BEHAVIORAL ECOLOGY OF *ORYZOMYS PALUSTRIS* (MARSH RICE RAT) IN COASTAL TEXAS: IMPLICATIONS FOR THE LIFE-HISTORY STRATEGY OF BAYOU VIRUS (BAYV)

by

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PHILOSOPHY

"When we are motivated by goals that have deep meaning, by dreams that need completion, by pure love that needs expressing -- then we truly live life."

~ Greg Anderson

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ABSTRACT

Prior to the 1990s, Hantaviruses (Family: Bunyaviridae) and their manifest human syndromes were viewed as public health concerns primarily for Eurasia. However, the Sin Nombre virus (SNV) outbreak of 1993-94 in the southwestern U. S. brought Hanta to North America's front door, fueling intense global efforts by mammalogists, ecologists, virologists, and epidemiologists to identify and characterize other potential, host-hantavirus associations. Since then, more than 21 pathogenic, hantaviral genotypes have been discovered that can induce human hantavirus pulmonary syndrome (HPS), with symptoms ranging from proteinuria to pulmonary edema to massive exsanguination, with up to 40% of clinical cases ending in death. Over time, researchers have linked specific large-scale, ecological disturbances (e.g., climate change) and habitat conversion to increases in rodent host abundances with concomitant increases in HPS cases.

However, the factors involved in maintenance of hantavirus seroprevalence levels in host populations at finer scales have proven elusive, as rodent phenotypic responses to environmental context and sympatric species can drive infection patterns, and these relationships are usually non-linear and often extremely complex.

The marsh rice rat (*Oryzomys palustris* Harlan, 1837) is the primary host for the hantavirus genotype Bayou (BAYV; discovered in 1994), the second-most common genotype in N. A. and so far, it is responsible for HPS cases in Louisiana and Texas. *O. palustris* has the capacity for life longevity and distance dispersing, and is

an integral marshland mammal, performing important roles in the riverine and coastal marsh, trophic webs along the Atlantic Coast and the northern Gulf of Mexico. Although macro- and microhabitat preferences of Texas *O. palustris* have been clarified, we know little of its intraspecific or heterospecific social behaviors or other life history traits that may either accelerate or decelerate BAYV dissemination in a rodent community, having resultant ramifications for human BAYV infections.

Therefore, the essence of this dissertation is to advance the general understanding of BAYV occurrence and perpetuity in a terrestrial, small mammal community in coastal Texas, through a multi-scaled evaluation of community biodiversity, habitat composition, and rodent population demography and movements, in order to garner a better understanding of the life history strategy of BAYV.

Unique approaches to describe the dynamical relationships of *O. palustris* and BAYV involved: (1) a melding of socioecological theory and GIS, and (2) the application of several robust regression techniques and The Dilution Effect. Using small mammal mark-recapture and harvest methods, specific emphases have been placed on filling research gaps in the transmission paradigm regarding: (1) the roles of breeding versus non-breeding females as potential infection drivers/mediators between seropositive and socially dominant, adult male *O. palustris*; (2) a possible "dilution effect" in this system, via a closer examination of the relative contributions of rodent species evenness, species richness, and true density of hosts on BAYV seroprevalence; and (3) discerning and describing infection costs in non-primary (male juveniles and subadults, and females of all age classes) *O. palustris* hosts by examining morphology, physiology, and behavior.

Contributions to the scientific community are as follows: (1) the first hantavirus study to investigate and show support for habitat selection in receptive females as a contributing factor to the spatiotemporal arrangement of socially dominant, seropositive males; (2) the first hantavirus study to parse out diversity effects on seroprevalence into species evenness and species richness and by using this approach, I determined that in this system, it is species richness (and not species evenness) that is the most reliable predictor of reducing BAYV seroprevalence in the rodent community; (3) the first empirical study to rigorously evaluate hantavirus infection costs in female and immature rodents, and to uncover that it is the females in all age groups, non-pregnant and pregnant, and their embryos and fetuses, which are negatively impacted by BAYV infection; and (4) the only robust dataset known to provide insights into rare life history information for Texas *O. palustris* populations.

For the global community, the value of this research lies in its illumination of potential evolutionary trade-offs in BAYV host selection, infection, and fitness, in which all are strongly influenced by habitat characteristics. With a clearer understanding of the life history strategy of BAYV and the behavioral ecology of Texas *O. palustris*, we are now better equipped to forecast potential BAYV outbreaks in human population centers along the Atlantic coastline and the northern Gulf of Mexico, areas crucial to the U. S. economy, but also some of the most fragile and abused habitats in North America.

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LIST OF ABBREVIATIONS

ADK	adaptive kernel density
ANDV	Andes virus
ANOVA	analysis of variance
ASL	above sea level
BAYV	Bayou virus
BCCV	Black Creek Canal virus
BSL3	Biosafety Level 3
CDC	Centers for Disease Control and Prevention
cDNA	complementary DNA
CDGV	Caño Delgadito virus
CI	confidence intervals
CV	cross-validation (bootstrap)
DBS	Department of Biological Sciences
DE	Dilution Effect
D.F.	degrees of freedom
DF	discriminant function
GCIW	Gulf Coast Intracoastal Waterway
GIS	Geographical Information Systems
GPS	global positioning system
HCPS	hantavirus cardiopulmonary syndrome
HFRS	hemorrhagic fever with renal syndrome
HPS	hantavirus pulmonary syndrome
IgG	immunoglobulin G
JHWMA	Justin Hurst Wildlife Management Area
LDA	linear discriminant function analysis
LNV	Laguna Negra virus
MANOVA	multivariate analysis of variance

MCP	minimum convex polygon
N. A.	North America
NE	nephropathia epidemica
NSRL	Natural Science Research Laboratory
O. palustris	*Oryzomys palustris*
P. maniculatus	*Peromyscus maniculatus*
PLS	partial least squares
PLSR	partial least squares regression, or projection on latent structure regression
PPWMA	Peach Point Wildlife Management Area
PUUV	Puumala virus
RMSEP	root mean squared error of prediction
RNA	ribonucleic acid
SEM	socio-ecological model
simPLS	simple partial least squares regression
SNV	Sin Nombre virus
TPWD	Texas Parks and Wildlife Department
TTU	Texas Tech University
U. S.	United States
VC	vagina closed
VO	vagina open
vRNA	viral RNA

CHAPTER I

INTRODUCTION AND OVERVIEW

Hantavirus background

Hantaviruses constitute a distinct genus (*Hantavirus*) of at least 39 antigenically and phylogenetically related viruses in the family *Bunyaviridae*; this family is comprised of more than 300 viruses in five genera, all characterized by lipid-enveloped, negative-sense, single-stranded RNA viruses with a tripartite genome (Klein and Calisher 2007), which encodes four structural proteins: the nucleoprotein (N), two glycoproteins (G1 and G2), and a viral polymerase. Unique to the other *Bunyaviridae* genera, hantaviruses are transmitted not by arthropod vectors but primarily by rodents and shrews; since 2007, moles and bats also have been implicated as infection reservoirs, sparking some rather important questions about host associations and adaptations, and the evolutionary history and trajectory of hantaviruses.

In Europe and Asia, Old World hantaviruses manifest as hemorrhagic fever with renal syndrome (HFRS) whereas in the Americas, New World hantaviruses cause hantavirus pulmonary syndrome (HPS), sometimes referred to as hantavirus cardiopulmonary syndrome (HCPS). HFRS encompasses several disorders, those formerly known as Korean hemorrhagic fever, epidemic hemorrhagic fever,

hemorrhagic nephrosonephritis, nephropathia epidemica (NE), a mild form of HFRS, and several other vernacular and area-specific names. HPS cases caused by the viruses Bayou (BAYV; Morzunov et al. 1995, Torrez-Martinez and Hjelle 1995), Black Creek Canal (BCCV; Ravkov et al. 1995), and Andes (ANDV; López et al. 1996) involve a higher incidence of renal failure and myositis, similar to characteristic symptoms of HFRS (Peters and Khan 2002). These hantavirus genotypes of North and South America are carried by sigmodontine rather than by peromyscine rodents.

Globally, as of 2012, more than 21 pathogenic, hantaviral genotypes have been discovered that can induce human symptoms ranging from proteinuria to pulmonary edema to massive blood loss with up to 40% of confirmed cases ending in death. More hantaviruses are likely to be identified because in many countries, hantaviral infections go undetected and/or unreported, especially in Africa, the Middle East, and subcontinental India (Jonsson et al. 2010). Excepting the Sin Nombre virus-*Peromyscus maniculatus* (deermouse) system indigenous to the southwestern U. S., for many of the documented hantaviral species, little information is available regarding their prevalence, geographic distribution, host ecological associations, or human disease potentials. And while hantaviruses themselves are not of recent origin (there is evidentiary support for their problematic presence from at least 1000 years ago; McKee et al. 1991), ecological drivers and outcomes of their re-emergences appear to be phenomenologically new.

Life history strategy of *Hantavirus*

Vectors of disease agents more easily facilitate intra- and interspecific transmission for hosts; however, their non-inclusion within this particular virus-reservoir system probably compelled hantaviruses to develop highly specialized host ranges. Therefore, the evolution of hantaviruses has been and continues to be strongly influenced by the ecological, genetic, and behavioral factors that form host populations. Topologically, hantaviruses and their rodent and insectivore hosts display similar phylogenies. Nevertheless, by applying cophylogenetic reconciliation analysis, nucleotide substitution rates, and TMRCA (time to most recent common ancestor), a more plausible explanation for what has appeared as cospeciation, is more likely preferential host switching events, dictated by geographical proximity and host-specific adaptation (Ramsden et al. 2009). Through a lengthy and sustained process, each hantavirus strain has adapted itself to the internal microenvironment of one (or a few closely related) rodent or insectivore species (Nemirov et al. 2004). A hantavirus that infects and exploits the predominant host species within a given ecosystem, consequently, has a better survival chance than would a non-selective virus or a virus that infects a rarely encountered or spatially disjunct rodent species; host specificity is, undoubtedly, an essential element of viral life-history strategy. And yet, because non-vectored microparasites display moderately low rates of transmission (due to a dependence upon local distributions of host populations) compared to those of flying, jumping, or even "sit-and-wait" (transported) vectors, both indirect (via environmental contamination) and direct (via aggressive interactions) modes of hantavirus

transmission among hosts are considered relatively inefficient methods of virus delivery and dissemination. So, how might BAYV and other hantaviruses compensate for perceived transmission shortcomings?

Extended phenotypes in the Bunyaviridae

Viruses of the Bunyaviridae infecting plants and animals can modify the behavior of their vectors. Like most members of the Bunyaviridae, Tospoviruses are amplified in their vectors and transmitted in the vector**'**s saliva during feeding (Elliott 1996). Tomato spotted wilt virus (genus *Tospovirus*) induces increased "probing" (analogous to biting), a feeding behavior, in its infected male vector, *Frankliniella occidentalis* (western flower thrips) resulting in enhanced virus transmission (Stafford et al. 2011). Increased biting rates are hallmark features in mosquito vectors infected with La Crosse virus, an Orthobunyavirus (Grimstad et al. 1980), and Rift Valley fever virus (Phlebovirus; Turell et al. 1985), both animal-infecting members of the Bunyaviridae. Evolutionarily, behavioral alteration of vectors may be a conserved trait within the Bunyaviridae extending across multiple genera (Stafford et al. 2011).

Behavioral modifications of hosts, such as inducing more frequent biting, maybe a plesiomorphic condition in the bunyaviruses, maintained because it provides a selective advantage by increasing virus dissemination, as virus is shed in salivating males. Seoul virus, another Bunyaviridae member, in *Rattus norvegicus* (Norway rat) males produces increased testosterone concentrations and increased intermale aggressive expressions (Klein et al. 2004). Other social responses (e.g., dispersal

movements, and changes in host reproductive patterns) are other logical candidates to be harnessed by members of the Bunyaviridae for increasing propagation opportunities. Whether *Hantavirus* species besides Seoul virus can implement and sustain manipulative survival mechanisms on their host, at multilevel processes rarely has been intimated (Klein 2003), although seemingly feasible. Generally, parasites of vertebrate hosts induce changes in behaviors through neuronal apoptosis (Dietzschold et al. 2001), by interrupting neurotransmissions (Lancaster et al. 2007), or by influencing steroid synthesis in peripheral organs (Larralde et al. 1995).

The transmission paradigm: rodent-rodent and environmental contamination

Numerous studies have shown that persistently infected males intermittently shed virus in saliva, urine, and feces. Therefore, it is widely accepted that hantaviruses are maintained in nature through male-male antagonism (saliva-borne) principally and male scent marking (by way of urine/feces) of territorial boundaries secondarily. Durability of hantaviruses outside their mammalian hosts is unknown, although Hutchinson et al. (1998) and Kallio et al. (2006) have shown support for external survivability of Black Creek Canal virus (BCCV) and Puumala virus (PUUV), respectively. Comparably virulent RNA viruses transmitted normally by some form of social or physical contact have evolved the capacity for prolonged survival in liquid media, the soil strata, or forest litter, such as rabies virus and various arenaviruses and enteroviruses (Garnett and Antia 1994).

Adult males: the major traffickers of Bayou virus (BAYV)

Dominance traits (of morphology, physiology, and behavior) appear to correlate with and define well the adult male *O. palustris* infected with BAYV (Holsomback et al. 2013, McIntyre et al. 2005, McIntyre et al. 2009). For many species of rodents, seropositive male hosts are distinguished by a combination of high-ranking, dominance features, i.e. they often are older, larger, and heavier, have larger testes, higher androgen levels (Glass et al. 1988, Abbott et al. 1999, Calisher et al. 1999, Mills et al. 1999, Douglass et al. 2001, Nisbett et al. 2001, Klein et al. 2004), and perhaps greater sperm production, characteristics also associated with mate acquisition and mating success (Bronson 1989, Adkins-Regan 2005); furthermore, for infected aging males, reproductive potentials could be improved vastly as life expectancies increase. Attainment of advanced age in male *O. palustris* has been noted (Bloch and Rose, unpub. data, Negus et al. 1961, Edmonds and Stetson 2001). Thus, seropositive males might be more likely to breed and may do so more frequently than their subordinate, seronegative counterparts; in turn, viral occurrence would be amplified as more susceptibles are introduced into the population. Although the association between male age and size with hantavirus infection has not gone unnoticed (Luong et al. 2011), no adaptive evolutionary explanations have been proposed for the relationship. Natural selection may favor high-ranking males to perpetuate hantaviruses in the wild because metabolically and behaviorally, they present the most lucrative transmission opportunities. Moreover, for these dominant males, the state of seropositivity itself possibly may convey an increased fitness

advantage in terms of lifespan and reproduction (Michalakis et al. 1992, Herre et al. 1999, van Baalen and Jansen 2001).

Socioecological theory: the neglected role of females in intermale transmission

The socioecological model (SEM; Crook and Gartlan 1966) provides a theoretical framework to link environmental factors and social systems characteristics (Emlen and Oring 1977), allowing for predictions about group (or sex) associations as a function of resource distribution, types of competition, social organizations, and structure (van Schaik and Kappeler 2006; Dammhahn and Kappeler 2009). An assumption of the SEM is that the spatiotemporal scattering of females is determined predominately by the distribution of habitat resources and risks (Dammhahn and Kappeler 2009). As the dispersive sex in mammals, males seek out reproductively receptive females (Altmann 1990), so their juxtaposition should be based primarily on the spatial arrangement of those females (Clutton-Brock 1989).

The SEM has gone unnoticed as an applied framework for studying direct disease transmission in small mammals, like hantaviruses in rodents. Hantavirus researchers also have overlooked the importance of females and their impact on male movements and virus dissemination. Application of the SEM to the host-hantavirus interface may reveal previously unknown aspects of transmission and, thence, human disease risk.

Biodiversity and BAYV transmission: the dilution effect (DE) hypothesis

Global losses of biological diversity are accelerating at alarming rates, with potentially irreversible consequences for ecosystems to replenish and restore themselves, resulting in hugely diminished productivity for a wealth of societal services. Ecosystem provisioning of human services and health needs (e.g., recreational activities, food production, medicinal harvesting, water purification, and protection from floods and droughts) is greatly reduced as biological diversity vanishes (Chan et al. 2006). The ability of biodiversity to dissuade disease effects is undeniably an indispensable ecosystem service (Ostfeld and LoGiudice 2003). Complex ecological systems can exert a hindrance on the incidence and distribution of diseases in plants (Pagán et al. 2012), and in wildlife and humans (Dobson et al. 2006, Keesing et al. 2006). Additional species within a host community are predicted to deflect pathogen transmission away from the competent, primary carriers and to secondary, dead-end hosts (encounter reduction, a direct effect of species diversity; Keesing et al. 2006), or to decrease primary host density and their physical contacts, thereby reducing transmission rates (susceptible host regulation, an indirect effect; Suzán et al. 2009). Elucidation of the underlying mechanisms at work in this 'dilution effect' (DE) remain a challenging frontier for disease ecologists (Johnson et al. 2012); nevertheless, in each of the dilution scenarios, high diversity is correlated with low disease risk or incidence in the natural host, with analogous reduced disease effects for humans.

A handful of hantavirus researchers have searched for a DE at work in their system of interest by using essentially the same diversity metric (Simpson's Index or of that family) and the same statistical approach repeatedly, i.e. linear regression analysis (e.g. Mills 2006, Dizney and Ruedas 2009, Suzán et al. 2009); for the most part, their results show a negative relationship between mammal diversity and seroprevalence (with and without statistical significance), thereby supporting the DE hypothesis. Recently however, the DE as a generalizable tenet in the transmission ecology paradigm of zoonotic diseases (and as a sweeping, biodiversity conservation tactic) has come under question (reviewed in Randolph and Dobson 2012): a meta-analysis revealed a standard normal distribution of *P*-values under the null from 16 studies, and a noticeable publication bias by researchers for reporting negative results almost exclusively (Salkeld et al. 2013).

Infection costs in secondary, *Oryzomys palustris* hosts

In the mid 1990s, several studies provided evidence that allowed the re-examination of the assumed "no effect" paradigm of hantaviral infection in rodents, while at the same time scrutinized infection outcomes in other cohorts arranged by sex and age. A southeastern Arizona study (Kuenzi et al. 1999) revealed Sin Nombre virus (SNV) antibody-positive brush mice (*Peromyscus boylii*) of both sexes had shorter lifespans than did antibody-negative conspecifics. Douglass et al. (2001), while conducting research on SNV in Montana deer mice (*P. maniculatus*), noted that survival rates of infected juveniles and subadults were lower than survival rates of

naive rodents in the same age classes. More recently, Luis et al. (2012) definitively showed that the "no effect" paradigm was no longer tenable, with more than 13% of Sin Nombre virus antibody-positive males exhibiting decreased survivorship.

Most recently, two studies of SNV-infected *P. maniculatus* afford unique insight into the rarely explored subject of unexplained or dismissed anomalies in infected rodents. Douglass et al. (2007) reported individual and population-level effects that may be linked to seroprevalence in rodent populations: recent seroconverters showed less weight gain compared with seronegatives, and seropositives had shorter lifespans than did seronegative mice. Lehmer et al. (2007) validated Douglass's field data with laboratory evidence linking lowered immune responses to overall diminished health seen in the antibody-positive male deer mice in the Douglass et al. (2007) study.

The opinion has been that because of the inferred pattern of codivergence, hantaviruses cause no detectable adverse effects in their rodent hosts with regard to the physiological processes governing reproduction, fecundity, longevity, or behaviors (Schmaljohn and Hjelle 1997, Bernshtein et al. 1999, Hjelle and Yates 2001). However, several lines of evidence indicate the contrary: a) the assumed pattern of long codivergence was debunked recently (Ramsden et al. 2009) and b) ample evidence suggests that in several hantavirus/reservoir systems, viral infection carries a fitness cost in life history traits (Kuenzi et al. 1999, Douglass et al. 2001; 2007, Luis et al. 2012, Holsomback et al., in review).

Dissertation essence and objectives

The importance of factors associated with the occurrence of hantavirus infection in natural rodent host populations is extremely complex. Because of a multitude of transmission availability constraints that are contingent upon environmental variables that in turn affect host demography, interactions, and movements, I hypothesize that hantaviruses have evolved the capacity to harness host physiology and behavior differentially by sex to balance maximized transmission probabilities with minimized risks of host (and thus viral) extinction. Until the mechanisms maintaining circulation of these viruses in wild hosts are fully understood, predictive modeling of disease outbreaks in human communities will remain problematic if not unattainable altogether.

Objective #1

To advance the understanding of BAYV occurrence and perpetuity in a terrestrial, small mammal community in southeastern Texas, through a detailed examination of the multidimensional effects of biodiversity, landscape structure at various spatial scales, rodent population demographies and movements, and viral life history strategy theory. Particular emphasis will be placed on filling research gaps in the BAYV transmission paradigm regarding the following:

A. *The roles of breeding versus non-breeding females as potential infection drivers/mediators between adult male* Oryzomys palustris*;*

B. *The possible existence of a dilution effect in this system, via a closer examination of the relative contribution of species evenness and species richness on BAYV maintenance; and lastly; and,*
C. *The potential costs of infection to non-primary* O. palustris*, i.e. juveniles, subadults, and adult females by examining morphology and behavior.*

Objective #2

To report the results gleaned (thus far) from a multiyear dataset, expanding the current sparse and conflicting knowledge base on the population ecology of O. palustris.

Finally, I will address the overarching question, "If natural selection has favored adult male *O. palustris* for the role of BAYV propagators in the wild, then is there an inherent trade-off when incidentals (i.e. female and young marsh rice rats) become infected?"

Study Site: Justin Hurst Wildlife Management Area (JHWMA)

History, Description, and Selection

The JHWMA (formerly Peach Point WMA) is a constituent of the Central Coast Wetlands Ecosystem Project, whose foremost objective is "to provide sound biological conservation of all wildlife resources within the central coast of Texas ... "

(http://www.tpwd.state.tx.us/wma/). Their secondary objective is to develop and manage habitats for indigenous and migratory wildlife species. Through the late 1800s, portions of JHWMA (known then as the Durazno Plantation) were used for cultivating cotton and sugar cane. Domestic cattle also occupied much of the lands during this time and into the early 20th century; today, a small portion of the land continues to be used for cattle grazing. Eventually, the Texas Parks and Wildlife Department acquired a tract of land known as Peach Point Plantation from The Nature Conservancy. Although mainly a refuge for waterfowl, JHWMA represents managed and manipulated state territory, as it experiences periodic prescribed burns and mechanical treatments to facilitate waterfowl hunting and cattle grazing. Other than the exceptions for Special Permit hunts and scheduled tours, portions of the park are open year-round to the public. Additionally, free-roaming populations of feral swine exacerbate existing ecological conditions caused by frequent anthropogenic disturbances in some park areas.

Our study was conducted at JHWMA (UTM: 15-3202562-262435; Figure A.1.) from March 2002 through May 2004. Representative of the Gulf Coast Prairies and Marshes Ecoregion, JHWMA is located approximately 100 km south of Houston, encompasses 4174.5 ha (10,315 acres), and is bordered southeasterly by the Gulf Coast Intracoastal Waterway (GCIW). Topographically, the landscape is relatively flat and low (0 – 5 m ASL), with clay soils composed of low-lying assemblages of brackish to saline coastal prairies, grading farther inland to upland habitats of freshwater marshes and old-fields with some trees (McIntyre et al. 2005). The area

also experiences episodic tropical storms, resulting in inundation of areas near the GCIW. Precipitation occurs throughout the year (with 60% falling between April and September), and average precipitation is 133.35 cm/yr (data from the National Oceanic and Atmospheric Administration Freeport 2NW weather station, located ~10 km from JHWMA; http://www.noaa.gov/). Average high temperatures range from 18°C in winter to 33°C in summer, with average lows above freezing (8°C) even in winter (McIntyre et al. 2005).

While conducting a study of the geographic distribution and natural host range of hantaviruses in Texas, Mantooth et al. (2001) uncovered a seroprevalence rate of 55.6% (using the highly cross-reactive Caño Delgadito virus; CDGV), the highest in their study, among *O. palustris* at JHWMA. This study site not only is illustrative of regional ecological diversity but also it sustains multiple populations of *O. palustris* with varying seroprevalencies.

Discovery of Bayou virus (BAYV); assignment of putative rodent host

Since its discovery in 1994 (Khan et al. 1995, Morzunov et al. 1995), BAYV has been implicated in several HPS cases from Louisiana and Texas. Although Bayou was described clinically in 1993 from a genetic sequence of cDNA amplified from lung tissue of a fatal human case from northern Louisiana (Torrez-Martinez and Hjelle 1995), the putative host remained elusive until 1997, when BAYV infection was detected in low numbers of *Oryzomys palustris* collected from Jefferson County, Texas (Torrez-Martinez et al. 1998). Using these data, Torrez-Martinez et al. (1998)

uncovered the reservoir relationship between the marsh rice rat (*O. palustris*) and BAYV by serologically cross-confirming rodent samples with BAYV-HPS patient tissue samples. Retrospective analyses by Torrez-Martinez and Hjelle (1995) of archived rodent kidney and liver samples collected from southern Louisiana *O. palustris* confirmed that, in actuality, rice rats had harbored BAYV since at least 1983, and most likely represented its natural reservoir. Indigenous to the region, BAYV poses a potential threat to humans residing within its range, particularly in and around the highly populated cities along the coastline from southern New Jersey to south Texas.

BAYV rodent host: *Oryzomys palustris* Harlan, 1837 (marsh rice rat)

Description

The most diverse tribe of sigmodontine rodents, the Oryzomyini (Order Rodentia: Family Cricetidae: Subfamily Sigmodontinae) are endemic to the Western Hemisphere, distributed in the Neotropical and Nearctic (southeastern section) regions from Tierra del Fuego to the southeastern U. S., in the Galápagos Archipelago, and on Trinidad and Tobago (Weksler and Percequillo 2011); extinct forms of oryzomyines are found on several Caribbean islands (Turvey et al. 2010). At present, 33 extant and extinct genera (with three undescribed genus-group taxa) and 130 valid species are recognized (Weksler and Percequillo 2011).

The marsh rice rat (*Oryzomys palustris*) is a medium-sized (40-68 g), semi-aquatic, generalized rat (Svihla 1931). This species is found from southeastern Pennsylvania and southern New Jersey to the tip of Florida (excluding the Florida Keys), and westward to Corpus Christi, Texas (Wolfe 1982; Figure B.1.) although the northern limit of its range varies with fluctuations in population densities and is correlated to precipitation levels (Hall 1981). It is doubtful that *O. palustris* occurs throughout its original historical range, and particularly not in the northern Georgia foothills of the Appalachian Mountains or in southwestern Tennessee (Eubanks et al. 2011). Marginal records for the marsh rice rat have been reported for southern Kentucky and Illinois, southwestern Missouri, and southeastern Oklahoma (Wolfe 1982). In addition to its coastal distribution, the marsh rice rat appears to be distributed in association with portions of large rivers across the southeastern United States. (Its sylvatic and obligate water rather than suburban habitat preferences are likely the reason why human fatalities from BAYV infection have remained low.) In Texas, rice rats are distributed throughout the east, west to Brazos County and into the deep south of two counties, Cameron and Willacy (Davis and Schmidly 1994; Figure B.2.); however, prolonged drought conditions have likely significantly reduced rodent abundances within pockets of these areas.

Habitat

Although *O. palustris* primarily occupies herbaceous wetland habitats (Negus et al. 1961), it also occurs in adjacent woodlands (Hamilton 1946). Upland usage

could be related to population demography, environmental factors, predator avoidance, or prey availability. Marsh rice rats also may utilize adjacent uplands as dispersal corridors and for nesting and refugial sites to escape the periodic flooding in tidal marshes (Kruchek 2004, Abuzeineh et al. 2007). To sustain viable populations, *O. palustris* generally requires large, contiguous areas of wetlands or marshlands with dense, emergent wetland vegetation (e.g. sedge, reed, cattail, and bulrush), or moist woodland habitats with at least vernal lakes, ponds, pools, or a stream or river. In addition to macrohabitat occupancy, some microhabitat selection preferences have been detected in rice rats, as well (Kincaid and Cameron 1982, McIntyre et al. 2005, Eubanks et al. 2011).

Behavior

Robust home range size information for *O. palustris* is limited to one study (based on the inclusive boundary-zone method) from the Everglades (Birkenholz 1963), in which ranges of 0.33-ha for females and 0.25-ha for males were observed. Average range length was reported as 74.7 m from a small Maryland study (Harris 1953), and Pournelle (1950) recorded range lengths of 67.7 m (males) and 82.3 m (females) in northern Florida using short-term data. In southern Illinois (where *O. palustris* is endangered), a study using radiotelemetry and at least 5 capture locations per individual, indicated the home ranges of 7 males averaged 0.68 + 0.25 ha with 4 females averaging 0.82 + 0.38 ha (Hofmann and Gardner 1992).

Svihla (1931) and Hamilton (1946) noted the predisposition of rice rats to dive, swim, and disperse over water, making them resourceful aquatic, BAYV disseminators throughout their riparian and coastal habitats. Esher et al. (1978) further supports these aquatic behaviors in a laboratory setting, during which the tendency for rice rats to enter water and swim was about 20 times that of cotton rats (*Sigmodon hispidus*). Not surprisingly, rice rats have been described as fastidious mammals, exhibiting intense self- and cross-grooming behaviors, most likely to maintain the water repellant nature of their pelage (Wolfe 1982). Allogrooming, an activity linked with agonistic displays in a controlled setting (Christensen 1980, unpub. diss.), also could serve as another avenue by which BAYV is trafficked by *O. palustris* because virions are shed in host saliva (McIntyre et al. 2005).

Density

In 1993, Forys and Dueser reported average *O. palustris* densities of 18 rice rats/ha in May through July on the Virginia Barrier Islands, similar to average densities reported previously by Negus et al. (1961) in Louisiana, by Smith and Vrieze (1979) in Florida, and also Chamberlain and Leopold (2003) in Mississippi. Seasonal fluctuations in overall density of rice rats were minor in Kruchek's study from Galveston Bay (2004), with density in wetlands (10.5 individuals/ha) greater than density in uplands (3 individuals/ha). In contrast, seasonal variation in density of rice rats was marked in Virginia (e.g., from 3-8 individuals/ha in late winter, to 15-87 individuals/ha in late fall on 2 plots – Bloch and Rose 2005) and Mississippi (from 5

individuals/ha in spring, to 25 individuals/ha in late fall/early winter – Wolfe 1985). Negus et al. (1961) suggested that density of rice rats was closely tied to the severity and duration of winter.

Reproduction

Reproductive biology studies of *O. palustris* have not provided a consensus on the timing or duration of its breeding season. In Mississippi, the marsh rice rat breeds throughout the year, with its predominant breeding season occurring from late spring to late autumn (Wolfe 1985). One laboratory study implies a similar pattern of occasional, year-round breeding: Conaway (1954) witnessed continual breeding for a captive population of Tennessee rice rats. And yet, evidence of reproductive behaviors were not observed by Negus et al. (1961) in 2:3 winters during which his team trapped rice rats in Louisiana. Edmonds and Stetson (1993) reported a breeding season from late winter (March) to late summer (September) in Delaware, although the typical breeding season may extend later and into autumn, if environmental conditions are favorable. From Bloch and Rose (2005) in Virginia, rice rats were found to be more active reproductively in the summer (June – August) than in the winter (December – February) months. Svihla (1931) and Worth (1950) described February to October breeding seasons in eastern Texas and Florida, respectively. Two studies in the Galveston Bay Region of Texas reveal data suggesting that rice rats breed year-round there, with a possible breeding peak in the spring (Hice and Schmidly 1999, Kruchek 2004).

The gestation period for *O. palustris* is 21 to 28 days (Park and Nowosielski-Slepowron 1972) with an average of 25 days (Svihla 1931). Average litter sizes (acquired from laboratory colony females) are 5 (Conaway 1954) and 3.6 (Park and Nowosielski-Slepowron 1972. Wolfe (1982) noted 1-7 pups per litter across its geographic range, but mostly 4-6 in Texas populations of *O. palustris*. The young are weaned at around 11 days after birth, both sexes reach sexual maturity at 45-60 days, and they usually breed during their first summer if they survive (Schmidly 2004).

Survival

Although the sex ratio at weaning has been reported to be 1:1 (Park and Nowosielski-Slepowron 1972, Wolfe 1982), several studies have indicated a male-biased sex-ratio from wild populations of rice rats caught in highly varied geographic regions and across all seasons (Birkenholz 1963, Wolfe 1985, Kruchek 2004). Social and other behavioral effects on trap response are well documented for several small mammal species, however (Tanaka 1980). For the rice rat, higher numbers of captured males have been attributed to a lower survival rate for females or decreased trapability of females, especially when they are pregnant or caring for their young (Wolfe 1985), although there are discrepancies in estimated longevities for both female and male rice rats. In a tidal marsh in the Gulf of Mexico, a 4-year study by Wolfe (1985) revealed a mortality rate of about 80% approximately every three months, with no significant differences between sex or age classes; maximum longevity in the field was 20 months (Svihla 1931) to 24 months (Wolfe 1985).

Edmonds and Stetson (2001) found an estimate of life expectancy in the field for female rice rats at ≤ 1 year, and even shorter than that for males. Immigration, emigration, and death undoubtedly all contribute to fluctuations in abundances and to clear disappearances, as marsh rice rats may exhibit unpredictably high levels of mobility and mortality (Smith and Vrieze 1979, Wolfe 1985).

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CHAPTER II

SOCIOECOLOGY OF *ORYZOMYS PALUSTRIS* AND THE SPATIOTEMPORAL DISTRIBUTION OF BAYOU VIRUS IN COASTAL TEXAS

ABSTRACT: Along the southeastern U. S. coast, the marsh rice rat (*Oryzomys palustris*) is the primary host for the hantavirus genotype Bayou. According to the socioecological model (SEM), for a territorial, polygamous species, females should be distributed across space and time by habitat resources and predation risks, whereas males should space themselves according to the degree of female aggregation and reproductive synchrony. Our objectives were to describe the socioecology of adult *O. palustris* at multiple spatial scales, and to investigate specifically how females affect the male-male transmission paradigm of Bayou virus (BAYV). Across a 30-month Texas study, rodents were captured, marked, and released in two macrohabitat types, and microhabitat cover variables were quantified around individual trap stations. A geodatabase was created from habitat and rodent capture data and analyzed in a Geographic Information System (GIS). The ratio of breeding to non-breeding females was ~1:1, with breeding females overly dispersed and non-breeding females randomly dispersed. Spatial analyses revealed both macro- and microhabitat preferences in females. Compared to seronegatives, higher proportions of seropositive adult males were found consistently within closer proximities to breeding females but not to non-breeding females, indicating that male locations were not driven simply by habitat

selection. Activities to acquire dispersed receptive females could be an important driver of Bayou virus transmission among male hosts. The SEM has been applied primarily to disease cycles of primates and other larger-bodied, social mammals; it has received much less attention as an investigative framework, particularly coupled with GIS, to study pathogen dynamics in small, solitary mammals. Herein, we describe an interdisciplinary effort providing a novel approach to elucidate the complexity of hantavirus trafficking and maintenance in rodent populations of a coastal marsh ecosystem.

INTRODUCTION

Hantaviruses constitute a distinct genus (*Hantavirus*) of currently 39 antigenically and phylogenetically related viruses of the family Bunyaviridae (Klein and Calisher 2007). At least 21 hantaviral species are known to cause fatal outcomes in humans, ranging from pulmonary edema manifested in hantavirus pulmonary syndrome (HPS) to massive exsanguination resulting from hemorrhagic fever with renal syndrome (HFRS). Unique within the Bunyaviridae, hantaviruses frequently exploit species-specific male rodents (Order Rodentia: Family Cricetidae) rather than arthropod vectors. For many of the known hantavirus-rodent associations, complete characterizations are still lacking with respect to the ecological and biological processes and spatial scales that determine viability and variability in transmission potentials.

Rodents of the tribe Oryzomyini (family Cricetidae; subfamily Sigmodontinae) are endemic to the Western Hemisphere, ranging from the Argentine Patagonia through the Galápagos and Central America, throughout Mexico and the Gulf and Atlantic coasts of the southeastern U. S. Oryzomyine rodents display an array of morphological adaptations (Carleton and Olson 1999) that enable them to thrive across a spectrum of vegetation types (Eisenberg 1999), including mixed forests and fresh and salt water marshes to sedge-shrub habitats (Wolfe 1985). Recent work has clarified the structure of this tribe (Weksler 2006, Hanson et al. 2010), but issues still remain at the species level, hampering identification of other potential reservoirs in epidemiologic and phylogeographic studies, among others (La Salle et al. 2009).

In the U. S., the oryzomyine rodent known as the marsh rice rat (*Oryzomys palustris* Harlan, 1837) is the primary host for the hantavirus genotype Bayou, the second-leading strain responsible for HPS in the U. S. (Torrez-Martinez et al. 1998). The marsh rice rat occurs from southern New Jersey southwestwardly to Kansas-Missouri-Illinois, through coastal Texas and down into peninsular Florida. It favors riparian and wetland habitats, is strictly nocturnal, and prefers a carnivorous diet of fish, crustaceans, insects, and the eggs and young of marsh birds and turtles, but will supplement its dietary needs with omnivory based on seasonal conspecific densities and plant phenologies. Empirical support for both macrohabitat and microhabitat selection in *O. palustris* has been described previously (Kincaid et al. 1983, McIntyre et al. 2005; 2009). Although little is known of the social relationships and behaviors

of the marsh rice rat, it is believed to be solitary, aggressive, and territorial, contingent upon population density and resource availability.

Hantaviruses are transmitted among hosts chiefly via antagonistic male-male interactions (Glass et al. 1988). Factors controlling the frequency of such interactions are poorly understood, however. Moreover, the nature of transmission between the sexes is also unclear. Our understanding of the factors that maintain the virus in wild populations thus hinges upon a better understanding of the social and environmental forces that drive potential hosts together or apart.

The socioecological model (SEM; Crook and Gartlan 1966) provides a theoretical link between environmental factors and characteristics of social systems (Emlen and Oring 1977), allowing for predictions about associations as functions of resource distributions, types of competition, and social organizations, relationships, and structure (van Schaik and Kappeler 2006, Dammhahn and Kappeler 2009). Sex-specific features limit the fitness of females and males resulting from intersexual differences in parental investment (Trivers 1972). An assumption of the SEM is that the spatiotemporal scattering of females is determined predominately by the distribution of habitat resources and risks (Dammhahn and Kappeler 2009). As the dispersive sex in mammals, males seek out reproductively receptive females (Altmann 1990), so their juxtaposition should be based primarily on the spatial arrangement of those females (Clutton-Brock 1989).

Socioecological thought has been invoked to comprehend and minimize effects of cyclic patterns of pathogens transmitted indirectly and directly among humans

(Parkes et al. 2003) and other social primates (Wlasiuk and Nachman 2010), but mainly for sexually transmitted diseases. Within the constructs of the SEM, no thorough attempt has been made to explain variation in general regarding the social systems of solitary species (Dammhahn and Kappeler 2009); furthermore, it has gone unnoticed as an applied framework for studying direct disease transmission in small mammals, like hantaviruses in rodents. Hantavirus researchers also have overlooked the importance of females and their impact on male movements and virus dissemination. Application of the SEM to the host-hantavirus system may reveal previously unknown aspects of transmission and, thence, human disease risk.

Geographic Information Systems (GIS) are used in hantavirus studies mainly from a human epidemiological perspective: (1) in retrospective, public health reports; and (2) for surveillance, to forecast outbreaks by spatial mapping of various risk criteria, including rodent host densities, conversion of landscape characteristics, and climatic variables (Ostfeld et al. 2005, Glass et al. 2006). However, we are unaware of other studies where GIS and the SEM have been converged and applied at the scales and towards the goals we have set forth here. Therefore, our objectives were to investigate the socioecology of adult *O. palustris*, using a GIS to evaluate the spatial relationships of females and males as a function of habitat as well as intraspecific attraction. Moreover, we specifically wanted to explore the role of females as potential infection drivers/mediators between males, based on spatial receptivity patterns. We therefore assessed habitat selection in adult breeding and non-breeding females, which was then associated with the distribution of adult males.

MATERIALS AND METHODS

Study site

Seasonal characterization of habitat and rodents was conducted at the Justin Hurst Wildlife Management Area (JHWMA) (UTM: 15-3202562-262435), formerly Peach Point WMA, in Brazoria County, Texas (Figure A.1.) from March 2002 through May 2004. Situated ~ 60 km south of Houston, JHWMA comprises 4174.5-ha and southeasterly is bordered by the Gulf Coast Intracoastal Waterway. Clay soils describe the landscape which is fairly low and flat (0 – 5 m ASL), and is composed of low-lying assemblages of brackish to saline coastal marshlands that grade farther inland to freshwater marshes and mixed uplands with trees. Precipitation occurs year round (60% falls from April to September), and average precipitation is 133.35 cm/yr (from the National Oceanic and Atmospheric Administration Freeport 2NW weather station, located ~10 km from JHWMA; http://www.noaa.gov/). High temperature averages range from 18°C (winter) to 33°C (summer); even in winter, average annual lows are well above freezing (8°C).

Mark-recapture grids

Sampling protocols were approved by the TTU Animal Care and Use Committee (permit #01134BX). Collection permits were granted by the Texas Parks and Wildlife Department (permit #APR-0498-944 and #SPR-0504-381). Rodents were live-trapped on 4 mark-recapture rectangular grids 7100-7700 m^2 in size (traps placed at 10 m

intervals and numbers of traps ranged from 93-113, contingent upon coastline topography and flooding) in two macrohabitat types (upland and coastal marshland, each with 1 replicate grid) for 4-6 consecutive nights for each of 4 seasons during the 30-month study period (mid-March, late May, late August, mid-December; McIntyre et al. 2005) (Figure 2.1, Figure A.2). Captured rodents were marked with a unique identifier, i.e. by either toe-clipping, or by subdermal insertion of a Passive Integrated Transponder [PIT] tag (Biomark, Inc., Boise, ID). Data recorded for each individual rodent included capture status (new or re-capture), its unique identifier, species, trap station, age class (juvenile, subadult, adult), sex, weight (using a Pesola spring scale), and reproductive status (testes position: abdominal, inguinal, or descended; vaginal patency: perforate or closed; pregnancy status: pregnant, recent parturition, or lactating). Capture coordinates were recorded using a handheld global positioning system (GPS). Using a sterile Pasteur pipet, a blood sample of several drops (0.1-0.5 mL) was extracted aseptically from the retro-orbital sinus and delivered to a sterile cryovial, after which the animal was released at the site of capture.

Anti-BAYV IgG determination by immunofluorescence microscopy (IFA)

Antibody detection assays using rodent blood tissues were conducted at the Southern Research Institute in Birmingham, AL, and adhered to CDC and BSL3 guidelines. Protocols were developed and validated previously (Chu et al. 1995; 2003). Stepwise details are in McIntyre et al. (2005).

Microhabitat composition

Based on capture, recruitment, and seropositivity rates being higher during one particular period on one trapping grid than on the other three grids in any other month, microhabitat selection analyses are from this month and grid (Grid 3, Aug. 2003), located within coastal marshland macrohabitat. However, the patterns discussed also hold true in other locations and time periods, but with smaller representative sample sizes of adult males and females (Table 2.1). Microhabitat composition (percent ground cover of 10 mutually exclusive categories: grass, herbaceous, bare ground, tree, shrub, litter [duff], vine, coarse woody debris, water, other) was quantified in a 3-m-radius circle centered on each trap station (N = 111 trap stations for Grid 3, ~7700 m^2) following Bullock's methodology (1996). The identity of plant species within each of these categories was also determined. These categorizations were utilized further to determine and compare microhabitat selection of adult breeding and non-breeding *O. palustris* females.

Spatial relationships, GIS

Location coordinates for the habitat and capture data had been recorded previously in a Microsoft Excel spreadsheet. To bring the coordinates into the GIS, the x-y values were converted to decimal degrees, with latitude values being positive and longitude values being negative. The spreadsheet was imported into ArcMap 9.3 (ESRI, Redlands, CA, USA) as an event layer and inspected for missing data. After the quality control check, the event layer was exported to a file geodatabase (Natural Resources

Conservation Service (NRCS), Geospatial Data Gateway 2008; Soil Data Mart 2009; U. S. Census Bureau, Census TIGER 2000 Data; USGS Seamless Server, Brazoria County, Texas and National Elevation Dataset), and saved as a feature class. The main feature class was sorted into separate categories and analyzed according to trapping session, habitat, sex, age class, reproductive condition, and serostatus. Spatial relationships between adult *O. palustris* males and females were evaluated using the 'Select by Location' tool in ArcMap, which includes a buffer option that allows records to be selected at varying distances. Each male *O. palustris* was categorized according to the following location criteria (i.e., spatial scales) in relation to a breeding female: detection within the same grid; within 30 meters, and in the same trap.

Statistical analyses

Spatial data were analyzed using the built-in statistical software in ArcToolbox to determine the spatial distribution of the sexes by reproductive status (for females) or serological status (for males). Chi-square Goodness-of-Fit tests were used to determine differences: (1) in microhabitat use versus availability for adult females by reproductive condition; and (2) between serological status of adult males and proximity to receptive females. The χ^2 tests were run using the function 'prop.test' (with the default continuity correction) in R version 2.2.1 (R Development Core Team 2005).

RESULTS

Macrohabitat description

From the vegetation and soil layers (Figure 2.1.), the upland grids (Grids 1, 2) are both in Bluestem Grassland upland habitat, with Grid 1 composed of Pledger Clay and Grid 2 composed of Asa Silty Clay Loam. Grids 3 and 4 are in Coastal Marshland ('Marsh Barrier Island'), both comprised by Harris Clay. Most recruitment of *O. palustris* young and immigrant adults occurred in the coastal marshland grids, with adult females detected more frequently in the Harris Clay-saltmarsh habitats (Table 2.1).

Microhabitat description

Because each trap station had both GPS coordinates and habitat data, these could be linked and brought into the GIS for spatial analysis (Figure 2.2). Thus, 90:111 trap stations were characterized by >75% grass cover (several species), with the remaining cover made up of sedge (*Carex* sp.). Going in descending order: 12 trap stations were characterized by 50-75% grass/remainder sedge; 3 trap stations described by 50-75% grass/remainder water; 3 trap stations described by 25-50% grass/remainder sedge; 1 trap station described by 25-50% grass/remainder water; 1 trap station with 50-75% grass/remainder saltwort (*Batis maritima*), and 1 trap station with >75% grass cover/remaining cover was reed (*Juncus effusus*). The most common plant species on Grid 3 (in descending order) were saltmarsh grass (*Distichlis spicata*), saltwort (*Batis maritima*), wiregrass (*Spartina patens*), and bulrush (*Scirpus robustus*).

Female microhabitat selection by reproductive condition

In a GIS, one can run a 'Location Query', use a 'Select by Location' tool, buffer the feature class of interest, and visualize the data (the steps followed in this section and the next, Figure 2.3 and 2.4, respectively). In Figure 2.3, non-receptive females (denoted by pink circles) primarily occupy habitat dominated by >75% grasses and ≤24% sedge. Buffering the layer containing the receptive females (purple circles) revealed that they utilize slightly more diverse areas: the >75% grass /≤24% sedge, but also the 50-75% grass/25-50% water, and >75% grass/≤24% reed traps. Nevertheless, χ^2 results indicated no significant association in female microhabitat selection by reproductive condition when compared to an equal number of randomly selected traps ($\chi^2 = 0.05$, $P = 0.819$). When the spatial-habitat data for both groups are compared (Figure 2.3, center), some overlap is noted between the two and also something interesting: apparent non-use of the grid interior and thus possible avoidance of the 50-75% grass/25-50% sedge cover (dark blue squares). Currently, the significance of this finding is unknown, but could be related to predator avoidance behavior.

Spatial relationships of females (based on breeding status) to males (based on serostatus)

For this analysis, breeding (vagina open; VO) females were buffered to include at least one seropositive male (Figure 2.4). Other results (not shown) are that no seropositive males were located within 30 m of a non-breeding (vagina closed; VC) female, and no seronegative males were found within 30 m of a breeding female. The

lack of opaque circles on Grid 4 is due to the absence (or our inability to detect the presence) of seropositive males there during the August 2003 trapping period. The fact that no seropositive males were captured on Grid 4 in August 2003 could be explained by low *O. palustris* population abundance generally, and by the presence of only one breeding female specifically.

Proportion of breeding to non-breeding females, degree of spatial clumping

We noted breeding throughout the year (Goldman 1918, McIntyre et al. 2005). An almost 1:1 ratio in terms of breeding and non-breeding females was revealed from both macrohabitats and on all 4 grids (data not shown). From the Nearest-Neighbor Ratio (Table 2.2), there was a random distribution for non-breeding females ($P = 0.7068$), and significant overdispersion of breeding females ($P = 0.0002$).

Association between male serostatus and proximity to a receptive female

At weaning, the sex ratio of *O. palustris* is roughly 1:1 (Park and Nowosielski-Slepowron 1972). We had a somewhat male-biased sex ratio in adults (1.8:1); in nearby Galveston, Texas, Kruchek (2004) noted strongly male-skewed, adult populations (2.3:1). Although adult males were 2.5x more likely to be seronegative than seropositive, χ^2 analysis results indicated a significant association between male seropositivity and spatial proximity to a breeding female (Table 2.3; data pooled for analysis to reduce the probability of an inflated Type 1 error). Compared to seronegatives, seropositive males were more frequently associated with receptive females by grid ($\chi^2 = 6.25$, $P = 0.012$),

within a 30-m radius ($\chi^2 = 9.84$, $P = 0.002$), and occupying the same trap ($\chi^2 = 11.57$, $P = 0.001$).

DISCUSSION

Although our study focused on a grass-dominated, coastal marshland macrohabitat, where only 4 out of 111 trap stations were surrounded by <50% grass cover, microhabitat selection in females was noted that could explain the distribution of males as well as the distribution of BAYV as assayed by serological status. Seropositive *O. palustris* males have been reported from JHWMA to avoid grasses on a microhabitat scale, which was attributed to the increased ranging seen in seropositive males (McIntyre et al. 2009). However, the pattern of habitat selection in males could instead be due to the fact that receptive (i.e., breeding) females tend to occur in areas with greater microhabitat diversity (e.g. in the >75% grass /≤24% sedge, the 50-75% grass/25-50% water, and in the >75% grass/≤24% reed traps) rather than in the areas with the greatest grass cover (as was the case for the non-receptive females). Seropositive males averaged significantly larger home ranges than seronegative males at our study site (McIntyre et al. 2009). A male that maintains a large home range increases the probability his range will include a receptive female; if he monitors his range vigilantly, then he has a better chance of crossing paths and copulating with her (Tew and Macdonald 1994). In light of our study, male home range sizes by serostatus make sense: diffuse, breeding females (overdispersed spatial distribution) induce larger home ranges for seropositive males, who tend to be socially dominant,

larger, and/or older (McIntyre et al. 2009). Socially dominant, seropositive males also were more frequently associated with breeding females by grid, within a 30-m radius, and by trap station. This socioecological explanation of host distributional patterns, based on female receptivity and habitat preferences *rather than just* male aggression, is an original approach to distill the links between hantaviral maintenance and circulation in natural host populations.

From his coastal Mississippi study dated 1981-1984, Wolfe (1990) states, "Documenting details of the . . . environmental influences on the distribution of [marsh rice rat] individuals is needed to understand the factors facilitating survival and resource use in this habitat." These words might resonate more truthfully today. *Oryzomys palustris* has been called 'an ecosystem engineer' in coastal marsh systems (Wolfe 1982), because it is often the only small mammal to occupy almost exclusively this vital yet vanishing habitat. Its major roles and low functional redundancy in this trophic web (Chabreck 1988) are highlighted by efficient extraction and recycling of marsh resources (Wolfe 1982). Reservoir competence in *O. palustris* also has been demonstrated for multiple parasites of community assemblage importance (Morlan 1951, Barnard et al. 1971, Levin et al. 1995). Lastly but not exhaustively, its long-distance, frequent dispersal behaviors (Esher et al. 1978) provide a flexible adaptation to survive periodic storm surges (Abuzeineh et al. 2007), minimize competition and inbreeding depression (Loxterman et al. 1998), and produce founder and rescue populations in nearby island systems (Forys and Dueser 1993).

Evidence of *Peromyscus maniculatus* (deer mouse; principal host for Sin Nombre virus and Monongahela virus) in or near human dwellings has been shown to be an unambiguous, HPS risk factor in southwestern Colorado (Calisher et al. 2011) and elsewhere. Unlike *P. maniculatus*, *O. palustris* normally does not invade human dwellings or co-habit with humans; rather, its behavioral ecology is one of a semi-aquatic, solitary species. Its riparian marsh-grassland as opposed to suburbia occupancy is likely one reason why BAYV occurrence in humans has remained low; however, it is possible that at least some HPS cases caused by BAYV are misdiagnosed and therefore underreported, as the complex symptoms of BAYV-HPS can involve multiple body systems either sequentially or simultaneously (Hjelle et al. 1996). For the handful of BAYV-HPS cases identified clinically, there is insufficient evidence to determine whether or not infections were acquired through occupational or recreational exposure to *O. palustris* and/or their excreta. Nevertheless, among BAYV-HPS patients, two noticeable commonalities are outdoor activities (near permanent water and dense grasses) during which large amounts of airborne dirt were inhaled repeatedly over time, and also fishing in areas with household refuse and visible rodents (Hjelle et al. 1996, Torrez-Martinez et al. 1998). Minimally, Gulf Coast fishermen and landfill laborers could be at particular risk in areas where their activities fall within the habitat range and preferences of *O. palustris* (Wolfe 1982; 1985, McIntyre et al. 2009).

The remaining riverine wetlands and tidal marshes of the northern Gulf of Mexico and southern Atlantic Coast approach unprecedented levels of contamination,

degradation, and discontinuity (Ketchum 1972, Bartlett and Smith 2005). The decay of this ecoregion is not expected to decelerate, further complicating hantaviral disease projections based on typical rodent life history traits and patterns, including preferential habitat uses, dispersal distances, and Hamiltonian sex ratios. It is risky to attempt response predictions of the marsh rice rat to continual loss of its natural habitats and looming "coastal squeeze" (Schleupner 2008). Nevertheless, if a change in population biology results in marsh rice rats subjected to artificial co-existence with dense populations of humans, then more cases of BAYV infection should be anticipated, especially where rodent densities are abnormally high (e.g., in remnant, high quality patches), and when male rodents travel and fight for breeding rights to spatially segregated, receptive females.

Although hantaviruses are not of recent origin (there is evidence for their problematic presence from at least 1000 years ago; in McKee et al. 1991), both drivers and outcomes of their "re-emergences" appear to be phenomenologically new. Deciphering the enigmatic conditions of how and why ancient hantaviruses only recently have become of epizootic importance has not proven easy. Regardless of the underlying mechanisms, rodent phenotypic and viral genetic plasticity coupled with anthropogenic landscape alterations can be expected to increase the liability and geographic scope of human hantavirus afflictions in the future. For example, shifts in host ecology are accompanied by compensatory shifts in virus ecology (owing to vicariance) and sometimes result in environmental release of more pernicious viral subtypes to humans following molecular reassortment events (Dragoo et al. 2006). To

disentangle these complex, non-linear disease transmission dynamics, we must employ all the techniques available. If the techniques are inadequate, new ones should be invented. Equally important, it might be fruitful to apply established theories and practices in a fresh way. With this, application of the sociecological framework and geographical information systems might provide powerful prediction tools for human hantavirus exposure and infection risk across coastal regions of North America and elsewhere.

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Table 2.1. Demographics of four populations of *O. palustris* at Justin Hurst WMA from May 2002 – May 2004. (In March 2002, pilot study did not include grid trapping, but see bottom of table for harvest trapping results.)

Grid	**1**			**2**			**3**			**4**		
Demography	**J/S**	**A**	**+**	**J/S**	**A**	**+**	**J/S**	**A**	**+**	**J/S**	**A**	**+**
May 2002	0	5	1	0	3	0	8	25	8	1	4	1
August 2002	0	4	0	0	3	0	4	12	0	5	15	0
December 2002	8	14	0	2	25	1	0	14	4	0	19	5
March 2003	1	4	0	4	5	1	5	8	6	2	6	0
May 2003	1	5	2	0	1	1	13	20	8	5	4	1
August 2003	0	2	2	1	2	0	14	40	11	3	8	0
May 2004	1	0	0	0	2	1	* N/A			6	16	2

Grid: 1, 2 - Bluestem Grassland (upland); 3, 4 - Coastal Marshland.

Demography: J/S = juvenile/subadult; A = adult; + = anti-BAYV IgG antibody positive.

March 2002: Harvest trapping conducted only (4 J/S, 37 A, 8 +).

* In May 2004, Grid 3 closed due to extreme coastal flooding.

Table 2.2. Results of K-parameterization, a measure of spatial aggregation, for non-breeding (vagina closed) and breeding (vagina open) females on Grid 3, August 2003. Equal sample sizes (total N = 26). Asterisks indicate level of statistical significance ($P \leq 0.0005$).

Vagina closed (non-receptive)		**Vagina open (receptive)**	
Observed mean distance	0.0014	Observed mean distance	0.0024
Expected mean distance	0.0015	Expected mean distance	0.0016
Nearest neighbour ratio	0.9536	Nearest neighbour ratio	1.4945
Z score	-0.3761	Z score	3.6637
P-value	0.707 (randomly distributed)	P-value	<0.001 **** (overly dispersed)

Table 2.3. Chi-square goodness-of-fit results for the association between male seropositivity and spatial proximity to a breeding female on the coastal marshland grids (3, 4) for study period (2002-2004).

	Location: same grid	**Location: ≤30 m**	**Location: same trap**
Sample size (n) by serostatus	(n) Neg = 73 (n) Pos = 31	(n) Neg = 39 (n) Pos = 22	(n) Neg = 2 (n) Pos = 5
χ^2 test statistic	6.25	9.84	11.57
P-value	0.012 *	0.002 **	0.001 ***
95% confidence interval	0.216, 0.390	0.249, 0.484	0.379, 0.935

All *d.f.* = 1.
Significant at level: * $P \leq 0.05$; ** $P \leq 0.005$; *** $P \leq 0.001$.

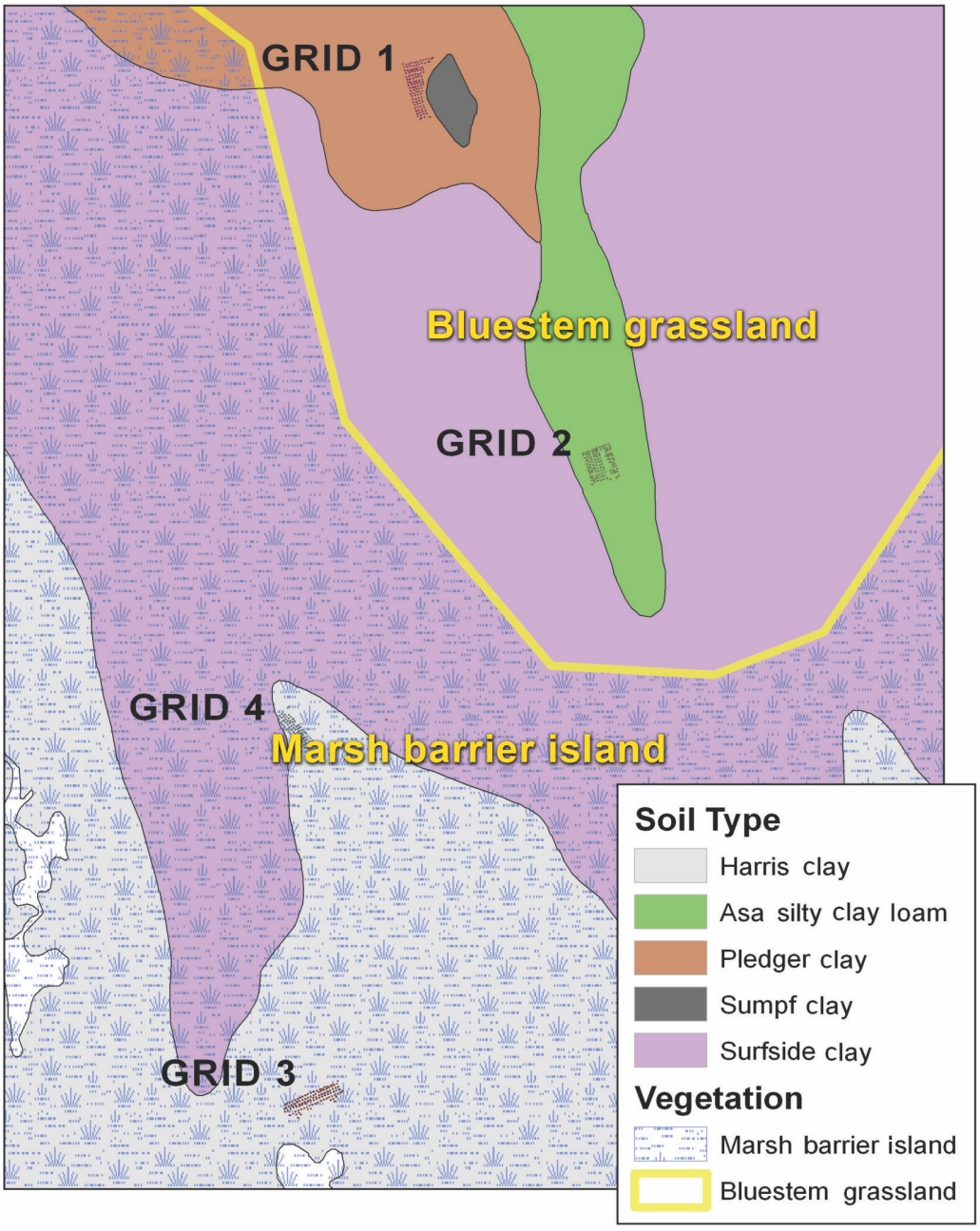

Figure 2.1. Map of predominant soils and vegetation at Justin Hurst WMA. Yellow line demarcates upland grids (1, 2). Stippling symbolizes coastal marshland grids (3, 4).

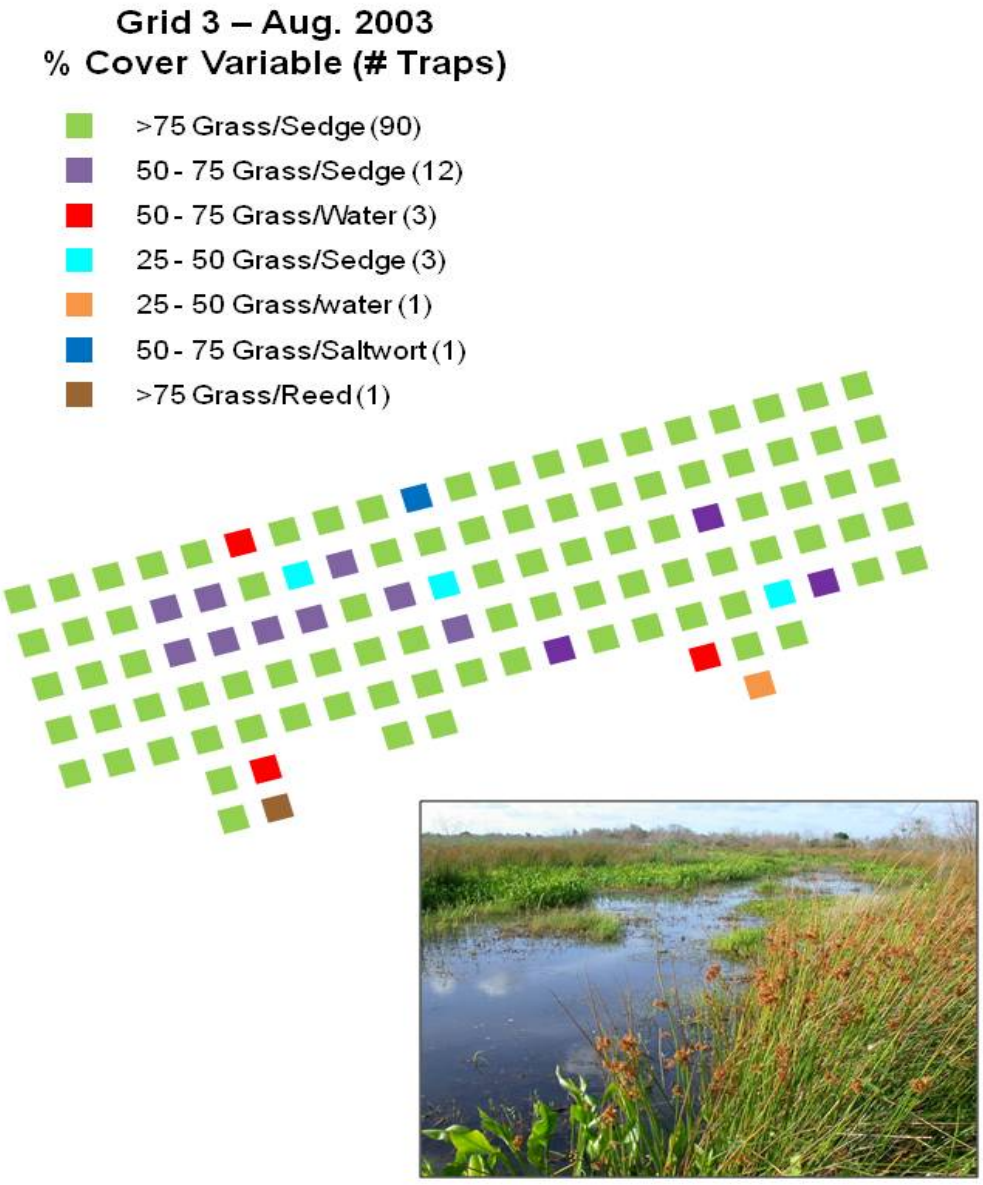

Figure 2.2. Percent habitat cover variables within 3 m of each trap; remaining cover follows slash. In parentheses are numbers of traps characterized by respective cover classes and percentages. Layout of Grid 3 (center). Total trap (n) = 111. Photo of typical marshland habitat, lower right (courtesy of NEM).

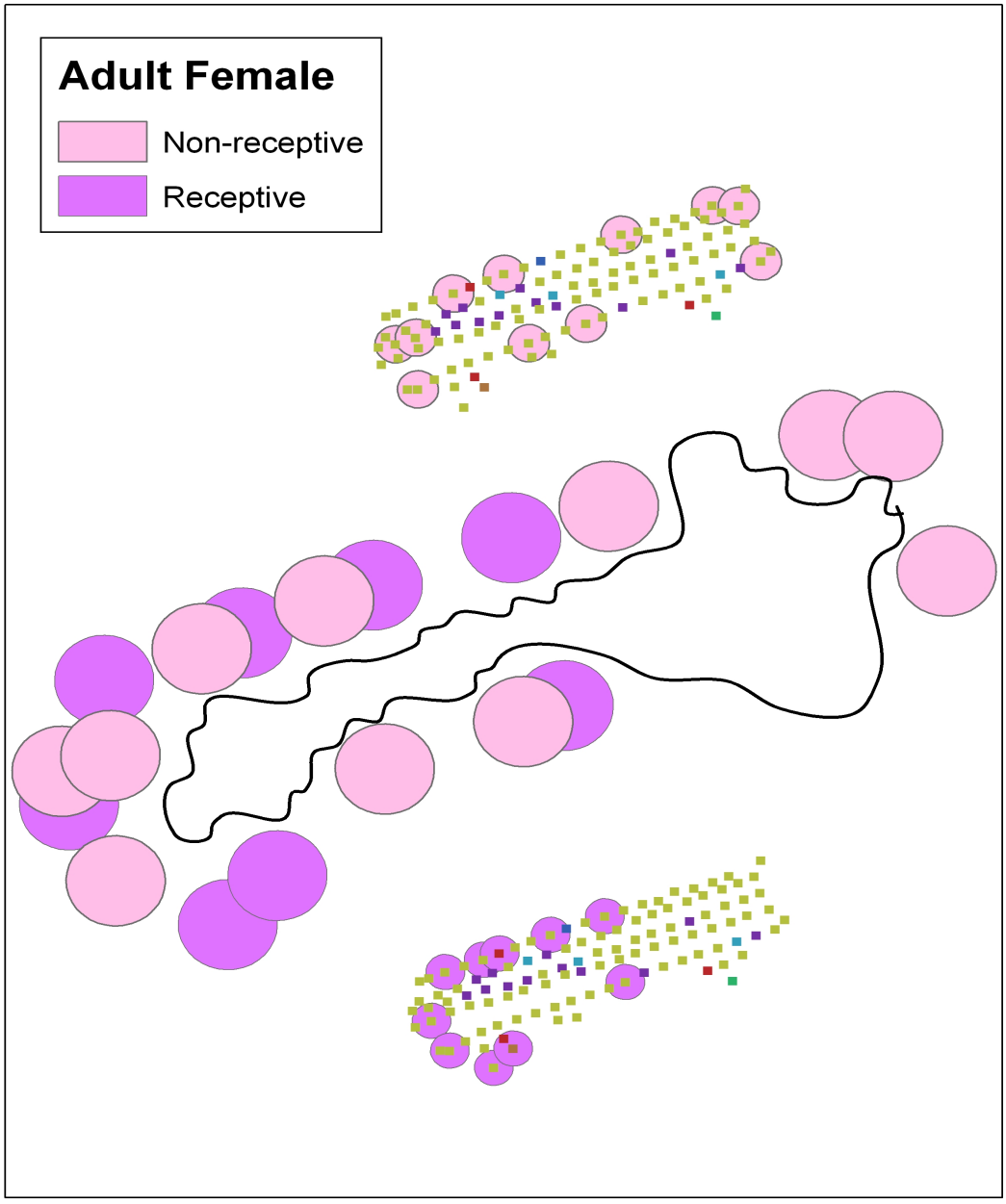

Figure 2.3. Habitat occupancies and spatial relationships of adult female *O. palustris* by reproductive condition. Non-receptive (non-breeding; pink circles) and receptive (breeding; purple circles) females are shown separately (non-receptive, top; receptive, bottom) and combined (center). Black line encloses area unused by both groups (grid removed for ease of viewing). Grid 3, August 2003.

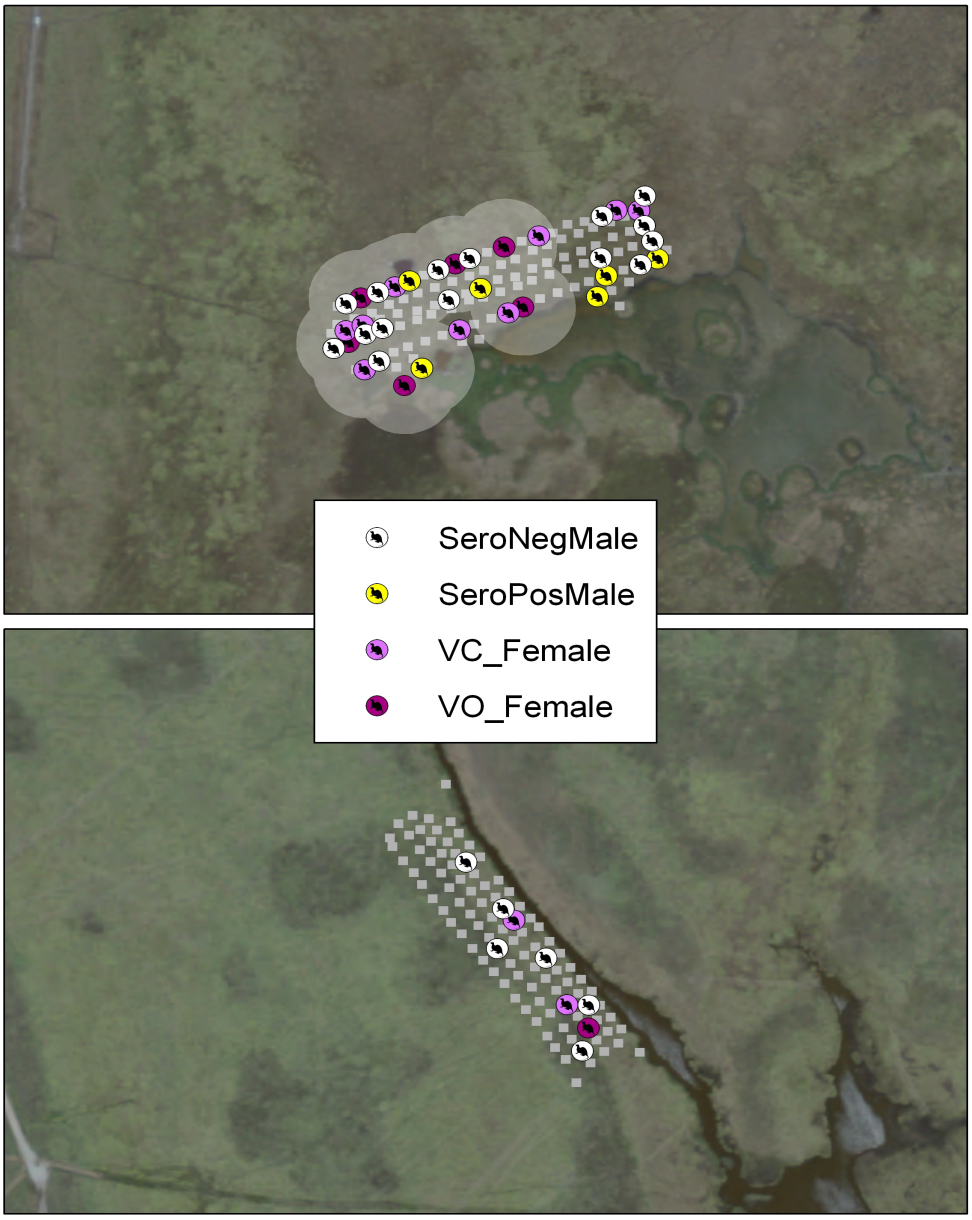

Figure 2.4. Map of *O. palustris* adult female-male spatial relationships using buffer tool in a GIS. Adult males have been added by serostatus. Grid 3 (top) and 4 (bottom), August 2003. Each buffer (opaque circle) represents a receptive female (VO = vagina open) found ≤30 m of a seropositive male. VC = vagina closed, or non-receptive female.

CHAPTER III

BAYOU VIRUS INFECTION COSTS IN FEMALE *ORYZOMYS PALUSTRIS*, WITH RARE LIFE-HISTORY DATA FOR TEXAS RICE RATS

ABSTRACT: A small but noteworthy body of evidence spanning 20 years indicates some primary (mainly adult males) and secondary, cricetid hosts can be affected adversely by hantavirus infections. And yet, hantavirus dogma perpetuates the position that rodent health, morphology, and behavior are uncompromised in primary or secondary host conspecifics. From a 30-month Texas study, empirical data signify that the *Hantavirus* genotype Bayou virus (BAYV) can effect costs in embryoid/fetal, juvenile, subadult, and adult *Oryzomys palustris* (marsh rice rat) females in terms of smaller body dimensions (average: 1mm x 1mm compared to seronegative dam embryos; 8mm x 13mm), increased trap myopathy ($X^2 = 4.07$, *d.f.* = 1, $P = 0.04$), decreased residency times and/or increased mortalities (recapture frequency ≤ 4 whereas seronegative recaptures were ≤ 6), and increased home range sizes (250.1 m^2 for seropositives, 91.0 m^2 for seronegatives; $F = 5.0517$, $P = 0.0324$). We noted fewer pups per litter (mean = 4.69, 95% CI [4.07, 5.32], $t = 2.42$, $P = 0.03$) compared with non-Texas populations in similar macrohabitat. Additionally, season and a conservative estimate of home range size may be useful in the prediction of serotatus

among recaptured rodents. Our BAYV results bolster related research as they underscore a need to no longer discount infection impacts on hosts, particularly in female and young rodents. Thus, these data impel a re-examination of the traditional Hanta community position of inconsequential infections in hosts of the Superfamily Muroidea.

INTRODUCTION

The biology of Old and New World hantaviruses indicates almost universally in host rodents, infection persistence is accomplished via transport of virions to seed satellite and peripheral tissue sites (Lee et al. 1981, Yanagihara et al. 1985, Gavrilovskaya et al. 1990, Hutchinson et al. 1998, Botten et al. 2003, Villarreal 2005). This pathophysiological state of chronic infection facilitates intermittent replication and environmental shedding of viral RNA in rodent excreta and secreta (Lee et al. 1981, Yanagihara et al. 1985, Gavrilovskaya et al. 1990, Hutchinson et al. 1998); the timing, duration, quantity, and mode (i.e., fluid, solid, or tissue type source) of shed virus can differ markedly by species (virus and host) and sex (Klein et al. 2001, Botten et al. 2003, Hinson et al. 2004, McIntyre et al. 2005). Although empirical support grows for rodent infection acquisition from environmental media (Kallio et al. 2006a, Hardestam et al. 2007), direct agonistic encounters among adult males still appear to most efficiently transmit hantaviral infections in wild populations (Glass et al. 1988, Hinson et al. 2004), including in *O.*

palustris (McIntyre et al. 2005; 2009). Even though infections are inveterate, it was initially accepted widely that hantaviruses cause no detectable adverse effects on host physiological processes governing reproduction, fecundity, longevity, or behaviors (Schmaljohn and Hjelle 1997, Bernshtein et al. 1999, Hjelle and Yates 2001).

Over the past 20 years, several field studies provide some evidence to the contrary, including potential effects on specific population segments and age groups, often trivialized in viral dynamics. For example, southeastern Arizona populations of brush mice (*Peromyscus boylii*) infected with Sin Nombre virus (SNV) showed small but significant differences in lifespan when compared to their antibody-negative conspecifics (Kuenzi et al. 1999). Likewise, Douglass et al. (2001), while conducting research on SNV in Montana deer mice (*P. maniculatus*), noted survival rates of infected juveniles and subadults were lower than survival rates of naive rodents in the same age classes.

Inquiries into the SNV–*P. maniculatus* study system in Montana by Douglass and collaborators (2001) indicated that seropositivity (a proxy for viral infection) was a reliable predictor, and in all probability, was associated with different types of fitness costs in SNV-infected *P. maniculatus*: from slower rates of weight gain after seroconverting, to shortened life spans, and also decreased survivorship compared to their uninfected counterparts (Douglass et al. 2007, Luis et al. 2012). Further support for the negative

effects of SNV in *P. maniculatus* hosts was presented by Lehmer et al. (2007), who validated Douglass's field data with laboratory evidence linking lowered immune responses to overall diminished health seen in the antibody-positive, male *P. maniculatus* in the Douglass et al. (2007) study.

Additionally, pulmonary edema, periportal hepatitis, and other histopathologic markers have been described in peromyscines seroreactive for either SNV (Lyubsky et al. 1996) or New York virus (Netski et al. 1999). Some cytopathologies have been indicated in cotton rats (*Sigmodon hispidus*) antigen-positive for Black Creek Canal virus (Hutchinson et al. 1998) and in bank voles (*Myodes glareolus*) infected with Puumala virus (PUUV; Gavrilovskaya et al. 1990). Overwinter survival was reduced significantly in PUUV-positive *M. glareolus* compared with seronegative *M. glareolus* (Kallio et al. 2007).

Although artificial introductions of SNV in *P. maniculatus* and Caño Delgadito virus (CDGV) in *S. alstoni,* Alston's cotton rat (both the respective natural hosts) produce infection persistence without explicit indictors of disease (Botten et al. 2000, Fulhorst et al. 2002), the literature abounds with examples whereby atypical inoculations (e.g., intracerebral or intraperitoneal) produce lethal outcomes in non-natural hosts (i.e., newborn and heterospecific rodents) (Tsai et al. 1982, Yamanouchi et al. 1984, Wichmann et al. 2002, to present a few); recently, however, evidence of Andes virus (ANDV) infection in a non-host species (*P. maniculatus*) manifesting asymptomatically and

immunogenically was publicized (Spengler et al. 2013). Certainly, the full range of symptomalogical features in host infections is still unfolding.

Of the hantaviral genotypes studied in any detail, apathogenicity in persistently infected, rodent reservoirs has been presupposed using few criteria; even still, it is reasonable to expect a similar silent disease state in other host-virus pairs not yet studied from this perspective, such as with *Oryzomys palustris* (marsh rice rat) and BAYV. We note, however, that in most Hanta systems investigated to date, the rodent reservoirs are Holarctic in distribution: *O. palustris* is a member of a predominantly Neotropical radiation, invading North America secondarily (Weksler 2006). And although *O. palustris* is an effective long-distance, aquatic disperser (Svihla 1931, Abuzeineh et al. 2007), some populations surrounding our study area may have experienced a genetic bottleneck during the late Pleistocene (Indorf 2010); as such, some individuals may be more susceptible to parasitic colonizations and pathologies (i.e. from sustained co-infections; Barnard et al. 1971, Levin et al. 1995, McIntyre et al. 2005, Carmichael et al. 2007) resulting from reduced genetic diversity, various metabolic trade-offs, and combined with continual immunological pressures (Lazzaro and Little 2009, Ruiz-López et al. 2012).

Therefore, the purpose of this research was to identify and describe potential infection costs in individual, non-primary *O. palustris* hosts (i.e., in the unborn, juvenile, and subadult males, and in the females of all age classes) using measurements of morphology (body sizes, mortality) and behavior

(recapture tendency, home range size, residency). If the null hypothesis is true, there should be no substantial differences in variable measurements of these *O. palustris* demographic groups regardless of serostatus.

MATERIALS AND METHODS

Field: Justin Hurst Wildlife Management Area (formerly Peach Point WMA) in Freeport, Texas

Study site description, mark-recapture design, and determination of antibody and viral positive rodents have been detailed elsewhere (McIntyre et al. 2005; 2009). Briefly, *O. palustris* were examined almost 3 years from four mark-recapture trapping grids (2 in coastal marshland, 2 in upland habitat) and from harvest traplines. Protocols for rodent capture, marking, handling, and euthanasia were approved by the Texas Tech University Animal Care and Use Committee (permit 03049-08) and followed ASM guidelines (1998). Human safety precautions, including the handling and disposition of biohazardous substances, were adopted from Mills et al. (1995). Age classifications of *O. palustris* (juvenile, subadult, adult) were assigned using pelage color stage and reproductive condition (testes position; nipple size, lactating, vaginal patency, recent parturition), then confirmed by weight (juvenile: ≤ 30 g; subadult: 31-49 g; and adult: > 50 g) and were based on ontogenetic and morphological data

from our live and harvested rodents compared with other recognized sources (Hamilton 1946, Hall 1981). Body weight is a reliable indicator of age in *O. palustris* up until adulthood (Negus et al. 1961). But to age long-lived rodents (i.e. > 5 months), total body length is a more precise metric, because quasi-indeterminate growth of advanced-age *O. palustris* (Negus et. al 1961) leads to a great deal of intrapopulation variation in other characters, like weight (Goldman 1918, Paradiso 1960). From harvested females, meristics (crown to rump length, mm) and counts of embryos and fetuses were recorded, along with other mensural characters.

Home range analyses based on residency

Home range sizes were estimated from mark-recapture data for grid 3 resident (i.e., no less than 3-6 recaptures) *O. palustris* with both the 100% minimum convex polygon (MCP; Mohr 1947) and 95% adaptive kernel (ADK; Worton 1989) methods in CALHOME software (Forestry Sciences Lab, Fresno, California, USA). The minimum convex polygon (MCP) method involves calculation of the smallest sized polygon encompassing all relocation points of the marked-recaptured rodent, with the resulting polygon representing the rodent's home range and core resource area. Despite unavoidable disadvantages (Nilsen et al. 2008), the MCP remains one of the most widely applied home range estimation techniques in use today. Worton's (1989) groundbreaking, adaptive kernel method (ADK) is also a commonly

used, nonparametric technique to estimate home range size and usage. Additionally, the ADK method has the advantages of robustness for outliers, spatially autocorrelated samples, and multiple centers of activity (Kernohan et al. 2001). Several bandwidths (smoothing parameters to compensate for non-normal data) were compared using a least-squares cross-validation (LSCV) score, a measure of how well the bandwidth fits the data; the bandwidth is reduced until the lowest LSCV score is achieved. The default bandwidth of 0 with a grid cell option of 30, or a 30 x 30 data matrix, resulted in the lowest LSCV score. Because of missing data and data redundancy in both the 75% MCP and ADK measurements, the 60% MCP (low MCP) and 95% ADK (high ADK) measurements were selected for the statistical analyses.

Descriptive statistics, statistical modeling

Field data were transferred from TK Data Sheets (NSRL of TTU) to a computer database using Excel 2007 (Microsoft Corp., Redmond, WA). All exploratory and descriptive statistics, statistical modeling and graphical output were accomplished with R version 2.12.2 (R Development Core Team 2008) with The R Manual (R Development Core Team 2011) used as a guide (http://cran.r-project.org/doc/manuals/R-intro.pdf). Two-sided, one sample t-tests were used to evaluate sample means for numbers of embryos, and weight and total length of pregnant seronegatives compared with mean values from the literature (Negus et al. 1961, Wolfe 1982) using function 't.test'. Pearson's

Chi-squared test with Yates's continuity correction ('fisher.test') was used to compare the odds ratio of the number of non-pregnant females by serostatus that expired in traps. To determine whether or not there was any difference in the weights of non-pregnant seronegatives versus seropositives, a Welch two-sample t-test ('t.test') assuming unequal variances was applied. Comparisons of the default distributions were made using ordinary nonparametric bootstrapping ('boot'), and bootstrap confidence level (CI) calculations were generated using function 'boot.ci', both based on 999 replicates.

Correlation analysis ('cor') was used to investigate a potential relationship between (1) weight and age and (2) weight and home range size (using estimate values from the 60% MCP and 95% ADK methods) in *O. palustris*. Additionally, correlation analyses were run to evaluate the correlation coefficients between (1) the numbers of embryos, and the weight and total length of dams, and (2) the dimensions (length x width in mm) of embryos and weights of dams. Pearson's product-moment correlation ('cor.test') provided levels of statistical significance (*P*-value) and 95% confidence intervals (CI).

Multivariate analysis of variance ('manova', test = 'Roy') was used to assess the significance in mean differences between the response variables sex, age, weight, season, recaptures, the home range estimates low MCP (conservative 60% use) and high ADK (95% activity kernel) and the predictor variable "sero", or serostatus (seronegative, seropositive). Linear discriminant

function analysis ('lda') with jackknifing (CV = 'TRUE') was run to cross-validate the MANOVA, by predicting group membership according to serostatus based on the most discriminating independent variables from the MANOVA (Tabachnick and Fidell 2007).

RESULTS

Specifics of morphological and behavioral costs of all seropositive female captures

Sample sizes and descriptive statistics of (mostly) seronegative *O. palustris* are in Table 3.1, with infection costs in seropositive females described in comparison to seronegative females provided in Table 3.2. (No viral or seropositive juvenile males were detected, and only 1 underweight seropositive, sub-adult male was detected; data not shown.)

Morphometrics of pregnant females by serostatus and of their embryos/fetuses

A one-sample t-test did not reveal any significant difference between the weights of 20 pregnant seronegatives (mean = 50.47 g, 95% CI [45.24, 55.70], t = -0.21, $d.f.$ = 19, P = 0.83) compared with an accepted, published weight for females (mean = 51.0 g in Schmidly 2004; BAYV serostatuses of females from this study are unknown). However, total length of 13 pregnant

seronegatives (mean = 229.15 mm, 95% CI [221.56, 236.75], t = -4.55, $d.f.$ = 12, P = 0.0007) was highly statistically different (shorter) based on the published total length (mean = 245.0 mm) in Schmidly (2004). A pregnant seropositive weighed substantially less and was quite smaller (weight: 34.9 g; total length = 214 mm) than the pregnant seronegatives (N = 20, mean weight = 50.47 g; N = 13, average total length = 229.15 mm) and also compared with Schmidly (2004; mean weight = 51.0 g, (mean total length = 245.0 mm), although a Welch's t-test did not indicate any detectable difference in mean weights of non-pregnant seropositives (44.96 g; t = -1.14, 95 % CI [-11.87, 3.50], P = 0.27) and seronegatives (40.77 g).

From other Texas *Oryzomys* populations, robust field data are lacking from which to draw any reasonably confident litter size comparisons. Nevertheless, based on very small sample sizes from field notes (Schmidly 2004 and pers. comm.), Texas litter sizes range from 2-7 averaging 4, values comparable to those from within the *O. palustris* complex (1-7 pups/litter averaging 4 or 5; Wolfe 1982). Our coastal marsh study indicated somewhat less variability in litter sizes (3-7 pups/litter), and slightly fewer pups per litter compared with more robust reproductive data from Louisiana populations (mean = 4.69, 95% CI [4.07, 5.32], t = 2.42, $d.f.$ = 12, P = 0.03; Negus et al. 1961), but comparable results to Virginia populations [4.63 ± 1.39 (mean ± SE) (Dreelin 1997)]. Pearson's product moment revealed a positive correlation between embryo/fetal widths and dam weights (t = 2.67, $d.f.$ = 7, P = 0.03, R^2

= 0.71, 95% CI [0.088, 0.934]). Other correlation analyses did not detect relationships between numbers of embryos/fetuses and weights of dams (R^2 = 0.46, P = 0.11) or numbers of embryos/fetuses and total length of dams (R^2 = 0.21, P = 0.49). Embryo/fetal lengths and dam weights were correlated but weakly (t = 2.02, $d.f.$ = 7, P = 0.08, R^2 = 0.61, 95% CI [-0.096, 0.906]).

Demography of recaptured adult females, and juveniles and subadults of both sexes

The most stable populations of *O. palustris* inhabited coastal prairie grid 3; therefore, grid 3 provided the majority of our most illuminating recapture data. Figure 3.1 shows *O. palustris* were recaptured more frequently in August than in other seasons, with the second highest rate of recapture occurring in May, and no recaptures recorded from December. Juvenile and subadult males were recaptured more often than adult females (Figure 3.2). Average recaptures for seronegative (n = 28) and seropositive (n = 3) rodents were the same ($x \approx 4$; Figure 3.3). However, seropositive rodents were recaptured up to 4 times, whereas seronegative rodents were recaptured up to 6 times (Figure 3.3). Weights of seronegative and seropositive recaptures were similar (Figure 3.3 insert). Juvenile females (FJ) were recaptured less frequently than all other groups in this study (Figure 3.4.1). Although there were more adult female (FA) recaptures (n = 19) than juvenile male (MJ) recaptures (n = 3), both groups were recaptured the same number of times on

average (x = 4; Figure 3.4.1). Both subadult females (FS) and males (MS) averaged higher rates of recapture compared to the other groups (Figure 3.4.1). Testes inguinal males (TIN) averaged higher recapture rates compared with pregnant females (PRG), who were recaptured rarely (Figure 3.4.2). Recapture trends were similar for testes abdominal males (TAB) and vagina-closed females (VAC) (Figure 3.4.2).

Rodent weight, home range size, and site fidelity

In *O. palustris*, weight and total body length are reliable indicators of age (Negus et al. 1961). Based on several criteria to assign age, we also found age and weight were strongly, positively correlated (t = 15.38, $d.f.$ = 29, P = 1.78^{-15}, R^2 = 0.94, 95% CI [0.886, 0.973). Rodent weights and recapture frequencies were uncorrelated (R^2 = -0.006, P = 0.98). Moreover, weight and home range size were <u>not</u> correlated variables (Figure 3.5; low MCP: R^2 = -0.02, P = 0.90; high ADK: R^2 = 0.17, P = 0.34), supporting further what was deduced for adult males in McIntyre et al. (2009), and providing some important physiological information previously lacking for *O. palustris*. Home range sizes were underestimated in 10 of the 62 (16.1%) home range measurements for 31 recaptured rodents because these rodents were recaptured several times at the same trap; these statistical interferences notwithstanding, site fidelity on grid 3 (between 1.0 m^2 to 27.03 m^2) could be approximated by combining these data with data from other low-movement, recaptured rodents.

Based on our findings, vagina closed (non-breeding) females displayed the strongest site fidelity (6:13 or 46.1%), with vagina open (breeding) females second (4:13 or 30.8%); testes abdominal males also displayed a proclivity to return to the same area, although not as often as the adult females (3:13 or 23.1%).

Multivariate analysis of variance (MANOVA) and Linear discriminant function analysis (LDA)

The multivariate F test revealed that *O. palustris* differed significantly by serostatus ($F = 3.0649$, $P = 0.0197$) (Table 3.3). ANOVA summaries indicated that out of the 7 response variables examined (sex, age, weight, season, recaptures, low MCP, and high ADK), season (August for seronegatives, May for seropositives; $F = 9.9576$, $P = 0.0037$) and low MCP (91.0 m^2 for seronegatives, 250.1 m^2 for seropositives; $F = 5.0517$, $P = 0.0324$) had the most significantly different means across the two groups (Table 3.3, Figure 3.6). Posterior probabilities of the LDA revealed classification accuracy was high (≈ 96.0%), indicating most sample observations were properly assigned to the correct group (Appendix E). The jackknifed validation showed predictive power as well (90.3%) and thus, overall model results of the MANOVA and LDA indicated strong support for utilizing the variables season and low MCP as useful and informative Bayou virus serostatus discriminators among resident *O. palustris* secondary hosts (Appendix E).

DISCUSSION

Consistently, we found that seropositive rather than seronegative *O. palustris* females fell at the lower end of the weight range for female captures. These data are in stark contrast to those ascertained for the main BAYV traffickers – the sero-/viral positive, *O. palustris* adult males (McIntyre et al. 2009). Most sample sizes were too small to analyze with any statistical rigor; however, the raw data can be interpreted straightforwardly (characterized in Table 3.2). From here, it is clear that infection costs from BAYV were detected in each and every seropositive female *O. palustris* capture (n = 14), and spanned all age classes, non-pregnant and pregnant, and their unborn and young progeny. Field data signify that compared to their seronegative conspecifics (i.e. the control groups), BAYV elicited costs in embryoid/fetal, juvenile, subadult, and adult *O. palustris* females in terms of: (1) lower body weights; (2) shorter total body lengths; (3) smaller embryoid/fetal body dimensions; (4) increased mortalities; (5) decreased residency and/or higher emigration, and (6) larger home range sizes. We observed larger litter sizes compared with small captive colonies of *O. palustris* (Svihla 1931, Worth 1950), but slightly fewer pups per litter compared with congeners (Goldman 1918) and a long-term *O. palustris* dataset from Louisiana (Negus et al. 1961), which could be indicative of overall diminished health in *O. palustris* caused

by poor resource availability, heavy parasite burdens, or both (Speakman 2008). Periodic high rodent densities may have affected some litter sizes, as well.

I did not analyze directly a possible relationship between habitat quality and morphological variation in *O. palustris* by serostatus based on season, population growth phase or population density. However, population stability markers, used as surrogates for habitat quality, were inferred based on Van Horne's criteria (1983) by evaluating temporal trends in relative density, recruitment, survival, and the percentage of resident mice based on recapture data (not shown). Again, grid 3 (coastal prairie) supported the most stable *O. palustris* populations. I did note, however, that the smaller-sized and lower weight, seropositive rodents were captured more often on coastal prairie grid 3 and from the harvest traplines, with these distinctions not necessarily explained by sample sizes alone. *O. palustris* comprised 55.8% of the grid captures and 41.0% of the harvest trapline captures (Holsomback et al. 2009). The highest seroprevalencies were detected from their preferred habitat, coastal prairie grid 3 (24.6%; McIntyre et al. 2005; 2009), even during periods of low rodent abundance, but the lowest seroprevalencies were seen on the harvest traplines (7.0%).

Combined with larger home range use estimates, seropositive non-breeding adult females may exhibit increased dispersal/emigration. These

behaviors could promote more efficient viral spreading through new and farther contacts with naïve rodents. The increased mortality rates proposed could cause shifts in population demography and breeding patterns, with more males fighting for fewer available females. Within this system, reduced abundances of seropositive reproductive females, due to higher rates of mortality and dispersal, may represent an unavoidable infection trade-off. On the flipside of the evolutionary coin, seropositivity in *O. palustris* females (5.7%; McIntyre et al. 2005), if even a fraction survives multiple seasons, provides BAYV with survival insurance against periodic population crashes or other stochastic events that may all but extinguish low densities of BAYV-infected adult males. Regardless, the situation could constitute a "win-win" for BAYV: high ranking males guarding large home ranges (McIntyre et al. 2009) to maximize overlap with as many breeding females as possible (Holsomback et al. 2013) could act to enhance BAYV virus dissemination via increased intermale aggression and virus excretion across expansive habitats.

From the 7 response variables (sex, age, weight, season, recaptures, low MCP, and high ADK) and a MANOVA, we reject the null hypothesis of no difference between serogroups (i.e. centroids). Season, the first discriminant function, represented the maximum differences between seronegative and seropositive recaptures. Low MCP, the second discriminant function, separated the serogroups using any residual variation. A jackknifed classification matrix was used to categorize each observation into 1 of the 2

serogroups, and classification success was high at > 90%. Seronegative *O. palustris* have smaller home range sizes and are more likely to be detected in August, whereas seropositive *O. palustris* have home range sizes nearly 3 times the size of seronegatives, with seropositives more often detected in May (Table 3.2, Figure 3.1).

With the discoveries of reduced weights and higher mortality probabilities in a portion of seropositive female *O. palustris*, we sought explanations for the low frequencies of seropositive young detected in this study. The traditional hantavirus community explanation of passive immunity via maternal antibodies does not apply completely in this case, as a portion of young rodents was indeed positive. Firstly, infected juveniles and subadults may be less trappable, perhaps because they invest more energy in growth, development, and immune defense and have less metabolic energy available for traversing and exploring the habitat; juveniles also are more likely to stay closer to the female parent until older. Secondly, true life history differences may have evolved between seropositive and seronegative non-primary *O. palustris* hosts (i.e., young mice and adult females), but our sample sizes were not large enough to detect them. Lastly, young *O. palustris* that succumb to adverse effects of infection at an early age, although possibly a small fraction of the populations, certainly will be less likely to be captured and especially recaptured. To reiterate, not all immature *O. palustris* will benefit automatically from trans-placental antibody transfer. Of importance to bear in

mind is the likelihood that among infected neonate, juvenile, and subadult rodents, a portion does not survive the initial phase of infection, due to trade-offs from loss of energy reserves for body maintenance functions and immune responses (Lochmiller and Deerenberg 2000). Along these lines, even subtle alterations in physiology or behaviors, resulting in diminished predator recognition or reaction times, could prove fatal for inexperienced, infected youngsters venturing away from the nest and their mother's sentry. Thus, these types of infection-related mortalities will be grossly underrepresented in the dataset, making those much-needed host measurements and comparisons with seronegatives rather rare opportunities to seize.

As described in McIntyre et al. (2009), adult rodents infected naturally with various hantaviral genotypes may show no pathologies in respiratory health (O'Connor et al. 1997) or in survivorship (Douglass et al. 2001) in *P. maniculatus* or in fecundity or survival rates, as seen in *Rattus norvegicus* (Norway rat; Childs et al. 1989). Moreover, Abbott et al. (1999) found that antibody-positive *P. boylii* males lived longer on average than either antibody-positive females or antibody-negative males. Even still, Kallio et al. (2006b) provide empirical support for possible individual and population-level *benefits* of PUUV infection in wild *M. glareolus*. In the present study, seropositive adult females weighed slightly more and utilized larger home ranges than did seronegative adult females, even though there was no statistically significant age or weight difference between the two groups; and, weight and home range

size are uncorrelated in *O. palustris* (this study and in McIntyre et al. 2009).

Compared to seronegatives, seropositives also showed less variability in recapture numbers and home range size estimates, even with smaller sample sizes. However, the significance of the consistency of these data is unknown. Spectral clarity of host infection outcomes requires unbiased scrutiny and the compilation of robust datasets. Long-term empirical studies of more muroid-virus pairs should help to fill some of the gaps in our current understanding of the impacts (negative, neutral, and beneficial) of hantavirus infection in both primary and non-primary traffickers, i.e. in juvenile, subadult, and female rodents and insectivores. Investigators largely dismiss how the latter demographic segments can affect and can be affected by the hantavirus enzootic cycle, despite cognition that the cycle is complex and incompletely characterized. Still open to discussion is the assumption that in other *Hantavirus* systems, non-target conspecific hosts - like females and young rodents - manifest no undesirable symptoms resulting from primary inter-host, infection transfer.

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Table 3.1. Sample sizes and descriptive statistics for demographic groups (coded variables) of *O. palustris* not described in text (mainly seronegatives). Numbers with two significant digits are followed by repeating zeros.

Variables (coded)	N	Null/ NaN	Min	Max	Range	Median	Mean	Std error mean	0.95 CI mean	Variance	Std dev	Coef vartn
NegJuve MaleWt	16	0/0	7.40	22.50	15.10	14.50	15.08	1.142	2.435	20.880	4.569	0.303
NegJuve FemaleWt	24	0/0	8.90	21.50	12.60	14.25	15.28	0.771	1.595	14.272	3.778	0.247
NegSub MaleWt	29	0/0	19.50	43.00	23.50	27.40	29.51	1.232	2.523	44.004	6.634	0.225
NegSub FemaleWt	46	0/1	15.00	46.00	31.00	25.75	27.04	1.134	2.284	59.157	7.691	0.284
PosNon-PregWt	9	0/0	36.00	60.00	24.00	44.40	44.96	2.771	6.391	69.120	8.314	0.185

Table 3.1. Continued

Variables (coded)	N	Null/ NaN	Min	Max	Range	Median	Mean	Std error mean	0.95 CI mean	Variance	Std dev	Coef vartn
NegPregWt	20	0/0	29.00	73.00	44.00	49.90	50.47	2.501	5.234	125.075	11.184	0.222
NegNon-Preg Total Length	52	0/0	184.0	256.0	72.0	219.5	220.2	2.385	4.788	295..82	17.20	0.078
NegPreg Total Length	13	0/0	210.00	249.00	39.00	226.00	229.15	3.486	7.595	157.97	12.57	0.055
DamNeg EmbNumber	13	0/0	3.00	7.00	4.00	5.00	4.69	0.286	0.623	1.064	1.031	0.220
DamNeg EmbWidth	9	0/0	2.00	18.00	16.00	9.00	8.44	1.591	3.669	22.778	4.773	0.565
DamNeg EmbLength	9	0/0	4.00	24.00	20.00	13.00	13.44	2.205	5.086	43.778	6.616	0.492

Table 3.2. *O. palustris* female comparisons by serostatus with implications of infection costs.

Seropositive	Comparison to seronegative	Descriptor	Possible implications
2 juveniles	10.0g 15.3g	Below-average weight	Lower immune defenses, Delayed time to reproduction, Reduced survival
1 subadult	18.0g, 187.0mm 27.0g, 191.9mm	Extremely low weight, shorter	Survival to first breeding season unlikely
2 non-pregnant adults	2:9 3:140	10x more likely to expire in trap	Altered population demography temporally
1 pregnant adult	34.9g, 214.0 mm 50.5g, 229.2 mm	Extremely low weight, much shorter	Reduced offspring survival
* 5 embryos from above dam	1mm x 1mm 8mm x 13mm	Much smaller body dimensions	Slower ontogeny; could be related to body size of dam
3 non-breeding adults	≤4 ≤6	Recaptured fewer times	Increased dispersal/emigration or Increased mortality
3 non-breeding adults	250.1 - 535.7 m^2 91.0 – 470.8 m^2	Larger home range size estimates	More efficient dissemination of virus to new hosts

* Serostatus unknown.

Table 3.3. ANOVA summaries of the 7 response variables (sex, age, weight, season, recaptures, low MCP, and high ADK) and the dependent variable, serostatus. Asterisks (*) emphasize statistical significance.

Independent Variable	Dependent Variable	*d.f.*	Sum of Squares	Mean Square	*F*	*P*
Sex	Serostatus	1	0.124	0.124	0.765	0.389
	Residuals	29	4.714	0.163		
Age	Serostatus	1	311.10	311.06	0.717	0.404
	Residuals	29	12585.7	433.99		
Weight	Serostatus	1	106.13	106.13	0.994	0.327
	Residuals	29	3094.92	106.72		
Season	Serostatus	1	19.788	19.788	9.958	0.004 **
	Residuals	29	57.631	1.987		
Recaptures	Serostatus	1	0.922	0.922	0.838	0.368
	Residuals	29	31.917	1.101		
Low MCP	Serostatus	1	68554	68554	5.052	0.033 *
	Residuals	29	393541	13570		
High ADK	Serostatus	1	11412	11412	0.035	0.854
	Residuals	29	9577198	330248		

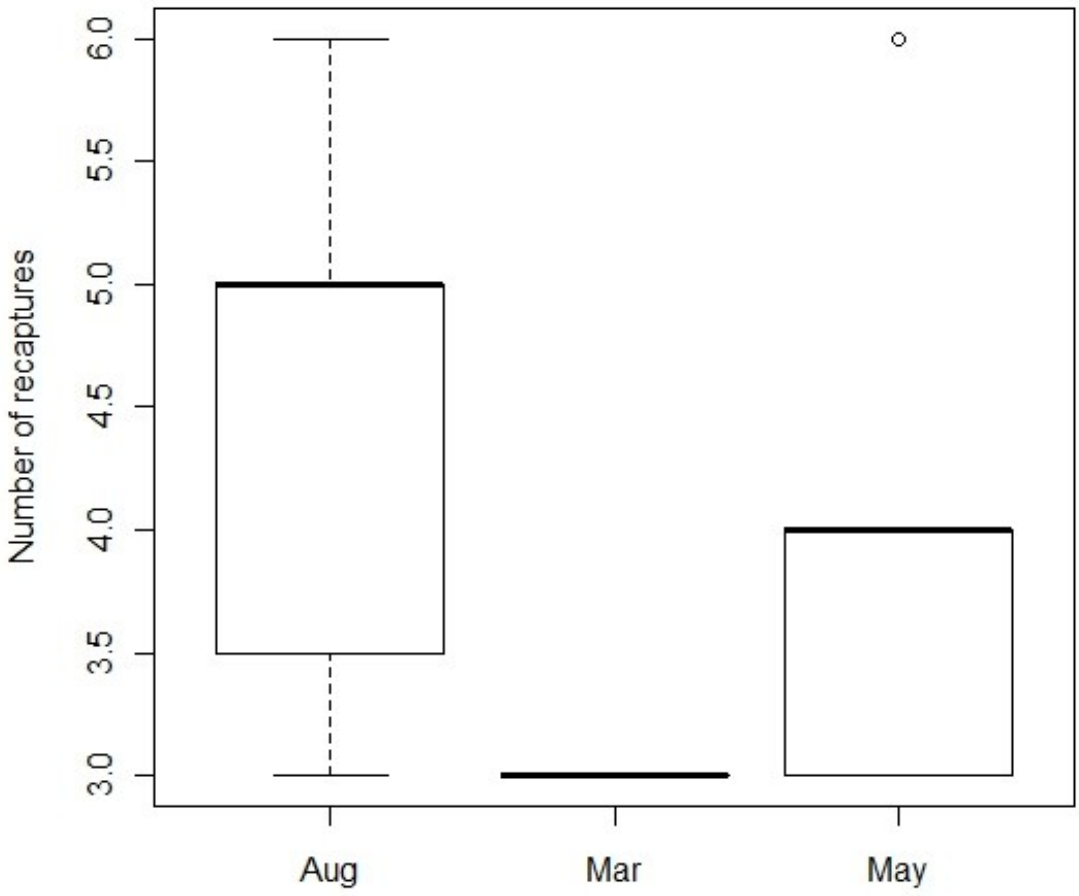

Figure 3.1. Recaptured *O. palustris* by season, represented by boxplots with medians, interquartile ranges, and 95% CI. Open circle denotes extreme value.

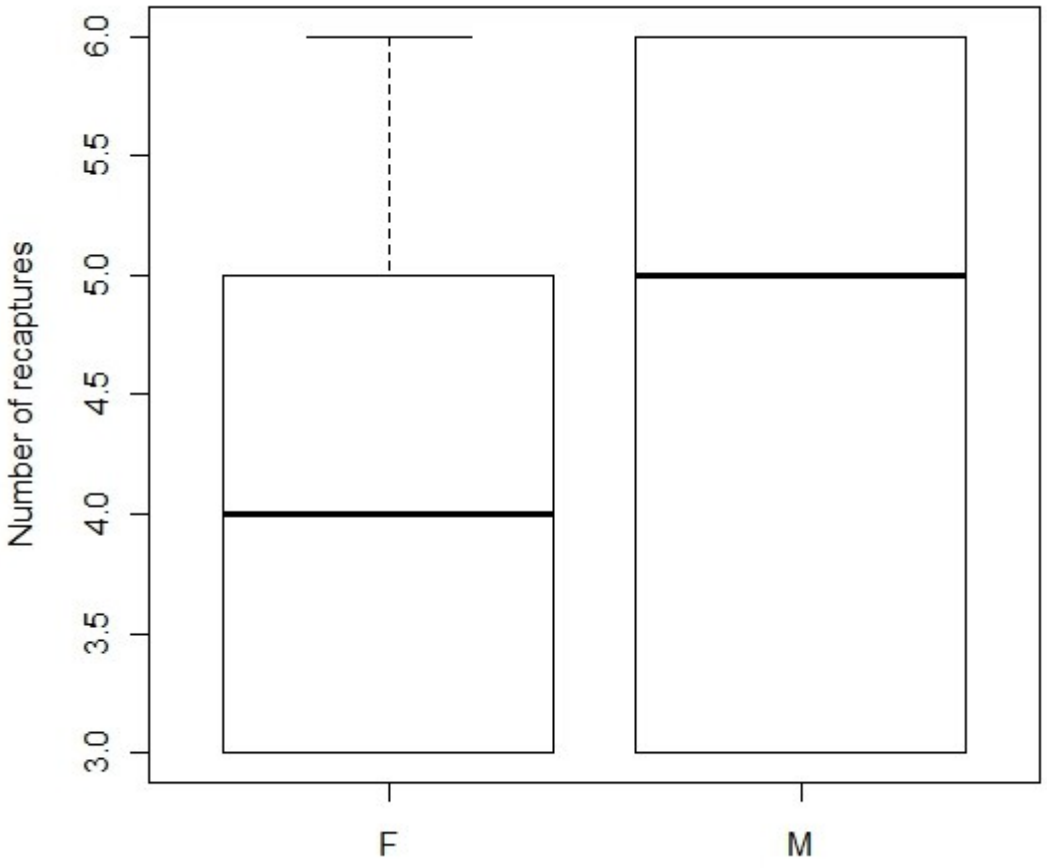

Figure 3.2. Recaptured *O. palustris* by sex (excluding male adults), represented by boxplots with medians, interquartile ranges, and 95% CI.

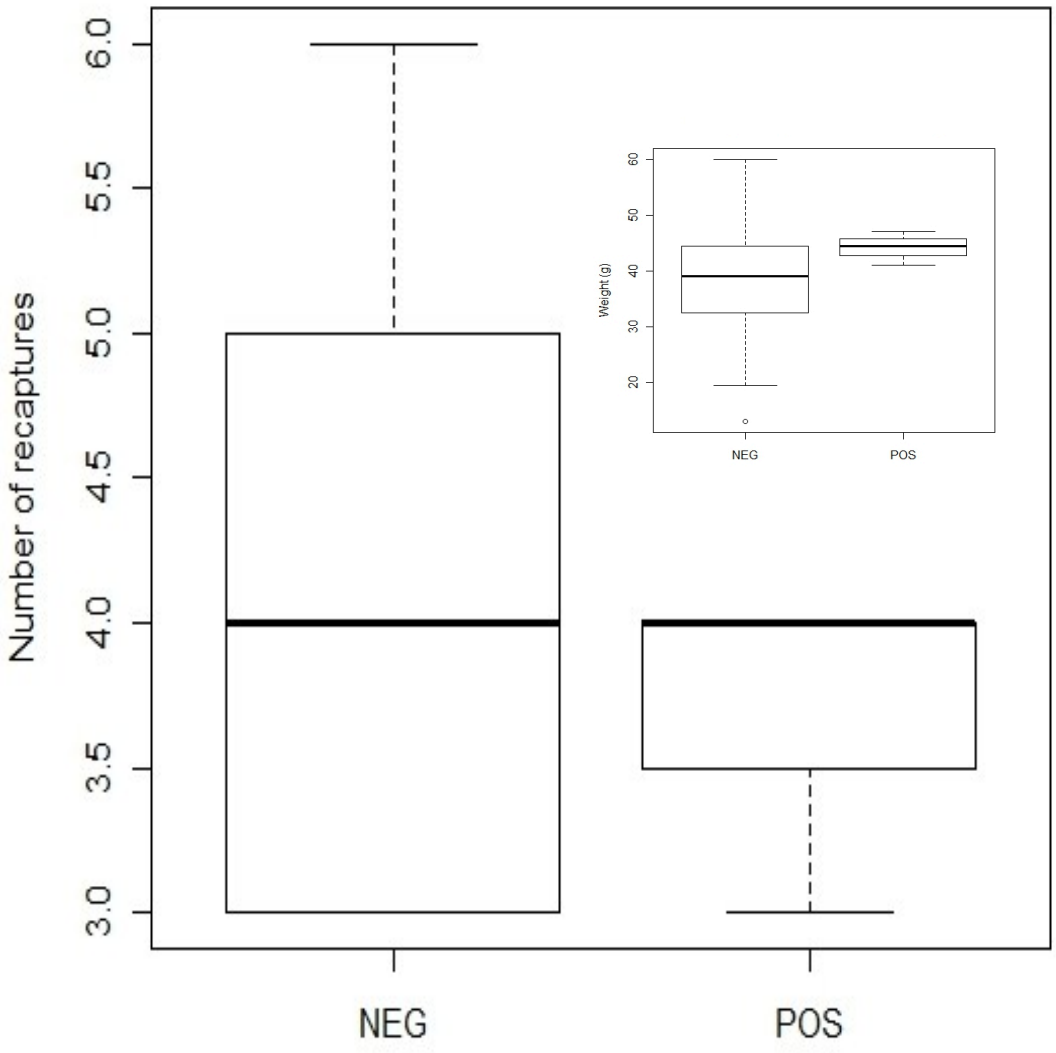

Figure 3.3. *O. palustris* recaptures by infection status, represented as boxplots with medians, interquartile ranges, and 95% CI. Insert: depicts no discernible difference in weights of recaptures by serostatus. Open circle denotes extreme value.

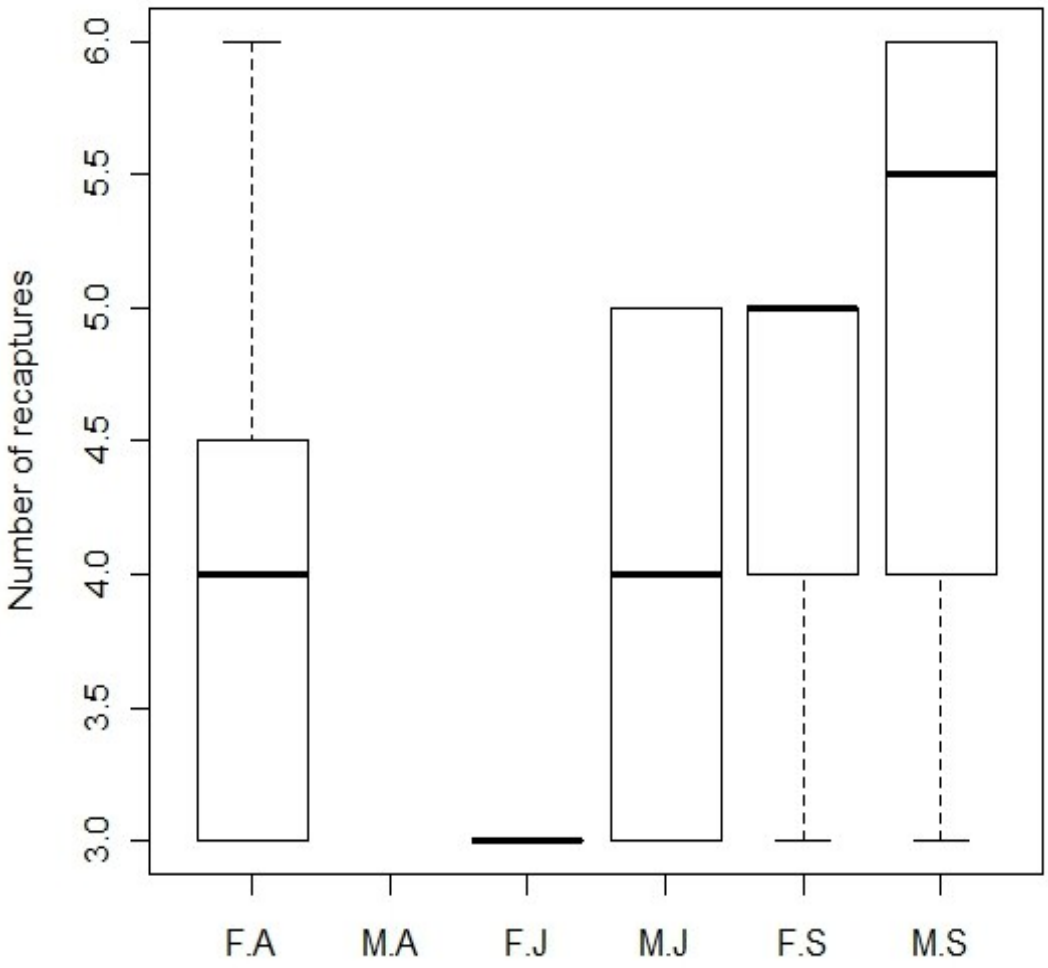

Figure 3.4.1. Boxplot representation with medians, interquartile ranges, and 95% CI of recaptured *O. palustris* by sex and age. A = Adult, J = Juvenile, and S = Subadult. Adult male (MA) data in McIntyre et al. (2009).

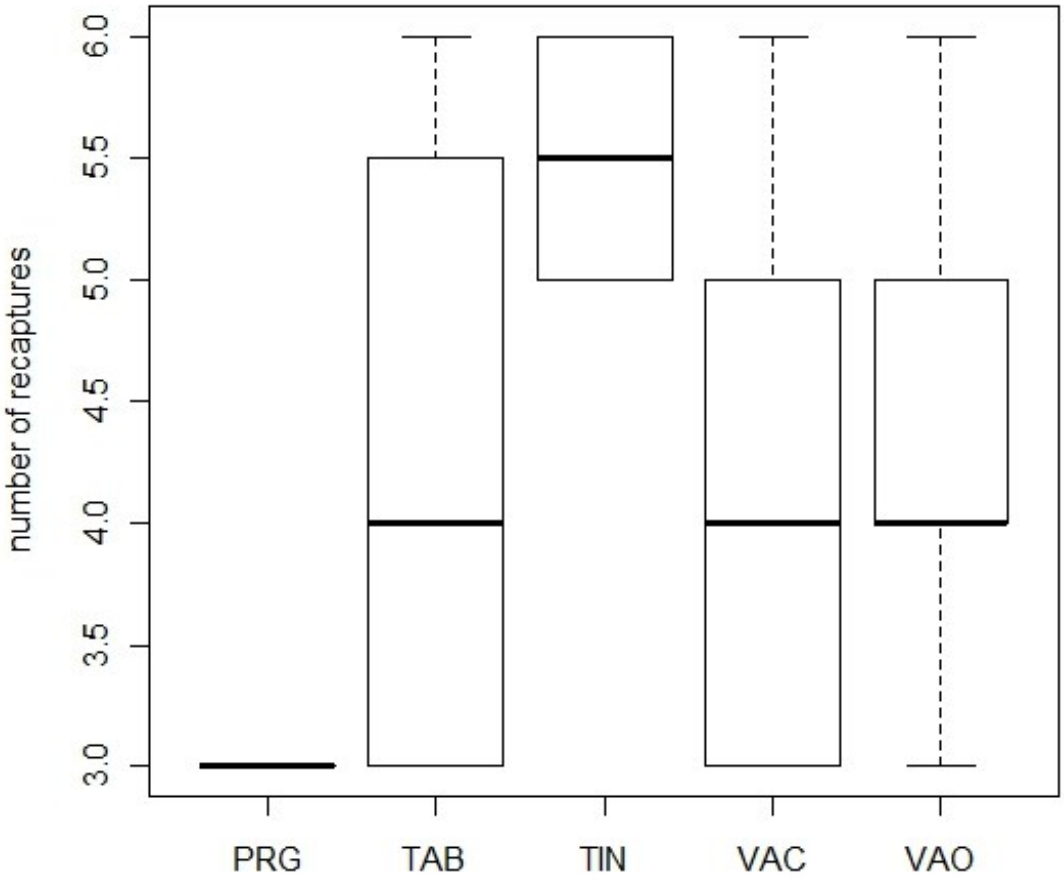

Figure 3.4.2. Boxplots with medians, interquartile ranges, and 95% CI of *O. palustris* recaptures by reproductive condition. PRG = Pregnant, TAB = Testes Abdominal, TIN = Testes Inguinal, VAC = Vagina Closed (non-receptive), and VAO = Vagina Open (receptive).

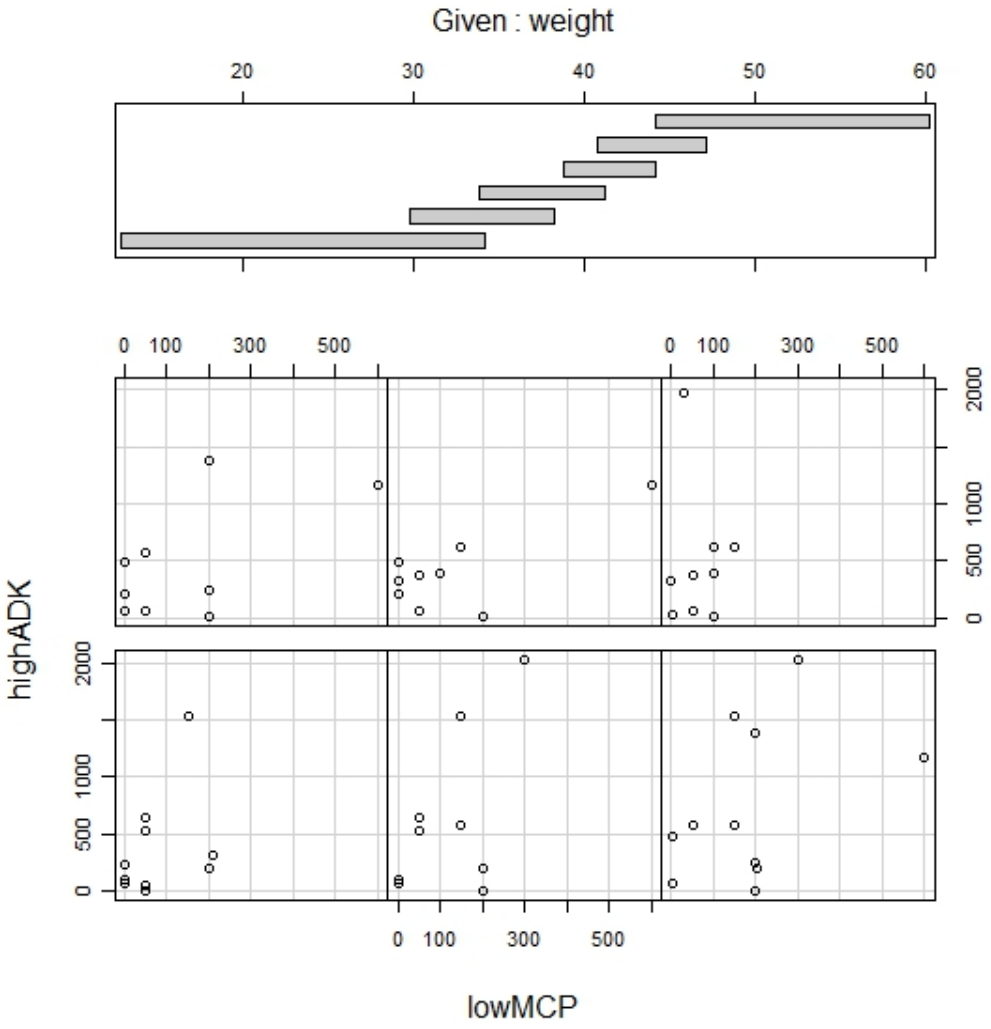

Figure 3.5. Coplot of no correlation between home range size (low MCP and high ADK scores) across weight spectrum for recaptured *O. palustris*.

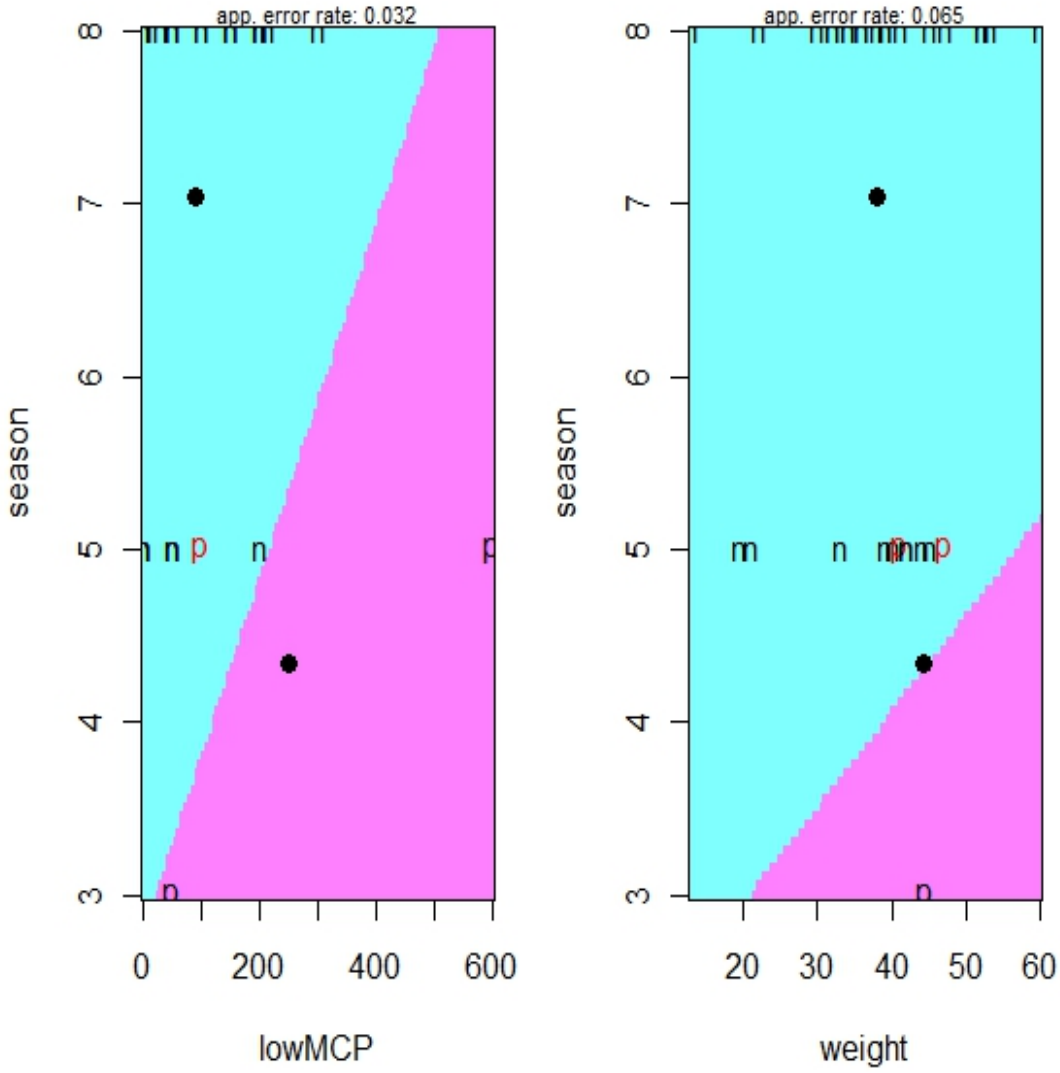

Figure 3.6. From the linear discriminant analysis, partition plots with centroids (solid black circles) and approximate classification error rates of *O. palustris* by serogroup (n = seronegative, p = seropositive) when season (DF1) and low MCP (DF2) are used as the most discriminating variables (left). Season and weight (DF3) are shown for comparison only (right), as using weight as a discriminator doubles the error rate of serogroup assignment.

CHAPTER IV

SPECIES RICHNESS: A PROMISING DILUTIVE PREDICTOR IN THE BAYOU VIRUS–*ORYZOMYS PALUSTRIS* INFECTION DUET

ABSTRACT: High biodiversity is correlated with low disease risk or incidence in hosts, with analogous reduced disease effects for humans, a pattern termed the 'dilution effect' (DE). Recently, however, the DE as a generalizable tenet in the transmission ecology paradigm of zoonotic diseases has come under closer scrutiny, for it is unknown whether it is species richness or evenness of biodiversity that plays the main role, and to what extent the effect is due to density rather than diversity. The goal of this investigation was to apply two regression techniques to determine whether or not a DE is probable in a coastal Texas rodent community where the *Hantavirus* genotype Bayou virus (BAYV) is maintained by its host, the marsh rice rat (*Oryzomys palustris*). Relative abundance of *O. palustris*, species evenness, species richness, and density of all rodents in the community were used as predictors and their influences on BAYV seroprevalencies were examined. Robust regression revealed that although species evenness had a negative effect on seroprevalence, species richness was a much more reliable transmission dilutor overall. From partial least squares regression (PLSR), we found that 12.47% of the variance in seroprevalence was accounted for using the predictor species evenness, 29.25% by combining species evenness and richness, and 47.42% explained by combining species richness, evenness, and density.

Regression techniques failed to detect statistical significance between BAYV seroprevalence and relative abundance or true density of *O. palustris* hosts or true density of heterospecifics. The 3 independent latent vectors from PLSR offer some insight into the structure of the data, although slightly more than 50% of the variance in seroprevalence remains unexplained in this system. Elucidation of other stable predictors as well as the underlying ecological mechanisms at work in this potential 'dilution effect' presents a crucial step towards a more comprehensive assessment of what weakens or strengthens BAYV intrahost transfer in *O. palustris*. Overall, species richness was the most supported component in terms of dilution potential of BAYV seroprevalence in this system.

INTRODUCTION

The beneficial effects of biodiversity and its abatement of disease risk in humans and wildlife are becoming increasingly recognized by epidemiologists, disease ecologists, and conservation biologists. Host community assemblages with high species richness and/or evenness are more likely to include a larger demographic of ineffectual hosts lacking in ability to transmit a particular pathogen (Matuschka et al. 1991, Matuschka and Spielman 1992). This 'dilution effect' or DE (Power 1987, Hochberg 1991) in the interplay of host diversity and transmission ecology has been explored extensively in the Lyme disease pathogen-vector-host system by employing an array of empirical and modeling approaches (van Buskirk and Ostfeld 1995,

Norman et al. 1999, Ostfeld and Keesing 2000a, Ostfeld and Keesing 2000b, Schmidt and Ostfeld 2001, Schauber and Ostfeld 2002, Dobson et al. 2006, LoGiudice et al. 2008). From these studies, reduction in rates of intraspecific contacts within the small mammal host communities (either by direct reduction in primary host abundance or through a tempering of intraspecific interactions) should weaken the disease potential of the numerically dominant host. For non-vectored pathogen systems, Ostfeld and Keesing (2000b) used data from Kosoy et al. (1997) for *Bartonella* spp. and Mills et al. (1998) for hantavirus antigens to demonstrate that infection frequency was reduced in more diverse host communities.

From these initial steps was borne the utility of how a DE could play a crucial role in affecting transmission outcomes of disease systems across a wide spectrum of landscape types and host communities. What is less clear, however, are the mechanisms behind dilution, which could be driven by either of the separate components of biodiversity--species richness (number of species) or evenness (relative abundances of each species)—or by total host density. If richness is the key factor, then identification of differences in disease susceptibility across host species should be possible, whereas if evenness is the key driver, then the availability of susceptible hosts as proportions of the total number of individuals would be more important than simple host presence (as would be the case for richness). Finally, if density is the primary factor rather than either biodiversity component, then the total number of animals that an area supports would be more important than which species were

present and in what proportions. Identification of which component(s) are responsible for a dilution effect is thus crucial in understanding the dynamics of disease ecology.

An examination of hantaviruses provides a test system to consider the relative roles of species richness, evenness, and density on the dilution effect because hantaviruses are not vectored (which simplifies matters because vector diversity and density do not have to be considered). Instead, hantaviruses are transmitted between rodents via contact with excreta (saliva, urine, feces) (Mills et al. 2008). Theoretically, only one competent host exists for each hantavirus genotype, but multiple rodent species can be infected; thus, a hantavirus should be transmitted most efficiently in a single host species community with maximal density of hosts (Dobson and Hudson 1995), plus other factors that create frequent and prolonged, physical contact between hosts, but with minimal host mortalities from the same high density effects (e.g., predator swarming, increased transmission of harmful microorganisms). Several theoretical and empirical examinations of dilution effects in various host-hantavirus systems have been conducted, with a variety of outcomes. For example, Peixoto and Abramson (2006) modeled the effects of host biodiversity on Hantavirus Pulmonary Syndrome dynamics in North America by comparing the transmission of Sin Nombre virus (SNV) in a community that consisted only of *Peromyscus maniculatus* (deer mouse) to more diverse communities that included several other rodent species. A major model assumption was that the other rodent species do not become infected with SNV, an assumption that would support the primacy of species richness in a dilution effect (if species do not differ in their susceptibility under this

factor, richness should amplify rather than dilute the disease prevalence). Using a two-species theoretical model, they proposed that species diversity dilutes SNV prevalence through competitive pressure exerted by non-carrier species on the primary host, *P. maniculatus*. The Peixoto and Abramson (2006) model differs from observational findings of Clay et al. (2009), as their model implicates the reduction of primary host density as the underlying mechanism by which species diversity mitigates SNV prevalence; Clay et al. (2009) detected no significant relationship between deer mouse density and the proportion of SNV-infected deer mice. However, the possibility exists that competitive pressures from non-host species alter retention (dispersal or survival) of deer mice in particular habitat types, in which case, non-host presence attenuates SNV prevalence by diluting deer mouse abundance (Clay et al. 2009).

Table 4.1 summarizes the empirical studies of hantaviruses from which dilution effects were reported. From this compilation, two features are noteworthy: (1) linear regression analysis was used in most studies (Yahnke et al. 2001, Mills 2005, Suzán et al. 2009, Carver et al. 2011, Orrock et al. 2011), whereby a biodiversity index (usually Simpson's *D*) was used as the predictor variable and antibody prevalence of the hantavirus genotype was regressed as the response variable; and, (2) most studies did not separate out the effects of species richness, evenness, or density (Simpson's *D* combines both richness and evenness into a single metric, for example; Magurran 2004).

These studies (Table 4.1) show little consensus as to the occurrence of a dilution effect or its mechanism, and this could possibly be related to conflating richness and evenness and to using a linear analytical technique to detect a pattern that is likely thresholded rather than linear. For example, using time series cross-correlation, Piudo et al. (2011) found no significant relationship between richness or evenness and seroprevalence in *Oligoryzomys longicaudatus*; likewise, Tersago et al. (2008) discovered a negative but statistically non-significant influence of the relative proportion of wood mice (*Apodemus sylvaticus*) on the occurrence of Puumala virus antibodies in *Myodes glareolus*, and in *Calomys laucha*, the seroprevalence of Laguna Negra virus was only slightly correlated with host density (Yahnke et al. 2001). Orrock et al. (2011) detected a significant negative relationship between Sin Nombre (SNV) seroabundance in *Peromyscus maniculatus* and predator richness, but no relationship between SNV seroabundance and the presence of heterospecific rodents; whereas the presence of two vole species exerted a negative effect on SNV antibody prevalence in *P. maniculatus* (Carver et al. 2011), with antibody prevalence not contingent on the density of *P. maniculatus* (also seen in Dizney and Ruedas 2009).

Thus, multiple hantavirus researchers have searched for a DE at work in their system by using essentially the same diversity metric (Simpson's Index or of that family) and the same statistical approach, i.e. linear regression analysis (Yahnke et al. 2001, Mills 2005, Suzán et al. 2009, Carver et al. 2011, Orrock et al. 2011); for the most part, their results show a negative relationship between small mammal diversity and seroprevalence but do not identify whether richness or evenness is the more

important component (Table 4.1). Moreover, a meta-analysis of 16 studies revealed a noticeable publication bias by researchers for reporting negative results almost exclusively (Salkeld et al. 2013). An example of a more appropriate statistical examination that parses out species richness, evenness, and density is thus warranted. I provide an example of using robust regression techniques to determine whether or not a DE is likely in a coastal Texas rodent community where the *Hantavirus* genotype Bayou virus (BAYV) is maintained by its host, *Oryzomys palustris*. Species evenness of *O. palustris*, species richness of all sympatric and potential, host rodent species, and density of all rodents in the community were used as predictors and their influences on Bayou virus seroprevalencies were examined.

MATERIALS AND METHODS

Field work, basic calculations

Field site and rodent community descriptions, in addition to field and laboratory methods, are described in McIntyre et al. (2005; 2009) and Holsomback et al. (2009). Briefly, seroprevalence was monitored over a three-year period (2002-2004) in a rodent community in Texas (Figure A.1) on four mark-recapture grids (Figure A.2), two grids each in uplands (Grids 1, 2) and in coastal marshes (Grids 3, 4). An eight-day pilot study conducted prior to establishment of our mark-recapture grids guided placement and size of the grids plus trapping regime (i.e., for diurnal or crepuscular species, etc.).

True density, *D*, was defined by the number of rodents per unit area (or ~7850 m^2, the average area of each grid). Species richness (*S*, number of rodent species present) and evenness (*E*, relative abundance of individuals from each rodent species) indices were calculated (Magurran 2004). Species evenness values range from 0 – 1 and describe negligible to maximal diversity levels, respectively. Average seroprevalence represents the total number of antigen and antibody positive *O. palustris* detected expressed as a percentage of the total number of rodents captured over a particular time period.

Regression models

Regression models with graphics were run using the R language, version 2.12.2 (R Development Core Team 2008). The R Manual (R Development Core Team 2011) was referenced extensively (http://cran.r-project.org/doc/manuals/R-intro.pdf). Several power transformations were considered initially for the Y-variable, seroprevalence. However, little improvement was detected in linearity or in offset of the 'mouse-elephant effect' (R. Strauss, pers. comm.), so transformations were not utilized during the functional form test processes; this decision also made more decipherable the true nature of the structural relationships within the data post-modeling.

Robust regression

To retain and examine all cases of an already modest dataset, robust regression was used to employ a fit criterion less-impacted by unusual observations compared to other regression methods (Huber and Ronchetti 2009). Three weight functions were compared (Bisquare, Huber, and Least-Squares) based on differing algorithms for down-weighting high leverage outliers. From the shape of the raw data distribution and preliminary diagnostics, the Least-Squares estimator was selected (from the linear model 'lm', running the Least-Squares model 'mod.ls.2' after selectively removing case 1, which not surprisingly presented the largest residual, as it was from our pilot data-collection period). Results of the robust regression were interpreted as statistically significant at the level of $0.05 \geq P$ with consideration of the test statistic values and how well the model fit the data (R^2 values).

Partial least squares regression (PLSR), a.k.a. projection on latent structure regression

Partial least squares regression (Wold et al. 1984) is used to construct parsimonious predictive models when predictors exhibit multicollinearity, and extends multiple linear regression without imposing the restrictions of other multivariate methods (de Jong 1993). The thrust of PLSR is to attempt extraction of the latent (i.e., underlying) factors, accounting for as much of the manifest factor variation as possible while modeling the responses well (Abdi 2010). Thus, the pragmatic beauty of PLSR lies in preservation of the asymmetry of the relationship between predictor and

dependent variables, while other multivariate techniques (discriminant function analysis, canonical correlation, etc.) treat these relationships symmetrically (Mevik and Wehrens 2007).

PLSR: Algorithm selection, running the latent variable model for structural detection

Using the simPLS algorithm in partial least squares regression, a covariance criterion was maximized, and successive PLS factors (i.e. the latent vectors) were forced into orthogonality. Overall, the simPLS solution is similar to principle component regression, where the aim is not to acquire precise answers from complex datasets but rather to identify stable predictors (Mevik and Cederkvist 2004). Although quite similar for single-response models, the simPLS (simple) algorithm was used rather than the kernel (default) algorithm because ours is not a long dataset; the orthogonal scores (nonlinear iterative partial least squares, NIPALS) algorithm was not selected because it deflates the data matrices (de Jong 1993). Predictive behaviors of the PLS estimators were assessed through two cross-validation (CV, or bootstrap) estimates: cross-validation is the ordinary CV estimate and adjCV is a bias-corrected CV estimate (Mevik and Cederkvist 2004). Validation criterion used was the root mean squared error of prediction (RMSEP) without trimming. Scores and loadings were extracted for all three X-variables and the Y-variable to assess the percentage of variance explained (Mevik and Cederkvist 2004).

Based on Euclidean distances, distance graphs show plot distances from the origin (zero for all dimensions) for the predicted and residual statistics, loadings, and weights for the number of components. These plots can be helpful in determining major contributors to the prediction of conceptual variables (plotting weights) as well as outliers that have a disproportionate influence, relative to other observations, on the results (plotting residual values) (Mevik and Wehrens 2007).

RESULTS

O. palustris relative abundance, species evenness and richness, and BAYV seroprevalence: robust regression

Summarized data used for this analysis can be found in Table 4.2. None of the three iterations - Bisquare, Huber, or Least-Squares - detected a significant relationship between the relative abundance of *O. palustris* hosts and seroprevalence, or any significance in the interaction of species richness, evenness, density, and seroprevalence. Using the Lease-Squares algorithm, species evenness had a negative, marginally significant effect on seroprevalence (Estimate = -40.627; Std. Error = 16.698; t value = -2.433; *P*-value = 0.07174), and species richness had a more reliably (based on standard error) significant negative effect on seroprevalence (Estimate = -4.918; Std. Error = 1.162; t value = -4.234; *P*-value = 0.01333). Overall model fit to the data was well supported (Residual standard error = 3.609 on 4 degrees of freedom;

Multiple R-squared = 0.8382, Adjusted R-squared = 0.7573; F-statistic = 10.36 on 2 and 4 DF, *P*-value = 0.02619).

Structure and relationships of species evenness, species richness, and true density (X-variables), and seroprevalence (Y-variable): PLS regression

A validation plot (Figure 4.1) shows a measure of prediction performance (RMSEP, root mean squared error of prediction) against the number of X components. Figure 4.2 depicts the estimated RMSEPs as functions of the number of X variables (3; species richness, evenness, true density), and clearly shows a nonlinear relationship between the three X-variables and seroprevalence plus 2 apparent outliers (which inflate RMSEP). A pairwise plot of the score values for the three components is displayed in Figure 4.3, representing the training step in the model where the percent variance is explained (Table 4.3). A distance graph (Figure 4.4) with its corresponding input (Table 4.4) can be used to interpret specific components with the most positive (augmenting) and negative (lessening) influences on seroprevalence. A loadings plot shows the correlations between each variable and the selected components, two in this case (Figure 4.5).

Taken together, the total sums of squares of the Y-variable, seroprevalence, was not explained maximally by the PLS latent vectors using all 3 components (Table 4.3, Figures 4.3 and 4.5). Only 12.47% of the variance in BAYV seroprevalence was accounted for using species evenness, 29.25% by species evenness and richness, and 47.42% explained by combining the 3 components - species evenness, richness, and

true density. Although these relationships offer some insight into the structure of the data, 52.58% of the variance in seroprevalence remains unexplained, meaning there are other undescribed predictor variables influencing seroprevalence levels in our BAYV- *O. palustris* system.

DISCUSSION

The Least-Squares algorithm and robust regression revealed that although species evenness had a negative effect on seroprevalence, species richness was a much more reliable transmission dilutor overall. From the steps of fitting the model and its validation, I found that 12.47% of the variance in seroprevalence was accounted for using the predictor of species evenness, 29.25% by combining species evenness and richness, and 47.42% explained by species richness, evenness, and density. The 3 independent (X-variable) latent vectors from PLSR offer some insight into the structure of the data, although slightly more than 50% of the variance in seroprevalence remains unexplained. Had a single, composite metric for biodiversity been used, these important nuances would have been missed. Both regression methods failed to detect statistical significance between BAYV seroprevalence and relative abundance and density of *O. palustris* or density of heterospecifics. Overall, species richness was the most reliable dilutive predictor of BAYV seroprevalence using these regression techniques. Similarly, Sin Nombre virus antibody prevalence in the deer mouse is not associated with host density (Dizney and Ruedas 2009, Carver et al. 2011).

There are at least 20 diversity indices, and almost innumerable methods to measure biodiversity at multiple levels (for example, from genes to biomes). Recently, much discussion has ensued over whether or not it would be better to reduce the large number of potential parameters by creating one currency for the measurement and comparison of biodiversity over multiple levels of observation (Natural History Museum 2011). This unified view of biodiversity measurement would benefit cross-discipline communications and endeavors, particularly in the area of species and ecosystem preservation. However, scientists, economists, and politicians are far from bringing this vision to reality. As for the diversity estimates used in other hantavirus DE studies, the Simpson's Diversity Index (Simpson 1949) seems to be the default method, possibly because of its usefulness for smaller datasets. However, there is less opacity when evenness, richness, and the relative contribution of each are analyzed as separate entities rather than combining them into one diversity measurement for analysis.

Linear regression analysis has been applied in most hantavirus DE studies (Yahnke et al. 2001, Mills 2005, Suzán et al. 2009, Carver et al. 2011, Orrock et al. 2011) whereby some index of biodiversity was used as the predictor variable and infection prevalence of the hantavirus genotype was regressed as the response variable. To select an analysis that depicts a relationship known (in advance) to be real does not add anything fresh to the story. Therefore, it is important that we do more than simply describe our own study system following in the analytical footprints of others. Rather than stopping at fitting the model to their dataset of interest,

hantavirus researchers should strive to predict future observations (model forecasting) and compare new observations with previous ones to reveal possibly unexpected features or relationships (structural-equation modeling) inherent in their respective system. Along these lines, an ongoing objective of the disease-diversity relationship is uncovering the distinct mechanisms that underpin the dilution effect. Although we now have a better understanding of how species richness can influence infection prevalence by way of several processes (Keesing et al. 2006), we still lack experimental verification of the DE mechanisms described from small field datasets and some modeling efforts, leaving us with a hazy view of how changes in host ecology and community structure and dynamics truly influence positive or negative shifts in infection rates, and whether such effects occur directly or indirectly (Johnson et al. 2012).

During this study, combined small mammal and plant species richness and evenness decreased from Grid $2 \geq 1 > 4 \geq 3$, with percent seroprevalence calculated overall at 9.2, 12.5, 12.0, and 24.6, respectively (McIntyre et al. 2005). Grid 2 was the most disturbed habitat, grid 1 the most diverse (*Reithrodontomys fulvescens* was the numerically dominant rodent inhabitant), grid 4 was an undisturbed, replicate grid for grid 3 in terms of macrohabitat and community composition), and grid 3 supported the most stable, abundant *Oryzomys palustris* population in this rodent community. Although grid 3 (highest average seroabundance, 24.6%) was the most homogeneous habitat with low species diversity, an inverse dilution effect (or amplification effect) is not necessarily the only plausible explanation for the high seroprevalences recorded.

In coastal Texas, *O. palustris* adults (the primary disseminators of BAYV) occur in marshlands and in upland habitats, with marshlands tending to be less structurally complex. Thus, high seroprevalence on grid 3 (but not on replicate grid 4 - half of grid 3, or 12.0%) could be an outcome related to population demography, microhabitat selection based on varying plant phenologies and with this, different predator evasion strategies, dominant-subordinate hierarchies, and resulting density disparities in more and less competent, *O. palustris* hosts. Furthermore, any potential role of susceptible host density controlling the abundance of infected hosts was supported by results of the complementary grid (grid 4), relatively independent of the presence of other species (*O. palustris* represented 77.2% of total captures), and about as close as we could get to host density manipulation in a controlled experiment. On its counterpart grid (grid 3), much higher abundances of *O. palustris* (97.6% of total community, probably from more preferred microhabitat) lead to concomitant increases in BAYV prevalence, most likely due to greater contact rates between infected and susceptible rodents.

Although there was a mere 0.5% difference in BAYV seroprevalence, it is an important distinction to consider the seroabundance difference estimated between the two demographically polar grids, grid 1 (12.5%; more disturbed old-field) and grid 4 (12.0%; less disturbed coastal prairie; McIntyre et al. 2005). Holsomback et al. (2009) noted the ratio of mammal species richness to plant species richness on these grids to be 5:34 (grid 1) and 3:11 (grid 4). Grid 1 (16.2% *O. palustris*) was the most diverse habitat, with the fulvous harvest mouse the most abundant rodent in the community,

and grid 4 (77.2% *O. palustris*) was less diverse, with the marsh rice rat the most frequently captured rodent. Certainly, we have yet to ascertain other variables affecting the spatiotemporal occurrence of BAYV-positive *O. palustris*. Other intriguing aspects recorded were low species richness and evenness and low seroprevalence on grid 4 (coastal prairie), with low species richness and high species evenness juxtaposed with the *lowest* seroprevalencies (~ 7.0%) found during the study.

A major assumption of the DE paradigm is that the mere presence of heterospecifics produces a reduction in encounters between individuals of the definitive host species. Certainly, this is not an absolute consequence. Investigators largely have failed to consider other potential disruptors in the transmission chain related to host-specific life history traits and population dynamics, e.g., asynchronous diel activity patterns, microhabitat partitioning, selective avoidance behaviors, home range expansion, and the degree of aggression/subordination, not to mention intrahost multi-parasite interactions, predator evasion tactics, and effects of varying levels and types of disturbances; human perturbations, in particular, are not often considered as important factors in interhost dynamics. Although it is not unfounded to suspect other species can decrease the frequency and duration of contacts between host conspecifics, it is unwise for researchers to assume a direct linear relationship is at work in all host systems at all times. Overall, however, correlational field data comparing seroprevalence and diversity suggest the DE hypothesis may be broadly applicative to hantaviruses and potentially useful across diverse ecosystems, community assemblages, and host-virus pairings.

Heterogeneities of host contact frequencies and durations are vital components of the dilution-amplification processes but are investigated rarely, most likely because host contacts are notoriously difficult to observe and quantify without biases and with true systematic rigor. Computer programs like MARK offer population size estimators with variable parameters including heterogeneity in behaviors by species. However, these kinds of programs require a fair amount of recapture data for estimates to be reliable. Although applied field technologies are improving with the advent of fluorescent powders and PIT tagging, both have unavoidable limitations and require further testing on a broader representation of mammal species to discern whether or not their use alters the behaviors under observation or diminishes test subject health. The availability of limited use techniques (knowing the inherent shortcomings in advance) is better than having no options whatsoever by which to observe rodent social behaviors resulting in bodily contact between hosts, a critical step to potentially track transmission events throughout host communities, especially as they may relate to Woolhouse's 20/80 Rule (1997). Theoretically, one 'super spreader' host in the acute phase of infection in a densely populated host community can be as potent an infection amplifier as 100s or possibly 1000s of chronically infected hosts (Botten et al. 2003, Lloyd-Smith et al. 2005).

Of course, physical contact between donor and recipient host rodents will not necessarily result in infection transfer because of individual differences in host physiology and susceptibility. But certainly the odds are more favorable for a greater percentage of successful infections in a new generation of hosts when the most

lucrative combination of host behavioral ecology and density, spatial proximity, and viral shedding is maximized. These types of influences (along with various levels of habitat disturbances), while not easily assimilated, must be kept in mind when attempting to model biologically realistic infection dynamics from within natural host populations to human rural and suburban population centers.

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Table 4.1. Summary of published, empirical hantavirus studies with proposed dilution effects. Unless noted otherwise, biodiversity was used as the predictor variable and antibody prevalence of the hantavirus genotype was regressed as the response variable.

Hantavirus	Host species	Diversity Metric	Results
Reference	**Location**	**Analysis**	**Comments**
Andes virus	*Oligoryzomys longicaudatus*	Shannon's *H'*, Evenness *J*	No significant correlation detected
Piudo et al. 2011	Patagonia, Argentina	Time series cross-correlation	
Sin Nombre virus	*Peromyscus maniculatus*	Resident predator richness	Significant negative relationship
Orrock et al. 2011	Channel Islands, California	OLS, multiple linear regression	No correlation with heterospecifics
Sin Nombre virus	*Peromyscus maniculatus*	N/A - Presence of voles (MNA)	Negative relationship
Carver et al. 2011	Cascade, Montana	Linear regression, GLM	Density-independent
Calabazo virus	*Zygodontomys brevicauda*	Simpson's Diversity Index, *D*	Negative relationship
Suzán et al. 2009	southwestern Panama	Linear regression	Removal of non-reservoir species

Table 4.1. Continued

Hantavirus	Host species	Diversity Metric	Results
Reference	**Location**	**Analysis**	**Comments**
Choclo virus	*Oligoryzomys fulvescens*	Simpson's Diversity Index, *D*	Negative relationship
Suzán et al. 2009	southwestern Panama	Linear regression	Removal of non-reservoir species
Sin Nombre virus	*Peromyscus maniculatus*	Simpson's Diversity Index, *D*	Significant negative relationship
Dizney and Ruedas 2009	Portland, Oregon	Non-linear regression analysis	Density-independent
Sin Nombre virus	*Peromyscus maniculatus*	Gini-Simpson Index	Negative relationship; no significance between deer mouse density & SNV
Clay et al. 2009	Great Basin Desert, Utah	Linear mixed model, PCA	
Puumala virus	*Myodes glareolus*	N/A	Non-significant, negative influence of relative proportion of wood mice (*Apodemus sylvaticus*)
Tersago et al. 2008	northeastern Belgium	Logistic regression	

Table 4.1. Continued

Hantavirus	Host species	Diversity Metric	Results
Reference	**Location**	**Analysis**	**Comments**
Sin Nombre virus	*Peromyscus maniculatus*	Simpson's Diversity Index, *D*	Highly significant, negative linear relationship from 10 sites
Mills 2005	southwestern U. S.	Linear regression	
Laguna Negra virus	*Calomys laucha*	N/A	Marginal non-significance with relative density of *C. laucha*; other factors
Yahnke et al. 2001	central Chaco, Paraguay	Linear regression	

Table 4.2. Results of grid trapping effort for 6 vertebrate species at Justin Hurst Wildlife Management Area (8 trapping periods from March 2002 – May 2004). More small mammal community and habitat characterizations are in McIntyre et al. (2005, 2009) and Holsomback et al. (2009).

Trap Session	*B. taylori	C. parva	O. palustris	P. leucopus	R. fulvescens	S. hispidus	**True density, D	Species evenness, E	Species richness, S	Seroprev-alence (%)
Mar 2002	8	0	40	5	42	21	163	0.09	5	20.0
May 2002	8	3	81	4	14	13	173	0.09	6	14.7
Aug 2002	16	3	56	1	7	15	138	0.08	6	3.7
Dec 2002	113	2	95	2	136	50	561	0.31	6	4.0
Mar 2003	14	0	64	2	116	39	331	0.18	5	16.4
May 2003	2	2	89	1	34	15	201	0.11	6	13.5
Aug 2003	0	2	99	0	16	12	182	0.10	4	24.8
May 2004	6	2	25	1	4	11	69	0.04	6	12.0

**Baiomys taylori, Cryptotis parva, Oryzomys palustris, Peromyscus leucopus, Reithrodontomys fulvescens, Sigmodon hispidus*

***D* = number of individuals/area

Table 4.3. Dilution effect model fit and validation results.

Validation: RMSEP				
Cross-validated using 8 leave-one-out (LOO) segments				
	(Intercept)	1 comp	2 comps	3 comps
CV	7.753	10.35	11.56	11.68
adjCV	7.753	10.04	11.16	11.24
Training: Percent (%) variance explained				
	No intercept	1 comp	2 comps	3 comps
X	—	99.99	100.00	100.00
Y (Seroprevalence)	—	12.47	29.25	47.42

Table 4.4. Input data for distance graph (Figure 4.4), Model name: Dil 1.

Numbers 1 – 8 correspond to trap session as in Table 4.2.

X PLS Latent Vector Scores				Y Scores (Seroprevalence)			
	Comp 1	Comp 2	Comp 3		Comp 1	Comp 2	Comp 3
1	43.1107	41.588	81.88	1	0.15813	-0.6004	0.08172
2	7.1992	1.238	8.85	2	0.13356	-0.1176	0.74580
3	-67.3339	-89.811	-71.63	3	0.21971	-0.3395	-0.48757
4	-65.3012	-31.988	-25.23	4	-0.82172	-0.1253	-0.21420
5	18.7180	35.316	19.46	5	-0.25541	0.2689	0.21932
6	-0.9317	-4.523	-11.70	6	0.06464	0.1088	0.05924
7	75.6342	81.802	45.88	7	0.11146	0.6105	-0.10176
8	-11.0953	-33.622	-47.50	8	0.38964	0.1947	-0.30255

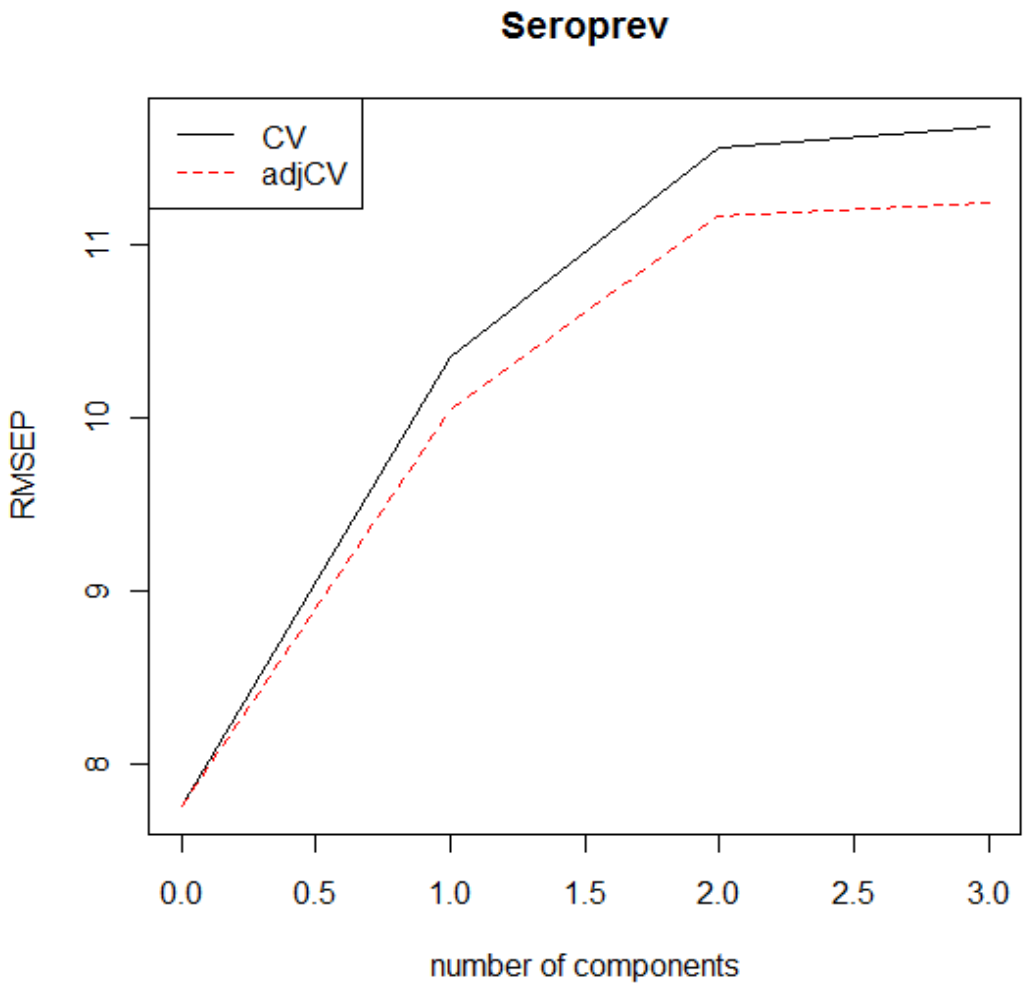

Figure 4.1. Cross-validated RMSEP (root mean squared error of prediction) using the 3 X-variables true density, species evenness and species richness and the Y-variable, seroprevalence.

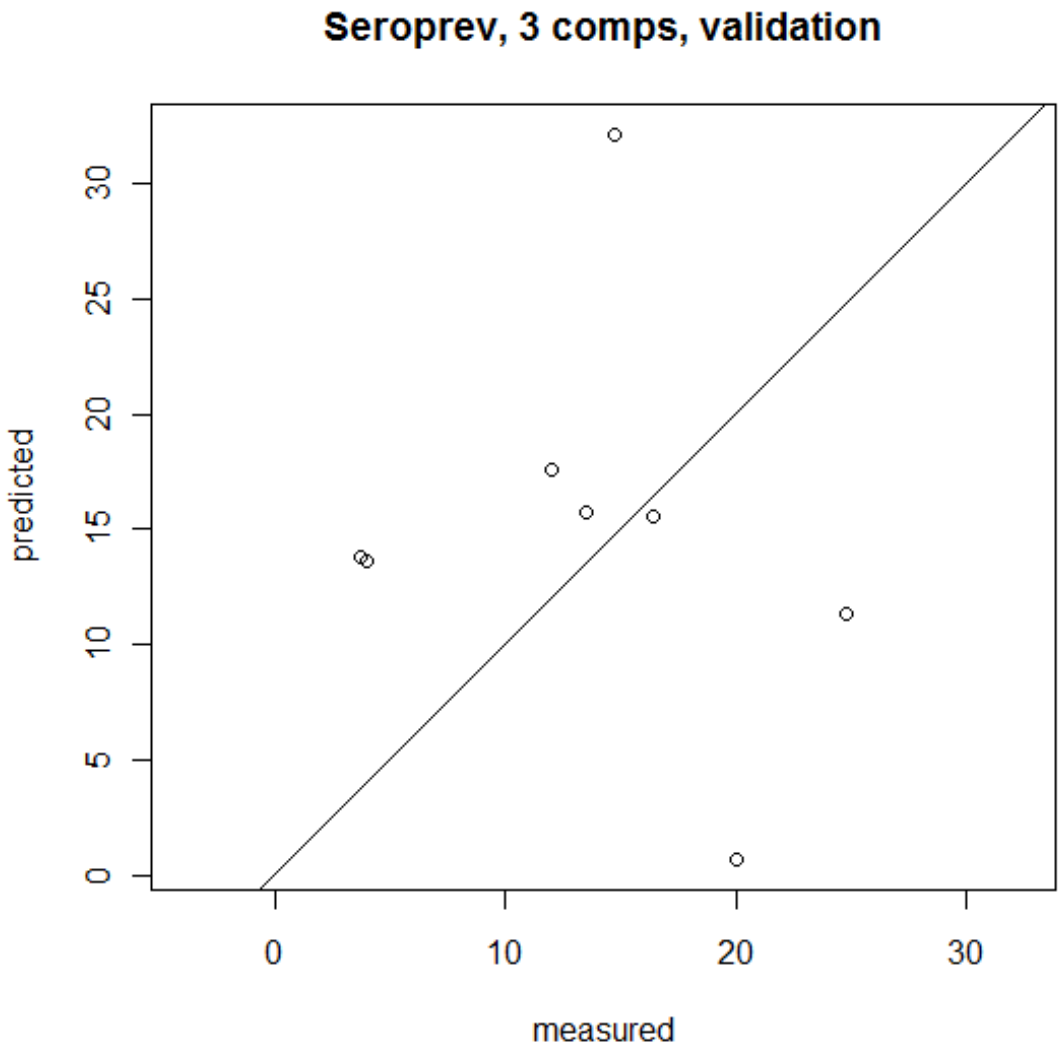

Figure 4.2. Cross-validated predictions for the 3 X-variables and 1 Y-variable.

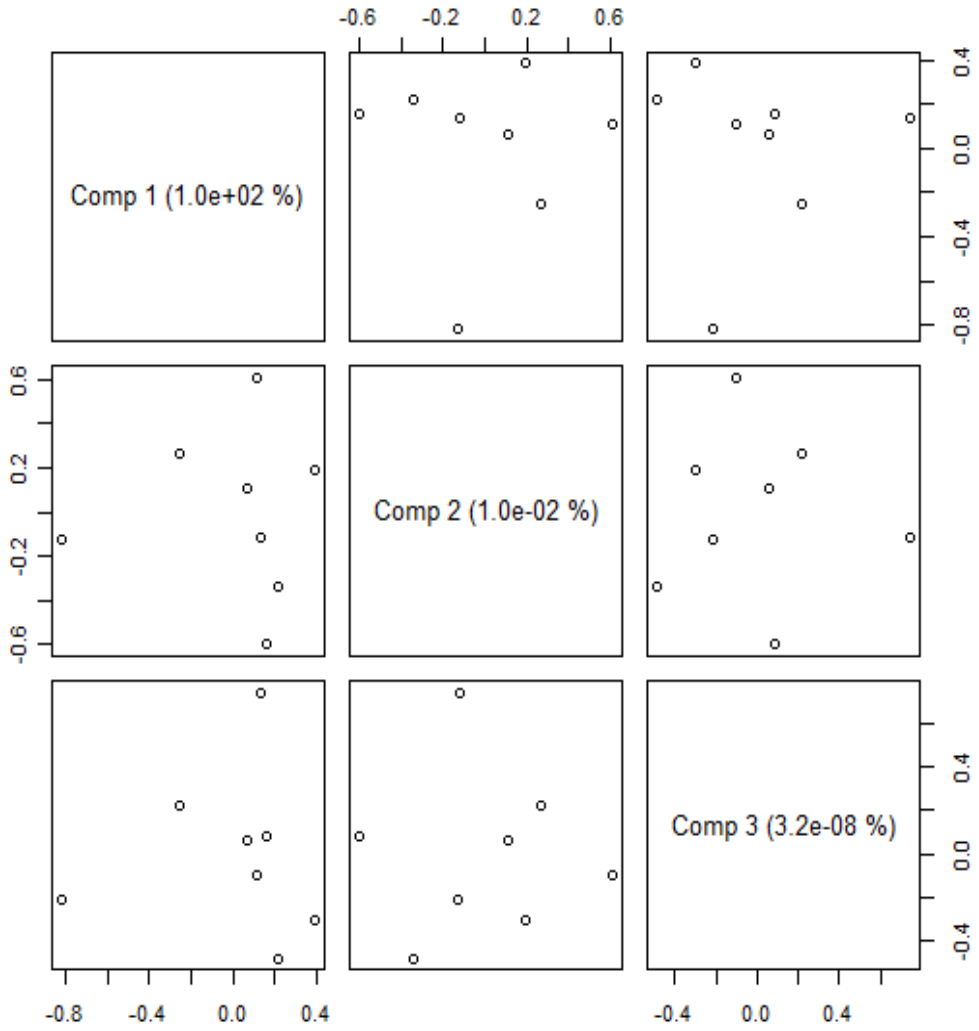

Figure 4.3. Pairwise plot of score values for the three components, representing the training step in the model where percent variance is explained (shown as extracted values along the diagonal).

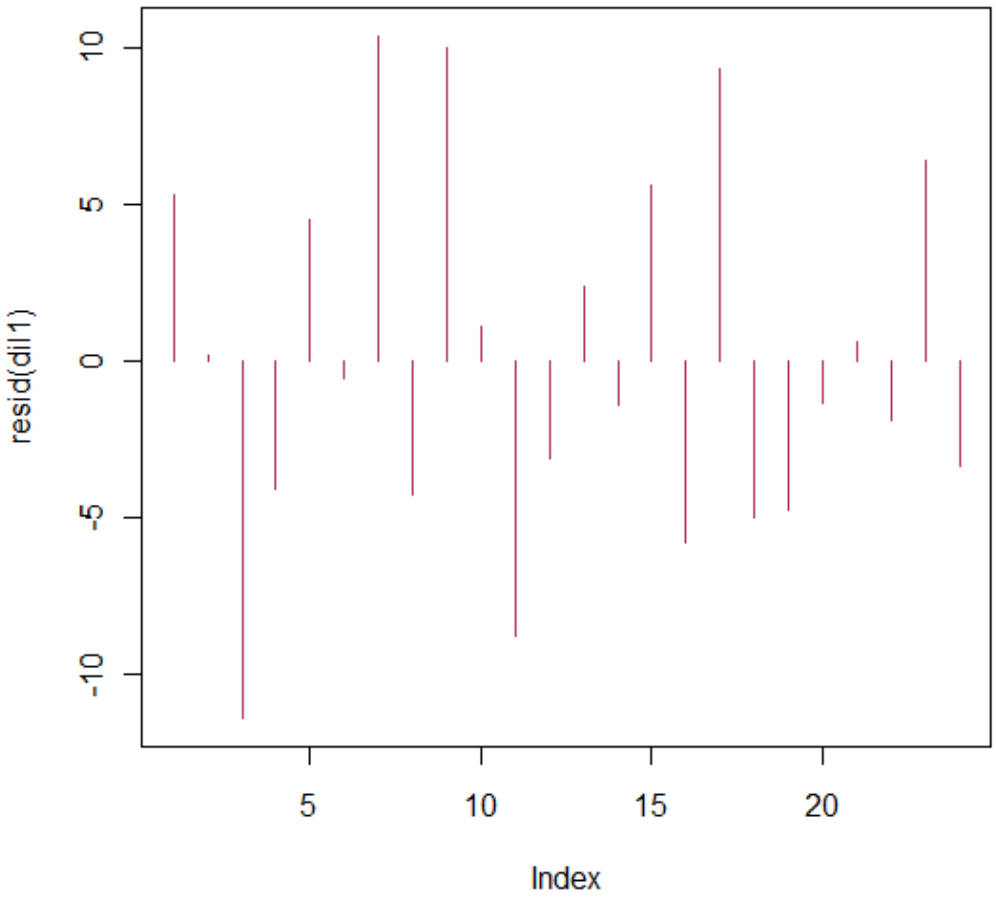

Figure 4.4. Distance graph of the residuals, loadings, and weights for Model Dil 1.

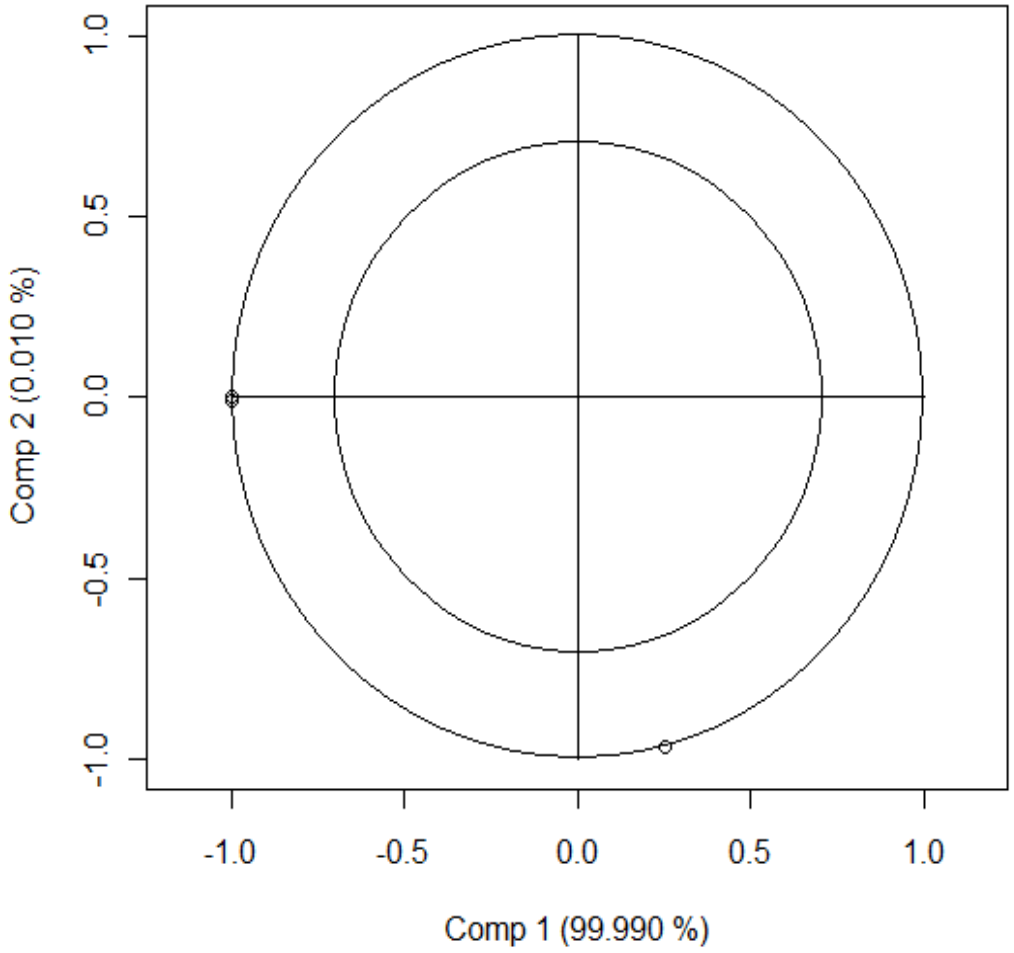

Figure 4.5. Correlations loadings plot for the first two components.

CHAPTER V

RESEARCH SYNTHESIS, AND BAYOU VIRUS LIFE HISTORY TRADE-OFFS AND BET HEDGING

Deciphering the enigmatic conditions of how and why ancient hantaviruses have become of contemporary epizootic importance has not proven easy (Holsomback et al. 2013). Hantaviruses are not novel pathogens, just as their cricetid hosts are not mammals recently derived. Although their phylogenetic footprints are almost parallel, there are some obvious geographical associations, in addition to co-infected rodents, non-selective, "promiscuous" viruses, and relatively high antibody levels detected periodically in a variety of companion animals, domestic livestock, and sympatric wildlife. Certainly, the one host-one virus codivergence paradigm is an oversimplification, at best (reviewed in Zeier et al. 2005). While hantaviruses themselves are not of recent origin (in fact, there is evidentiary support for their problematic presence from at least 1000 years ago; McKee et al. 1991), both drivers and consequences of their re-emergences appear to be phenomenologically new (Holsomback et al. 2013). To date, what remains elusive is a clearer picture of the global complexity of the ecologic, phenotypic, phylogenetic, and phylogeographic relationships between these viruses and their hosts. It is therefore imperative that we seek to identify potential mechanistic harbingers to human hantaviral disease emergence. And this means invoking multidisciplinary approaches and devising

innovative tools, including the resurrecting and application of perhaps less common but useful tools, to expand our understanding of the behavioral ecology and interaction transmission of vertebrate hosts.

Long-term studies in natural settings (regarded widely by ecologists as essential for comprehension of the temporal dynamics of vertebrate communities; Cody 1996) are useful in particular for investigating rare but impactful events, and for discerning and assessing processes that may unfold gradually in populations or communities of vertebrates (Mills et al. 1999). Despite obvious benefits, multi-year investigations of reservoir populations associated with zoonotic agents are rather sparse. If conducted properly, these types of studies are labor intensive and financially costly; consequently, they require not only substantive, stable funding for several years, but also a level of human dedication and systematic implementation not always mustered easily. Furthermore, if they are not replicated stringently for a long enough duration, sheer noise or erroneous results may be the only end products, otherwise known as GIGO - the "Garbage in, garbage out " phenomenon (George Fuechsel, IBM).

Efforts from this Texas study revealed that in preferred grass-dominated, coastal prairie macrohabitat, microhabitat selection in *O. palustris* adult females could explain the distribution of conspecific adult males and the distribution of BAYV (as assayed by serological status) – a unique, explanatory stance to interpret the spatial arrangement of hantavirus antibody-positive hosts from within any Hanta system. Seropositive *O. palustris* males have been reported from JHWMA to avoid grasses on

a microhabitat scale, which at the time was attributed to the increased ranging seen in seropositive males (McIntyre et al. 2009). However, the pattern of habitat selection in males could instead be due to the fact that receptive (i.e., breeding) females tended to occur in areas with the greatest habitat diversity rather than the areas with the greatest grass cover (as was the case for the non-receptive females). Seropositive males averaged significantly larger home range size estimates than seronegative males at our study site (McIntyre et al. 2009). A male that maintains a larger territory increases the probability it will include a receptive female; if he monitors his range vigilantly, he has a better chance of crossing paths and copulating with her (Tew and Macdonald 1994). In light of our study, male home range sizes by serostatus make sense: diffuse (i.e. overdispersed, spatial distribution) breeding females induce larger home ranges in seropositive males, who tend to be socially dominant, larger, and/or older (McIntyre et al. 2009). This socioecological explanation of host distributional patterns, based on female receptivity and habitat preferences *rather than just* male aggression, is an original approach to distill the logical links between hantaviral maintenance and circulation in natural host populations (Holsomback et al. 2013).

Seropositive adult females weighed more and had larger home ranges than did seronegative adult females, even though there was no statistically significant age or weight difference between the two groups. Moreover, we showed that weights and home range sizes were uncorrelated variables in this species (McIntyre et al. 2009, Holsomback et al., in review). Compared to seronegatives, seropositives also showed less variability in recapture numbers and home range size estimates, i.e. these data are

more consistent, even with smaller sample sizes. The import of these results is unknown, currently.

With respect to characterizing complex systems like zoonoses, non-significant data are just as important to disclose as are the significant data. In this case, the MANOVA and LDA detected no significant differences between recaptured seronegative and seropositive *O. palustris* juveniles, subadults, and female adults with regard to sex, age, weight, recapture numbers, and high ADK (upper range of home range use). However, season (August for seronegatives, May for seropositives) and low MCP (91.0 m^2 for seronegatives, 250.1 m^2 for seropositives) were found to be reliable serostatus discriminators of *O. palustris* hosts, and may prove useful for researchers attempting to differentiate other New World host-virus associations. Although the LDA was highly accurate in predicting group membership, it is likely other variables are in need of inclusion to more precisely demarcate potential hantavirus hosts by serogroup markers.

As for the diversity metrics and estimates used in other hantavirus dilution effect studies, the Simpson Diversity Index (Simpson 1949) seems to be the default method, possibly because of its usefulness for smaller datasets. However, there is less opacity when evenness, richness, and the relative contribution of each are analyzed as separate entities rather than combining them into one diversity measurement for analysis. Robust regression revealed that although species evenness had a somewhat negative effect on seroprevalence, species richness was a much more reliable transmission dilutor overall utilizing several estimators. However, no significant

relationship was detected between the relative abundance of *O. palustris* hosts and seroprevalence. By applying the simPLS algorithm in partial least squares regression, we find that seroprevalence is not explained maximally even using all 3 components, or 100.00% of the X variance. Only 12.47% of the variance in BAYV seroprevalence in *O. palustris* was accounted for using species evenness, 29.25% by species evenness and richness, and 47.42% explained by combining the 3 components, species evenness, richness, and true density. And although these relationships offer some insight into the structure of the data, slightly more than 50% of the variance in seroprevalence remains unexplained. Hence, we should strive to characterize the other impactful predictor variables that may dilute BAYV transmission among *O. palustris*. Partial LSR and other nontraditional regression methods (like robust and ridge regression) should be considered as viable alternatives in other Hanta studies when there exists a need to retain all the data points (including non-normal and high impact outliers), particularly when the dataset has few entries already, and for a more complete and honest representation of the respective system. As always, researchers have the option of running the models with and without the polar data points, and drawing comparisons between the two. Inherently, the essence here would be to describe the zoonotic system to the best of one's ability with the resources at hand, rather than to report the most stonkeringly smallest *P*-value generated.

Naturally in studies like this one, researchers should attempt to address at least two other nagging issues: (1) spatial scale, and (2) individual versus population-level regulation/outcomes. Most hantavirus studies of reservoir-virus ecology in natural

settings have focused on large-scale patterns of transmission dynamics (Root et al. 1999, Biggs et al. 2000, Boone et al. 2000, Glass et al. 2000, Olsson et al. 2002, Yates et al. 2002, Armién et al. 2004, Olsson et al. 2005, Goodin et al. 2006). And yet, within broad habitat types associated with higher viral prevalences, the distributions of hosts and viruses are unlikely to be uniform because of microhabitat selection in the host species (McIntyre et al. 2009). Spatial scale is unmistakably crucial to our understanding of the environmental variables exerting effects on rodent physiologies and behaviors associated with human hantaviral exposure and risk (Giuggioli et al. 2005). Therefore, we applied a multi-scaled approach in examining hantavirus-rodent relationships at both coarse and finer scales (McIntyre et al. 2005). More *O. palustris* of both sexes were captured in coastal prairie than in upland macrohabitat, and the abundance of seropositive rodents was correspondingly higher in coastal prairie (McIntyre et al. 2005). Significant differences in microhabitat selection by serological status were detected for the three most commonly used microhabitat variables: seropositive rodents were more likely to avoid grasses and forbs compared to seronegatives, which more often selected microhabitats characterized mostly by coarse woody debris cover (McIntyre et al. 2009). Using GIS technology, I found that in preferred coastal prairie habitats, no seropositive males were located within 30 m of non-breeding females, and no seronegative males were found within 30 m of breeding females. Moreover, compared to seronegatives, seropositive males were more frequently associated with receptive females by grid, within a 30 m radius, and even occupying the same trap. Ours is one of a few studies to have incorporated a multi-

tiered approach to analyze host habitat selection, the spatial relationships of adult males and females, and hantavirus presence and prevalence (McIntyre et al. 2009, Holsomback et al., in review).

In natural settings, the impact of a parasite on its host population potentially can affect the perpetuity of the parasite itself and moreover, the infection risk to sympatric wildlife and to humans. Endemicity of parasites can be characterized by a tendency towards long periods of stable prevalence, during which time these largely avirulent parasites do not produce obvious decreases in reproduction, fecundity, or survival of their host populations (Anderson and May 1979, Grenfell and Dobson 1995). Even still, infected hosts may incur some detrimental effects and thus, the fitness of individual hosts may be compromised. Certainly, these deleterious end products may prove impossible to distinguish from other natural influential factors on fitness of individuals within host populations (Telfer et al. 2002; 2005).

Consistently, I found that seropositive rather than seronegative *O. palustris* females fell at the lower end of the weight range for female captures. These data are in stark contrast to those ascertained for the main BAYV traffickers – the sero-/viral positive, *O. palustris* adult males (McIntyre et al. 2009). Most sample sizes were too small to analyze with any statistical rigor; however, the raw data can be interpreted straightforwardly (characterized in Table 3.2). From here, it is clear that infection costs from BAYV were detected in each and every seropositive female *O. palustris* capture (n = 14), and spanned all age classes, non-pregnant and pregnant, and their unborn and young progeny. Field data signify that compared to their seronegative

conspecifics (i.e. the control groups), BAYV elicited costs in embryoid/fetal, juvenile, subadult, and adult *O. palustris* females in terms of: (1) lower body weights; (2) shorter total body lengths; (3) smaller embryoid/fetal body dimensions; (4) increased mortalities; (5) decreased residency and/or higher emigration, and (6) larger home range sizes. We observed larger litter sizes compared with small captive colonies of *O. palustris* (Svihla 1931, Worth 1950), but slightly fewer pups per litter compared with congeners (Goldman 1918) and a long-term *O. palustris* dataset from Louisiana (Negus et al. 1961). Periodic high rodent densities may have affected some litter sizes, as well. Compared to Holarctic hosts, these data may be met with skepticism and/or criticism; nevertheless, all things being equal for females regardless of serostatus, they are indicative of very real costs of BAYV infection in the secondary host females.

For population-level regulation, the parasite must exert influence on host reproduction or survival via a density-dependent mode (Gulland 1995, Tompkins and Begon 1999). This controversial, yet rarely traversed topic of population-level regulation by directly transmitted pathogens (although daunting to show clear, direct causation let alone assess mathematically) is well within the realm of pertinent speculation as it relates to the life history strategy of viruses (Villarreal 2009) such as Bayou. From a reasonable sample size however (n = 61), we failed to detect the presence of anti-BAYV IgG antibodies in either embryos or fetuses sacrificed at any gestational stage (data not shown); as of this writing, we have found no empirical support for transplacental vertical transmission of BAYV from dams to pups within

these sample populations of *O. palustris* in southeast Texas.

But, has Bayou virus evolved the capacity to regulate *O. palustris* hosts at the population level? Possibly. A nebulous answer I know, however do read on and ruminate. Pathophysiologic characteristics seen across seropositive females (low weights, smaller sizes, fewer recaptures, increased mortalities) stir uneasy questions about whether such commonalities in pathogenesis are chance similarities in the phenotypic immune responses of a random subset, or instead reflect very real and clearly negative consequences of infection in the non-primary host sex; therein, may also lie one intriguing, evolutionary trade-off in BAYV host selection, infection, and fitness. Seropositive males averaged significantly larger home ranges than seronegative males at our study site (McIntyre et al. 2009). A male that maintains larger areas of home range usage increases the probability his territory will include a receptive female. From our results, male home range sizes by serostatus make sense: breeding females that are spatially overdispersed induce larger home ranges in seropositive males, who tend to be socially dominant, larger, and/or older with much larger testes (McIntyre et al. 2009). Recall also that no statistically significant relationship has been detected between home range size and weight in either sex of any age class in *O. palustris* (McIntyre et al. 2009, Holsomback et al., in review). Combined with larger home range size estimates, seropositive non-breeding, adult females may exhibit increased dispersal and/or emigration. These behaviors could promote more efficient viral spread through new and farther contacts with naïve rodents. The increased mortality rates detected in seropositive adult females could

cause shifts in breeding patterns and population demography, with more males fighting for less available, female mates. Any of these dynamics could generate profound effects in populations of *O. palustris*, with possibly a negative cascade for human hantavirus epidemiology. Moreover, higher ranking males guarding large home ranges and fighting for possibly fewer breeding females could create a domino effect in terms of Bayou virus spreading, while at the same time, providing more opportunities for these dominant (and more likely seropositive) males to reproduce. Adult males and females respond differently to photoperiodic effects, whereby females are less reproductively active than males during the autumn and winter seasons (Edmonds and Stetson 2001). The adaptive utility of a sexually dimorphic response to shorter photoperiods in aged male and female rice rats has eluded researchers. However, it is conceivable that if older males remain reproductively active, then they may have the advantage (over individuals that undergo a cycle of gonadal regression and recrudescence) of being the first ones to breed in the spring when more females become reproductively active (Edmonds and Stetson 2001); they may also be able to sire young year round in areas with milder winters and where at least some females remain receptive, like in south Texas (McIntyre et al. 2005). With apparent costs of BAYV infection in young mice (Holsomback et al., in review), the increased mobility observed in seropositive males may be a way to disrupt relatedness with susceptible progeny.

Within this directly transmitted, zoonotic disease system, reduced abundances of seropositive reproductive females, due to higher rates of mortality and dispersal

(both temporary and permanent), may represent an unavoidable evolutionary trade-off. On the flipside of the evolutionary coin, an adequate rate of seropositivity in *O. palustris* females (5.7 %; McIntyre et al. 2005), if even only a few survive multiple seasons, provides some virus insurance against periodic population crashes or other stochastic events that may all but extinguish low densities of BAYV-infected, adult males.

Applying the benefits of hindsight, what would I have done differently during the course of this research? Well, several things, actually. (Some are best not to immortalize here.) Perhaps most importantly, we should have taken greater advantage of the resources before us, and collected everything from the rodents sacrificed, and I mean everything. For example, if we had collected the entire reproductive tracts and a single eye (to have the lens) from each sacrificed animal, these tissues would have afforded much greater precision, and not just accuracy, in age determinations. We did collect the skulls and mandibles, however. Much more information regarding individual rodent health and immunological status could have been gleaned potentially from several straightforward analyses of host spleens, reproductive organs, and nervous system tissues. With new technological insights shedding light on host ecological immunology and physiological markers of 'superspreaders', we might have been able to illuminate some other fascinating and useful information from the predominant hosts of Bayou virus. Retained blood samples remain available for most of the rodents captured, and it might prove illuminating to determine the demography of rodents co-infected with other endemic zoonotics, namely species of *Borrelia*,

Mycobacterium (particularly given the semi-aquatic nature of *O. palustris*), and *Rickettsia*, for starters.

Zoonotic pathogen emergences and resurgences are almost always coupled to human-invoked destabilization of natural environmental landscapes and both local and large-scale climatic cycles. Human illnesses and deaths linked to zoonoses are increasing at alarming rates worldwide (Cutler et al. 2010), throwing into sharp relief an indisputable need for reweighing the costs to benefits ratio regarding manipulation of the biosphere. Are the trade-offs from exponential population growth, overwhelming agricultural and industrial demands, invasive species, biodiversity losses, and diminished ecosystem functioning and resilience unavoidable, and do we just accept wildlife disease spill-back to humans as an inescapable consequence of our choices and activities? If so, then the most pragmatic shield is a full comprehension of how we create these multi-leveled ecological and climatological cascades, and how to predict what variables are likely to induce the most directional change, and to what magnitude. Hantaviruses and their associated syndromes did not appear overnight; humans, through the course of years of systematic habitat abuses, have paved the way for a hantaviral renaissance of sorts, providing not only the means (environmental change) but also the ends (themselves) – fertile ground too for a resultant antigenic shift – avirulence in rodents transformed from localized human infections to large-scale outbreaks spanning an unpredictable gamut from mild to mortal.

Unfortunately, it is likely we have just barely "scratched the surface" with respect to identifying and describing hantaviruses and other zoonotic diseases of the

21st century. Zoonoses and their related human afflictions are on the rise globally, causing undetermined numbers of human casualties and suffering (particularly among children, the elderly, and otherwise immunocompromised peoples in poverty-stricken, rural areas), skyrocketing medical costs in at-risk regions, and creating enormous financial losses to ranchers and legal tradesmen, who have been forced to destroy animals suspected to be potential carriers. Wildlife diseases transmissible to humans also have had a negative impact on tourism throughout much of the world, owing to increased public fear of certain outdoor activities equated with higher risks of exposure to reservoirs. As of this writing and if estimated, the economic fallout resulting from all these lost revenues combined has not been made available to the mainstream media outlets.

Adding to the current problem of undiscovered zoonoses, there exists the ominously unpredictable, future issue of zoonotic disease outbreaks arising through mechanisms of habitat adulteration, disconnection, and obliteration, host and pathogen genetic mutations, overzealous antibiotic therapies, international travel, culture-specific practices, and so on. These forces combined with the impacts of exponential human population growth and fluxing regional and broad-scale climate patterns create complex ecological scenarios requiring transdisciplinary approaches to even speculate on human epizootiological outcomes. Short-term, single-scaled, monodisciplinary methods will not suffice to elucidate the full mechanistic spectrum at work in the dynamics of hantavirus trafficking and epidemiology.

At the frontier of disease ecology research is the challenge to synthesize

information from ecology, physiology, and life history theory to better understand and predict patterns of disease risk among hosts. A broader integration between physiological and ecological studies of disease has applied importance for identifying species that are either at risk of disease-driven population declines, or those that function as reservoir or 'superspreader' hosts with the potential to influence epidemics or epizootics (Blaustein et al. 2012).

Hopefully, the data acquired from this relatively long-term, multi-scaled, cross-disciplinary study of BAYV hosts and habitat will prove useful to the global scientific community, while never being needed by the public health community. I have endeavored to more thoroughly identify and quantify key environmental and behavioral variables that may facilitate and further augment virus trafficking by *O. palustris*. Therefore, the groundwork has been established to build a generalized, biologically realistic and meaningful, predictive model of human BAYV risk across *O. palustris* habitats in the southeastern United States.

A large part of the process of science is open sharing of information. Rather than this dissertation just existing as the means to my own ends, my desire is for the fruits of these labors to afford other spatial disease ecologists new cerebral grist so they may find insights into their own projects. Perhaps other hantavirus researchers, theoretical ecologists, medical virologists, and wildlife epidemiologists will benefit from the information here, and they will build upon our methods and create or fine-tune models of their respective disease systems. Hopefully, new vistas of exploration will open for fellow scientists by a spark of exciting questions with resounding "Ah-

ha!" moments, too.

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APPENDIX A

STUDY SITE

Figure A.1. Justin Hurst Wildlife Management Area (JHWMA), formerly Peach Point WMA, in Brazoria County (at arrow), Freeport, Texas, USA. Reproduced from McIntyre et al. 2009.

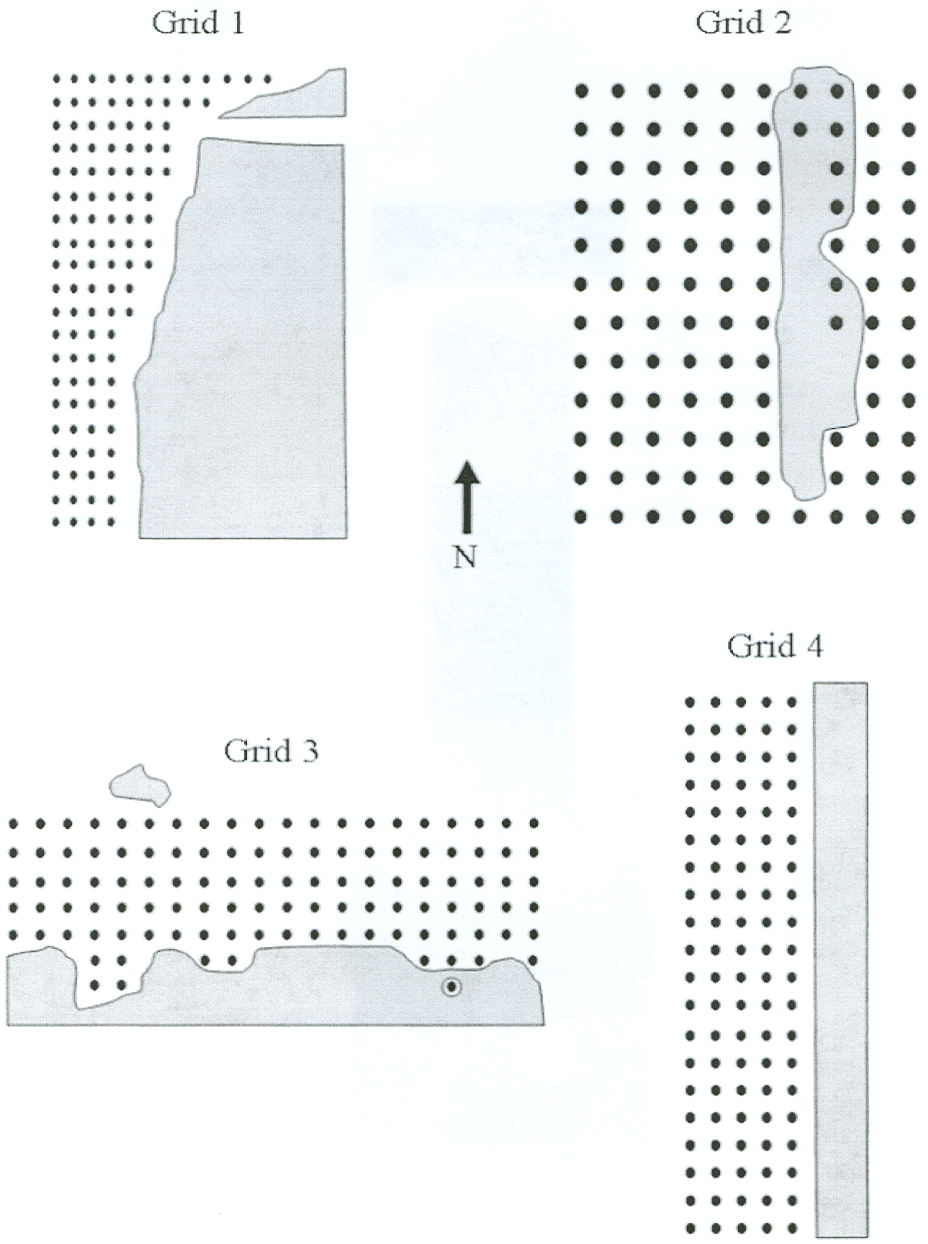

Figure A.2. The four mark-recapture grids at Justin Hurst WMA. Upland grids (1, 2) above, coastal prairie grids (3, 4) below. Figure credited to Nancy E. McIntyre.

APPENDIX B

RANGE OF *ORYZOMYS PALUSTRIS*

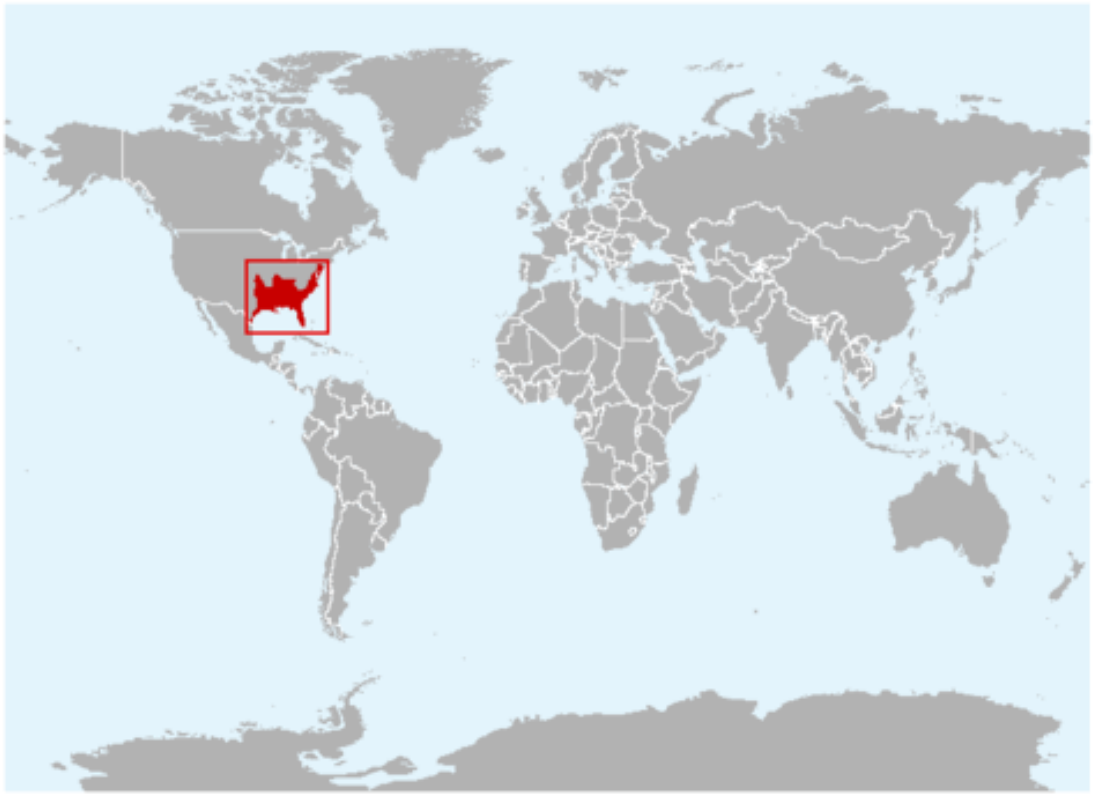

Figure B.1. Distribution map of *Oryzomys palustris* in North America (http://www.discoverlife.org/mp/).

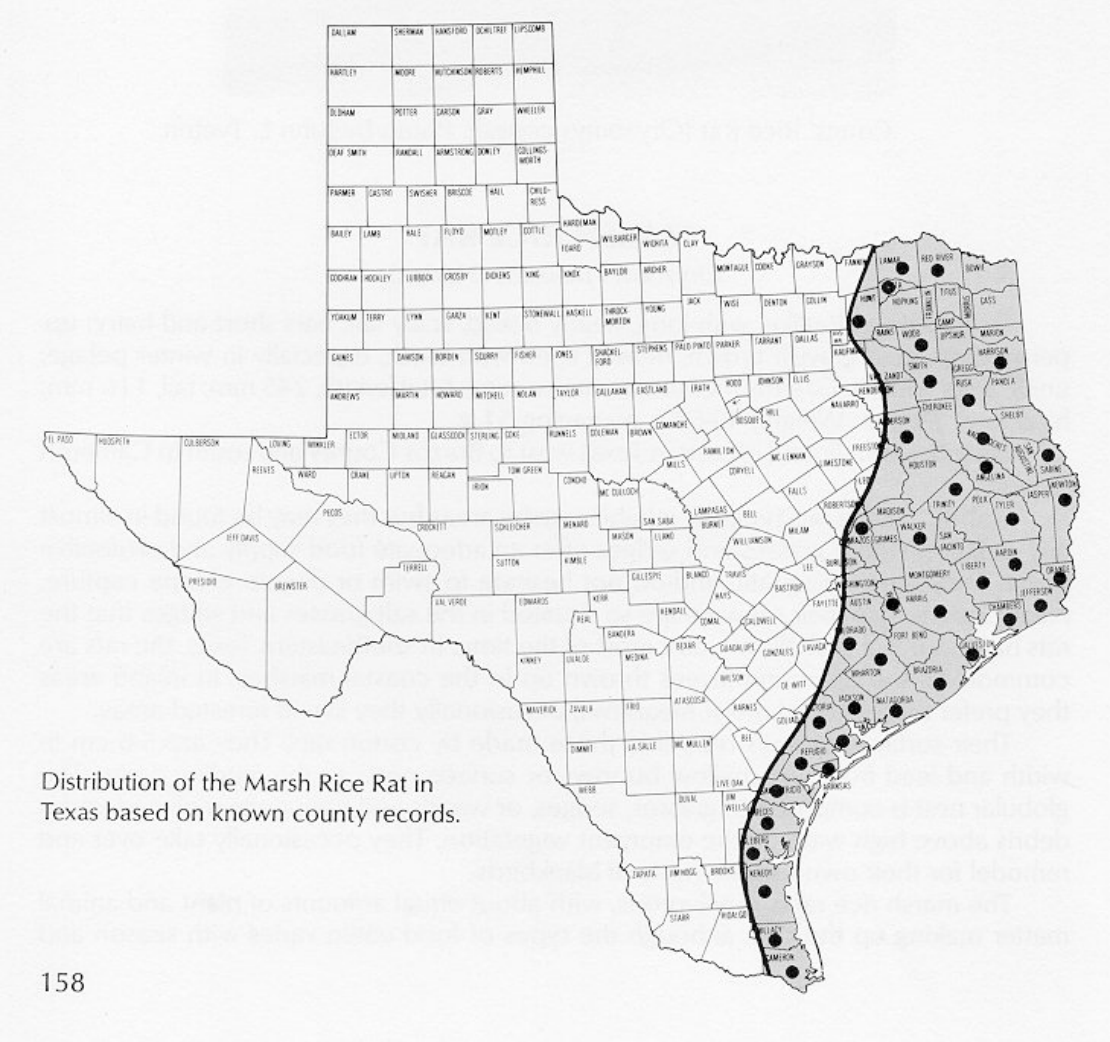

Figure B.2. Known distribution of Texas *Oryzomys palustris* by county.

From *The Mammals of Texas*, Davis and Schmidly (2004).

APPENDIX C

PLANT SPECIES BY MACROHABITAT TYPE

Table C.1. Dominant plant species by grid and macrohabitat type, based on percent cover, listed in decreasing order. Common names are parenthetical. Asterisk (*) denotes non-native.

Grid 1 Old-Field	Grid 2 Old-Field	Grid 3 Coastal Prairie	Grid 4 Coastal Prairie
Spartina spartinae (cordgrass)	*Rubus trivialis* (dewberry)	*Distichlis spicata* (saltgrass)	*Borrichia frutescens* (sea daisy)
Iva annua (sumpweed)	*Iva annua* (sumpweed)	*Batis maritima* (saltwort)	*Spartina patens* (wiregrass)
Sapium sebiferum (chinese tallow tree)	*Sapium sebiferum* (chinese tallow tree)	*Spartina patens* (wiregrass)	*Iva frutescens* (marsh elder)
Juncus effusus (reed)	*Carex* sp. (small sedge)	*Scirpus robustus* (bulrush)	*Salicornia* spp. (pickleweed)
Mimosa strigillosa (powderpuff mimosa)	* *Verbena brasiliensis* (Brazilian verbena)		*Scirpus robustus* (bulrush)
Rubus trivialis (dewberry)	*Juncus effusus* (reed)		*Spartina spartinae* (cordgrass)

Table C.1. Continued

Grid 1 Old-Field	Grid 2 Old-Field	Grid 3 Coastal Prairie	Grid 4 Coastal Prairie
Lepidium virginicum (Virginia peppergrass)	*Sambucus canadensis* (elderberry)		*Distichlis spicata* (saltgrass)
Ambrosia trifida (giant ragweed)	*Rumex crispus* (curly dock)		*Batis maritima* (saltwort)
Andropogon sp. (bluestem/redweed)	*Cardiospermum halicacabum* (balloonvine)		*Juncus effusus* (reed)
	Ambrosia trifida (giant ragweed)		

APPENDIX D

HABITAT INTERACTIONS OF *ORYZOMYS PALUSTRIS*

Table D.1. Species-habitat influence table for *Oryzomys palustris* based on several Key Environmental Correlates (KECs) and Key Ecological Functions (KEFs). Modified from *Wildlife-Habitat Relationships: Concepts and Applications*, Morrison et al. 2006, p. 390.

Key Environmental Correlates (KECs)	Key Ecological Functions (KEFs)
Prefers open water, woodlands, riparian zones, meadows, wetlands, marshes, emergent vegetation, mixed forests, sedge-shrub habitat, dense herbaceous cover[1]	Importance of historic, phylogeographic range across the Americas and endemicity of Bayou virus (and other hantaviruses associated with *Oryzomys* spp.)
Avoidance of grasses and forbs[2] - possibly where more easily spotted by predators (raptors, snakes, mesocarnivores)	Ecosystem engineer and nutrient cycler[3]; displaces *Ammospiza maritima* (seaside sparrow) over nesting site competition[4]

Table D.1. Continued

Key Environmental Correlates (KECs)	Key Ecological Functions (KEFs)
Efficient long-distance, terrestrial and aquatic disperser; can produce founder and rescue populations[5]	May persist as metapopulation[6] (sensu lato), potentially trafficking Bayou virus between source / sink populations
Little known of sociality, but believed to be solitary, polygynous, and territorial; for a small mammal, can have long lifespan in the lab and field (up to 24 months)[7]	Demonstrated high reservoir competency: carrier of Bayou virus[8,9]; host to other endo-[10] and ectoparasites[11] of community assemblage importance
As of 2011, state-threatened species in Illinois[12], the northernmost boundary of its U.S. range	Indicator species for historic wetland losses and future habitat conservation concerns, public land-use policies and permits[12]

[1] Svihla, A. 1931. Life history of the Texas rice rat (*Oryzomys palustris texensis*). J. Mammal. 12: 238-242.

[2] McIntyre, N. E., R. A. Nisbett, A. A. Abuzeineh, T. S. Holsomback, Y.-K. Chu, J. A. Carmichael, N. de la Sancha, C. W. Dick, C. Jonsson, and R. D. Owen. 2009. Ecological correlates of serological status for Bayou virus in *Oryzomys palustris* (RODENTIA: SIGMODONTINAE). Mastozool. Neotrop 16: 83-94.

Table D.1. Continued

[3] Wolfe, J. L. 1982. *Oryzomys palustris*. Am. Soc. Mammal., Mammalian Species No 176: 1-5.

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[6] Kruchek, B. L. 2004. Use of tidal marsh and upland habitats by the marsh rice rat (*Oryzomys palustris*). J. Mammal. 85: 569-575.

[7] Wolfe, J. L. 1985. Population ecology of the Rice rat (*Oryzomys palustris*) in a coastal marsh. J. Zool. 205: 235-244.

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[10] Barnard, W. P., J. V. Ernst, and R. O. Stevens. 1971. *Eimeria palustris sp.* N. and *Isospora hammondi sp*. N. (Coccidia: Eimeriidae) from the marsh rice rat, *Oryzomys palustris* (Harlan). J. Parasitol. 57: 1293-1296.

[11] Morlan, H. B. 1951. Notes on the genus *Gigantolaelaps* and description of a new species, *Gigantolaelaps cricetidarum* (Acarina: Laelaptidae). J. Parasitol. 37: 273-279.

[12] Eubanks, B. W., E. C. Hellgren, J. R. Nawrot, and R. D. Bluett. 2011. Habitat associations of the marsh rice rat (*Oryzomys palustris*) in freshwater wetlands of southern Illinois. J. Mammal. 92: 552-560.

APPENDIX E

R STATISTICAL OUTPUT

- R functions and pre-modeling, data quality check
  - test statistics for evaluations of sample distribution normality and autocorrelation of residuals, goodness of fit criteria, independence of observations, linearity, singularity, multicollinearity, high leverage outliers, and homogeneity of variance-covariance matrices
    - most graphical output omitted to reduce appendix length and printing consumables and costs

Chapter III - BAYV infection costs: Dataset 'Manrecap'

SeroNegJuveMaleWt:

```
> mean(juvewts)
[1] 15.08125

> sd(juvewts)
[1] 4.569496

> summary(juvewts)
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
   7.40   11.07   14.50   15.08   18.25   22.50

> var(juvewts)
```

Appendix E. Continued

[1] 20.88029

Shapiro-Wilk normality test

data: SeroNegJuveMaleWt

W = 0.9633, p-value = 0.722

ORDINARY NONPARAMETRIC BOOTSTRAP

Call:

boot(data = SeroNegJuveMaleWt, statistic = mean.w, R = 500, stype = "w")

Bootstrap Statistics :

	original	bias	std. error
t1*	15.08125	-0.0200875	1.097317

stat.desc(juvewts)

nbr.val	nbr.null	nbr.na	min	max	range
16.0000000	0.0000000	0.0000000	7.4000000	22.5000000	15.1000000

sum	median	mean	SE.mean	CI.mean.0.95	var
241.3000000	14.5000000	15.0812500	1.1423739	2.4349124	20.8802917

std.dev	coef.var
4.5694958	0.3029918

SeroNegJuvFemTotalLength:

Appendix E. Continued

```
negjuvfemTL <- c(138,151,162,171,169,150,162,169,175,173)

> stat.desc(negjuvfemTL)

     nbr.val     nbr.null       nbr.na          min          max        range

1.000000e+01 0.000000e+00 0.000000e+00 1.380000e+02 1.750000e+02
3.700000e+01

         sum       median         mean      SE.mean CI.mean.0.95          var

1.620000e+03 1.655000e+02 1.620000e+02 3.815174e+00 8.630524e+00
1.455556e+02

     std.dev     coef.var

1.206464e+01 7.447309e-02

> summary(negjuvfemTL)

   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.

  138.0   153.8   165.5   162.0   170.5   175.0

ORDINARY NONPARAMETRIC BOOTSTRAP

Call:

boot(data = negjuvfemTL, statistic = mean.w, R = 500, stype = "w")

Bootstrap Statistics :

    original  bias    std. error

t1*      162 -0.1506    3.524586
```

Appendix E. Continued

```
> boot.ci(boot.out=negjuvfemTL.boot,type=c("basic","perc"),h=exp)

BOOTSTRAP CONFIDENCE INTERVAL CALCULATIONS
Based on 500 bootstrap replicates

CALL :
boot.ci(boot.out = negjuvfemTL.boot, type = c("basic", "perc"),
    h = exp)

Intervals :
Level      Basic              Percentile
95%   (-1.662901e+73,  4.534870e+70 )   ( 1.788497e+67,  1.667438e+73 )

Calculations and Intervals on Transformed Scale

> boot.ci(boot.out=negjuvfemTL.boot,type=c("basic","perc"),h=exp,R=1000)
BOOTSTRAP CONFIDENCE INTERVAL CALCULATIONS
Based on 500 bootstrap replicates

SeroNegJuvFem:

summary(negjuvfemwt)
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
   8.90   12.50   14.25   15.28   17.60   21.50
> stat.desc(negjuvfemwt)
```

Appendix E. Continued

```
    nbr.val     nbr.null       nbr.na          min          max        range
 24.0000000    0.0000000    0.0000000    8.9000000   21.5000000   12.6000000
       sum       median         mean      SE.mean CI.mean.0.95          var
366.8000000   14.2500000   15.2833333    0.7711432    1.5952313   14.2718841
    std.dev     coef.var
  3.7778147    0.2471853

      Shapiro-Wilk normality test

data:  njfwt
W = 0.9404, p-value = 0.1665

> anova(lm(wtposjuvfem ~ negjuvfemwt, femwtAVA))
Analysis of Variance Table

Response: wtposjuvfem
             Df Sum Sq Mean Sq F value Pr(>F)
negjuvfemwt  1   3.75  3.7543   0.219 0.6444
Residuals   22 377.17 17.1442

> stat.desc(femwtAVA)
             negjuvfemwt wtposjuvfem
nbr.val       24.0000000  24.0000000
```

Appendix E. Continued

nbr.null	0.0000000	0.0000000
nbr.na	0.0000000	0.0000000
min	8.9000000	9.0000000
max	21.5000000	22.0000000
range	12.6000000	13.0000000
sum	366.8000000	369.9000000
median	14.2500000	16.0000000
mean	15.2833333	15.4125000
SE.mean	0.7711432	0.8307128
CI.mean.0.95	1.5952313	1.7184604
var	14.2718841	16.5620109
std.dev	3.7778147	4.0696451
coef.var	0.2471853	0.2640483

NegSubadultMales:

stat.desc(nsubmtol)

nbr.val	nbr.null	nbr.na	min	max	range
12.0000000	0.0000000	0.0000000	175.0000000	231.0000000	56.0000000

sum	median	mean	SE.mean	CI.mean.0.95	var
2440.0000000	205.5000000	203.3333333	4.4743940	9.8480749	240.2424242

std.dev	coef.var

Appendix E. Continued

15.4997556 0.0762283

Min. 1st Qu. Median Mean 3rd Qu. Max.

175.0 195.5 205.5 203.3 212.5 231.0

Shapiro-Wilk normality test

data: nsubmwt

W = 0.939, p-value = 0.09418

nbr.val nbr.null nbr.na min max range

29.0000000 0.0000000 0.0000000 19.5000000 43.0000000 23.5000000

sum median mean SE.mean CI.mean.0.95 var

855.7000000 27.4000000 29.5068966 1.2318228 2.5232747 44.0042365

std.dev coef.var

6.6335689 0.2248142

Min. 1st Qu. Median Mean 3rd Qu. Max.

19.50 24.00 27.40 29.51 34.00 43.00

NegSubadultFemales:

Total Length

Min. 1st Qu. Median Mean 3rd Qu. Max.

166.0 182.0 185.5 191.9 201.5 225.0

Appendix E. Continued

```
> stat.desc(nsubftl)

    nbr.val     nbr.null       nbr.na          min          max        range

1.800000e+01 0.000000e+00 0.000000e+00 1.660000e+02 2.250000e+02
5.900000e+01

        sum       median         mean      SE.mean CI.mean.0.95          var

3.455000e+03 1.855000e+02 1.919444e+02 3.835227e+00 8.091622e+00
2.647614e+02

    std.dev     coef.var

1.627149e+01 8.477188e-02


summary(nsubfwt)

   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.    NA's

  15.00   22.02   25.75   27.04   31.75   46.00    1.00

> stat.desc(nsubfwt)

    nbr.val     nbr.null       nbr.na          min          max        range

 46.0000000    0.0000000    1.0000000   15.0000000   46.0000000   31.0000000

        sum       median         mean      SE.mean CI.mean.0.95          var

1244.0000000   25.7500000   27.0434783    1.1340307    2.2840551   59.1571787

    std.dev     coef.var

  7.6913704    0.2844076


       Shapiro-Wilk normality test


data:  nsubfwt
```

Appendix E. Continued

W = 0.9525, p-value = 0.05859

NegNonPregFem:

summary(negnonpreg)

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
  18.00   33.50   39.00   39.07   44.00   65.00
```

> stat.desc(negnonpreg)

```
     nbr.val     nbr.null       nbr.na          min          max        range
 140.0000000    0.0000000    0.0000000   18.0000000   65.0000000   47.0000000
         sum       median         mean      SE.mean  CI.mean.0.95          var
5469.9000000   39.0000000   39.0707143    0.7484074    1.4797343   78.4158988
     std.dev     coef.var
  8.8552752    0.2266474
```

Shapiro-Wilk normality test

data: negnonpreg

W = 0.9929, p-value = 0.7123

summary(negnonpregTL)

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
  184.0   211.8   219.5   220.2   232.2   256.0
```

> stat.desc(negnonpregTL)

Appendix E. Continued

```
    nbr.val     nbr.null       nbr.na          min          max
5.200000e+01 0.000000e+00 0.000000e+00 1.840000e+02 2.560000e+02
      range          sum       median         mean      SE.mean
7.200000e+01 1.145100e+04 2.195000e+02 2.202115e+02 2.385119e+00
CI.mean.0.95          var      std.dev     coef.var
4.788325e+00 2.958171e+02 1.719933e+01 7.810370e-02
```

PosNonPregFem:

```
summary(posnonpregwt)
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
  36.00   37.10   44.40   44.96   51.00   60.00
> stat.desc(posnonpregwt)
     nbr.val     nbr.null       nbr.na          min          max
   9.0000000    0.0000000    0.0000000   36.0000000   60.0000000
      range          sum       median         mean      SE.mean
  24.0000000  404.6000000   44.4000000   44.9555556    2.7712869
CI.mean.0.95          var      std.dev     coef.var
   6.3905990   69.1202778    8.3138606    0.1849351

 Shapiro-Wilk normality test

data:  posnonpregwt
```

Appendix E. Continued

W = 0.9244, p-value = 0.4301

PregNegFem:

summary(pregnegTL)

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
  210.0   220.0   226.0   229.2   237.0   249.0
```

stat.desc(pregnegTL)

```
     nbr.val     nbr.null       nbr.na          min          max
1.300000e+01 0.000000e+00 0.000000e+00 2.100000e+02 2.490000e+02
       range          sum       median         mean      SE.mean
3.900000e+01 2.979000e+03 2.260000e+02 2.291538e+02 3.485954e+00
CI.mean.0.95          var      std.dev     coef.var
7.595241e+00 1.579744e+02 1.256879e+01 5.484868e-02
```

summary(pregnegwt)

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
  29.00   41.73   49.90   50.47   60.00   73.00
> stat.desc(pregnegwt)
     nbr.val     nbr.null       nbr.na          min          max
  20.0000000    0.0000000    0.0000000   29.0000000   73.0000000
       range          sum       median         mean      SE.mean
  44.0000000 1009.4000000   49.9000000   50.4700000    2.5007483
```

Appendix E. Continued

```
CI.mean.0.95         var      std.dev     coef.var
  5.2341264  125.0748421   11.1836864    0.2215908

EmbryoNumbers:
summary(embnum)
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
  3.000   4.000   5.000   4.692   5.000   7.000
> stat.desc(embnum)
     nbr.val     nbr.null       nbr.na          min          max
  13.0000000    0.0000000    0.0000000    3.0000000    7.0000000
       range          sum       median         mean      SE.mean
   4.0000000   61.0000000    5.0000000    4.6923077    0.2861015
CI.mean.0.95         var      std.dev     coef.var
   0.6233615    1.0641026    1.0315535    0.2198393
> t.test(pregnegwt, mu=51, alternative="two.sided")

        One Sample t-test

data:  pregnegwt
t = -0.2119, df = 19, p-value = 0.8344
alternative hypothesis: true mean is not equal to 51
95 percent confidence interval:
```

Appendix E. Continued

45.23587 55.70413

sample estimates:

mean of x

50.47

> t.test(embnum, mu=4, alternative="two.sided")

One Sample t-test

data: embnum

t = 2.4198, df = 12, p-value = 0.03233

alternative hypothesis: true mean is not equal to 4

95 percent confidence interval:

4.068946 5.315669

sample estimates:

mean of x

4.692308

> t.test(pregnegTL, mu=245, alternative="two.sided")

One Sample t-test

data: pregnegTL

t = -4.5457, df = 12, p-value = 0.0006711

Appendix E. Continued

alternative hypothesis: true mean is not equal to 245

95 percent confidence interval:

221.5586 236.7491

sample estimates:

mean of x

229.1538

NonPregFemales by Serostatus (Dead in Trap):

> DITdata

[,1] [,2]

[1,] 140 9

[2,] 3 2

> fisher.test(DITdata)

Fisher's Exact Test for Count Data

data: DITdata

p-value = 0.04137

alternative hypothesis: true odds ratio is not equal to 1

95 percent confidence interval:

0.7487583 99.6022670

sample estimates:

odds ratio

Appendix E. Continued

10.01093

> chisq.test(DITdata)

Pearson's Chi-squared test with Yates' continuity correction

data: DITdata

X-squared = 4.0706, df = 1, p-value = 0.04364

EmbryoDimensions:

Call:

line(embW, embL)

Coefficients:

[1] 0.2308 1.5385

> plot(embL,embW)

> cor(embL,embW)

[1] 0.7371553

summary(embW)

Min. 1st Qu. Median Mean 3rd Qu. Max.

2.000 5.000 9.000 8.444 10.000 18.000

> stat.desc(embW)

Appendix E. Continued

```
    nbr.val     nbr.null       nbr.na          min          max
  9.0000000    0.0000000    0.0000000    2.0000000   18.0000000
      range          sum       median         mean      SE.mean
 16.0000000   76.0000000    9.0000000    8.4444444    1.5908690
CI.mean.0.95          var      std.dev     coef.var
  3.6685505   22.7777778    4.7726070    0.5651771
> summary(embL)
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
   4.00   11.00   13.00   13.44   18.00   24.00
> stat.desc(embL)
    nbr.val     nbr.null       nbr.na          min          max
  9.0000000    0.0000000    0.0000000    4.0000000   24.0000000
      range          sum       median         mean      SE.mean
 20.0000000  121.0000000   13.0000000   13.4444444    2.2054926
CI.mean.0.95          var      std.dev     coef.var
  5.0858750   43.7777778    6.6164777    0.4921347

> cor(pregnegREG, method = "pearson")
            embnum    weight pregnegTL
embnum    1.0000000 0.4622087 0.2096313
weight    0.4622087 1.0000000 0.7481320
```

Appendix E. Continued

pregnegTL 0.2096313 0.7481320 1.0000000

> cor.test(embnum, weight, method="pearson")

Pearson's product-moment correlation

data: embnum and weight

t = 1.7287, df = 11, p-value = 0.1118

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

-0.1191105 0.8075381

sample estimates:

cor

0.4622087

Pearson's product-moment correlation

data: embnum and pregnegTL

t = 0.7111, df = 11, p-value = 0.4918

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

-0.3859304 0.6818593

sample estimates:

cor

Appendix E. Continued

0.2096313

> cor.test(weight, pregnegTL, method="pearson")

Pearson's product-moment correlation

data: weight and pregnegTL

t = 3.7394, df = 11, p-value = 0.003270

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

0.3354030 0.9199181

sample estimates:

cor

0.748132

pregnegcorr

embW embL wtembdim

1 5 11 29.0

2 12 15 37.4

3 2 4 38.5

4 4 5 39.8

5 10 13 42.0

6 7 11 49.0

Appendix E. Continued

7 9 24 49.8

8 9 18 52.8

9 18 20 66.9

Correlation between embryo width and weight of dam:

> cor.test (embW, wtembdim, method="pearson")

Pearson's product-moment correlation

data: embW and wtembdim

t = 2.6707, df = 7, p-value = 0.03197

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

0.08764356 0.93391410

sample estimates:

cor

0.7104149

> cor.test(embL, wtembdim, method="pearson")

Pearson's product-moment correlation

data: embL and wtembdim

Appendix E. Continued

t = 2.0194, df = 7, p-value = 0.0832

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

-0.09612968 0.90584630

sample estimates:

cor

0.6067265

> cor(pregnegcorr, method="pearson")

	embW	embL	wtembdim
embW	1.0000000	0.7371553	0.7104149
embL	0.7371553	1.0000000	0.6067265
wtembdim	0.7104149	0.6067265	1.0000000

HomeRange Data:

summary(homerange)

TKnum	sex	age	repcon	sero	weight	season
Min. :110903	F:25	A :19	PRG: 1	NEG:28	Min. :13.00	Aug:19
1st Qu.:115800	M: 6	J : 3	TAB: 4	POS: 3	1st Qu.:33.50	Mar: 1
Median :116099	S : 2	TIN: 2			Median :39.00	May:11
Mean :114892	SA: 7	VAC:14			Mean :38.48	

Appendix E. Continued

```
3rd Qu.:116114                VAO:10          3rd Qu.:44.70
 Max.   :116197                                Max.   :60.00

    recaps          lowMCP          highADK
 Min.   :3.000   Min.   :  0.298   Min.   :  27.03
 1st Qu.:3.000   1st Qu.: 50.000   1st Qu.: 146.56
 Median :4.000   Median :100.000   Median : 378.90
 Mean   :4.194   Mean   :131.718   Mean   : 547.64
 3rd Qu.:5.000   3rd Qu.:200.000   3rd Qu.: 620.70
 Max.   :6.000   Max.   :600.000   Max.   :2027.00
                 NA's   :  6.000   NA's   :   4.00
```

```
> stat.desc(homerange)
                TKnum sex age repcon sero       weight season      recaps
nbr.val     3.100000e+01  NA  NA     NA   NA   31.0000000     NA  31.0000000
nbr.null    0.000000e+00  NA  NA     NA   NA    0.0000000     NA   0.0000000
nbr.na      0.000000e+00  NA  NA     NA   NA    0.0000000     NA   0.0000000
min         1.109030e+05  NA  NA     NA   NA   13.0000000     NA   3.0000000
max         1.161970e+05  NA  NA     NA   NA   60.0000000     NA   6.0000000
range       5.294000e+03  NA  NA     NA   NA   47.0000000     NA   3.0000000
sum         3.561666e+06  NA  NA     NA   NA 1192.9000000     NA 130.0000000
median      1.160990e+05  NA  NA     NA   NA   39.0000000     NA   4.0000000
```

Appendix E. Continued

mean	1.148925e+05	NA	NA	NA	NA	38.4806452	NA	4.1935484
SE.mean	3.871232e+02	NA	NA	NA	NA	1.8552594	NA	0.1879107
CI.mean.0.95	7.906110e+02	NA	NA	NA	NA	3.7889452	NA	0.3837649
var	4.645795e+06	NA	NA	NA	NA	106.7016129	NA	1.0946237
std.dev	2.155411e+03	NA	NA	NA	NA	10.3296473	NA	1.0462426
coef.var	1.876025e-02	NA	NA	NA	NA	0.2684375	NA	0.2494886

	lowMCP	highADK
nbr.val	25.000000	2.700000e+01
nbr.null	0.000000	0.000000e+00
nbr.na	6.000000	4.000000e+00
min	0.298000	2.703000e+01
max	600.000000	2.027000e+03
range	599.702000	1.999970e+03
sum	3292.938000	1.478615e+04
median	100.000000	3.789000e+02
mean	131.717520	5.476352e+02
SE.mean	25.146744	1.103452e+02
CI.mean.0.95	51.900328	2.268178e+02
var	15808.968030	3.287536e+05
std.dev	125.733719	5.733704e+02

Appendix E. Continued

No correlation b/t recap # and weight:

```
 cor.test(recaps,weight, method="pearson")

        Pearson's product-moment correlation

data:  recaps and weight
t = -0.0296, df = 29, p-value = 0.9766
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.3591420  0.3495195
sample estimates:
         cor
-0.005502047
coef.var         0.954571 1.046993e+00
```

No correlation b/t weight and home range sizes:

```
summary(wtHRcorrel)
    weight          lowMCP          highADK
 Min.   :13.00   Min.   :  0.298   Min.   :   1.00
 1st Qu.:33.50   1st Qu.: 15.925   1st Qu.:  55.19
 Median :39.00   Median : 50.250   Median : 312.80
```

Appendix E. Continued

```
Mean   :38.48   Mean   :106.417   Mean   : 477.10
 3rd Qu.:44.70   3rd Qu.:175.000   3rd Qu.: 600.55
 Max.   :60.00   Max.   :600.000   Max.   :2027.00
> stat.desc(wtHRcorrel)

                weight       lowMCP      highADK
nbr.val       31.0000000    31.000000 3.100000e+01
nbr.null       0.0000000     0.000000 0.000000e+00
nbr.na         0.0000000     0.000000 0.000000e+00
min           13.0000000     0.298000 1.000000e+00
max           60.0000000   600.000000 2.027000e+03
range         47.0000000   599.702000 2.026000e+03
sum         1192.9000000  3298.938000 1.479015e+04
median        39.0000000    50.250000 3.128000e+02
mean          38.4806452   106.417355 4.771016e+02
SE.mean        1.8552594    22.290714 1.015398e+02
CI.mean.0.95   3.7889452    45.523711 2.073720e+02
var          106.7016129 15403.153462 3.196203e+05
std.dev       10.3296473   124.109441 5.653497e+02
coef.var       0.2684375     1.166252 1.184967e+00

> cor(lowMCP,weight)
```

Appendix E. Continued

```
[1] -0.02402984
> cor(weight,lowMCP)
[1] -0.02402984
> cor(highADK,weight)
[1] 0.1753935
> plot(lowMCP,weight)
> plot(weight,lowMCP)
> biplot(weight,lowMCP)
Error in 1L:n : argument of length 0
> plot(weight,highADK)
> cor(wtHRcorrel,method="pearson")

            weight      lowMCP   highADK
weight   1.00000000 -0.02402984 0.1753935
lowMCP  -0.02402984  1.00000000 0.4023145
highADK  0.17539352  0.40231450 1.0000000
> cor.test(weight ~ lowMCP + highADK, method="pearson")
Error in cor.test.formula(weight ~ lowMCP + highADK, method = "pearson") :
  'formula' missing or invalid
> cor.test(weight,lowMCP, method="pearson")

        Pearson's product-moment correlation
```

Appendix E. Continued

data: weight and lowMCP

t = -0.1294, df = 29, p-value = 0.8979

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

-0.3751754 0.3331469

sample estimates:

cor

-0.02402984

> cor.test(weight,highADK,method="pearson")

Pearson's product-moment correlation

data: weight and highADK

t = 0.9594, df = 29, p-value = 0.3453

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

-0.1908049 0.4987376

sample estimates:
cor

0.1753935

Weight is highly correlated with age:

Appendix E. Continued

```
 cor.test(weight,age, method="pearson")

        Pearson's product-moment correlation

data:  weight and age
t = 15.3796, df = 29, p-value = 1.776e-15
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.8856692 0.9728146
sample estimates:
     cor
0.943814

MANOVA results:

manrecap
   sex age repcon sero weight season recaps  lowMCP highADK
1    1  60    VAC    0   44.0      5      3  50.250   50.25
2    1  50    VAC    0   40.0      5      4   1.000   62.80
3    2  20    TAB    0   21.0      5      3   1.000  235.00
4    2  20    TAB    0   19.5      5      3  49.160   49.16
5    1  90    PRG    0   60.0      8      3  99.120  618.40
```

Appendix E. Continued

6	1	90	VAO	0	52.0	8	3	3.640	27.03
7	1	10	VAC	0	13.0	8	3	211.400	312.80
8	1	60	VAC	1	44.4	3	3	50.250	50.25
9	1	60	VAO	1	47.0	5	4	100.000	387.90
10	1	60	VAO	0	45.0	5	6	0.298	314.90
11	1	60	VAC	1	41.0	5	4	600.000	1169.00
12	1	60	VAC	0	42.0	5	4	1.000	206.60
13	1	60	VAC	0	42.0	5	4	200.000	1.00
14	1	40	VAO	0	33.0	5	3	49.610	648.90
15	2	40	TIN	0	39.0	5	6	50.000	571.50
16	1	60	VAC	0	47.0	8	5	150.000	623.00
17	1	90	VAC	0	52.0	8	5	100.000	1.00
18	1	40	VAC	0	34.0	8	6	150.000	1528.00
19	1	40	VAO	0	32.0	8	5	50.000	523.70
20	1	40	VAO	0	39.0	8	4	200.000	241.70
21	1	90	VAC	0	53.0	8	3	28.210	1965.00
22	1	60	VAO	0	41.0	8	4	1.000	480.40
23	1	40	VAO	0	36.0	8	4	150.000	582.70
24	2	40	TAB	0	30.0	8	6	1.000	92.13
25	1	40	VAC	0	38.0	8	5	300.000	2027.00
26	1	60	VAO	0	45.0	8	5	50.000	378.90

Appendix E. Continued

27 2 40 TIN 0 39.0 8 5 200.000 1378.00

28 1 40 VAC 0 32.0 8 5 1.000 60.13

29 2 20 TAB 0 22.0 8 5 50.000 1.00

30 1 40 VAC 0 34.0 8 3 201.000 201.00

31 1 40 VAO 0 36.0 8 4 200.000 1.00

(Removed repcon (categorical variable)) –

> summary(manova(cbind(sex,age, weight,season,recaps,lowMCP,highADK) ~ sero, data=manrecap), test = "Roy")

	Df	Roy	approx F	num Df	den Df	Pr(>F)
sero	1	0.93281	3.0649	7	23	0.01969 *
Residuals	29					

Signif. codes:

> tapply(manrecap$sex, manrecap$sero, mean)

0 1

1.214286 1.000000

> tapply(manrecap$age, manrecap$sero, mean)

0 1

49.28571 60.00000

> tapply(manrecap$weight, manrecap$sero, mean)

0 1

Appendix E. Continued

```
37.87500 44.13333
> tapply(manrecap$season, manrecap$sero, mean)
      0       1
7.035714 4.333333
> tapply(manrecap$recaps, manrecap$sero, mean)
      0       1
4.250000 3.666667
> tapply(manrecap$lowMCP, manrecap$sero, mean)
       0        1
 91.02457 250.08333
> tapply(manrecap$highADK, manrecap$sero, mean)
      0       1
470.8214 535.7167

 summary(homerange_manova, test="Roy")
         Df     Roy approx F num Df den Df  Pr(>F)
sero      1 0.93281   3.0649      7     23 0.01969 *
Residuals 29
---
Signif. codes:  0 ë***í 0.001 ë**í 0.01 ë*í 0.05 ë.í 0.1 ë í 1
> summary.aov(homerange_manova)
 Response sex :
```

Appendix E. Continued

```
          Df Sum Sq Mean Sq F value Pr(>F)
sero       1 0.1244 0.12442  0.7654 0.3888
Residuals 29 4.7143 0.16256

 Response age :
          Df  Sum Sq Mean Sq F value Pr(>F)
sero       1   311.1  311.06  0.7167 0.4041
Residuals 29 12585.7  433.99

 Response weight :
          Df  Sum Sq Mean Sq F value Pr(>F)
sero       1  106.13  106.13  0.9945 0.3269
Residuals 29 3094.92  106.72

 Response season :
          Df Sum Sq Mean Sq F value   Pr(>F)
sero       1 19.788 19.7884  9.9576 0.003716 **
Residuals 29 57.631  1.9873
---
Signif. codes:  0 ë***í 0.001 ë**í 0.01 ë*í 0.05 ë.í 0.1 ë í 1

 Response recaps :
          Df Sum Sq Mean Sq F value Pr(>F)
```

Appendix E. Continued

sero 1 0.922 0.92204 0.8378 0.3676

Residuals 29 31.917 1.10057

Response lowMCP :

Df Sum Sq Mean Sq F value Pr(>F)

sero 1 68554 68554 5.0517 0.03238 *

Residuals 29 393541 13570

Response highADK :

Df Sum Sq Mean Sq F value Pr(>F)

sero 1 11412 11412 0.0346 0.8538

Residuals 29 9577198 330248

DFA Results:

$posterior probabilities:

$posterior

n p

1 0.94309208 5.690792e-02

2 0.98663340 1.336660e-02

3 0.99820259 1.797405e-03

4 0.99519145 4.808552e-03

Appendix E. Continued

5 0.99723668 2.763323e-03

6 0.99991042 8.958405e-05

7 0.99993115 6.884735e-05

8 0.80679519 1.932048e-01

9 0.97387090 2.612910e-02

10 0.97546348 2.453652e-02

11 0.02075085 9.792492e-01

12 0.98302917 1.697083e-02

13 0.36892810 6.310719e-01

14 0.98340554 1.659446e-02

15 0.96882861 3.117139e-02

16 0.99857044 1.429559e-03

17 0.99911196 8.880375e-04

18 0.99968770 3.123027e-04

19 0.99997723 2.277488e-05

20 0.99835053 1.649467e-03

21 0.99981217 1.878259e-04

22 0.99997829 2.170791e-05

23 0.99960529 3.947146e-04

24 0.99999585 4.153497e-06

25 0.98477818 1.522182e-02

Appendix E. Continued

26 0.99987882 1.211776e-04

27 0.99835053 1.649467e-03

28 0.99999415 5.854243e-06

29 0.99999603 3.970176e-06

30 0.99904091 9.590859e-04

31 0.99882505 1.174947e-03

ct <- table(dfmanrecap$sero, fit$class)

> diag(prop.table(ct, 1))

n p

0.9642857 0.3333333

> sum(diag(prop.table(ct)))

[1] 0.9032258

Chapter IV – Biodiversity and the Dilution Effect: Dataset 'Dileff'

StudRes Hat CookD

1 6.49836840 0.5171676 1.00003282

2 -1.57628462 0.7128813 1.06056464

3 -0.99484930 0.5262539 0.52494116

4 -0.46184791 0.8618203 0.64344203

5 0.08791359 0.3106498 0.03402924

Appendix E. Continued

6 -0.26069114 0.1445286 0.06117522

7 1.55695666 0.5204449 0.69643438

8 -0.56655307 0.4062536 0.25716

Suggested power transformation for heterosced (i.e. non-constant error variance):

0.9441339

Variance inflation factors (multi-collinearity)

Tdens Richn Evenn

960.208827 1.113128 956.889625

Test for autocorrelation (non-indep) of errors:

durbinWatsonTest(fitdil)

lag Autocorrelation D-W Statistic p-value

1 -0.2701362 2.032079 0.61

Alternative hypothesis: rho != 0

Appendix E. Continued

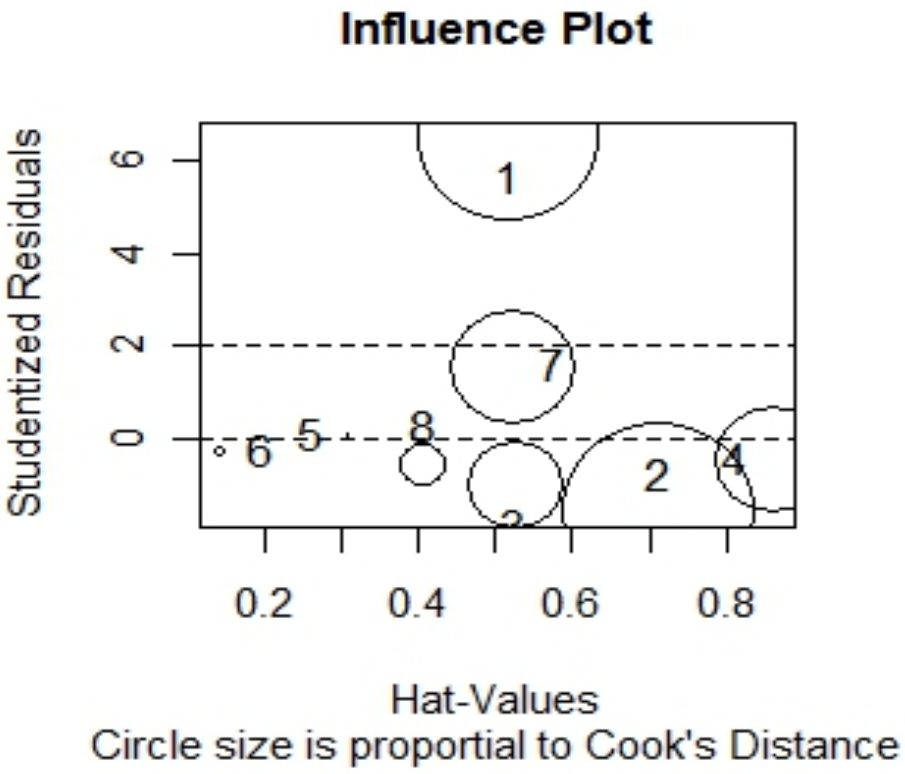

Figure E.1. Influential observations in Dataset 'Dileff'.

Appendix E. Continued

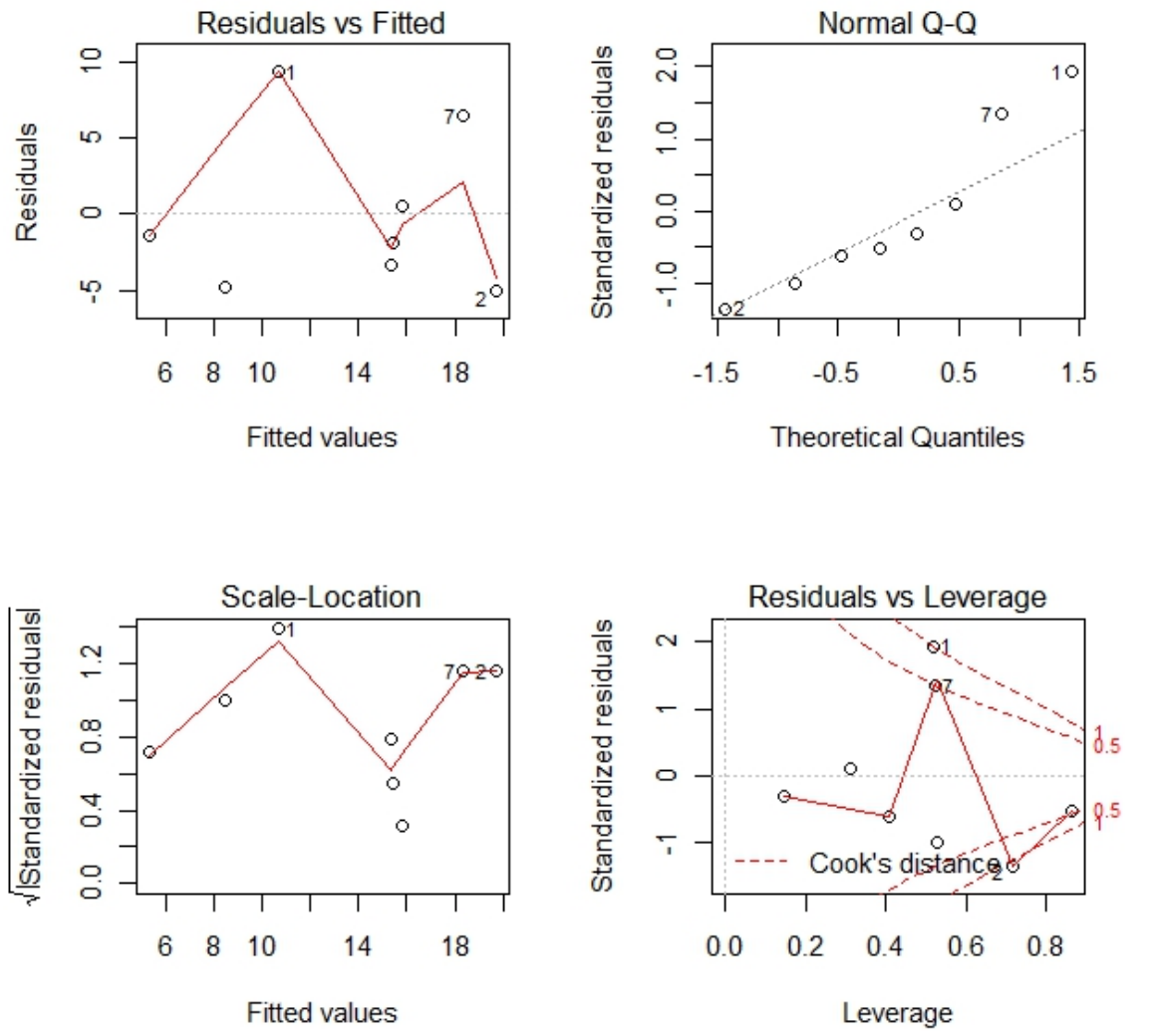

Figure E.2. Pre-regression diagnostics plots for Dataset 'Dileff'.

ROBUST REGRESSION RESULTS:

Call:

lm(formula = Seroprev ~ Richn + Evenn, data = dileff)

Appendix E. Continued

```
Residuals:
     1      2      3      4      5      6      7      8
 9.9863  0.8362 -8.6472 -2.8038  2.3618 -1.4720  5.5695 -5.8309

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   50.265     30.012   1.675    0.155
Richn         -1.925      1.747  -1.102    0.321
Evenn        -40.841     33.096  -1.234    0.272

Residual standard error: 7.153 on 5 degrees of freedom
Multiple R-squared: 0.3052,     Adjusted R-squared: 0.02723
F-statistic: 1.098 on 2 and 5 DF,  p-value: 0.4024

> mod.ls.2 <- update(mod.ls, subset=-c(1))
> summary(mod.ls.2)

Call:
lm(formula = Seroprev ~ Richn + Evenn, data = dileff, subset = -c(1))

Residuals:
      2       3       4       5       6       7       8
 5.26449 -1.22363 -1.41566  0.78464 -0.04112  1.01630 -4.38503
```

Appendix E. Continued

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	96.701	19.160	5.047	0.00725	**
Richn	-4.918	1.162	-4.234	0.01333	*
Evenn	-40.627	16.698	-2.433	0.07174	.

Residual standard error: 3.609 on 4 degrees of freedom

Multiple R-squared: 0.8382, Adjusted R-squared: 0.7573

F-statistic: 10.36 on 2 and 4 DF, p-value: 0.02619

shapiro.test(resid(mod.ls.2))

Shapiro-Wilk normality test

data: resid(mod.ls.2)

W = 0.9495, p-value = 0.725

HUBER ESTIMATOR (graph not shown):

Call: rlm(formula = Seroprev ~ Richn * Evenn, data = dileff)

Residuals:

1	2	3	4	5	6	7	8
13.0605	2.4135	-6.7728	-1.8089	0.5128	-0.6281	4.4651	-5.0399

Coefficients:

Appendix E. Continued

	Value	Std. Error	t value
(Intercept)	29.5239	160.3110	0.1842
Richn	-0.6763	9.9054	-0.0683
Evenn	313.4697	1597.6753	0.1962
Richn:Evenn	-22.1916	100.0621	-0.2218

Residual standard error: 5.099 on 4 degrees of freedom

RIDGE REGRESSION RESULTS:

Call:

lm(formula = Seroprev ~ Richn * Evenn, data = dileff, subset = -c(1))

Residuals:

2	3	4	5	6	7	8
5.36602	-1.46429	-1.11671	0.06139	0.17009	1.18802	-4.20453

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	77.866	90.485	0.861	0.453
Richn	-3.751	5.599	-0.670	0.551
Evenn	148.457	881.457	0.168	0.877
Richn:Evenn	-11.845	55.206	-0.215	0.844

Appendix E. Continued

```
Residual standard error: 4.136 on 3 degrees of freedom
Multiple R-squared: 0.8406,     Adjusted R-squared: 0.6812
F-statistic: 5.274 on 3 and 3 DF,  p-value: 0.1027

> mod.ridge <- lm(Seroprev ~ Richn * Evenn, data=dileff, subset=-c(1,2))
> summary(mod.ridge)
Call:
lm(formula = Seroprev ~ Richn * Evenn, data = dileff, subset = -c(1,
   2))

Residuals:
     3      4      5      6      7      8
 0.9349 -0.5468 -0.4675  1.5082  1.1687 -2.5975
Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   69.270     53.203   1.302    0.323
Richn         -3.324      3.290  -1.010    0.419
Evenn        332.326    522.112   0.637    0.590
Richn:Evenn  -23.097     32.686  -0.707    0.553
Residual standard error: 2.427 on 2 degrees of freedom
Multiple R-squared: 0.9629,     Adjusted R-squared: 0.9072
F-statistic:  17.3 on 3 and 2 DF,  p-value: 0.05515
```