

University of Montana

ScholarWorks at University of Montana

Graduate Student Theses, Dissertations, &
Professional Papers

Graduate School

2021

LINKS BETWEEN INTERSPECIFIC COMPETITION AND INFECTIOUS DISEASE: A MECHANISTIC INVESTIGATION WITH A DIRECTLY-TRANSMITTED ZOO NOTIC SYSTEM

Andreas Eleftheriou

Follow this and additional works at: <https://scholarworks.umt.edu/etd>

Let us know how access to this document benefits you.

Recommended Citation

Eleftheriou, Andreas, "LINKS BETWEEN INTERSPECIFIC COMPETITION AND INFECTIOUS DISEASE: A MECHANISTIC INVESTIGATION WITH A DIRECTLY-TRANSMITTED ZOO NOTIC SYSTEM" (2021). *Graduate Student Theses, Dissertations, & Professional Papers*. 11742.

<https://scholarworks.umt.edu/etd/11742>

This Dissertation is brought to you for free and open access by the Graduate School at ScholarWorks at University of Montana. It has been accepted for inclusion in Graduate Student Theses, Dissertations, & Professional Papers by an authorized administrator of ScholarWorks at University of Montana. For more information, please contact scholarworks@mso.umt.edu.

**LINKS BETWEEN INTERSPECIFIC COMPETITION AND INFECTIOUS
DISEASE: A MECHANISTIC INVESTIGATION WITH A DIRECTLY-
TRANSMITTED ZOO NOTIC SYSTEM**

By

ANDREAS ELEFTHERIOU

Bachelor of Arts, Boston University, Boston, MA, 2006
Master of Science, University of Massachusetts, Lowell, MA, 2008
Doctor of Veterinary Medicine, Tufts University, North Grafton, MA, 2013

Dissertation presented in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy
in Fish and Wildlife Biology

The University of Montana
Missoula, MT

May 2021

Approved by:

Scott Whittenburg,
Graduate School Dean

Angela D. Luis, Ph.D., Chair
Wildlife Biology

Amy J. Kuenzi, Ph.D.
Department of Biological Sciences, Montana Tech University

Creagh W. Breuner, Ph.D.
Wildlife Biology, Division of Biological Sciences

Dean E. Pearson, Ph.D.
Division of Biological Sciences, USDA Forest Service

Mike F. Minnick, Ph.D.
Division of Biological Sciences

© COPYRIGHT

by

Andreas Eleftheriou

2021

All Rights Reserved

Links between interspecific competition and infectious disease: A mechanistic investigation with a directly-transmitted zoonotic system

Chairperson: Angela D. Luis, Ph.D.

Globally, biodiversity is declining while emerging infectious diseases are on the rise. To explain this pattern, the “dilution effect” hypothesis predicts that with lower diversity, disease increases, because diversity “dilutes” disease. Although there is strong support for this hypothesis, there is evidence to support the “amplification effect” hypothesis, which predicts lower disease with lower diversity. Recently, there has been a push to investigate underlying mechanisms than general patterns to create a more mechanistic framework across disease systems. However, mechanisms have been mostly examined with indirectly-transmitted disease systems (e.g., Lyme disease), while less attention has been directed towards directly-transmitted systems, such as rodent-hantavirus systems.

For my dissertation, I investigated mechanisms behind diversity-disease patterns in directly-transmitted disease systems, using the North American deer mouse (*Peromyscus maniculatus*)-Sin Nombre hantavirus (SNV) system as a model. SNV is an emerging zoonosis with high human fatality. A dilution effect has been primarily reported for this well-studied system, which exhibits density-dependent transmission. Because community composition than diversity *pe se*, is most likely responsible for a dilution effect, in this dissertation I primarily considered heterospecific competitors as the component of the community responsible for affecting disease.

In Chapter 1, I briefly introduce my dissertation. In Chapter 2, I discuss theory and empirical evidence of mechanisms that underlie patterns between disease and diversity (or community composition) across rodent-hantavirus systems through a systematic literature review. I conclude that host density is most likely the primary mechanistic driver, with density-independent mechanisms (i.e. contact rates, probability of transmission given contact and infectious period) requiring further investigation. In Chapter 3, I experimentally validate the use of two enzyme immunoassays for measuring fecal corticosterone metabolites in deer mice, as a measure of stress. In Chapter 4, I present observational data that voles and shrews have negative impacts on deer mouse stress physiology but differentially influence scar numbers (a proxy for contact rates). In Chapter 5, I use a mathematical model with interspecific competition and a deconstructed transmission rate (i.e. contact rates and probability of transmission) to show that non-monotonic patterns (increase then decrease) between competitor density and disease prevalence represent most of the realistic parameter space examined.

ACKNOWLEDGEMENTS

Thank you to my advisor, Angie Luis for her great mentorship and willingness to take me on as her first graduate student and guide me through the incredible world of infectious disease ecology. I am very grateful to Amy Kuenzi for her vast knowledge on small mammals and hantaviruses and I am extremely thankful for all the field and laboratory training she selflessly provided. Thank you to the rest of my committee, Creagh Breuner, Dean Pearson and Mike Minnick for their wonderful support during my tenure at University of Montana.

I am thankful to Michael Sheriff and Rudy Boonstra, who welcomed me into their labs and introduced me to the field of stress physiology. I am especially grateful to Cate Pritchard for training and supporting me through the technical aspects of stress physiology. Rupert Palme, thank you for sharing your stress physiology wisdom.

Thank you to all the Luis lab members, faculty, staff, and students of the Wildlife Biology and OBEE programs, past and present, who provided me with some form of assistance or entertainment throughout the years. I am especially grateful and appreciative for all the help from undergraduate students that made the field and laboratory work possible. Thank you to Joyce Knoll and Pam Shaw for helping me set up the clinical pathology aspects of my project. Thank you to John Grant for helping me select and gain access to my field sites at Ninepipe WMA.

Lastly, thank you to my dearest friend, Gloria DeWalt, who has consistently inspired me and offered a voice of support. Importantly, I thank my parents and sisters with all my heart, without whom I would not have pursued a Ph.D. degree. I am forever indebted to you and I truly thank you for your support and your love that you never stop giving.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	iv
LIST OF TABLES	viii
LIST OF FIGURES	ix
CHAPTER 1 OVERVIEW OF DISSERTATION	1
BACKGROUND	1
RESEARCH OBJECTIVES	1
RESULTS	2
CONCLUSIONS	3
CHAPTER 2 MECHANISTIC LINKS BETWEEN BIODIVERSITY AND DIRECTLY- TRANSMITTED DISEASES: A REVIEW OF THEORY AND EMPIRICAL EVIDENCE FROM RODENT-HANTAVIRUS SYSTEMS	5
ABSTRACT	5
THE RELATIONSHIP BETWEEN DIVERSITY AND DISEASE	5
<i>DIVERSITY-DISEASE PATTERNS IN DIRECTLY-TRANSMITTED DISEASE SYSTEMS</i>	6
DIVERSITY-DISEASE PATTERNS IN RODENT-HANTAVIRUS SYSTEMS	7
<i>HANTAVIRUSES AND THEIR RESERVOIR HOSTS</i>	7
<i>EVIDENCE FOR DIVERSITY-DISEASE RELATIONSHIPS IN RODENT-HANTAVIRUS SYSTEMS</i>	8
DIVERSITY-DISEASE MECHANISMS: THEORY AND EVIDENCE	9
<i>DETERMINANTS OF DISEASE PREVALENCE</i>	9
<i>HOST DENSITY</i>	11
<i>DENSITY-INDEPENDENT CONTACT RATES</i>	14
<i>DENSITY-INDEPENDENT PROBABILITY OF TRANSMISSION GIVEN CONTACT</i>	16
<i>DENSITY-INDEPENDENT INFECTIOUS PERIOD</i>	17
EFFECTS OF MULTIPLE MECHANISMS ON DISEASE OUTCOMES	17
<i>CONCURRENT DILUTION AND AMPLIFICATION EFFECTS</i>	17
<i>SIMULATION EXERCISES WITH THE DEERMOUSE-SNV SYSTEM</i>	18
CONCLUSIONS AND FUTURE DIRECTIONS	20
ACKNOWLEDGEMENTS	21
LITERATURE CITED	21
SUPPLEMENTARY INFORMATION	31

CHAPTER 3 ASSESSMENT OF THE STRESS RESPONSE IN NORTH AMERICAN DEERMICE: LABORATORY AND FIELD VALIDATION OF TWO ENZYME IMMUNOASSAYS FOR FECAL CORTICOSTERONE METABOLITES	39
ABSTRACT	39
INTRODUCTION	40
MATERIALS AND METHODS	42
<i>ACQUISITION AND HUSBANDRY OF LABORATORY DEERMICE</i>	42
<i>FECAL SAMPLE COLLECTION</i>	43
<i>DEXAMETHASONE SUPPRESSION CHALLENGE</i>	43
<i>ACTH STIMULATION CHALLENGE</i>	44
<i>FIELD VALIDATION</i>	44
<i>PROCESSING OF FECES AND EXTRACTION OF FCMs</i>	45
<i>IMMUNOASSAY METHODS</i>	46
<i>STATISTICAL ANALYSES</i>	47
RESULTS	48
<i>DEXAMETHASONE SUPPRESSION CHALLENGE</i>	48
<i>ACTH STIMULATION CHALLENGE</i>	49
<i>FIELD VALIDATION</i>	49
DISCUSSION	50
<i>DIURNAL RHYTHM AND SEX EFFECTS</i>	51
<i>SUPPRESSION OF ADRENOCORTICAL ACTIVITY</i>	52
<i>STIMULATION OF ADRENOCORTICAL ACTIVITY</i>	52
<i>TRAP-INDUCED EFFECTS ON FCMs</i>	53
<i>DRYING EFFECTS ON FCMs</i>	54
<i>CONCLUSIONS</i>	55
ACKNOWLEDGEMENTS	55
LITERATURE CITED	56
CHAPTER 4 HETEROSPECIFIC COMPETITORS AND SEASONALITY CAN AFFECT HOST PHYSIOLOGY AND BEHAVIOR: KEY FACTORS IN DISEASE TRANSMISSION	66
ABSTRACT	66
INTRODUCTION	67
MATERIALS AND METHODS	71
<i>STUDY SYSTEM</i>	71
<i>VARIABLES MEASURED</i>	72

<i>SITE DESCRIPTION AND LIVE-TRAPPING</i>	72
<i>ANIMAL SAMPLING</i>	73
<i>FCM ANALYSES</i>	74
<i>WBC COUNT ANALYSES</i>	75
<i>SNV ANTIBODY DETECTION</i>	75
<i>STATISTICAL ANALYSES</i>	75
RESULTS	76
<i>LIVE-TRAPPING</i>	76
<i>STRESS PHYSIOLOGY MEASURES</i>	76
<i>IMMUNITY</i>	78
<i>SCAR NUMBERS</i>	78
<i>SNV INFECTION</i>	78
DISCUSSION	79
<i>INTERSPECIFIC COMPETITION</i>	80
<i>SEASONALITY</i>	82
<i>CONCLUSIONS</i>	84
ACKNOWLEDGMENTS	85
LITERATURE CITED	85
SUPPLEMENTARY INFORMATION	102
CHAPTER 5 INTERSPECIFIC COMPETITION CAN LEAD TO NON-MONOTONIC DISEASE OUTCOMES: A THEORETICAL INVESTIGATION WITH A DIRECTLY-TRANSMITTED ZONOSIS.....	103
ABSTRACT	103
INTRODUCTION	104
METHODS	108
<i>DECONSTRUCTING TRANSMISSION RATE</i>	109
<i>ESTIMATING CRITICAL HOST DENSITY</i>	110
<i>RUNNING MODEL SIMULATIONS FOR ALL SCENARIOS</i>	111
RESULTS	111
<i>COMPETITOR AFFECTS EACH MECHANISM INDIVIDUALLY</i>	111
<i>COMPETITOR AFFECTS VARIOUS MECHANISMS SIMULTANEOUSLY</i>	113
DISCUSSION	115
LITERATURE CITED	119
SUPPLEMENTARY INFORMATION	130

LIST OF TABLES

Table 2. 1 Diversity-disease studies with rodent-hantavirus systems. We list data on the disease system, diversity-disease outcome, empirical support (with directionality) for density-dependent and -independent mechanisms (contact rates, probability of transmission, and host survival), metrics of diversity and disease, and study reference.....	29
Table S2. 1 Variables and parameters used in model with descriptions and estimates.	35
Table 3. 1 Timeline of treatments and samples used in statistical analyses for evaluation of fecal corticosterone metabolites in deermice.....	59
Table 3. 2 Summary of demographic factors and fecal cortisol/corticosterone metabolite (FCM) values from free-ranging deermice captured in MT, USA in August 2017 for field study 2.	60
Table 4. 1 List of hypotheses for how heterospecific competitors and seasonality will associate with stress physiology, immunity, and contact rates of deermice, with corresponding predictions and empirical support for each measured variable. Seasonality hypotheses received support if there was consistency across both years.	95
Table 5. 1 Variables and parameters used in the models with descriptions and estimates.....	126
Table S5. 1 Model-estimated β values generated from all possible simulations compared to published β values from Montana, USA.....	137
Table S5. 2 Results of broad elasticity analyses where the effect of competitor density on intraspecific contact rates, β_c , (ψ), was kept at zero (scenario in center of Figure 5.3 in main text). Parameters and variables were changed $\pm 30\%$ to visualize the percentage change in disease outcomes compared to their fixed values (Table 5.1 in main text). For each figure inset, the horizontal axis, represents γ , the degree of stress-induced immunosuppression (range; 0-0.3) while the vertical axis represents α , the strength of regulatory competition (range; 0-2), both of which are effects exerted on hosts by competitor density. Dark purple signifies an amplification effect, light purple, a dilution effect, light green, no change in prevalence (i.e. represents baseline when ψ , α & γ are set to zero), and dark green, a non-monotonic relationship.	138
Table S5. 3 Results of elasticity analyses where the effect of competitor density on contact rates, β_c , (ψ), was kept at zero (scenario in center of Figure 5.3). Parameters and variables were changed to document the percentage change in non-monotonic outcomes compared to their fixed values. Red signifies increase, blue decrease, and gray no change.	139
Table S5. 4 Results of elasticity analyses where the effect of competitor density on contact rates, β_c , (ψ), was kept at zero (scenario in center of Figure 5.3). Parameters and variables were	

changed to document the percentage change in dilution outcomes compared to their fixed values. Red signifies increase, blue decrease, and gray no change..... 139

Table S5. 5 Results of elasticity analyses where the effect of competitor density on contact rates, β_c , (ψ), was kept at zero (scenario in center of Figure 5.3). Parameters and variables were changed to document the percentage change in amplification outcomes compared to their fixed values. Red signifies increase, blue decrease, and gray no change. 140

LIST OF FIGURES

Figure 2. 1 Conceptual diagram describing possible relationships between diversity and prevalence for a directly-transmitted disease with density-dependent (DD) transmission and one primary reservoir host. Arrows propose hypothetical effects that diversity may have on mechanisms that determine disease prevalence. Diversity can indirectly determine prevalence via DD mechanisms (blue arrows), or directly (gray arrows) via density-independent mechanisms (contact rates, probability of transmission given contact, and infectious period). Numbers in brackets illustrate number of studies across rodent-hantavirus systems that found support for each mechanism (studies included in multiple counts if multiple mechanisms were examined). 27

Figure 2. 2 Simulation outcomes for three scenarios where density-dependent and density-independent mechanisms varied in their relationship with diversity, using a mathematical model of the deer mouse-Sin Nombre virus system. Scenario 1 (blue): When diversity has a negative effect on host density, a dilution effect emerges. Scenario 2 (red): When diversity has a positive effect on transmission rate for a given density, an amplification effect results. Scenario 3 (black): When diversity affects both mechanisms in opposing directions, there is a non-monotonic outcome..... 28

Figure S2. 1 : Color-coded relationships used in simulation scenarios whose outcomes are shown in figure 2 of the main text. Top left panel: Relationships between diversity and host density. Top right panel: Relationships between diversity and transmission rate for a given host density (contact rates \times probability of transmission given contact). 35

Figure S2. 2 Conceptual representations of simulation results of diversity-disease outcomes for three scenarios where density-dependent (host density) and density-independent (transmission rate for a given host density) mechanisms were allowed to vary in their relationship with species diversity, using a Susceptible-Infected (SI) model of the deer mouse-Sin Nombre virus (SNV) system. Instead of antibody prevalence, two other metrics employed by a minority of studies found by our review were used: density of infected individuals, and force of infection (i.e. probability of infection). Across both disease metrics, scenario 1 (blue) generated dilution, scenario 2 (red) amplification, and scenario 3 (black), a non-monotonic relationship, consistent with findings when antibody prevalence was used instead. 37

Figure 3. 1 Parallelism curves for pooled fecal extract from deermice measured with (A) corticosterone enzyme immunoassay (EIA) and (B) group-specific EIA. Standard curves for each EIA are shown in black with each concentration as an open diamond. Parallelism is shown in red with serial dilutions of pooled fecal extract as open red circles. 61

Figure 3. 2 Changes in corticosterone baseline fecal cortisol/corticosterone metabolites (FCMs) from laboratory-bred deermice across the dark cycle measured with (A) corticosterone enzyme immunoassay (EIA) and (B) group-specific EIA. Lines connect means from each sampling time and circles indicate data points whereby an individual is denoted with a different color. Males are shown in blue and green colors, whereas females are shown in red and tan. 62

Figure 3. 3 Corticosterone baseline fecal cortisol/corticosterone metabolites (FCMs) (solid lines and circles) versus post dexamethasone FCMs (dashed lines and triangles) in laboratory-bred deermice measured with (A) corticosterone enzyme immunoassay (EIA) and (B) group-specific EIA. Lines connect means from each sampling time. Circles and triangles indicate individual data points whereby an individual is denoted with a different color. Males are shown in blue and green colors, whereas females are shown in red and tan. Dexamethasone was administered at ~20:00 h (not shown). Asterisk (*) denotes significant differences at $\alpha = 0.05$ 63

Figure 3. 4 Corticosterone baseline fecal cortisol/corticosterone metabolites (FCMs) (solid lines and circles) versus post adrenocorticotrophic hormone (ACTH) FCMs (dashed lines and triangles) in laboratory-bred deermice measured with (A) corticosterone enzyme immunoassay (EIA) and (B) group-specific EIA. Lines connect means from each sampling time. Circles and triangles indicate individual data points whereby an individual is denoted with a different color. Males are shown in blue and green colors, whereas females are shown in red and tan. ACTH was administered at ~20:00 h (not shown). 64

Figure 3. 5 Fecal cortisol/corticosterone metabolites (FCMs) across different trap confinement times in free-ranging deermice. FCMs were measured with the group-specific EIA. Boxplots display the median (line), 25–75% interquartile range (boxes) and the full range (whiskers). In the 0–2 h group, there is a large outlier. Circles indicate individual data points whereby blue denotes males and red denotes females. 65

Figure 4. 1 Small mammal densities for each grid (A-D) across study period (October 2016 to August 2018). Deermouse and vole densities were estimated using Minimum Number Alive (MNA) index. Shrew densities were estimated using unique captures per trapping session. 96

Figure 4. 2 Regression tree for stress response fecal corticosterone metabolites (FCMs) of deermice. Each terminal node provides a sample size with a boxplot for that subgroup. Density is per hectare. Statistical significance was set to 0.05. 97

Figure 4. 3 Regression tree for ln neutrophil to lymphocyte (N/L) ratio of deermice. Each terminal node provides a sample size with a boxplot for that subgroup. Grids A and B were trapped October'16 – July'18. Grids C and D were trapped October'17 – August'18. Juveniles and subadults were grouped into “Non-Adult”. F1, S1, Su1, F2, S2, and Su2 represent

Fall/Winter'16, Spring'17, Summer'17, Fall/Winter'17, Spring'18, and Summer'18, respectively. Statistical significance was set to 0.05. 98

Figure 4. 4 Regression tree for body condition score (BCS) of deermice. Each terminal node provides a sample size with a boxplot for that subgroup. "ReproStatus" stands for reproductive status. Juveniles and subadults were grouped together into "Non-Adult". F1, S1, Su1, F2, S2, and Su2 represent Fall/Winter'16, Spring'17, Summer'17, Fall/Winter'17, Spring'18, and Summer'18, respectively. Density is per hectare. Statistical significance was set to 0.05..... 99

Figure 4. 5 Regression tree of white blood cell (WBC) counts of deermice. Each terminal node provides a sample size with a boxplot for that subgroup. Grids A and B were trapped October'16 – July'18. Grids C and D were trapped October'17 – August'18. F1, S1, Su1, F2, S2, and Su2 represent Fall/Winter'16, Spring'17, Summer'17, Fall/Winter'17, Spring'18, and Summer'18, respectively. Statistical significance was set to 0.05. 100

Figure 4. 6 Regression tree for scar numbers of deermice. Each terminal node provides a sample size with a boxplot for that subgroup. Density is per hectare. "ReproStatus" stands for reproductive status. S1, F2, Su1, S2, and Su2 represent Spring'17, Fall/Winter'17, Summer'17, Spring'18, and Summer'18, respectively. Statistical significance was set to 0.05..... 101

Figure 5. 1 Relationships of density-independent contact rate (β_c) (A) and probability of transmission (β_p) (B) with competitor density (V). **A.** The effect of V on β_c , (ψ), was set to either zero (—) to denote constant β_c , or 0.001064445 (—) and - 0.001064445 (—), to denote increasing or decreasing β_c with V , respectively. The intercept was set to 0.02128889. **B.** The effect of V on β_p , (γ), was set to either 0.03 (—), 0.15 (—), or 0.30 (—), to develop relationships that ranged from linear to more exponential with V . The intercept was set to 0.50. 127

Figure 5. 2 Relationships between Sin Nombre virus (SNV) prevalence and vole competitor density. The effect of the competitor on host density (α) and probability of transmission (γ) via stress-induced immunosuppression, were varied as shown, while assuming constant density-independent contact rates between hosts ($\psi = 0$). 128

Figure 5. 3 Parameter space of 5859 combinations of α , γ and ψ values corresponding to their respective outcome for a fixed competitor density, V , (0-20). Dark purple signifies an amplification effect or a sole increase in disease prevalence, light purple, a dilution effect or a sole decline in prevalence, light green, no change in prevalence (i.e. represents baseline when all parameters are set to zero), and dark green, a non-monotonic relationship between prevalence and competitor density. The percentage values indicate the proportion of parameter space represented by each outcome. 129

Figure S5. 1 Total deermouse (host) density, N , (— — —), and critical host density, N_c , (—) relationships with vole competitor density, V , (top row), and respective outcome between Sin Nombre virus (SNV) prevalence (bottom row) and V . The effect of competitors on contact rates, (ψ), and probability of transmission, (γ), were kept at zero. The effect of competitors on host density, α , was varied in strength as displayed. 130

Figure S5. 2 Total deer mouse (host) density, N , (---), and critical host density, N_c , (—) relationships with vole competitor density, V , (top row), and respective outcome between Sin Nombre virus (SNV) prevalence (bottom row) and V . The effect of competitors on host density (α), and contact rates (ψ) were kept at zero. The effect of competitors on the probability of transmission, (γ), was varied in strength as displayed..... 131

Figure S5. 3 Total deer mouse (host) density, N , (---), and critical host density, N_c , (—) relationships with vole competitor density, V , (top row), and respective outcome between Sin Nombre virus (SNV) prevalence (bottom row) and V . The effect of competitors on host density (α) and on the probability of transmission (γ) were kept at zero. The effect of competitors on contact rates (ψ) was varied as displayed..... 132

Figure S5. 4 Total deer mouse (host) density, N , (---), and critical host density, N_c , (—) relationships with vole competitor density, V , to complement figure 2 in the main text. The effect of competitors on host density (α) and on the probability of transmission (γ) were varied as shown. The effect of competitors on contact rates (ψ) was kept at zero..... 133

Figure S5. 5 Total deer mouse (host) density, N , (---), and critical host density, N_c , (—) relationships with vole competitor density, V , (top row), and respective outcome between Sin Nombre virus (SNV) prevalence (bottom row) and V . The effect of competitors on host density (α) and probability of transmission (γ) were varied as shown, while the effect of competitors on contact rates, (ψ), was fixed at 0.001064445. 134

Figure S5. 6 Total deer mouse (host) density, N , (---), and critical host density, N_c , (—) relationships with vole competitor density, V , (top row), and respective outcome between Sin Nombre virus (SNV) prevalence (bottom row) and V . The effect of competitors on host density (α) and probability of transmission (γ) were varied as shown, while the effect of competitors on contact rates, (ψ), was fixed at - 0.001064445. 135

Figure S5. 7 Relationships between Sin Nombre virus (SNV) prevalence and vole competitor density as modeled with linear regression (—) when competitors have a weak effect on host density ($\alpha = 0.8$) and no effect on contact rate ($\psi = 0$), a scenario shown in Figure 5.2 of the main text. When we add a degree of stochasticity to the simulated data, an obvious dilution effect can only be seen when a monotonic relationship is predicted ($\gamma = 0.03$). Otherwise, no pattern is found with linear regression due to the non-monotonic shape of the predicted relationship..... 136

CHAPTER 1 OVERVIEW OF DISSERTATION

BACKGROUND

Globally, biodiversity is declining while emerging infectious diseases are on the rise. To explain this pattern, the “dilution effect” hypothesis predicts that with lower diversity, there will be higher disease risk because diversity “dilutes” disease. However, community composition (e.g., competitors) may be more important than diversity *per se*. Although the “dilution effect” hypothesis has been tested across various disease systems, its underlying mechanisms have been studied mostly in vector-borne (e.g., Lyme disease) and environmentally-transmitted (e.g., amphibian trematodes) disease systems. However, mechanisms in directly-transmitted disease systems, such as rodent-hantavirus systems, have been relatively understudied. This is an important knowledge gap because mechanisms will differ between directly-transmitted and other disease systems. A mechanistic approach can help us better understand and predict how losses in biodiversity can impact disease outcomes in people, animals, and plants, across disease systems.

RESEARCH OBJECTIVES

Although rodent-hantavirus systems are commonly cited as examples of directly-transmitted disease systems that support the “dilution effect” hypothesis, we know little about the underlying mechanisms. The overall goal of this dissertation was to investigate the mechanisms that drive patterns between disease and diversity (or community composition) in directly-transmitted disease systems, using rodent-hantavirus systems as models. Through a systematic literature review, I collated the existing evidence on diversity-disease patterns across rodent-hantavirus systems, identified and synthesized mechanistic evidence, and demonstrated diversity-disease outcomes in a realistic modeling framework that incorporated opposing mechanisms. To empirically collect my own mechanistic evidence, I used Sin Nombre hantavirus (SNV), and its reservoir host, the North American deermouse (*Peromyscus maniculatus*, hereafter deermouse),

as a model system in western Montana grasslands. In this system, voles (*Microtus* spp.) are the dominant competitor of deermice with shrews (*Sorex* spp.) serving as an additional member in species-poor small mammal communities. Based on evidence from the literature, I hypothesized that voles would influence contact rates, induce chronic stress, and suppress immunity in deermice, with shrews having a lesser effect. To empirically quantify how competitors impacted stress physiology of deermice, I conducted experimental studies in the laboratory and field to validate two enzyme immunoassays for measuring fecal metabolites of corticosterone (the primary glucocorticoid hormone in deermice), as a measure of stress. Over approximately two years, I performed an observational study at Ninepipe WMA where I trapped small mammals monthly and collected measures of contact rates (scar numbers), immunity, and stress physiology from deermice (using one of the immunoassays from Chapter 3 in addition to other measures). Motivated by the theoretical results of Chapter 2 and empirical results of Chapter 4, I modified an existing mathematical model of the deermouse-SNV model to run simulations where I incorporated effects of vole competitors on deermouse density, intraspecific contact rates, and immunity via stress physiology, to examine single and interactive effects on disease outcomes.

RESULTS

In Chapter 2, I described all the possible density-dependent and -independent (contact rates, probability of transmission given contact, infectious period) mechanistic links between disease prevalence and species diversity (or community composition) for directly-transmitted diseases with one primary reservoir and density-dependent transmission, a combination representative of rodent-hantavirus systems. Through a systematic review, I found evidence for dilution, amplification, and neutral outcomes across rodent-hantavirus systems. Most importantly, mechanistic studies primarily examined host density, with fewer investigating density-independent mechanisms. A modeling exercise inspired by empirical data, with the deermouse-

SNV system, demonstrated a non-monotonic outcome between diversity and disease when density-dependent and -independent mechanisms competed within the same system. In Chapter 3, I provided experimental evidence that physiologically validated two different enzyme immunoassays for quantifying fecal corticosterone metabolites (FCMs), a measure of stress in deermice and showed that confinement in a live-trap for more than four hours can increase FCMs in free-ranging deermice. In Chapter 4, I demonstrated associations between heterospecific competitors (voles and shrews) and measures of contact rates (scar numbers) and stress physiology (FCMs, body condition). In particular, only shrews decreased stress response FCMs in deermice whereas both shrews and voles decreased body condition scores. I also found that deermice had higher neutrophil/lymphocyte ratios (a measure of stress) during spring/summer, and lower white blood cell counts (a measure of immunity), with higher scar numbers (a measure of contact rates) in summer. In Chapter 5, I demonstrated that a non-monotonic outcome between competitor density and disease prevalence, a result of competing density-dependent and -independent mechanisms, occupied most of the parameter space examined, which was kept realistic for the deermouse-SNV system.

CONCLUSIONS

The systematic review suggests that host density is most likely the primary mechanism by which diversity can indirectly impact disease prevalence, and that density-independent mechanisms (contact rates, probability of transmission given contact, and infectious period) still require further empirical investigation. The latter is especially important because non-monotonic outcomes between diversity and disease could occur when density-independent mechanisms compete with host density in the same disease system. Given the results from the observational study at Ninepipe WMA, stress physiology could perhaps be an important mediator by which diversity (or community composition) may impact probability of transmission given contact,

thereby increasing the host's susceptibility to infection or infectiousness. Lastly, non-monotonic outcomes stemming from competing mechanisms, occupied most of the parameter space examined for the deermouse-SNV system. This is crucial because non-monotonic outcomes could be misinterpreted since researchers typically look only for linear relationships in field studies. Indeed, such misinterpretations could help explain why some studies report no relationships between diversity and disease.

CHAPTER 2 MECHANISTIC LINKS BETWEEN BIODIVERSITY AND DIRECTLY-TRANSMITTED DISEASES: A REVIEW OF THEORY AND EMPIRICAL EVIDENCE FROM RODENT-HANTAVIRUS SYSTEMS

ABSTRACT

Globally, biodiversity is declining while emerging infectious diseases are on the rise. To explain this pattern, the “dilution effect” hypothesis predicts that with lower diversity, there will be higher disease risk because diversity “dilutes” disease. The mechanisms behind this hypothesis have been relatively understudied in directly- transmitted disease systems, such as orthohantaviruses (hereafter hantaviruses) and their rodent reservoirs. Here, we aim to: (1) characterize mechanisms underlying diversity-disease relationships in rodent-hantavirus systems, (2) review the literature to identify and synthesize mechanistic evidence, and (3) demonstrate relationships that could occur from competing mechanisms within a modeling framework. Our findings suggest that host density is the primary mechanistic driver of a diversity-disease pattern, and density-independent mechanisms (intraspecific contact rates, probability of transmission, and infectious period) still require further investigation. Our modeling exercises demonstrate that a decrease (dilution) or increase (amplification) in disease prevalence could occur, as well as a non-monotonic pattern (increase then decrease), depending on mechanisms considered (density-dependent and -independent) and their relationship with diversity. The modeling findings advocate for the examination of understudied mechanisms and could explain the idiosyncratic nature of diversity-disease relationships. Although this review focuses on directly-transmitted diseases, our mechanistic approach and findings can be extended to other diseases.

THE RELATIONSHIP BETWEEN DIVERSITY AND DISEASE

Globally, anthropogenic actions have led to a rapid loss in species diversity [1] while emerging infectious diseases of humans [2] and wildlife [3] have been on the rise. To explain

this alarming pattern, the “dilution effect” hypothesis postulates that as species diversity decreases, disease risk increases (e.g. higher disease prevalence or transmission), because diversity “dilutes” disease [4]. This hypothesis has attracted a great deal of attention because if true and universal, conserving biodiversity would be a win-win scenario for both conservation and public health. However, there has been a heated debate about its generality and applicability across host-parasite systems [5-8]. This is partially because of evidence that supports an alternative hypothesis, the “amplification effect” hypothesis, which predicts that as diversity decreases, disease risk decreases [4,9]. The debate resulted in a call to move beyond simply describing correlations between diversity and disease, to investigating and identifying the mechanisms behind these patterns [10-12]. Such an approach can generate mechanistic models that can guide useful strategies for mitigation and prevention of infectious diseases [13].

Of the empirical studies focused on testing mechanisms that lead to disease dilution or amplification [4], most have been with vector-borne parasites, such as *Borrelia burgdorferi* (causative agent of Lyme disease) (e.g. [14]), and parasites with complex life cycles, such as amphibian trematodes (e.g. [15]). Although most infectious diseases are transmitted directly, relatively few studies have examined the mechanisms that underlie relationships between such diseases and biodiversity [11,13]. This is problematic because mechanisms can differ between directly-transmitted and other systems. Therefore, more empirical studies that investigate mechanisms in directly-transmitted disease systems are urgently needed.

DIVERSITY-DISEASE PATTERNS IN DIRECTLY-TRANSMITTED DISEASE SYSTEMS

Orthohantaviruses (*Orthohantavirus*; Hantaviridae; Bunyavirales; hereafter hantaviruses), and their rodent hosts, [16] are examples of directly-transmitted disease systems, which have received considerable attention from a diversity-disease perspective. Indeed, these systems are

commonly cited as examples of directly-transmitted diseases that support the “dilution effect” hypothesis. The focus on these systems is likely because of two reasons. Firstly, because several hantaviruses can cause fatal disease in humans, there is an urgency to investigate their epidemiology and ecology to help inform public health [17]. Secondly, these systems meet the criteria necessary for diversity to “dilute” disease because other species do not typically serve as viable hosts and hantavirus reservoirs are likely to persist as species diversity declines [18].

To various degrees, six recent reviews have synthesized evidence about diversity-disease relationships across rodent-hantavirus systems [17,19-23]. However, there has been no systematic review delving into the mechanisms that drive these relationships, the focus of this review. Here, we summarize what is presently known about diversity-disease patterns. Next, we summarize and synthesize empirical evidence about the underlying mechanisms. Lastly, we demonstrate the significance of considering simultaneous mechanisms within the modeling framework of a directly-transmitted disease system.

Although we focus on rodent-hantavirus systems, our mechanistic approach of reviewing theory and evidence can be valuable across host-parasite systems. Importantly, our quantitative approach of incorporating multiple mechanisms within a modeling framework can be customized and extended to other systems.

DIVERSITY-DISEASE PATTERNS IN RODENT-HANTAVIRUS SYSTEMS

HANTAVIRUSES AND THEIR RESERVOIR HOSTS

Hantaviruses are found in Eurasia, Africa, and the Americas [24]. Several hantaviruses have been classified as emerging zoonoses, including Sin Nombre virus (SNV) and Andes virus (ANDV) in the Americas, Puumula virus (PUUV) in Europe, and Hantaan virus (HTNV) in Asia [25]. SNV and ANDV cause hantavirus cardiopulmonary syndrome with up to a 40% fatality rate in humans. In contrast, HTNV and PUUV cause hemorrhagic fever with renal syndrome

with lower fatality rates [17,26]. Humans are typically exposed to hantaviruses indirectly via aerosolized excreta and secreta of infected rodents [17].

Hantaviruses are primarily transmitted between hosts during aggressive encounters [17,27], although indirect transmission may also occur [28-29]. Hantaviruses typically cause a chronic infection in their hosts despite life-long production of antibodies [17]. Although infection does not typically lead to overt clinical signs [30], some studies with free-ranging hosts have found negative effects, such as lower survival [31] and reduced fecundity [32].

EVIDENCE FOR DIVERSITY-DISEASE RELATIONSHIPS IN RODENT-HANTAVIRUS SYSTEMS

Our literature review identified 35 studies (Table 2.1), including studies that did not test for mechanisms (see supplementary information about search process). However, two were meta-analyses [63-64] that included studies our search had already found. Consequently, these were not included in Table 2.1, but we discuss them further in the supplementary information. Out of the remaining 33 studies, three were experimental [39,54,59]. We also note that a few studies used subsets of the same datasets ($N = 8$, Table 2.1), which we counted only once if they generated identical outcomes. In the end, most studies identified a dilution effect ($N = 12$ [18,33,36-38,41,43,46,48,54,58,60]). Others found either no effect ($N = 6$ [49,51,53,55,57,59]), amplification ($N = 2$ [42,50]), or a non-monotonic outcome ($N = 1$, [62]). The remaining studies reported conditional outcomes: a “component amplification” effect with overall dilution or no effect depending on geographic location ($N = 1$, [11]), amplification or a non-monotonic relationship depending on the diversity metric ($N = 1$, [61]), and dilution or no effect depending on the diversity ($N = 1$, [35]) or disease ($N = 2$, [47,50]) metric.

Most studies were done on a small spatial scale (i.e. field plots), which is the scale where we expect diversity to affect disease spread. Two studies were performed at a larger spatial scale (i.e. municipality or district) [61-62], which we revisit in the modeling section.

DIVERSITY-DISEASE MECHANISMS: THEORY AND EVIDENCE

Rodent-hantavirus studies have used various metrics to quantify disease, including prevalence of hantavirus antibody (i.e., seroprevalence), number of infected individuals, and individual probability of infection (i.e., force of infection). Because most studies used antibody prevalence, we focused on this metric.

DETERMINANTS OF DISEASE PREVALENCE

For directly-transmitted diseases with one primary reservoir host, such as hantaviruses, there are three determinants of R_0 , which dictates disease prevalence at equilibrium [4]. Therefore, when antibody prevalence is the metric used, there are three mechanisms that can directly drive diversity-disease relationships (Figure 2.1, gray arrows). The first is contact rates between infected and susceptible hosts, which diversity can affect directly (*sensu* encounter reduction, [4], Figure 2.1, gray arrow) or indirectly via host density (*sensu* susceptible host regulation, [4], Figure 2.1, blue arrow). The second is probability of transmission given contact (*sensu* transmission reduction, [4], Figure 2.1, gray arrow), representing host infectiousness and susceptibility to infection [65], which can also be affected indirectly via density (*sensu* susceptible host regulation, [4], Figure 2.1, blue arrow). The third is the infectious period of hosts (*sensu* infected host mortality, recovery augmentation, or both [4], Figure 2.1, gray arrow), which can be affected indirectly via density as well (*sensu* susceptible host regulation, [4], Figure 2.1, blue arrow). For rodent-hantavirus systems, the infectious period is determined by host

survival because hosts never recover from infection. If diversity affects any of these mechanisms, it can alter disease prevalence and lead to dilution or amplification.

As mentioned above, host density can play a powerful role in how the three determinants of R_0 can influence disease prevalence. However, we do not consider host density as a direct determinant of R_0 because it can only affect disease indirectly. Importantly, the indirect effect of density on disease via intraspecific contact rates, will only apply when disease transmission is density-dependent. With density-dependent transmission, contact rates are expected to increase directly with host density (Figure 2.1, blue arrow)—that is, the true contact rate will be determined by κN , where κ is a constant and N is host density [66]. For example, an increase in diversity could affect density-dependent contact rates by reducing host density and leading to lower overlap of home ranges, thereby reducing contact rates. For diversity to directly affect contact rates, it needs to do so independent of host density (Figure 2.1, gray arrow). For example, an increase in diversity could affect density-independent contact rates by changing host behavior, such as restricting movement in the presence of competitors. However, with frequency-dependent transmission, contact rates are independent of density [66]; therefore, diversity cannot indirectly impact contact rates via density.

In contrast to contact rates, mode of transmission does not matter when host density affects the other two determinants of R_0 — probability of transmission given contact and infectious period (Figure 2.1, blue lines). Empirical evidence suggests that a higher host density, could increase the probability of transmission given contact (hereafter, probability of transmission), via stress-induced suppression of immunity (e.g. [67-68]). Alternatively, higher host density could reduce probability of transmission if it leads to enhanced immunity (e.g. [69]). Furthermore, evidence suggests that higher density could increase the infectious period by

increasing host survival if access to food resources is increased (e.g. [70]). However, higher density could also decrease the infectious period if it leads to a decline in food availability (e.g. [71]). Therefore, whenever we examine relationships between diversity and disease, there is evidence to suggest the need to consider the effects of density, not only on contact rates but also on the probability of transmission and infectious period.

In the end, it is important to consider that multiple mechanisms, acting independent (Figure 2.1, gray lines) or dependent on host density (Figure 2.1, blue lines), may be at play at any given time, which may lead to opposing, additive, or synergistic effects on outcomes.

We found 14 studies that examined mechanisms behind diversity-disease relationships for rodent-hantavirus systems. Studies have paid most attention to host density, and to a lesser extent, contact rates independent of density as hypothetical mechanisms, whereas probability of transmission, and infectious period, have been neglected (Table 2.1). Below, we review existing evidence to provide a strong foundation for future studies. We focus on the deer mouse-SNV system because it was the most commonly investigated (Table 2.1). However, we provide some evidence for the bank vole-PUUV system in the supplementary information.

HOST DENSITY

Host density can have a central role in driving disease prevalence by potentially affecting each of three mechanisms: contact rates, probability of transmission, and infectious period. Consequently, when diversity is negatively or positively associated with host density, there will be fewer or more hosts, respectively, with higher diversity, which can indirectly influence contact rates, probability of transmission, and infectious period (Figure 2.1).

For host density to indirectly drive diversity-disease relationships, two conditions must be met: (1) host density must associate with species diversity, and (2) transmission must be density-

dependent for contact rates to serve as a mechanism (Figure 2.1, blue line). However, even if transmission is density-dependent, density-mediated changes in contact rates are not the only possible mechanism; probability of transmission and infectious period could also serve as possible mechanisms (Figure 2.1, blue lines). Generally, when diversity negatively affects density, dilution would be expected. Alternatively, if diversity positively affects density, amplification would be predicted. Below, we examine evidence for the two conditions listed above.

There is compelling evidence that host density is a key mechanism driving the relationship between diversity and disease. This is exemplified by four empirical studies that identified dilution, and also a negative relationship between diversity and deer mouse density (Table 2.1, [11, 33, 36-37]). However, depending on the ecological scenario, diversity does not always lead to a decrease in host density, and subsequently dilution is not observed. For example, Luis et al. [11] found a dilution effect in the southwest U.S.A., where they also saw a negative relationship between small mammal diversity and deer mouse density; however, they found no evidence for dilution in Montana, U.S.A., where there was no relationship between diversity and density (Table 2.1). Although the meta-analysis by Vadelin et al. [63] merged deer mouse-SNV with other systems, they found that host density was the main predictor of hantavirus prevalence, and once accounted for, there was no significant effect of diversity on disease prevalence, and only weak evidence for a negative relationship between diversity and density. Taken together, these studies strongly suggest that density is a strong indirect determinant of disease, and when diversity decreases host density, there is a dilution effect, but when diversity does not decrease host density, there is no effect.

A positive relationship between host density and disease prevalence is expected if there is density-dependent transmission. In the deermouse-SNV system, researchers have not consistently found a simultaneous positive relationship between host density and disease prevalence ([33, 36-37] but see [40]), and some have concluded that diversity could not affect contact rates via density. However, Luis et al. [72] showed that deermouse density does indeed correlate positively with SNV prevalence, but with a variable time lag, which is most apparent when using a dynamical mathematical model with density-dependent transmission. Because SNV prevalence lags behind deermouse density, testing for a simultaneous relationship between current deermouse density and SNV prevalence can lead to misinterpretations. Given the rising support for density-dependent transmission, host density may indeed serve as a key indirect determinant of disease prevalence.

However, there are two studies that contradict this line of reasoning. The first is by Dizney & Dearing [38], from Utah U.S.A., who found a dilution effect at two sites varying in diversity but not current deermouse density. However, because of inherent time lags in how SNV prevalence manifests, it is unclear if the two sites were indeed similar. The second study is by Carver et al. [41], from one site in Montana, U.S.A, who found a positive relationship between vole and deermouse densities. Although the authors found no relationship between SNV prevalence and vole density (i.e. no effect), they did find a dilution effect when voles were present (i.e. a temporal dilution).

Despite their contradictory findings, these two studies used sites included in the more extensive studies by Clay et al. [33] and Luis et al. [11]. Although Clay et al. [33] did find dilution, they also found a negative relationship between diversity and density by using data collected over a longer time span from 16 sites. In contrast to Carver et al. [41], Luis et al. [11]

detected no dilution and no relationship between density and diversity when they analyzed five more sites from Montana. However, Luis et al. [11] used density and diversity values that were averaged across time, whereas Carver et al. [41] examined average values from month-to-month. Therefore, it will be important to further examine how incorporating temporal fluctuations in diversity, density and disease metrics could affect study conclusions. In summary, differences in study design and data analysis could be why these two studies do not fit the overall pattern.

DENSITY-INDEPENDENT CONTACT RATES

For directly-transmitted diseases with density-dependent transmission, intraspecific contact rates could be affected by species diversity in two ways— indirectly, through changes in host density (Figure 2.1, blue arrow), and directly, independent of density (Figure 2.1, gray arrow). To determine if diversity affects density-independent contact rates, studies must ensure that contact rates are not changing due to density (Figure 2.1, blue arrow). When diversity directly reduces contact rates (e.g. via changes in host behavior), dilution can emerge, whereas amplification can occur, when diversity directly increases contact rates (Figure 2.1, gray arrow).

Only five studies tested contact rates as a hypothetical mechanism. Four directly evaluated contact rates through observational or experimental design setups, whereas one did so indirectly, as a component of the transmission rate (Table 2.1).

Two of the five studies were performed at sites naturally varying in deer mouse density. Therefore, density was not manipulated but accounted for during analyses. The first study is from Utah, U.S.A., which found lower intraspecific contact rates at more diverse sites [34]. The authors used foraging arenas and infrared cameras to document the frequency and duration of contacts. Their findings suggested that a higher diversity decreases frequency of contacts but not their duration. Although interesting, two limitations were the inclusion of an influential outlier,

and the foraging arenas may have artificially modified behavior. The second study from Montana, U.S.A., found that the number of scars (a proxy for contact rates) was differentially associated with densities of shrews (*Sorex* spp.) and voles (*Microtus* spp.), but not deermice [42]. Despite intriguing conclusions, number of scars is unlikely to be a very precise measure. Taken together, although these studies provide motivating evidence, it is challenging to definitively make conclusions about the role of density-independent contact rates because density was not controlled experimentally.

Two other studies controlled for the effects of density more reliably. One of these two studies was done in Utah, U.S.A., where their two sites naturally varied in rodent diversity, but not current deermouse density [38]. Although there was no site replication, deermice at the less diverse site exhibited behaviors that could potentially increase contact rates (e.g. time spent foraging). However, because they used foraging arenas, it is unclear if behaviors were contrived, and whether they would lead to higher contact rates. The second study by Rubio et al. [39] used experimental enclosures in Mexico and found that rodent diversity did not influence deermouse contact rates. Although this study did not test deermice for SNV, used foraging arenas to record behaviors, and included treatments with only one or two nonhost species, they ensured that host density was held constant, making it more likely they measured density-independent contacts.

The last study by Luis et al. [11] found that for a given host density, the SNV transmission rate (product of contact rates and probability of transmission), increased with higher small mammal diversity in Montana, and Southwest U.S.A. The authors called this pattern a “component amplification” because the net effect was either dilution or no effect. However, they could not disentangle contact rates from probability of transmission.

Although the aforementioned studies have strengths and weaknesses, they provide intriguing, yet inconclusive evidence, about whether diversity can affect contact rates independent of density. Nevertheless, Rubio et al. [39] may have most reliably examined density-independent contact rates by experimentally controlling for density in replicated treatment groups. However, community composition was different between their study in Mexico and studies in U.S.A., which makes definitive conclusions more difficult.

DENSITY-INDEPENDENT PROBABILITY OF TRANSMISSION GIVEN CONTACT

The probability of transmission will depend on host susceptibility to infection, and infectiousness, both of which are regulated by host immunity. However, studies that aim to test probability of transmission independent of density, must ensure that they account for changes via density (Figure 2.1, blue arrow) regardless of transmission mode. When diversity directly decreases or increases the probability of transmission, dilution or amplification can emerge, respectively (Figure 2.1, gray arrow).

There are two studies that investigated probability of transmission as a hypothetical mechanism (Table 2.1). Because intricate physiological processes (e.g. endocrine) will typically affect host susceptibility or infectiousness, this mechanism can be difficult to estimate from field data [73]. Such complexity may explain why this mechanism has been rarely studied.

The first study by Luis et al. [11], mentioned previously, could not identify whether contact rates, probability of transmission, or both, were responsible for a “component amplification”. The second study by Eleftheriou et al. [42], also mentioned previously, reported that densities of shrews and voles, but not deermice, were associated with measures of stress physiology in deermice. Although their findings come with limitations, they are relevant because changes in stress could negatively impact immunity, thereby increasing host susceptibility or

infectiousness. Therefore, there is some evidence to suggest that probability of transmission, independent of density, could potentially play an influential role. Indeed, both aforementioned studies suggest that diversity (or community composition) may have a positive impact on probability of transmission, an effect potentially mediated via stress physiology.

DENSITY-INDEPENDENT INFECTIOUS PERIOD

Because hosts never recover from hantavirus infection, host survival determines the infectious period. Therefore, when diversity directly reduces the survival of infected hosts, by either increasing natural and/or disease-induced mortality, it can reduce their infectious period and cause dilution. Alternatively, diversity could increase host survival and lead to amplification (Figure 2.1, gray arrow). In either case, density-independent infectious period could serve as a hypothetical mechanism. However, if diversity also regulates host density, it could become difficult to unravel the indirect effects of density (Figure 2.1, blue arrow).

Only one study has examined density-independent infectious period as a potential mechanism. This study used deermouse persistence as a measure of infectious period, which makes separating survival from dispersal challenging [33]. Although deermouse persistence was lower with higher rodent diversity, this conclusion did not account for density effects (Figure 2.1, blue arrow). Thus, even if we assumed that dispersal was minimal, it would be misleading to state that dilution was due to a reduction in infectious period independent of density.

EFFECTS OF MULTIPLE MECHANISMS ON DISEASE OUTCOMES

CONCURRENT DILUTION AND AMPLIFICATION EFFECTS

Although researchers typically search for dilution or amplification, Luis et al. [11] demonstrated that both can occur at the same time via different mechanisms in the deermouse-SNV system.

Therefore, researchers should consider whether concurrent effects could exist in their own systems as they examine all relevant mechanisms at play. To motivate this idea, we investigate through simulations how interactive mechanisms could modify outcomes using the deermouse-SNV system as a model.

SIMULATION EXERCISES WITH THE DEERMOUSE-SNV SYSTEM

Here, we use a simpler version of the Susceptible-Infected (SI) model from [11], which assumes density dependence (supplementary information). There is no recovered class because deermice never recover. We first generated linear relationships between host density and species diversity, and between transmission rate (the product of density-independent contact rates and probability of transmission) and species diversity (supplementary information, Figure S2.1). These relationships were inspired by empirical patterns [11].

By incorporating different relationships between each mechanism and diversity, we completed three scenarios to visualize patterns that could manifest at equilibrium (supplementary material, Table S2.1). Firstly, diversity influenced only host density (scenario 1, Figure 2.2). Secondly, diversity affected only the transmission rate for a given density (scenario 2, Figure 2.2). Thirdly, diversity impacted both density and transmission rate (scenario 3, Figure 2.2).

In scenario 1, where diversity negatively affected density, dilution occurred (Figure 2.2, blue line). In scenario 2, where diversity positively affected the transmission rate, amplification occurred instead (Figure 2.2, red line). In scenario 3, where diversity negatively affected density, but positively influenced transmission rate, our findings showed a non-monotonic relationship, where prevalence peaked at intermediate levels of diversity (Figure 2.2, black line). Collectively, we show that dilution, amplification, or a non-monotonic relationship, could theoretically

develop within the same system. These results were robust to the disease metric used (supplementary material, Figure S2.2).

A non-monotonic relationship has previously been described as a potential pattern across systems, but under a different framework dependent on scales of biodiversity [13,74-75]. Specifically, at lower diversity, there could be no host species, so disease risk would be zero (e.g., urban centers). However, as diversity increases (e.g., urban to rural gradient), host species would be more likely to occur, thereby increasing disease risk. Conversely, at higher diversity, non-competent species would also be likely to exist (e.g., forests), thereby “diluting” disease. Such non-monotonic patterns have been described by studies we found during our search [61-62]. These two studies, done at broad spatial scales, found non-monotonic patterns between predator diversity and cases of human infections.

However, here, we show that a non-monotonic relationship between diversity and disease, can also occur independent of scale, when diversity affects determinants of prevalence in opposing directions. This pattern occurs because the dominant mechanism changes across values of diversity. At lower diversity, the transmission rate is the primary mechanism that increases disease whereas at higher diversity, host density predominates to decrease disease because density becomes too low to sustain transmission, despite a maximum transmission rate. Results from more complex models with the same system, where the transmission rate was deconstructed into its two components, demonstrated similar patterns (Eleftheriou & Luis, Chapter 5). Those findings suggest that non-monotonic outcomes could be misinterpreted as no effect, potentially helping to explain why some studies may report no pattern (Table 2.1).

Given that we used a simple mathematical model with density-dependent transmission, our modeling approach can be applied to other systems, as long as additional mathematical

equations are incorporated that address system specifics. Using this approach, researchers can determine how mechanisms that vary in their relationship to diversity could alter final outcomes. This approach can also inform prospective studies, to help guide what mechanisms will be the most critical to evaluate empirically.

CONCLUSIONS AND FUTURE DIRECTIONS

In the context of the “dilution effect” hypothesis, we conclude that host density is the primary mechanism that has received the most attention from empirical studies using rodent-hantavirus systems as models of directly-transmitted diseases. Indeed, several studies have provided support for host density as the strongest determinant of hantavirus prevalence, whereby dilution, amplification, or neutral outcomes can culminate according to the relationship between density and diversity. If there is a negative relationship between diversity and density, we would expect to see dilution as exemplified by several studies (Table 2.1). Alternatively, if there is a positive relationship, we would expect amplification, as shown by one study from Argentina (Table 2.1). However, if there is no relationship, we would expect no effect, a prediction supported by two studies (Table 2.1). Further support comes from studies that did not report a disease outcome but found a negative relationship between density and diversity (Table 2.1). Similarly, a meta-analysis by Rubio et al. [76] found lower small mammal diversity but higher host density in fragmented habitats. We acknowledge that the small number of studies highlights the need for further investigation to verify and generalize these conclusions.

Despite our claim that host density appears to be the primary mechanistic driver, we know little about which mechanisms are actually affected (Figure 2.1, blue lines). Broadly, we also know much less about the roles of density-independent mechanisms: contact rates, probability of transmission, and infectious period. The latter becomes significant when we

consider studies that find dilution or amplification in the absence of the expected relationship between diversity and density. For example, Khalil et al. [46] found a positive relationship between nonhost and host densities despite finding a dilution effect (Table 2.1), suggesting that density-independent mechanisms may be at play. Therefore, we require more empirical studies to further characterize understudied mechanisms.

When testing for relationships between diversity and disease, it will be crucial to consider the likelihood of competing dilution and amplification within the same system. Our simulation exercises emphasize that contrasting outcomes can result, depending on the relationship between each mechanism and diversity. These outcomes further highlight the need to expand our knowledge about understudied mechanisms because they could compete with density to affect the net outcome. Consequently, to best inform disease control and biological conservation, we suggest that researchers use similar exercises with other systems to elucidate theoretical outcomes that could potentially manifest in nature from competing mechanisms.

ACKNOWLEDGEMENTS

The authors acknowledge funding from the National Science Foundation under Grant No. 1836793, Wildlife Disease Association and University of Montana (W.A. Franke Endowed Fellowship in Wildlife Biology, Bertha Morton Fellowship, Clancy Gordon Environmental Scholarship).

LITERATURE CITED

- [1] Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., Narwani, A., Mace, G. M., Tilman, D. & Wardle, D. A. 2012 Biodiversity loss and its impact on humanity. *Nature*. 486, 59-67.
- [2] Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L. & Daszak, P. 2008 Global trends in emerging infectious diseases. *Nature*. 451, 990-993.
- [3] Daszak, P., Cunningham, A. A. & Hyatt, A. D. 2000 Emerging infectious diseases of wildlife--threats to biodiversity and human health. *Science*. 287, 443-449.

- [4] Keesing, F., Holt, R. D. & Ostfeld, R. S. 2006 Effects of species diversity on disease risk. *Ecology Letters* 9, 485-498.
- [5] Randolph, S. E. & Dobson, A. 2012 Pangloss revisited: a critique of the dilution effect and the biodiversity-buffers-disease paradigm. *Parasitology*. 139, 847-863.
- [6] Ostfeld, R. S. 2013 A Candid response to Panglossian accusations by Randolph and Dobson: biodiversity buffers disease. *Parasitology*. 140, 1196-1198.
- [7] Wood, C. L. & Lafferty, K. D. 2013 Biodiversity and disease: a synthesis of ecological perspectives on Lyme disease transmission. *Trends in Ecology & Evolution*. **28**, 239-247.
- [8] Ostfeld, R. S. & Keesing, F. 2013 Straw men don't get Lyme disease: response to Wood and Lafferty. *Trends in Ecology & Evolution* 28, 502-503.
- [9] Salkeld, D. J., Padgett, K. A. & Jones, J. H. 2013 A meta-analysis suggesting that the relationship between biodiversity and risk of zoonotic pathogen transmission is idiosyncratic. *Ecology Letters* 16, 679-686.
- [10] Johnson, P. T., Ostfeld, R. S. & Keesing, F. 2015 Frontiers in research on biodiversity and disease. *Ecology Letters* 18, 1119-1133.
- [11] Luis, A. D., Kuenzi, A. J. & Mills, J. N. 2018 Species diversity concurrently dilutes and amplifies transmission in a zoonotic host-pathogen system through competing mechanisms. *Proc. Natl. Acad. Sci. U. S. A.* 115, 7979-7984.
- [12] Rohr, J. R., Civitello, D. J., Halliday, F. W., Hudson, P. J., Lafferty, K. D., Wood, C. L. & Mordecai, E. A. 2020 Towards common ground in the biodiversity–disease debate. *Nature Ecology & Evolution*, 1-10.
- [13] Kilpatrick, A. M., Salkeld, D. J., Titcomb, G. & Hahn, M. B. 2017 Conservation of biodiversity as a strategy for improving human health and well-being. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 372, 20160131.
- [14] LoGiudice, K., Duerr, S. T., Newhouse, M. J., Schmidt, K. A., Killilea, M. E. & Ostfeld, R. S. 2008 Impact of host community composition on Lyme disease risk. *Ecology*. 89, 2841-2849.
- [15] Orlofske, S. A., Jadin, R. C., Preston, D. L. & Johnson, P. T. 2012 Parasite transmission in complex communities: predators and alternative hosts alter pathogenic infections in amphibians. *Ecology*. 93, 1247-1253.
- [16] Adams, M. J., Lefkowitz, E. J., King, A. M., Harrach, B., Harrison, R. L., Knowles, N. J., Kropinski, A. M., Krupovic, M., Kuhn, J. H. & Mushegian, A. R. 2017 Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2017). *Arch. Virol.* 162, 2505-2538.
- [17] Jonsson, C. B., Figueiredo, L. T. & Vapalahti, O. 2010 A global perspective on hantavirus ecology, epidemiology, and disease. *Clin. Microbiol. Rev.* 23, 412-441.
- [18] Mills, J. N. 2006 Biodiversity loss and emerging infectious disease: an example from the rodent-borne hemorrhagic fevers. *Biodiversity*. 7, 9-17.
- [19] Dearing, M. D. & Dizney, L. 2010 Ecology of hantavirus in a changing world. *Ann. N. Y. Acad. Sci.* 1195, 99-112.
- [20] Heyman, P., Thoma, B. R., Marié, J., Cochez, C. & Essbauer, S. S. 2012 In search for factors that drive hantavirus epidemics. *Frontiers in physiology*. 3, 237.
- [21] Khalil, H., Hörnfeldt, B., Evander, M., Magnusson, M., Olsson, G. & Ecke, F. 2014 Dynamics and Drivers of Hantavirus Prevalence in Rodent Populations. *Vector-Borne and Zoonotic Diseases*. 14, 537-551.
- [22] Guterres, A. & de Lemos, Elba Regina Sampaio. 2018 Hantaviruses and a neglected environmental determinant. *One Health*. 5, 27-33.

- [23] Forbes, K. M., Sironen, T. & Plyusnin, A. 2018 Hantavirus maintenance and transmission in reservoir host populations. *Current opinion in virology*. 28, 1-6.
- [24] Laenen, L., Vergote, V., Calisher, C. H., Klempa, B., Klingström, J., Kuhn, J. H. & Maes, P. 2019 Hantaviridae: Current classification and future perspectives. *Viruses*. 11, 788.
- [25] Kruger, D. H., Figueiredo, L. T. M., Song, J. & Klempa, B. 2015 Hantaviruses—globally emerging pathogens. *Journal of clinical virology*. 64, 128-136.
- [26] Brocato, R. L. & Hooper, J. W. 2019 Progress on the prevention and treatment of hantavirus disease. *Viruses*. 11, 610.
- [27] Warner, B. M., Stein, D. R., Griffin, B. D., Tierney, K., Leung, A., Sloan, A., Kobasa, D., Poliquin, G., Kobinger, G. P. & Safronetz, D. 2019 Development and Characterization of a Sin Nombre Virus Transmission Model in *Peromyscus maniculatus*. *Viruses*. 11, 183.
- [28] Madrières, S., Castel, G., Murri, S., Vulin, J., Marianneau, P. & Charbonnel, N. 2019 The needs for developing experiments on reservoirs in hantavirus research: Accomplishments, challenges and promises for the future. *Viruses*. 11, 664.
- [29] Kallio, E. R., Klingström, J., Gustafsson, E., Manni, T., Vaheeri, A., Henttonen, H., Vapalahti, O. & Lundkvist, Å. 2006 Prolonged survival of Puumala hantavirus outside the host: evidence for indirect transmission via the environment. *J. Gen. Virol.* 87, 2127-2134.
- [30] Ermonval, M., Baychelier, F. & Tordo, N. 2016 What do we know about how hantaviruses interact with their different hosts? *Viruses*. 8, 223.
- [31] Luis, A. D., Douglass, R. J., Hudson, P. J., Mills, J. N. & Bjørnstad, O. N. 2012 Sin Nombre hantavirus decreases survival of male deer mice. *Oecologia*. 169, 431-439.
- [32] Kallio, E. R., Helle, H., Koskela, E., Mappes, T. & Vapalahti, O. 2015 Age-related effects of chronic hantavirus infection on female host fecundity. *J. Anim. Ecol.* 84, 1264-1272.
- [33] Clay, C. A., Lehmer, E. M., Jeor, S. S. & Dearing, M. D. 2009 Sin Nombre virus and rodent species diversity: a test of the dilution and amplification hypotheses. *PloS One*. 4, e6467.
- [34] Clay, C. A., Lehmer, E. M., Jeor, S. S. & Dearing, M. D. 2009 Testing mechanisms of the dilution effect: deer mice encounter rates, Sin Nombre virus prevalence and species diversity. *EcoHealth*. 6, 250-259.
- [35] Orrock, J. L., Allan, B. F. & Drost, C. A. 2011 Biogeographic and ecological regulation of disease: prevalence of Sin Nombre Virus in island mice is related to island area, precipitation, and predator richness. *Am. Nat.* 177, 691-697.
- [36] Dizney, L. J. & Ruedas, L. A. 2009 Increased host species diversity and decreased prevalence of Sin Nombre virus. *Emerg. Infect. Dis.* 15, 1012-1018.
- [37] Lehmer, E. M., Korb, J., Bombaci, S., McLean, N., Ghachu, J., Hart, L., Kelly, A., Jara-Molinar, E., O'Brien, C. & Wright, K. 2012 The interplay of plant and animal disease in a changing landscape: The role of sudden aspen decline in moderating Sin Nombre virus prevalence in natural deer mouse populations. *EcoHealth*. 9, 205-216.
- [38] Dizney, L. & Dearing, M. D. 2016 Behavioural differences: a link between biodiversity and pathogen transmission. *Anim. Behav.* 111, 341-347.
- [39] Rubio, A. V., Castro-Arellano, I., Mills, J. N., List, R., Ávila-Flores, R. & Suzán, G. 2017 Is species richness driving intra-and interspecific interactions and temporal activity overlap of a hantavirus host? An experimental test. *PloS One*. 12, e0188060.
- [40] Calisher, C. H., Root, J. J., Mills, J. N. & Beaty, B. J. 2002 Assessment of ecologic and biologic factors leading to hantavirus pulmonary syndrome, Colorado, USA. *Croat. Med. J.* 43, 330-337.

- [41] Carver, S., Kuenzi, A., Bagamian, K. H., Mills, J. N., Rollin, P. E., Zanto, S. N. & Douglass, R. 2011 A temporal dilution effect: hantavirus infection in deer mice and the intermittent presence of voles in Montana. *Oecologia*. 166, 713-721.
- [42] Eleftheriou, A., Kuenzi, A.J., & Luis, A.D. 2021. Heterospecific competitors and seasonality affect host physiology and behavior; key determinants of disease transmission. *Ecosphere*. In Press.
- [43] Holsomback, T. S., McIntyre, N. E., Nisbett, R. A., Strauss, R. E., Chu, Y., Abuzeineh, A. A., De La Sancha, N., Dick, C. W., Jonsson, C. B. & Morris, B. E. 2009 Bayou virus detected in non-oryzomyine rodent hosts: an assessment of habitat composition, reservoir community structure, and marsh rice rat social dynamics. *Journal of Vector Ecology*. 34, 9-21.
- [44] Thoma, B. R., Müller, J., Bäessler, C., Georgi, E., Osterberg, A., Schex, S., Bottomley, C. & Essbauer, S. S. 2014 Identification of Factors Influencing the Puumala Virus Seroprevalence within Its Reservoir in a Montane Forest Environment. *Viruses*. 6, 3944-3967.
- [45] Ecke, F., Angeler, D. G., Magnusson, M., Khalil, H. & Hörnfeldt, B. 2017 Dampening of population cycles in voles affects small mammal community structure, decreases diversity, and increases prevalence of a zoonotic disease. *Ecology & Evolution*. 7, 5331-5342.
- [46] Khalil, H., Ecke, F., Evander, M., Magnusson, M. & Hörnfeldt, B. 2016 Declining ecosystem health and the dilution effect. *Scientific reports*. 6, 31314.
- [47] Tersago, K., Schreurs, A., Linard, C., Verhagen, R., Van Dongen, S. & Leirs, H. 2008 Population, environmental, and community effects on local bank vole (*Myodes glareolus*) Puumala virus infection in an area with low human incidence. *Vector-Borne and Zoonotic Diseases*. 8, 235-244.
- [48] Voutilainen, L., Savola, S., Kallio, E. R., Laakkonen, J., Vaheri, A., Vapalahti, O. & Henttonen, H. 2012 Environmental change and disease dynamics: effects of intensive forest management on Puumala hantavirus infection in boreal bank vole populations. *PLoS One*. 7, e39452.
- [49] Tersago, K., Verhagen, R., Vapalahti, O., Heyman, P., Ducoffre, G. & Leirs, H. 2011 Hantavirus outbreak in Western Europe: reservoir host infection dynamics related to human disease patterns. *Epidemiology & Infection*. 139, 381-390.
- [50] Piudo, L., Monteverde, M. J., Walker, R. S. & Douglass, R. J. 2011 Rodent community structure and Andes virus infection in sylvan and peridomestic habitats in northwestern Patagonia, Argentina. *Vector-borne and Zoonotic Diseases*. 11, 315-324.
- [51] Rubio, A. V., Fredes, F. & Simonetti, J. A. 2019 Exotic *Pinus radiata* Plantations do not Increase Andes Hantavirus Prevalence in Rodents. *EcoHealth*. 16, 659-670.
- [52] Polop, F. J., Provencal, M. C., Pini, N., Levis, S. C., Priotto, J. W., Enría, D., Calderón, G. E., Costa, F. & Polop, J. J. 2010 Temporal and spatial host abundance and prevalence of Andes Hantavirus in Southern Argentina. *EcoHealth*. 7, 176-184.
- [53] Raharinosy, V., Olive, M., Andriamiarimanana, F. M., Andriamandimby, S. F., Ravalohery, J., Andriamamonjy, S., Filippone, C., Rakoto, D. A. D., Telfer, S. & Heraud, J. 2018 Geographical distribution and relative risk of Anjozorobe virus (Thailand orthohantavirus) infection in black rats (*Rattus rattus*) in Madagascar. *Virology journal*. 15, 1-11.
- [54] Suzán, G., Marce, E., Giermakowski, J. T., Mills, J. N., Ceballos, G., Ostfeld, R. S., Armien, B., Pascale, J. M. & Yates, T. L. 2009 Experimental evidence for reduced rodent diversity causing increased hantavirus prevalence. *PLoS One*. 4, e5461.

- [55] Eastwood, G., Camp, J. V., Chu, Y. K., Sawyer, A. M., Owen, R. D., Cao, X., Taylor, M. K., Valdivieso-Torres, L., Sage, R. D. & Yu, A. 2018 Habitat, species richness and hantaviruses of sigmodontine rodents within the Interior Atlantic Forest, Paraguay. *PloS One*. 13, e0201307.
- [56] Suzán, G., Armién, A., Mills, J. N., Marcé, E., Ceballos, G., Ávila, M., Salazar-Bravo, J., Ruedas, L., Armién, B. & Yates, T. L. 2008 Epidemiological considerations of rodent community composition in fragmented landscapes in Panama. *J. Mammal*. 89, 684-690.
- [57] Milholland, M. T., Castro-Arellano, I., Arellano, E., Nava-García, E., Rangel-Altamirano, G., Gonzalez-Cozatl, F. X., Suzán, G., Schountz, T., González-Padrón, S. & Viguera, A. 2017 Species identity supersedes the dilution effect concerning hantavirus prevalence at sites across Texas and Mexico. *ILAR journal*. 58, 401-412.
- [58] Blasdel, K., Cosson, J. F., Chaval, Y., Herbreteau, V., Douangboupha, B., Jittapalapong, S., Lundqvist, A., Hugot, J., Morand, S. & Buchy, P. 2011 Rodent-borne hantaviruses in Cambodia, Lao PDR, and Thailand. *EcoHealth*. 8, 432-443.
- [59] Camp, J. V., Spruill-Harrell, B., Owen, R. D., Solà-Riera, C., Williams, E. P., Eastwood, G., Sawyer, A. M. & Jonsson, C. B. 2021 Mixed Effects of Habitat Degradation and Resources on Hantaviruses in Sympatric Wild Rodent Reservoirs within a Neotropical Forest. *Viruses*. 13, 85.
- [60] Ruedas, L. A., Salazar-Bravo, J., Tinnin, D. S., Armién, B., Cáceres, L., García, A., Diaz, M. Á., Gracia, F., Suzán, G. & Peters, C. 2004 Community ecology of small mammal populations in Panama following an outbreak of Hantavirus pulmonary syndrome. *Journal of Vector Ecology*. 29, 177-191.
- [61] Min, K., Schneider, M. C. & Cho, S. 2020 Association between predator species richness and human hantavirus infection emergence in Brazil. *One Health*. 11, 100196.
- [62] Min, K., Kim, H., Hwang, S., Cho, S., Schneider, M. C., Hwang, J. & Cho, S. 2020 Protective effect of predator species richness on human hantavirus infection incidence. *Scientific reports*. 10, 1-7.
- [63] Vadell, M. V., Villafañe, I. E. G. & Carbajo, A. E. 2020 Hantavirus infection and biodiversity in the Americas. *Oecologia*. 192, 169-177.
- [64] Milholland, M. T., Castro-Arellano, I., Garcia-Peña, G. E. & Mills, J. N. 2019 The Ecology and Phylogeny of Hosts Drive the Enzootic Infection Cycles of Hantaviruses. *Viruses*. 11, 671.
- [65] O'Regan, S. M., Vinson, J. E. & Park, A. W. 2015 Interspecific Contact and Competition May Affect the Strength and Direction of Disease-Diversity Relationships for Directly Transmitted Microparasites. *Am. Nat.* 186, 480-494.
- [66] Begon, M., Bennett, M., Bowers, R. G., French, N. P., Hazel, S. & Turner, J. 2002 A clarification of transmission terms in host-microparasite models: numbers, densities and areas. *Epidemiology & Infection*. 129, 147-153.
- [67] Dhabhar, F. S. & McEwen, B. S. 1997 Acute stress enhances while chronic stress suppresses cell-mediated immunity in vivo: A potential role for leukocyte trafficking. *Brain Behav. Immun.* 11, 286-306.
- [68] Harper, J. M. & Austad, S. N. 2004 Fecal corticosteroid levels in free-living populations of deer mice (*Peromyscus maniculatus*) and southern red-backed voles (*Clethrionomys gapperi*). *The American midland naturalist*. 152, 400-409.
- [69] Wilson, K., Thomas, M. B., Blanford, S., Doggett, M., Simpson, S. J. & Moore, S. L. 2002 Coping with crowds: density-dependent disease resistance in desert locusts. *Proc. Natl. Acad. Sci. U. S. A.* 99, 5471-5475.

- [70] Clutton-Brock, T., Gaynor, D., McIlrath, G., Maccoll, A., Kinsky, R., Chadwick, P., Manser, M., Skinner, J. & Brotherton, P. 1999 Predation, group size and mortality in a cooperative mongoose, *Suricata suricatta*. *J. Anim. Ecol.* 68, 672-683.
- [71] Mduma, S. A., Sinclair, A. & Hilborn, R. 1999 Food regulates the Serengeti wildebeest: a 40-year record. *J. Anim. Ecol.* 68, 1101-1122.
- [72] Luis, A. D., Douglass, R. J., Mills, J. N. & Bjørnstad, O. N. 2015 Environmental fluctuations lead to predictability in Sin Nombre hantavirus outbreaks. *Ecology*. 96, 1691-1701.
- [73] Craft, M. E. 2015 Infectious disease transmission and contact networks in wildlife and livestock. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 370, 20140107.
- [74] Wood, C. L., Lafferty, K. D., DeLeo, G., Young, H. S., Hudson, P. J. & Kuris, A. M. 2016 Does biodiversity protect humans against infectious disease? Reply. *Ecology*. 97, 543-546.
- [75] Halliday, F. W. & Rohr, J. R. 2019 Measuring the shape of the biodiversity-disease relationship across systems reveals new findings and key gaps. *Nature communications*. 10, 1-10.
- [76] Rubio, A. V., Ávila-Flores, R. & Suzán, G. 2014 Responses of small mammals to habitat fragmentation: epidemiological considerations for rodent-borne hantaviruses in the Americas. *EcoHealth*. 11, 526-533.

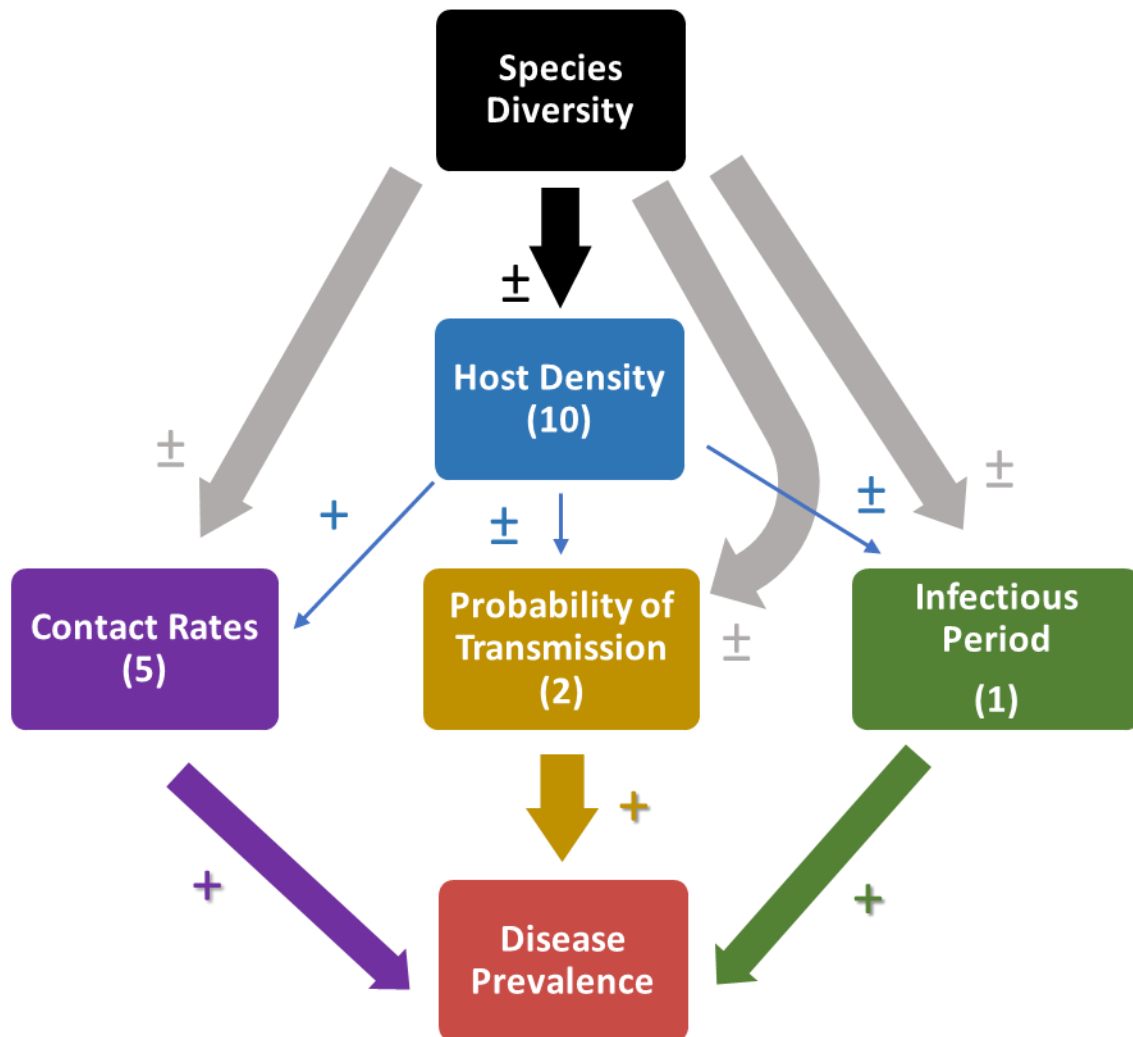


Figure 2. 1 Conceptual diagram describing possible relationships between diversity and prevalence for a directly-transmitted disease with density-dependent (DD) transmission and one primary reservoir host. Arrows propose hypothetical effects that diversity may have on mechanisms that determine disease prevalence. Diversity can indirectly determine prevalence via DD mechanisms (blue arrows), or directly (gray arrows) via density-independent mechanisms (contact rates, probability of transmission given contact, and infectious period). Numbers in brackets illustrate number of studies across rodent-hantavirus systems that found support for each mechanism (studies included in multiple counts if multiple mechanisms were examined).

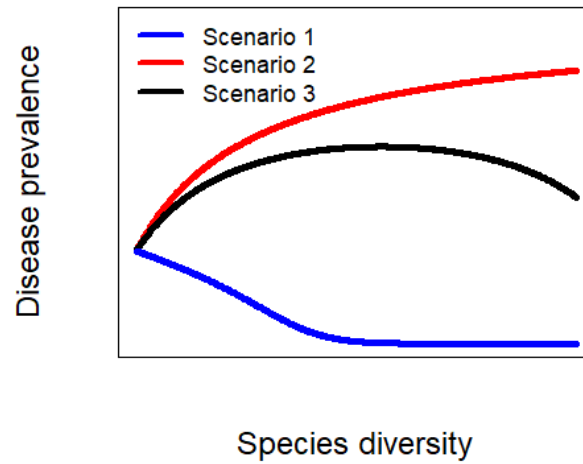


Figure 2. 2 Simulation outcomes for three scenarios where density-dependent and density-independent mechanisms varied in their relationship with diversity, using a mathematical model of the deermouse-Sin Nombre virus system. Scenario 1 (blue): When diversity has a negative effect on host density, a dilution effect emerges. Scenario 2 (red): When diversity has a positive effect on transmission rate for a given density, an amplification effect results. Scenario 3 (black): When diversity affects both mechanisms in opposing directions, there is a non-monotonic outcome.

Table 2. 1 Diversity-disease studies with rodent-hantavirus systems. We list data on the disease system, diversity-disease outcome, empirical support (with directionality) for density-dependent and -independent mechanisms (contact rates, probability of transmission, and host survival), metrics of diversity and disease, and study reference.

Host-virus system	Outcome	Host density	Host density-independent			Diversity metric	Disease metric	Ref.
			Contact rates	Prob. of transmission	Host survival			
<i>P. maniculatus</i> –SNV	D ^a	-	.	.	-?	RDY	AP	[33]
	D ^a	-	-?	.	.	RDY	AP	[34]
	N	SDY	AP	[35]
	D	PDY	AP	[35]
	D	-	.	.	.	SDY	AP	[36]
	D	-	.	.	.	SDY	AP/NI	[37]
	D	NA	-	.	.	RDY	AP	[38]
	.	NA	N	.	.	RDY	.	[39]
	D ^b	-	NA	NA	.	SDY	AP/NI	[11]
	N ^c	N	.	.	.	SDY	AP/NI	[11]
	CA ^{b,c}	NA	+?	+?	.	SDY	AP	[11]
	D ^b	RDY	AP	[40]
	TD ^c	+	.	.	.	NHP	AP	[41]
	D ^b	SDY	AP	[18]
	.	.	+ or NM	+	.	NHD	.	[42]
<i>O. palustris</i> – BAYV	D	SDY	AP	[43]
<i>M. glareolus</i> – PUUV	A	SDY	AP	[44]
	D ^d	SDY	AP/NI	[45]
	D ^d	+	.	.	.	NHD	IP	[46]
	. ^d	-	.	.	.	NHD	.	[46]
	D	NHPr	AP	[47]
	N	NHPr	NI	[47]
	D	NHD	IP	[48]
	N	NHPr	AP/NI	[49]
<i>O. longicaudatus</i> – ANDV	D	RDY	NI	[50]

Host-virus system	Outcome	Host density	Host density-independent			Diversity metric	Disease metric	Ref.
			Contact rates	Prob. of transmission	Host survival			
<i>R. rattus</i> – THAIV Various	N	RDY	AP	[50]
	N	N	.	.	.	SDY	AP/NI	[51]
	A	+	.	.	.	RDY	NI	[52]
	N	SDY	IP	[53]
	D	-	.	.	.	RDY	AP	[54]
	N	RDY	AAP	[55]
	.	-	.	.	.	SDY	.	[56]
	N	SDY	AP	[57]
	D	RDY	NI	[58]
	N	RDY	AP	[59]
	D	SDY	DP/HI	[60]
	A	NPDY	HI	[61]
	NM	DPDY	HI	[61]
	NM	PDY	HI	[62]

CATEGORIES: Prob. of transmission = probability of transmission given contact, Ref. = reference, **HOST-VIRUS SYSTEM:** SNV = Sin Nombre virus, BAYV = Bayou virus, PUUV = Puumala virus, ANDV = Andes virus, THAIV = Thailand virus **OUTCOME:** D = dilution, A = amplification, CA = component amplification (i.e. not the main effect), TD = temporal dilution. **DISEASE METRIC:** AP = antibody prevalence, NI = number of infected hosts, IP = infection probability, AAP = antibody and/or antigen prevalence, DP = disease present (i.e. presence of infected rodents), HI = human infection cases, **DIVERSITY METRIC:** RDY= rodent diversity, SDY = small mammal diversity, PDY = predator diversity, NHP= nonhost presence, NHD = nonhost density, NHPr = nonhost proportion, NPDY = nocturnal predator diversity, DPDY = diurnal predator diversity. **ALL:** ‘.’ = not explicitly examined, ‘+’ = positive relationship, ‘-’ = negative relationship, N = no relationship, NM = non-monotonic relationship, NA= not applicable (variable accounted for or controlled).

SUPPLEMENTARY INFORMATION

LITERATURE REVIEW METHODS

We searched the literature for studies across rodent-hantavirus systems with the Google Scholar search engine using the following key words: “dilution effect” OR “amplification effect”, “hantavirus”, “disease”, “infection”, and “species diversity”. We restricted our literature search to peer-reviewed field studies (observational or experimental), and published in English, until March 28th, 2021. We also searched through reference lists of the six reviews mentioned in the main text, to identify studies our original search may have missed. We aimed to include only empirical studies that examined relationships between disease and diversity (or community composition) across rodent-hantavirus systems, with or without mechanistic evidence.

This search approach generated 344 results when “dilution effect” was included and 87 results when “amplification effect” was included. We selected relevant articles for further examination by reading through every article’s title. The abstract was reviewed only when there was not enough information to decide from the title alone. Articles with titles and abstracts deemed relevant were reviewed further to evaluate if they should be included, as stated above.

EVIDENCE FROM META-ANALYSES

During our literature search, we found two meta-analyses that focused on studies primarily not testing for a diversity-disease relationship. The first is by Vadell et al. [1], with disease systems from 22 studies in the Americas, and the second by Milholland et al. [2], with disease systems from 67 studies in Asia, Europe and the Americas. The first meta-analysis investigated the effect of rodent diversity on hantavirus prevalence and found no patterns after accounting for host density. The second meta-analysis examined the effect of small mammal richness and phylogeny on hantavirus prevalence and found that both were negatively associated with prevalence. However, host density was not included in their models. In summary, these two studies came to

contrasting conclusions, most likely because they used different studies (shared only six) and analytical approaches (e.g. accounting for density).

HOST DENSITY: BANK VOLE-PUUV SYSTEM

Several studies have found evidence that supports density-dependent transmission in the bank vole-PUUV system [3-5] suggesting that indirect effects via host density could result in a dilution effect. Density-dependent transmission has been echoed by Voutilainen et al. [6] and Khalil et al. [7], who found that bank vole density was important in predicting the probability of PUUV infection. However, not all studies found such a relationship. For example, although Tersago et al. [8] identified a critical threshold density required for PUUV persistence in vole populations, they did not find a relationship between vole density and PUUV prevalence during their two-year study. Similarly, Thoma Palo et al. [9] found no density-disease relationship over a three-year period. However, given that time lags may play a significant role in rodent-hantavirus dynamics (e.g., [10]), a shorter temporal window of data collection could make density-dependent transmission more challenging to detect.

Although most mechanistic studies that used the bank vole-PUUV system did not always examine relationships between host density and diversity *per se*, studies did look at relationships between host and nonhost densities (or their proportions relative to total captures) and found some support. For example, Khalil et al. [7], who observed a dilution effect found negative relationships between densities of field voles (*Microtus agrestis*) and bank voles, but only in the fall, and only in core field vole habitat. Interestingly, Ecke et al. [11] who observed no effect with α diversity (local diversity) but a dilution effect with β and γ diversity, found that when the community contribution of bank voles was higher, those of gray-sided voles (*Myodes rufocanus*) were lower. However, not all studies that reported a dilution effect found negative relationships.

Tersago et al. [8] detected no relationship between the densities of voles and wood mice (*Apodemus sylvaticus*), although they used proportion of wood mice (instead of their density) as their diversity measure (Table 2.1 in main text). Khalil et al. [7] found positive associations between densities of voles and other small mammals. In particular, there was a positive relationship with field vole density only in spring, and only in certain habitat types. However, the positive relationship they detected with density of common shrews (*Sorex araneus*) was irrespective of habitat, or season, suggesting that a dilution effect via shrews is more likely to be density-independent.

When considering all the evidence, the empirical support for host density acting as an indirect driver of a dilution effect in the bank vole-PUUV system is not as strong compared to the deermouse-SNV system. However, it is likely this is the result of having fewer studies available for examination (Table 2.1 in main text). Most of the bank vole-PUUV studies do highlight an interesting point, however. Given that particular nonhost species are more likely to be the actual drivers of dilution or amplification effects than diversity *pe se*, it may be better in future studies to employ densities of relevant nonhost species (or functional groups of species), than broader diversity measures (e.g. [12]), such as small mammal diversity, especially in species-poor communities.

MODELING METHODS

The Model:

$$\frac{dS}{dt} = N \left(b - ar \left(\frac{N}{K} \right) \right) - S \left(d + (1 - a)r \left(\frac{N}{K} \right) \right) - \beta SI \quad \text{Equation (1a)}$$

$$\frac{dI}{dt} = \beta SI - I \left(\mu + d + (1 - a)r \left(\frac{N}{K} \right) \right) \quad \text{Equation (1b)}$$

where S is the number of susceptible hosts, I is the number of infected hosts, N is the total host number ($S+I$), K is the number of hosts the environment can sustain, b is the maximum birth rate, d is the minimum death rate, a is the proportion of density-dependence on birth rates, r is the intrinsic growth of the population ($b-d$), μ is the disease-induced mortality rate, and β is the transmission rate.

Below are the mathematical equations for the two linear regressions (Figure S2.1) that were used in simulations with the susceptible-infected (SI) model above (Equations 1a and b). The linear regressions were constructed, as such, to generate realistic values of K and β as reported by Luis et al. [13].

$$K = 30 - 5.5 SDI \quad \text{Equation (2)}$$

where K is the environmental carrying capacity of deer mice at equilibrium (and thus the density that can be sustained), and SDI is the Simpson's Diversity Index as calculated by Luis et al. [13].

$$\beta = 0.01 + 0.01 SDI \quad \text{Equation (3)}$$

where β is the transmission rate of SNV for a given deer mouse density (the product of contact rates and probability of transmission given contact).

We parameterized the SI model using values estimated previously by Luis et al. [10] (Table S2.1) and we tested diversity (Simpson's Index) values ranging from 1 to 4 as to remain within a realistic context [13] and to demonstrate the array of possible outcomes. We used R (version 4.0.3, [14]) within RStudio [15] to run simulations over 100 time steps to reach equilibrium using the package "deSolve" for generating solutions of differential equations [16].

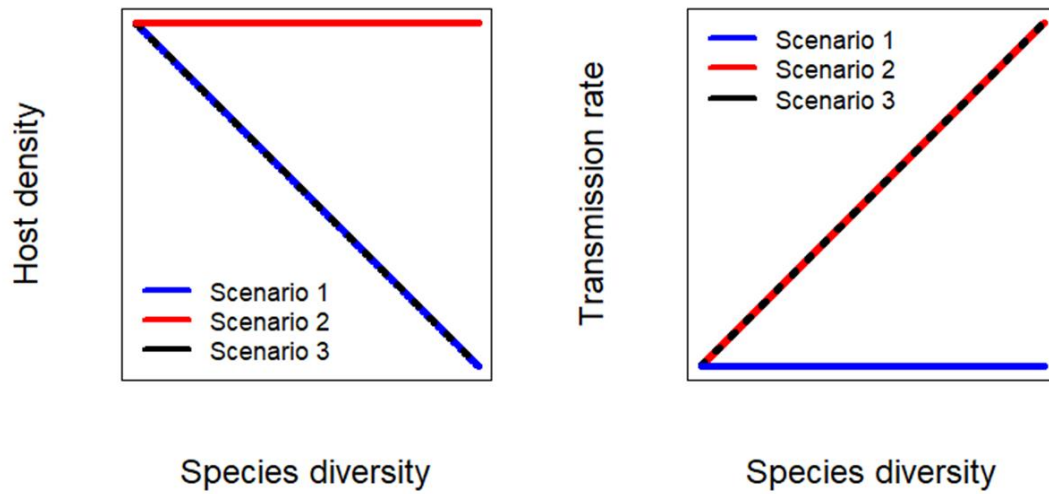


Figure S2. 1 : Color-coded relationships used in simulation scenarios whose outcomes are shown in figure 2 of the main text. Top left panel: Relationships between diversity and host density. Top right panel: Relationships between diversity and transmission rate for a given host density (contact rates \times probability of transmission given contact).

Table S2. 1 Variables and parameters used in model with descriptions and estimates.

Parameter/Variable	Value	Description
a	0.6142470	Density-dependent effect on births [10].
b	0.3154089	Birth rate of deer mouse per capita [10].
d	3.655774e-05	Death rate of deer mouse per capita [10].
μ	0.085	Disease-induced mortality [10].
SDI	1 – 4	Average Simpson's Diversity Index. Values selected to represent range reported in [13].

CONSIDERING DISEASE METRICS OTHER THAN ANTIBODY PREVALENCE

During our literature search, we found that a minority of studies reported two disease metrics

other than antibody prevalence, including the density of infected hosts and the probability of

infection for a given host (i.e. force of infection). In particular, two studies identified contrasting relationships between diversity and disease, based on what metric they used [8,17]. Given this disagreement, we examined the relationships between diversity and disease, under all simulation scenarios, by using these two disease metrics instead of antibody prevalence. Overall, we found similar patterns to what we observed with antibody prevalence as the disease metric (Figure 2.1). However, we did find that disease risk peaked at lower diversity values with density of infected hosts under scenario 3 (Figure 2.2 in main text versus Figure S2.2 below). One of the studies [17] found a dilution effect with number of infected hosts but no effect with antibody prevalence. Our findings under scenario 3 suggest that, where a dilution effect could be seen with density of infected hosts, a neutral relationship could be observed instead with antibody prevalence if there was enough stochasticity in the data. Therefore, it is theoretically possible for the same study to report two contrasting disease outcomes depending on the disease metric used. However, our simulation findings do not explain the opposing conclusions generated by Tersago et al. [8] that reported a dilution effect with antibody prevalence but no effect with density of infected hosts.

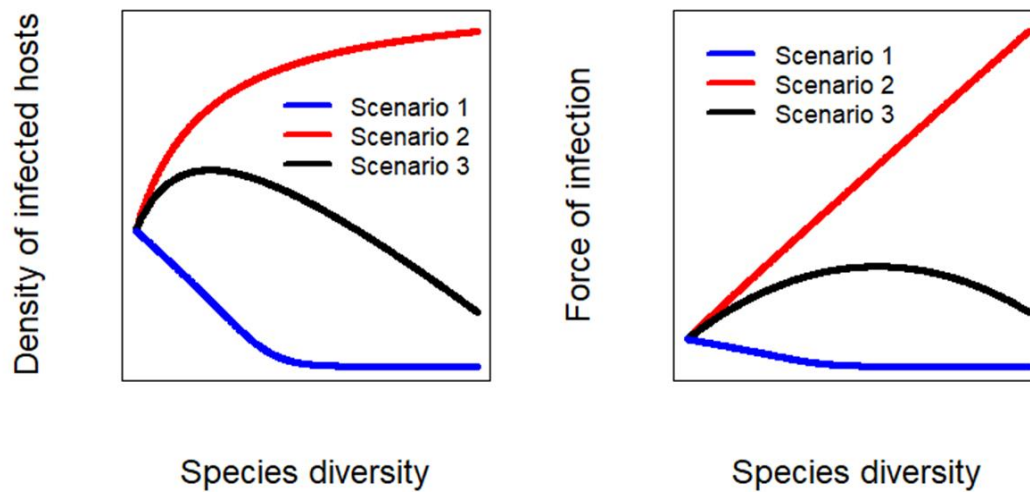


Figure S2. 2 Conceptual representations of simulation results of diversity-disease outcomes for three scenarios where density-dependent (host density) and density-independent (transmission rate for a given host density) mechanisms were allowed to vary in their relationship with species diversity, using a Susceptible-Infected (SI) model of the deer-mouse-Sin Nombre virus (SNV) system. Instead of antibody prevalence, two other metrics employed by a minority of studies found by our review were used: density of infected individuals, and force of infection (i.e. probability of infection). Across both disease metrics, scenario 1 (blue) generated dilution, scenario 2 (red) amplification, and scenario 3 (black), a non-monotonic relationship, consistent with findings when antibody prevalence was used instead.

LITERATURE CITED

- [1] Vadell, M. V., Villafañe, I. E. G. & Carbajo, A. E. 2020 Hantavirus infection and biodiversity in the Americas. *Oecologia*. 192, 169–177.
- [2] Milholland, M. T., Castro-Arellano, I., Garcia-Peña, G. E. & Mills, J. N. 2019 The Ecology and Phylogeny of Hosts Drive the Enzootic Infection Cycles of Hantaviruses. *Viruses*. 11, 671.
- [3] Thomas Palo, R. 2009 Time series analysis performed on nephropathia epidemica in humans of northern Sweden in relation to bank vole population dynamic and the NAO index. *Zoonoses and public health*. 56, 150-156.
- [4] Dobly, A., Yzord, C., Cochez, C., Ducoffre, G., Aerts, M., Roels, S. & Heyman, P. 2012 Spatiotemporal dynamics of Puumala hantavirus in suburban reservoir rodent populations. *Journal of Vector Ecology*. 37, 276-283.
- [5] Haredasht, S. A., Taylor, C. J., Maes, P., Verstraeten, W., Clement, J., Barrios, M., Lagrou, K., Van Ranst, M., Coppin, P. & Berckmans, D. 2013 Model-based prediction of Nephropathia epidemica outbreaks based on climatological and vegetation data and bank vole population dynamics. *Zoonoses and public health*. 60, 461-477.
- [6] Voutilainen, L., Savola, S., Kallio, E. R., Laakkonen, J., Vaheri, A., Vapalahti, O. & Henttonen, H. 2012 Environmental change and disease dynamics: effects of intensive forest

- management on Puumala hantavirus infection in boreal bank vole populations. *PloS One*. 7, e39452.
- [7] Khalil, H., Ecke, F., Evander, M., Magnusson, M. & Hornfeldt, B. 2016 Declining ecosystem health and the dilution effect. *Sci. Rep.* 6, 31314.
- [8] Tersago, K., Schreurs, A., Linard, C., Verhagen, R., Van Dongen, S. & Leirs, H. 2008 Population, environmental, and community effects on local bank vole (*Myodes glareolus*) Puumala virus infection in an area with low human incidence. *Vector-Borne and Zoonotic Diseases*. 8, 235-244.
- [9] Thoma, B. R., Müller, J., Bässler, C., Georgi, E., Osterberg, A., Schex, S., Bottomley, C. & Essbauer, S. S. 2014 Identification of Factors Influencing the Puumala Virus Seroprevalence within Its Reservoir in a Montane Forest Environment. *Viruses*. 6, 3944-3967.
- [10] Luis, A. D., Douglass, R. J., Mills, J. N. & Bjørnstad, O. N. 2015 Environmental fluctuations lead to predictability in Sin Nombre hantavirus outbreaks. *Ecology*. 96, 1691-1701.
- [11] Ecke, F., Angeler, D. G., Magnusson, M., Khalil, H. & Hörnfeldt, B. 2017 Dampening of population cycles in voles affects small mammal community structure, decreases diversity, and increases prevalence of a zoonotic disease. *Ecology and evolution*. 7, 5331-5342.
- [12] LoGiudice, K., Duerr, S. T., Newhouse, M. J., Schmidt, K. A., Killilea, M. E. & Ostfeld, R. S. 2008 Impact of host community composition on Lyme disease risk. *Ecology*. 89, 2841-2849.
- [13] Luis, A. D., Kuenzi, A. J. & Mills, J. N. 2018 Species diversity concurrently dilutes and amplifies transmission in a zoonotic host-pathogen system through competing mechanisms. *Proc. Natl. Acad. Sci. U. S. A.* 115, 7979-7984.
- [14] R Core Team. 2018. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria.
- [15] RStudio Team. 2015. RStudio: Integrated Development for R; RStudio, Inc.: Boston, MA, USA.
- [16] Soetaert, K., Petzoldt, T., Setzer, R.W. 2010. Solving Differential Equations in R: Package deSolve. *Journal of Statistical Software*, 33, 1–25.
- [17] Piudo, L., Monteverde, M. J., Walker, R. S. & Douglass, R. J. 2011 Rodent community structure and Andes virus infection in sylvan and peridomestic habitats in northwestern Patagonia, Argentina. *Vector-borne and Zoonotic Diseases*. 11, 315-324.

CHAPTER 3 ASSESSMENT OF THE STRESS RESPONSE IN NORTH AMERICAN DEERMICE: LABORATORY AND FIELD VALIDATION OF TWO ENZYME IMMUNOASSAYS FOR FECAL CORTICOSTERONE METABOLITES¹

ABSTRACT

Stress physiology is commonly employed in studies of wildlife ecology and conservation. Accordingly, we need robust and suitable methods to measure stress physiology in the field. Fecal cortisol/corticosterone metabolites (FCMs) are now increasingly being used to non-invasively evaluate adrenocortical activity; a measure of stress physiology. However, immunoassays that measure FCMs must be appropriately validated prior to their use and factors that can influence FCMs, such as trap-induced stress, must be considered. Deermice (*Peromyscus maniculatus*) are widely used in scientific studies so that developing methods that appropriately measure their adrenocortical activity is critical. In the laboratory, we tested the suitability of two enzyme immunoassays (EIAs; a corticosterone EIA, and a group-specific 5 α -pregnane-3 β ,11 β ,21-triol-20-one EIA) in deermice by challenging individuals with dexamethasone and adrenocorticotrophic hormone (ACTH). We found that dexamethasone suppressed FCM levels within ~10 h post injection whereas ACTH increased FCM levels within ~2 h post injection. In the field, we found that FCM levels generally increased with more time in trap confinement when using both EIAs. Although we acknowledge low sample sizes (N = 4), our results validated the two EIAs for use with FCMs from deermice.

¹ This chapter has been published as A. Eleftheriou, R. Palme, and R. Boonstra. 2020 *Assessment of the Stress Response in North American Deermice: Laboratory and Field Validation of Two Enzyme Immunoassays for Fecal Corticosterone Metabolites*. *Animals*. 10 (7): 1120.

INTRODUCTION

In recent decades, researchers have started to employ stress physiology more often as a tool to evaluate how natural and anthropogenic stressors can affect survival and reproductive success of wildlife populations. Given the widespread use of stress physiology in managing and conserving wildlife, identifying suitable and robust methods for evaluating stress physiology in every species is of paramount importance. This is critical because anthropogenic stressors can induce chronic stress in wildlife, which can lead to pathological perturbations [1,2].

Adrenocortical activity, a measure of stress physiology, is typically evaluated via blood glucocorticoids (GCs) and more recently via fecal cortisol/corticosterone metabolites (FCMs), which are metabolized GCs excreted in feces [2–4]. Evaluation of stress physiology via FCMs is non-invasive and avoids the acute stress effects of capture, handling, and venipuncture [1]. Although researchers are increasingly using FCMs, there are concerns about the methodology used to measure them, such as lack of validation [4]. Immunoassays are validated when they can detect expected changes in FCM levels [4]. Without validation, inference becomes less robust. Thus, immunoassays need to undergo analytical, physiological, and biological validations before they are used to measure FCMs in field settings [4]. Analytical validation may include intra- and inter-assay coefficients of variation and parallelism tests [4]. Validations also must be performed in every species because suitable immunoassays for measuring FCMs can vary even between closely related species [4–6].

North American deermice (*Peromyscus maniculatus*, hereafter deermice) are widely used in biomedical (e.g., [7]), physiological (e.g., [8]), and ecological (e.g., [9]) research. Hence, it may not be surprising that FCM evaluation in deermice has already been investigated by others where a corticosterone radioimmunoassay (RIA; [10,11]) and two corticosterone enzyme

immunoassays (EIAs; [12,13]) have been used. Although the RIA was validated before use, the EIAs were not, which makes their use questionable [4]. In addition, all assays used antibodies that bind to corticosterone, which is the predominant GC in *Peromyscus* (e.g., [14]). However, intact corticosterone is essentially absent from feces so their use may be suboptimal [4]. For example, an EIA that used an antibody, which detects FCMs with a 5α - 3β , 11β -diol structure, demonstrated improved FCM detection in house mice (*Mus musculus*) compared with corticosterone EIAs [15]. Nevertheless, corticosterone immunoassays may still detect FCMs, albeit to a lesser degree, because of cross-reactivity between the corticosterone antibody and FCMs [4]. Commercial corticosterone immunoassays are also relatively easy to acquire and use, although expensive [4]. However, commercial RIAs, unlike EIAs, may be less appealing because they use radioactive materials and require a licensed laboratory for their use [4,16]. To the best of our knowledge, there have been no studies that compared or validated EIAs for measuring FCMs in deermice.

We can physiologically validate immunoassays by using adrenocorticotrophic hormone (ACTH) and dexamethasone (a synthetic steroid), which increase and decrease endogenous GC production, respectively [4]. However, to biologically validate immunoassays, we need to use stressors that are biologically relevant to the species of interest. Although immunoassays can be validated biologically if they can track diurnal rhythm changes in FCMs, this should be done in addition to other biological validations [4], such as live trapping, which can increase FCMs (e.g., [17,18]). Knowing when these trap-induced rises in FCMs manifest in feces is also of practical use because they can artificially increase FCMs and lead to erroneous results about baseline adrenocortical activity [19]. This time delay between blood GCs and the appearance of metabolites in feces is species-specific [20]. Specifically, in deermice, [21] found a delay of 4 h

before there were trap-induced effects on FCMs, but as they pointed out, the effects could have appeared sooner because they did not sample during a shorter interval (<4 h).

In this study, we had three objectives. Firstly, in the laboratory, we wanted to validate two different EIAs in measuring FCMs using physiological challenges (i.e., dexamethasone and ACTH injections). The immunoassays were a corticosterone EIA [12] and a 5 α -pregnane-3 β ,11 β ,21-triol-20-one EIA (hereafter referred to as the group-specific EIA, [15]). Secondly, again in the laboratory, we wanted to use the diurnal rhythm in GC secretion to biologically validate both EIAs. Thirdly, in the field, we wanted to investigate temporal effects of trap confinement on FCMs using both EIAs to provide additional biological validation.

MATERIALS AND METHODS

ACQUISITION AND HUSBANDRY OF LABORATORY DEERMICE

We acquired 4 adult deermice (2 females: 2 males) from McMaster University, Ontario, Canada, and transported them to the animal holding facility at the University of Toronto—Scarborough, ON, Canada, in April 2016. These deermice were F1 generation offspring from wild deermice that were originally captured from Nebraska, USA [8]. All were ear-tagged, weighed, and sexed. All deermice were non-reproductive (males were non-scrotal, and females had non-perforate vaginas). They were individually housed in polypropylene cages (47 cm \times 26 cm \times 20 cm) that were equipped with a wire bottom and a glass water bottle with a stainless-steel nipple. All cages were mounted within a second same-sized cage that was equipped with a fine metal mesh. The wire bottom allowed feces and urine to fall through the bottom of the first cage. Though urine continued to pass through the fine mesh, the feces did not. This arrangement minimized urine contamination of feces and disturbance to the animals [22]. Male and female deermice were kept separate on two different but opposite racks within the same animal facility to limit exposure to odor from the opposite sex. We provided them with *ad libitum* water, rodent chow (LabDiet, St.

Louis, Missouri, USA) and ample cotton bedding as nesting material. Deermice were housed under a 12:12 h dark-light cycle (lights on at 08:00 h) at room temperature (20 ± 5 °C).

Ventilation fans were positioned in the wall at either end of the holding facility and operated in a push-pull method (the fan at one end pulled air out of the facility and the other pushed outside air into the facility). This method changed the air in the room 13 times/h. The direction of air flow was parallel to cage racks, which prevented cross contamination between cages that held males and females [23].

FECAL SAMPLE COLLECTION

We collected fecal samples by following Table 3.1 and discarded feces contaminated with urine. Forceps were disinfected between individuals during sample collection. If fecal pellets were in excess for an individual, we subsampled to get a representative pooled sample from all areas where the individual had defecated. We then stored samples at -20°C until analyses. During the acclimation period, fecal samples were collected every 2 h (during the dark and light cycles) whereas during the challenge experiments, samples were generally collected every 2 h during the dark cycle and every 4 h during the light cycle (Table 3.1). This sampling change occurred due to personnel constraints.

ACCLIMATION PERIOD

Fecal samples were collected for ~92 h after the animals were transferred to our facility (Table 3.1). We assumed that samples collected on the 3 last day of acclimation reflected baseline FCMs given that previous work with wild meadow voles (*Microtus pennsylvanicus*) found that FCMs were the lowest by the end of the third day of captivity [24].

DEXAMETHASONE SUPPRESSION CHALLENGE

To test whether EIAs could detect an expected decrease in FCM levels, we injected all deermice with 2.5 mg/kg dexamethasone sodium phosphate (Vétoquinol, Québec, Canada)

diluted in sterile 0.9% saline intraperitoneally at ~20:00 h. In this way, each individual was used as its own control. Because dexamethasone doses have not been reported for deermice, we formulated this dose based on studies with other small rodents (e.g., [25–27]). We started to collect samples at 22:00 h for ~48 h although we were unable to analyze samples 12 h post injection (Table 3.1). No samples were collected at the time of injection.

ACTH STIMULATION CHALLENGE

To test whether EIAs could detect an expected increase in FCM levels, we injected all deermice with 250 µg/ kg ACTH (Cortrosyn, Amphastar Pharmaceuticals, Inc., Rancho Cucamonga, CA, USA) mixed in 0.9% sterile saline solution intraperitoneally at ~20:00 h. Again, each individual was used as its own control and because ACTH doses have not been reported for deermice, we formulated this dose based on above cited studies. As above, we started to collect samples at 22:00 h for ~48 h although we were unable to analyze samples 12 h post injection (Table 3.1). No samples were collected at the time of injection.

FIELD VALIDATION

We carried out two field studies to assess the temporal effect of trap confinement on FCM levels. In both studies, deermice were captured in individual non-folding Sherman traps (H.B. Sherman Traps, Tallahassee, FL, USA) baited with oats and peanut butter, and provided with cotton bedding. In field study 1 (for group-specific EIA), apple slices were also provided. For field study 1, we trapped 20 adult deermice (7 males, 13 females) near Drummond, MT, USA, in June 2017. Only three were non-reproductive. Once trapped, we confined deermice in a trap for either 0–2, 4–6 or 8–10 h prior to processing. To do this, we set traps around dusk and checked them after 2 h. Trapped deermice were either processed for the 0–2 h treatment or left in the trap to be processed for the 4–6 h and 8–10 h treatments, where they spent an additional 4 h and 8 h in the trap, respectively. Deermice were removed from traps by “emptying” contents into a

plastic bag. We then sexed, weighed, ear-tagged, and evaluated them for reproductive status. Age was determined based on mass (<14 g = juvenile, 14–17 g = subadult, >17 g = adult, [28]). Reproductive status was determined by the presence of scrotal testes in males, and presence of a perforate vagina, lactation, or pregnancy in females.

In field study 2 (for corticosterone EIA), seven adult deermice (4 males, 3 females) were trapped near Charlo, MT, USA, in August 2017. Only two were non-reproductive. We checked traps after ~4 h of setup when we ear-tagged and collected feces from deermice. Afterwards, all deermice were returned to their respective clean traps where they spent the night until dawn (an additional ~7 h). At that time, they were sexed, weighed, evaluated for reproductive status, and sampled a second time for feces. Age and reproductive status were determined as above.

In both field studies, we collected feces from restrained animals and/or their trap and released individuals on site after processing. Feces contaminated with urine were not collected. We followed field protocols to avoid accidental hantavirus infection [29]. All fecal samples were stored at –80 °C until analysis. All procedures involving animal use were approved by the University of Toronto (protocol # 20011602) and/or by the University of Montana Institutional Animal Care and Use Committees (protocol #s 024-16ALDECS-042616, 027-16ALDECS-051016, 028-16ALDECS-051016). Field work was approved by Montana Fish, Wildlife and Parks (permit #2017-029-W).

PROCESSING OF FECES AND EXTRACTION OF FCMs

Laboratory and field study 1 fecal pellets were first oven-dried for 1 h at ~60 °C to heat-inactivate hantavirus (if present) and then lyophilized (Labconco Corp., Kansas City, MO, USA) for at least 15.5 h at Pennsylvania State University, State College, Pennsylvania, USA. Laboratory study fecal samples were not pulverized; they remained in pelleted form. However,

in field study 1 we did pulverize, using a mortar and pestle, because there were fewer total samples (20 samples). To extract FCMs, we weighed 0.05 g (± 0.005 g) of dried pellets/powder. Then, we added 1 mL of 80% methanol to each sample suspension, vortexed at 1500 RPM for 30 min, and centrifuged at $2500\times g$ at 22 °C for 20 min [4,15]. Supernatants were decanted and frozen at -20 °C. The extraction of field study 2 samples was slightly different and performed at the University of Montana, Missoula, MT, USA. Fecal pellets (14 samples total) were heat-inactivated and oven-dried for 2 h at ~63 °C to ensure elimination of water, because a lyophilizer was unavailable. We pulverized dried pellets and weighed out 0.04 g (± 0.005 g) of powder. The lower threshold weight was chosen because sample weights were generally lower in this field study. The rest of the extraction procedure remained unchanged.

IMMUNOASSAY METHODS

For the analysis of FCMs two different EIAs were used. The immunoassays were a corticosterone EIA (commercial kit #K014-H1 or H5, provided by Arbor Assays, Ann Arbor, MI, USA) [12] and a 5α -pregnane- $3\beta,11\beta,21$ -triol-20-one EIA (group-specific EIA, measuring FCMs with a 5α - $3\beta,11\beta$ -diol configuration [15]). Details of the EIAs including cross-reactions are given by Arbor Assays and Touma et al. [15], respectively. All fecal extracts were diluted with EIA buffer prior to being analyzed. The dilution factor was determined by running pooled sample extract at different dilutions against the standard curve, to identify the one that resulted in ~50% binding for the corticosterone EIA (Figure 3.1A) and the group-specific EIA (Figure 3.1B). Through parallelism tests, we showed that serial dilutions of pooled extract tracked the EIA's standard curve (Figure 3.1). These findings demonstrated that methanol residue in sample extracts did not interfere with assay performance. Consequently, sample extracts were diluted 1:10 for the corticosterone EIA, and 1:200 for the group-specific EIA. However, extracts from field study 2

analyzed with the corticosterone EIA had to be diluted 1:80 instead of 1:10 (change discussed later).

We followed manufacturer's instructions for the corticosterone EIA. However, when we analyzed fecal samples with this EIA for field study 2, we also used a wavelength of 650 nm (reference wavelength) in addition to 450 nm. We followed the protocol described by Touma et al. [15] for the group-specific EIA. All samples were assayed in duplicate. Intra-assay coefficients of variation (CVs) were calculated by averaging all sample CVs and inter-assay CVs were calculated by averaging CVs of low and high concentration controls for all plates (except for field study 2, we could only calculate average of low concentration controls). Intra-assay and inter-assay CVs for the corticosterone EIA were ($n = 9$) 5.7% and 12.7% (field study 2: 12.8%) and for the group-specific EIA ($n = 6$) 8.6% and 8.6%, respectively.

STATISTICAL ANALYSES

All data analyses were done in R [30] within RStudio [31]. We used linear mixed effect models (LMMs) from R packages “lme4” [32] and “lmerTest” [33] to test for diurnal patterns and how each treatment (dexamethasone suppression and ACTH stimulation) affected FCM levels of laboratory-bred deermice compared to baseline. We considered FCMs collected on the last day of acclimation as baseline FCMs. Because deermice were sampled repeatedly, individual identification was included as a random effect, where the model structure was sex + treatment \times sampling time, except for when testing for diurnal patterns, which was sex + sampling time. P-values were calculated using Satterthwaite's method [33]. We used the R package “emmeans” [34] to perform post hoc pairwise comparisons where p -values were adjusted accordingly.

For field study 1, we used a one-way ANOVA with post hoc Tukey's Honest Significant Differences to compare FCM levels across trap confinement treatments (i.e., 0–2, 4–6 and 8–10 h), sex and reproductive status, where the model structure was treatment + sex + reproductive

status. For field study 2, we used a LMM to examine the difference between FCMs across two sampling times (i.e., 0–4 h vs. overnight), sex, and reproductive status, where the model structure was treatment + sex + reproductive status. Because each deermouse was sampled twice, individual identification was included as a random effect.

FCM data were ln-transformed prior to all analyses to meet the assumptions of normality and homoscedasticity. Below, we present ln-transformed means with standard errors (ln ng/g of dry feces) where we considered results statistically significant at $\alpha = 0.05$. However, in the figures we present non-transformed data to ease comparisons with other studies.

RESULTS

ACCLIMATION PERIOD

We did not find changes across sex ($p = 0.16$) or time ($p = 0.10$) with the corticosterone EIA (Figure 3.2A). Similarly, there were no changes across sex ($p = 0.60$) or time ($p = 0.22$) with the group-specific EIA (Figure 3.2B). However, it is noteworthy that the variability in FCMs appeared smaller towards the end of the dark cycle compared to the beginning. FCMs collected during the third day of acclimation were considered as baseline when we evaluated the effects of treatments (dexamethasone and ACTH).

DEXAMETHASONE SUPPRESSION CHALLENGE

We found a sampling time by treatment effect for the corticosterone EIA ($F_{4, 18.31} = 3.73, p = 0.02$; Figure 3A). Deermice had lower FCM levels ($n = 4, 6.23 \pm 0.26$ ln ng/g) ~10 h post injection than baseline (06:00 h, $n = 3, 7.54 \pm 0.30$ ln ng/g, post hoc, $t_{18.32} = -3.37, p = 0.003$). We also found a sampling time by treatment effect for the group-specific EIA ($F_{4, 21} = 3.39, p = 0.03$; Figure 3B). Deermice had lower FCMs ($n = 4, 7.03 \pm 0.15$ ln ng/g) than baseline ~10 h post injection (06:00 h, $n = 3, 7.60 \pm 0.17$ ln ng/g, post hoc, $t_{19.46} = -2.47, p = 0.02$). Interestingly, deermice showed a marginal increase in FCMs ($n = 4, 8.16 \pm 0.15$ ln ng/g) ~4 h

post injection compared to baseline ($n = 2$, 7.63 ± 0.22 ln ng/g, $t_{20.42} = 1.96$, $p = 0.06$). We note that baseline FCM levels at 06:00 h represent a 2 h interval (04:00–06:00 h) whereas treatment FCM levels at the same time represent a 4 h interval (02:00–06:00 h; Table 3.1).

ACTH STIMULATION CHALLENGE

We found an effect of treatment for the corticosterone EIA ($F_{1, 19.22} = 11.94$, $p = 0.003$; Figure 3.4A) where deermice had consistently higher FCM levels (7.77 ± 0.19 ln ng/g) post injection than baseline (7.27 ± 0.20 ln ng/g, post hoc, $t_{19.17} = 3.44$, $p = 0.003$). We also found an effect of sampling time ($F_{4, 19.19} = 5.42$, $p = 0.004$) where deermice had higher FCM levels at 22:00 h (7.84 ± 0.23 ln ng/g; $t_{19.07} = 4.06$, $p = 0.005$), 00:00 h (7.74 ± 0.24 ln ng/g; $t_{19.17} = 3.45$, $p = 0.02$), and 06:00 h (7.62 ± 0.22 ln ng/g; $t_{19.05} = 3.14$, $p = 0.04$) compared to 08:00 h (6.99 ± 0.22 ln ng/g) regardless of treatment. Similarly, using the group-specific EIA, we found an effect of treatment ($F_{1, 20.25} = 14.16$, $p = 0.001$; Figure 3.4B) where deermice had higher (7.99 ± 0.12 ln ng/g) FCM levels consistently post injection than baseline (7.58 ± 0.12 ln ng/g, $t_{20.16} = 3.75$, $p = 0.001$; Figure 3.4B). Again, we also found an effect of sampling time ($F_{4, 20.28} = 3.42$, $p = 0.03$) where deermice had higher FCM levels at 22:00 h (8.02 ± 0.15 ln ng/g) compared to 08:00 h (7.48 ± 0.14 ln ng/g, $t_{20.06} = 3.50$, $p = 0.02$) regardless of treatment. We note that baseline FCM levels at 06:00 h represent a 2 h interval (04:00–06:00 h) whereas treatment FCM levels at the same time represent a 4 h interval (02:00–06:00 h; Table 3.1).

FIELD VALIDATION

For field study 1 (group-specific EIA), we found that there was a marginal effect of confinement time on FCM levels ($F_{2,15} = 3.38$, $p = 0.06$), which we still elected to explore because of likely biological relevance. We found no effect of sex ($p = 0.56$) or reproductive status ($p = 0.39$). Deermice confined for 4–6 h had marginally higher FCM levels ($n = 6$, 4 females and 2 males, 8.33 ± 0.08 ln ng/g) compared to those confined for 0–2 h ($n = 7$, 4 females

and 3 males, 7.90 ± 0.11 ln ng/g, $p = 0.07$). However, deermice confined for 0–2 h had no differences in their FCM levels compared to those of deermice confined for 8–10 h ($n = 7$, 5 females and 2 males, $p = 0.16$) or 4–6 h versus 8–10 h ($p = 0.85$; Figure 3.5). For field study 2 (corticosterone EIA), we found a significant effect of confinement time on FCM levels ($F_{1, 10} = 23.21$, $p = 0.001$). Deermice had lower FCMs (9.12 ± 0.32 ln ng/g) when confined for 0–4 h compared to after short-term restraint and overnight confinement ($n = 7$, 11.10 ± 0.32 ln ng/g, $t_6 = 4.82$, $p = 0.003$; Table 3.2). Sex ($p = 0.60$) and reproductive status ($p = 0.75$) were not significant.

DISCUSSION

We provided evidence that validates the use of both the corticosterone and the group-specific EIAs with FCMs in deermice. In the laboratory studies, both EIAs showed a similar decrease and increase in FCMs post dexamethasone and ACTH injections, respectively. Despite the group-specific EIA's ability to detect particular corticosterone metabolites in feces, FCM values were comparable to the ones we detected with the corticosterone EIA. Field study 1 (group-specific EIA) showed that deermice had marginally higher FCM levels when confined for 4–6 h versus 0–2 h. Field study 2 (corticosterone EIA) more strongly echoed these results where deermice had higher FCMs after short-term restraint and confinement more than 0–4 h. Although we did not verify whether the stressors we used increased blood corticosterone, we do not think this affects our conclusions because of two main reasons. Firstly, the stressors we used have been known to influence blood GCs in other species (e.g., [18,35]), and secondly, many other validation studies for FCMs were successful without performing this type of verification (e.g., [15,36–38]). Although comparing between studies with different extraction and assay protocols

is difficult, both EIAs we used consistently detected higher values compared to the corticosterone RIA used in previous deermouse studies (e.g., [10,11]).

DIURNAL RHYTHM AND SEX EFFECTS

We detected no effects of diurnal rhythm or sex on FCMs with either EIA when using only data from the third day in captivity. However, the variability in FCM data was smaller towards the end of the dark cycle compared to the beginning, suggesting that if we had FCM data from each deermouse for each time point, a significant change over time may have manifested. Regardless, we did find higher FCMs at 22:00 h compared to 08:00 h when using pooled data from the ACTH challenge for both EIAs. In fact, FCMs were also higher at 00:00 h and 06:00 h compared to 08:00 h for the corticosterone EIA. This finding from pooled data is most likely because, although treatment FCMs were relatively higher than baseline, they still showed a declining trend, similar to baseline FCMs, across the dark cycle. Previous studies with small mammals found either a presence or absence of a diurnal rhythm in FCMs (e.g., [24,25]). However, when a diurnal rhythm is found, FCM levels will typically rise before the period of highest activity and start to decrease closer to the period of inactivity, which is similar to what we found [25,39]. This reflects the dynamics of blood GCs before they appear in feces, which is governed by a species-specific time delay [4]. This delay can range from 4 h in small mammals (e.g., house mice, [15]) to ~24 h in larger mammals [20]. Similarly, [40] found no effect of sex on FCM levels in deermice, although [25] detected sex differences in house mice where females had higher FCM levels. Due to low sample size of females and males, there may have been an effect of sex on FCMs, which we were unable to detect.

SUPPRESSION OF ADRENOCORTICAL ACTIVITY

We found that FCM levels decreased significantly ~10 h post dexamethasone injection with both EIAs. FCMs decreased on average by ~73% and ~43% for the corticosterone and group-specific EIAs, respectively. This however, could have happened sooner (i.e., ~8 h post injection) since we lacked FCM data at 04:00 h. Nevertheless, other rodent studies found FCM levels decreased 8-10 h post dexamethasone in house mice [25] and 10–12 h in Norway rats (*Rattus norvegicus*) [41], although injections were given during the light cycle. However, the percentage decreases we observed were lower than in Norway rats (~86%; [41]) but higher than in Columbian ground squirrels (*Urocitellus columbianus*) (~33%; [36]), both of which used the same group-specific EIA. This could suggest that a higher dexamethasone dose could be used to more strongly suppress FCMs in deermice. It is noteworthy that the group-specific EIA did detect a marginal increase in FCM levels ~4 h post dexamethasone injection (~69% average increase), most likely due to restraint/injection stress. Even if a higher dose may have resulted in a larger effect size, both EIAs tracked the expected suppression in FCMs post dexamethasone.

STIMULATION OF ADRENOCORTICAL ACTIVITY

FCM levels increased ~2 h post ACTH injection and remained elevated when using both EIAs. In particular, FCMs on average increased by ~65% and ~50% with the corticosterone and group-specific EIAs, respectively. Given that other rodent studies found longer time delays than 2 h post ACTH injection, such as 5–7 h in Egyptian spiny mice (*Acomys cahirinus*) [42] and 6–8 h in bank voles (*Myodes glareolus*) [43], this finding was unexpected. However, in these studies the ACTH injections were given during the light cycle in rodents that are mostly nocturnal, which could have affected time delays [15]. Regardless, [44] did find that brown lemmings (*Lemmus trimucronatus*) reached their half maxima FCM values within 2 h of capture, anesthesia, and transportation in the field. Similarly, [26] found that fecal radioactivity appeared

as early as 2 h in a radiometabolism study of California mice (*Peromyscus californicus*). Nonetheless, in our study, it is still possible that capture and restraint for injection significantly decreased gut passage time [4]. The percentage increases we found are much lower than previous mammal studies. For example, [36] found ~255% increase post ACTH in Columbian ground squirrels using the group-specific EIA. However, [24] found ~56% increase in meadow voles using the group-specific EIA. Therefore, the ACTH dose we used may not have been high enough to reach a stronger effect. Regardless, the modest yet significant increase in FCM levels post ACTH injection provides validation evidence for both EIAs.

TRAP-INDUCED EFFECTS ON FCMs

Trap confinement for 4–6 h marginally increased FCM levels in free-ranging deermice, compared to confinement for 0–2 h but not compared to 8–10 h (field study 1). Similarly, deermice confined for 0–4 h had lower FCM levels compared to additional confinement of ~7 h and after short-term restraint (field study 2). Although we cannot easily tease apart effects from restraint and trap confinement time in field study 2, the findings still provide biological validation. Because FCM levels tend to decrease shortly into the active phase [25,39], the elevations we observed after 4 h would most likely have been due to trap-induced stress. The lack of difference between 0–2 h and 8–10 h could stem from how the stressor of trap confinement remained consistent over time so that FCMs eventually returned to baseline. Alternatively, it could be that the natural decline of FCMs overnight conflicted with the increase in FCMs from trap-induced stress, thereby leading to a lower average FCMs and a larger variability in the data for the 8–10 h group (Figure 3.5). Similarly, [21] found no differences in FCM levels between deermice in traps for 4–8 h versus overnight, although FCM levels did continue to increase with more trap confinement in another deermouse population. Based on field study 1 findings, the lag time

between corticosterone in the blood to excretion in the feces may be ~4 h during the period of highest activity (i.e., dark cycle). This is similar to what has been reported in another deermouse study that used a corticosterone RIA [21]. Although sex and reproductive status can influence stress physiology [4], we did not find any effects on FCMs from sex or reproductive status. However, this may have been due to low sample sizes, and not a limitation of the EIAs. Nevertheless, our findings suggest that trap-induced stress may affect FCM levels even within 4 h of confinement so that earlier fecal collection may better capture baseline adrenocortical activity and unmask individual heterogeneity.

DRYING EFFECTS ON FCMs

Although samples oven-dried for 1 h and then lyophilized were diluted 1:10 for the corticosterone EIA, samples oven-dried for 2 h with no lyophilization had to be diluted 1:80 instead. This increase in the dilution factor could be the result of additional drying time where further alteration of FCMs can affect actual FCM levels and influence antibody binding. Similar heat effects on FCM levels were reported by [45] where autoclaving ungulate feces artificially increased FCM levels. However, an alternative reason could be the origin of the samples because those that were diluted more came from free-ranging deermice whereas those that were diluted less came from laboratory deermice on rodent chow diet. Because diet can affect FCM levels, the diet of free-ranging deermice may have led to artificially higher FCM levels [46]. However, because the group-specific EIA did not detect differences between laboratory and wild deermice (i.e., same dilution factor), it is most likely that an additional hour of oven-drying induced structural changes to FCMs that were detected by the corticosterone EIA antibody, thereby increasing FCM levels (e.g., [47]). Therefore, the drying protocol needs to remain consistent

throughout a study, (e.g., for multiple samples from one individual) if valid FCM comparisons are to be made.

CONCLUSIONS

We analytically, physiologically, and biologically validated two EIAs for measuring FCMs in deermice. Although we used identical sample processing and extraction methods for laboratory samples, this was not the case with field samples so direct comparisons should be made with caution. Nevertheless, both field studies demonstrated similar temporal patterns, so they provided biological validation for the two EIAs. Although we acknowledge low sample sizes, our study is the first to provide validation evidence for EIAs that can quantify FCMs in deermice.

ACKNOWLEDGEMENTS

We thank Grant McClelland and Graham Scott of McMaster University for providing us with the laboratory-bred deermice that originated from Nebraska, USA. We thank Michael Sheriff for all his advice in setting up the study, for partially funding laboratory analyses and for allowing the primary author to complete assay work at his laboratory space at Pennsylvania State University. We also thank Catharine E. Pritchard for her laboratory and editorial assistance, Ben Dantzer from the University of Michigan for shipping samples to us at Pennsylvania State University and Angela Luis from the University of Montana in Missoula for her general feedback and support during the study. Additionally, we thank the private cattle ranch owners and Montana Fish, Wildlife and Parks for allowing us access to their lands where the fieldwork took place, and all the volunteers that assisted with field and lab work at the University of Montana. Lastly, this research was partially funded by the Wildlife Biology program at the University of Montana,

Interdisciplinary Collaborative Network, Montana Institute on Ecosystems and the Associated Students of the University of Montana.

LITERATURE CITED

1. Sheriff, M.J.; Dantzer, B.; Delehanty, B.; Palme, R.; Boonstra, R. Measuring stress in wildlife: Techniques for quantifying glucocorticoids. *Oecologia* **2011**, *166*, 869–887.
2. Dantzer, B.; Fletcher, Q.E.; Boonstra, R.; Sheriff, M.J. Measures of physiological stress: A transparent or opaque window into the status, management and conservation of species? *Conserv. Physiol.* **2014**, *2*, cou023.
3. Touma, C.; Palme, R. Measuring fecal glucocorticoid metabolites in mammals and birds: The importance of validation. *Ann. N. Y. Acad. Sci.* **2005**, *1046*, 54–74.
4. Palme, R. Non-invasive measurement of glucocorticoids: Advances and problems. *Physiol. Behav.* **2019**, *199*, 229–243.
5. Fanson, K.V.; Best, E.C.; Bunce, A.; Fanson, B.G.; Hogan, L.A.; Keeley, T.; Narayan, E.J.; Palme, R.; Parrott, M.L.; Sharp, T.M. One size does not fit all: Monitoring faecal glucocorticoid metabolites in marsupials. *Gen. Comp. Endocrinol.* **2017**, *244*, 146–156.
6. Heistermann, M.; Palme, R.; Ganswindt, A. Comparison of different enzyme immunoassays for assessment of adrenocortical activity in primates based on fecal analysis. *Am. J. Primatol.* **2006**, *68*, 257.
7. Schountz, T.; Prescott, J.; Cogswell, A.C.; Oko, L.; Mirowsky-Garcia, K.; Galvez, A.P.; Hjelle, B. Regulatory T cell-like responses in deer mice persistently infected with Sin Nombre virus. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 15496–15501.
8. Mahalingam, S.; McClelland, G.B.; Scott, G.R. Evolved changes in the intracellular distribution and physiology of muscle mitochondria in high-altitude native deer mice. *J. Physiol. (Lond)* **2017**, *595*, 4785–4801.
9. Luis, A.D.; Douglass, R.J.; Mills, J.N.; Bjørnstad, O.N. Environmental fluctuations lead to predictability in Sin Nombre hantavirus outbreaks. *Ecology* **2015**, *96*, 1691–1701.
10. Harper, J.M.; Austad, S.N. Fecal glucocorticoids: A noninvasive method of measuring adrenal activity in wild and captive rodents. *Physiol. Biochem. Zool* **2000**, *73*, 12–22.
11. Hayssen, V.; Harper, J.; DeFina, R. Fecal corticosteroids in agouti and non-agouti deer mice (*Peromyscus maniculatus*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **2002**, *132*, 439–446.
12. Pedersen, A.B.; Greives, T.J. The interaction of parasites and resources cause crashes in a wild mouse population. *J. Anim. Ecol.* **2008**, *77*, 370–377.
13. Fredebaugh-Siller, S.; Suski, C.; Zuckerman, Z.; Schooley, R. Ecological correlates of stress for a habitat generalist in a biofuels landscape. *Can. J. Zool* **2013**, *91*, 853–858.
14. Bradley, E.L.; Terman, C.R. A comparison of the adrenal histology, reproductive condition, and serum corticosterone concentrations of prairie deer mice (*Peromyscus maniculatus bairdii*) in captivity. *J. Mammal.* **1981**, *62*, 353–361.
15. Touma, C.; Sachser, N.; Möstl, E.; Palme, R. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. *Gen. Comp. Endocrinol.* **2003**, *130*, 267–278.

16. Wielebnowski, N.; Watters, J. Applying fecal endocrine monitoring to conservation and behavior studies of wild mammals: Important considerations and preliminary tests. *Isr. J. Ecol. Evol.* **2007**, *53*, 439–460.
17. Delehanty, B.; Boonstra, R. Impact of live trapping on stress profiles of Richardson's ground squirrel (*Spermophilus richardsonii*). *Gen. Comp. Endocrinol.* **2009**, *160*, 176–182.
18. Bosson, C.; Islam, Z.; Boonstra, R. The impact of live trapping and trap model on the stress profiles of North American red squirrels. *J. Zool* **2012**, *288*, 159–169.
19. Millspaugh, J.J.; Washburn, B.E. Use of fecal glucocorticoid metabolite measures in conservation biology research: Considerations for application and interpretation. *Gen. Comp. Endocrinol.* **2004**, *138*, 189–199.
20. Palme, R.; Rettenbacher, S.; Touma, C.; El-Bahr, S.; Möstl, E. Stress hormones in mammals and birds: Comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. *Ann. N. Y. Acad. Sci.* **2005**, *1040*, 162–171.
21. Harper, J.M.; Austad, S.N. Effect of capture and season on fecal glucocorticoid levels in deer mice (*Peromyscus maniculatus*) and red-backed voles (*Clethrionomys gapperi*). *Gen. Comp. Endocrinol.* **2001**, *123*, 337–344.
22. Montiglio, P.; Pelletier, F.; Palme, R.; Garant, D.; Réale, D.; Boonstra, R. Noninvasive monitoring of fecal cortisol metabolites in the eastern chipmunk (*Tamias striatus*): Validation and comparison of two enzyme immunoassays. *Physiol. Biochem. Zool* **2012**, *85*, 183–193.
23. Fletcher, Q.E.; Boonstra, R. Impact of live trapping on the stress response of the meadow vole (*Microtus pennsylvanicus*). *J. Zool* **2006**, *270*, 473–478.
24. Edwards, P.D.; Dean, E.K.; Palme, R.; Boonstra, R. Assessing space use in meadow voles: The relationship to reproduction and the stress axis. *J. Mammal.* **2019**, *100*, 4–12.
25. Touma, C.; Palme, R.; Sachser, N. Analyzing corticosterone metabolites in fecal samples of mice: A noninvasive technique to monitor stress hormones. *Horm. Behav.* **2004**, *45*, 10–22.
26. Harris, B.N.; Saltzman, W.; de Jong, T.R.; Milnes, M.R. Hypothalamic–pituitary–adrenal (HPA) axis function in the California mouse (*Peromyscus californicus*): Changes in baseline activity, reactivity, and fecal excretion of glucocorticoids across the diurnal cycle. *Gen. Comp. Endocrinol.* **2012**, *179*, 436–450.
27. Hare, J.F.; Ryan, C.P.; Enright, C.; Gardiner, L.E.; Skyner, L.J.; Berkvens, C.N.; Anderson, W.G. Validation of a radioimmunoassay-based fecal corticosteroid assay for Richardson's ground squirrels *Uroditellus richardsonii* and behavioural correlates of stress. *Curr. Zool* **2014**, *60*, 591–601.
28. Fairbairn, D.J. The spring decline in deer mice: Death or dispersal? *Can. J. Zool* **1977**, *55*, 84–92.
29. Mills, J.N.; Yates, T.L.; Childs, J.E.; Parmenter, R.R.; Ksiazek, T.G.; Rollin, P.E.; Peters, C. Guidelines for working with rodents potentially infected with hantavirus. *J. Mammal.* **1995**, *76*, 716–722.
30. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2018.
31. RStudio Team. *RStudio: Integrated Development for R*; RStudio, Inc.: Boston, MA, USA, 2015.
32. Bates, D.; Maechler, M.; Bolker, B.; Walker, B. Fitting Linear Mixed-Effects Models using lme4. *J. Stat. Soft.* **2015**, *67*, 1–48.

33. Kuznetsova, A.; Brockhoff, P.B.; Christensen, R.H.B. lmerTest Package: Tests in Linear Mixed Effects Models. *J. Stat. Soft.* **2017**, *82*, 1–26.
34. Lenth, R. Emmeans: Estimated Marginal Means aka Least-Squares Means. 2018. R Package Version 1.3.1. Available online: <https://CRAN.R-project.org/package=emmeans> (accessed on 1 May 2020).
35. Siswanto, H.; Hau, J.; Carlsson, H.E.; Goldkuhl, R.; Abelson, K.S. Corticosterone concentrations in blood and excretion in faeces after ACTH administration in male Sprague-Dawley rats. *In Vivo* **2008**, *22*, 435–440.
36. Bosson, C.O.; Palme, R.; Boonstra, R. Assessment of the stress response in Columbian ground squirrels: Laboratory and field validation of an enzyme immunoassay for fecal cortisol metabolites. *Physiol. Biochem. Zool.* **2009**, *82*, 291–301.
37. Sheriff, M.J.; Bosson, C.O.; Krebs, C.J.; Boonstra, R. A non-invasive technique for analyzing fecal cortisol metabolites in snowshoe hares (*Lepus americanus*). *J. Comp. Physiol. B* **2009**, *179*, 305–313.
38. Dantzer, B.; McAdam, A.G.; Palme, R.; Fletcher, Q.E.; Boutin, S.; Humphries, M.M.; Boonstra, R. Fecal cortisol metabolite levels in free-ranging North American red squirrels: Assay validation and the effects of reproductive condition. *Gen. Comp. Endocrinol.* **2010**, *167*, 279–286.
39. Bauer, B.; Palme, R.; Machatschke, I.H.; Dittami, J.; Huber, S. Non-invasive measurement of adrenocortical and gonadal activity in male and female guinea pigs (*Cavia aperea f. porcellus*). *Gen. Comp. Endocrinol.* **2008**, *156*, 482–489.
40. Harper, J.M.; Austad, S.N. Fecal corticosteroid levels in free-living populations of deer mice (*Peromyscus maniculatus*) and southern red-backed voles (*Clethrionomys gapperi*). *Am. Midl. Nat.* **2004**, *152*, 400–409.
41. Lepschy, M.; Touma, C.; Hruby, R.; Palme, R. Non-invasive measurement of adrenocortical activity in male and female rats. *Lab. Anim.* **2007**, *41*, 372–387.
42. Nováková, M.; Palme, R.; Kutalová, H.; Janský, L.; Frynta, D. The effects of sex, age and commensal way of life on levels of fecal glucocorticoid metabolites in spiny mice (*Acomys cahirinus*). *Physiol. Behav.* **2008**, *95*, 187–193.
43. Sipari, S.; Ylönen, H.; Palme, R. Excretion and measurement of corticosterone and testosterone metabolites in bank voles (*Myodes glareolus*). *Gen. Comp. Endocrinol.* **2017**, *243*, 39–50.
44. Fauteux, D.; Gauthier, G.; Berteaux, D.; Bosson, C.; Palme, R.; Boonstra, R. Assessing stress in Arctic lemmings: Fecal metabolite levels reflect plasma free corticosterone levels. *Physiol. Biochem. Zool.* **2017**, *90*, 370–382.
45. Millspaugh, J.J.; Washburn, B.E.; Milanick, M.A.; Slotow, R.; van Dyk, G. Effects of heat and chemical treatments on fecal glucocorticoid measurements: Implications for sample transport. *Wildl. Soc. Bull.* **2003**, 399–406.
46. Goymann, W. On the use of non-invasive hormone research in uncontrolled, natural environments: The problem with sex, diet, metabolic rate and the individual. *Methods Ecol. Evol.* **2012**, *3*, 757–765.
47. Lexen, E.; El-Bahr, S.; Sommerfeld-Stur, I.; Palme, R.; Möstl, E. Monitoring the adrenocortical response to disturbances in sheep by measuring glucocorticoid metabolites in the faeces. *Wien. Tierarztl. Monat.* **2008**, *95*, 64.

Table 3. 1 Timeline of treatments and samples used in statistical analyses for evaluation of fecal corticosterone metabolites in deermice.

Date	Treatment ¹	Immunoassay	Sample Collection ² Schedule (h Post Treatment)
May 2–3	Acclimation	Corticosterone EIA	70, 72, 74, 76, 80, 82
		Group-specific EIA	70, 72, 74, 76, 78, 80, 82
May 3–4	Adrenal suppression	Corticosterone EIA	2, 4, 6, 10, 12
		Group-specific EIA	2, 4, 6, 10, 12
May 5–6	Adrenal stimulation	Corticosterone EIA	2, 4, 6, 10, 12
		Group-specific EIA	2, 4, 6, 10, 12

¹ Injections administered at ~20:00 h, ² Acclimation lasted for ~92 h whereas adrenal treatments lasted for ~48 h (see text for details).

Table 3. 2 Summary of demographic factors and fecal cortisol/corticosterone metabolite (FCM) values from free-ranging deermice captured in MT, USA in August 2017 for field study 2.

Sex	Reproductive ¹	FCMs at 0–4 h (ng/g)	FCMs Overnight (ng/g)	FCM Difference ²
Male	No	21034	49709	28676
Male	No	5407	76071	70663
Male	Yes	11224	185928	174704
Male	Yes	4649	91514	86865
Female	Yes	37172	37776	604
Female	Yes	3611	41678	38067
Female	Yes	9704	82299	72595

¹ Reproductive status was determined via scrotal testes in males and presence of a perforate vagina, lactation, or pregnancy in females, ² FCM difference was calculated by subtracting FCMs at 0–4 h from FCMs overnight.

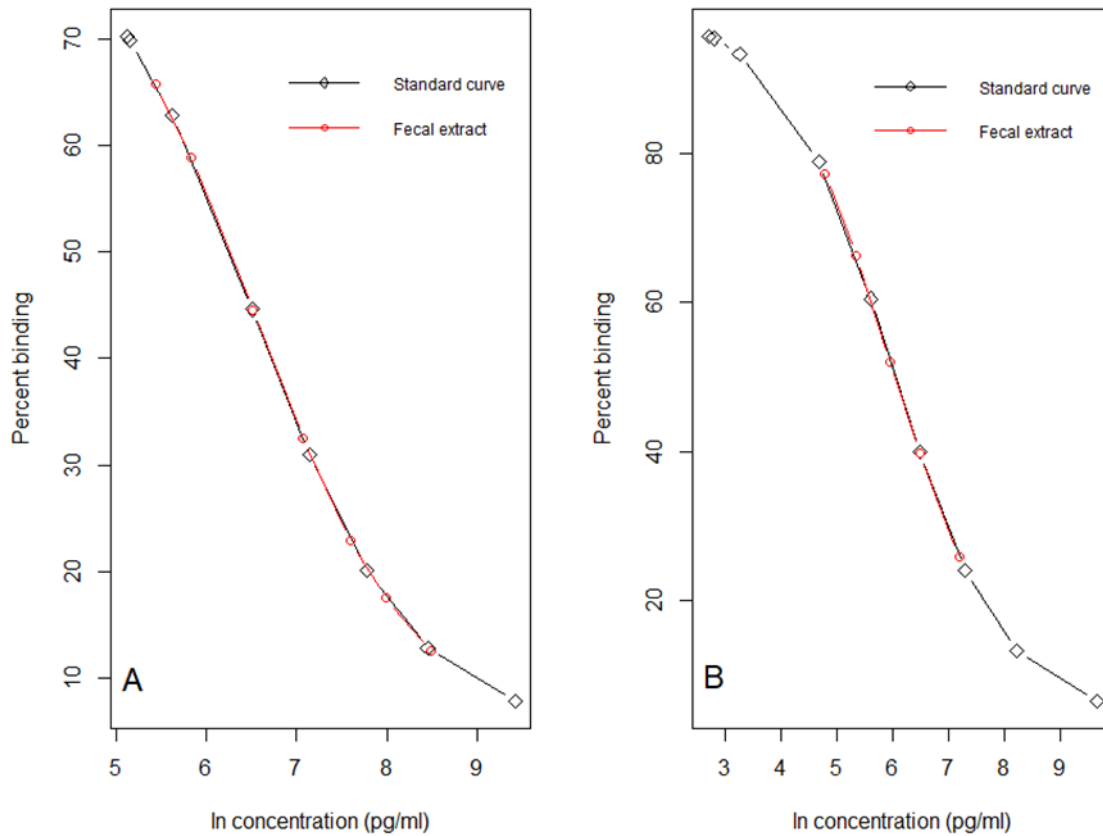


Figure 3. 1 Parallelism curves for pooled fecal extract from deermice measured with (A) corticosterone enzyme immunoassay (EIA) and (B) group-specific EIA. Standard curves for each EIA are shown in black with each concentration as an open diamond. Parallelism is shown in red with serial dilutions of pooled fecal extract as open red circles.

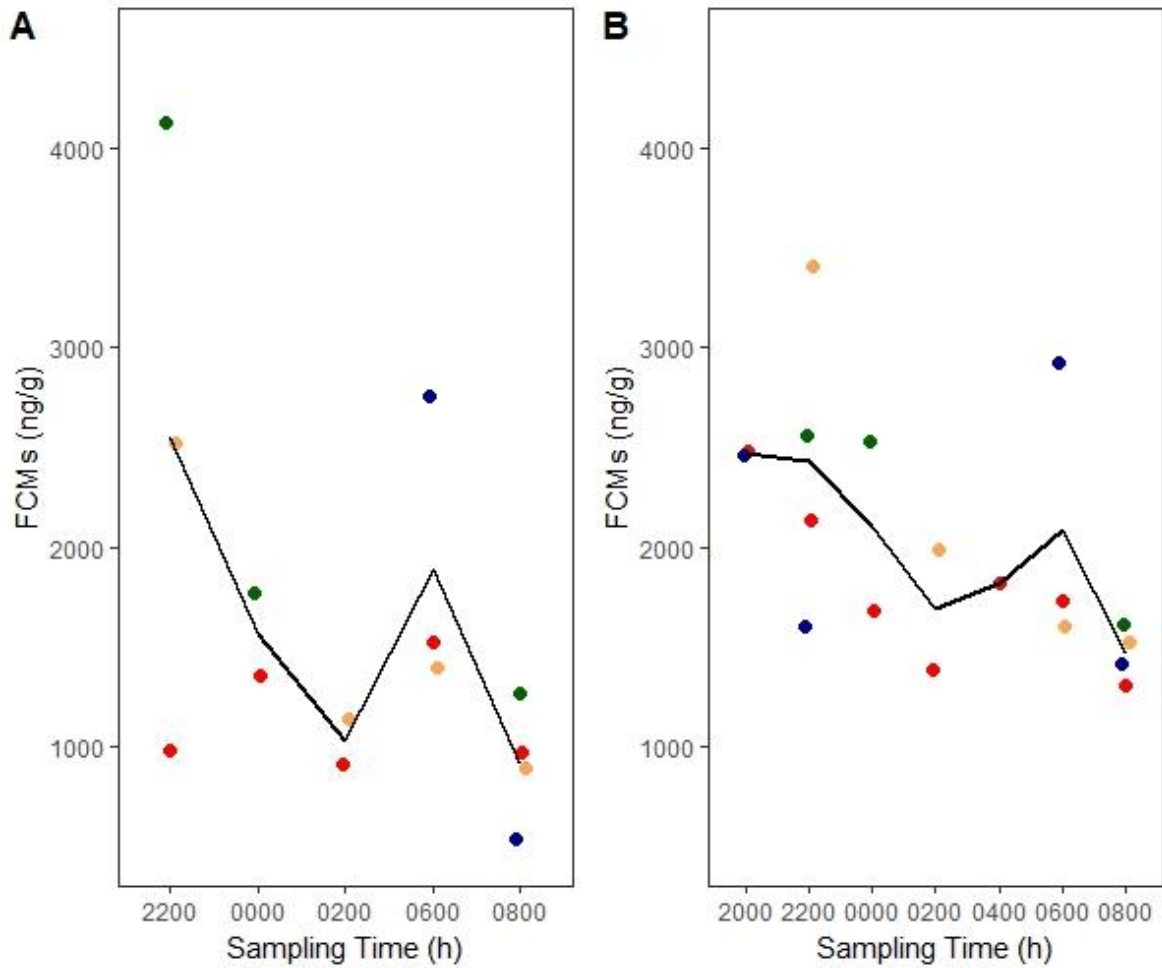


Figure 3. 2 Changes in corticosterone baseline fecal cortisol/corticosterone metabolites (FCMs) from laboratory-bred deermice across the dark cycle measured with (A) corticosterone enzyme immunoassay (EIA) and (B) group-specific EIA. Lines connect means from each sampling time and circles indicate data points whereby an individual is denoted with a different color. Males are shown in blue and green colors, whereas females are shown in red and tan.

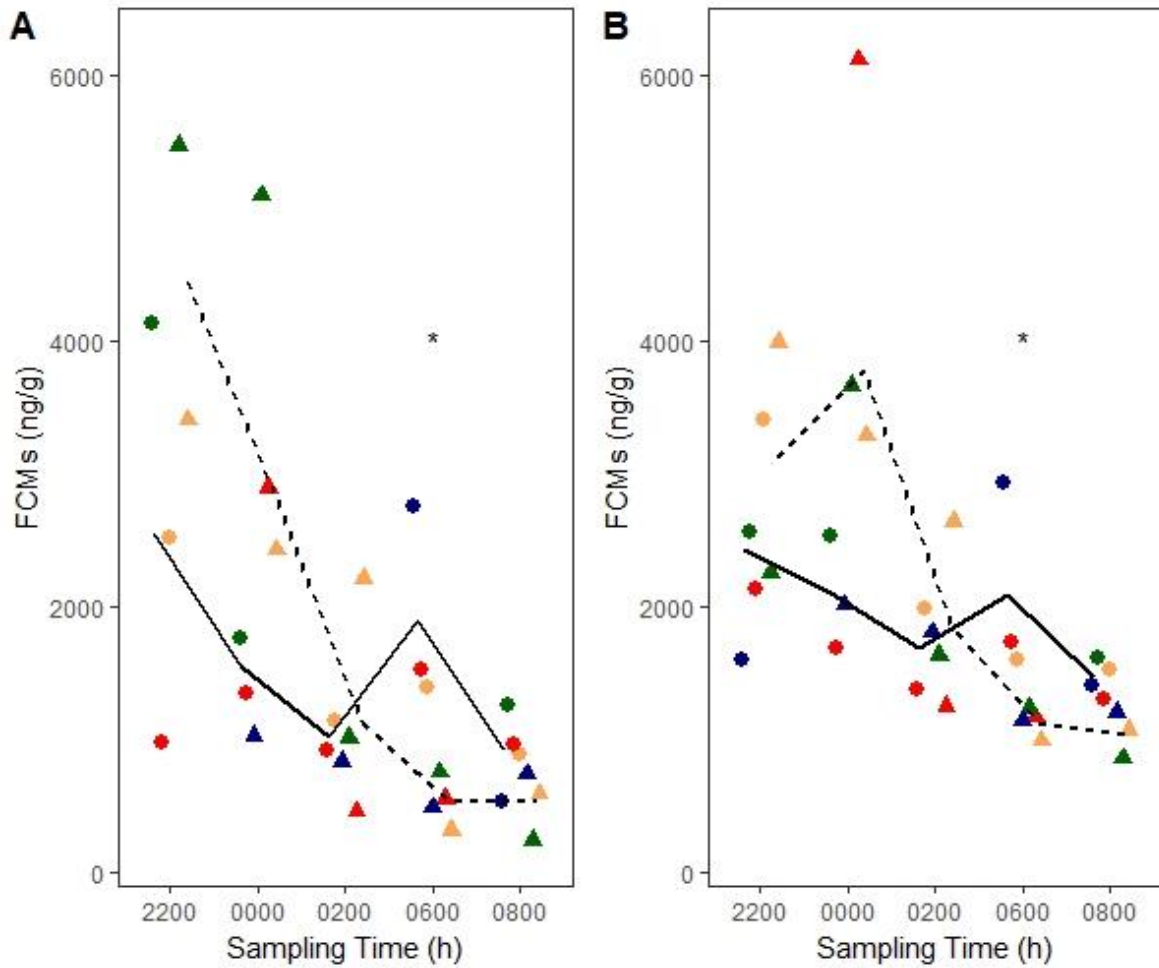


Figure 3. 3 Corticosterone baseline fecal cortisol/corticosterone metabolites (FCMs) (solid lines and circles) versus post dexamethasone FCMs (dashed lines and triangles) in laboratory-bred deermice measured with (A) corticosterone enzyme immunoassay (EIA) and (B) group-specific EIA. Lines connect means from each sampling time. Circles and triangles indicate individual data points whereby an individual is denoted with a different color. Males are shown in blue and green colors, whereas females are shown in red and tan. Dexamethasone was administered at ~20:00 h (not shown). Asterisk (*) denotes significant differences at $\alpha = 0.05$.

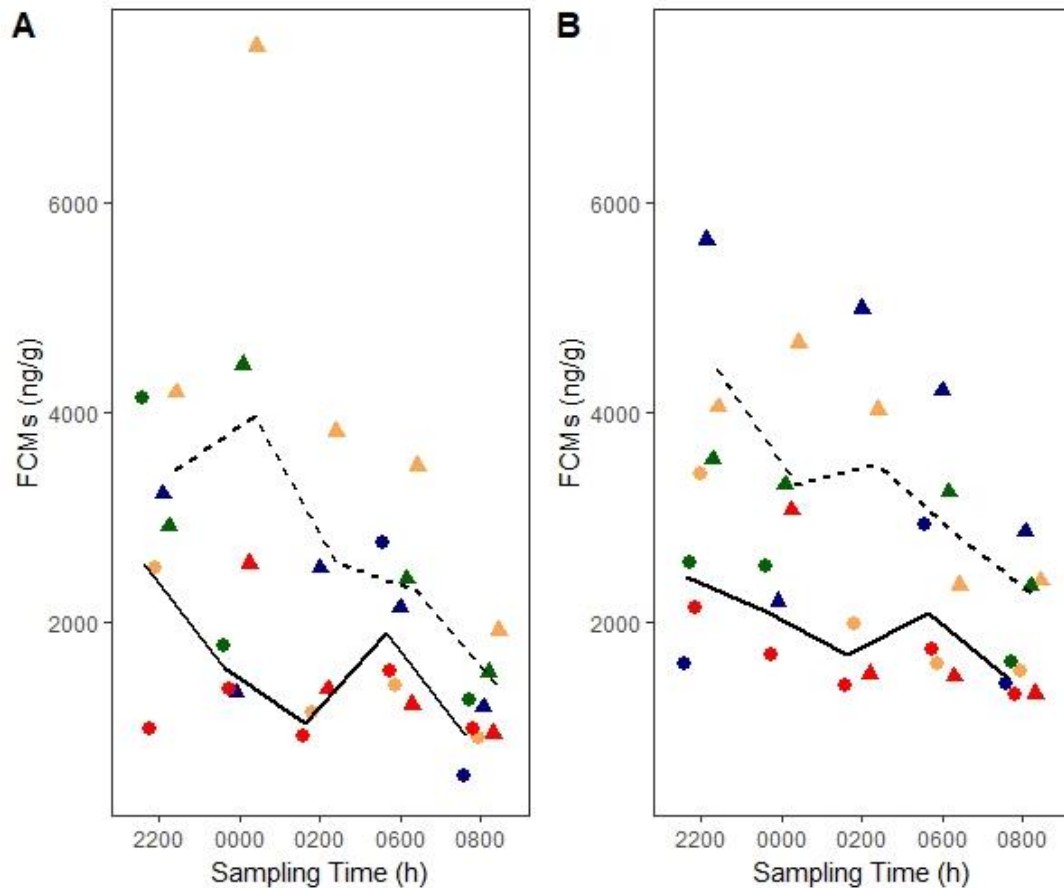


Figure 3. 4 Corticosterone baseline fecal cortisol/corticosterone metabolites (FCMs) (solid lines and circles) versus post adrenocorticotrophic hormone (ACTH) FCMs (dashed lines and triangles) in laboratory-bred deermice measured with (A) corticosterone enzyme immunoassay (EIA) and (B) group-specific EIA. Lines connect means from each sampling time. Circles and triangles indicate individual data points whereby an individual is denoted with a different color. Males are shown in blue and green colors, whereas females are shown in red and tan. ACTH was administered at ~20:00 h (not shown).

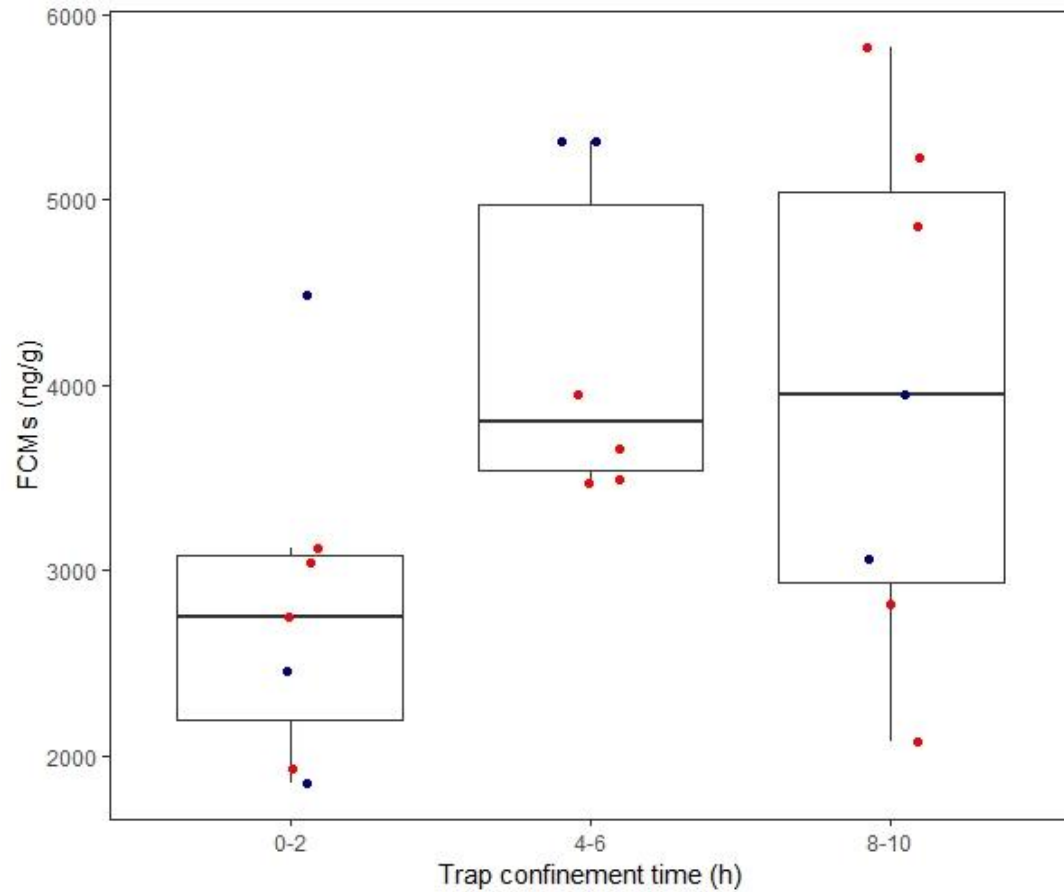


Figure 3. 5 Fecal cortisol/corticosterone metabolites (FCMs) across different trap confinement times in free-ranging deermice. FCMs were measured with the group-specific EIA. Boxplots display the median (line), 25–75% interquartile range (boxes) and the full range (whiskers). In the 0–2 h group, there is a large outlier. Circles indicate individual data points whereby blue denotes males and red denotes females.

CHAPTER 4 HETEROSPECIFIC COMPETITORS AND SEASONALITY CAN AFFECT HOST PHYSIOLOGY AND BEHAVIOR: KEY FACTORS IN DISEASE TRANSMISSION²

ABSTRACT

Ecological and environmental factors can influence the transmission of infectious diseases. They can accomplish this via effects on host susceptibility and exposure to infection, which are governed by host physiology and behavior, respectively. To better inform disease control, more information is needed about how extrinsic factors affect physiological and behavioral processes that determine transmission. We investigated how heterospecific competitors and seasonality may influence host susceptibility and intraspecific contact rates using a directly-transmitted disease system, the North American deermouse (*Peromyscus maniculatus*) - Sin Nombre hantavirus (SNV) system. In grasslands of western Montana, USA, deermice compete with dominant voles (*Microtus* spp.) and shrews (*Sorex* spp.) and experience a seasonal temperate climate. Higher SNV transmission occurs primarily during spring/summer, when changes in physiology and behavior may serve as influential contributors. We hypothesized that: (1) voles, and to a lesser extent shrews, will induce chronic stress, suppress immunity, and may change contact rates of deermice, and (2) during spring/summer, deermice may experience chronic stress, suppressed immunity, and higher contact rates, which may help explain the reported seasonality in SNV transmission. Over two years, we trapped small mammals at four grids in western Montana. Deermice were sampled for feces and blood and evaluated for scar numbers, demography and body condition scores (BCSs). We evaluated stress physiology with fecal

² This paper is in press in "Ecosphere". A. Eleftheriou, A. J. Kuenzi and A.D. Luis. 2021. Heterospecific competitors and seasonality can influence host physiology and behavior: key factors in disease transmission. Ecosphere. In Press.

corticosterone metabolites (FCMs), neutrophil/lymphocyte (N/L) ratios and BCSs, immunity with white blood cell (WBC) counts, and contact rates with scar numbers. We found that shrew density was negatively associated with stress response FCMs, suggestive of chronic stress. Additionally, although complex interactions existed, shrew and vole densities were negatively associated with BCSs, but differentially with scar numbers. N/L ratios were higher in spring/summer whereas WBC counts were lower in summer, suggestive of chronic stress and suppressed immunity, respectively. Our results suggest that (1) heterospecific competitors may differentially influence disease transmission via stress physiology and contact rates, and that (2) chronic stress, suppressed immunity, and higher contact rates may help explain why higher SNV transmission has been previously reported during spring/summer in Montana. Our findings may extend to other directly-transmitted disease systems.

INTRODUCTION

Globally, emerging infectious diseases are causing wildlife populations to decline and species to go extinct (Daszak et al. 1999, Williams et al. 2002, Smith et al. 2006). Extrinsic (i.e., ecological and environmental) factors may drive a rise in disease emergence, because they can influence two key determinants of disease transmission. These include host susceptibility and exposure to infection, which are governed by host physiology and behavior, respectively (Hawley et al. 2011). Therefore, to better inform wildlife conservation and disease management, it is imperative that we identify physiological and behavioral mechanisms that link extrinsic factors to disease transmission so we can better understand and predict their impacts on wildlife disease dynamics.

Extrinsic factors may impact host physiology via glucocorticoid (GC) hormones, crucial regulators of the stress response. Although an acute rise in GCs from a stressor is considered adaptive, persistently elevated GCs (i.e., chronic stress) are maladaptive because they can lower

immunity and increase susceptibility to infection (Dickens and Romero 2013, Dantzer et al. 2014). Stress physiology is typically evaluated with blood GCs but also with metabolized GCs in feces called fecal cortisol/corticosterone metabolites (FCMs) (Palme 2019). When vertebrates experience chronic stress, their baseline FCMs can rise and their stress response to an acute challenge (i.e., stress response FCMs) can decline (Busch and Hayward 2009). However, GC secretion patterns can be highly variable, which can make interpretation of hormone data a cumbersome task. Therefore, it is best practice to employ several measures that span GC metabolism (i.e., downstream effects of GCs) to attain a more comprehensive evaluation of stress physiology (Breuner et al. 2013, Dantzer et al. 2014).

There are various downstream measures of GCs (reviewed in Breuner et al. 2013). For example, because GCs increase neutrophils and decrease lymphocytes in the peripheral circulation, individuals under chronic stress are expected to have higher neutrophil/lymphocyte (N/L) ratios (Davis et al. 2008), while they are expected to have lower body condition scores (BCSs) from GC-induced breakdown of fat and muscle reserves (Sapolsky et al. 2000). However, the effect of GCs on immunity is much less straightforward because components of the immune system (e.g., humoral versus cellular) may respond differently to chronic stress (Sapolsky et al. 2000). Despite this inherent complexity, total white blood cell (WBC) counts are largely expected to decline with chronic stress (Martin 2009).

Heterospecific competitors may influence host susceptibility and exposure to infection. In particular, competitors may increase host susceptibility to infection via GCs. For example, Santicchia et al. (2018) found that invasive grey squirrels (*Sciurus carolinensis*) increased baseline FCMs in Eurasian red squirrels (*Sciurus vulgaris*), and Narayan et al. (2015) showed that invasive cane toads (*Rhinella marina*) decreased body condition in Fijian ground frogs

(*Platymantis vitiana*). Therefore, interspecific competition can influence stress physiology, and presumably, host immunity. Additionally, heterospecific competitors may alter host behavior, thereby affecting exposure to infection. For example, Glass and Slade (1980) found that prairie voles (*Microtus ochrogaster*) avoided space that was used by reproductive cotton rats (*Sigmodon hispidus*), and Gutman and Dayan (2005) found that common spiny mice (*Acomys cahirinus*) inhibited the nocturnal activity of golden spiny mice (*A. russatus*). Taken together, heterospecific competitors may influence disease transmission via both host physiology and behavior.

Environmental factors that fluctuate seasonally, such as food availability and ambient temperature, may influence stress physiology, immunity, and behavior, which may explain why disease prevalence can vary seasonally in wildlife hosts (Altizer et al. 2006). Particularly in temperate habitats, seasonal environmental factors can affect stress physiology and immunity of wildlife, which may have ramifications for disease transmission (Nelson and Demas 1996). In fact, most vertebrates undergo seasonal changes in baseline and stress-induced GCs, with higher baseline GCs expected during the breeding period, although this can be species-specific (Reeder et al. 2005, Romero et al. 2008). Similarly, vertebrates undergo seasonal changes in immunity. For example, birds and small mammals enhance their immunity in winter (Martin et al. 2008). Wildlife can also exhibit seasonal changes in behavior that may affect disease transmission. For example, by aggregating in flocks during winter, house finches may experience seasonal exposure to *Mycoplasma gallisepticum* (Altizer et al. 2004), and by engaging in aggressive interactions during their breeding season, bank voles (*Myodes glareolus*) may experience seasonal exposure to Puumala hantavirus (Escutenaire et al. 2002). In summary, seasonality in environmental factors may impact disease transmission via host physiology and behavior.

To examine physiological and behavioral links between extrinsic factors and mechanisms affecting disease transmission, we conducted an observational study in grasslands of western Montana, USA, with the North American deermouse (*Peromyscus maniculatus*; hereafter deermouse), the reservoir host for the directly-transmitted Sin Nombre hantavirus (SNV). This is an appropriate system for examining how physiology and behavior may link extrinsic factors to transmission for two main reasons. Firstly, there has been considerable research regarding the “diluting” effect of species diversity on disease risk (i.e. dilution effect; *sensu* Keesing et al. 2006) across rodent-hantavirus systems (e.g., Suzán et al. 2009, Dearing et al. 2015, Khalil et al. 2016). However, the dilution effect observed may not be due to diversity *per se* but due to ecological processes, such as interspecific competition between host and nonhost species, which may happen to correlate with diversity (Johnson et al. 2015). Regardless, we still require more information about the physiological and behavioral mechanisms behind the dilution effect that may be applicable to other directly-transmitted disease systems (Rubio et al. 2017, Luis et al. 2018). Secondly, although seasonality has been extensively studied as a crucial driver for disease dynamics across rodent-hantavirus systems (Luis et al. 2015, Voutilainen et al. 2016), potential physiological and behavioral mechanisms have received comparatively less attention. Therefore, we used the deermouse-SNV system as a model to investigate how ecological and environmental factors affect physiological and behavioral mechanisms of disease transmission.

At our study site, deermice experience a temperate climate with strong seasonality and coexist with voles (*Microtus* spp.) and shrews (*Sorex* spp.). This study system allowed us to examine how heterospecific competitors (i.e., voles and shrews) and seasonality associated with measures of deermouse physiology (i.e., stress physiology and immunity) and behavior (i.e., contact rates), which has implications for SNV transmission. Given that demography, parasites,

and habitat may influence physiology and behavior, they were also accounted for in our analyses (Dantzer et al. 2014).

Voles are considered dominant competitors of deermice. For example, Grant (1971) found that meadow voles excluded deermice from grasslands through aggressive interactions. However, we found no evidence to suggest that shrews are also dominant over deermice, and because of their much smaller size, we reasoned that they are most likely less dominant. Consequently, we hypothesized that dominant voles will induce chronic stress, depress immunity and/or alter contact rates of deermice, with shrews having a lesser effect (Table 4.1). We did not have *a priori* hypotheses as to how contact rates may be altered because previous field studies generated inconsistent findings (Clay et al. 2009, Rubio et al. 2017, Luis et al. 2018).

In Montana, SNV transmission is typically highest during spring/summer, which coincides with the breeding season of deermice during which they will engage in aggressive intraspecific encounters, leading to the accumulation of scars (Douglass et al. 2001, Bagamian et al. 2012). Field studies from nearby Idaho, suggest that stress physiology of deermice varies seasonally given that deermice were found to have lower FCMs during fall than summer (Harper and Austad 2001). Deermouse immunity may also vary seasonally as indicated through laboratory experiments where deermice under short-day conditions (i.e., mimicking winter conditions in North America), had higher WBC counts compared to long day conditions (Blom et al. 1994). Given all the evidence above, we also hypothesized that deermice will experience chronic stress, suppressed immunity, and higher contact rates during spring/summer (Table 4.1).

MATERIALS AND METHODS

STUDY SYSTEM

Deermice are the primary reservoir for SNV in North America (Childs et al. 1994). At grasslands in western Montana, deermice coexist with few other small mammals, which include meadow

(*Microtus pennsylvanicus*) and montane voles (*Microtus montanus*), and vagrant (*Sorex vagrans*) and montane shrews (*Sorex monticolus*; Carson et al. 2006). None of these small mammals are considered to be competent SNV hosts (Mills et al. 2010).

VARIABLES MEASURED

We evaluated stress physiology with four measures that span GC metabolism: (1) baseline FCM levels, (2) stress response FCM levels (FCMs after overnight trap confinement minus baseline), (3) N/L ratio, and (4) BCS. Immunity was evaluated with total WBC counts. We used number of scars to evaluate contact rates because positive correlations between presence of scars and SNV infection in deermice have been found (e.g., Douglass et al. 2001). Despite its limitations, we thought number of scars was suitable for two reasons: (1) SNV is primarily directly transmitted via bites that can result in scars (Mills et al. 1999, Warner et al. 2019), and (2) it allowed us to incorporate a measure of contact rates, which are notoriously difficult to quantify in the field.

SITE DESCRIPTION AND LIVE-TRAPPING

Four 1-hectare grids were established, where each grid had 100 trap stations 10 m apart in a 10 X 10 array (Kuenzi et al. 2001). These were located at the Ninepipe Wildlife Management Area, Montana, USA, and were at least 1600 meters away from each other. The most common grasses were intermediate wheatgrass (*Agropyron intermedium*), smooth brome (*Bromus* sp.) and timothy grass (*Phleum pratense*) (see Supplementary Information for more grid descriptions).

Small mammals were live-trapped November 2016 - August 2018. Grids A and B were trapped October - November 2016, February - December 2017 (excluding June and July for A, and July for B) and March - July 2018. Grids C and D were trapped from October - November 2017 and March - August 2018. We trapped two grids concurrently at a time (i.e., grids A and B together, grids C and D together) once a month for three nights. We initially removed voles from grid B by euthanasia August 2017 - April 2018, so we could assess removal effects on deermice

(Vole N removed = 25). However, we considered this approach to be unsuccessful because voles were trapped consistently despite removal. Although removal may have influenced vole densities, we still typically trapped voles most often at this grid (Fig. 1). Therefore, we still had enough variation in vole densities to address our competitor hypotheses. We speciated euthanized or dead voles by examining their dentition; meadow voles have an extra cusp on their upper middle molar (Hall and Kelson 1959). We did not speciate shrews but, most likely, they were vagrant and montane shrews (Carson et al. 2006).

We baited non-folding Sherman live traps (H. B. Sherman, Tallahassee, Florida, USA) with peanut butter and oats and supplied them with polyester bedding. Traps were opened around dusk and checked approximately four hours later when trap-induced stress is less likely to influence baseline FCMs (Harper and Austad 2001, Eleftheriou et al. 2020). Voles and shrews were released after processing, whereas deermice were returned to their traps so they could be processed again around dawn before release on site. This allowed evaluation of the FCM response to an acute stressor (i.e. trap confinement; Eleftheriou et al. 2020).

ANIMAL SAMPLING

At the initial check, traps that contained animals were taken to a central station for processing. We tagged deermice with metal ear tags (National Band and Tag Co., Newport, Kentucky, USA), collected feces and returned them to their traps until around dawn, which is when we collected more feces and blood, and also weighed them. Sex, presence of fleas and reproductive status were also noted. A BCS was estimated between one minus and five plus by palpation of tissue at the base of the tail, with five plus being extremely obese (Ullman - Culleré and Foltz 1999). BCS estimation was performed by the same experienced investigator throughout the entire course of the study. We also examined deermice for scars, which we counted, if present. We assigned deermice to four scar categories starting in April 2017: (0) no scars, (1) ≤ 4 , (2) 5-8

and $(3) \geq 9$ scars. Age was estimated from weight (juveniles < 14 g, subadults 14-17 g and adults > 17 g; Fairbairn 1977). Voles were ear tagged, weighed, sexed and evaluated for BCS and reproductive status. Given that shrews cannot be ear tagged, we began marking them in April 2017 with a permanent marker. Because recaptures of marked shrews were rare (two recaptured April 2017 - August 2018), we are confident we did not count the same animals twice within the same trapping session. Active reproductive status in female rodents was determined via the presence of a perforate vagina, pregnancy and/or lactation, and in male rodents via the presence of scrotal testes. Blood was collected with heparinized capillary tubes (Fisher Scientific, Pittsburgh, Pennsylvania, USA) from the retroorbital capillary sinus, after topical anesthesia with proparacaine (Akorn, Inc., Lake Forest, Illinois, USA). Blood and feces were immediately frozen. We followed safety guidelines for working with animals potentially infected with hantavirus (Mills et al. 1995). All animal procedures were approved by the Institutional Animal Care and Use Committee (# 027-16ALDECS-051016) at the University of Montana, Missoula, MT. Land access was granted by Montana Fish, Wildlife and Parks.

FCM ANALYSES

We heated feces in a laboratory oven within a biosafety cabinet at $\sim 63^{\circ}\text{C}$ for two hours to inactivate any SNV (J.N. Mills, personal communication) and reach constant weight. Dried feces were ground into powder and 0.040 (± 0.005) g was weighed out for extraction. One ml of 80% methanol was added to powdered samples, vortexed for 30 minutes at 1500 rpm, and centrifuged for 20 minutes at $\sim 2500g$ (Eleftheriou et al. 2020). Supernatants were frozen until analyses. Because corticosterone is the main glucocorticoid in *Peromyscus maniculatus* (e.g., Bradley and Terman 1981), we quantified FCMs using a corticosterone enzyme immunoassay (EIA) after supernatants were diluted (typically 1:80). We followed manufacturer's instructions (Assay Designs, Ann Arbor, Michigan, USA) but also used a reference wavelength of 650 nm. This EIA

has been validated with deermouse feces (Eleftheriou et al. 2020). Intra-assay and inter-assay coefficients of variation were less than 15% and 20%, respectively.

WBC COUNT ANALYSES

In healthy deermice, the most common WBCs in circulation are lymphocytes and neutrophils (Schountz et al. 2014). To evaluate WBCs, we followed methodology by Eleftheriou and Luis (2020). We stained blood smears with Modified Wright stain (Sigma-Aldrich, St. Louis, Missouri, USA) and counted WBCs using light microscopy. Total WBC counts were estimated by counting cells from the feathered edge towards the smear's center for 20 fields at 400X. The mean count of 20 fields was multiplied by 2000 to get an estimate of cells/ μ L. At 1000X, we counted lymphocytes, neutrophils, monocytes, eosinophils and basophils out of 100 WBCs.

SNV ANTIBODY DETECTION

Because deermice never resolve SNV infections, antibodies are a reliable marker of infection (Mills et al. 1999). Therefore, we detected infected deermice by the presence of SNV antibodies in blood samples. We coated 96-well plates with SNV recombinant nucleocapsid antigen and followed an enzyme-linked immunosorbent assay (ELISA) protocol (Schountz et al. 2007) to determine antibody presence.

STATISTICAL ANALYSES

To examine seasonal effects, months were grouped to create a season variable where we assigned October - February to fall/winter, March - May to spring, and June - August to summer. We also grouped juveniles with sub-adults into one group we called non-adults to increase sample size. Minimum number alive (MNA) was used as an index of deermouse and vole densities (*sensu* Krebs 1966) because it works well for small mammals (e.g., Luis et al. 2010). Number of unique captures was used as an index for shrew densities. We used linear and generalized mixed effect regression trees, which use model-based recursive data partitioning, where each tree node is a regression coefficient (Fokkema et al. 2018). When individuals were

sampled more than once, tag number was a random effect. We considered regression trees as appropriate because they can easily handle and identify complex interactions among many potential predictor variables that could be overlooked with traditional statistical approaches that use stepwise variable selection (Strobl et al. 2009). We used linear mixed effect trees for each of these response variables: baseline FCMs, BCSs, N/L ratios, and WBC counts. For scar numbers, we used a generalized linear mixed effect tree with a Poisson error structure (Fokkema et al. 2018). However, for stress response FCMs, we used a generalized linear tree with a Gaussian error structure and no random effects because deermice were sampled only once (Hothorn and Zeileis 2015). The trees included the following predictor variables: deermouse, vole, and shrew densities, trapping grid, season, reproductive status, age, sex, flea presence and SNV infection status. Statistical significance was set to $\alpha = 0.05$. Back transformed means with standard errors are presented. We performed analyses in R (R Core Development Team 2018) within R Studio (RStudio Team 2015), using R package “glmertree” (Fokkema et al. 2018) to build mixed effect regression trees and R package “partykit” (Hothorn and Zeileis 2015) to build generalized linear trees. All response variables except stress response FCMs, BCSs and scar numbers were natural log transformed to meet normality and homoscedasticity assumptions. We excluded any extreme outliers that were identified during this process and noted below whenever this was done.

RESULTS

LIVE-TRAPPING

We captured 289 individual deermice (1028 captures) and 152 individual voles (211 captures). Additionally, we had 131 shrew captures. There was variability in animal densities across space and time (Figure 4.1). Nearly all voles identified to species were meadow voles (~93%, $n = 27$).

STRESS PHYSIOLOGY MEASURES

We only found a temporal effect on baseline FCMs. Deermice had higher FCMs in fall/winter 2017 (12030.13 ± 1503.06 ng/g, $n = 149$, $P < 0.001$) compared to other times ($7547.49 \pm$

1112.90 ng/g, $n = 307$). Initially, only presence of shrews was associated with lower stress response FCMs (19526 ± 2614 ng/g, $n = 61$, $P < 0.001$). However, after removing one extreme outlier (174703.9 ng/g) the more nuanced effect of shrew density was apparent (Figure 4.2). Shrew density $> 4/\text{ha}$ was then associated with lower stress response FCMs (10565 ± 3201 ng/g, $n = 14$, $P = 0.01$). When shrew density was 1- 4/ha, deermice had higher stress response FCMs (21537 ± 3071 ng/g, $n = 49$). However, deermice had even higher stress response FCMs when shrews were absent (31612 ± 3471 ng/g, $n = 67$, $P = 0.04$). In summary, as shrew density increased, stress response FCMs decreased, indicative of chronic stress with a higher shrew density.

Significant predictors for N/L ratio were season, grid, and age (Figure 4.3). N/L ratios were lowest in deermice at grid A (0.42 ± 0.05 , $n = 61$) compared to other grids (0.70 ± 0.10 , $n = 115$, $P = 0.002$), only in fall/winter. In spring/summer, adults had higher N/L ratios (1.43 ± 0.18 , $n = 279$, $P < 0.001$) compared to non-adults (0.69 ± 0.15 , $n = 22$).

Significant predictors for BCS were reproductive status, shrew and vole densities, season, and age (Figure 4.4). Non-reproductive deermice had higher BCSs (3.07 ± 0.05 , $n = 138$, $P < 0.001$) when shrew density was $\leq 1/\text{ha}$. When shrew density was $> 1/\text{ha}$, BCSs were lower (2.79 ± 0.09 , $n = 76$). Reproductive non-adults had higher BCSs (3.18 ± 0.12 , $n = 27$, $P = 0.001$) than reproductive adults. For adults, the presence of voles had a significant effect on BCSs ($P < 0.001$), and was associated with a temporal effect, where deermice had lower BCSs in spring and summer 2018 (2.34 ± 0.08 , $n = 116$), compared to other times (2.64 ± 0.08 , $n = 109$, $P = 0.002$). When voles were absent, higher shrew density ($> 1/\text{ha}$) was associated with lower BCSs (2.28 ± 0.12 , $n = 37$), compared to lower density ($\leq 1/\text{ha}$; 2.92 ± 0.11 , $n = 46$, $P < 0.001$). In summary,

deermice had lower BCSs when heterospecific competitors were present, and reproductive adults had lower BCSs compared to other age/reproductive classes.

IMMUNITY

Significant predictors for WBC counts were grid and season. Deermice at grids A and B had lower WBC counts compared to grids C and D ($P < 0.001$; Figure 4.5), which is most likely a temporal artifact of which grids were trapped together every month. For all grids, WBC counts were higher (A and B: 2239.37 ± 139.00 / μ l, $n = 230$; C and D: 3273.81 ± 340.23 / μ l, $n = 118$) in fall/winter and spring, and lower in summer (A and B: 1511.72 ± 146.38 / μ l, $n = 81$; C and D: 2271.31 ± 295.96 / μ l, $n = 52$, $P = 0.002$). To meet the normality assumption, we had to exclude two outliers that had counts of 100 WBCs each from grids A and B.

SCAR NUMBERS

Significant predictors for scar numbers were season, vole and shrew densities, and reproductive status. Deermice had more scars in spring 2018 and across both summers, compared to spring 2017 and fall/winter 2017 ($P < 0.001$; Figure 4.6). During spring and fall/winter 2017, reproductive deermice had more scars (1.20 ± 0.17 , $n = 94$) than non-reproductive individuals (0.85 ± 0.09 , $n = 115$, $P = 0.04$). In spring 2018 and both summers, intermediate vole density (1-3/ha) was associated with more scars (2.33 ± 0.28 , $n = 96$, $P < 0.001$), compared to when voles were absent or at a higher density (> 3 /ha; 1.59 ± 0.20 , $n = 101$, $P = 0.001$). Shrews only mattered when voles were absent; then, with a shrew density > 1 /ha, deermice had more scars (2.13 ± 0.30 , $n = 45$) than when shrew density was ≤ 1 /ha (1.53 ± 0.22 , $n = 58$, $P = 0.013$). Taken together, the identity of heterospecific competitors was important in how number of scars in deermice were affected.

SNV INFECTION

We detected 23 infected deermice with most of them ($n=21$) from grids where voles were also trapped. All, except for one, were trapped August 2017 - August 2018 (Figure 4.1). Because of a

low number of infected deermice, there was not enough power to perform formal statistical tests for evaluating competition or seasonality effects on SNV prevalence.

DISCUSSION

We showed that two key determinants of SNV transmission, host susceptibility and exposure to infection, were associated with ecological and environmental factors. In particular, we examined how heterospecific competitors and seasonality associated with: (1) stress physiology and immunity (i.e., host susceptibility), and (2) scar numbers (i.e., host exposure to infection) in deermice. Broadly, we found significant associations between both factors and select measures of stress physiology, immunity, and contact rates (summarized in Table 4.1).

Unfortunately, a low number of SNV-infected deermice, made it difficult to evaluate direct relationships between SNV prevalence or transmission with any of our physiological and behavioral measures. This was not surprising given the low deermouse densities at our site during the study. Deermouse density varies widely over space and time and has been shown to be the main driver of SNV prevalence (Luis et al. 2015; 2018). The densities at our field site ranged between 2 and 32 deermice/ha (Figure 4.1), with most hovering around the critical host density needed for SNV to invade (estimated at ~ 17 deermice/ha at another site in Montana; Luis et al 2015). Given the deermouse densities observed, we would expect to see, at most, one infected individual when using the epidemiological model of Luis et al. (2015), which is similar to what we observed (maximum of three infected individuals per any given month). Although deermouse density appears to be the most important driver of SNV prevalence, Luis et al. (2018) showed that species diversity can also affect the SNV transmission rate (for a given deermouse density), with SNV transmission rate higher in communities with more diverse heterospecific competitors. However, Luis et al. (2018) could not discern if this increase in transmission rate was due to changes in host susceptibility or contact rates. Here, we present evidence that

heterospecific competitor densities may induce chronic stress, which could potentially result in suppressed immunity and increased susceptibility to infection. We also present evidence that competitors may potentially alter contact rates, which could impact exposure to infection, as evidenced by changes in scar numbers.

INTERSPECIFIC COMPETITION

We found evidence that heterospecific competitor densities were associated with chronic stress in deermice. Shrews were associated with two stress measures (stress response FCMs and BCSs) and voles were associated with one (BCSs). Although we hypothesized, based on support from the literature, that voles are dominant over deermice, we reasoned that shrews are most likely less dominant given the lack of evidence to suggest otherwise. Thus, we expected shrews to minimally influence deermouse stress physiology, but we found that shrew density, and not vole density, was negatively associated with stress response FCMs, indicative of chronic stress at a higher shrew density. Because shrew and deermouse diets can overlap (i.e., insects, Rychlik and Jancewicz 2002, Witmer and Moulton 2012), exploitative competition may be intense enough to induce chronic stress in deermice. In contrast to our findings, we expected to see an effect of voles on deermouse baseline FCMs because: (1) they are dominant over deermice, and (2) a positive correlation between vole densities and deermouse baseline FCMs has been found previously (Fredebaugh et al. 2013). However, we may have missed an effect because we did not sample frequently enough (e.g., weekly) or the effect was too subtle to detect. The absence of changes in N/L ratios was also unexpected but may have been due to the aforementioned reasons.

Both vole and shrew densities were negatively associated with body condition scores (BCSs) in deermice. BSCs are one measure of chronic stress. However, they could indicate lower food availability; another potential result of interspecific competition. Regardless of the

cause, individuals with lower BCSs may be more susceptible to infection (Beldomenico et al. 2009). We found that the presence of voles, and higher shrew density, were associated with lower BCSs in reproductive adults only. However, shrew density was associated with larger changes (Figure 4.4). This pattern may ensue if reproductive adults compete with voles and shrews relatively more, perhaps due to their higher energetic demands.

Despite associations between heterospecific competitors and stress physiology, we found no association with WBC counts, the immunity measure. The complex and multifaceted relationship between stress physiology and immunity makes it difficult to choose the best suited immune measure that can capture the desired response (Martin 2009). Although relatively cheap and simple to attain, WBC counts may increase in response to infection or inflammation, potentially masking stress-induced reductions (Davis et al. 2008). Nevertheless, our findings suggest that we may need to explore other immunity measures that are more functional in nature, such as lymphocyte proliferation assays, which allow for a real-time immune challenge (Demas et al. 2011).

We also found that vole and shrew densities were differentially associated with deer mouse scar numbers, primarily during the breeding period. Interestingly, the relationship between vole density and scar numbers was non-monotonic. Deer mice had the most scars at intermediate vole densities (between 1 and 3/ha), with fewer scars either when voles were absent or present at densities $> 3/\text{ha}$ (Figure 4.6). However, at higher shrew densities ($>1/\text{ha}$), deer mice had more scars than when shrews were absent. Although we did not have clear *a priori* predictions on how contact rates might change in response to interspecific competition, our findings suggest that deer mice may change their behavior based on what competitor is present. Such behavioral changes were reported by Clay et al. (2009) in deer mice from Utah, USA, at

sites with high small mammal diversity, although Rubio et al. (2017) found no change in deer mice kept in outdoor enclosures with dominant Merriam's kangaroo rats (*Dipodomys merriami*). We acknowledge that using scar numbers as a proxy for contact rates has limitations (e.g., wounds healed with no scars), so our findings related to this measure need to be evaluated with caution. Thus, controlled experiments where technology is used to better estimate contact rates are needed (e.g., Dearing et al. 2015).

In summary, our findings suggest that identity of competitor species may matter in how transmission is affected because deer mouse physiology and behavior responded differently to vole and shrew densities. Studies of other disease systems have demonstrated the importance of species identity in affecting disease transmission in the focal host, such as with Lyme disease (vector-borne transmission, LioGuidice et al. 2003) and chytridiomycosis (environmental transmission, Venesky et al. 2013). However, our study is one of the few to examine consequences of competitor species on both physiological and behavioral traits of the host that have implications for transmission of directly-transmitted diseases. Given our results, it is crucial that we continue to test how heterospecific competitors can differentially affect host physiology and behavior so we can improve how we understand and predict disease dynamics across diverse communities.

SEASONALITY

There was no clear seasonality (consistent between years) in baseline or stress response FCMs. Although seasonal variation in photoperiod necessarily affected how long deer mice were in overnight confinement, this did not appear to be influential for stress response FCMs because season was not selected as a predictor (Figure 4.2). Our findings are not entirely surprising because a review by Romero (2002) found no consistent seasonal patterns in baseline or stress-induced FCMs (i.e., after trap confinement) in mammals.

We found seasonality in N/L ratios, where deermice had higher N/L ratios in spring/summer compared to fall/winter. Seasonal environmental factors that influence GCs may also affect N/L ratios, although these two measures may evaluate the stress response differently across time (Davis et al. 2008, Goessling et al. 2015). However, inflammation and infection may also affect N/L ratios (Davis et al. 2008). Given that deermice were not tested for infection other than with SNV, we cannot discern if the seasonality observed was explicitly due to GCs. However, given the large sample size and consistent pattern across years, seasonality in stress physiology is a more parsimonious explanation. It is noteworthy that seasonal variation in photoperiod necessitated that time in confinement also varied by season. Although this may have influenced the findings, seasonality in N/L ratios (or heterophil to lymphocyte ratio in birds and reptiles) has been observed in other vertebrates (e.g., Norte et al. 2009, Goessling et al. 2016). In contrast to N/L ratios, we did not find clear seasonality in BCSs, despite seasonal changes in body condition occurring in other vertebrates (e.g., Milenkaya et al. 2013, Pokharel et al. 2017).

There was clear seasonality in the immunity measure, WBC counts, which were higher in fall/winter and spring, but lower in summer. Similar to N/L ratios, inflammation or infection may also influence WBC counts and possibly time in confinement as well (Davis et al. 2008). However, evidence consistent with our field data from previous studies of small mammals, including deermice, propose that seasonality is the most likely parsimonious explanation for our findings (Blom et al. 1994, Martin et al. 2008).

Aggressive encounters between deermice that lead to scars are associated with breeding and typically occur in spring/summer (Bagamian et al. 2012). Similar changes in scarring from intraspecific fights occur seasonally in other vertebrates (Christian 1970, Woodroffe and Macdonald 1995). Such seasonal changes in aggressive encounters can result from changes in

home range dictated by reproduction and food availability (e.g., Perelberg et al. 2003). For the most part, our study confirms this pattern. We also observed that deermice had more scars across summers and spring 2018 compared to fall/winter. However, lower scar numbers in spring 2017 did not fit this pattern. Perhaps food availability, which we did not measure, was greater in spring 2017, which may have led to fewer aggressive encounters.

Taken together, higher N/L ratios in spring/summer, lower WBC counts in summer, and more scar numbers in summer, may provide valuable insight as to why previous studies in Montana found that SNV transmission is higher in spring/summer. However, experimental studies would be needed to causally link these findings to SNV transmission in deermice.

CONCLUSIONS

Overall, we found evidence that heterospecific competitors and seasonality were associated with select physiological and behavioral measures in deermice. However, we found no effects of deermouse density, which was surprising given that density has been associated with stress physiology and intraspecific contact rates in rodents (Harper and Austad 2004, Ostfeld and Keesing 2019). Similarly, there were no effects from flea infestation or SNV infection (although we very likely lacked power to detect an effect). Absence of these associations suggests that neither deermouse density nor parasitism were as important as heterospecific competitors and seasonality in affecting physiological and behavioral measures. However, if deermouse density were high enough for a long enough duration to sustain SNV transmission and increase infection, perhaps those associations could manifest. We should note that because we did not measure temporal or spatial heterogeneity in vegetation cover across grids, we cannot ascertain its consequences on physiological and behavioral measures. However, because grid was not a significant predictor in most models, it seems likely that vegetation cover itself did not play a widely influential role.

The mechanistic approach of our study can inform other disease ecology studies that seek to identify potential physiological and behavioral links between extrinsic factors and disease transmission. Going forward, conducting controlled experiments in outdoor enclosures where host densities are held constant will allow for more robust evaluations of the effects of interspecific competition and seasonality on stress physiology, immunity and intraspecific contact rates in our system and other directly-transmitted disease systems.

ACKNOWLEDGMENTS

The authors thank Montana Fish, Wildlife and Parks for land access, the numerous students that assisted in the field and laboratory, T. Schountz for SNV antigen, C. Breuner, M. Minnick and D. Pearson for advice, J. del Sol for plant surveys and identification. Funding was provided from University of Montana Graduate School and Wildlife Biology program (George and Mildred Cirica Scholarship, Mary Jane Landt Memorial Scholarship), Montana Institute on Ecosystems, Montana Chapter of The Wildlife Society, Montana Academy of Sciences, Society of Integrative and Comparative Biology and National Science Foundation under Grant No. 1836793.

LITERATURE CITED

Altizer, S., A. Dobson, P. Hosseini, P. Hudson, M. Pascual, and P. Rohani. 2006. Seasonality and the dynamics of infectious diseases. *Ecology Letters* 9:467-484.

Altizer, S., W. M. Hochachka, and A. A. Dhondt. 2004. Seasonal dynamics of mycoplasmal conjunctivitis in eastern North American house finches. *Journal of Animal Ecology* 73:309-322.

Bagamian, K. H., R. J. Douglass, A. Alvarado, A. J. Kuenzi, B. R. Amman, L. A. Waller, and J. N. Mills. 2012. Population density and seasonality effects on Sin Nombre virus transmission in North American deermice (*Peromyscus maniculatus*) in outdoor enclosures. *PloS one* 7:e37254.

- Beldomenico, P. M., S. Telfer, L. Lukomski, S. Gebert, M. Bennett, and M. Begon. 2009. Host condition and individual risk of cowpox virus infection in natural animal populations: cause or effect? *Epidemiology & Infection* 137:1295-1301.
- Blom, J. M., J. M. Gerber, and R. J. Nelson. 1994. Day length affects immune cell numbers in deer mice: interactions with age, sex, and prenatal photoperiod. *The American Journal of Physiology* 267:R596-601.
- Bradley, E. L., and C. R. Terman. 1981. A comparison of the adrenal histology, reproductive condition, and serum corticosterone concentrations of prairie deermice (*Peromyscus maniculatus bairdii*) in captivity. *Journal of Mammalogy* 62:353-361.
- Breuner, C. W., B. Delehanty, and R. Boonstra. 2013. Evaluating stress in natural populations of vertebrates: total CORT is not good enough. *Functional Ecology* 27:24-36.
- Busch, D. S., and L. S. Hayward. 2009. Stress in a conservation context: a discussion of glucocorticoid actions and how levels change with conservation-relevant variables. *Biological Conservation* 142:2844-2853.
- Carson, S., H. Welsh, and B. Murray. 2006. Ninepipes Wildlife Management Area. Small Mammal Trapping and Species Inventory. Montana Fish, Wildlife, and Parks, Montana, USA.
- Childs, J. E., T. G. Ksiazek, C. F. Spiropoulou, J. W. Krebs, S. Morzunov, G. O. Maupin, K. L. Gage, P. E. Rollin, J. Sarisky, and R. E. Enscore. 1994. Serologic and genetic identification of *Peromyscus maniculatus* as the primary rodent reservoir for a new hantavirus in the southwestern United States. *The Journal of Infectious Diseases* 169:1271-1280.
- Christian, J. J. 1970. Social subordination, population density, and mammalian evolution. *Science* 168:84-90.

- Clay, C. A., E. M. Lehmer, S. S. Jeor, and M. D. Dearing. 2009. Testing mechanisms of the dilution effect: deer mice encounter rates, Sin Nombre virus prevalence and species diversity. *EcoHealth* 6:250-259.
- Dantzer, B., Q. E. Fletcher, R. Boonstra, and M. J. Sheriff. 2014. Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species? *Conservation Physiology* 2:cou023.
- Daszak, P., L. Berger, A. A. Cunningham, A. D. Hyatt, D. E. Green, and R. Speare. 1999. Emerging infectious diseases and amphibian population declines. *Emerging infectious diseases* 5:735-748.
- Davis, A., D. Maney, and J. Maerz. 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Functional Ecology* 22:760-772.
- Dearing, M. D., C. Clay, E. Lehmer, and L. Dizney. 2015. The roles of community diversity and contact rates on pathogen prevalence. *Journal of Mammalogy* 96:29-36.
- Demas, G. E., D. A. Zysling, B. R. Beechler, M. P. Muehlenbein, and S. S. French. 2011. Beyond phytohaemagglutinin: assessing vertebrate immune function across ecological contexts. *Journal of Animal Ecology* 80:710-730.
- Dickens, M. J., and L. M. Romero. 2013. A consensus endocrine profile for chronically stressed wild animals does not exist. *General and Comparative Endocrinology* 191:177-189.
- Douglass, R. J., T. Wilson, W. J. Semmens, S. N. Zanto, C. W. Bond, R. C. Van Horn, and J. N. Mills. 2001. Longitudinal studies of Sin Nombre virus in deer mouse-dominated ecosystems of Montana. *The American Journal of Tropical Medicine and Hygiene* 65:33-41.
- Eleftheriou, A. and A.D. Luis. 2020. Leukocyte Evaluation of the Free-Ranging Deermouse (*Peromyscus maniculatus*) from Montana, USA. *Journal of Wildlife Diseases* 56: 717-720.

- Eleftheriou, A., R. Palme, and R. Boonstra. 2020. Assessment of the Stress Response in North American Deermice: Laboratory and Field Validation of Two Enzyme Immunoassays for Fecal Corticosterone Metabolites. *Animals* 10:1120.
- Escutenaire, S., P. Chalon, F. De Jaegere, L. Karelle-Bui, G. Mees, B. Brochier, F. Rozenfeld, and P. P. Pastoret. 2002. Behavioral, physiologic, and habitat influences on the dynamics of Puumala virus infection in bank voles (*Clethrionomys glareolus*). *Emerging infectious diseases* 8:930-936.
- Fairbairn, D. J. 1977. The spring decline in deer mice: death or dispersal? *Canadian Journal of Zoology* 55: 84-92.
- Fokkema, M., N. Smits, A. Zeileis, T. Hothorn, and H. Kelderman. 2018. Detecting treatment-subgroup interactions in clustered data with generalized linear mixed-effects model trees. *Behavior Research Methods* 50:2016-2034.
- Fredebaugh-Siller, S., C. Suski, Z. Zuckerman, and R. Schooley. 2013. Ecological correlates of stress for a habitat generalist in a biofuels landscape. *Canadian Journal of Zoology* 91:853-858.
- Glass, G. E., and N. A. Slade. 1980. The effect of *Sigmodon hispidus* on spatial and temporal activity of *Microtus ochrogaster*: evidence for competition. *Ecology* 61:358-370.
- Goessling, J. M., C. Guyer, and M. T. Mendonça. 2016. Seasonal acclimation of constitutive immunity in gopher tortoises *Gopherus polyphemus*. *Physiological and Biochemical Zoology* 89:487-497.
- Goessling, J. M., H. Kennedy, M. T. Mendonça, and A. E. Wilson. 2015. A meta-analysis of plasma corticosterone and heterophil: lymphocyte ratios—is there conservation of physiological stress responses over time? *Functional Ecology* 29:1189-1196.

- Grant, P. 1971. Experimental studies of competitive interaction in a two-species system. *The Journal of Animal Ecology* 40:323-350.
- Gutman, R., and T. Dayan. 2005. Temporal partitioning: an experiment with two species of spiny mice. *Ecology* 86:164-173.
- Hall, E.R. and K.R. Kelson. 1959. *The mammals of North America*. Ronald Press, New York, USA.
- Harper, J. M., and S. N. Austad. 2004. Fecal corticosteroid levels in free-living populations of deer mice (*Peromyscus maniculatus*) and southern red-backed voles (*Clethrionomys gapperi*). *The American Midland Naturalist* 152:400-409.
- Harper, J. M., and S. N. Austad. 2001. Effect of capture and season on fecal glucocorticoid levels in deer mice (*Peromyscus maniculatus*) and red-backed voles (*Clethrionomys gapperi*). *General and Comparative Endocrinology* 123:337-344.
- Hawley, D. M., R. S. Etienne, V. O. Ezenwa, and A. E. Jolles. 2011. Does animal behavior underlie covariation between hosts' exposure to infectious agents and susceptibility to infection? Implications for disease dynamics. *Integrative & Comparative Biology* 51: 528-539.
- Hothorn, T. and A. Zeileis. 2015. partykit: A modular toolkit for recursive partytioning in R. - *The Journal of Machine Learning Research* 16: 3905-3909.
- Johnson, P. T., R. S. Ostfeld, and F. Keesing. 2015. Frontiers in research on biodiversity and disease. *Ecology Letters* 18:1119-1133.
- Keesing, F., R. D. Holt, and R. S. Ostfeld. 2006. Effects of species diversity on disease risk. *Ecology Letters* 9:485-498.
- Khalil, H., F. Ecke, M. Evander, M. Magnusson, and B. Hörnfeldt. 2016. Declining ecosystem health and the dilution effect. *Scientific reports* 6:31314.

- Krebs, C. J. 1966. Demographic changes in fluctuating populations of *Microtus californicus*. Ecological Monographs 36:239-273.
- Kuenzi, A. J., R. J. Douglass, D. White Jr, C. W. Bond, and J. N. Mills. 2001. Antibody to Sin Nombre virus in rodents associated with peridomestic habitats in west central Montana. The American Journal of Tropical Medicine and Hygiene 64:137-146.
- LoGiudice, K., R. S. Ostfeld, K. A. Schmidt, and F. Keesing. 2003. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. Proceedings of the National Academy of Sciences of the United States of America 100:567-571.
- Luis, A. D., R. J. Douglass, J. N. Mills, and O. N. Bjørnstad. 2015. Environmental fluctuations lead to predictability in Sin Nombre hantavirus outbreaks. Ecology 96:1691-1701.
- Luis, A. D., R. J. Douglass, J. N. Mills, and O. N. Bjørnstad. 2010. The effect of seasonality, density and climate on the population dynamics of Montana deer mice, important reservoir hosts for Sin Nombre hantavirus. Journal of Animal Ecology 79:462-470.
- Luis, A. D., A. J. Kuenzi, and J. N. Mills. 2018. Species diversity concurrently dilutes and amplifies transmission in a zoonotic host-pathogen system through competing mechanisms. Proceedings of the National Academy of Sciences of the United States of America 115:7979-7984.
- Martin, L. B. 2009. Stress and immunity in wild vertebrates: timing is everything. General and Comparative Endocrinology 163:70-76.
- Martin, L. B., Z. M. Weil, and R. J. Nelson. 2008. Seasonal changes in vertebrate immune activity: mediation by physiological trade-offs. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 363:321-339.

- Milenkaya, O., N. Weinstein, S. Legge, and J. R. Walters. 2013. Variation in body condition indices of crimson finches by sex, breeding stage, age, time of day, and year. *Conservation physiology* 1: cot020.
- Mills, J. N., B. R. Amman, and G. E. Glass. 2010. Ecology of hantaviruses and their hosts in North America. *Vector-Borne and Zoonotic Diseases* 10:563-574.
- Mills, J. N., T. G. Ksiazek, C. J. Peters, and J. E. Childs. 1999. Long-term studies of hantavirus reservoir populations in the southwestern United States: a synthesis. *Emerging infectious diseases* 5:135-142.
- Mills, J. N., T. L. Yates, J. E. Childs, R. R. Parmenter, T. G. Ksiazek, P. E. Rollin, and C. Peters. 1995. Guidelines for working with rodents potentially infected with hantavirus. *Journal of mammalogy* 76:716-722.
- Narayan, E. J., T. S. Jessop, and J. Hero. 2015. Invasive cane toad triggers chronic physiological stress and decreased reproductive success in an island endemic. *Functional Ecology* 29:1435-1444.
- Nelson, R. J., and G. E. Demas. 1996. Seasonal changes in immune function. *The Quarterly Review of Biology* 71:511-548.
- Norte, A., J. Ramos, J. Sousa, and B. Sheldon. 2009. Variation of adult great tit *Parus major* body condition and blood parameters in relation to sex, age, year and season. *Journal of Ornithology* 150:651.
- Ostfeld, J. K., and F. Keesing. 2019. Impacts of large mammals on movements of the pouched mouse (*Saccostomus mearnsi*) in central Kenya. *African Journal of Ecology* 57:2-9.
- Palme, R. 2019. Non-invasive measurement of glucocorticoids: Advances and problems. *Physiology & Behavior* 199:229-243

- Perelberg, A., D. Saltz, S. Bar-David, A. Dolev, and Y. Yom-Tov. 2003. Seasonal and circadian changes in the home ranges of reintroduced Persian fallow deer. *The Journal of Wildlife Management* 67:485-492.
- Pokharel, S. S., P. B. Seshagiri, and R. Sukumar. 2017. Assessment of season-dependent body condition scores in relation to faecal glucocorticoid metabolites in free-ranging Asian elephants. *Conservation Physiology* 5: cox039.
- R Core Team. 2018. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria.
- RStudio Team. 2015. RStudio: Integrated Development for R; RStudio, Inc.: Boston, MA, USA.
- Reeder, D. M., and K. M. Kramer. 2005. Stress in free-ranging mammals: integrating physiology, ecology, and natural history. *Journal of Mammalogy* 86:225-235.
- Romero, L. M. 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *General and Comparative Endocrinology* 128:1-24.
- Romero, L. M., C. J. Meister, N. E. Cyr, G. Kenagy, and J. C. Wingfield. 2008. Seasonal glucocorticoid responses to capture in wild free-living mammals. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 294: R614-R622.
- Rubio, A. V., I. Castro-Arellano, J. N. Mills, R. List, R. Ávila-Flores, and G. Suzán. 2017. Is species richness driving intra-and interspecific interactions and temporal activity overlap of a hantavirus host? An experimental test. *PloS one* 12:e0188060.
- Rychlik, L., and E. Jancewicz. 2002. Prey size, prey nutrition, and food handling by shrews of different body sizes. *Behavioral Ecology* 13:216-223.

Santicchia, F., B. Dantzer, F. van Kesteren, R. Palme, A. Martinoli, N. Ferrari, and L. A. Wauters. 2018. Stress in biological invasions: Introduced invasive grey squirrels increase physiological stress in native Eurasian red squirrels. *Journal of Animal Ecology* 87:1342-1352.

Sapolsky, R. M., L. M. Romero, and A. U. Munck. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions 1. *Endocrine reviews* 21:55-89.

Schountz, T., S. Quackenbush, J. Rovnak, E. Haddock, W. C. Black 4th, H. Feldmann, and J. Prescott. 2014. Differential lymphocyte and antibody responses in deer mice infected with Sin Nombre hantavirus or Andes hantavirus. *Journal of Virology* 88:8319-8331.

Schountz, T., C. H. Calisher, T. R. Richens, A. A. Rich, J. B. Doty, M. T. Hughes, and B. J. Beaty. 2007. Rapid field immunoassay for detecting antibody to Sin Nombre virus in deer mice. *Emerging infectious diseases* 13:1604-1607.

Smith, K. F., D. F. Sax, and K. D. Lafferty. 2006. Evidence for the role of infectious disease in species extinction and endangerment. *Conservation Biology* 20:1349-1357.

Strobl, C., J. Malley, and G. Tutz. 2009. An introduction to recursive partitioning: rationale, application, and characteristics of classification and regression trees, bagging, and random forests. *Psychological methods* 14:323-348.

Suzán, G., E. Marce, J. T. Giermakowski, J. N. Mills, G. Ceballos, R. S. Ostfeld, B. Armien, J. M. Pascale, and T. L. Yates. 2009. Experimental evidence for reduced rodent diversity causing increased hantavirus prevalence. *PloS one* 4: e5461.

Ullman-Culleré, M. H., and C. J. Foltz. 1999. Body condition scoring: a rapid and accurate method for assessing health status in mice. *Comparative Medicine* 49:319-323.

- Venesky, M. D., X. Liu, E. L. Sauer, and J. R. Rohr. 2014. Linking manipulative experiments to field data to test the dilution effect. *Journal of Animal Ecology* 83:557-565.
- Voutilainen, L., E. R. Kallio, J. Niemimaa, O. Vapalahti, and H. Henttonen. 2016. Temporal dynamics of Puumala hantavirus infection in cyclic populations of bank voles. *Scientific Reports* 6:1-15.
- Warner, B. M., D. R. Stein, B. D. Griffin, K. Tierney, A. Leung, A. Sloan, D. Kobasa, G. Poliquin, G. P. Kobinger, and D. Safronetz. 2019. Development and Characterization of a Sin Nombre Virus Transmission Model in *Peromyscus maniculatus*. *Viruses* 11:183.
- Williams, E., T. Yuill, M. Artois, J. Fischer, and S. Haigh. 2002. Emerging infectious diseases in wildlife. *Revue Scientifique et Technique-Office International des Epizooties* 21:139-158.
- Witmer, G. W. and R. S. Moulton. 2012. Deer mice (*Peromyscus* spp.) biology, damage and management: a review. Pages 213-219 in Robert M. Timm, editor. *Proceedings of the 25th Vertebrate Pest Conference*, University of California, Davis, California, USA.
- Woodroffe, R. and D. Macdonald. 1995. Costs of breeding status in the European badger, *Meles meles*. *Journal of Zoology* 235: 237-245.

Table 4. 1 List of hypotheses for how heterospecific competitors and seasonality will associate with stress physiology, immunity, and contact rates of deermice, with corresponding predictions and empirical support for each measured variable. Seasonality hypotheses received support if there was consistency across both years.

Hypothesis		Prediction	Support?
Heterospecific competitors (voles, and to a lesser extent, shrews)	↑ stress	↑ Baseline FCMs † ↓ Stress response FCMs † ↑ N/L ratio ‡ ↓ BCS §	No Yes (shrews) No Yes (both)
	↓ immunity	↓ WBC counts ¶	No
	- or Δ contacts	- or Δ scar numbers	Yes Δ (both)
Spring/summer seasons	↑ stress	↑ Baseline FCMs † ↓ Stress response FCMs † ↑ N/L ratio ‡ ↓ BCS §	No No Yes (both) No
	↓ immunity	↓ WBC counts ¶	Yes (summer)
	↑ contacts	↑ scar numbers	Yes (summer)

† Fecal corticosterone metabolites

‡ Neutrophil/lymphocyte ratio

§ Body condition score

¶ White blood cell counts

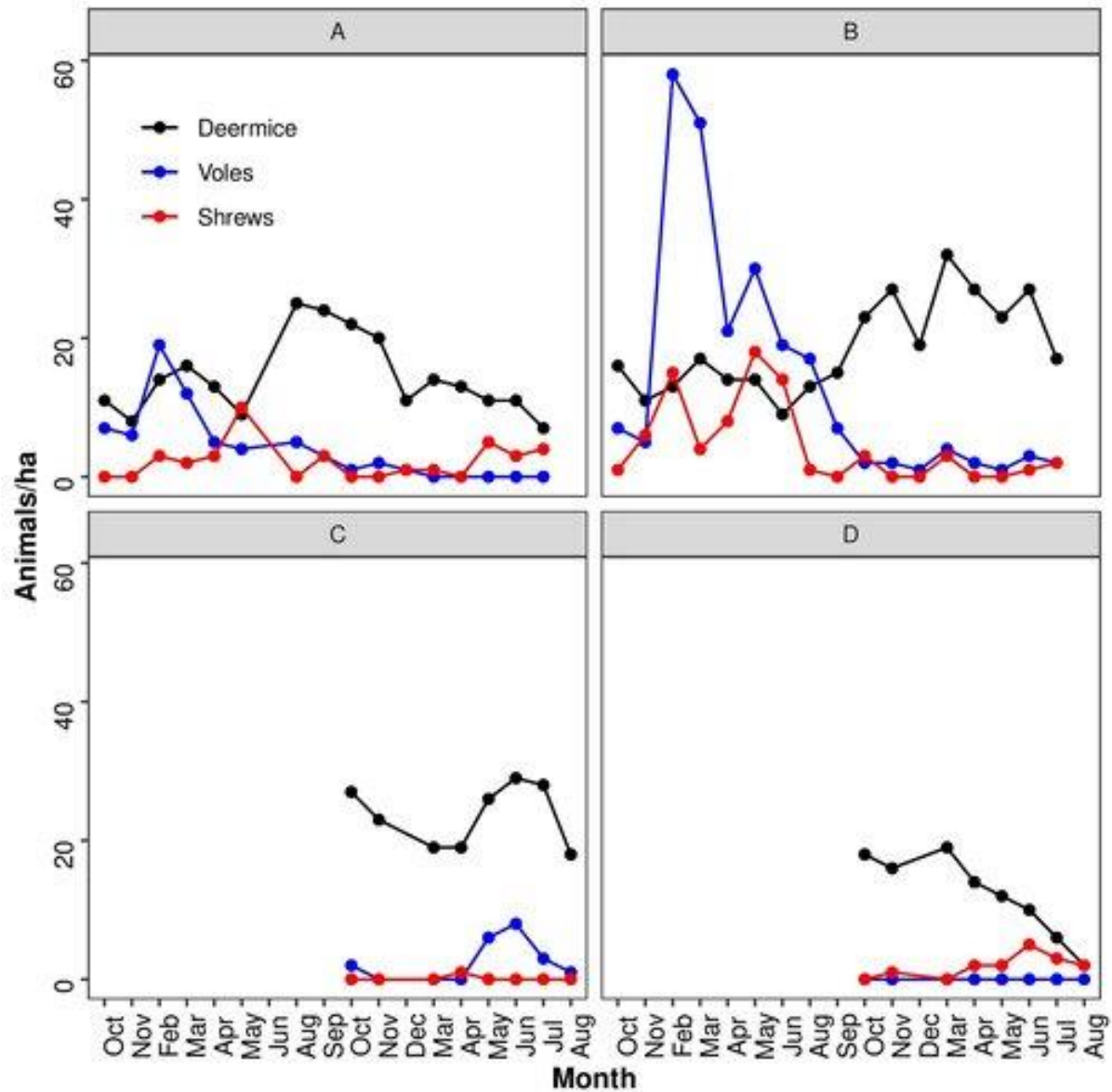


Figure 4. 1 Small mammal densities for each grid (A-D) across study period (October 2016 to August 2018). Deermouse and vole densities were estimated using Minimum Number Alive (MNA) index. Shrew densities were estimated using unique captures per trapping session.

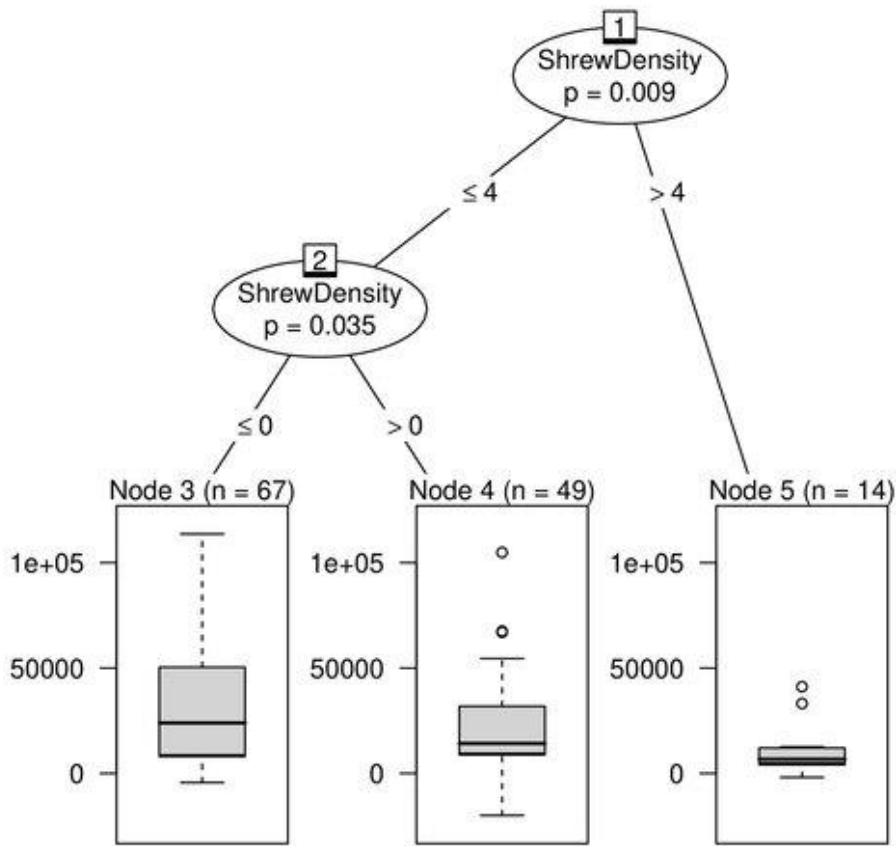


Figure 4. 2 Regression tree for stress response fecal corticosterone metabolites (FCMs) of deermice. Each terminal node provides a sample size with a boxplot for that subgroup. Density is per hectare. Statistical significance was set to 0.05.

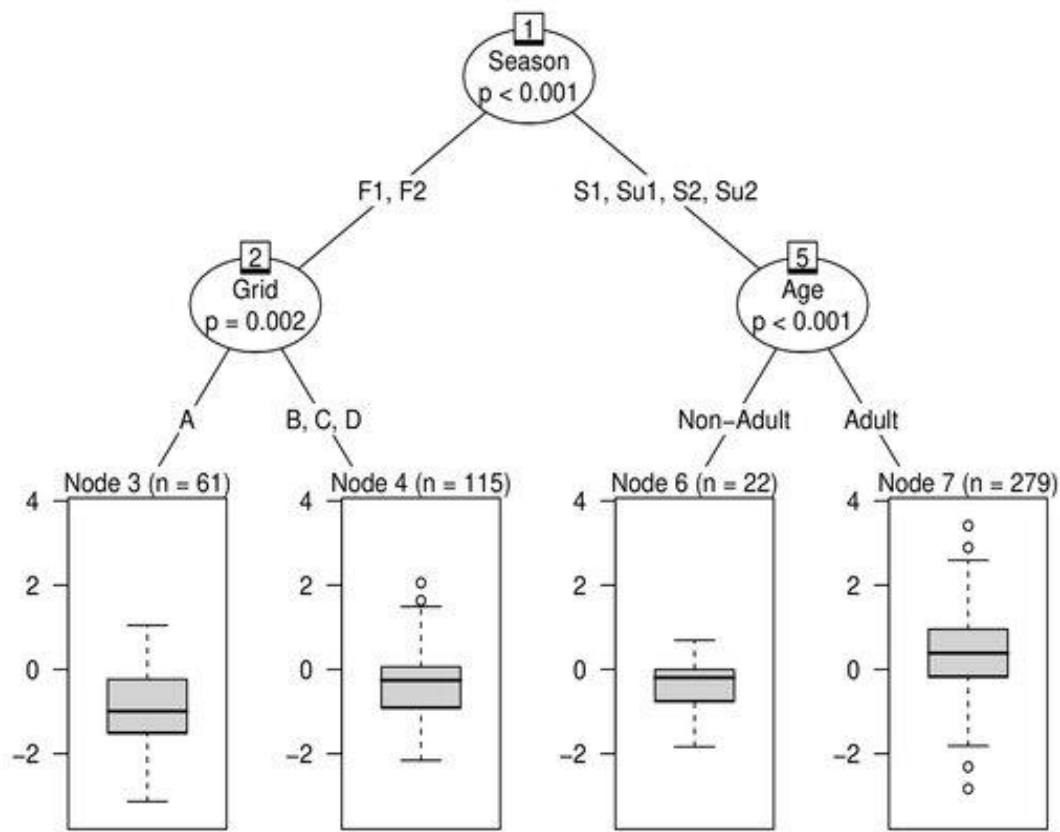


Figure 4. 3 Regression tree for ln neutrophil to lymphocyte (N/L) ratio of deermice. Each terminal node provides a sample size with a boxplot for that subgroup. Grids A and B were trapped October'16 – July'18. Grids C and D were trapped October'17 – August'18. Juveniles and subadults were grouped into “Non-Adult”. F1, S1, Su1, F2, S2, and Su2 represent Fall/Winter'16, Spring'17, Summer'17, Fall/Winter'17, Spring'18, and Summer'18, respectively. Statistical significance was set to 0.05.

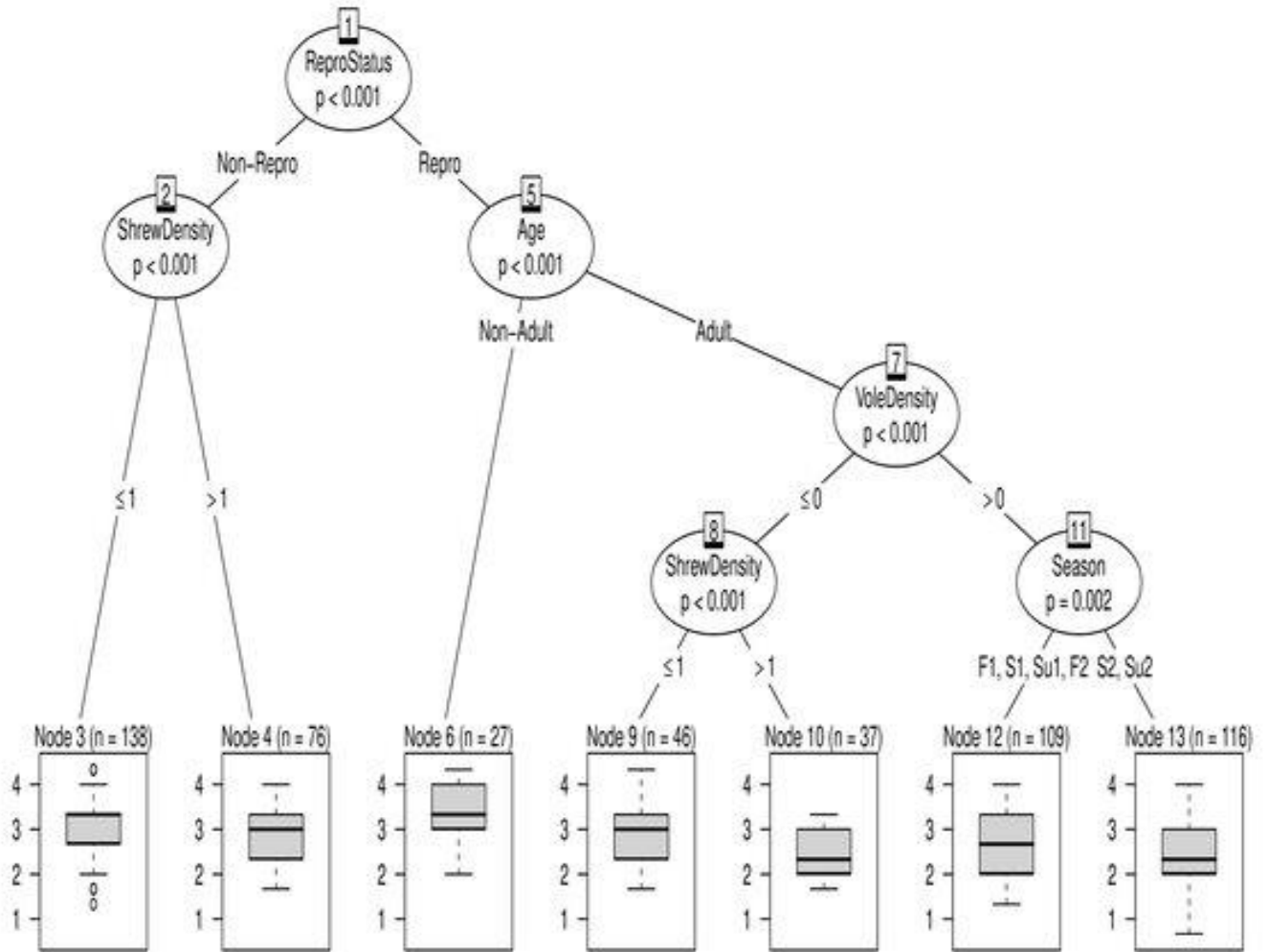


Figure 4. 4 Regression tree for body condition score (BCS) of deermice. Each terminal node provides a sample size with a boxplot for that subgroup. “ReproStatus” stands for reproductive status. Juveniles and subadults were grouped together into “Non-Adult”. F1, S1, Su1, F2, S2, and Su2 represent Fall/Winter’16, Spring’17, Summer’17, Fall/Winter’17, Spring’18, and Summer’18, respectively. Density is per hectare. Statistical significance was set to 0.05.

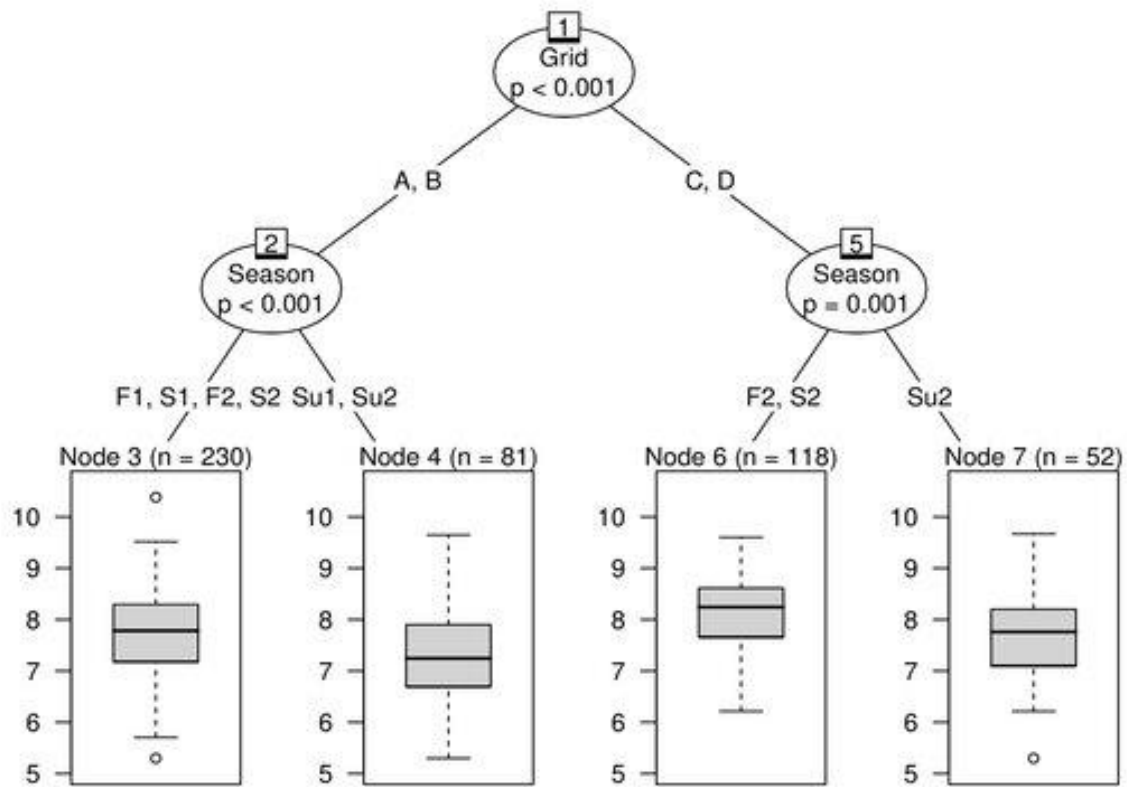


Figure 4. 5 Regression tree of white blood cell (WBC) counts of deermice. Each terminal node provides a sample size with a boxplot for that subgroup. Grids A and B were trapped October'16 – July'18. Grids C and D were trapped October'17 – August'18. F1, S1, Su1, F2, S2, and Su2 represent Fall/Winter'16, Spring'17, Summer'17, Fall/Winter'17, Spring'18, and Summer'18, respectively. Statistical significance was set to 0.05.

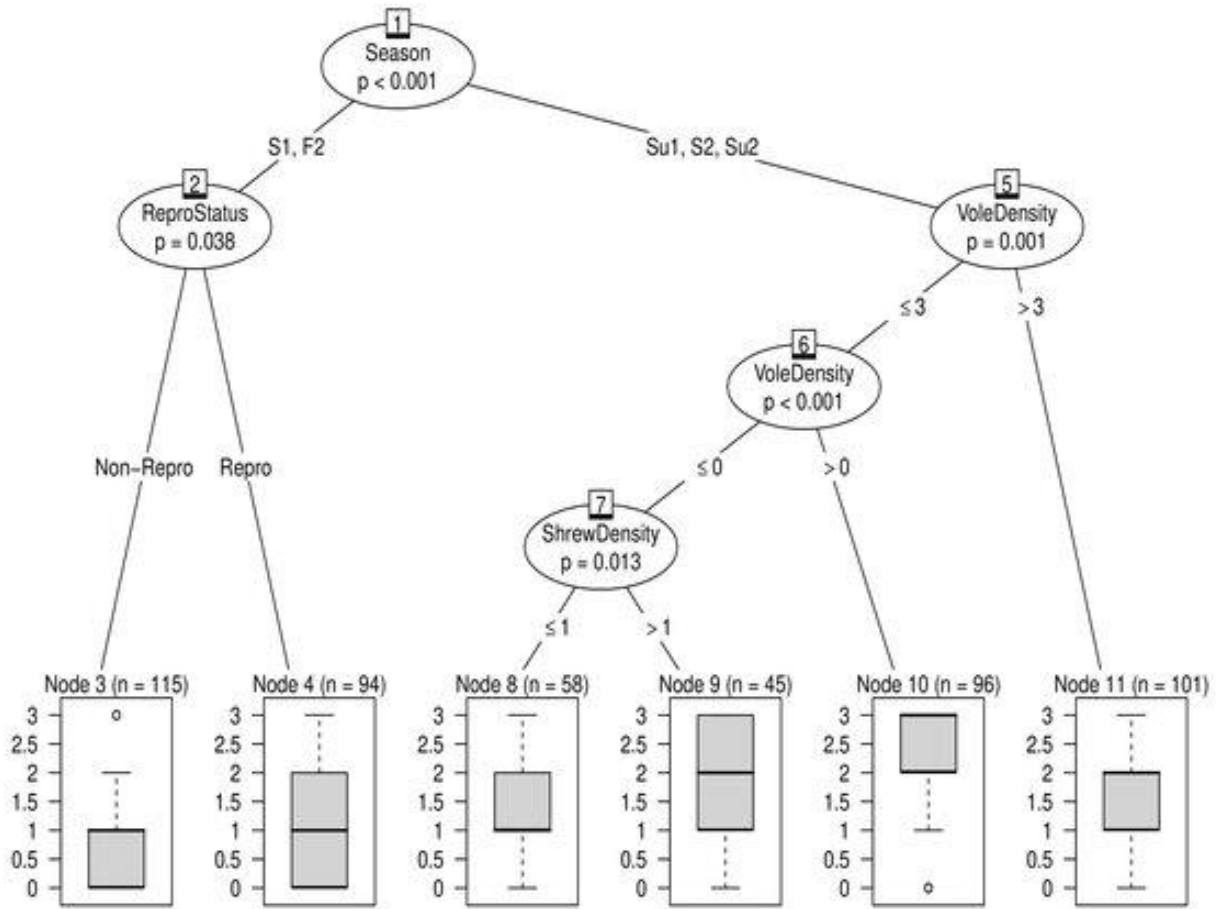


Figure 4. 6 Regression tree for scar numbers of deermice. Each terminal node provides a sample size with a boxplot for that subgroup. Density is per hectare. “ReproStatus” stands for reproductive status. S1, F2, Su1, S2, and Su2 represent Spring’17, Fall/Winter’17, Summer’17, Spring’18, and Summer’18, respectively. Statistical significance was set to 0.05.

SUPPLEMENTARY INFORMATION

Grid descriptions: Most common grasses at grid A were intermediate wheatgrass (*Agropyron intermedium*) and smooth brome (*Bromus* sp.), at grid B, slender wheatgrass (*Elymus trachycaulus*) and timothy grass (*Phleum pratense*), at grid C, smooth brome and timothy grass, and at grid D, intermediate wheatgrass and wild oat (*Avena fatua*). Grid B had the most forbs including mustards (*Brassica* spp.), thistles (*Cirsium* spp.) and whitetop (*Lepidium draba*), and grid D the least.

CHAPTER 5 INTERSPECIFIC COMPETITION CAN LEAD TO NON-MONOTONIC DISEASE OUTCOMES: A THEORETICAL INVESTIGATION WITH A DIRECTLY-TRANSMITTED ZONOSIS

ABSTRACT

The “dilution effect” and “amplification effect” hypotheses predict negative and positive monotonic relationships, respectively, between biodiversity and disease. However, non-monotonic patterns have also received support, and community composition, such as heterospecific competitors, has been argued to be more important than biodiversity *per se*. Because underlying mechanisms of dilution and amplification have been studied less with directly-transmitted disease systems, here, we theoretically investigated how a heterospecific competitor could affect disease outcomes via disparate mechanisms in such a system. To mimic a natural system, we used realistic parameters for Sin Nombre virus, hosted by the North American deer mouse (*Peromyscus maniculatus*), with the vole (*Microtus* spp.) serving as the competitor. Because such competitors could affect host density, behavior, and physiological stress, we used a mathematical model with density-dependent transmission, to explore how competitor density could influence disease prevalence through these transmission mechanisms (either singly or combined). Our results indicated that, although dilution was more commonly predicted than amplification, a non-monotonic relationship (increase then decrease), was the most commonly predicted outcome in the parameter space we examined. Taken together, our findings broadly highlight that heterospecific competitors could, via competing mechanisms, generate non-monotonic disease outcomes within the same system, which could indeed be common and subject to misinterpretation in natural systems.

INTRODUCTION

Globally, species diversity has been declining, while emerging infectious diseases of humans and animals have been increasing (Daszak et al. 2000; Jones et al. 2008; Cardinale et al. 2012). To explain this pattern, the “dilution effect” hypothesis proposes that as species diversity decreases, infectious disease risk increases because diversity “dilutes” disease (Keesing et al. 2006). Although most empirical evidence suggests that the dilution effect is prevalent in nature (Civitello et al. 2015), many have argued against its generality across disease systems (Randolph & Dobson 2012; Wood et al. 2014). This is partially because of evidence supporting the “amplification effect” hypothesis, which proposes that as diversity decreases, disease risk also decreases (Keesing et al. 2006; Salkeld et al. 2013). However, community composition (e.g., heterospecific competitors), and not diversity *per se*, has been argued to be more relevant (Randolph & Dobson 2012; Johnson et al. 2015), a concept termed the “identity effect” (Huang et al. 2016).

Regardless of whether species diversity or community composition is considered, there has been more attention given towards the shapes of the patterns present (i.e. dilution versus amplification), instead of the underlying mechanisms that dictate those patterns. Such critical mechanistic work has been performed predominantly with vector-borne diseases, such as Lyme disease (e.g., Ostfeld & Keesing 2000), and environmentally-transmitted diseases, such as chytridiomycosis (e.g., Venesky et al. 2014), with directly-transmitted diseases receiving comparatively less attention (Luis et al. 2018). If we can broadly document how key mechanistic drivers interact together to govern the net disease outcome, we can better understand the shapes of the outcomes observed in natural systems (Wood et al. 2016; Halliday & Rohr 2019; Rohr et al. 2020). Consequently, we will be better equipped to predict the shapes of disease outcomes across host-parasite systems and environmental contexts.

Based on epidemiological theory, diversity can affect prevalence of a directly-transmitted disease, with one primary reservoir host, via three key mechanisms: intraspecific contact rates, probability of transmission given contact (hereafter probability of transmission), and infectious period, which is determined by infected host mortality and recovery (Keesing et al. 2006). The transmission rate of a disease, commonly referred to as β , is the product of contact rates, β_c (behavioral component), and probability of transmission, β_p (physiological component), for a given host density (Hawley et al. 2011). With density-dependent transmission, intraspecific contact rates are expected to increase directly with host density, N , that is the true contact rate is equal to $\beta_c N$, where β_c is a constant (Begon et al. 2002). Therefore, when diversity affects host density, it will indirectly affect intraspecific contact rates. For diversity to directly affect the true contact rate, it needs to do so via β_c , independent of host density, N (e.g. via causing hosts to behaviorally aggregate in refugia). Because β_c and β_p are difficult to estimate from empirical data (Hawley et al. 2011), they are often collapsed to the single parameter, β . However, this has received valid criticism because crucial information about transmission could be otherwise lost (McCallum et al. 2017; White et al. 2018). In order to clearly understand the effects of diversity on disease outcomes, we need to examine how diversity can affect each of the key mechanisms independently and ascertain the potential for interactions (synergistic or antagonistic) among mechanisms that could impact the net outcome.

Rodent-hantavirus systems have been employed as models of directly-transmitted disease systems for studying relationships between diversity (or community composition) and disease. Hantaviruses are primarily transmitted between hosts via bites and are closely associated with one primary reservoir species that once infected, never recovers (Jonsson et al. 2010). An example of such a system is the Sin Nombre virus (SNV) and its primary host, the North

American deermouse (*Peromyscus maniculatus*, hereafter deermouse), which has received considerable attention in the dilution effect literature (e.g., Mills 2006; Clay et al. 2009a; Rubio et al. 2017; Luis et al. 2018). The deermouse is the primary reservoir for SNV in North America (Childs et al. 1994), and this system exhibits direct (Warner et al. 2019) and density-dependent transmission (Luis et al. 2015). Although dilution is the effect primarily found in this system (e.g., Calisher et al. 2002; Mills 2006; Clay et al. 2009a; Dizney & Ruedas 2009; Lehmer et al. 2012), neutral relationships between diversity and disease have also been identified (Orrock et al. 2010; Milholland et al. 2017; Vadell et al. 2020). However, the mechanisms responsible for these patterns have been studied comparatively less (Clay et al 2009b; Dizney & Dearing 2016; Rubio et al. 2017). Most importantly, Luis et al. (2018) found that dilution and amplification can occur concurrently within the same system and are driven by different mechanisms. In particular, the authors found that an overall dilution effect in the Southwest United States was driven by deermouse density, despite a concurrent “component amplification effect”—a positive relationship between diversity and SNV transmission rate, β , at a given deermouse density. Therefore, the deermouse-SNV system presents an ideal opportunity to examine how key mechanisms could work in tandem to influence the net disease outcome; a concept that applies across host-parasite systems.

Studies testing the “dilution effect” hypothesis in the deermouse-SNV system have largely focused on small mammal diversity instead of broader diversity measures. Consequently, the patterns observed between disease and diversity (or community composition) in this system, most likely stem from interspecific competition exerted on deermice, by one or more small mammal competitors. Therefore, the deermouse-SNV system allows us to tease apart how and why a heterospecific competitor can alter the spread of a directly-transmitted disease. In

particular, the deermouse-SNV system in grasslands of western Montana, USA, provides a suitable framework. There are two main reasons why this is the case. Firstly, deermice primarily compete with voles (*Microtus* spp.) in simple small mammal communities (Eleftheriou et al. 2021). Unlike deermice, voles are considered dead-end hosts for SNV (Mills 2006). Secondly, voles are dominant competitors of deermice and could regulate their density (e.g., Redfield et al. 1977), alter their intraspecific contact rates (i.e. interference competition; Grant 1971), and suppress their immunity via a rise in physiological stress, thereby potentially increasing probability of transmission (Dickens & Romero 2013; Fredebaugh-Siller et al. 2013, Eleftheriou et al. 2021). Therefore, voles could influence SNV prevalence via multiple mechanisms, and the simpler community in western Montana allows us to disentangle their relative importance on disease outcomes.

Our study objective was to broadly examine, in a theoretical yet realistic framework, how community composition, represented here by a heterospecific competitor, could affect disease prevalence, via multiple key mechanisms, within a directly-transmitted disease system that exhibits density-dependent transmission. To accomplish this, we modified a deterministic model previously validated for the deermouse-SNV system (Luis et al. 2015) to include a heterospecific competitor. Because we wanted to evaluate an array of potential, yet realistic, disease outcomes, we varied the strength of vole-induced effects on deermouse density (i.e. regulatory interspecific competition), contact rates (β_c), and probability of transmission (β_p), as best informed by published data. Importantly, although our study focused on the deermouse-SNV system within a simple small mammal community, we emphasize that our modeling approach could be modified, and broadly applied to other disease systems that experience interspecific competition.

METHODS

Susceptible-infected-recovered (SIR) models are compartmentalized models developed to track disease dynamics within a host population. The compartments are connected through parameters that allow one host to leave one compartment and enter another (Anderson & May 1979). For example, a host can leave the susceptible compartment and enter the infected compartment, through transmission from an infected individual. Our model incorporates background birth and death rates of all deer mouse classes irrespective of age or sex. This approach has been successful in predicting SNV dynamics in deer mouse populations (Luis et al. 2018). Our model also incorporates logistic growth where there is density-dependence in background birth and death rates, and assumes density-dependent transmission (Luis et al. 2015). Because deer mice never recover from infection, there is no recovered compartment (Mills et al. 1999). Our model also incorporates disease-induced mortality (Luis et al. 2012). The following model is based on the model from Luis et al. (2018), with the addition of vole density (V), a competition coefficient (α), β split into its behavioral and physiological components, and removal of immigration of infected individuals:

$$\frac{dS}{dt} = N \left(b - cr \left(\frac{N+\alpha V}{K} \right) \right) - S \left(d + (1-c)r \left(\frac{N+\alpha V}{K} \right) \right) - \beta_c \beta_p SI \quad (1a)$$

$$\frac{dI}{dt} = \beta_c \beta_p SI - I \left(\mu + d + (1-c)r \left(\frac{N+\alpha V}{K} \right) \right) \quad (1b)$$

where N is the total deer mouse density ($S+I$), S is the susceptible deer mouse density, I is the infected deer mouse density, K is the fixed carrying capacity for deer mice, b is the maximum birth rate (in absence of density-dependence), d is the minimum death rate, c is the proportion of

density dependence attributed to density dependence in birth rates, r is the intrinsic growth of the population ($b-d$), μ is the disease-induced mortality rate, α is the Lotka-Volterra competition coefficient that measures the effect of vole density on deermouse density in the absence of disease, V is vole density, β_c is the contact rate, and β_p is the probability of transmission (Table 1).

In order to theoretically examine a comprehensive range of the regulatory pressure deermice could experience from voles, we initially selected three α values that spanned from weak competition ($\alpha = 0.8$) to competitive exclusion ($\alpha = 2$). Later, we explored the full range from 0 to 2, in a broader analysis.

DECONSTRUCTING TRANSMISSION RATE

Given that no previous studies have estimated β_c and β_p separately, we explored a range of plausible values for β informed by Luis et al. (2018). These authors estimated a range of values from various Montana field sites (Table S5.1). We used this range to first calculate a mean transmission rate. Given that β_p can only take values between 0 and 1, we assumed an arbitrary value of 0.5 in the absence of effects from competitor density (β_{p0}); we later performed an elasticity analysis to test how this parameter affects the outcome. A value for a contact rate in the absence of effects from competitors (β_{c0}) was calculated by dividing the mean transmission rate by 0.5. This approach allowed our simulations to generate β estimates within the reported range (Luis et al. 2018) and thus, keep our findings realistic (Table S5.1).

Because of ambiguity as to how deermouse contact rates change with diversity or competitor density (Clay et al. 2009b; Rubio et al. 2017; Luis et al. 2018; Eleftheriou et al. 2021), we either allowed density-independent contact rates to stay constant ($\beta_c = \beta_{c0}$), increase, or decrease with competitor density using the equation below:

$$\beta_c = \beta_{c0} + \psi V, \quad (2)$$

where ψ is the effect of vole density, V (Table 2). At the highest simulated V ($V = 20$), β_c reaches its lowest value, 0, when $\psi = -\frac{\beta_{c0}}{\max(V)}$ (Figure 5.1A, Table 5.1). However, this changes when $\psi = \frac{\beta_{c0}}{\max(V)}$, where β_c reaches its highest value, 0.04257778, at the highest V . We decided on this mathematical expression for ψ because we wanted to incorporate scenarios where the competitor could completely block intraspecific contact rates when at their highest density ($V = 20$).

Previous evidence suggests that vole density can impact measures of physiological stress in deermice (Fredebaugh-Siller et al. 2013, Eleftheriou et al. 2021). A sustained increase in physiological stress could suppress host immunity, which could increase probability of transmission as a result, here modeled by:

$$\text{logit}(\beta_p) = \beta_{p0} + \gamma V, \quad (3)$$

where γ is the effect from vole density, V . The probability of transmission, β_p , increases with vole density but as a probability it is constrained between 0 and 1 with the logit function. In the absence of vole-induced effects, probability of transmission remains constant ($\beta_p = \beta_{p0}$). We elected to model the relationship between β_p and V using three different γ values (0.03, 0.15, 0.30), which allowed us to explore how a range in the rates of increase in physiological stress could affect the final outcome (Figure 5.1B, Table 5.1).

ESTIMATING CRITICAL HOST DENSITY

Because SNV has density-dependent transmission, we can calculate a critical host density (N_c), below which SNV cannot persist. We can examine how voles could influence N_c , and thus, SNV prevalence indirectly, by:

$$N_c = \frac{\mu + d + (1 - c)r}{\beta_c \beta_p}, \quad (4)$$

RUNNING MODEL SIMULATIONS FOR ALL SCENARIOS

We used R (version 4.0.3, R Core Team 2018) within RStudio (RStudio Team 2015) to run all scenario simulations where we initially tested three α values (0.8, 1.2, 2), three γ values (0.03, 0.15, 0.30) and three ψ values (0, ± 0.001064445) across a range of V ($V = 0-20$) (Figure 5.1). To solve differential equations, we used the R package “deSolve” (Soetaert et al. 2010). We introduced one infected deermouse in a population of 29 susceptible deermice, while keeping environmental carrying capacity, K , constant at 30 deermice. In the end, we ran each of the scenarios for 100 time steps (months), which was enough to reach equilibrium in all simulations. SNV prevalence was estimated as density of infected deermice divided by the total density of deermice at equilibrium. We ensured that our simulation results remained within a realistic context by confirming that the β values we generated were within or near the range previously reported (Table S5.1).

To demonstrate in a larger parameter space what outcomes would be possible, we ran another set of simulations with a wider range of α , γ and ψ values that generated 5859 possible combinations. Additionally, to better generalize our findings to other disease systems, we ran a broader sensitivity analysis where we varied system-specific parameters or variables that were typically kept fixed in our model simulations, to understand their effect on disease outcomes (fixed parameters and variables in Table 5.1). To do this, we varied fixed parameter or variable values by $\pm 10\%$ or 30% and generated outcomes for the simulation scenario where ψ was kept at zero (center square in Figure 5.3).

RESULTS

COMPETITOR AFFECTS EACH MECHANISM INDIVIDUALLY

Firstly, we examined the impact of competitor density on disease outcomes via each individual mechanism of transmission (host density, density-independent contact rates and probability of

transmission) separately, by varying α , ψ , and γ , one at a time, and evaluating how each parameter, in isolation, influenced the relationship between competitor density and disease prevalence. If disease prevalence (at equilibrium) declined as competitor density increased, we considered this a “dilution effect”, i.e. the competitor “diluted” disease. Alternatively, if disease prevalence increased with competitor density, we considered this an “amplification effect”.

We found that as the strength of interspecific competition (α) increased, the competitor more strongly regulated host density, leading to a steeper decline in density. We observed extinction of hosts (competitive exclusion) at $\alpha = 2$ at intermediate competitor densities, for our simulated scenario of $K = 30$. This reduction in host density led to a decrease in disease prevalence (due to density-dependent transmission) and disease extinction when host density (N) declined below the critical host density (N_c) (Figure S5.1). Thus, as the strength of interspecific competition (α) increased, disease extinction happened at lower competitor densities (V) and a dilution effect occurred.

As the strength of stress-induced immunosuppression (γ) increased, competitor density (V) had a greater effect on the probability of transmission (β_p), leading to higher disease prevalence at lower competitor densities and, thus, an amplification effect (Figure S5.2).

When we allowed the competitor to influence contact rates (β_c) between hosts, we found that a positive ψ (competitor increases β_c) led to an amplification effect, whereas a negative ψ (competitor decreases β_c) generated a dilution effect (Figure S5.3).

Taken together, our findings demonstrate that dilution or amplification can occur in this system, depending on the mechanism working in isolation and its relationship with competitor density.

COMPETITOR AFFECTS VARIOUS MECHANISMS SIMULTANEOUSLY

Next, we explored how the combination of competitor regulation (α) and stress-induced immunosuppression (γ) could affect disease outcomes by simultaneously varying α and γ , while holding ψ at zero (no direct effect of competitor on density-independent contact rates). With stronger interspecific competition ($\alpha = 2$), the negative effect of competitor density (V) on host density (N), and thus, density-dependent transmission, outweighed the positive effect of V on the probability of transmission (β_p), leading to dilution. The only exception was when V had a stronger effect on β_p ($\gamma = 0.3$) where a non-monotonic relationship occurred (figure 2, figure A4). A dilution effect was also predicted when the effect of competitor density (V) on β_p was weak ($\gamma = 0.03$). However, the combination of weaker competition ($\alpha < 2$) and intermediate to strong effect of V on β_p ($\gamma > 0.03$), led to a non-monotonic relationship between competitor density (V) and disease prevalence, such that prevalence increased at lower competitor densities (V), was highest at low to intermediate V , and then decreased at higher V (Figure 5.2).

Next, we added the potential effect of the competitor on intraspecific contact rates (β_c) by varying the effect of competitor density (V) on β_c (while varying α and γ). When competitor density, V , increased intraspecific contact rates, β_c (positive ψ), a wider parameter space generally led to a non-monotonic relationship between competitor density and disease prevalence (Figure S5.4). The only exception was when V had a stronger regulatory effect ($\alpha = 2$) on host density but a weaker effect on probability of transmission, β_p ($\gamma = 0.03$), resulting in dilution (Figure S5.5). However, when competitor density, V , decreased β_c (negative ψ), a wider parameter space most often led to a dilution effect, whereas a non-monotonic relationship was only observed when the effect of V on probability of transmission, β_p , was stronger ($\gamma = 0.30$) (Figure S5.6).

Lastly, we explored a broader parameter space to characterize which parameter combinations led to dilution (decrease), amplification (increase), or a non-monotonic (increase followed by decrease) relationship between competitor density, V , and disease prevalence. Only when $\psi < -5e-04$ (i.e. V decreases β_c), a greater portion of parameter space predicted a dilution effect (light purple, Figure 5.3). Indeed, this effect gradually comprised a narrower parameter space as the effect of V on contact rates (β_c), ψ , reached its maximum value (parameter space from 61.9% to 3.8%). A non-monotonic relationship was predicted most commonly when α was lower (i.e. weaker regulatory strength) and γ was higher (i.e., greater stress-induced immunosuppression). This relationship gradually comprised a wider parameter space (increased from 38.1% to 70.5%) as the effect of V on contact rates (β_c), ψ , reached its maximum value (dark green, Figure 5.3). Importantly, a non-monotonic relationship was predicted even when there was no regulatory effect on host density ($\alpha = 0$), suggestive that the sole interactions between components of the transmission rate (β_c and β_p) can still manifest in non-monotonic outcomes. Additionally, an amplification effect emerged (dark purple, Figure 5.3), although across a narrower parameter space (ranged from 9.2% to 25.7%), when $\psi \geq 0$ (i.e. V has no effect or increases β_c) and interspecific competition was weaker ($\alpha < 0.5$). Taken together, dilution became less prominent across the parameter space we examined as the effect of competitor density on contact rates, ψ , switched from negative to positive values, whereas a non-monotonic relationship became the most commonly predicted outcome. Overall, an amplification effect was the least predicted outcome, which only appeared under restricted conditions given realistic parameter values for this system.

In order to extend our findings beyond the deer-mouse-SNV system, we conducted a broader elasticity analysis where we varied parameters or variables previously assumed to be

fixed (Table 5.1) in a standardized manner (Tables S5.2-S5.5). These findings did not indicate any significant deviations from our conclusions suggesting that our simulation findings are robust to the fixed parameter and variable values we used in this study. Indeed, the greatest change that we observed was relatively small, ~ 12% reduction in dilution for 30% decrease in the intercept for contact rates, β_{c0} (Tables S5.2, S5.4).

DISCUSSION

Using a directly-transmitted disease system, we theoretically examined possible effects of interspecific competition on three mechanisms of transmission (i.e. host density, density-independent contact rates, and probability of transmission). We show that monotonic (amplification or dilution) and, most importantly, non-monotonic relationships between competitor density and disease prevalence can occur under a range of realistic parameter values. Non-monotonic relationships between diversity and disease have been previously considered, typically across a land-type gradient (e.g. urban-forest). However, here we show that non-monotonic relationships could also occur due to antagonistic interactions of key mechanisms within the same study system.

The most intriguing finding of our study was a non-monotonic relationship that was the most commonly predicted outcome in the parameter space we examined (~60%, Figure 5.3). This relationship was characterized by an increase in disease prevalence at lower competitor densities (V), a peak at intermediate V , and a decline at higher V (Figure 5.2). Regardless of an effect on contact rates (β_c), this outcome was most evident when the competitor (1) weakly regulated host density (N), and (2) had a greater effect on the probability of transmission (β_p) via stress-induced immunosuppression.

Such non-monotonic relationships have been previously considered between diversity and disease because when there are no host or vector species present (e.g., a parking lot or urban center), no to little disease risk would be expected, and therefore amplification would need to occur prior to any dilution (Wood et al. 2016; Kirkpatrick et al. 2017). This concept has been exemplified with the Lyme disease system, which requires the presence of multiple host and vector species for sustained transmission (Wood & Lafferty 2013). For instance, in urban areas with zero diversity, there will be no hosts (e.g. mice or deer) or vectors (i.e. ticks), and thus, Lyme disease cannot persist. However, diversity is expected to increase when moving across different land-types, such as from urban to rural as suggested for the Lyme disease system. As competent hosts and vectors become more likely to be present along this gradient, an amplification effect can manifest. However, in areas with higher levels of diversity, represented by forests in the Lyme disease system, more non-competent hosts are also likely to be present, which can lead to a dilution effect.

However, our study suggests that non-monotonic relationships between diversity and disease could occur for another reason, the result of competing mechanisms within the same study system. This novel result could explain why non-monotonic relationships between diversity and disease have been reported in various systems (Halliday & Rohr 2019). Importantly, our findings could explain why some studies have found no diversity-disease patterns in the deer-mouse-SNV system (Orrock et al. 2010, Milholland et al. 2017; Vadell et al. 2020). To demonstrate this concept, we added stochasticity to simulation findings where non-monotonic patterns were most notable (e.g., when $\alpha = 0.8$ and $\psi = 0$), where we show that fitted linear regressions can only find neutral patterns as they are inherently unable to identify non-monotonic relationships (figure A7). Given that linear relationships are typically the patterns

searched for in field studies, non-monotonic outcomes could be easily mistaken for the absence of a pattern in the presence of stochasticity. Although findings similar to our own were also shown by a recent theoretical study (Lee & Mohd 2020), the transmission rate, (β), was not deconstructed into its two components. Therefore, our study further extends their findings, introduces guidelines for when a non-monotonic relationship could occur in the presence of interspecific competition, and provides theoretical support for why empirical studies may find no diversity-disease patterns in natural systems.

Dilution was the second most commonly predicted outcome (~29%) in the parameter space we examined, with amplification rarely predicted (~11%). Conversely, dilution was the sole outcome found by theoretical studies that examined the regulatory role of a non-competent competitor on host density in a directly-transmitted disease system with density-dependent transmission (Peixoto & Abramson 2006; O'Regan et al. 2015). Importantly, dilution is often reported in empirical studies with the deer-mouse-SNV system (e.g., Mills 2006; Clay et al. 2009a; Disney & Ruedas 2009). In contrast, amplification has received limited support and only as a weak effect (Luis et al. 2018). Indeed, our findings support that amplification could be less common in nature given that it occurred in a narrower parameter space, where the relationship between contact rates (β_c) and competitor density (V) was primarily neutral or positive, and when the regulatory effect (α) on host density (N) was weak or absent. Therefore, our findings provide theoretical support for a more common dilution, than amplification effect in nature, at least within the parameter space used for this disease system.

Our findings of the great potential for competitor density to lead to a non-monotonic relationship with disease prevalence is contingent upon the parameter space examined. We informed our model with realistic parameter values for the deer-mouse-SNV system with vole

competitors, and the results of our broader elasticity analysis suggest that our conclusions are largely robust to the fixed parameter/variable values that we used to inform the model.

Therefore, our conclusions likely apply to other directly-transmitted disease systems that experience interspecific competition. Despite uncertainties in parameter values, we are confident that a non-monotonic outcome between competitor density and disease prevalence could arise from a wide range of parameter space, especially since it was by far, the most commonly predicted relationship in the parameter space we examined.

Although we acknowledge that a two-species framework implies that we did not examine diversity *per se*, as would be expected for placing our findings within a dilution effect context, there are three primary reasons as to why our approach and findings are still relevant to the “dilution effect” hypothesis. Firstly, community composition (e.g., heterospecific competitors) has been suggested to be more relevant (Randolph & Dobson 2012). Secondly, a simple two-species system has been exploited by others when asking similar questions (Peixoto & Abramson 2006; O’Regan et al. 2015). Lastly, competitive interactions are most likely the primary ecological process by which small mammal diversity (typically used as the diversity index in deer-mouse-SNV studies) can actually impact hantavirus prevalence.

It is important to note that our findings are based on equilibrium conditions, which are not always found in natural disease systems, such as the deer-mouse-SNV system (Luis et al. 2015). Although rodent densities and their environmental carrying capacities will vary across time and space, we elected to keep these fixed to allow for a more tangible examination of our research objective. Additional layers of complexity where rodent densities and their carrying capacities can vary across time and space will be useful, but these were beyond the scope of this study. Furthermore, we did not examine infectious period as a mechanism to keep our modeling

framework more tractable and because this mechanism has received scant empirical attention. Therefore, future studies can investigate how non-equilibrium conditions could affect our findings and incorporate competitor-induced effects on infectious period in more complex modeling frameworks.

A passionate debate has been ongoing (Randolph & Dobson 2012; Ostfeld 2013; Ostfeld & Keesing 2013; Wood & Lafferty 2013) since the “dilution effect” hypothesis was first formally proposed (Ostfeld & Keesing 2000). Although we did not focus on species diversity *per se*, it is more likely that community composition (e.g., competitor species) is more relevant in how disease outcomes manifest (Randolph & Dobson 2012; Johnson et al. 2015; Kilpatrick et al. 2017). Therefore, focusing on the density of relevant species, such as heterospecific competitors, may provide more explanatory or predictive power than broad measures of diversity (e.g. species richness or evenness). By placing our study within the dilution effect framework, we propose that monotonic and non-monotonic relationships can theoretically occur within the same system depending on the interaction strengths of mechanisms that work in tandem. Therefore, to advance the field forward, we need to consider the co-occurring effects of relevant mechanisms under realistic ecological scenarios. We predict that our findings and approach can extend diversity-disease theory, stimulate novel empirical studies, and inform disease management in applied settings.

LITERATURE CITED

Anderson, R. M., and R. M. May. 1979. Population biology of infectious diseases: Part I. *Nature* 280:361-367.

- Begon, M., M. Bennett, R. G. Bowers, N. P. French, S. Hazel, and J. Turner. 2002. A clarification of transmission terms in host-microparasite models: numbers, densities and areas. *Epidemiology & Infection* 129:147-153.
- Calisher, C. H., J. J. Root, J. N. Mills, and B. J. Beaty. 2002. Assessment of ecologic and biologic factors leading to hantavirus pulmonary syndrome, Colorado, USA. *Croatian Medical Journal* 43:330-337.
- Cardinale, B. J., J. E. Duffy, A. Gonzalez, D. U. Hooper, C. Perrings, P. Venail, A. Narwani, G. M. Mace, D. Tilman, and D. A. Wardle. 2012. Biodiversity loss and its impact on humanity. *Nature* 486:59-67.
- Childs, J. E., T. G. Ksiazek, C. F. Spiropoulou, J. W. Krebs, S. Morzunov, G. O. Maupin, K. L. Gage, P. E. Rollin, J. Sarisky, and R. E. Enscoe. 1994. Serologic and genetic identification of *Peromyscus maniculatus* as the primary rodent reservoir for a new hantavirus in the southwestern United States. *The Journal of Infectious Diseases* 169:1271-1280.
- Civitello, D. J., J. Cohen, H. Fatima, N. T. Halstead, J. Liriano, T. A. McMahon, C. N. Ortega, E. L. Sauer, T. Sehgal, S. Young, and J. R. Rohr. 2015. Biodiversity inhibits parasites: Broad evidence for the dilution effect. *Proceedings of the National Academy of Sciences of the United States of America* 112:8667-8671.
- Clay, C. A., E. M. Lehmer, S. S. Jeor, and M. D. Dearing. 2009a. Sin Nombre virus and rodent species diversity: a test of the dilution and amplification hypotheses. *PloS one* 4:e6467.
- Clay, C. A., E. M. Lehmer, S. S. Jeor, and M. D. Dearing. 2009b. Testing mechanisms of the dilution effect: deer mice encounter rates, Sin Nombre virus prevalence and species diversity. *EcoHealth* 6:250-259.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife--threats to biodiversity and human health. *Science* 287:443-449.

Dickens, M. J., and L. M. Romero. 2013. A consensus endocrine profile for chronically stressed wild animals does not exist. *General and comparative endocrinology* 191:177-189.

Dizney, L., and M. D. Dearing. 2016. Behavioural differences: a link between biodiversity and pathogen transmission. *Animal Behaviour* 111:341-347.

Dizney, L. J., and L. A. Ruedas. 2009. Increased host species diversity and decreased prevalence of Sin Nombre virus. *Emerging infectious diseases* 15:1012-1018.

Eleftheriou, A., A. J. Kuenzi, and A. D. Luis. 2021. Heterospecific competitors and seasonality can affect physiology and behavior, key determinants of disease transmission. *Ecosphere*. In Press.

Fredebaugh-Siller, S., C. Suski, Z. Zuckerman, and R. Schooley. 2013. Ecological correlates of stress for a habitat generalist in a biofuels landscape. *Canadian Journal of Zoology* 91:853-858.

Grant, P. 1971. Experimental studies of competitive interaction in a two-species system. *The Journal of Animal Ecology* 40:323-350.

Halliday, F. W., and J. R. Rohr. 2019. Measuring the shape of the biodiversity-disease relationship across systems reveals new findings and key gaps. *Nature communications* 10:1-10.

Hawley, D. M., R. S. Etienne, V. O. Ezenwa, and A. E. Jolles. 2011. Does animal behavior underlie covariation between hosts' exposure to infectious agents and susceptibility to infection? Implications for disease dynamics. *Integrative and Comparative Biology* 51:528-539.

Huang, Z., F. Van Langevelde, A. Estrada-Peña, G. Suzán, and W. De Boer. 2016. The diversity-disease relationship: evidence for and criticisms of the dilution effect. *Parasitology* 143:1075-1086.

- Johnson, P. T., R. S. Ostfeld, and F. Keesing. 2015. Frontiers in research on biodiversity and disease. *Ecology Letters* 18:1119-1133.
- Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. *Nature* 451:990-993.
- Jonsson, C. B., L. T. Figueiredo, and O. Vapalahti. 2010. A global perspective on hantavirus ecology, epidemiology, and disease. *Clinical Microbiology Reviews* 23:412-441.
- Keesing, F., R. D. Holt, and R. S. Ostfeld. 2006. Effects of species diversity on disease risk. *Ecology Letters* 9:485-498.
- Kilpatrick, A. M., D. J. Salkeld, G. Titcomb, and M. B. Hahn. 2017. Conservation of biodiversity as a strategy for improving human health and well-being. *Philosophical Transactions of the Royal Society B: Biological Sciences* 372:20160131.
- Lee, L. W. F., and M. H. Mohd. 2020. The Effects of Amplification and Dilution Agent in Transmitting Sin Nombre Virus (SNV) in Deer Mouse Population. *MATEMATIKA: Malaysian Journal of Industrial and Applied Mathematics* 36:85-98.
- Lehmer, E. M., J. Korb, S. Bombaci, N. McLean, J. Ghachu, L. Hart, A. Kelly, E. Jara-Molinar, C. O'Brien, and K. Wright. 2012. The interplay of plant and animal disease in a changing landscape: The role of sudden aspen decline in moderating Sin Nombre virus prevalence in natural deer mouse populations. *EcoHealth* 9:205-216.
- Luis, A. D., A. J. Kuenzi, and J. N. Mills. 2018. Species diversity concurrently dilutes and amplifies transmission in a zoonotic host-pathogen system through competing mechanisms. *Proceedings of the National Academy of Sciences of the United States of America* 115:7979-7984.

- Luis, A. D., R. J. Douglass, J. N. Mills, and O. N. Bjørnstad. 2015. Environmental fluctuations lead to predictability in Sin Nombre hantavirus outbreaks. *Ecology* 96:1691-1701.
- Luis, A. D., R. J. Douglass, P. J. Hudson, J. N. Mills, and O. N. Bjørnstad. 2012. Sin Nombre hantavirus decreases survival of male deer mice. *Oecologia* 169:431-439.
- McCallum, H., A. Fenton, P. J. Hudson, B. Lee, B. Levick, R. Norman, S. E. Perkins, M. Viney, A. J. Wilson, and J. Lello. 2017. Breaking beta: deconstructing the parasite transmission function. *Philosophical transactions of the Royal Society of London. Series B, Biological Sciences* 372:20160084.
- Milholland, M. T., I. Castro-Arellano, E. Arellano, E. Nava-García, G. Rangel-Altamirano, F. X. Gonzalez-Cozatl, G. Suzán, T. Schountz, S. González-Padrón, and A. Vigueras. 2017. Species identity supersedes the dilution effect concerning hantavirus prevalence at sites across Texas and Mexico. *ILAR Journal* 58:401-412.
- Mills, J. N. 2006. Biodiversity loss and emerging infectious disease: an example from the rodent-borne hemorrhagic fevers. *Biodiversity* 7:9-17.
- Mills, J. N., T. G. Ksiazek, C. J. Peters, and J. E. Childs. 1999. Long-term studies of hantavirus reservoir populations in the southwestern United States: a synthesis. *Emerging Infectious Diseases* 5:135-142.
- O'Regan, S. M., J. E. Vinson, and A. W. Park. 2015. Interspecific Contact and Competition May Affect the Strength and Direction of Disease-Diversity Relationships for Directly Transmitted Microparasites. *The American Naturalist* 186:480-494.
- Orrock, J. L., B. F. Allan, and C. A. Drost. 2011. Biogeographic and ecological regulation of disease: prevalence of Sin Nombre Virus in island mice is related to island area, precipitation, and predator richness. *The American Naturalist* 177:691-697.

- Ostfeld, R. S. 2013. A Candide response to Panglossian accusations by Randolph and Dobson: biodiversity buffers disease. *Parasitology* 140:1196-1198.
- Ostfeld, R. S., and F. Keesing. 2013. Straw men don't get Lyme disease: response to Wood and Lafferty. *Trends in Ecology & Evolution* 28:502-503.
- Ostfeld, R. S., and F. Keesing. 2000. Biodiversity and disease risk: the case of Lyme disease. *Conservation Biology* 14:722-728.
- Peixoto, I. D., and G. Abramson. 2006. The effect of biodiversity on the hantavirus epizootic. *Ecology* 87:873-879.
- R Core Team. 2018. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria.
- Randolph, S. E., and A. Dobson. 2012. Pangloss revisited: a critique of the dilution effect and the biodiversity-buffers-disease paradigm. *Parasitology* 139:847-863.
- Redfield, J., C. Krebs, and M. Taitt. 1977. Competition between *Peromyscus maniculatus* and *Microtus townsendii* in grasslands of coastal British Columbia. *The Journal of Animal Ecology* 46:607-616.
- Rohr, J. R., D. J. Civitello, F. W. Halliday, P. J. Hudson, K. D. Lafferty, C. L. Wood, and E. A. Mordecai. 2020. Towards common ground in the biodiversity–disease debate. *Nature Ecology & Evolution* 4:24-33.
- RStudio Team. 2015. RStudio: Integrated Development for R; RStudio, Inc.: Boston, MA, USA.
- Rubio, A. V., I. Castro-Arellano, J. N. Mills, R. List, R. Ávila-Flores, and G. Suzán. 2017. Is species richness driving intra-and interspecific interactions and temporal activity overlap of a hantavirus host? An experimental test. *PloS one* 12:e0188060.

- Salkeld, D. J., K. A. Padgett, and J. H. Jones. 2013. A meta-analysis suggesting that the relationship between biodiversity and risk of zoonotic pathogen transmission is idiosyncratic. *Ecology Letters* 16:679-686.
- Soetaert K., T. Petzoldt, R.W. Setzer. 2010. Solving Differential Equations in R: Package deSolve. *Journal of Statistical Software* 33: 1–25.
- Vadell, M. V., I. E. G. Villafañe, and A. E. Carbajo. 2020. Hantavirus infection and biodiversity in the Americas. *Oecologia* 192:169-177.
- Venesky, M. D., X. Liu, E. L. Sauer, and J. R. Rohr. 2014. Linking manipulative experiments to field data to test the dilution effect. *Journal of Animal Ecology* 83:557-565.
- Warner, B. M., D. R. Stein, B. D. Griffin, K. Tierney, A. Leung, A. Sloan, D. Kobasa, G. Poliquin, G. P. Kobinger, and D. Safronetz. 2019. Development and Characterization of a Sin Nombre Virus Transmission Model in *Peromyscus maniculatus*. *Viruses* 11:183.
- White, L. A., J. D. Forester, and M. E. Craft. 2018. Covariation between the physiological and behavioral components of pathogen transmission: host heterogeneity determines epidemic outcomes. *Oikos* 127:538-552.
- Wood, C. L., and K. D. Lafferty. 2013. Biodiversity and disease: a synthesis of ecological perspectives on Lyme disease transmission. *Trends in Ecology & Evolution* 28:239-247.
- Wood, C. L., K. D. Lafferty, G. DeLeo, H. S. Young, P. J. Hudson, and A. M. Kuris. 2016. Does biodiversity protect humans against infectious disease? Reply. *Ecology* 97:542-546.
- Wood, C. L., K. D. Lafferty, G. DeLeo, H. S. Young, P. J. Hudson, and A. M. Kuris. 2014. Does biodiversity protect humans against infectious disease? *Ecology* 95:817-832.

Table 5. 1 Variables and parameters used in the models with descriptions and estimates.

Parameter	Value	Description
K	30	Carrying capacity of deermice (per hectare) (Eleftheriou et al. 2021)
V	0-20	Vole competitor density (per hectare) (Eleftheriou et al. 2021)
α	0.8, 1.2, 2	Competition coefficient
c	0.6142470	Density-dependent effect on birth rates (Luis et al. 2015)
b	0.3154089	Maximum birth rate of deermice (Luis et al. 2015)
d	3.655774e-05	Minimum death rate of deermice (Luis et al. 2015)
μ	0.085	Disease-induced mortality rate (Luis et al. 2015)
β_{c0}	0.02128889	Density-independent contact rate in the absence of voles
β_{p0}	0.5	Probability of transmission in the absence of voles
ψ	0, ± 0.001064445	Effect on intraspecific contact rate from vole density
γ	0.03, 0.15, 0.3	Effect on probability of transmission from vole density

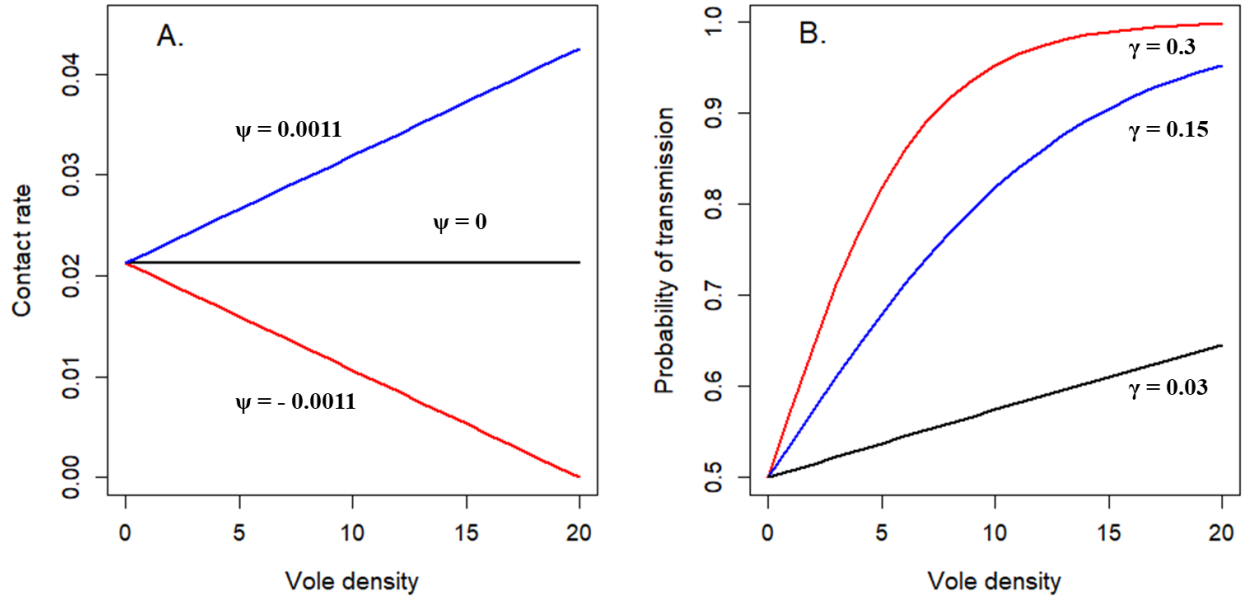


Figure 5.1 Relationships of density-independent contact rate (β_c) (A) and probability of transmission (β_p) (B) with competitor density (V). **A.** The effect of V on β_c , (ψ), was set to either zero (—) to denote constant β_c , or 0.001064445 (—) and - 0.001064445 (—), to denote increasing or decreasing β_c with V , respectively. The intercept was set to 0.02128889. **B.** The effect of V on β_p , (γ), was set to either 0.03 (—), 0.15 (—), or 0.30 (—), to develop relationships that ranged from linear to more exponential with V . The intercept was set to 0.50.

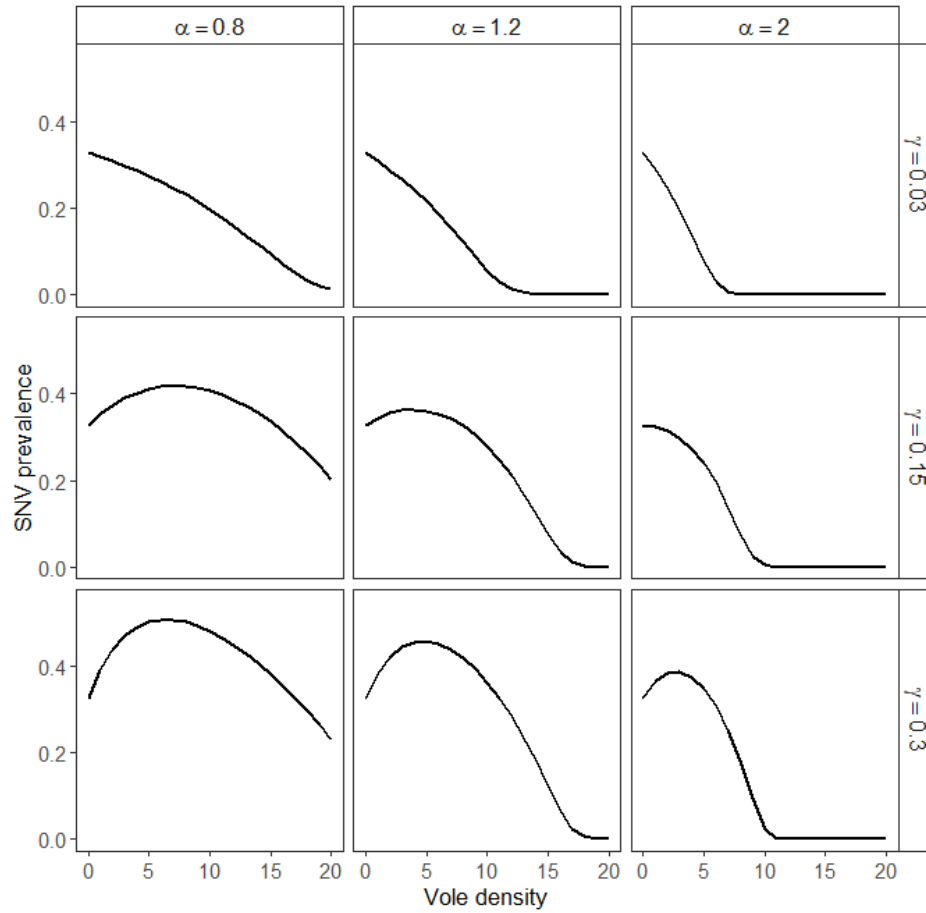


Figure 5. 2 Relationships between Sin Nombre virus (SNV) prevalence and vole competitor density. The effect of the competitor on host density (α) and probability of transmission (γ) via stress-induced immunosuppression, were varied as shown, while assuming constant density-independent contact rates between hosts ($\psi = 0$).

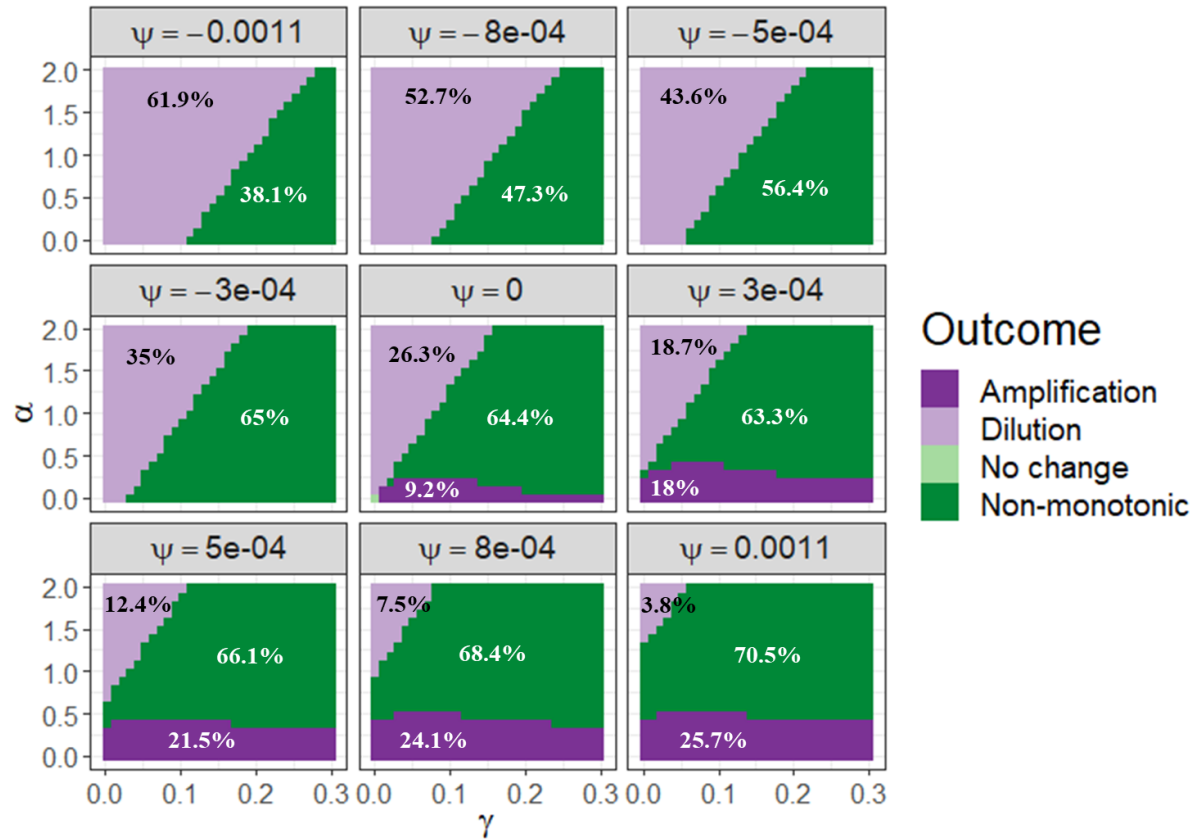


Figure 5. 3 Parameter space of 5859 combinations of α , γ and ψ values corresponding to their respective outcome for a fixed competitor density, V , (0-20). Dark purple signifies an amplification effect or a sole increase in disease prevalence, light purple, a dilution effect or a sole decline in prevalence, light green, no change in prevalence (i.e. represents baseline when all parameters are set to zero), and dark green, a non-monotonic relationship between prevalence and competitor density. The percentage values indicate the proportion of parameter space represented by each outcome.

SUPPLEMENTARY INFORMATION

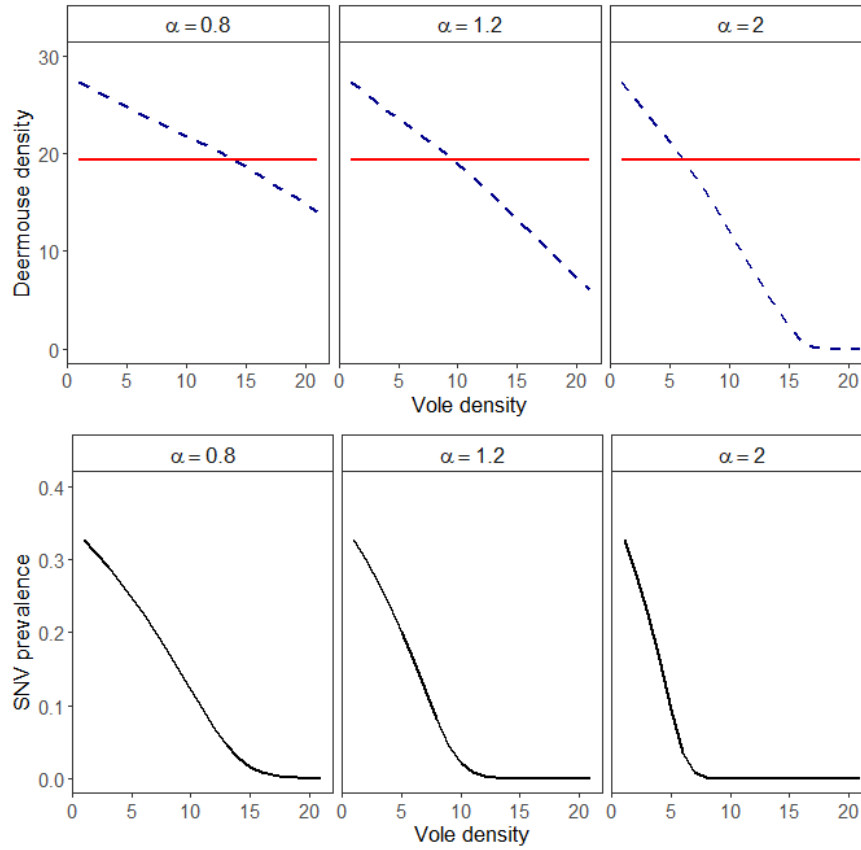


Figure S5. 1 Total deer mouse (host) density, N , (— — —), and critical host density, N_c , (—) relationships with vole competitor density, V , (top row), and respective outcome between Sin Nombre virus (SNV) prevalence (bottom row) and V . The effect of competitors on contact rates, (ψ), and probability of transmission, (γ), were kept at zero. The effect of competitors on host density, α , was varied in strength as displayed.

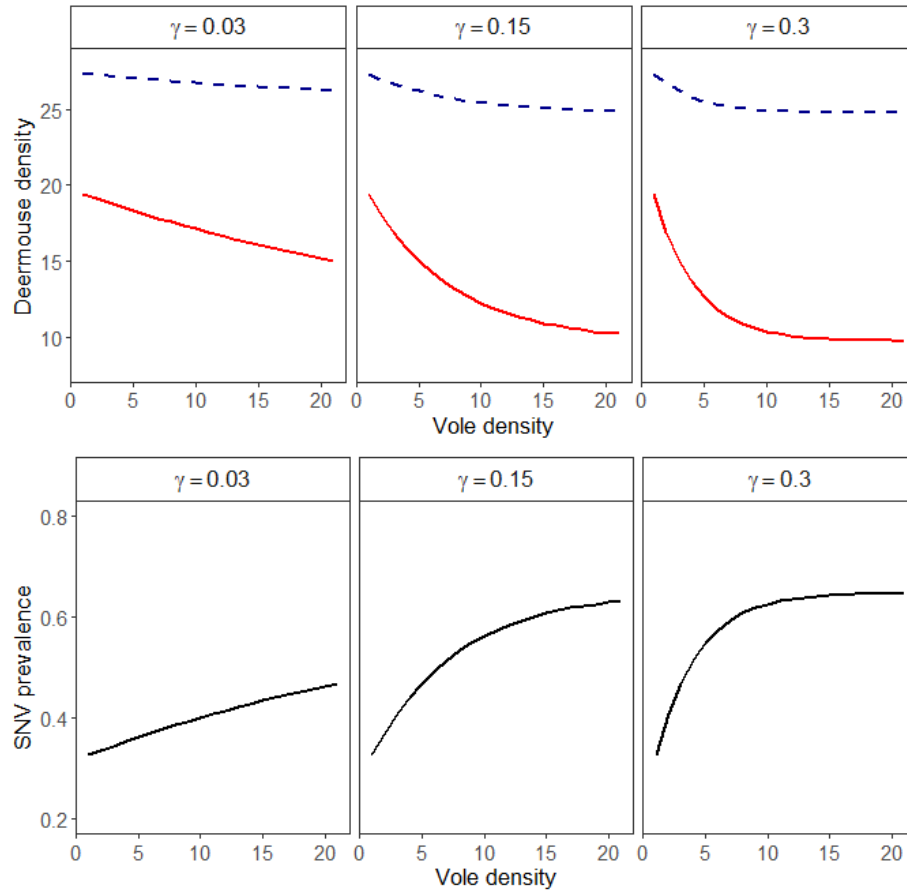


Figure S5. 2 Total deermouse (host) density, N , (— — —), and critical host density, N_c , (—) relationships with vole competitor density, V , (top row), and respective outcome between Sin Nombre virus (SNV) prevalence (bottom row) and V . The effect of competitors on host density (α), and contact rates (ψ) were kept at zero. The effect of competitors on the probability of transmission, (γ), was varied in strength as displayed.

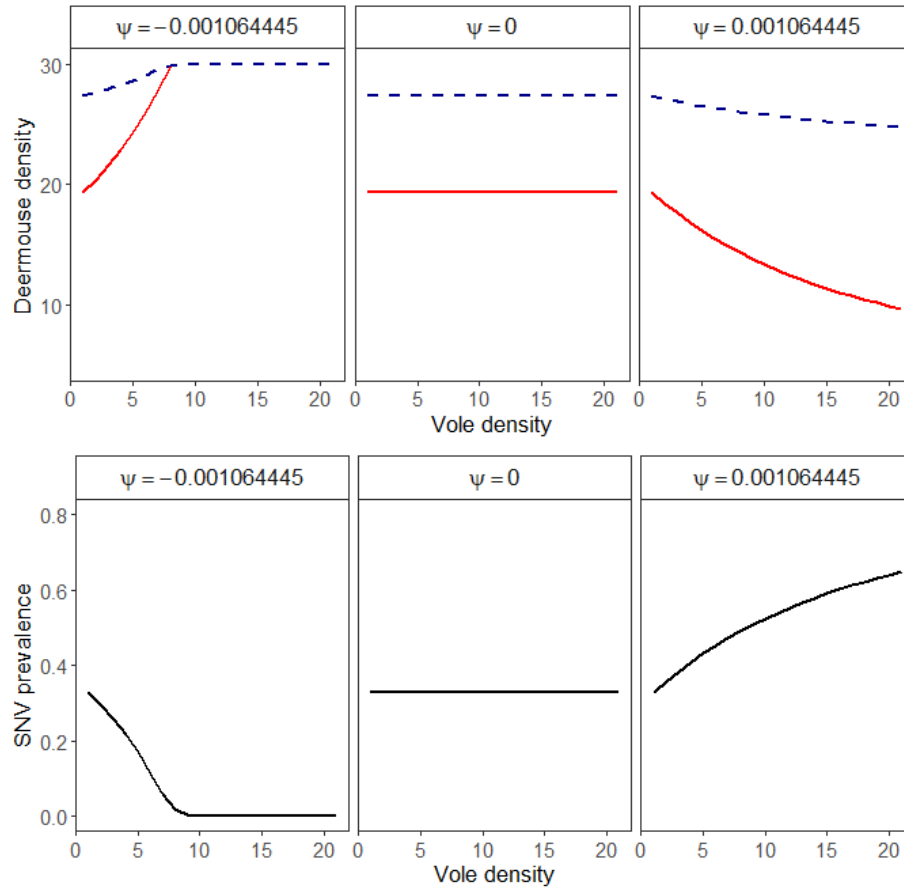


Figure S5. 3 Total deermouse (host) density, N , (— —), and critical host density, N_c , (—) relationships with vole competitor density, V , (top row), and respective outcome between Sin Nombre virus (SNV) prevalence (bottom row) and V . The effect of competitors on host density (α) and on the probability of transmission (γ) were kept at zero. The effect of competitors on contact rates (ψ) was varied as displayed.

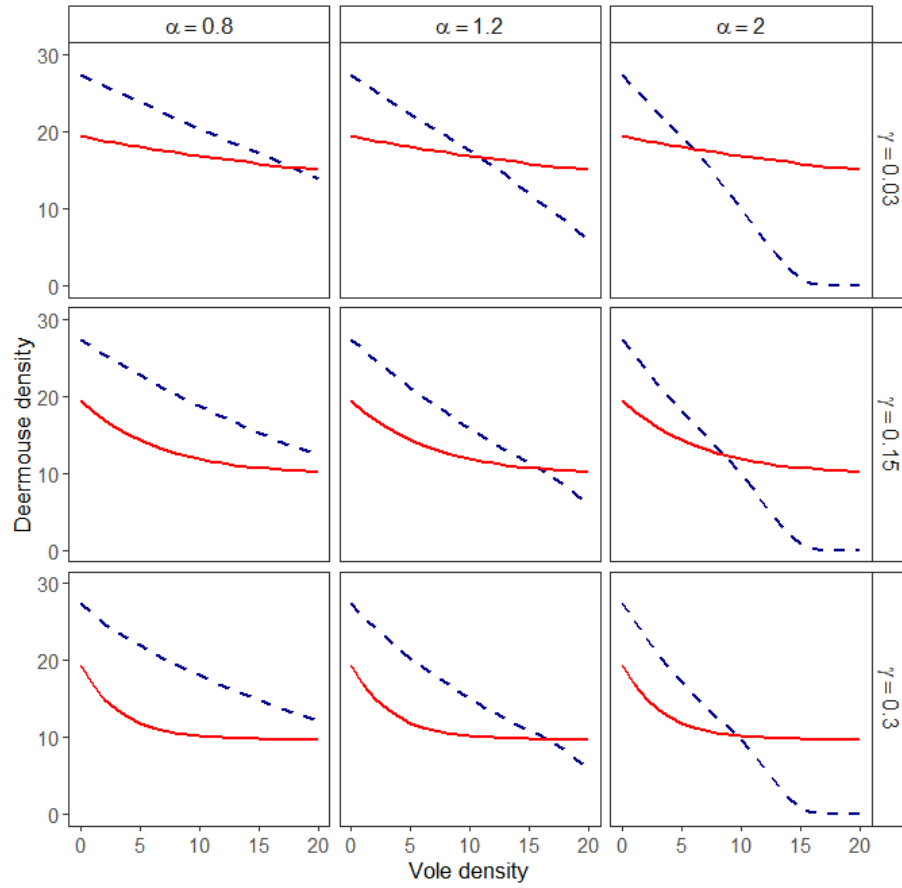


Figure S5. 4 Total deermouse (host) density, N , (— —), and critical host density, N_c , (—) relationships with vole competitor density, V , to complement figure 2 in the main text. The effect of competitors on host density (α) and on the probability of transmission (γ) were varied as shown. The effect of competitors on contact rates (ψ) was kept at zero.

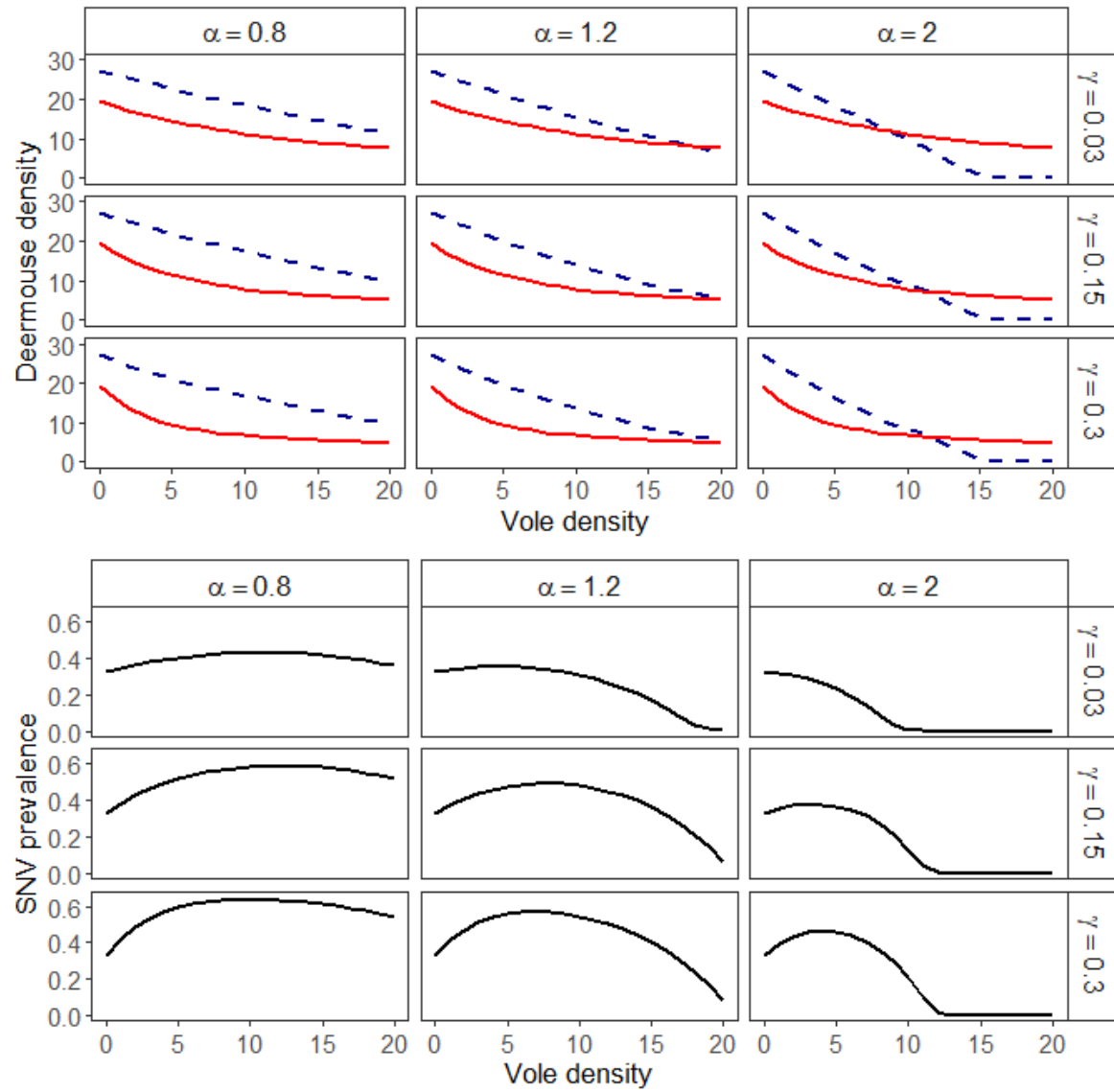


Figure S5. 5 Total deer mouse (host) density, N , (— — —), and critical host density, N_c , (—) relationships with vole competitor density, V , (top row), and respective outcome between Sin Nombre virus (SNV) prevalence (bottom row) and V . The effect of competitors on host density (α) and probability of transmission (γ) were varied as shown, while the effect of competitors on contact rates, (ψ), was fixed at 0.001064445.

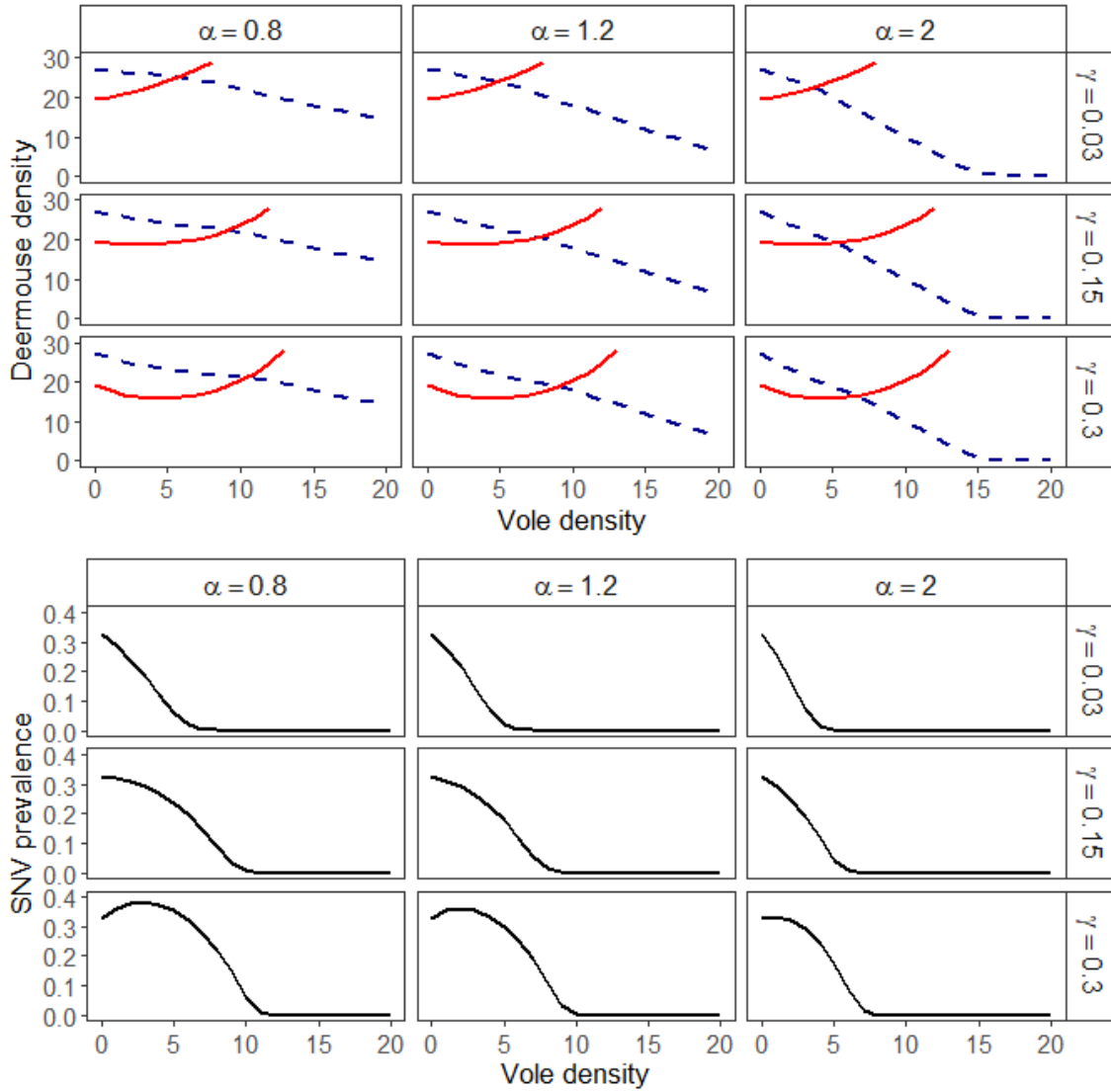


Figure S5. 6 Total deermouse (host) density, N , (- - -), and critical host density, N_c , (—) relationships with vole competitor density, V , (top row), and respective outcome between Sin Nombre virus (SNV) prevalence (bottom row) and V . The effect of competitors on host density (α) and probability of transmission (γ) were varied as shown, while the effect of competitors on contact rates, (ψ), was fixed at - 0.001064445.

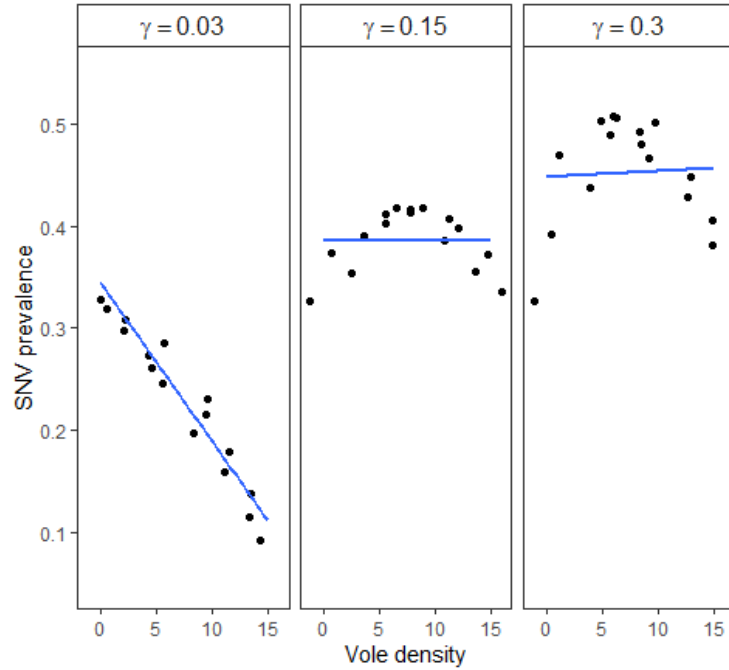


Figure S5. 7 Relationships between Sin Nombre virus (SNV) prevalence and vole competitor density as modeled with linear regression (—) when competitors have a weak effect on host density ($\alpha = 0.8$) and no effect on contact rate ($\psi = 0$), a scenario shown in Figure 5.2 of the main text. When we add a degree of stochasticity to the simulated data, an obvious dilution effect can only be seen when a monotonic relationship is predicted ($\gamma = 0.03$). Otherwise, no pattern is found with linear regression due to the non-monotonic shape of the predicted relationship.

Table S5. 1 Model-estimated β values generated from all possible simulations compared to published β values from Montana, USA.

Parameter	Mean	Standard deviation	Range
β (this study)	0.01592162	0.00772337	0 – 0.0424725
β (Luis et al. 2018)	0.01064	0.006088331	0.00270 – 0.02020

Table S5. 2 Results of broad elasticity analyses where the effect of competitor density on intraspecific contact rates, β_c , (ψ), was kept at zero (scenario in center of Figure 5.3 in main text). Parameters and variables were changed $\pm 30\%$ to visualize the percentage change in disease outcomes compared to their fixed values (Table 5.1 in main text). For each figure inset, the horizontal axis, represents γ , the degree of stress-induced immunosuppression (range; 0-0.3) while the vertical axis represents α , the strength of regulatory competition (range; 0-2), both of which are effects exerted on hosts by competitor density. Dark purple signifies an amplification effect, light purple, a dilution effect, light green, no change in prevalence (i.e. represents baseline when ψ , α & γ are set to zero), and dark green, a non-monotonic relationship.

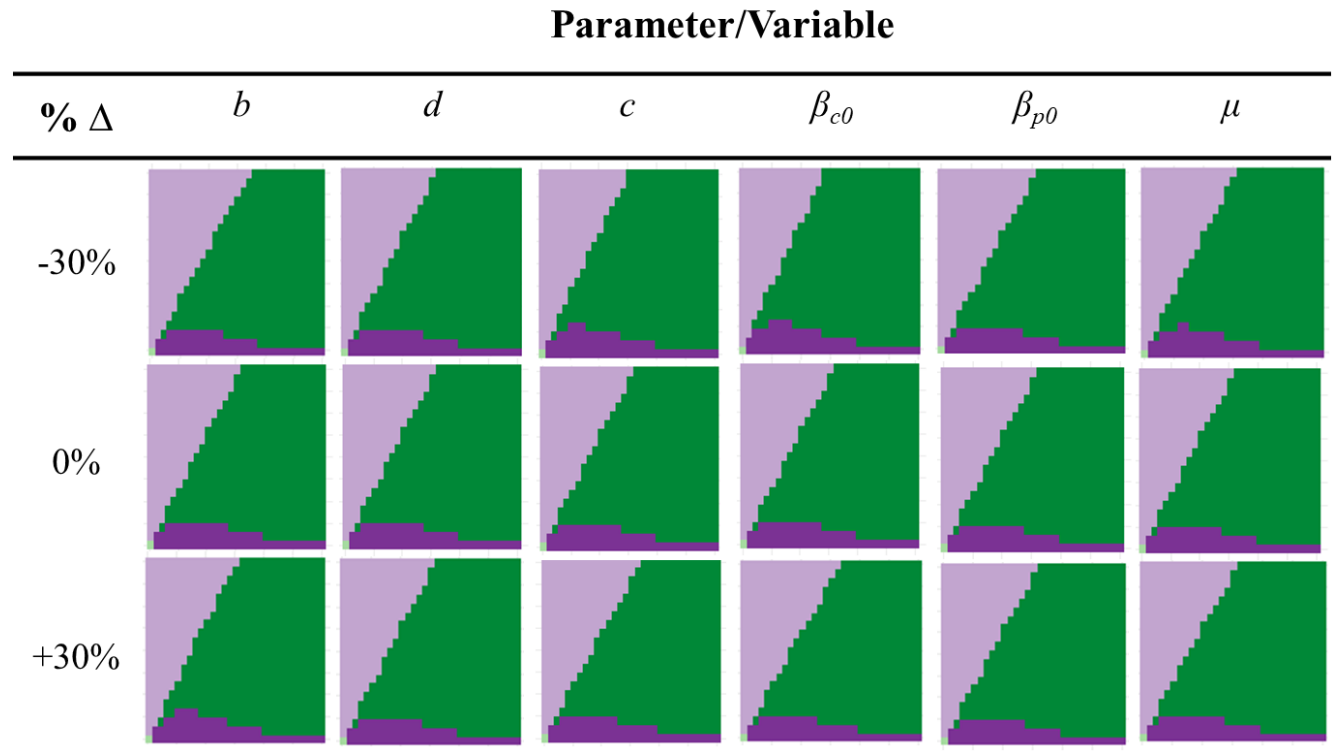


Table S5. 3 Results of elasticity analyses where the effect of competitor density on contact rates, β_c , (ψ), was kept at zero (scenario in center of Figure 5.3). Parameters and variables were changed to document the percentage change in non-monotonic outcomes compared to their fixed values. Red signifies increase, blue decrease, and gray no change.

% Δ	β_{p0}	β_{c0}	c	b	d	μ
+30	0	-1.70	-2.20	0.48	0	0.24
+10	0	-0.96	-0.96	0.24	0	0
-10	0	0.71	0.48	-1.21	0	0
-30	0	3.01	1.41	-3.46	0	0

Table S5. 4 Results of elasticity analyses where the effect of competitor density on contact rates, β_c , (ψ), was kept at zero (scenario in center of Figure 5.3). Parameters and variables were changed to document the percentage change in dilution outcomes compared to their fixed values. Red signifies increase, blue decrease, and gray no change.

% Δ	β_{p0}	β_{c0}	c	b	d	μ
+30	0	4.47	5.52	-3.64	0	-0.59
+10	0	2.29	2.29	-1.18	0	0
-10	0	-2.40	-1.18	2.84	0	0
-30	0	-11.76	-5.56	8.56	0	-1.18

Table S5. 5 Results of elasticity analyses where the effect of competitor density on contact rates, β_c , (ψ), was kept at zero (scenario in center of Figure 5.3). Parameters and variables were changed to document the percentage change in amplification outcomes compared to their fixed values. Red signifies increase, blue decrease, and gray no change.

% Δ	β_{p0}	β_{c0}	c	b	d	μ
+30	0	-1.69	-1.69	6.25	0	0
+10	0	0	0	1.64	0	0
-10	0	1.64	0	0	0	0
-30	0	7.69	0	-3.45	0	3.23