly-described host-parasite system, and provide valuable context for understanding the spatial and environmental variables that promote infection and transmission dynamics in this system.

Materials and Methods

Methodological Approach

Our objective was to test the relative roles of spatial and environmental variables in explaining patterns of community dissimilarity. I carried out our analyses at two community scales: infracommunity and component community (Bush et al. 1997) with two different community dissimilarity measures (compositional and taxonomic) as dependent variables within each scale. Compositional dissimilarity represents the amount of species overlap between two study sites based on the number of both unique and shared species within them (Chao et al. 2005). Taxonomic or phylogenetic dissimilarity represents the amount of relatedness between two study sites based on the presence or absence of species within the same clade. A high degree of relatedness indicates that two sites share the same taxonomic groupings (e.g. same genera present in both) but two sites that have many different taxonomic groupings (e.g. no shared genera) would have lower relatedness and hence higher taxonomic dissimilarity (Webb 2000). For each scale and dissimilarity measure, I collected data on independent variables related to land use, climate, distance to water, and geographic distance between sites (Table 1), as well as location data allowing us to test for the effect of geographic distance and spatial scale. In addition, for infracommunities only, I included temporal (month and year of sampling) and host (age and sex of host) as independent variables in the analysis. These independent variables then underwent analysis by at least two different sets of redundancy analysis (RDA) for each dissimilarity measure and community scale. The first set included forward selection and resulted in a parsimonious model of spatial and non-spatial independent variables that best explained helminth community dissimilarity.

Table 1. Subsets of non-spatial explanatory variables considered in redundancy analysis. All landcover variables are in units of km^2 except for distance to nearest water which is in km. Temperatures were measured in degrees celsius and precipitation in centimeters. Summer precipitation included rainfall during the months of June, July, and August. Winter precipitation included rainfall during the months of December, January, and February.

Landcover	Climatic	Temporal	Host
Open Water	Mean annual temperature	Month of Capture	Age
Open Developed Spaces	Mean January temperature	Year of Capture	Sex
Low Developed Spaces	Mean July temperature		
Moderately Developed Spaces	Annual precipitation		
High Developed Spaces	Summer precipitation		
Barren Land	Winter precipitation		
Deciduous Forest			
Evergreen Forest			
Mixed Forest			
Shrub/Scrub			
Grassland/Herbaceous			
Pasture/Hay			
Cultivated Crops			
Woody Wetlands			
Emergent Herbaceous Wetlands			
Distance to Nearest Water			

After completing this first set of analyses, I were able to carry the parsimonious models through to the second set of analyses, a variance-partitioning RDA. These analyses determined how much variation was contributed by spatial or non-spatial classes of variables by holding the other classes constant. As there were multiple subsets of non-spatial variables (landcover, climate, temporal, and host) a third set of analyses, a partial RDA, was performed on the parsimonious non-spatial model if it included more than one of these subsets. Although this partial RDA was not carried through to any other analyses, it helped determine which subset of non-spatial variables was contributed the most variation while holding all other subsets constant.

Host and parasite collection

Two hundred sixty *E. fuscus* from 13 colonies in the midwestern USA (Michigan, Indiana, and Kentucky, Figure 1) were captured with mist nets as they emerged from their roosts or were hand-caught in the roost prior to nightly emergence. Bats captured in the same roost were considered to belong to the same site. Colonies in Michigan (MI-2 through MI-6) were sampled a single time in 2008. Most colonies in Michigan, Kentucky, and Indiana were subsequently sampled twice per year for two years (2009-2010), with the exceptions of MI-1 and KY-2 which were sampled a single time in 2009 (bats were excluded from the roosts by landowners after initial sampling).

Figure 1. Map showing locations of the thirteen study sites.

I recorded host sex, host age, capture date, and capture location via handheld GPS for all captured bats. Bats were euthanized by cervical dislocation followed by exsanguination via cardiac puncture, and then were frozen at -20 °C. Helminth burden was then assessed by necropsy after thawing overnight in a 4 °C refrigerator. The entire carcass and viscera were examined separately using a stereoscope. Trematodes and cestodes were counted, collected, stained with Semichon's acetocarmine, mounted, and examined with a compound light microscope. Nematodes were stored in glycerine-alcohol, cleared, and examined as temporary mounts with a phase-contrast microscope. Then, worms were identified using dichotomous keys (Anderson et al. 2009, Bray et al. 2008, Schmidt 1970) and original species descriptions.

Quantification of Community Dissimilarity

Prevalence, mean abundance, and mean intensity (sensu Bush et al. 1997) were calculated for each helminth species at every host colony. In addition, species richness and diversity (1- Simpson's D) were calculated for each study site. As the number of hosts examined varied among colonies, I confirmed that host sample size was not correlated to helminth species richness at our study sites prior to any other analyses.

I used Bray-Curtis distances to measure compositional dissimilarity, which takes the presence or absence of a species into account as well as the number of individuals of each species. Sites will have perfect Bray-Curtis similarity if they share all species and the abundance of each species is identical at both sites (Bloom 1981). All component communities shared at least one helminth species (Table 2), however not all infracommunities shared species. Rather than exclude uninfected hosts and loose valuable information about infection patterns of individual hosts, I addressed zero

Table 2. Prevalence (Prev.) and mean intensity (Intensity) of helminth fauna found at each study site.

	P. naviculum		*P. swansoni*		*P. transversum*		*P. macnabi*		*A. pipistrelli*		*A. microacanthum*	
Site	Prev.	Intensity	Prev.	Intensity	Prev.	Intensity	Prev.	Intensity	Prev.	Intensity	Prev.	Intensity
IN-1	11.1%	9.0	-------	-------	33.3%	5.0	-------	-------	-------	-------	33.3%	6.0
IN-2	20.0%	4.5	10.0%	49.0	60.0%	3.5	-------	-------	-------	-------	20.0%	12.0
IN-3	18.2%	33.5	9.1%	1.0	18.2%	6.5	9.1%	6.0	18.2%	24.5	-------	-------
KY-1	8.3%	1.0	-------	-------	25.0%	2.0	-------	-------	-------	-------	-------	-------
KY-2	12.5%	4.3	9.4%	4.3	43.8%	4.6	-------	-------	3.1%	3.0	3.1%	3.0
KY-3	-------	-------	8.3%	7.0	8.3%	4.0	-------	-------	-------	-------	-------	-------
KY-4	20.0%	17.0	-------	-------	26.7%	7.3	13.3%	1.5	20.0%	28.0	-------	-------
MI-1	20.0%	6.0	-------	-------	48.0%	79.8	8.0%	6.5	-------	-------	-------	-------
MI-2	20.0%	4.5	20.0%	34.0	60.0%	8.8	10.0%	58.0	10.0%	7.0	-------	-------
MI-3	48.8%	45.8	7.0%	2.3	-------	-------	9.3%	56.8	7.0%	36.0	2.3%	9.0
MI-4	38.9%	40.3	16.7%	0.3	38.9%	22.0	-------	-------	11.1%	12.0	5.6%	9.0
MI-5	31.6%	7.2	5.3%	5.0	36.8%	7.1	15.8%	4.0	-------	-------	-------	-------
MI-6	25.0%	17.7	-------	-------	25.0%	3.0	8.3%	52.0	-------	-------	-------	-------

Table 2—Continued

A. eptesici		*A. alicatai*		*G. noctophilus*		*O. breckenridgei*		*Pl. vespertilionis*		*H. roudabushi*	
Prev.	Intensity	Prev.	Intensity	Prev.	Intensity	Prev.	Intensity	Prev.	Intensity	Prev.	Intensity
11.1%	18.0	-------	-------	-------	-------	-------	-------	22.2%	2.0	11.1%	1.0
10.0%	1.0	-------	-------	-------	-------	-------	-------	20.0%	1.5	10.0%	4.0
9.1%	3.0	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------
8.3%	12.0	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------
6.3%	11.5	-------	-------	3.1%	4.0	-------	-------	3.1%	36.0	3.1%	1.0
-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	8.3%	1.0
-------	-------	-------	-------	-------	-------	6.7%	16.3	-------	-------	6.7%	1.0
8.0%	59.0	4.0%	31.0	-------	-------	-------	-------	12.0%	1.7	-------	-------
20.0%	16.0	-------	-------	10.0%	11.0	-------	-------	10.0%	2.0	30.0%	16.3
16.3%	22.1	-------	-------	2.3%	2.0	-------	-------	34.9%	5.2	16.3%	2.1
5.6%	44.0	-------	-------	-------	-------	-------	-------	16.7%	3.7	-------	-------
-------	-------	-------	-------	-------	-------	-------	-------	15.8%	1.7	-------	-------
-------	-------	-------	-------	8.3%	5.0	-------	-------	8.3%	5.0	-------	-------

dissimilarity values by adding one member of a dummy helminth species to every host in infracommunity analyses. I did this because dissimilarity indices are undefined in hosts with no parasites and the inclusion of a single dummy helminth prevents that issue (Locke et al. 2012). As redundancy analysis preserves Euclidian distances between sites and species abundance data are not Euclidean in nature, I transformed raw data using the Hellinger formula recommended by Legendre and Gallagher (2001) and then computed Euclidian distances of the transformed data, thus ensuring that our data would be compatible with redundancy analysis.

I calculated taxonomic dissimilarity following a procedure similar to that of Locke et al. (2013) by calculating Γ^{+}, or the average shortest distance to a common ancestor in a taxonomic tree, for every helminth in two different component or infracommunities that were infected with at least one helminth species. Essentially, Γ^{+} represents the mean number of all taxonomic steps between each parasitic species in one host and its closest related parasite in the next host (Clarke et al. 2006). As I did not have complete phylogenies for all helminths in our dataset, I used taxonomic designations of Anderson et al. (2009), Bray et al. (2008), and Schmidt (1970) as an approximation of phylogenetic relatedness. I set taxonomic distances along a zero to six scale based on whether helminths were conspecifics (0), congeners (1), or belonged to different families (2), superfamilies (3), orders (4), classes (5), or phyla (6).

Quantification of Independent Variables

Given that bat helminths use arthropod intermediate hosts, our environmental dataset included variables that could influence the presence of these arthropods surrounding the roost site. These variables included distance of roost to nearest water source, type of landcover surrounding the roost, and climatic conditions in the area where the roost was located (Table 1). GIS layers of the study sites with a 1:24,000 scale were obtained from the United States Fish and

Wildlife Service National Wetlands Inventory. I used these layers to determine the distance from the roost to nearest body of water within a 12km radius of the roost (based on maximum foraging distances recorded for *E. fuscus*; Arbuthnott and Brigham 2007, Wilkinson and Barclay 1997) via ArcGIS 10 (ESRI). I also used GIS layers from the National Landcover Database (Fry et al. 2009) to determine categories of land usage and the amount of area covered by each category in km^2. Land use categories describing human development typically consisted of a mixture of vegetation and impervious surface (artificial structures such as pavement, concrete, or buildings). These categories fell into four designations: 1) developed open spaces (e.g. lawn grasses, large-lot single family housing developments, parks, golf courses) containing less than 20% impervious surface; 2) low intensity developed areas (e.g., single-family housing with larger lots) with 20-49% impervious surface; 3) moderately developed areas with impervious surfaces ranging from 50%-79% (e.g., single-family housing with smaller lot size); and 4) highly developed areas with 80%-100% impervious surfaces (e.g. apartment complexes, industrial areas, row houses). Barren land included areas covered with earthen material and vegetation accounted for less than 15% of total cover. Forest categories consisted of areas with more than 20% of total vegetation coverage by trees more than 5 meters tall. Deciduous forest was identified as areas with over 75% deciduous foliage, evergreen forest was identified as areas with over 75% evergreen foliage, and mixed forest was identified as areas where neither tree type consisted of more than 75% of landcover. Shrub/scrub consisted of woody plants less than 5 meters tall that made up more than 20% of total vegetation (e.g. true shrubs, young trees). Grassland/herbaceous areas were dominated by herbaceous vegetation that made up more than 80% of total vegetation. These areas were tilled but may have been grazed by livestock. Pasture/hay included areas where grasses and legumes planted for grazing or hay production accounted for more than 20% of total

vegetation. Cultivated crops included land used for the production of annual crops (e.g. corn, tobacco) and perennial woody crops (e.g. orchards, vineyards) where crops accounted for more than 20% of total vegetation as well areas that were actively tilled. Open water consisted of areas of open water with less than 25% cover by vegetation or soil. Woody wetlands were areas where forest or shrub accounted for more than 20% of vegetation and the soil was often saturated with or covered with water. Emergent herbaceous wetlands were lands where perennial herbaceous plants were more than 80% of vegetative cover and the soil was often saturated with or covered with water.

To assess variation among sites in climate I used the National Atmospheric and Oceanic Administration's Quality Controlled Local Climatological Data to determine annual mean temperature, annual mean precipitation, mean winter temperature (December, January, February), mean summer temperature (June, July, August), mean winter precipitation, and mean summer precipitation for the weather station closest to each roost site. I chose these parameters because they characterize the climate of a given area (Krasnov et al. 2005) and are likely to affect arthropod intermediate hosts. For analyses at the infracommunity scale, I included additional independent variables related to time of sampling (month and year of capture) and host characteristics (host sex and host age). Age was determined by the degree of ossification of phalanges (Adams 1998), and I could distinguish young of the year (juveniles) from adults (two years and older).

Assessing the Relative Importance of Predictors

To select independent variables that explained a significant proportion of variation in community dissimilarity, I first employed RDA with a forward selection procedure. The procedure first tests the significance of variables within pre-defined groups or classes of

variables. In our case I defined three classes of spatial variables (see below) and a class of non-spatial variables. As multicollinearity was a concern, I ensured that all variables had variance inflation factors below 10. I then tested the relative importance of spatial and non-spatial classes of variables on community dissimilarity using a variance-partitioning RDA following Borcard et al. (2011) using the R packages vegan and pcnm.

To carry out the RDA and forward selection procedure I used R function rda for compositional dissimilarity, and for taxonomic dissimilarity I used R function capscale because it can handle any dissimilarity matrix, even one that is user-generated. Latitude and longitude of our study sites were converted to Cartesian coordinates and then I created three classes of spatial independent variables using these coordinates. The first class of spatial independent variables was a set of matrices of straight-line distances (x,y distances), longitudinal distances (distances along the x-axis only), and latitudinal distances (y-axis only) between all pairs of sites. This allowed us to test for a simple linear trend between geographic distance and our measures of community dissimilarity. The second and third classes of spatial independent variables were a series of distance matrices defining spatial subsets of sites, defined using the principal coordinates of neighbor matrices technique (PCNM) in the pcnm R package. PCNM is useful because it produces linearly independent spatial variables over wide scales, can model any type of spatial structure, and is appropriate for irregular sampling designs (Borcard et al. 2011). Using measures of community dissimilarity as the dependent variable, this technique heuristically defines all possible subsets of locations and retains subsets with greater than expected Moran's I values, a measure of spatial autocorrelation in the dependent variable. The matrices defining subsets of sites with significant spatial autocorrelation are then ordered in size from largest to smallest spatial extent, with the largest 50% of matrices used to examine broad spatial-scale

patterns of community dissimilarity (broad-scale class of spatial variables) and the smallest 50% to examine fine spatial-scale patterns (fine-scale class of spatial variables).

Results of the within-class RDA for non-spatial variables were visualized using triplots that display explanatory variables and helminth species scores (based on either compositional or taxonomic distances as appropriate) as vectors and sites or individuals as points. On the plots, the distances between points for sites or individuals represent distances between observations based on compositional or taxonomic dissimilarity. Explanatory variable vectors are scaled according to their magnitude, where arrows point to the direction of the gradient and length indicates the strength of the variable. Similarly, vectors for helminth species scores indicate directions of larger abundances. Sites should be perpendicularly projected onto the explanatory variable vectors to predict the value of the variable; values increase towards the arrow head, and decrease to the opposite direction. Angles between points representing sites or individuals and arrows representing explanatory variable vectors illustrate a two-dimensional approximation of correlations.

After the significance of variables within pre-defined classes of variables was tested using RDA and forward selection, the next step was the variance-partitioning analysis that tested the relative importance of each class in explaining patterns of community dissimilarity by holding all other classes of variables constant while assessing the focal class of interest. Thus, I could, for example, test whether the non-spatial class explained more variation than the linear trend class. Explanatory variables fell into four classes: non-spatial (a), linear trend (b), broad-scale spatial structure (c), and fine-scale spatial structure (d; see Figure 2). I then performed an ANOVA-like permutation test with 1,000 permutations to determine significance of the relative contribution of each class in explaining helminth community dissimilarity.

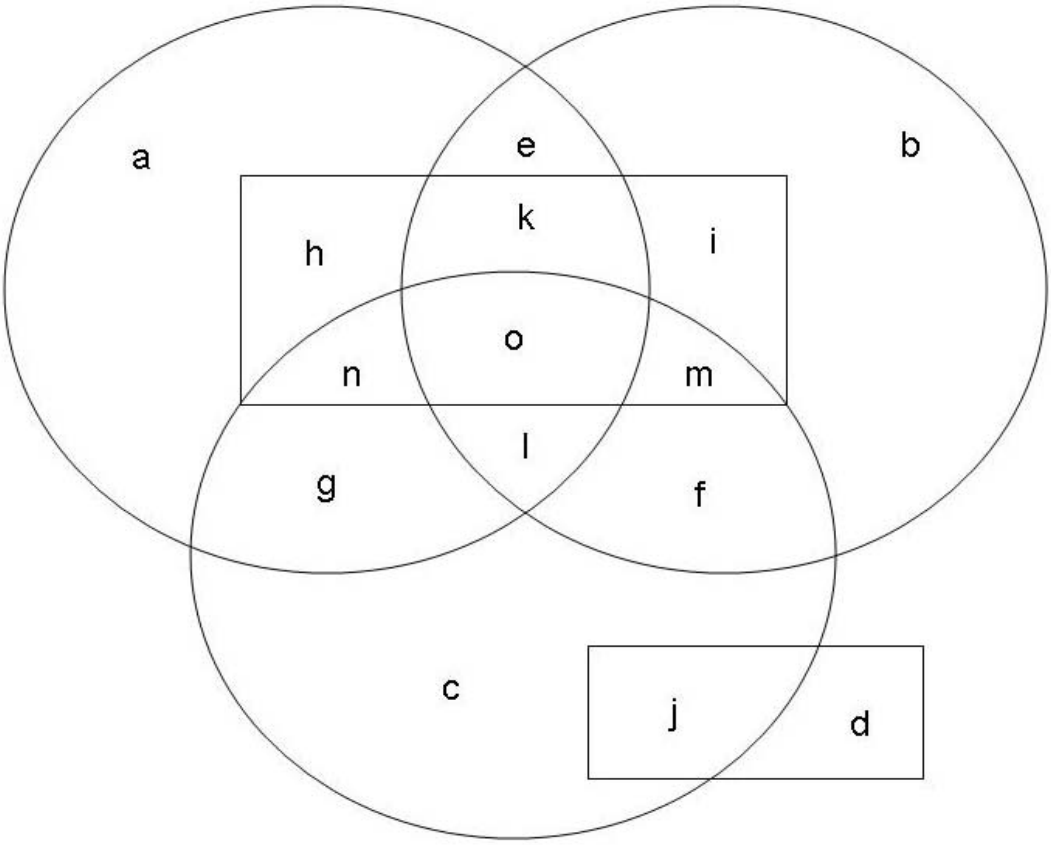


Figure 2. Generalized model used in variance partitioning analysis to examine the relative contribution of different classes of explanatory variables. It includes non-spatial (upper left-hand circle; a), linear trend (upper right-hand circle; b), broad-scale spatial (lower circle; c), and fine-scale spatial (disjoined rectangles; d in the lower rectangle) classes. Various combinations of classes are shown by overlaps among shapes (e-j). For example, the fine-scale spatial class is shown on its own (d) and in combination with the broad-scale class (j) in the lower rectangle, the upper rectangle shows the fine-scale spatial class in combination with the non-spatial and linear trend classes (h,i,k,m,n,o), and overlapping circles represent combined influences of the other classes. In total then, this procedure tests the explanatory power of each class of variables on its own as well as in a series of nested combinations with all other classes in the analysis. Each fraction can then be tested for significance via an ANOVA-like permutation test.

Visualizing Community Dissimilarity

To visualize whether component and infracommunities fell into clearly delineated geographical groups based on either compositional or taxonomic dissimilarity, I applied nonmetric multidimensional scaling (NMDS) in the R package vegan in R version 2.13.1 (R Development Core Team) to our raw, untransformed data. To create visual representations of helminth community and infracommunity dissimilarity among sites I employed the wrapper function metaMDS that utilized the Bray-Curtis dissimilarity index for compositional dissimilarity or the dissimilarity index of Locke et al. (2013) for taxonomic dissimilarity. This wrapper function used R's default NMDS settings of a Wisconsin double standardization, where abundance values are first standardized by species maximum in order to create relative abundances and then by sample total because the number of samples varied by site, along with 50 random starts (Borcard et al. 2011).

Results

Helminth species richness and diversity (1-Simpson's D) within component communities ranged from 4-11 and 0.30-0.79, respectively (Table 3). The component communities with the highest species richness (MI-3) and diversity (MI-2) as well as the least diverse site (MI-1), were located in Michigan. Site KY-3, located in Kentucky, had the lowest species richness. Species richness was not significantly correlated with number of hosts captured at each site (Spearman $r = 0.4658$, d.f. = 11, $p = 0.1113$) and species accumulation curves were sufficiently saturated at each site; therefore, differences among sites were not an artifact of sampling. Fourteen different species of helminths were identified from the 13 roost sites (Table 2). Digenetic trematodes (11 species) dominated the parasitic fauna, followed by nematodes (2 species) and cestodes (1 species). Two

trematodes, *Paralecithodendrium naviculum* and *P. transversum*, were found at 12 of the 13 sites, usually with relatively high prevalence and mean intensity. Three species were only found at one site each (*Acanthatrium eptesici*, *Ochoterenatrema breckenridgei*, and *Rictularia lucifugus*), all with relatively low prevalence and mean intensity.

Table 3. Study sites located in Michigan (MI), Indiana (IN), or Kentucky (KY) with site helminth species richness and helminth species diversity (1-Simpson's D).

Site	Richness	Diversity	Years Sampled
IN-1	6	0.77	2009, 2010
IN-2	7	0.71	2009, 2010
IN-3	6	0.64	2009, 2010
KY-1	6	0.50	2009
KY-2	10	0.79	2009, 2010
KY-3	4	0.67	2009, 2010
KY-4	6	0.66	2009, 2010
MI-1	6	0.30	2009
MI-2	10	0.83	2009, 2010
MI-3	11	0.41	2008, 2009, 2010
MI-4	7	0.62	2008, 2009, 2010
MI-5	6	0.67	2008, 2009, 2010
MI-6	5	0.41	2008, 2009, 2010

Proportion of landcover categories varied among sites (Table 4). Open water was extremely high at three Michigan sites (MI-2, MI-3, MI-4) but several orders of magnitude lower at sites IN-2 and KY-2. Categories of developed land were consistently higher at sites MI-4, MI-5, and MI-6 but lowest at IN-2 and IN-3. Forested land categories were highest at KY-4 and KY-1, followed by sites MI-2 through MI-5; forest cover was relatively low at the remaining six sites. Pasture/hay was consistently highest at Kentucky sites but relatively low at all Michigan sites except for MI-1. The amount of land occupied by cultivated crops was lowest at all Kentucky sites and highest in all

Table 4. Landcover categories considered and the number of square kilometers of each landcover type within a 12km radius of each study site. Categories included open water (OW), open developed spaces (OD), low intensity developed areas (DL), moderately developed areas (DM), highly developed areas (DH), barren land (BL), deciduous forest (DF), evergreen forest (EF), mixed forest (MF), shrub/scrub (SS), grassland/herbaceous (GH), pasture/hay (PH), cultivated crops (CC), woody wetlands (WW), and emergent herbaceous woodlands (HW).

Cover	IN-1	IN-2	IN-3	KY-1	KY-2	KY-3	KY-4	MI-1	MI-2	MI-3	MI-4	MI-5	MI-6
OW	4398	246	9447	6543	778	1567	3951	2635	21462	21968	20527	3443	6597
DO	26890	24702	22749	36270	33967	31453	25040	26015	21038	41446	49762	41778	99740
DL	11179	3781	3865	23412	14785	3721	8309	14052	13230	16215	40791	26391	71078
DM	1671	490	1566	8301	8834	1396	1885	3659	2283	3542	13611	7905	13285
DH	665	50	484	2397	3676	869	344	1568	553	1460	7706	2368	6391
BL	347	0	1176	5773	374	36	1636	738	2774	1112	1434	1114	702
DF	57244	16345	81765	188580	19574	64615	237150	98833	110453	119996	109376	118102	52350
EF	2194	33	1002	4176	371	1149	12479	6348	8015	6237	3796	4240	1333
MF	32	0	16	4980	12	481	81421	5450	7756	5296	5187	4207	1618
SS	4200	56	137	1023	1490	790	2164	810	1997	995	1384	2338	376
GH	1582	5699	750	12951	888	1805	31508	5855	5378	5687	7088	10459	6082
PH	28844	14110	13985	149280	347366	314926	46016	105917	71968	65518	49218	32575	21400
CC	297828	386674	306491	8644	20190	28921	464	118488	137034	104698	110676	137592	139364
WW	12951	198	8530	0	0	0	0	54617	46121	54655	30521	58688	30589
HW	2363	14	419	48	78	663	21	7419	2332	3559	1305	1179	472

Indiana sites. Finally, both herbaceous and woody wetlands were much more abundant in all Michigan sites whereas these wetlands were relatively low in Indiana sites and extremely low in Kentucky sites.

Compositional Dissimilarity

Component community analyses

After performing forward selection, the only class of variables that explained a significant portion of the variation in component community compositional dissimilarity was the non-spatial class (Table 5); no linear trend or spatial structure variables were significant in within-class RDA ($F_{2,10}$ = 0.7395, p = 0.85 and $F_{2,10}$ = 0.1412, p = 0.87, respectively). Thus, a subsequent variance partitioning RDA was not performed because variation was sufficiently explained by non-spatial variables. To confirm and visualize this lack of spatial structure, I performed NMDS (stress = 0.13) that showed that geographic nearest neighbors did not necessarily cluster together in homogenous groups (Figure 3A). Instead, geographic nearest neighbors varied widely in their helminth community dissimilarity, and some component communities in different regions were more similar to one another than to component communities in the same region.

Table 5. Variance partitioning indicated significant variable classes for each scale.

Community Scale	Dissimilarity	Variable Classes	F-value	Significance	R^2_{adj}
Component	Compositional	Non-spatial	$F_{2,10}$ = 2.3565	p = 0.003	0.501
Infracommunity	Compositional	Non-spatial, Fine-scale spatial	$F_{8, 207}$ = 5.5282	p = 0.001	0.126
Component	Taxonomic	Linear trend	$F_{1, 11}$ = 9.6460	p = 0.01	0.279
Infracommunity	Taxonomic	Non-spatial	$F_{2, 152}$ = 3.9524	p = 0.01	0.073

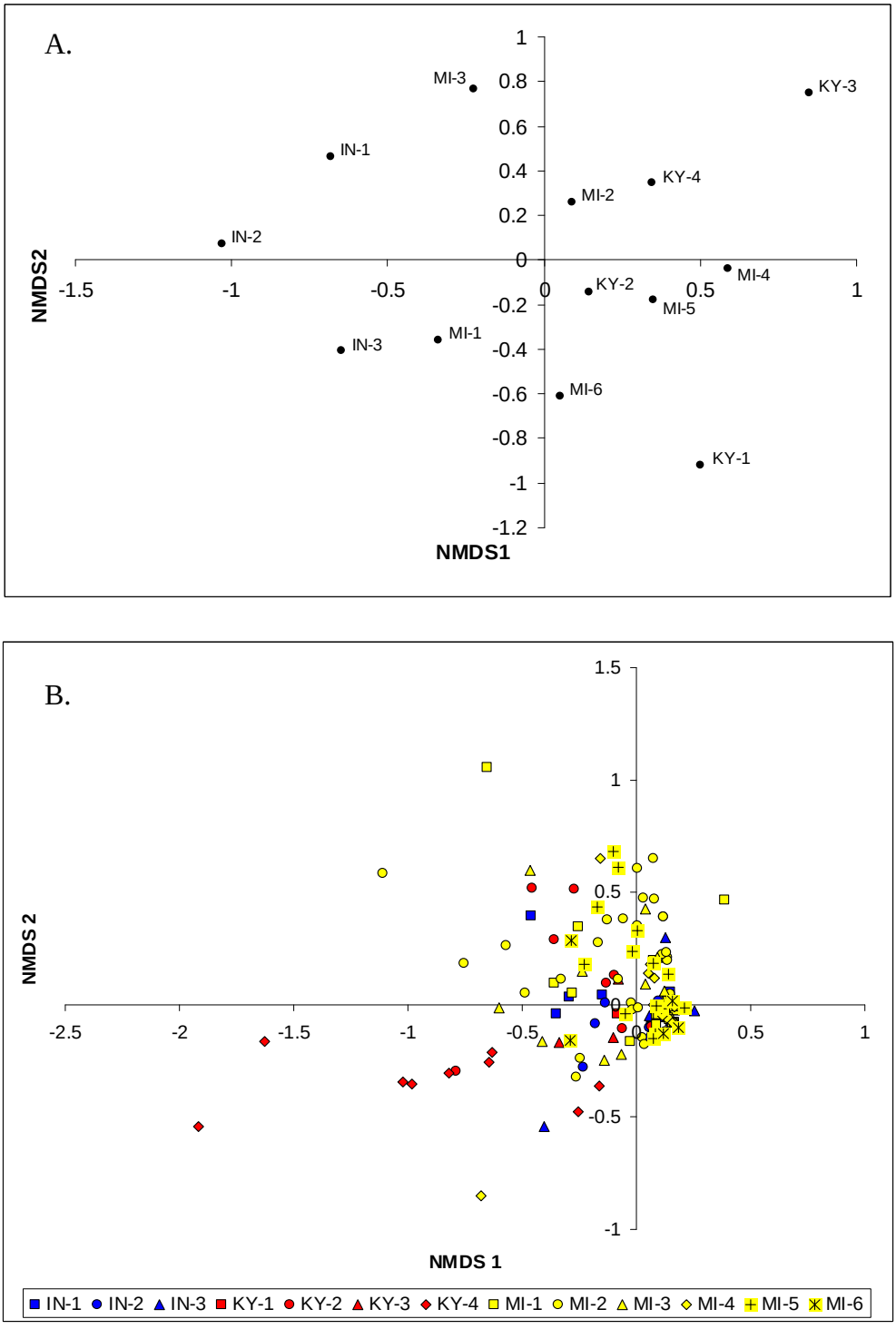


Figure 3. Nonmetric multidimensional scaling of compositional dissimilarity revealed that sites did not form distinct clusters based on geographical location for component communities (A) or infracommunities (B).

Within-class RDA for non-spatial variables did determine the factors that significantly explained compositional dissimilarity among sites. I discovered that the global component community model that included all possible non-spatial variables (Table 1) did not have high explanatory power ($R^2_{adj} = 0.206$) because of the large number of variables in this model. Obtaining a parsimonious model (Table 5) through forward-selection resulted in much higher explanatory power ($R^2_{adj} = 0.501$) and according to the ANOVA-like permutation test, it significantly accounted for helminth community dissimilarity ($F_{2,10} = 2.3565$, $p = 0.003$). After forward selection, two significant land use variables (Table 6) were significant (developed open spaces, $F_{2,10} = 6.1895$, $p = 0.003$ and high intensity developed spaces, $F_{2,10} = 5.1701$, $p = 0.0031$) and an additional two land use variables that approached significance (cultivated crops, $F_{2,10} = 4.2238$, $p = 0.0504$ and woody wetlands, $F_{2,10} = 1.8643$, $p = 0.078$) were retained. This model's corresponding triplot, which allows us to visualize landcover variables most closely associated with each helminth species and each study site, shows three general clusters of helminth species grouped around developed spaces, woody wetlands, or cultivated crops (Figure 4). Species such as *P. swansoni* and *H. roudabushi* cluster around developed landcover wheras *Glyptoporus noctophilus*, *A. alicatai*, *A. pipistrelli*, *O. breckenridgei*, *R. lucifugus*, and *Litomosa americana* cluster near cultivated crops indicating that those species are more abundant in those landcover types (Figure 4). *Acanthatrium eptesici* was more abundant near woody wetlands whereas abundances of *A. microacanthum* and *Plagiorchis vespertilionis* were better explained by a combination of woody wetlands and developed areas. However, *P. macnabi* and *P. naviculum* abundances were inversely

Table 6. Redundancy analysis indicated which variables should be included in models describing each community scale and dissimilarity measure.

Community Scale	Dissimilarity Measure	Variables in Model	F-value	Variable Significance
Component	Compositional	Developed open spaces	$F_{2,10} = 6.1895$	$p = 0.003$
		High intensity developed spaces	$F_{2,10} = 5.1701$	$p = 0.0031$
		Cultivated crops	$F_{2,10} = 4.2238$	$p = 0.0504$
		Woody wetlands	$F_{2,10} = 1.8643$	$p = 0.078$
Infracommunity	Compositional	Barren land	$F_{8,207} = 6.8972$	$p = 0.001$
		Shrub/scrub	$F_{8,207} = 5.4962$	$p = 0.001$
		Moderately developed open spaces	$F_{8,207} = 4.6708$	$p = 0.001$
		Open Water	$F_{8,207} = 4.4755$	$p = 0.002$
		High intensity developed spaces	$F_{8,207} = 1.8643$	$p = 0.002$
		Evergreen Forest	$F_{8,207} = 3.2122$	$p = 0.005$
		Year of capture	$F_{8,207} = 5.0933$	$p = 0.001$
		Fine-scale spatial	$F_{2,207} = 2.9299$	$p = 0.005$
Component	Taxonomic	Latitude	$F_{1,11} = 9.6460$	$p = 0.01$
Infracommunity	Taxonomic	Developed open spaces	$F_{2,152} = 7.152$	$p = 0.0004$
		Emergent herbaceous wetlands	$F_{2,152} = 6.043$	$p = 0.0017$

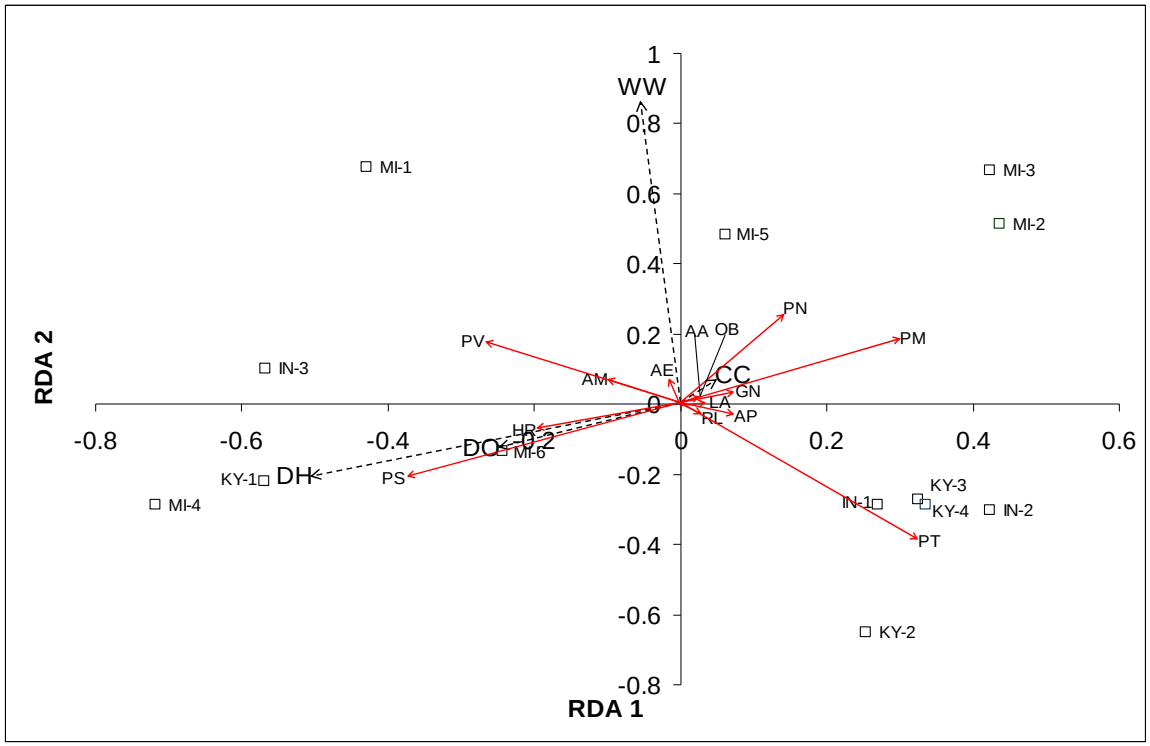


Figure 4. Redundancy analysis triplot for component community compositional dissimilarity. Sites and species cluster around different explanatory variables according to their abundance. For example, helminth species that cluster around cultivated crops are more abundant in that landcover, whereas sites that cluster around cultivated crops have a higher abundance of that landcover. Explanatory variables include highly developed spaces (DH), developed open spaces (DO), woody wetlands (WW), and cultivated crops (CC). Helminth species include *A. eptesici* (AE), *A.alicatai* (AA), *G. noctophilus* (GN), *O. breckenridgei* (OB), *Pl. vespertilionis* (PV), *H. roundabushi* (HR), *L. Americana* (LA), and *R.lucifugus* (RL).

related to developed areas. Finally, *P. transversum* abundance was not associated with any of the significant explanatory variables.

Infracommunity analyses

Variables in all three spatial classes, linear trend (latitudinal distance, $F_{1,218}$ = 8.6949, p = 0.001), broad-scale spatial structure (2 PCNMs, $F_{2,207}$ = 2.7367, p = 0.001 and $F_{2,207}$ = 2.7239, p = 0.001), and fine-scale spatial structure (2 PCNMs, $F_{2,207}$ = 2.7203, p = 0.001 and $F_{2,207}$ = 2.0037, p = 0.019), were significant in their within-class RDAs and were subsequently carried through forward selection to a variance-partitioning RDA (Table 5). The within-class RDA model of non-spatial variables was highly significant according to the ANOVA-like permutation test ($F_{8,207}$ = 5.5282 , p = 0.001; Table 6) but had low explanatory power (R^2_{adj} = 0.126). After forward selection, this model shared the land use variable highly developed open spaces ($F_{8,207}$ = 3.5149, p = 0.002) with the component community model, but also included shrub/scrub ($F_{8,207}$ = 5.4962, p = 0.001), barren land ($F_{8,207}$ = 6.8972, p = 0.001), moderately developed open spaces ($F_{8,207}$ = 4.6708, p = 0.001), open water ($F_{8,207}$ = 4.4755, p = 0.002), and evergreen forest ($F_{8,207}$ = 3.2122, p = 0.005). This model also included a temporal variable, year of host capture ($F_{8,207}$ = 5.0933, p = 0.001). As two different subsets of non-spatial variables (landcover, temporal) were significant, I performed a subsequent partial RDA to determine the relative contribution of each subset. When landcover was constrained, year of capture accounted for 27.8% of the variation in the infracommunity model, indicating that landcover variables accounted for 72.2% of variation.

The within-class RDA triplot for infracommunities (Figure 5) revealed that individuals from different regions did not form uniform groups around any particular explanatory variable; instead multiple individuals from multiple regions were clustered together, which is indicative of sites in different regions having similar proportions.

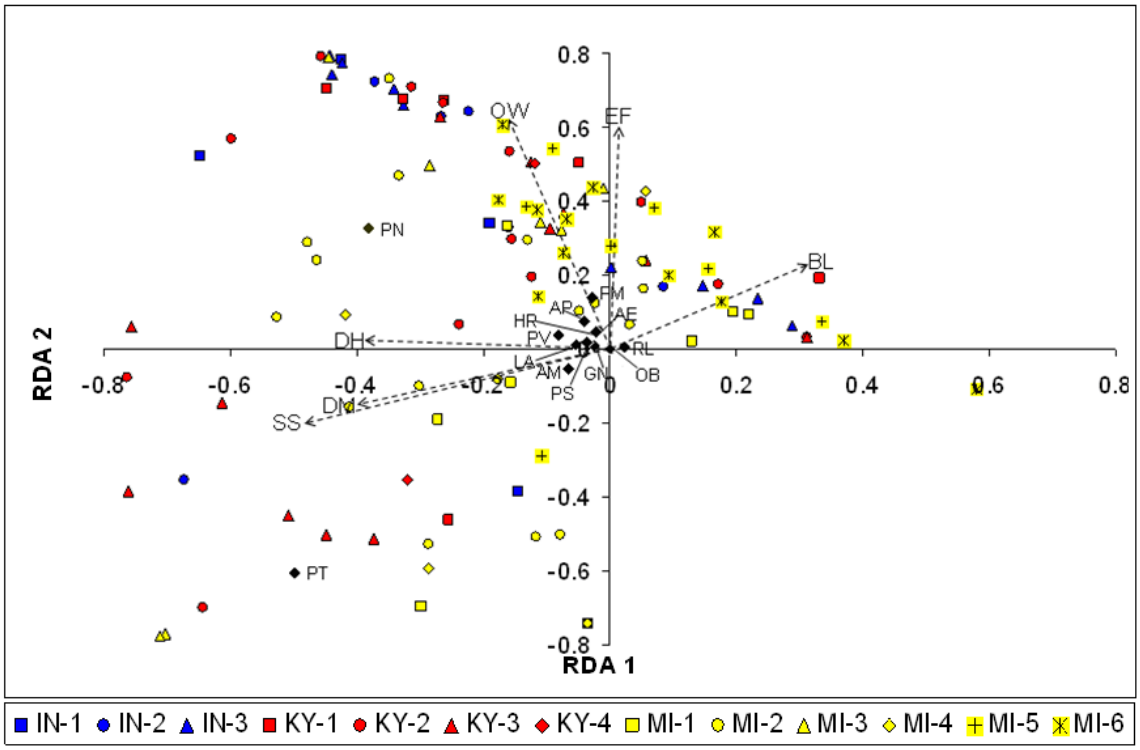


Figure 5. Redundancy analysis triplot for infracommunity compositional dissimilarity. Sites and species cluster around landcover variables according to their abundance. Explanatory variables include highly developed areas (DH), moderately developed areas (DM), shrub/scrub (SS), barren land (BL), evergreen forest (EF), open water (OW).

Most helminth species clustered around the variables moderately developed spaces, highly developed spaces, and shrub/scrub (*A. alicatai*, *P. macnabi*, *P. swansoni*, *H. roudabushi*), or open water (*A. eptesici*, *O. breckenridgei*), or between them (*P. naviculum*; Figure 4). *Reticularia lucifugus* was more closely associated with barren land, while *P. transversum* was not associated with any of the significant explanatory variables (Figure 4).

I assessed the relative importance of different classes of variables (linear trend, broad-scale spatial, fine-scale spatial and non-spatial) to infracommunity dissimilarity

using a variance- partitioning RDA (as in Figure 2). While holding all other variation constant, non-spatial variables explained a significant portion of variation in infracommunity dissimilarity ($R^2_a = 0.082$, $F_{8,207} = 3.5793$, $p = 0.005$), as did fine-scale spatial structure ($R^2_d = 0.017$, $F_{2,207} = 2.9299$, $p = 0.005$), but the overall amount of variation explained by the model was low. Neither linear trend ($F_{1,207} = 1.8488$, $p = 0.105$) nor broad-scale spatial structure ($F_{1,207} = 1.8027$, $p = 0.091$) significantly explained variation in community dissimilarity in the variance partitioning analysis after variation in other classes of variables was held constant (Figure 6A). I used NMDS to visualize and confirm this trend in spatial structure. NMDS (stress = 0.16) showed that infracommunities from the same study site were clustered together (Figure 3B), whereas infracommunities from neighboring colonies were not, and this accounted for fine-scale spatial structure in the data. This pattern indicates that although individuals from the same colony tended to have similar helminth infracommunities, this did not necessarily extend to hosts from neighboring sites within regions.

Taxonomic Dissimilarity

Component community analyses

After performing within-class RDAs, the only class with a significant variable that explained variation in taxonomic dissimilarity among component communities was linear trend (Table 5) and forward selection (Table 6) specifically identified latitudinal distance as the only significant variable in the model ($F_{1,11} = 9.6460$, $p = 0.01$; $R^2_{adj} = 0.28$). A variance partitioning RDA could not be performed on component community taxonomic dissimilarity because only one class of explanatory variable (linear trend) significantly

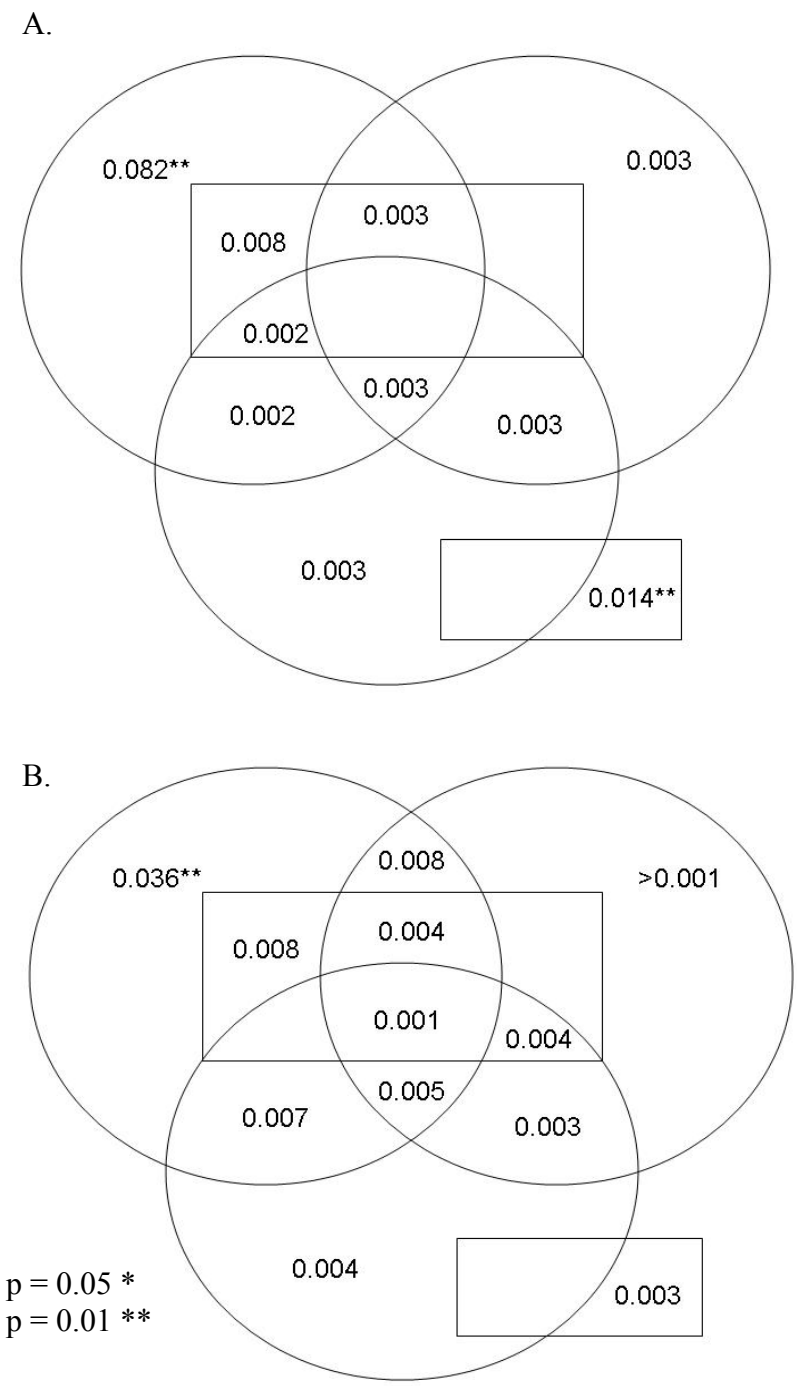


Figure 6. Visual representation of the variance partitioning RDA analysis on infracommunity compositional dissimilarity (A) and infracommunity taxonomic dissimilarity (B). Partial R^2 values are provided for each fraction (negative numbers omitted), and significance is indicated with asterisks.

explained the variation in that dataset. NMDS was unable to find a convergent solution and thus, I could not visually represent these data with this method.

Infracommunity analyses

After performing within-class RDAs, variables in all three spatial classes (Table 5), linear trend (latitudinal distance, $F_{1,152} = 4.477$, $p = 0.008$), broad-scale spatial structure (1 PCNM, $F_{1,152} = 3.907$ and $p = 0.011$), and fine-scale spatial structure (1 PCNM, $F_{1,152} = 2.841$ and $p = 0.039$), were significant and carried through forward selection to a variance partitioning RDA. Two non-spatial variables (Table 6) were significant in the within-class RDA, emergent herbaceous wetland ($F_{2,152} = 7.152$, $p = 0.0004$) and highly developed spaces ($F_{2,152} = 6.043$, $p = 0.0017$), but overall the model explained a small proportion of variation ($R^2 = 0.079$) in taxonomic dissimilarity. Of the four classes entered into the subsequent variance-partitioning RDA, only the non-spatial class consisting of the two landcover variables, explained a significant portion of the variation in infracommunity taxonomic dissimilarity while holding all other classes constant ($F_{2,152} = 3.9524$, $p= 0.01$; Figure 6B). NMDS found a convergent solution (stress = 0.15) for infracommunity taxonomic dissimilarity and individuals from the same study sites grouped loosely together without forming distinct geographical sets (Figure 7A). However, all individuals were tightly clustered around the origin (Figure 7B) compared to other NMDS plots.

Discussion

Our results did not support distance decay of community dissimilarity in our host-parasite system and suggest that environmental processes rather than dispersal ability were more important in structuring these helminth communities. I discovered that landcover most

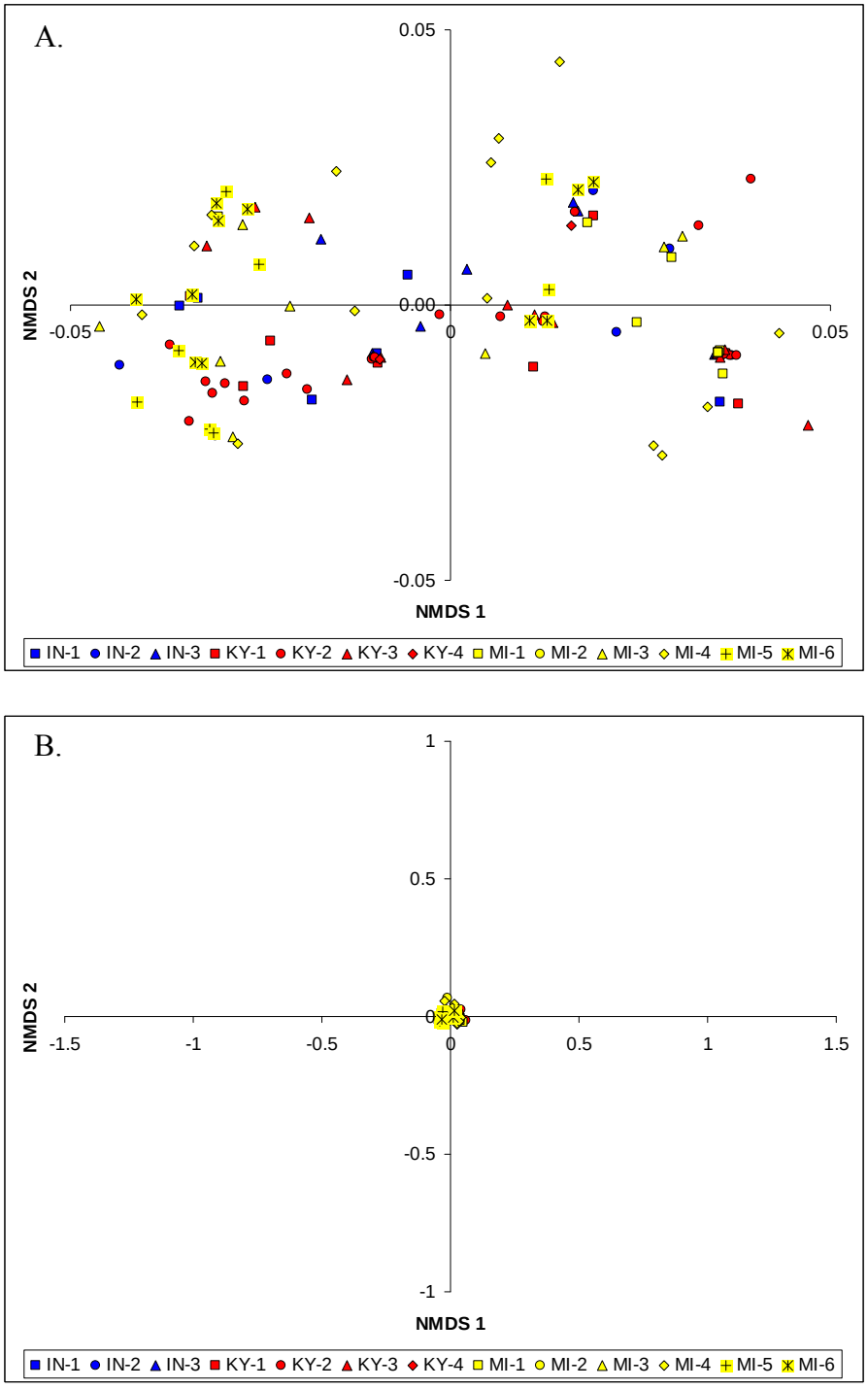


Figure 7. NMDS of taxonomic dissimilarity revealed did not form distinct sets based on geographical location. Although infracommunities from the same site exhibited some similarity, infracommunities from different states were interspersed with each other (A) and all infracommunties essentially clustered together around the origin (B).

frequently shaped helminth community dissimilarity over multiple community scales. By simultaneously examining relative contributions of various spatial and non-spatial variables to the variation in our three-state dataset, our novel approach frequently identified a significant link between landcover classes related to anthropogenic impact (e.g. highly developed spaces, cultivated crops) and helminth community dissimilarity. These findings highlight possible downstream effects of human land use on host-parasite systems.

I found that environmental factors, especially landcover, strongly influenced helminth community dissimilarity at three separate levels: component community compositional dissimilarity, infracommunity compositional dissimilarity, and infracommunity taxonomic dissimilarity. With the exception of infracommunities from hosts using the same roost site, both component and infracommunities that were spatially close did not necessarily have similar helminth communities. Although evidence exists for distance decay of community dissimilarity in multiple host-parasite systems (e.g. Poulin 2003, Felis and Esch 2005, Oliva and Gonzalez 2005, Locke et al. 2012), when included, environmental factors typically explains more variation in parasite community dissimilarity than distance alone (e.g. Poulin and Morand 1999, Krasnov et al. 2005, Thieltges et al. 2010, Timi et al. 2010). Our results agree with these latter studies as landcover surrounding the roost site better predicted helminth community dissimilarity than any other class of variables, especially in compositional component community dissimilarity where it explained about 50% of variation in the dataset. The compositional models at both the component and infracommunity levels shared highly developed spaces while some terms were unique to each (cultivated crops; barren land and moderately

developed open spaces, respectively). Developed open spaces, although unique to the taxonomic infracommunity model, also stressed the relative influence of anthropogenically disturbed areas on community dissimilarity. The ubiquity of human land use throughout these models further highlights anthropogenic disturbance as a key driver of community dissimilarity in this host-parasite system.

Anthropogenic landscape changes could have serious implications for intermediate host communities and host exposure to parasites. I expect that parasites with complex lifecycles, like those in our system, exhibit community dissimilarity over large spatial scales due to the narrow ecological requirements of intermediate stages combined with extensive environmental variation (Pietrock and Marcogliese 2003, Thieltges et al. 2009, Thieltges et al. 2010). Human activity could cause downstream effects on parasite communities by driving changes in landcover and interfering with complex parasite lifecycles. Changes in communities of parasites that use arthropod intermediate hosts are likely related to changes in intermediate host communities that correspond to human activity. Helminth community structure within definitive hosts is recruitment-driven and can reflect the community structure found in intermediate hosts (Lotz et al. 1995, Vickery and Poulin 2002). Our helminth communities were mainly comprised of digenean trematodes that use freshwater aquatic snails as a first intermediate host and insects with aquatic stages as second intermediate hosts. In the absence of urbanization, environmental variables (e.g. canopy cover, hydroperiod), significantly structure snail communities in the Midwestern USA and their community dissimilarity is dependent on environmental dissimilarity rather than spatial distance (Hoverman et al. 2011). Undisturbed communities of aquatic insects, such as caddisflies, that act as intermediate

hosts of trematodes such as *Paralecithodendrium* spp. and *Acanthatrium* spp. (Etges 1960), are also structured with regard to local environmental conditions, such as stream size, temperature, water flow, substratum type and macrophyte cover, rather than spatial processes (Mykra et al. 2007, Landeiro et al. 2012, Heino et al. 2013). These intermediate host communities are strongly influenced by environmental processes in the presence of urbanization and the drivers structuring these communities shift from variables such as stream size and water flow to proximity to roadways (Galbraith et al. 2008), levels of anthropogenic organic matter (e.g. sewage sludge; Johnson et al. 2009, Arimoro et al. 2012), eutrophication (Couceiro et al. 2007, Trigal et al. 2009), altered pH (Clements 2006, Couceriro et al. 2007), and changes in land use (Couceiro et al. 2007, Galbraith et al. 2008, Johnson 2009). Thus, intermediate host community structure changes with urbanization, which, in turn, changes parasite communities within definitive hosts.

Fine-scale spatial structure did influence compositional dissimilarity among infracommunities even though environmental variables consistently explained the most variation. Infracommunities from the same site clustered together, while infracommunities from different sites did not group together based on geography and there was a lack of broad-scale spatial structure. Bats are central-place foragers, with members of the same colony leaving the shared roost-site, dispersing across the landscape to forage, and then returning to the roost. Although they may fly up to 12 km from the roost site, individual big brown bats typically fly within several kilometers of their roost during foraging bouts (Kurta and Baker 1990). Thus, their typical foraging home range will likely be small relative to the scale over which there is significant environmental variation. Individuals from the same colony are likely to be exposed to the

same set of habitats and environmental conditions over the course of a season, thus individuals from the same colony are likely exposed to the same parasite species pool and share similar parasite infracommunities.

I found evidence of a latitudinal trend in which component communities that were close to each other along a north-south gradient were more taxonomically similar than more distant communities. Species richness and taxonomic distinctiveness of parasite communities can change along a latitudinal gradient that reflects changes in climate, and, as with compositional dissimilarity, environmental gradients can shape spatial structure in parasite community taxonomic dissimilarity (Krasnov et al 2005, Vinarski et al 2007) through effects on abundance of intermediate hosts or parasites (e.g. Krasnov et al. 2005, Thielges et al 2010). However, our analyses indicated that climatic variables did not significantly influence taxonomic dissimilarity among sites, and it is likely that some other correlate of latitude other than climate itself produced the pattern I observed. There was not a broad-scale pattern in taxonomic dissimilarity nor significant variation explained by longitude, and hence it is unlikely that dispersal-related processes are driving the observed pattern of latitudinal variation only. Processes related to environmental variables (e.g. climate, rainfall) influence spatial taxonomic composition of highly vagile invertebrates, like the flying insects that bats prey upon, much more strongly than dispersal ability (Bonada et al. 2012). Certain genera in our dataset, especially *Paralecithodendrium*, exhibited higher prevalence at northern sites and I suspect that factors influencing intermediate host assemblages have downstream effects on taxonomic dissimilarity of parasite communities in definitive hosts. A number of insect orders that have aquatic stages and may act as helminth intermediate hosts (Bray et

al. 2008), such as Ephemeroptera, Plecoptera, and Trichoptera, exhibit peaks of global richness in northern latitudes, especially around 40^{o}N, due to favorable conditions (e.g. hydrology, winter temperatures, precipitation; Boyero et al. 2011, Pearson and Boyero 2009, Vinson and Hawkins 2003). Our northern sites clustered between 41^{o}N and 42^{o}N, very close to this peak and a wider pool of intermediate hosts that results in a wider pool of helminth taxa at these latitudes could account for our latitudinal trend in taxonomic dissimilarity.

In our host-parasite system, non-spatial variables, especially landcover, strongly influenced both compositional and taxonomic parasite community dissimilarity over multiple community scales. I found little evidence for spatial processes such as distance decay of community dissimilarity, and the evidence of latitudinal variation in taxonomic component community dissimilarity could ultimately result from latitudinal variation in environmental characteristics rather than dispersal-related processes. Our investigation was unique in that I simultaneously quantified the relative contribution of both non-spatial and spatial variables to parasite community dissimilarity. Landcover, especially those categories marked by anthropogenic disturbance, played a critical role in shaping the amount of dissimilarity in these parasite communities. Given that urbanization can change the presence and abundance of disease vectors within a given area (Bradley and Altizer 2007) and alter parasite transmission by changing hosts' exposure to infective propagules (Taylor and Merriam 1996) I must continue to examine this anthropogenic impact at various scales in host-parasite systems. Landscape changes, such as deforestation, riparian disturbance, and agricultural practices, readily modify the transmission dynamics of helminths and other pathogens (Bonett et al. 2011, Davidson

1991, Molyneux 1998, Hulbert and Boag 2001, Schotthoefer et al. 2011, Sehgal 2010), and these changes ultimately alter environmental processes that control parasite communities.

Even though I investigated many non-spatial and spatial variables, I could only quantify a relatively small amount of the variation present in our infracommunity datasets (12.6% for compositional infracommunity data and 7.3% for taxonomic infracommunity data as compared to over 50% for compositional component community data). Although community structure could be more stochastic at the infracommunity level, perhaps other factors, such as host characteristics (e.g. host habitat use, genetic distance; Locke et al. 2012, Locke et al. 2013), exert control over dissimilarity in these infracommunities. I investigated effects of sex and age at both compositional and taxonomic levels but found that neither explained dissimilarity in our infracommunities, thus suggesting that these characteristics do not influence the diversity of helminth infections in big brown bats. In the case of these infracommunities, future work should shift its focus to greater consideration of environmental conditions that exist inside of the host and their influence on parasite establishment within the host environment. More detailed assessments of variation between hosts, such as genetic heterozygosity (e.g. Coltman et al. 1999), especially at immunogenetic loci (e.g. Schad et al. 2005), and immune function (e.g. Galvani 2003), could ultimately better explain differences in infracommunity dissimilarity than either external environmental conditions or spatial processes.

CHAPTER II

THE ROLES OF HOST SEX, BODY CONDITION, AND IMMUNE FUNCTION IN PREDICTING HELMINTH BURDENS OF BIG BROWN BATS (*EPTESICUS FUSCUS*)

Introduction

Sex-biased parasitism highlights the potential for sexually dimorphic approaches to host fitness that result in differing energetic trade-offs for males and females. Parasites use hosts as patches of favorable habitat; however, hosts are not passive and may attempt local extinction of their parasites by mounting an energetically costly immune response. Resources that might otherwise be used for self-maintenance or reproduction must be transferred into immune investment (Lochmiller and Deerenberg 2000, Schmid-Hempel and Ebert 2003, Moreno-Rueda 2011). Thus, host fitness could decrease due to increasing energy allocation to immunity in response to parasites (Sheldon and Verhulst 1996, Hanssen et al. 2003, Hasu et al. 2006, Honkavaara et al. 2009, Allen and Little 2011, Gooderham and Schulte-Hostedde 2011). Although faced with similar challenges, the sexes may approach the consequences of parasitism with differential investment of their resources in order to attain their own optimal fitness outcome.

Males are often expected to harbor more parasites than females, either due to higher exposure to parasites and/or greater susceptibility to parasites (Moore and Wilson 2002). Males are larger than females in many vertebrate species, making them larger targets for arthropods and anthropod-vectored parasites (Moore and Wilson 2002). Males often have larger home ranges due to foraging or mate-seeking behaviors and engage in riskier behavior than females resulting in higher parasite exposure (Tälleklint-Eisen and Eisen 1999, Ferrari et al. 2004, Hillegass et al.

2008, Perkins et al. 2008). Conversely, exposure rates for males and females could be similar but parasites establish more readily in males due to some intrinsic characteristics that impact host susceptibility. The immunocompetence handicap hypothesis, a possible mechanism for male-biased parasitism, contends that production of androgen steroid hormones suppresses immune responses that would normally protect against infection (Folstad and Karter 1992, Poulin 1996). Male-biased parasitism occurs in multiple vertebrate taxa (Moore and Wilson 2002) and there is some support for androgen suppression of immunity (Roberts et al. 2004); however, male-biased parasitism is not universal. Some female hosts are more heavily infected than their male counterparts (Nakazawa et al. 1997, Khan et al. 2010, Rossin et al. 2010), infection bias between the sexes can vary widely with differing host-parasite systems (Zuk and McKean 1996, Kurtz et al. 2000, Rolff 2001, MacIntosh et al. 2010, Robinson et al. 2010, Arizza et al. 2013, Kiffner et al. 2013, Waterman et al. 2014), and the immune response could suppress testosterone production instead due to trade-offs between male investment in self-maintenance or reproductive effort (Degen, 2006, Marzal et al. 2007, Boonekamp et al. 2008, Cai et al. 2009, Mills et al. 2009, 2010). Thus, sex differences in immune function could result from differences in life histories and sexual behavior with males investing more resources to mating success rather than immunity while females invest more energy into immunity to increase survival (Rolff 2002, Zuk 2009, Nunn et al. 2009).

If energy limits immune function and reproduction or growth, then investment into parasite tolerance, rather than investment into parasite resistance could be an optimal strategy. Resistance involves energetic investment into a costly immune response but it ultimately increases host fitness by eliminating parasites (Bordes et al. 2012). Conversely, tolerance is less costly in terms of energy because it does not require parasite elimination but rather the host maintains fitness by

limiting pathology caused by parasites and prioritizing self-maintenance (Bordes et al. 2012). Thus, limited host resources create energetic trade-offs that may favor immune responses when body condition is high (resistance) or limit the immune response if body condition is low (tolerance; Norris and Evans 2000, Martin et al. 2006, 2008, Ujvari and Madsen 2006, Forsman et al. 2008). Males and females regularly exhibit differing investment into life-history traits such as offspring size and number (Kindsvater and Alonzo 2014), lifespan and reproductive status (Lee et al. 2014), nutritional status and immunocompetence (Kelly et al. 2014), and immunity and reproduction (McNamara et al. 2013). It is plausible that males and female address parasitic infection differently, with one sex preferring resistance over tolerance or vice-versa, to optimize individual fitness.

Sexually dimorphic approaches that result in either resistance or tolerance may result in differing fitness trade-offs in response to parasitic infection. Therefore, our investigation focused on the relationships between parasite burden, sex, immune function, and body condition within a wild mammal population. I used the big brown bat (*Eptesicus fuscus*) and its helminths as our model host-parasite system. Helminths are easily counted discrete units that spend their adult lives in one single host. Big brown bats are relatively common, widely distributed throughout most of North America, and form aggregations in buildings or trees during the summer (Kurta and Baker 1990). Males may be found in the same roosts as females or may roost solitarily. These bats exhibit little physical sexual dimorphism, although females tend be slightly larger than males (Ralls 1976); however, male and female bats differ in their foraging behavior (Kurta 1995). During pregnancy and lactation, female bats will forage heavily throughout the night as they require much more energy during this period (Kurta et al. 1990) while males will often forage for much shorter periods 1-2 hours after sunset (Altringham 1996, Kurta 1995, Neuweiler

2000). Given that bats often contract helminths via ingestion of infected prey (Kumar 1999, Schell 1985) typical foraging behaviors would put female bats at higher risk of helminthiasis than males. Thus, our overall goals were to determine if: 1) sex-biased parasitism occurred in our host-parasite system, and 2) the sexes were investing equally into parasite resistance or parasite tolerance by analyzing their immune responses (a measure of resistance) and body condition indices (a measure of tolerance). I predicted that: 1) if sex-biased parasitism occurs in our system, females rather than males would bear heavier worm burdens, due to elevated exposure, and 2) females will invest more resources into parasite resistance whereas males will employ tolerance. Ultimately, uncovering these relationships would allow us to determine if one sex disproportionately maintains helminth infection in a host population and if the sexes cope with parasitic infection differently, possibly due to sex-specific pressures in either exposure to parasites or establishment of parasites within the host.

Materials and Methods

Host and Parasite collection

One hundred forty *E. fuscus* (75 females, 65 males) were captured from 7 colonies in Michigan, USA (Figure 8) with mist nets as they emerged from their roosts or were hand-caught in the roost prior to nightly emergence. Bats captured in the same roost were considered to belong to the same site. Colonies 2 through 6 were sampled a single time in 2008. Most colonies in were subsequently sampled twice per year for two years (2009), with the exception of site 1 which was sampled a single time because bats were excluded from the roost by the landowner after initial sampling.

Figure 8. Seven study sites located in the lower peninsula of Michigan.

I recorded host sex, host age, capture date, and capture location via handheld GPS for all captured bats. I also recorded mass and right forearm length for each individual host. Bats were euthanized by cervical dislocation followed by exsanguination via cardiac puncture, and then were frozen at -20°C. Helminth burden was then assessed by necropsy after and thawing overnight in a 4°C refrigerator. The entire carcass and viscera were examined separately using a stereoscope. Trematodes and cestodes were counted, collected, stained with Semichon's acetocarmine, mounted, and examined with a compound light microscope. Nematodes were stored in glycerine-alcohol, cleared, and examined as temporary mounts with a phase-contrast microscope. Then, worms were identified using dichotomous keys (Schmidt 1970, Bray et al. 2008, Anderson et al. 2009) and original species descriptions.

Assessing parasite resistance

I used three immune assays as proxies for parasite resistance: bactericidal assay, lytic assay, and agglutination assay. After obtaining blood from cardiac puncture, it was used in a bacterial killing assay as described by Millet et al. (2007). To perform this procedure, two tubes of 1.5 µL of fresh whole blood from each bat were diluted with a combination of 135.7µL of CO_2-independent medium (Gibco-Invitrogen) and 2.8µL of 200 micromolar L-glutamine (Sigma) that

was warmed to 40°C. I used two bacterial strains, *Escherichia coli* (ATCC 8739) and *Staphylococcus aureus* (ATCC 6538), reconstituted in sterile phosphate buffered saline from lyophilized pellets (Epower Assayed Microorganism Preparation, Microbiologics Inc.) following the manufacturer's instructions to determine the bactericidal activity of blood leukocytes. After reconstitution, 10µL of *E. coli* or *S. aureus* (approximately 200 bacteria) were each added to an appropriately labeled tube containing the blood/CO_2-independent medium/ L-glutamine solution and the tubes were vortexed. Then, 50µL of the resulting solution were plated onto an LB agar plate under a horizontal flow clean bench (EdgeGUARD HF, The Baker Company). After initial plating (T_0), the blood/CO_2-independent medium/ L-glutamine/bacteria solution was allowed to incubate at room temperature for 30 min in order to allow leukocytes in the blood to kill bacteria. Then, the plating procedure was repeated (T_{30}) and plates were incubated at 37°C for 24 hours. After incubation, the number of colony forming units on each plate were counted and a bactericidal index (antimicrobial activity = $1-(T_{30}/T_0) \times 100$) was calculated for each individual bat.

Whole blood was then centrifuged to separate plasma from red blood cells (RBCs). This plasma was used to assess lysis and agglutination by performing hemolysis/hemagglutination assays following a technique similar to Matson et al. (2005) with a few modifications. In short, 25µL of plasma from each individual was pipetted into wells in the first and second column of a 96 well round-bottom polystyrene tissue culture plate (Costar 3790, Corning). Plasma in the first column remained undiluted and served as a positive control. Then, 25µL microliters of phosphate buffered saline (PBS) were then pipetted into the well in the second through twelfth columns of the plate. This created a 1:1 dilution of plasma to saline in the second well that was serially diluted through to the eleventh well. The twelfth well contained only PBS and served as

a negative control. After dilution, 25 microliters of washed, laboratory-grade rabbit RBCs (Hemostat Laboratories) were added to each well to serve as foreign antigen. Plates were incubated at 37^{o}C for 90 min and then scanned as a full size image at 600 pixels per inch using an Epson Perfection flatbed scanner to record lytic activity. After leaving the plate at room temperature for an additional 20 min I recorded agglutination using the same procedure. All digital images were then scored manually by the same individual to determine the relative roles of complement and native antibodies (NAbs) in functional immunocompetence. Individuals were scored as negative $\log_2$ of the last plasma dilution exhibiting each behavior with lysis reflecting the interaction of complement and NAbs while agglutination reflected the activity of NAbs only.

Assessing parasite tolerance

Parasite tolerance can be illustrated as the slope of the relationship between parasite burden and some host fitness characteristic (Bordes et al. 2012). A steep slope indicates low tolerance because fitness declines sharply as worm burden increases; however, a shallow slope indicates high tolerance because fitness declines slowly as worm burden increases. I did not have access to information about host reproductive fitness; therefore, I used body condition as a measure of health and a proxy for fitness following Bordes et al. (2012). I employed two methods to determine body condition: body mass to forearm length index and neutrophil to lymphocyte (N:L) ratio. Body condition index was calculated as the residual of as significant ordinary least squares regression analysis ($p<0.0001$) of right forearm length on mass (Schulte-Hostedde et al. 2005, Pearce et al. 2008). Therefore, individuals with a negative index value possessed lower body condition for their size whereas individuals with a positive index value enjoyed higher body condition for their size. The ratio of neutrophils to lymphocytes provides useful information on host condition with higher ratios indicating increased physiological stress (Davis

et al. 2008). To determine N:L ratio whole blood obtained from cardiac puncture was used to make at least two blood smears from each individual bat. These blood smears were examined via light microscopy to assess number and type of circulating leukocytes and the ratio of neutrophils to lymphocytes was calculated (Superina and Sierra, 2008). As helminth burdens are count data that included zeros, I applied a $\sqrt{(x+1)}$ transformation to normalize the data. I then plotted N:L ratios and body condition values against transformed worm burden to obtain a best-fit line for males and females. I compared the slopes of these lines via ANCOVA using the aov command in R. If these slopes differed significantly, then one sex was judged to have significantly greater parasite tolerance than the other.

Model building procedure

In addition to analyzing sexual dimorphism in host parasite resistance and tolerance, I wanted to model overall host worm burdens as functions of host sex, resistance, and tolerance. To determine which variables best predicted worm burden I utilized the generalized linear models function in SPSS 17.0 to create candidate models via maximum likelihood estimation. As our data were overdispersed our models used a negative binomial distribution, log link, and a dispersion parameter, which describes a spread of values around the central tendency, estimated at 2.3. Candidate models included combinations of all predictor variables and all interactions between them. I used sex, lysis, agglutination, body condition index, and N:L ratio as predictors of worm burden; I also included year of capture to account for possible temporal variation in worm burden. As multicollinearity was a concern, I confirmed that variance inflation factors for each term were lower than two. I also used an intercept-only model as a null model to provide a reference for our candidate models. The best-fit models were identified using Akaike's

information criterion (AIC; Mazerolle 2006) and then independently confirmed for fit using the omnibus log-likelihood test.

If any significant interactions consistently appeared in our best-fit models, I used the SPSS macro PROCESS (Hayes 2013) to perform tests for moderation in order to uncover the relationships between interacting variables. Moderation is the combined effect of two variables, a predictor and a moderator, on an outcome variable (Figure 9). Tests for moderation essentially use linear regression; however, the data are centered, or transformed into deviations around the mean (Field 2013). This allowed us to test for significant relationships between the predictor and the outcome variables with different values of the moderator. Thus, I could determine if the significance of the relationship between predictor and outcome variables changed as the moderator increased or decreased. Again, I applied a $\sqrt{(x+1)}$ transformation to normalize overdispersed helminth burdens prior to performing tests of moderation.

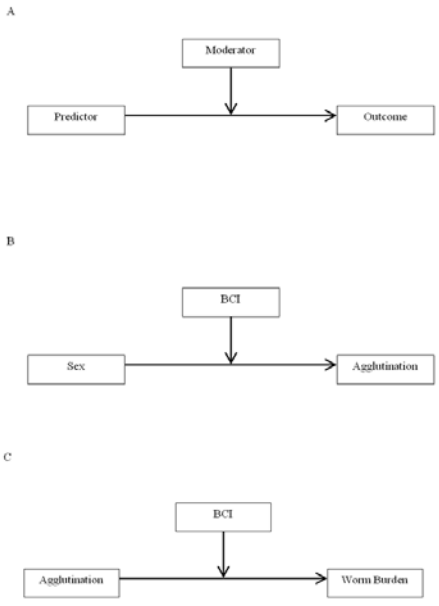


Figure 9. Models of moderation. Comparison of a conceptual model (A) of moderation with proposed models of body condition acting as a moderator between sex and agglutination (B) as well as between agglutination and worm burden (C).

Results

Digenetic trematodes from the family Lecithodendriidae were by far the most common helminths in our study (Table 7). Some lecithodendriids had relatively high prevalence (e.g. *Paralecithodendrium naviculum*, *P. transversum*) while others had very low prevalence (e.g. *Acanthatrium microacanthum*, *Glyptoporus noctophilus*). I indentified only one non-lecithodendriid trematode (*Plagiorchis vespertilionis*) as well as only one cestode (*Hymenolepis roudabushi*) and one nematode (*Litomosa americana*). These three species had relatively low prevalence, mean intensity, and mean abundance. Mean intensity and mean abundance were highest among species belonging to *Paralecithodendrium* with *P. naviculum* exhibiting the highest mean intensity and *P. macnabi* having the highest mean abundance.

I found no evidence for sex-biased parasitism in our system. Male and female hosts had comparable worm burdens (Figure 10). The sexes also had no significant difference in lysis scores (Figure 11), N:L ratios (Figure 12), body condition indices (Figure 13), and antimicrobial activity (Figure 14). However, females possess significantly higher agglutination scores (Figure 15) suggesting that females invested more in that measure of parasite resistance than males. I also discovered that males and females did not significantly differ in parasite tolerance. Slopes for male and female body condition index ($F_{1, 136} = 0.700$, $p = 0.404$) or N:L ratio ($F_{1, 136} = 0.696$, 136, $p = 0.405$) in relationship to worm burden did not decline at significantly different rates (Figure 16). Individual hosts with high body condition regardless of sex, possessed significantly lower worm burdens ($F_{1, 138} = 4.331$, $p = 0.04$) and agglutination scores ($F_{1, 138} = 5.086$, $p = 0.026$) however, they did not possess higher lysis scores ($F_{1, 138} = 1.130$, $p = 0.29$)

Table 7. Taxonomic designation, prevalence, mean intensity, and mean abundance of helminths infecting *E. fuscus*. Note that standard error (SE) terms accompany both mean intensity and mean abundance.

Species	Higher Taxonomy	Prevalence	Mean Intensity (±SE)	Mean Abundance (±SE)
A. eptesici	Trematoda: Lecithodendriidae	10.64%	1.30 (±0.80)	12.27 (±7.09)
A. microacanthum	Trematoda: Lecithodendriidae	2.13%	0.15 (±0.09)	7.00 (±0.63)
A. pipestrelli	Trematoda: Lecithodendriidae	3.55%	0.32 (±0.15)	9.00 (±0.79)
P. macnabi	Trematoda: Lecithodendriidae	5.67%	2.48 (±1.22)	38.89 (±4.83)
P. naviculum	Trematoda: Lecithodendriidae	38.30%	9.84 (±2.43)	25.25 (±3.89)
P. swansoni	Trematoda: Lecithodendriidae	8.51%	2.32 (±1.55)	27.25 (±17.22)
P. transversum	Trematoda: Lecithodendriidae	43.97%	3.66 (±0.77)	8.46 (±1.57)
G. noctophilus	Trematoda: Lecithodendriidae	2.13%	0.19 (±0.13)	9.00 (±3.61)
Pl. vespertilionis	Trematoda: Plagiorchiidae	14.18%	0.62 (±0.26)	4.35 (±1.66)
H. roudabushi	Cestoda: Hymenolepididae	9.22%	0.52 (±0.28)	5.62 (±2.76)
L. americana	Nematoda: Dipetalonematidae	9.93%	0.13 (±0.34)	1.29 (±0.16)

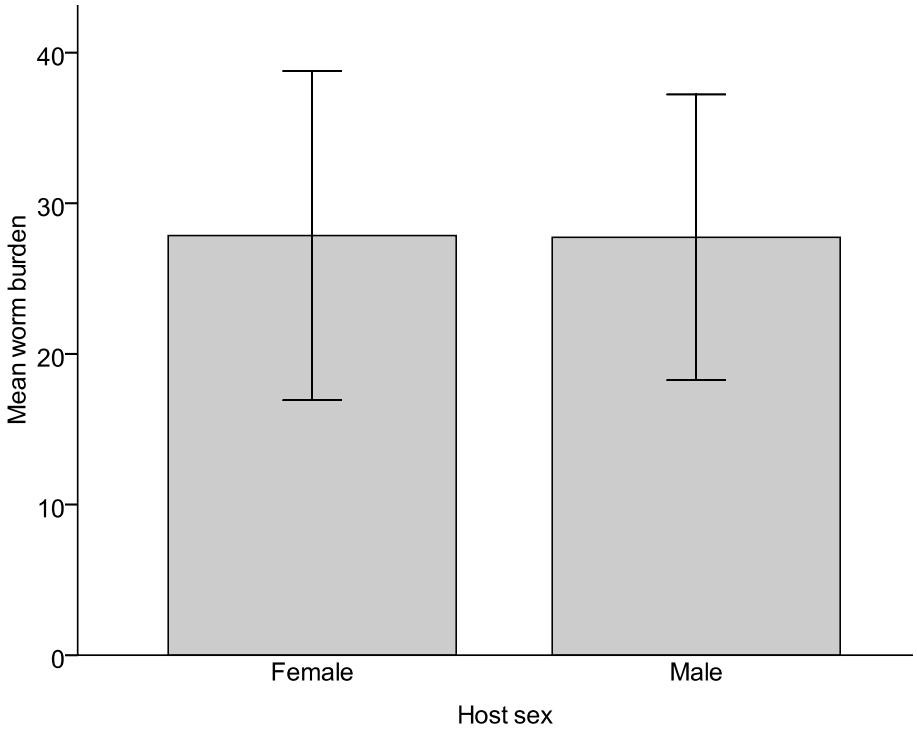


Figure 10. Mean number of worms per host did not differ significantly for male and female hosts. Error bars represent 95% confidence intervals.

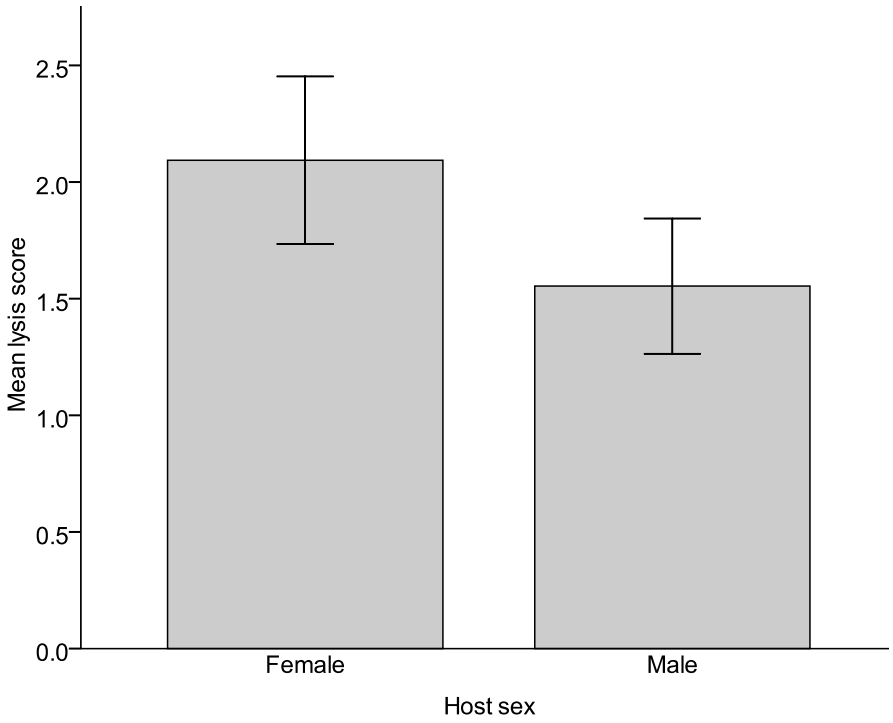


Figure 11. Mean lysis score did not differ significantly for male and female hosts. Error bars represent 95% confidence intervals.

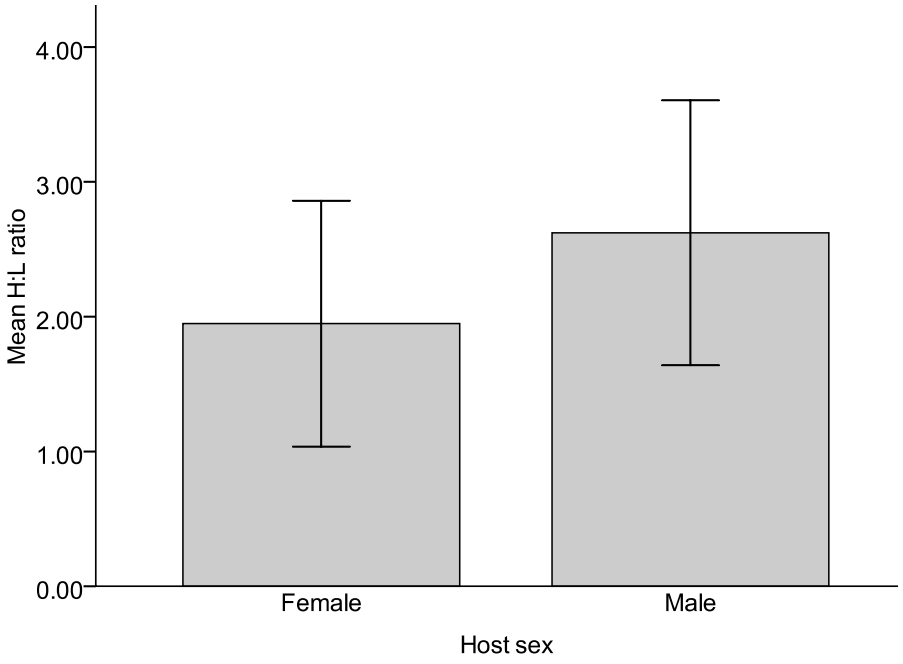


Figure 12. Mean N:L ratio was not significantly different for males and females. Error bars represent 95% confidence intervals.

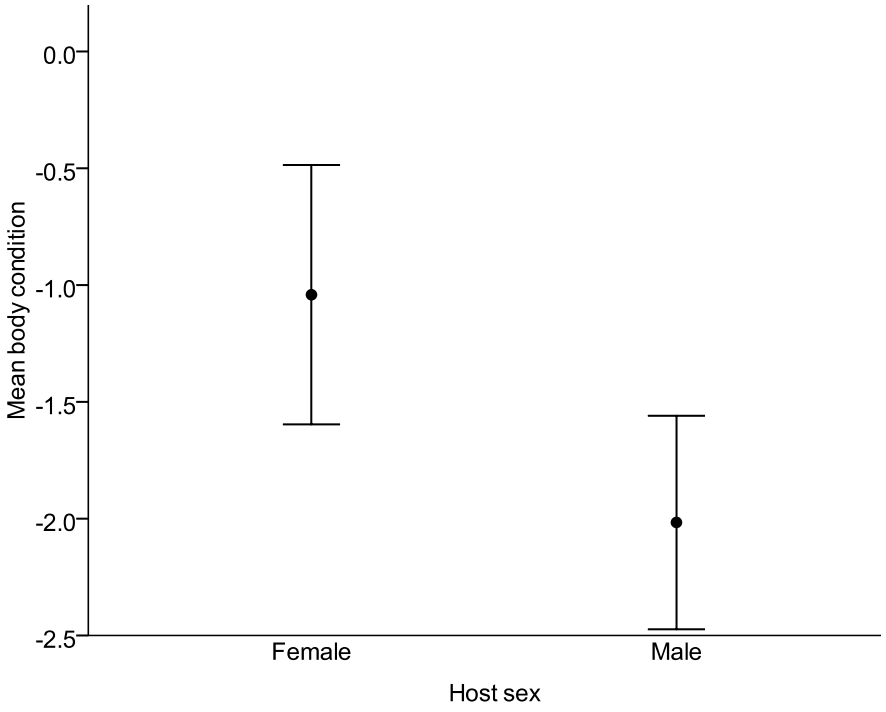


Figure 13. Females had slightly higher but non-significant mean body condition compared to males. Error bars represent 95% confidence intervals.

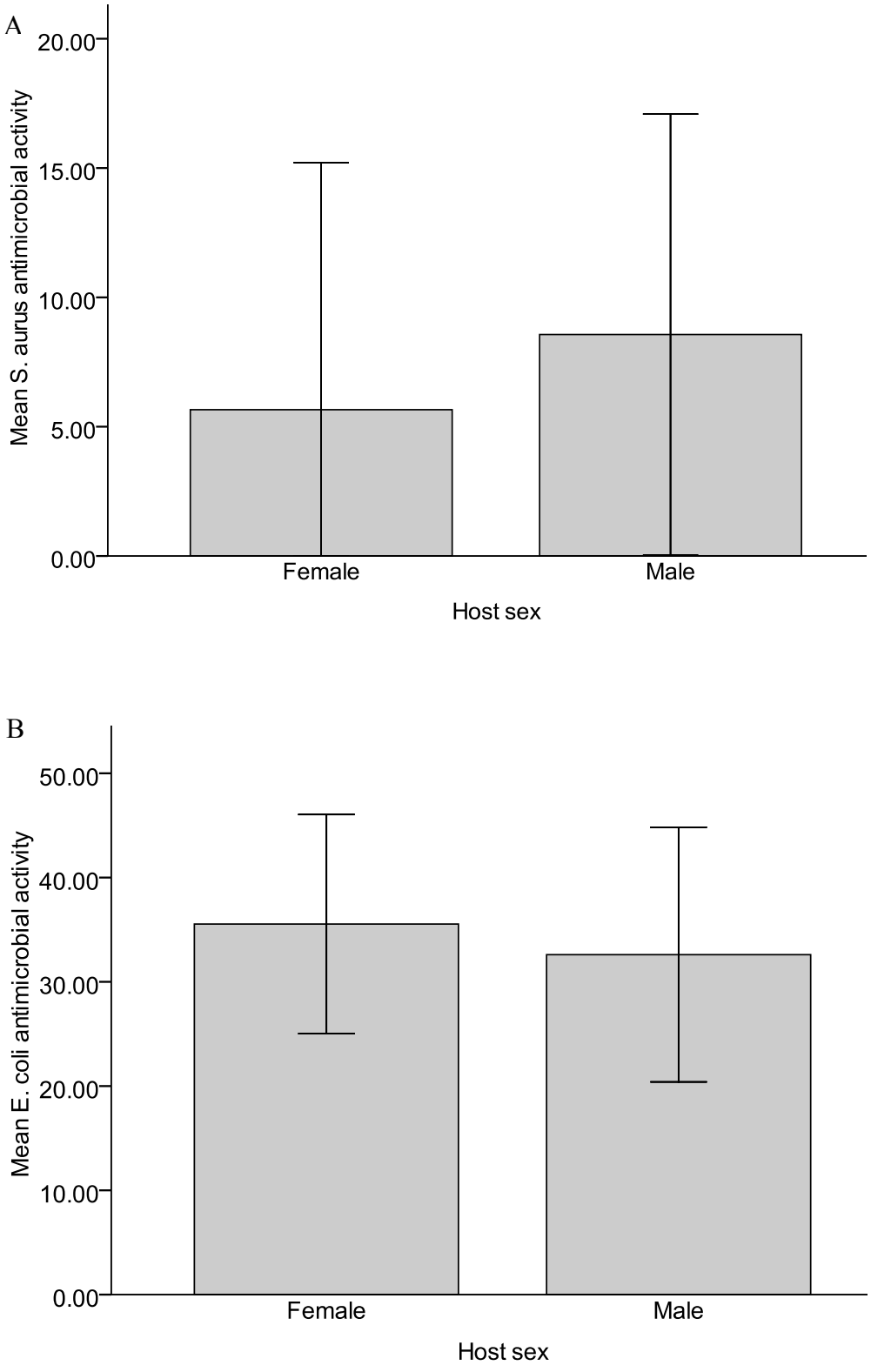


Figure 14. Males and females did not differ significantly in mean antimicrobial activity for either *S. aureus* (A) or *E. coli* (B). Error bars represent 95% confidence intervals.

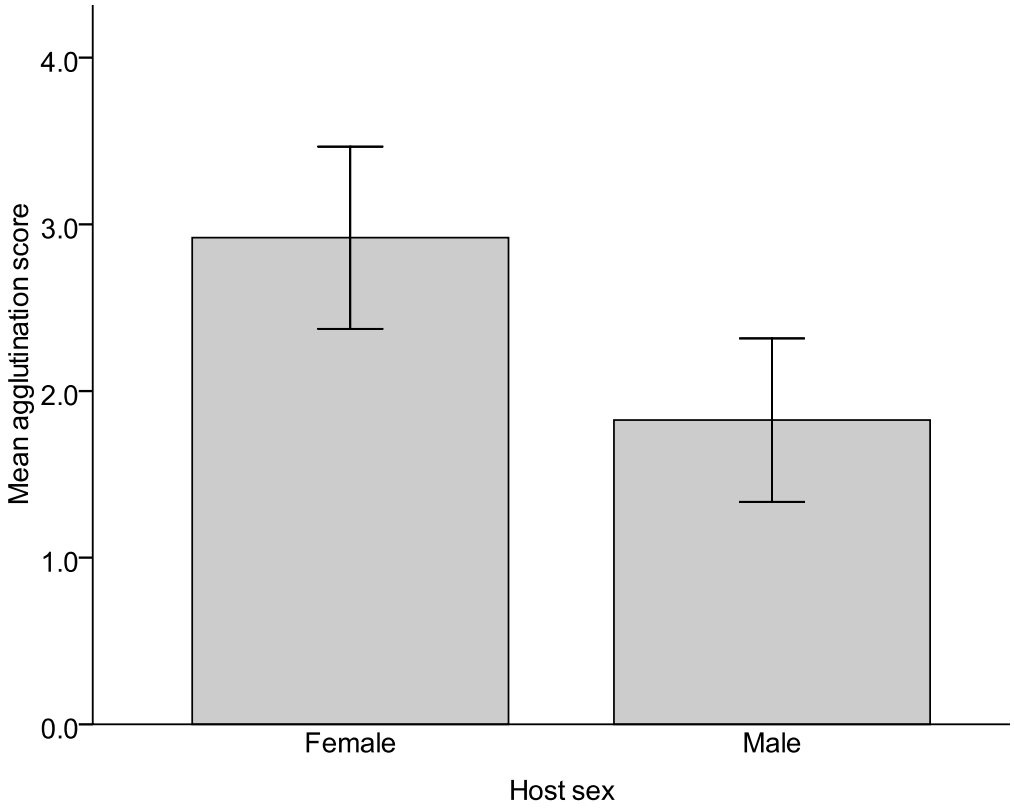


Figure 15. Mean agglutination score was significantly higher for females than males. Error bars represent 95% confidence intervals.

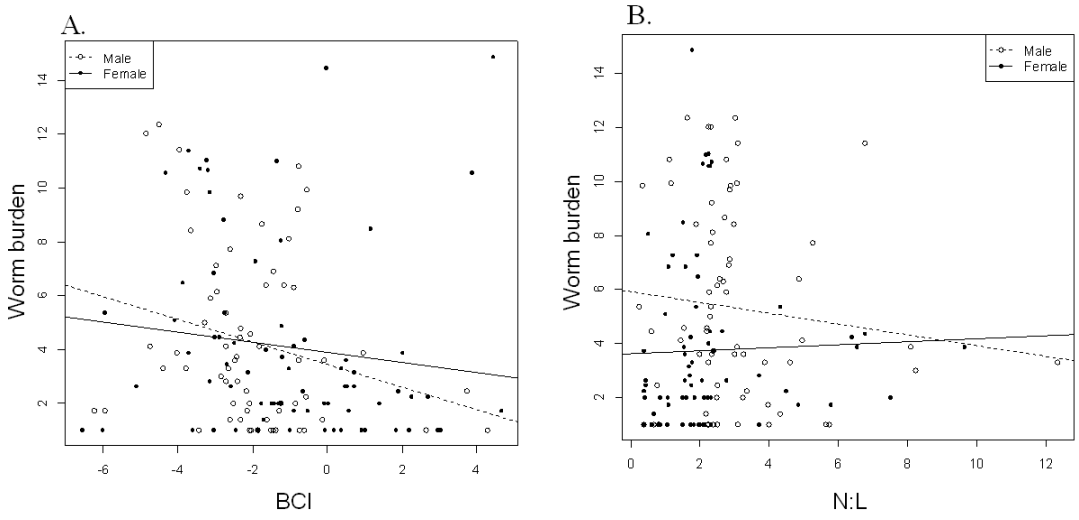


Figure 16. Slopes indicating tolerance via BCI (A) and N:L ratio (B) did not significantly differ between males and females. Note that the √(x+1) transformation was applied to normalize worm burden.

Five models of helminth burden had better fit than the intercept-only model (Table 8). All five models included agglutination, body condition index, lysis, and year as well as the interaction between agglutination and body condition index. In addition, four of the five models included host sex and three of the five included the interaction between agglutination and sex. For top candidate model, the coefficient for year ($b = -0.773$) indicated that bats caught in earlier (e.g. 2008) had heavier worm burdens than bats caught later (e.g. 2010). The coefficient for lysis score ($b = -0.360$) indicated that individuals with lower lysis scores also had higher worm burdens.

To understand interactions between sex, BCI, and agglutination as well as their influence on worm burden I performed two tests for moderation. Given the best-fit models, I proposed that BCI could be moderating the effect of host sex on agglutination (Figure 9B). I determined that significance of the conditional effect of sex on agglutination score changed depending on the values for BCI (Table 9). Body condition indices 2.22 standard deviations below the mean had no significant effect ($p = 0.0605$); however, body condition indices at the mean ($p = 0.0027$) or 2.22 standard deviations above the mean had a significant conditional effect of sex on agglutination score ($p = 0.0251$). I proposed that, while holding the effect of sex constant, BCI could moderate the effect of agglutination score on worm burden (Figure 9C). I determined that significance of the conditional effect of agglutination score on worm burden changed depending on the values for BCI (Table 10). Body condition indices at the mean ($p = 0.2269$) or 2.24 standard deviations below the mean ($p = 0.8423$) had no significant effect; however, body condition indices from 2.24 standard deviations above the mean had a significant conditional effect ($p = 0.0132$) of agglutination score on worm burden.

Table 8. Terms and AIC values of the top five candidate models compared to the intercept-only model. Note that agglutination, body condition (BCI), lysis, and year, as well as the interaction between agglutination and BC, appeared in all five top models. All models had an omnibus log-likelihood p-value of 0.001.

Model Terms								AIC
Agglutination	BCI	Lysis	Sex	Year	Agglutination*BCI	Agglutination *Sex		1089.588
Agglutination	BCI	Lysis	Sex	Year	Agglutination*BCI			1089.872
Agglutination	BCI	Lysis	Sex	Year	Agglutination*BCI	Agglutination *Sex	Lysis*Sex	1089.978
Agglutination	BCI	Lysis	Sex	Year	Agglutination*BCI	Agglutination *Sex	Agglutination*Sex*BCI	1090.073
Agglutination	BCI	Lysis		Year	Agglutination*BCI			1090.637
Intercept-only								1101.455

Table 9. Results from the test for moderation of body condition index on the relationship between host sex and agglutination at the mean as well as $^{+}$/-2.22 standard deviations from the mean.

BCI values	Effect	Standard Error	*t*	*p*-value
-2.22 S.D.	0.97	0.51	1.89	0.0605
Mean	1.08	0.35	3.05	0.0027
2.22 S.D.	1.19	0.52	2.26	0.0251

Table 10. Results from the test for moderation of body condition index on the relationship between agglutination and worm burden at the mean as well as $^{+}$/-2.24 standard deviations from the mean.

BCI values	Effect	Standard Error	*t*	*p*-value
-2.24 S.D.	-0.06	0.31	-0.20	0.8423
Mean	-0.23	0.18	-1.22	0.2269
2.24 S.D.	-0.39	0.15	-2.51	0.0132

Discussion

I found that no evidence for sex-biased parasitism in our host-parasite system. Male and female hosts had similar worm burdens with females experiencing slightly heavier infections. I also found no evidence for sex differences in parasite tolerance, and female bats scored higher than males in only one measure of parasite resistance. Our top models of helminth burden suggested that a BCI—agglutination score interaction and an agglutination score—sex interaction were driving the presence of sex in four of the five

models' main effects. Moreover, our discoveries that BCI was moderating the effect of sex on agglutination score and that agglutination score on worm burden support this contention. Lysis score, another measure of parasite resistance, was also present in all five top models and without interactions in four of those five models. Lysis score did not differ significantly between the sexes or interact with measures of body condition in any of the top models. This suggests that individuals could be investing in different facets parasite resistance of the immune response based not on sex but on body condition. All individuals invested in lysis while only those with higher body condition invested resources in agglutination. Immune system trade-offs are well-documented (Mallon et al. 2003, Schmid-Hempel 2003, Downs et al. 2013); therefore, individuals could invest in different facets of the immune system, depending on their body condition and the relative costs of each strategy, that optimize their fitness at that particular moment.

Mounting an immune response requires energy (Muehlenbein et al. 2010). Lysis is an evolutionarily ancient method of parasite resistance that pre-dates adaptive immunity (Fujita et al. 2004); therefore, it could be a default resistance strategy, regardless of body condition. Complement is a key component of lysis score; however, investment in complement-mediated responses, especially the alternative complement pathway, is less costly than antibody-mediated defenses (Lee et al. 2008). Complement readily recognizes and eliminates pathogens via either direct killing by rupturing foreign cell membranes or initiating phagocytosis of foreign cells by other immune cells (Chen et al. 2009). Complement proteins are an especially important element of host the host innate immune response to helminths because they aid in leukocyte-mediated immunity. These proteins mediate recruitment of leukocytes to the target by generating chemotactic

factors C3a and C5a, then promote leukocyte attachment by depositing factors C3b and iC3b on the surface of the helminth (Gasque et al. 2004, Giacomin et al. 2004). Complement proteins then initiate anti-helminth defenses via immune effector cell functions like eosinophil-mediated killing, mast cell degranulation, phagocytosis, and antigen presentation (Meeusen and Balic 2000).

Employing agglutination in addition to lysis could be a more effective strategy if individuals have more energy available for immune investment and require more than a default method of parasite resistance. Unlike lysis, agglutination depends on the ability of NAbs, essentially non-adaptive immunoglobulins, to recognize foreign antigens and mark them for phagocytosis by other immune cells (Boyden 1965). Despite being part of the innate immune response, NAbs are strongly associated with B-cell functions necessary for acquired immunity, possibly because of a common genetic mechanism (Palacios et al. 2012). Therefore, NAbs, like other immunoglobulins, could be more energetically expensive than other components of innate immunity, such as complement. Considering that trade-offs between facets of the immune system become increasingly apparent as body condition deteriorates (Muehlenbein et al. 2010) and body condition itself can influence the type of immune response a host deploys (Russo and Madec 2013), infected individuals may have a suite of ideal defenses that differ depending on their energy reserves. Thus, all hosts may be able to invest in lysis while only a host with good body condition could invest in agglutination.

Higher male exposure to parasites or energetic trade-offs between the production of testosterone and immune defenses may explain male-biased parasitism (Moore and Wilson 2002, Schroderus et al. 2010); however, I found that male and female bats had

similar levels of helminthiasis. One possible caveat, at least in our host-parasite system, could be that males are exposed to fewer helminths than females. Female big brown bats typically forage more than males because they undergo pregnancy and lactation; thus requiring more food (Kurta and Baker 1990). All but one of the helminths in our study (*L. americana*) is acquired by ingestion; therefore, increased exposure rate could conceivably compensate for increased female immune defense. Conversely, male bats in our host-parasite system produce high amounts of testosterone (Mendonca et al. 1996) and increased female exposure could be counteracted by increased parasite establishment within males, if testosterone were immunosuppressive. There is no empirical evidence of higher female exposure rate or testosterone-mediated immunosupression in males for our host species; however, of both these topics warrant further study.

Overall, our investigation found no evidence for sex-biased parasitism in our host-parasite system. None of our data supported the contention that males invested more in parasite tolerance than females and only weakly supported increased female investment in parasite resistance. Instead, I discovered that individual body condition was more likely to influence parasite resistance. This suggests that overall host health or nutritional status, rather than sex, regulates trade-offs between self-maintenance and immunity. Thus, individuals with similar worm burdens could be managing their infections via different facets of the immune response depending on available energy reserves. Our results highlight the need for more investigation into interactions between sex, immune response, and body condition in order to better understand how individual hosts manage fitness trade-offs and respond to the pressures exerted by exposure to parasites or establishment of parasites

CHAPTER III

RELATIVE ROLES OF EXPOSURE AND ESTABLISHMENT IN PRODUCING PATTERNS OF PARASITE AGGREGATION IN BIG BROWN BATS (*EPTESICUS FUSCUS*)

Introduction

Variation in parasite burden among hosts is a key feature in transmission dynamics in most parasitic species (Anderson and May 1982, Anderson and May 1985a, Anderson and May 1985b). Parasites are usually aggregated in a host population and a minority of individuals bear the bulk of the parasitic burden, while most individuals have few or no parasites (Crofton 1971, Wakelin 1985, Sréter et al. 1994, Shaw and Dobson 1995, Shaw et al. 1998, Galvani 2003, Poulin 2004, Craig et al. 2007, Poulin 2007, Marques et al. 2010). More heavily-infected individuals introduce a disproportionate number of infective propagules into the host population, playing a vital role in maintaining a pathogens ability to reproduce and infect new hosts (Anderson et al. 1991, McCallum et al. 2001). These individuals may act as super-spreaders (cf. Lloyd-Smith et al. 2005) by disproportionately infecting other individuals in the population, leading to sharp increases in the frequency of infection within a population (Fujie and Odagaki 2007). Whether or not individuals will become infected with parasites and become super-spreaders will be determined by ecological and behavioral factors that determine exposure to infective stages along with intrinsic factors such as host immunocompetence and genetic diversity that influence susceptibility to infection (Galvani 2003, Craig et al. 2007). Clear understanding of factors that lead to heterogeneous exposure to and establishment of parasites among potential hosts is therefore critical for understanding variation in parasite burden, and hence transmission dynamics, among hosts.

Heterogeneous exposure assumes that, although all hosts are suitable for colonization, only a subset of the hosts encounter infective propagules. Individuals with higher activity rates or that range over a wider number of habitats could encounter more indirectly-transmitted parasites or their intermediate hosts (Tälleklint-Eisen and Eisen 1999, Ferrari et al. 2004, Hillegass et al. 2008, Perkins et al. 2008) and various social behaviors could also increase contact rates with directly-transmitted parasites among potential hosts (Lyons et al. 2001, Krkosek et al. 2007, Young et al. 2013). Variation in landscape characteristics, such as availability of water and landcover type, may shape parasite communities at both regional and local scales (Poulin and Mouillot 2003, Marques et al. 2006, Luque and Poulin 2007, Davies and Pedersen 2008) and often correlates with intraspecific and interspecific differences in parasite prevalence, abundance, and diversity (Esteban et al. 2001, Landry and Bernatchez 2001, Brooker et al. 2002, Wegner et al. 2003, Kabatereine et al. 2005, Schad et al. 2005, Kalbe and Kurtz, 2006, Knopp et al. 2008, Steinmann et al. 2007, Koroma et al. 2010, Hill et al. 2010, Siers et al. 2010). Similarly, exposure could vary temporally if the number of infective propagules that potential hosts encounter varies across seasons or years (Nickel and Hansen 1966, Blankenspoor and Ulmer 1972, Coggins et al. 1982, Getachew et al. 2008, Hamilton et al. 2009, Sabri et al. 2010).

Heterogeneous establishment of parasites assumes that all hosts encounter infective propagules but conditions within the host itself either promote or deter parasite establishment making some hosts more suitable for colonization than others. A host's ability to resist or tolerate infections depends on their energy reserves and trade-offs between mounting an immune response and other processes such as growth and reproduction. Host populations represent immunologically heterogeneous groups (Green et al. 2006) and immune function could covary with a number of other intrinsic characteristics such as host sex, age, body condition, and genetic

heterozygosity. Male and female hosts experience differing trade-off between reproduction and immune defense; therefore, these sexes could possess different worm burdens (Folstad and Karter 1992, Abu-Madi et al. 2005, Cowan et al. 2007, Stothard et al. 2009). Young or elderly individuals possess decreased immune function that could leave them more parasitized than reproductive adults (Abu-Madi et al. 2005, Brown et al. 2006, Craig et al. 2007, Eira et al. 2007, Hakkarainen et al. 2007). Individuals with poor condition have less energy to devote toward immunocompetence and are therefore more likely to be parasitized than conspecifics in good condition (Norris and Evans 2000, Irvine et al. 2006, Ujvari and Madsen 2006, Beldomenico et al. 2008, Forsman et al. 2008). Increasingly homozygous individuals are considered more parasitized than those that are genetically diverse due to decreased ability to recognize or destroy foreign antigens (Howard and Lively 1998, Coltman et al. 1999, Hedrick et al. 2001, Arkush et al. 2002, Acevedo-Whitehouse et al. 2003, 2006, Hawley et al. 2005, Luong et al. 2006, Whiteman et al. 2006, Charpentier et al. 2008, Ilmonen et al. 2008, Luikart et al. 2008, Rijks et al. 2008). Therefore, all these intrinsic host characteristics are potential predictors of worm burden.

Variation in parasite burden within a host population represents a combination of heterogeneity in parasite exposure and heterogeneity in parasite establishment produces. Exposure variables like host density and establishment variables like host age can significantly impact the abundance of directly-transmitted parasites in empirical studies (Abu-Madi et al. 2005). Exposure regime and intrinsic immune variation can produce variable parasite burdens within host populations in theoretical models of directly-transmitted helminths as well (Fox et al. 2013, Morrill and Forbes 2012). However, few empirical studies examine the relative roles of extrinsic variables, characteristics of the environment (e.g. habitat descriptors and temporal variables), as well as intrinsic variables, characteristics of the host itself (e.g. immune function

and genetic diversity), that likely influence parasite exposure and establishment. Here I utilize structural equation modeling with a multi-year, multi-region set of predictive variables to identify extrinsic and intrinsic variables associated with increased helminth burdens in the big brown bat, *Eptesicus fuscus* (Chiroptera: Vespertilionidae) and its helminths as a model system. This novel approach allowed us to model latent, unobservable variables by building indices from observed variables. Quantifying the mechanisms producing variation in helminth burdens allow us to identify which individuals are more likely to bear heavy infections and thereby influence parasite transmission dynamics. Extension of our methods into other host-parasite systems and subsequent identification of individuals with increased infection risk may allow for targeted interventions to manage disease in wildlife and human populations (e.g. Van Riper 1986, Daszak et al. 2000, Cleaveland et al. 2001, Schloegel et al. 2006, Hill et al. 2010, Alum et al. 2010). Further, as parasites have evolved within the ecological context of aggregation, uncovering the mechanisms behind this process would advance our understanding of general parasite biology (Poulin 2006).

Methods and Materials

Host-parasite system

The big brown bat, *E. fuscus*, is a large (15-24 g) insectivorous bats species found throughout temperate North America. Females aggregate in buildings or trees during the summer while males may be found in the same roosts as females or may roost solitarily (Kurta 1995, Altringham 1996, Neuweiler 2000, Lausen and Barclay 2006). Colony members typically forage for insects within the vicinity of the roost site (Kurta and Baker 1990); however, bats have been observed foraging 11-13km from their colony (Wilkinson and Barclay 1997, Arbuthnott and Brigham 2007). During pregnancy and lactation, female bats will forage heavily throughout the

night as they require much more energy during this period (Kurta et al. 1990). Conversely, males and non-reproductive females will often forage for much shorter periods, usually for one to two hours after sunset (Kurta 1995, Altringham 1996, Neuweiler 2000).

Although some bat nematodes are blood-borne (Esslinger 1973), *E. fuscus* typically becomes infected by helminths after ingesting an arthropod acting as an intermediate host. Most of these helminths are digenetic trematodes (Platyhelminthes: Trematoda) with a lifecycle that includes three hosts. Two of these hosts are likely aquatic: snails act as a first intermediate host while insects with aquatic stages act as a second intermediate host (Schell 1985, Kumar 1999). Helminths, in general, are valuable for tools of assessing infection risk because they are discrete units that can easily be counted. With other pathogens, like bacteria or viruses, an individual's infection status is either "infected" or "uninfected" and the actual pathogen burden is usually ignored (Fenton 2008). Thus, helminths allow for easy identification of hosts with heavy burdens without specialized techniques and may be more useful in assessing transmission dynamics within the host population than bacteria or viruses.

Host capture and helminth collection

Four hundred twenty-two *E. fuscus* (55% female, 45% male and 64% adult, 36% juvenile) were captured from 16 colonies in the midwestern USA (Michigan, Indiana, and Kentucky, Figure 17) with mist nets as they emerged from their roosts or were hand-caught in the roost prior to nightly emergence. Bats captured in the same roost were considered to belong to the same site. The majority of colonies in Michigan (2 through 6) were sampled a single time in 2008 (Table 11). Most colonies in were subsequently sampled twice per year for two years (2009-2010) with the exceptions of 1 and 15 which were sampled a single time only in 2009 because bats were excluded from their roosts by homeowners after initial sampling.

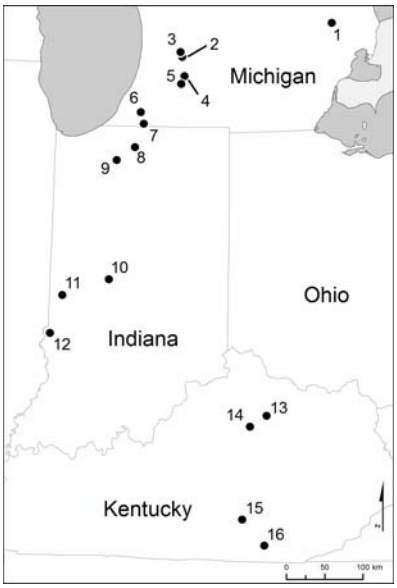


Figure 17. Sixteen study sites from three states.

Table 11. Sampling regime of study sites. Note that after 2009 bats were excluded by the homeowners from sites 1 and 15. Also, colonies not sampled in 2008 were unknown to us until 2009.

Site	Years Sampled
1	2009
2	2008, 2009, 2010
3	2009, 2010
4	2008, 2009, 2010
5	2009, 2010
6	2008, 2009, 2010
7	2008, 2009, 2010
8	2009, 2010
9	2008, 2009
10	2009, 2010
11	2009, 2010
12	2009, 2010
13	2009, 2010
14	2009, 2010
15	2009
16	2009, 2010

I recorded host sex, host age based on the ossification of wing phalanges, capture date, and capture location via handheld GPS for all captured bats. Bats were euthanized by cervical dislocation followed by exsanguination via cardiac puncture, and then were frozen at -20°C. Helminth burden was then assessed by necropsy after and thawing overnight in a 4°C refrigerator. The entire carcass and viscera were examined separately using a stereoscope. Trematodes and cestodes were counted, collected, stained with Semichon's acetocarmine, mounted, and examined with a compound light microscope. Nematodes were stored in glycerine-alcohol, cleared, and examined as temporary mounts with a phase-contrast microscope. Worms were identified using dichotomous keys (Schmidt 1970, Bray et al. 2008, Anderson et al. 2009) and original species descriptions.

Extrinsic variables

Variables external host, usually describing environmental conditions or a host's placement in time and space, were classified as extrinsic variables. I used roost site, host month of capture, and host year of capture as extrinsic variables. In addition, GIS layers of the study sites with a 1:24,000 scale were obtained from the United States Fish and Wildlife Service National Wetlands Inventory. I used these layers to determine the distance from the roost to nearest body of water and the total amount of surface area covered by water within a 12 km radius of the roost (based on maximum foraging distances recorded for *E. fuscus*) via ArcGIS 10 (ESRI). I also used GIS layers from the National Landcover Database (Fry et al. 2009) to determine categories of land usage (Table 12) within 12km of the roost and then recorded the dominant landcover type that surrounded each colony. In total, I utilized six extrinsic variables in our model.

Table 12. Landcover categories as defined in the National Landcover Database and provided by the United States Geological Survey (http://www.mrlc.gov/nlcd06_leg.php).

Landcover Category	Description
Developed, Open Space	Mostly lawn areas with less than 20% of total cover as impervious surfaces. Examples: Parks and golf courses
Developed, Low Intensity	Land with vegetation and impervious surfaces that account for 20% to 49% percent of total coverage. Example: Single-family housing units with large lots
Developed, Medium Intensity	Land with vegetation and impervious surfaces that account for 50% to 79% of the total cover. Example: Single-family housing units with small lots
Developed High Intensity	Highly developed areas where impervious surfaces account for 80% to 100% of the total cover. Examples: Apartment complexes, row houses, and commercial complexes.
Barren Land	**A**reas of earthen material where vegetation accounts for less than 15% of total cover. Examples: Sand dunes, strip mines, and gravel pits
Deciduous Forest	Land where trees greater than 5 meters tall comprise more than 20% of total vegetation cover. More than 75% of tree species must be deciduous.
Evergreen Forest	Land where trees greater than 5 meters tall comprise more than 20% of total vegetation cover. More than 75% of tree species must be evergreen.
Mixed Forest	Land where trees greater than 5 meters tall comprise more than 20% of total vegetation cover. Neither deciduous nor evergreen species are greater than 75% of total tree cover.
Shrub/Scrub	Areas where true shrubs, young trees, and stunted trees, all less than 5 meters tall, make up more than 20% of total vegetation.
Grassland/Herbaceous	Land where more than greater than 80% of total vegetation is herbaceous. These areas are not subject are not tilled but may be use for grazing.
Pasture/Hay	Areas of grasses or legumes planted for livestock grazing or hay production that accounts for more than 20% of total vegetation.
Cultivated Crops	Land used for the production of annual crops and perennial woody crops where crop vegetation accounts for more than 20% of total vegetation as well as all land being actively tilled. Examples: Corn, soybeans, vegetables, tobacco, cotton, orchards and vineyards
Woody Wetlands	Land where forested or shrub vegetation accounts for more than 20% of vegetative cover and the soil or substrate is periodically saturated with or covered with water.
Emergent Herbaceous Wetlands	Land where perennial herbaceous vegetation accounts for more than 80% of vegetative cover and the soil or substrate is periodically saturated with or covered with water.
Open Water	Areas of open water with less than 25% cover of vegetation or soil.

Intrinsic variables

Variables that are a product of the host itself, not the external environment, are classified as intrinsic variables. I used host age, sex, body condition, two measures of immunocompetence, and neutral genetic heterozygosity as intrinsic variables. I employed two methods to determine body condition: body mass to forearm length index and neutrophil to lymphocyte (N:L) ratio. Body condition index was calculated as the residual of a significant regression ($F_{1, 420}$=59.261, p<0.001) of right forearm length on mass by ordinary least squares regression (Schulte-Hostedde et al. 2005, Pearce et al. 2008). Therefore, individuals with a negative index value possessed lower body condition for their size whereas individuals with a positive index value enjoyed higher body condition for their size. The ratio of neutrophils to lymphocytes provides useful information on host condition with higher ratios indicating increased physiological stress (Davis et al. 2008). To determine N:L ratio whole blood obtained from cardiac puncture was used to make at least two blood smears from each individual bat. These blood smears were examined via light microscopy to assess number and type of circulating leukocytes and the ratio of neutrophils to lymphocytes was calculated (Superina and Sierra 2008).

To determine immune function I performed hemolysis/hemagglutination assays following a technique similar to Matson et al. (2005) with a few modifications. In these assays, lysis of foreign antigen reflected the interaction of complement and native antibodies (NAbs) while agglutination of foreign antigen reflected the activity of NAbs only. In short, whole blood resulting from cardiac puncture of each bat was centrifuged to separate plasma from red blood cells (RBCs) and 25µl of resulting plasma were pipetted into wells in the first and second column of a 96 well round-bottom polystyrene tissue

culture plate (Costar 3790, Corning). Plasma in the first column remained undiluted and served as a positive control. Twenty-five µl of phosphate buffered saline (PBS) were then pipetted into the well in the second through twelfth columns of the plate. This created a 1:1 dilution of plasma to saline in the second well that was serially diluted through to the eleventh well. The twelfth well contained only PBS and served as a negative control. After dilution, 25 µl of washed, laboratory-grade rabbit RBCs (Hemostat Laboratories) were added to each well to serve as foreign antigen. Plates were incubated at 37°C for 90 minutes to and then scanned as a full size image at 600 pixels per inch using an Epson Perfection flatbed scanner to record lytic activity. After leaving the plate at room temperature for an additional 20 minutes I recorded agglutination using the same procedure. All digital images were then scored manually by the same individual to determine the relative roles of complement and NAbs in functional immunocompetence. Individuals were scored as negative $\log_2$ of the last plasma dilution exhibiting each behavior.

I genotyped a subset of 200 bats originating from Michigan colonies at 11 autosomal microsatellites using primers developed for *E. fuscus* or other vespertilionid bats (Table 13). Pectoral muscle obtained at necropsy and stored in 5M NaCl with 20% dimethyl sulfoxide was used for extraction of DNA using a DNAeasy kit (Qiagen). Multiple loci were co-amplified in multiplex reactions of 3 to 4 loci and subsequently pooled for loading. Resulting products from were sent to Vanderbilt University for microsatellite genotyping on an ABI 3730 capillary electrophoresis system with an internal size standard. GeneMarker 1.9 software (Softgenetics) was used for to visualize and score allele sizes so I could assess heterozygosity of each individual. I then tested

observed allele frequencies at each locus for deviation from Hardy–Weinberg equilibrium in Arlequin version 3.5.1.2. Satisfied that our data did not differ significantly from expected values, I then calculated standardized multilocus heterozygosity (MHL), or the ratio of heterozygous loci to total number of loci that is then divided by the population mean heterozygosity at typed loci (Slate et al. 2004), for each individual bat.

Table 13. Microsatellite loci used to determine neutral genetic diversity along with primer sequences and their sources.

Locus	Primers	Source
EPFU-C33-F/R	FAM-GCA CCG GGC CTC TAG TAT TT GGG ATG ATT AAT GGG CAA GA	Vonhof unpublished data
EPFU-C209-F/R	NED-GGG TCC TTT CTG GGT TTT TC CCC ACC GAC TCC ACT ACT GT	Vonhof unpublished data
Cora-F11-C04-F/R	VIC-AAG CTC AGA GAC TGC TCC TTC ATC CAT TAT GTT TGC TGA TGT TC	Piaggio et al. 2009a
Coto-H10-E11R-F/R	NED-AGG CAA ACT TTC TTA CAG TTG A TCT TCT TCC ATT TTC CTT CAC	Piaggio et al. 2009b
MMG9-F/R	FAM-AGG GGA CAT ACA AGA ATC AAC C TAA TTT CTC CAC TGA ACT CCC C	Castella and Ruedi 2000
MS3D02-F/R	PET-CTA AGA CCC TTT CCA GCT CTC A GAT ACC ATC ACT CTT TCC CCT G	Trujillo and Amelon 2009
Coto-F09F-F10R-F/R	VIC-GAG AAG GAA GAG AAA CTG GTG TT TAC TAA AGA ACC TTG ACA GTG GC	Piaggio et al. 2009b
EF14B-F/R	NED-ATC ATA TAT TTG TGT TCT GG TGC AAG CTC TTT GAA	Vonhof et al. 2002
EF6-F/R	NED-ATC ACA TTT TTG AAG CAT ATC TGT TTT TCT CTC CTT AT	Vonhof et al. 2002
EF15B-F/R	FAM-AGC AGC AAA GGG GAC TCA GA GAG AAG CAG GGA GGG CAT TT	Vonhof et al. 2002
EF20C-F/R	PET-TTA TCT TTG CCG TGG TT CCC CAC AAT GCC ATT A	Vonhof et al. 2002

Statistical approach

Our objective was to create a predictive model of helminth burden using structural equation modeling (SEM) for both total number of worms and number of worms belonging to each major taxonomic designation (trematodes, cestodes, nematodes). This

multivariate procedure can be used for exploratory model development or confirmatory testing. It goes beyond regression modeling in that can incorporate hypothesized latent, or unobserved, variables that can only be measured indirectly (Shipley, 2000). As helminth burdens are count data that included zeros, I applied a $\sqrt{(x+1)}$ transformation to normalize the collected data. As genotyping all 422 bats was cost-prohibitive, I genotyped a subset of 200 bats. This approach resulted in one set of data that included genetic heterozygosity as an intrinsic variable and one set that did not. I performed the following procedures identically on each of these sets so I could compare models with and without genetic data and assess the relative fit of each. I performed two rounds of SEM in the program AMOS: an exploratory round for model creation and a confirmatory round to test our model with novel data. Our exploratory SEM analyses were performed with 70% of the collected data in order to determine which independent variables explained the most variation in the dependent variables of total helminth burden, trematode burden, cestode burden, or nematode burden. An initial *a priori* model based on results from previous chapters and the literature provided a basis for data analysis (Figure 18). However, this model was considered exploratory and was modified to improve fit by manually removing variables that contributed little explanatory value to the model but only if removal made biological sense in our system. Corresponding AIC (Mazerolle 2006) values were used to rank competing exploratory models whose x^2 values indicated good fit. After determining the top models, I performed our confirmatory SEM analyses with the remaining 30% of the data to verify that the best-fit models generalized to novel data. This approach was used for both the overall dataset and the subset of data that focused on neutral genetic heterozygosity.

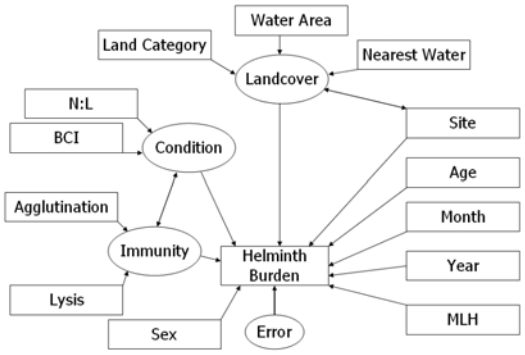


Figure 18. Exploratory SEM of helminth burden.

Results

Distribution of helminth species infecting big brown bats

I found that helminths were highly aggregated within the host population (Figure 19). Many hosts were uninfected (127 bats) and most infected individuals bore light helminth burdens of less than 25 worms (186 bats). Very few hosts were infected with more than

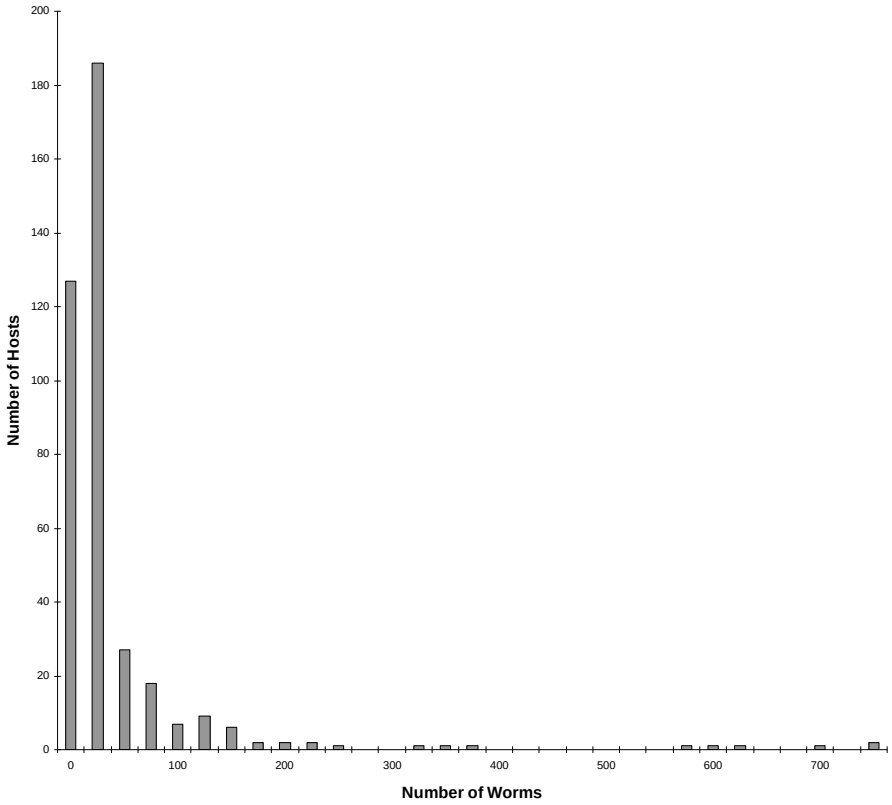


Figure 19. Helminths were aggregated in the host population.

200 worms (14 bats). I found 10 different species of trematodes, one species of cestode, and two species of nematode for 13 total species of helminths (Table 14). Members of the genus *Paralecithodendrium* encountered at most study sites. *Paralecithodenrium transversum* was found at 15 of the 16 study sites while its congeners, *P. naviculum* and *P. swansoni*, were found at 14 and 8 sites, all with relatively high prevalence and intensity (Table 15). The trematodes *Ochoterenatrema breckenridgei* and *Acanthatrium alacati* as well as the nematode *Rictularia lucifugus* were rare and found at only one site each with low prevalence and intensity (Table 15). Trematodes had the highest prevalence (0.63, Figure 20) and mean intensity (27.96, Figure 21). Cestodes and nematodes had very low prevalence (both 0.038, Figure 20) and mean intensity (0.22 and 0.19, respectively, Figure 21).

Table 14. Helminth species along with prevalence, mean intensity, and mean abundance.

Species	Higher Taxonomy	Prevalence	Mean Intensity	Mean Abundance
Acanthatrium alicatai	Trematoda: Lecithodendriidae	0.002	0.078283	31
Acanthatrium. eptesici	Trematoda: Lecithodendriide	0.035	0.906566	25.64286
Acanthatrium microacanthum	Trematoda: Lecithodendriidae	0.027	0.209596	9.222222
Acanthatrium pipestrelli	Trematoda: Lecithodendriidae	0.032	0.462121	14.07692
Glyptoporus noctophilus	Trematoda: Lecithodendriidae	0.010	0.078283	7.75
Paralecithodendrium macnabi	Trematoda: Lecithodendriidae	0.038	1.333333	35.2
Paralecithodendrium naviculum	Trematoda: Lecithodendriidae	0.149	3.911616	26.25424
Paralecithodendrium swansoni	Trematoda: Lecithodendriidae	0.038	0.381313	10.06667
Paralecithodendrium transversum	Trematoda: Lecithodendriidae	0.212	4.065657	19.16667
Plagiorchis vespertilionis	Trematoda: Plagiorchiidae	0.080	0.393939	4.875
Hymenolepis roudabushi	Cestoda: Hymenolepididae	0.043	0.391414	9.117647
Litomosa americana	Nematoda: Dipetalonematidae	0.053	0.088384	1.666667
Rictularia lucifugus	Nematoda: Reticulariidae	0.003	0.002525	1

Table 15. Prevalence (Prev.) and mean intensity (Inten.) of helminths found at each study site.

	P. naviculum		*P. swansoni*		*P. transversum*		*P. macnabi*		*A. pipistrelli*		*A. microacanthum*		*A. eptesici*	
Site	Prev.	Inten.	Prev.	Inten.	Prev.	Inten.	Prev.	Inten.	Prev.	Inten.	Prev.	Inten.	Prev.	Inten.
1	20.0%	6.0	-------	-------	48.0%	79.8	8.0%	6.5	-------	-------	-------	-------	8.0%	59.0
2	48.8%	45.8	7.0%	2.3	-------	-------	9.3%	56.8	7.0%	36.0	2.3%	9.0	16.3%	22.1
3	20.0%	4.5	20.0%	34.0	60.0%	8.8	10.0%	58.0	10.0%	7.0	-------	-------	20.0%	16.0
4	38.9%	40.3	16.7%	0.3	38.9%	22.0	-------	-------	11.1%	12.0	5.6%	9.0	5.6%	44.0
5	31.6%	7.2	5.3%	5.0	36.8%	7.1	15.8%	4.0	-------	-------	-------	-------	-------	-------
6	12.5%	14	-------	-------	12.5%	1	12.5%	157	-------	-------	-------	-------	-------	-------
7	25.0%	17.7	-------	-------	25.0%	3.0	8.3%	52.0	-------	-------	-------	-------	-------	-------
8	11.1%	9.0	-------	-------	33.3%	5.0	-------	-------	-------	-------	33.3%	6.0	11.1%	18.0
9	-------	-------	-------	-------	11.1%	5.5	-------	-------	11.1%	5.0	-------	-------	-------	-------
10	20.0%	4.5	10.0%	49.0	60.0%	3.5	-------	-------	-------	-------	20.0%	12.0	10.0%	1.0
11	10.0%	2.0	-------	-------	20.0%	22.5	-------	-------	-------	-------	20.0%	14.5	-------	-------
12	18.2%	33.5	9.1%	1.0	18.2%	6.5	9.1%	6.0	18.2%	24.5	-------	-------	9.1%	3.0
13	-------	-------	8.3%	7.0	8.3%	4.0	-------	-------	-------	-------	-------	-------	-------	-------
14	12.5%	4.3	9.4%	4.3	43.8%	4.6	-------	-------	3.1%	3.0	3.1%	3.0	6.3%	11.5
15	8.3%	1.0	-------	-------	25.0%	2.0	-------	-------	-------	-------	-------	-------	8.3%	12.0
16	20.0%	17.0	-------	-------	26.7%	7.3	13.3%	1.5	20.0%	28.0	-------	-------	-------	-------

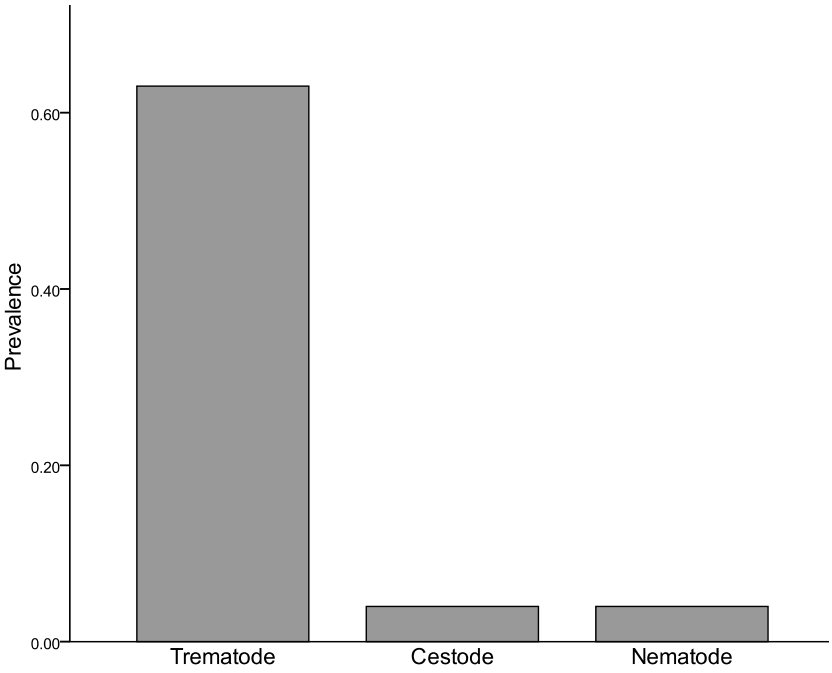


Figure 20. Prevalence of major helminth groups in the study population.

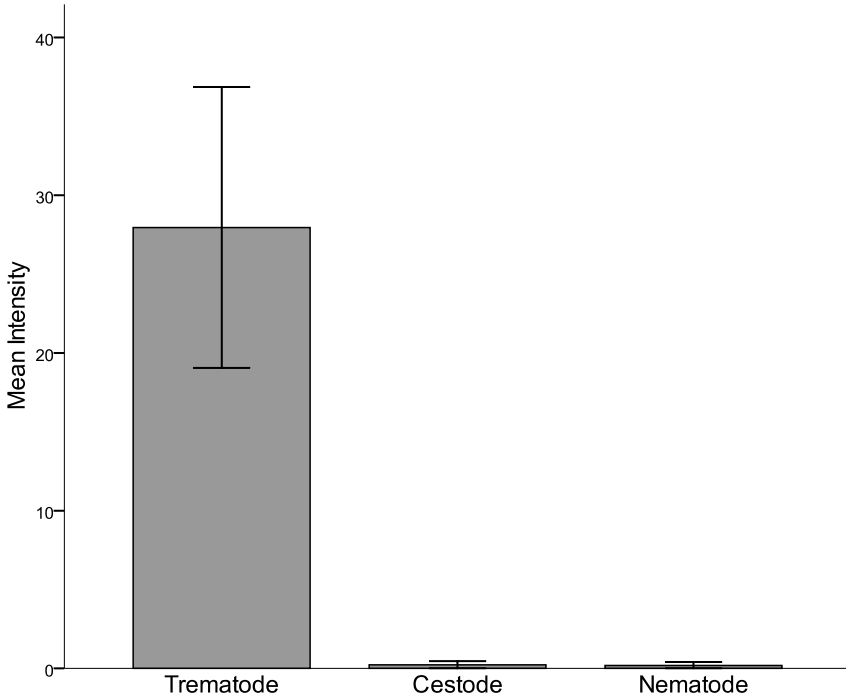


Figure 21. Mean intensity of major helminth groups in the study population. Note that error bars represent 95% confidence intervals.

Modeling overall helminth burden

Without including genetic data, the model that best predicted helminth burdens for all bats (AIC=16.193; Table 16; Figure 22A) included distance from the roost to the nearest water source (standardized estimate=-0.33) and year of capture (standardized estimate=-0.09). There was an inverse relationship between both variables with overall helminth burden and distance to nearest water source being a stronger predictor of helminth burden. However, the model with genetic data ultimately had better fit (AIC=11.714; Figure 22B) and included standardized multilocus heterozygosity (MLH; standardized estimate=-0.14) and distance from roost to nearest water (standardized estimate=-0.06). Overall helminth burden decreased as MLH and distance from the roost to nearest water increased. Confirmatory SEM analyses determined that both of these models generalized to novel data, indicating good fit (AIC=11.934 without MLH, AIC=10.353 with MLH). All models had x^2 goodness-of-fit p-value greater than p=0.30 indicating the models fit the data well.

Table 16. Summary of predictors and fit (AIC value) of each model of helminth burden.

Model	Predictors	Exploratory Fit	Confirmatory Fit
Overall Burden	Distance to Water, Year of Capture	16.193	11.934
Overall Burden	Distance to Water, MLH	11.714	10.353
Trematode Burden	Distance to Water, Year of Capture	16.193	11.934
Trematode Burden	Distance to Water, MLH	11.714	10.353
Cestode Burden	Month of Capture, Roost Site	16.943	10.662
Cestode Burden	Month of Capture, MLH	11.704	12.770
Nematode Burden	Month of Capture, Roost Site	25.916	10.616
Nematode Burden	Month of Capture, MLH	10.006	12.727

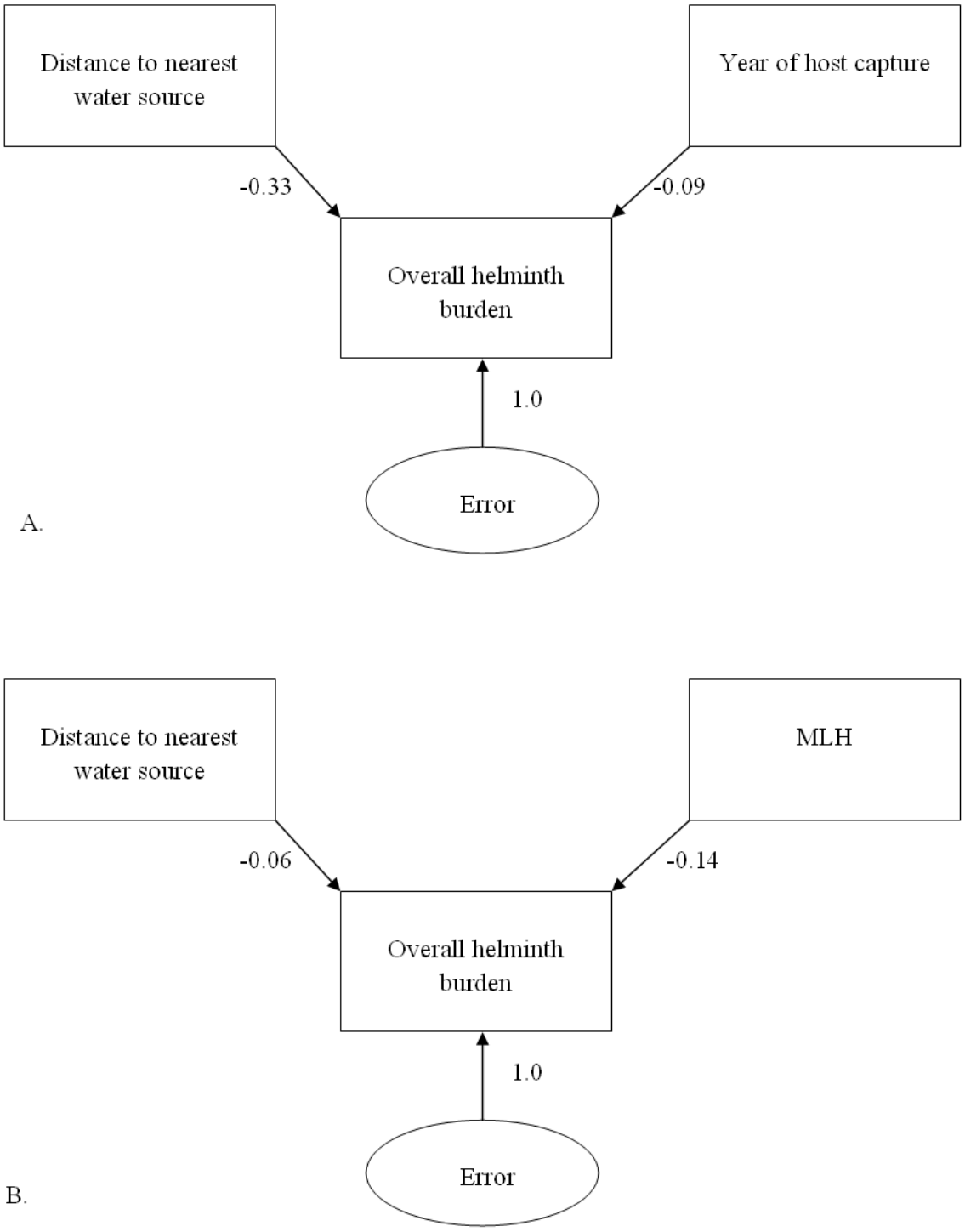


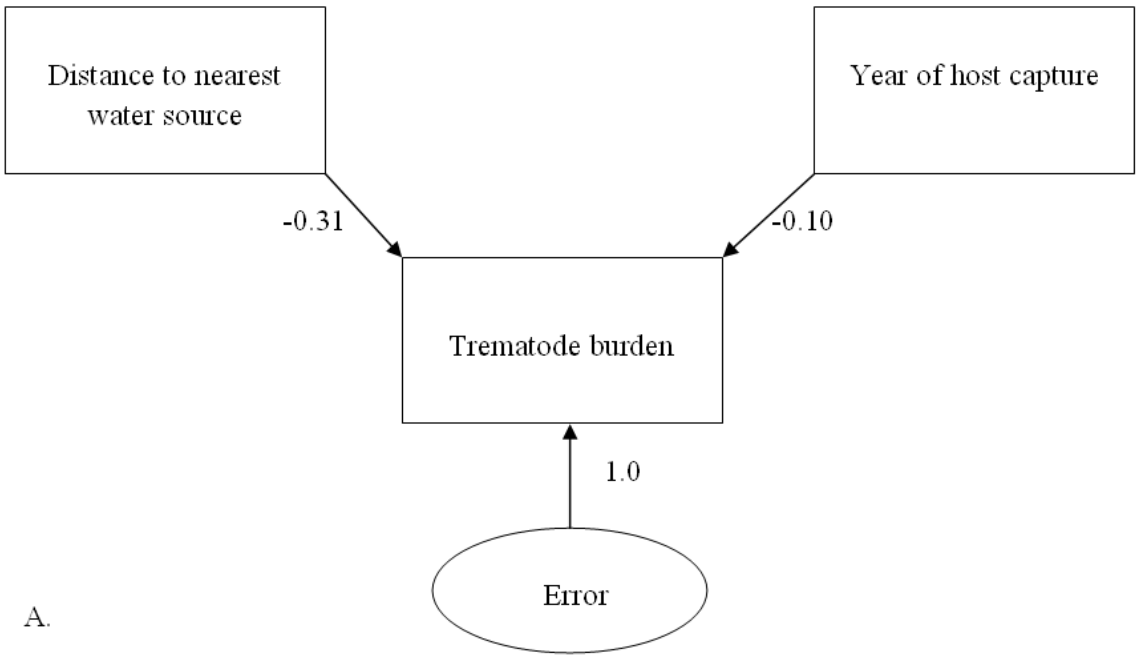
Figure 22. Best-fit SEM models of overall helminth burden without (A) and with (B) multilocus heterozygosity included as a predictor.

Modeling trematode burden

The model that best predicted trematode burden with no genetic data (AIC=16.193; Table 6; Figure 23A;) consisted of distance from the roost to the nearest water source (standardized estimate=-0.31) and year of capture (standardized estimate=-0.10). Trematode burden decreased as distance from the water and year of capture decreased. The model including genetic data ultimately had better fit than the one without (AIC=11.714; Figure 23B) and consisted of MLH (standardized estimate=-0.14) and distance from roost to nearest water (standardized estimate=-0.06). Bats living close to water and with lower genetic diversity had increased worm burdens. These models also generalized to novel data in confirmatory analyses and were considered appropriately robust (AIC=11.934 without genetic data, AIC=10.353 with genetic data). Note that the models for overall helminth burden and the model for trematode burden were nearly identical. Trematodes were overwhelmingly the most prevalent and most intense group of helminths in our study; therefore, the model for overall helminth burden was essentially a model of trematode burden. All models had x^2 goodness-of-fit p-value greater than p=0.30 indicating the models fit the data well.

Modeling cestode burden

Without including genetic data, the best-fit model for cestode burden (AIC=16.943; Table 16; Figure 24A) included month of host capture (standardized estimate=0.21) and roost site (standardized estimate=-0.15). The model including genetic data had better fit (AIC=11.704; Figure 24B) and consisted of month of host capture (standardized estimate=0.21) and MLH (standardized estimate=-0.09). Cestode burden decreased as roost site changed and MHL decreased while cestode burden increased as



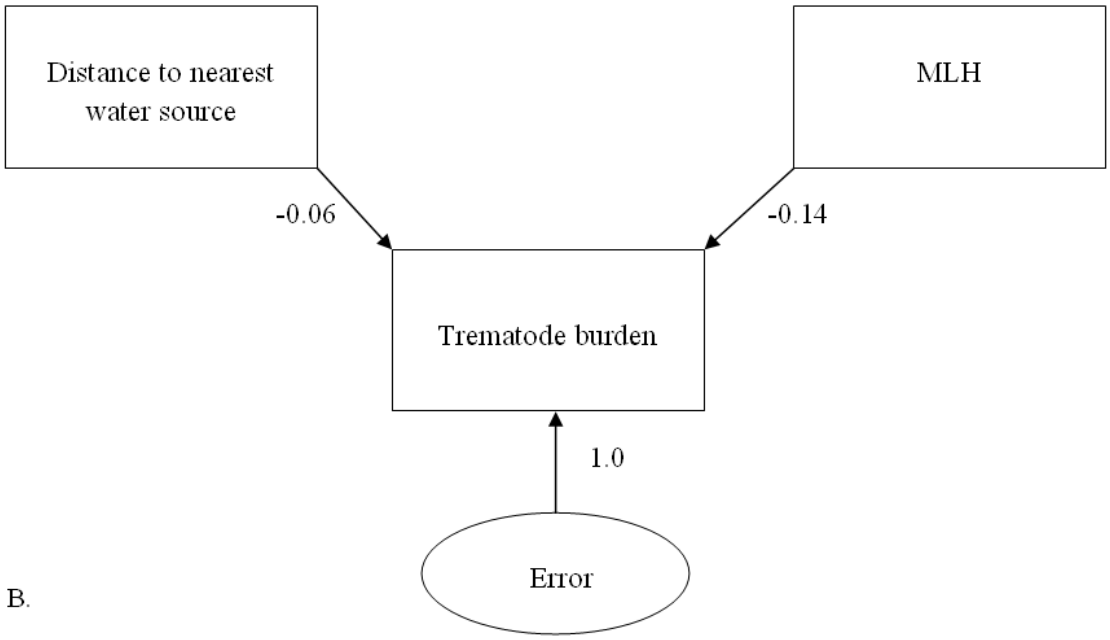
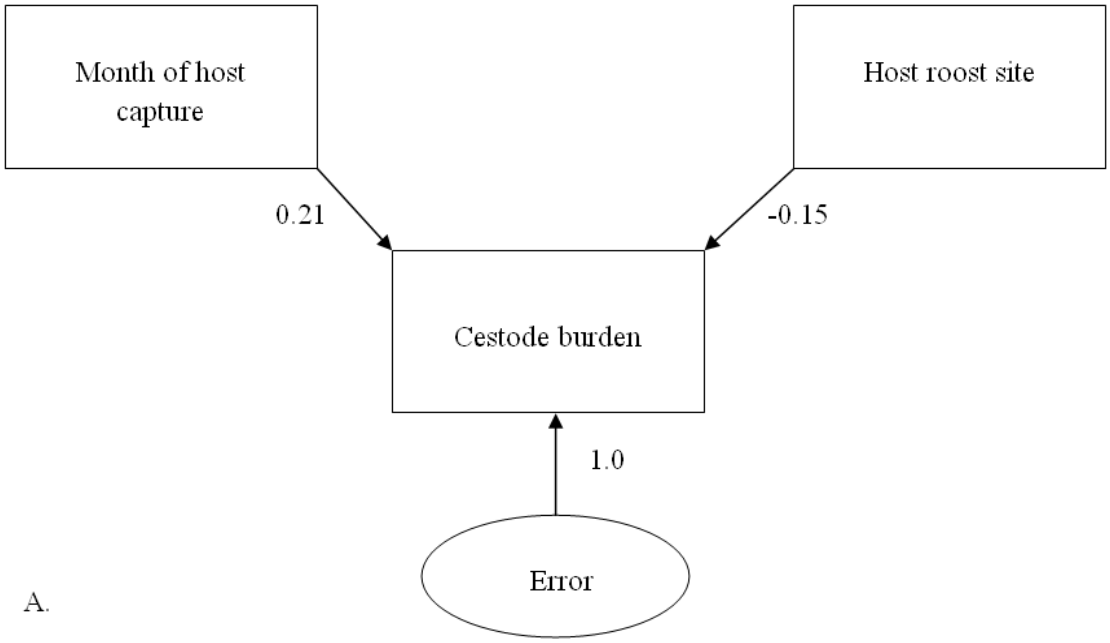


Figure 23. Best-fit SEM models of trematode burden without (A) and with (B) multilocus heterozygosity included as a predictor.



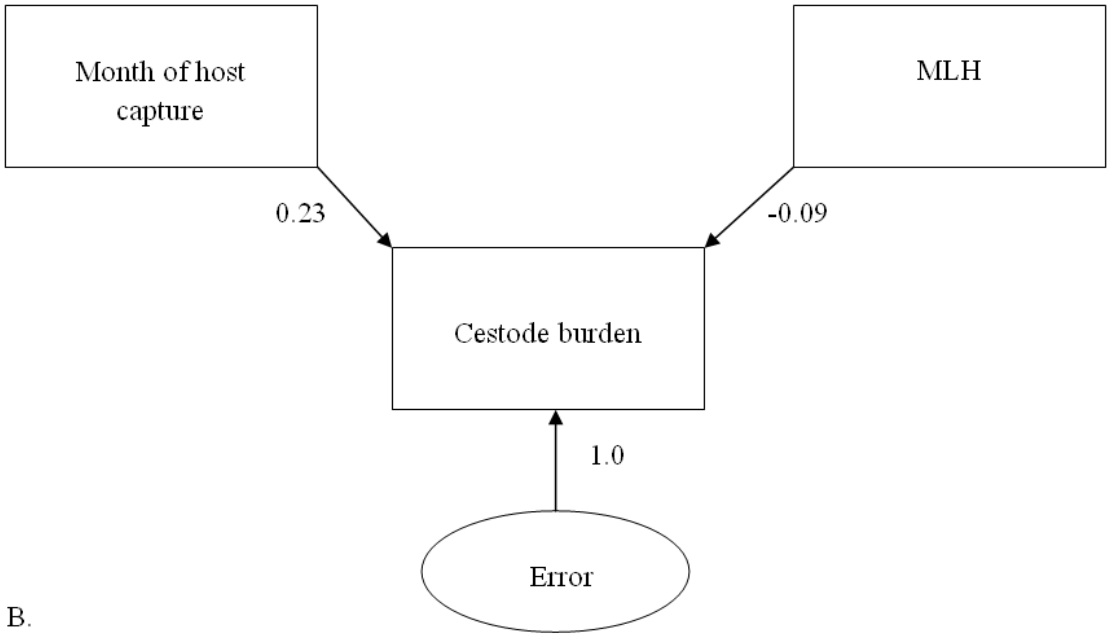


Figure 24. Best-fit SEM models of cestode burden without (A) and with (B) multilocus heterozygosity included as a predictor.

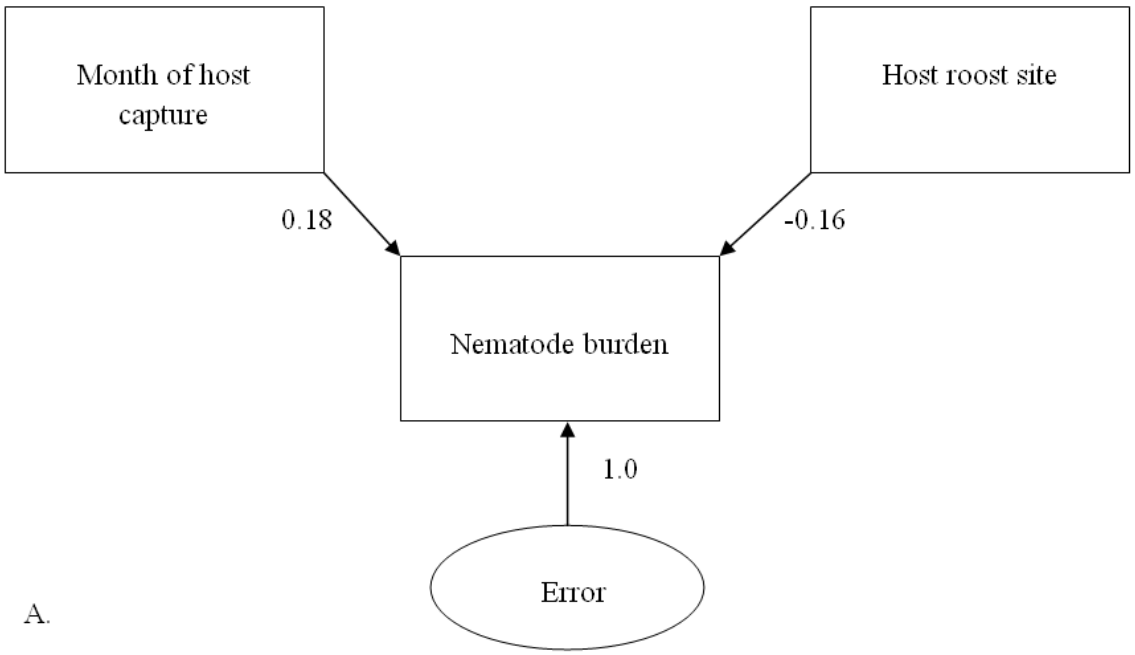
month of host capture increased. These results indicate that bats captured in late summer and those with low genetic diversity had heavier cestode burdens. Confirmatory SEM analyses determined that these models generalized to novel data indicating that they fit the data in an appropriate manner (AIC=10.662 without genetic data, AIC=12.770 with genetic data). All models had x^2 goodness-of-fit p-value greater than p=0.30 indicating the models fit the data well.

Modeling nematode burden

Without genetic data the best-fit model for nematode burden (AIC=25.916; Table 16; Figure 25A), included month of host capture (standardized estimate=0.18) and host roost site (standardized estimate=-0.16). However, with genetic data, the best-fit model (AIC=10.006; Figure 25B) consisted of month of host capture (standardized estimate=0.14) and MLH (standardized estimate=-0.01). These results suggest that bats captured in late summer and those with low genetic diversity had heavier nematode burdens. Confirmatory SEM analyses determined that these best-fit models generalized to novel data and indicated good fit (AIC=10.616 without genetic data, AIC=12.727 with genetic data). All models had x^2 goodness-of-fit p-value greater than p=0.30 indicating the models fit the data well.

Discussion

Our predictive models highlight the roles of both exposure and establishment variables in producing patterns of parasite aggregation. Models without genetic data highlighted extrinsic variables that were likely related to exposure; however, when genetic data were included, the exposure variable of MLH was present in models for each. Genetic diversity tended to have a stronger association with trematode burden than



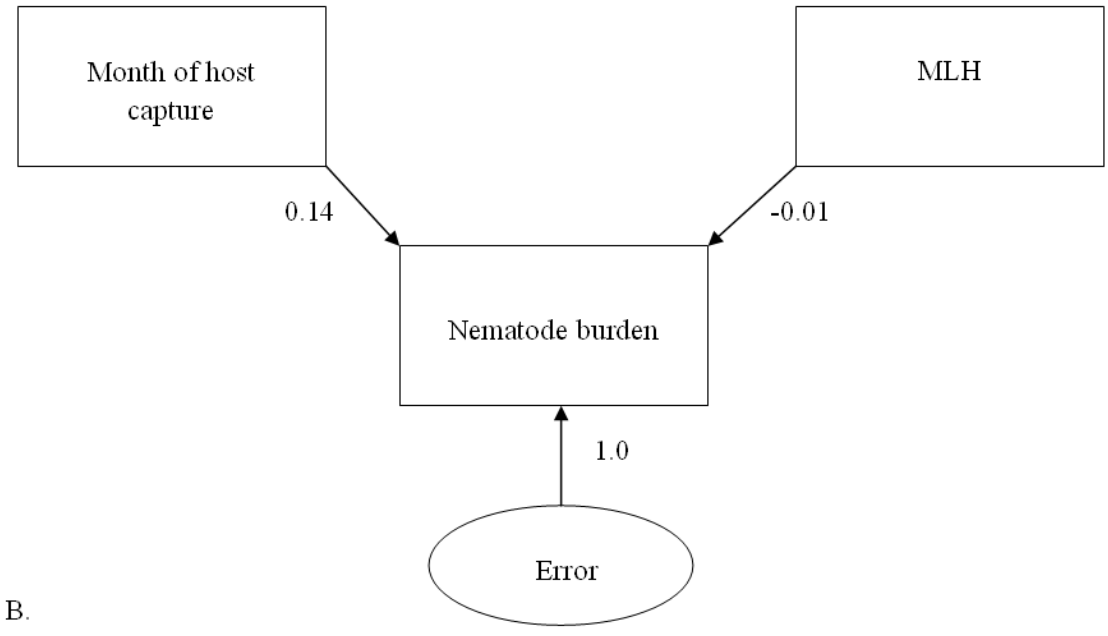


Figure 25. Best-fit SEM models of nematode burden without (A) and with (B) multilocus heterozygosity included as a predictor.

distance to nearest water source; however, this trend was reversed in models for cestode and nematode burden which were more strongly associated with month of host capture. Although strength of relationships between predictors and helminth burden varied across parasitic taxa, variables related to host exposure as well as host genetic diversity consistently played critical roles in predicting helminth burden.

Elements of time and space were vital to understanding host exposure and uncovering its relationship with worm burden. The spatial aspect of a roost's proximity to water is directly tied to the lifecycle of trematodes within our host-parasite system because a number of insects with aquatic stages may act as helminth intermediate hosts (Bray et al. 2008). Species richness, abundance, and biomass of adult insects with aquatic stages significantly decrease with greater distance from water (Jackson and Resh 1989); therefore, bats living close to water will be exposed to a larger pool of insects and have greater risk of an infected intermediate host. The lack of water contact variables in models for fully terrestrial helminths, such as cestodes and nematodes, also indicates that lifecycle influences parasite burdens within definitive hosts (Esteban et al. 2001). Temporal variation, such as month or year of capture, could affect either the availability of intermediate hosts or the accumulation of helminths by definitive hosts. Processes related to climate cause changes in arthropod abundance, dispersal, and diversity over time (Taylor and Merriam 1996, Krasnov et al. 2005, Bonada et al. 2012). Thus, I would expect hosts who pass through seasonal or yearly peaks of arthropod activity to have heavier parasite burdens than those that did not (Lourenco et al. 2008).

Our models indicated that low genetic diversity consistently predicted high helminth burdens in all major helminth groups. Increased genetic heterozygosity often

significantly correlates with to increased immune function (Reid et al. 2003, 2007, Hawley et al. 2005, Whiteman et al. 2006) and decreased parasite burden in wild hosts (Hedrick et al. 2001, Acevedo-Whitehouse et al. 2003, 2006, Hawley et al. 2005, Luong et al. 2006, Ortego et al. 2007, Luikart et al. 2008, Meyer-Lucht and Sommer, 2009, Isomursu et al. 2012, Zhang and He 2013). Even though our eleven loci represent a small proportion of the host genome and use of microsatellites could underestimate genomic diversity (Vali et al. 2008), our results suggest a pattern of high MLH that deters helminth establishment. Given the evidence that MLH relates to both adaptive (Arkush et al. 2002, Charpentier et al. 2008) and innate immunity (Townsend et al. 2010), overall genetic heterozygosity likely provides general protection against a wide range of pathogens. Host populations tend to exhibit greater genome-wide heterozygosity in older age classes, suggesting that homozygous individuals die earlier than their heterozygous cohorts and that the protection conferred by genetic heterozygosity increases the likelihood of host survival (Coltman et al. 1999, Charpentier et al. 2008, Rijks et al. 2008). This maintenance of genetic heterozygosity within a host population by eliminating potentially super-spreading homozygotes could limit disease spread and deter future pathogen invasion of that population (King and Lively 2012). This protection could derive from genetic diversity itself (Spielman et al. 2004, Ebert et al. 2007) or heterozygous individuals could be more likely to possess certain parasite resistance alleles (Schwensow et al. 2010, Zhang and He 2013) and as newer DNA sequencing technology becomes more cost-effective I may be able to definitively determine which mechanism is at work (Vali et al. 2008). Either way, the implication that greater host genetic heterozygosity correlates with lower parasite burdens remains the same for our

current investigation.

Although I examined a comprehensive set of predictive variables, relatively few of them impacted helminth burden in a meaningful way. I found no evidence for sex- or age-biased parasitism, indicating that these variables contribute little variation to host helminth burden. Host sex and age are popular predictors of parasitism (e.g. Moore and Wilson 2002, Abu-Madi et al. 2005, Ortego et al. 2007); however, unless there is significant evidence that they correlate with markedly dimorphic behaviors that impact host exposure (Lyons et al. 2001, Krkosek et al. 2007, Hillegass et al. 2008, Perkins et al. 2008), these variables may not cause enough intrinsic host heterogeneity to be as useful in predicting helminth burdens as genetic diversity. Similarly, unless there is good empirical evidence that parasite resistance is highly correlated with selected measures of host immune function and condition (e.g. Nagasawa et al. 1992, Schopf et al. 2002, Ganley-Leal et al. 2006, Idika et al. 2012), including these variables in a predictive model of infection may not be useful when compared to more meaningful measures of host exposure and genetic diversity.

Overall, I discovered both host exposure and parasite establishment play vital roles in predicting high helminth burdens. Individuals with these high burdens are a source of parasite aggregation and impact transmission dynamics by maintaining infection in a population (Anderson et al. 1991, McCallum et al. 2001). I must consider sources heterogeneous exposure and heterogeneous establishment, especially variation in time, space, and genetic diversity, when attempting to predict parasite burdens in order to obtain the clearest picture possible. Given that uncovering transmission dynamics requires knowledge of host exposure patterns through time and space, specific variables

within these categories of predictors would likely vary depending on the host—parasite system in question; however, models that do not simultaneously examine meaningful aspects of host exposure along with genetic diversity are incomplete. Including every possible predictor of parasite burden is intractable; therefore, I suggest winnowing out variables such as host age and sex, unless there is strong system—specific evidence for their inclusion, to focus on meaningful estimates of host—parasite contact and host genetic heterozygosity. Including these classes of variables would ultimately result in more informative predictive models of infection that could better direct targeted interventions such as selective chemotherapy, changing land use patterns, or altering host behavior in order to disrupt parasite transmission.

APPENDIX

IACUC REVIEW AND APPROVAL

WESTERN MICHIGAN UNIVERSITY

Institutional Animal Care and Use Committee

ANNUAL REVIEW OF VERTEBRATE ANIMAL USE

PROJECT OR COURSE TITLE: Mechanisms Influencing Aggregated Distributions Of Helminths In Vespertilionid Bats

IACUC Protocol Number: 10-01-05

Date of Review Request: 01/31/12 Date of Last Approval: 02/19/11

Purpose of project (select one): ☐Teaching ☒Research ☐Other (specify):

PRINCIPAL INVESTIGATOR OR ADVISOR

Name: Maarten Vonhof Title: Assoc./Assist. Professor

Department: BIOS Electronic Mail Address: maarten.vonhof@wmich.edu

CO-PRINCIPAL OR STUDENT INVESTIGATOR

Name: Elizabeth Warburton Title: Select one

Department: BIOS Electronic Mail Address: elizabeth.warburton@wmich.edu

1. The research, as approved by the IACUC, is completed:
   ☐Yes (Continue with items 4-5 below.) ☒No (Continue with items 2-5 below.)

If the answer to any of the following questions (items 2-4) is "Yes," please provide a detailed explanation on an attached sheet of paper. Include details of any modifications made to the protocol based on new findings or publications, adverse events or mortalities.

2. Have there been any changes in Principal or Co-Principal Investigators? ☐Yes ☒No
3. Have there been any new findings or publications relative to this research? ☐Yes ☒No
   Describe the sources used to determine the availability of new findings or publications:
   - ☐No search conducted (Please provide a justification on an attached sheet.)
   - ☐Animal Welfare Information Center (AWIC)
   - ☐Search of literature databases (select all applicable)
     - ☐AGRICOLA
     - ☐Biological Abstracts
     - ☐Current Research Information Service (CRIS)
     - ☐Medline
     - ☒Other (please specify):

     Date of search: 01/31/12 Years covered by the search: 2008-12

     Key words: bats, biopsy, blood sampling, euthanasia, capture
   - ☐Additional search strategy narrative:
4. Are there any adverse events, in terms of animal well being, or mortalities to report as a result of this research? ☐Yes ☒No

   Cumulative number of mortalities: 0
5. Animal usage: Number of animals used during this quarter (3 months): 0 Cumulative number of animals used to date: 298

✏ Maarten Vonhof 2/1/12

Principal Investigator/Faculty Advisor Signature Date

✏ Elizabeth Warburton 2/1/12

Co-Principal or Student Investigator Signature Date

IACUC REVIEW AND APPROVAL

Upon review of the relevant information regarding this protocol, the IACUC approval for this project has been extended for one year from the date of this signature.

✏ 2/1/12

IACUC Chair Signature Date

Revised 10/01 WMU IACUC
All other copies obsolete.

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