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Rodent assemblages and rodent associated pathogens in a counter-urbanizing landscape

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I am submitting herewith a dissertation written by Anna Christine Peterson entitled "Rodent assemblages and rodent associated pathogens in a counter-urbanizing landscape." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

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Rodent assemblages and rodent associated pathogens in a counter-urbanizing landscape

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DEDICATION

I would like to dedicate this dissertation to the people of New Orleans. Especially those whose lives have been personally touched by the events discussed throughout this document. May we all demonstrate such resiliency and courage.

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This work was conducted in accordance with Tulane University IACUC approved protocols 0451 and 0460.

ABSTRACT

Human population declines in urban centers (also known as counter-urbanization) can result in increased levels of vacancy and infrastructure loss, though relatively little is known regarding the ecological outcomes of this type of landscape change. The abundance and diversity of pest and zoonotic pathogen hosts are predicted to increase in counter-urbanizing environments, giving rise to a novel human-animal interface. Furthermore, the human-animal interface is a key location for zoonotic pathogen emergence, thus, understanding how host communities shift in regard to counter-urbanization can lend insight into risk of zoonotic pathogens in these areas.

In this dissertation, I investigate the abundance and diversity of rodent hosts across a counter-urbanizing environment in the city of New Orleans, Louisiana, USA to understand how features of the urban environment shape rodent assemblages and the risk of zoonotic pathogens. I demonstrate that rodent abundance and diversity increase in areas with higher levels of vacancy, and that increased rodent abundance and diversity also translate to increased risk from some zoonotic pathogens. However, this work also indicates that not all zoonotic pathogens show similar patterns across the landscape. In this work I provide practical insight into specific environmental and sociological risk factors associated with rodent abundance and zoonotic pathogens, while also leveraging the gradient of rodent abundance and diversity present in the counter-urbanizing environment of New Orleans to test predictions regarding relationships between host diversity, host abundance, pathogen prevalence, and diversity.

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INTRODUCTION

In 2012, the global population surpassed the threshold at which more people live in urban centers than outside of them, with over 66% of all people expected to reside in cities by the year 2050 (United Nations, Department of Economic and Social Affairs, Population Division, 2015). Given the drastic concentration of the global population in cities, the need to better understand the sociological and ecological interactions that occur in these human-modified environments is also of increasing importance.

However, global urban expansion is also heterogeneous. On more localized scales, some regions, particularly in Europe and North America, are undergoing de-population. Also known as urban shrinkage or counter-urbanization, this phenomenon is typified by declining infrastructure and increases in abandonment (Gulachenski et al. 2016, Lima and Escheid 2017). In the United States, for example, some well-known examples of cities undergoing decline include Detroit, Michigan, where the population has fallen by ~61% from its peak population in 1950; Saint Luis, Missouri, and New Orleans, Louisiana, where the population has decreased by ~45% since 1960 (US Census, 2010). The drivers of urban population declines are complex and vary among cities (Haase et al. 2014). For example, economic and socioeconomic factors can drive de-urbanization, as is the case with Detroit, where reduction in regional industry contributed to urban flight over the past half-century (Reiniets 2009). In Europe, shifting demographics resulting from decreasing birth rates and increased life expectancy are the main driver in population loss in many urban areas (Haase et al. 2014). Additionally, acute events, such as natural disasters, can result in rapid abandonment of urban areas. While the immediate impacts are short-term, recovery from extreme natural disaster events can take decades.

Such is the case with the city of New Orleans, Louisiana, where, in 2005, Hurricane Katrina resulted in levee failures that flooded the city and drove the exodus of 80% of the human population in the immediate aftermath of the storm. Today, over a decade after the event, the legacy of Hurricane Katrina remains on the landscape. Human population returns have been heterogeneous across neighborhoods, with some areas of the city still well below pre-Katrina levels (Fussel et al. 2014, Lewis et al. 2017). While Hurricane Katrina was a singular event, the frequency and severity of extreme weather-related events is predicted to increase as a result of global climate change (Webster et al. 2005, Elsner et al. 2008, Rahmstorf and Coumou 2011). Given that cities located with 100 km of coastlines are experiencing disproportionately large growth rates relative to other regions, the risk of extreme weather events impacting large urban populations, such as occurred in New Orleans, is only predicted to increase (Small and Nicholls 2003).

Regardless of cause, counter-urbanization is a relatively understudied form of landscape change that can result in ecological shifts (Gulachenski et al. 2017, Nassaur and Raskin, 2014, Lewis et al. 2017) with potential consequences for zoonotic disease and human health (Rael et al. 2016, Eskew and Olival 2018). Areas undergoing counter-urbanization are predicted to exhibit increased risk from zoonotic pathogens relative to highly modified and maintained urban areas, for several reasons. First, commensal animal diversity and abundance are expected to increase in areas undergoing declines as a result of loss of decreased disease

management efforts and increased resource provisioning (Bradley and Altizer 2008, Rael et al. 2016, Eskew and Olival 2018). Increased diversity in these areas, is then, in turn, predicted to support an increased pool of pathogens (Eskew and Olival 2018), a key feature in predicting hazards related to zoonotic pathogen emergence at that human-animal interface (Jones et al. 2008, Hosseini et al. 2017, Eskew and Olival 2018).

In this document, I will outline several studies undertaken to test these predictions to identify ecological outcomes and associated risk from zoonotic pathogens in the counter-urbanizing environment of New Orleans, Louisiana. I focus on rodent assemblages, as they are ubiquitous across urban environments, are economically important pest species, and are also hosts for several zoonotic pathogens, including several of global importance.

In my first chapter, I highlight work done to clarify how rodent assemblage structure varies according to the prevalence of abandoned, unmaintained properties present in post-Katrina New Orleans. This work will address one predicted outcome of counter-urbanization: if areas undergoing decline support increased abundance and richness of rodent hosts (Eskew and Olival 2018). Furthermore, by collecting data on several environmental characteristics that also vary across New Orleans, including vegetation data and features related to infrastructure loss (trash and unmaintained vegetation), this work also identifies the specific features of counter-urbanizing environments that may support rodent abundance. Lastly, I draw comparisons among rodent assemblages in abandoned areas that are undergoing differing vacant lot management strategies, with the aim to provide practical insight that may facilitate the control of problematic pest species.

In my second chapter, I build on these findings to clarify how rodent assemblage structure can influence the risk of the zoonotic multi-host bacterial pathogen *Leptospira*. I utilize the natural gradient of rodent diversity and abundance present across the urban and peri-urban areas of New Orleans to address theoretical predictions regarding the relationship between host diversity, abundance and pathogen risk. I provide evidence that rodent diversity, *Leptospira* infection prevalence and carriage loads in rodent hosts parallel abandonment. Indicating that counter-urbanization can elevate zoonotic disease risk within cities, particularly in underserved communities that are burdened with disproportionate concentrations of abandoned and vacant properties.

However, in my third chapter I demonstrate that different pathogens can show different patterns across a counter-urbanizing environment. We compared infection with the bacterial vector-borne pathogen *Bartonella* in New Orleans to that in New York City. With the aim to identify if infection differs according to the (co)occurrence of rat hosts across New Orleans, where both Norway (*Rattus norvegicus*) and roof rats (*Rattus rattus*) are found in comparison to *Bartonella* infection in New York City, which only harbors Norway rats. While we did find differences in the diversity of *Bartonella* present both within and across these cities, flea infestation appears to most clearly relate to *Bartonella* infection in both cities.

Pathogen diversity is hypothesized to reflect free-living host diversity (Hechinger and Lafferty 2005, Kimiya et al. 2014), and areas undergoing counter-urbanization are predicted to support increased diversity of pathogens by supporting an increased diversity of hosts (Eskew and Olival 2018). In my fourth chapter, I utilize genomic sequencing techniques to identify the

suite of viruses infecting rodent hosts in New Orleans. In particular I investigate the extent to which viral communities show host species specificity and geographic spatial structuring, while also identifying if viral community diversity reflects rodent host diversity and/or de-urbanization.

As a whole, the work presented in the following chapters addresses both applied and foundational questions in the realm of the ecology of infectious diseases. This work also provides practical insight into features of counter-urbanizing landscapes that may support populations of problematic pest species.

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CHAPTER I
RODENT ASSEMBLAGE STRUCTURE REFLECTS SOCIOECOLOGICAL MOSAICS OF COUNTER-URBANIZATION ACROSS POST-KATRINA NEW ORLEANS

A version of this chapter by Anna Peterson, Bruno Ghersi, Richard Campanella, Joshua Lewis, and Michael Blum is in review for publication in the journal Landscape and Urban Planning,

This article was reformatted from the submitted version as part of this dissertation. M. Blum, C. Rigel, R. Campanella, and A. Peterson conceived of the study. B. Ghersi and A. Peterson collected rodent samples. R. Campanella contributed GIS analyses, J. Lewis contributed vegetation survey data. A. Peterson conducted all other analyses and wrote the manuscript. All authors provided edits and comments to the manuscript.

Abstract

Often overshadowed by global trends in urbanization, counter-urbanization is also on the rise worldwide. Left unaddressed, counter-urbanization can result in conditions that imperil human well-being. For example, counter-urbanization may increase the prevalence of ecological hazards like synanthropic pest and pathogen host species by shifting habitat and resource availability. In this study, we examined whether the abundance or diversity of rodents varies according to the prevalence of abandoned, unmaintained properties across a mosaic of counter-urbanization in post-Katrina New Orleans (Louisiana, USA). We found that total rodent abundance was highest in areas with increased tree cover and lower population densities. Additionally, we found that areas with more vacant lots and debris support a higher abundance and richness of rodents, especially in winter. While these results highlight that property abandonment can augment populations of pest and pathogen host species, our findings also indicate that management of abandoned areas can potentially mitigate public health concerns in counter-urbanizing landscapes.

Introduction

Global demographic shifts are giving rise to two seemingly contradictory outcomes; an increasing proportion of the world's population resides in cities, and an increasing number of cities are experiencing population loss. Often overshadowed by countervailing trends like the growth of mega-cities, counter-urbanization is also on the rise worldwide. In the United States, for example, 13% of cities with ≥100,000 inhabitants have recently declined in population size (Wiechmann et al. 2012, Grobmann et al. 2013). Counter-urbanization can result from a range of concurrent and successive factors including economic decline, disasters, and shifting demography, that determine the pace and magnitude of population loss. While counter-urbanization can be a slow progression–for example, decreasing population growth rates underlie steady declines that are unfolding in cities across a number of European countries (Nassauer and Raskin 2014)– chronic population loss can be punctuated by acute disruptions. For instance, a decadal progression of population loss in Detroit (Michigan, USA) has recently been exacerbated by a severe economic recession (Ryzner and Wagner 2007).
Similarly, chronic population loss in New Orleans (Louisiana, USA) spiked in 2005, when levee failures and flooding triggered by Hurricane Katrina resulted in a mass exodus.

Landscapes can be transformed by counter-urbanization. Often considered to be synonymous with idled and derelict infrastructure, counter-urbanization also can lead to higher rates of land abandonment (Nassauer and Raskin 2014; Gulachenski et al. 2016). Land abandonment can result in ecological shifts, including biotic changes like increased vegetation growth (i.e., 'greening'). In Detroit, for example, vegetation has increased in areas with greater abandonment and vacancy rates (e.g. Ryzner and Wagner 2007). Similarly, the composition of plant communities in post-Katrina New Orleans reflects socioeconomically stratified patterns of abandonment (Lewis et al. 2017). Unmanaged greening, which is sometimes referred to as 'green blight' (Lewis et al. 2017), can lead to conditions that are generally considered beneficial, such as greater shading and elevated biological diversity (Riley et al. 2018, Kattwinkel et al. 2011), but it can also generate ecological disservices, including conditions that are of concern to human well-being (e.g., Troy et al. 2012, Katz et al. 2014, Gulachenski et al. 2016, Rael et al. 2016, Troy et al. 2016, Branas et al. 2018, Eskew and Olival 2018). While this is receiving greater attention by those charged with safe-guarding public health in cities (e.g., Branas and Beyer 2014, Garvin et al. 2012, Bogar and Beyer 2016, Troy et al. 2016, Branas et al. 2018), ecological interpretations of 'green blight' remain focused on highlighting possible benefits, with little consideration given to how it might imperil the well-being of affected communities (Lewis et al. 2017).

Counter-urbanization can imperil human health by creating conditions that favor pests and pathogen vectors (Rael et al. 2016, Gulachenski et al. 2016, Eskew and Olival 2018), which can potentially elevate zoonotic disease risk, especially across novel human-environment interfaces where occupancy becomes juxtaposed with abandonment (Despommier et al. 2006). Idled or degraded infrastructure can, for example, increase the availability of habitat supporting pathogen vectors like mosquitos. This is well illustrated by conditions in Baltimore (Maryland, USA), where disinvestment in housing and associated infrastructure has allowed mosquitos to become hyper-abundant, increasing the risk of mosquito-borne pathogen transmission to local residents (LaDeau et al. 2013). Similarly, areas of New Orleans that have experienced greater levels of abandonment since Hurricane Katrina appear to harbor larger commensal rodent populations (Rael et al. 2016). Like mosquitos, commensal rodents in New Orleans are known to carry zoonotic pathogens of concern such as *Bartonella* sp., *Angiostrongylus* sp., and Hantaviruses (Cross et al. 2014, Peterson et al. 2017, Rael et al. 2018). Evidence that abandonment can lead to hyper-abundance of zoonotic pathogen hosts (Rael et al. 2016) highlights the possibility that commensal rodents may drive zoonotic disease outbreaks in areas experiencing urbanization (Bordes et al. 2015, Han et al. 2015) and counter-urbanization. It is unclear, however, whether the factors driving rodent-associated pathogen transmission risk in urbanizing landscapes (Bordes et al. 2015, Han et al. 2015) also determine transmission risk in counter-urbanizing landscapes. Determining the factors that shape rodent diversity and abundance in counter-urbanizing areas thus represents a key step towards preventing zoonotic disease outbreaks worldwide.

Because rodents generally exhibit strong site fidelity, species diversity and abundance often reflects local habitat characteristics (Cavia et al. 2009). In rural and natural areas, rodent species richness is positively associated with habitat heterogeneity (Horvath et al. 2001).

Similar trends have been found in urbanizing landscapes (Cavia et al. 2009). For example, the abundance of particular rodent species in Buenos Aires (Argentina) varies across a gradient of urbanization, where the greatest diversity occurs in less urbanized areas (Cavia et al. 2009). Rodent presence and abundance in cities also reflects socioeconomic conditions and factors like accessibility to structures and human-derived food resources (Himsworth et al. 2013, Feng and Himsworth 2014). This is well illustrated by rodent control efforts that aim to limit access to public trash receptacles, which can reduce rodent abundance (Lambropoulos et al. 1999). Some evidence also suggests that habitat and resource heterogeneity elevate local richness and turnover across urban environments by limiting movement of some rodents (Combs et al. 2017). It is not known, however, whether and how rodent abundance, diversity, and co-occurrence varies across habitat mosaics in counter-urbanizing landscapes, which can exhibit starkly different configurations than those that occur in urban and suburban landscapes (*reviewed in* Gulachenski et al. 2016).

In this study, we assessed the diversity, abundance, and co-occurrence of rodent species across post-Katrina New Orleans. We focus specifically on a subset of rodents (rats and mice) within the broader community (hereafter referred to as the rodent assemblage) to determine the socio-environmental features that shape the abundance and diversity of commensal rodents in a counter-urbanizing city. Our objectives were to first elucidate how rodent assemblages vary over time and across residential urban neighborhoods. This involved drawing comparisons among neighborhoods within counter-urbanizing areas that have experienced recent and acute population decline, neighborhoods in areas that have not experienced population loss, and a natural area adjacent to the city that is devoid of human residency. Our second objective was to identify the socio-environmental features associated with rodent diversity and abundance. We hypothesize that rodent abundance and diversity vary in relation to land cover, vegetation, as well as human sociodemography (Cavia et al. 2009, Feng and Himsworth 2014, Walsh 2014). We predict that rodents are more abundant in areas with greater abandonment or features indicative of infrastructure decline (Eskew and Olival 2018), as has been observed with other pests and vectors elsewhere (e.g., LaDeau et al. 2013). We also predicted that rodent diversity would increase in areas with greater habitat diversity. We met our study objectives and evaluated our predictions by characterizing rodent assemblage structure across the city, with comparisons drawn among the study areas, which exhibit varying levels of abandonment. We also included a comparison of the rodent assemblage present in two spatially proximate neighborhoods with contrasting municipal policies on post-Katrina vacant lot management to shed light on how interventions can shape health risks in counter-urbanizing cities.

Methods

Study area and study design

Hurricane Katrina, considered to be one of the deadliest and most destructive hurricanes in U.S. history, transformed the sociocultural, built, and ecological features of the New Orleans metropolitan area. Storm surge and the failure of levees flooded over 80% of the

urbanized East Bank of New Orleans, displacing approximately 86% of the human population in its immediate aftermath. While the population of the city has rebounded since the storm, population recovery has been heterogeneous, with some areas remaining well below pre-Katrina levels (Fussel et al. 2014, Lewis et al. 2017). The storm also reconfigured ecological communities in New Orleans. For example, pre- and post-Katrina surveys indicate that flooding reduced the abundance of birds and mammals across the city (Yukey 2008). Comparisons of land cover (Gotham et al. 2014) also indicate that Katrina-related flooding reduced landscape diversity across the city. Plot-based plant surveys indicate, however, that post-Katrina vegetation communities reflect post-disaster landscape management as much or more than Katrina-related flooding (Lewis et al. 2017). Post-Katrina management also has resulted in mosaics of abandonment that have reinforced legacies of sociodemographic disparities (Gulachenski et al. 2016, Rael et al. 2016, Lewis et al. 2017).

To meet our study objectives, we assessed rodent abundance and diversity in five focal study areas across the greater New Orleans metropolitan region. Study area boundaries were set based on the 2010 US Census data and historical neighborhood boundaries as described in Lewis et al. (2017). The study areas capture variation in income, post-Katrina population recovery, and land management (Table S1.1, Figure 1.1) that has given rise to differences in vegetation characteristics (Lewis et al. 2017). We trapped rodents in three Orleans Parish neighborhoods: Uptown, Gentilly, and the Lower 9th Ward. The Uptown neighborhood largely escaped Katrina-related flooding, and consistent with this, the predominantly higher income neighborhood exhibits the lowest level of vacancy relative to all other study areas (Figure 1.1). Both Gentilly and the Lower 9th Ward experienced extensive flooding, though population recovery has been greater in Gentilly, where median household income is higher, and vacancy is lower relative to the Lower 9th Ward (Figure 1.1). Comparisons across the three neighborhoods thus offer perspectives on whether rodent communities differ according to human sociodemography and vacancy. We also trapped in the Arabi and Chalmette neighborhoods of St. Bernard Parish (hereafter referred to as St. Bernard) (Figure 1.2), which are adjacent to the Lower 9th Ward neighborhood. While both of these areas experienced similar levels of flooding and vacancy (Figure 1.1), vegetation in the Lower 9th Ward stands in stark contrast to vegetation in the adjacent St. Bernard Parish neighborhoods (Lewis et al. 2017, Figure 1.1). Institutional programs intended to foster population recovery created striking mosaics of abandonment across public and privately-owned vacant properties in Orleans Parish (Lewis et al. 2017). This is particularly evident in the Lower 9th Ward, where publicly owned lots have been mowed and maintained, while ruderal vegetation growth has been left relatively unchecked on many privately-owned vacant lots (Lewis et al. 2017). Nearly all vacant properties in the adjacent St. Bernard Parish neighborhoods, on the other hand, have been subject to strict management practices regardless of ownership, including regular mowing (Lewis et al. 2017). Comparisons across the municipal boundary thus offer perspectives on whether rodent communities differ according to municipal land management policies. Lastly, we collected rodents in a non-residential 'natural' area located to the north of the Lower 9th Ward and Chalmette, adjacent to East New Orleans, which enabled us to compare rodent assemblage structure across a full spectrum of land use.

Rodent trapping

We estimated rodent abundance and diversity across 48 study blocks between May 2015 and February 2017. As described in Lewis et al. (2017), we selected ten study blocks within each of the four residential focal study areas by overlaying a 500 m x 500 m grid generated in ArcGIS over the metropolitan area of New Orleans and surrounding areas. We then selected a random subset of 10 blocks within each focal residential area that fell at the intersection of the grid-lines for inclusion in our study. We similarly selected eight equally-sized trapping sites in the non-residential 'natural area'. With the exception of the sites in St. Bernard Parish, we trapped at all sites in the summer (May-August) of 2015, winter (November-February) of 2015/2016, summer of 2016, and winter of 2016/2017. We only trapped at sites in St. Bernard Parish during the summer of 2016 and winter of 2016/2017.

During each trapping bout, we placed 30 live Tomahawk traps (Tomahawk Live-trap Co., WI, USA) to target larger bodied rodents (i.e., rats) and 30 live Sherman traps (H.B. Sherman Traps, Tallahassee, FL, USA) to target smaller bodied rodents (i.e., mice) within each trapping block. Pairs of Sherman and Tomahawk traps were placed within 1 meter of each other. We placed all traps outside in areas of observed or potential rodent activity (e.g., near visible runways, trash bins, compost, debris piles, etc.) in yards, alleys, and in vacant lots whenever present. The placement of traps within each block was dependent on property access. In residential areas, all trapping occurred within the boundaries of each study block, unless we were not able to obtain access to a sufficient number of properties to place all traps. When access was limited, we placed traps on properties that directly faced the focal study block. We set all Tomahawk traps for a minimum of three continuous nights. Tomahawk trapping was sustained at each site until the trap rate reached an asymptote (i.e., we "trapped out" a block). For Sherman traps, we completed trapping for a minimum of 3 continuous nights, but limited trapping to 4 nights total. To ensure estimates of rodent abundance from the Sherman and Tomahawk trapping are equivalent across all sites, we have limited our analyses to data collected within the first four nights of Tomahawk trapping. Abundance estimates from Tomahawk traps as measured in the first 4 nights of trapping are reflective of the full asymptotic trapping estimates (Pearson's correlation: $r=0.94$, $p < 0.001$). For both Sherman and Tomahawk traps, we set and baited traps with a mixture of peanut butter and bacon bits each afternoon and checked and closed all traps the following morning. Each morning we counted the number of traps that were positive for rodents, positive for non-target (i.e., non-rodent) species, and sprung but empty traps. We released non-target animals in the area of capture, and all rats and mice were euthanized and necropsied at the City of New Orleans Mosquito, Termite, Rodent Control Board facilities in accordance with Tulane-approved IACUC protocols 0451 and 0460.

Socio-environmental habitat and vegetation assessments

We used four methods to assess habitat, vegetation cover and sociodemographic variables at each trapping location: 1) on-the-ground estimates of percent cover within each trapping area; 2) on-the-ground plant diversity data from vegetation plots within each trapping block; 3) land cover categories from satellite imagery; and 4) sociodemographic variables from

the 2010 US Census. On-the-ground estimates of percent cover were obtained for each trapping bout at each location. We first demarcated the trapping area boundaries within a block, which typically aligned with property boundaries. We then visually estimated the proportion of coverage within each trap area that corresponded to the following attributes: unmaintained vegetation (grass taller than 15 cm and bushes that were not trimmed within 15 cm from the base), bare dirt (including unpaved areas underneath raised homes), and impervious surfaces (concrete and asphalt). We also counted the total number of unmaintained buildings (identified as buildings that were missing major structural features such as the roof or windows), and the number of debris piles (food waste, compost, and miscellaneous trash such as tires and construction debris). Following Lewis et al. (2017), measures of plant diversity- including shrub, tree, and herbaceous species- in each trapping area were estimated by surveying a 400 m^2 circular vegetation plot in accordance with US Forest Service protocols (Nowak et al. 2008, USDA-FS 2016). We completed vegetation surveys at all trapping sites in the Gentilly, Lower 9^{th} and Uptown neighborhoods during the summer of 2015, though surveys in St. Bernard and the natural area were completed in the summer of 2016 only.

We characterized land cover according to high-resolution satellite imagery for each year of the study. To do this, we acquired two Pleiades satellite images of the greater New Orleans metropolitan area, each with four multispectral bands and 0.5-meter spatial resolution, captured on 17 March 2015 and 28 March 2016. Using ESRI ArcGIS 10.3.1, we completed a supervised classification of five land cover categories: mature trees, open grass, urban surfaces (impervious surfaces and bare soil), buildings, and open water. We validated and improved classifications through visual inspection as well as the inclusion of rasterized GIS layers of building footprints and GIS layers of open water bodies such as canals. This reduced uncertainties that can arise when features are obscured by trees and other similar aspects of the landscape. We implemented the same process for characterizing the 'natural area' sites, but rather than using US Census block boundaries, we instead bounded land cover data within a 250 m x 250 m polygon, which corresponds to the average size of the census blocks with our trapping sites. Lastly, we intersected the trapping blocks with US Census block boundaries to derive sociodemographic attributes of the trapping blocks according to the 2010 US Census (Gotham et al. 2014). Finally, we determined the proportion of vacant lots on each trapping block using satellite imagery. We obtained spatial layers of parcel boundaries for both Orleans and St. Bernard Parishes (htpps://www.gis.nola.gov: https://gis-stbernard.opendata.arcgis.com, respectively), which we overlaid onto Google Earth satellite imagery to count the total number of lots and number of vacant lots on each census block. We considered a lot to be vacant if a home, shed or other man-made structure (e.g., swimming pool) did not fall within its boundaries. We considered all lots in trapping locations outside of residential areas (all sites in the 'natural area', one location within a city park, and one area fully located in a roadside median) as 100% vacant. The availability of true-color historical imagery available at multiple time points through Google Earth enabled us to estimate annual variation in vacancy over the course of the study period.

Study area characterization

To understand how socio-environmental features of interest varied among the five focal study areas, we calculated the mean and standard deviation of all variables across all sites located within a given area (Table S1.1). The sociodemographic factors of interest included: the proportion of vacant lots at each site and US Census based estimates of median household income and total human population. The environmental features of interest included remotely sensed measures of proportional coverage of mature trees, grass, urban surfaces, and buildings. Additional environmental features of interest also included on-the-ground measures of proportional coverage of bare dirt, impervious surfaces, and unmaintained vegetation, as well as the number of unmaintained buildings and debris piles within each specific trapping site. For the variables that were normally distributed, we used repeated-measures ANOVA followed by pairwise comparisons of least-square means (Tukey HSD) to determine if socio-environmental features differed temporally and spatially among the study areas (Figure 1.1). For the variables that were measured as proportions (e.g. the satellite-derived land cover estimates), we used beta regression to determine how each varied across the study areas (Eskelson et al. 2011) using the betareg package in R (R development core team, 2008). In preparation for further analysis, we then checked all of these variables for collinearity. Several of the socio-environmental variables were highly correlated, and thus we selected only a subset for use in further analyses.

Statistical analysis of spatiotemporal characterization of rodent assemblage structure

We first assessed how the composition of rodent assemblages varies among the study areas through non-metric multi-dimensional scaling (NMDS) based on Bray-Curtis dissimilarity index values using the vegan package in R. The NMDS plot displays the rank-order position of communities within non-dimensional space (Figure 1.2). To determine if spatially proximate assemblages are more similar than spatially disparate assemblages, we calculated a Mantel correlogram and Mantel's r values across a progression of spatial lags (Mantel 1967) by comparing the community dissimilarity matrix to a matrix of pairwise distances between sampling sites in the R package ade4 (Legendre and Fortin 1989, Chessel et al. 2004, Bougeard and Dray et al. 2018) (Figure S1.1). We utilized a probabilistic model of species co-occurrence (Veech 2013) to determine if some rodent species were more or less likely to co-occur with one another than would be expected by chance. These analyses were completed using the presence-absence matrix from all trapping sites and all years, using the 'cooccur' package in R (Griffith et al. 2016).

To assess how rodent assemblages varied over time and across gradients of urbanization and vacancy, we constructed three generalized linear mixed models (glmm) to determine whether: 1) overall rodent abundance; 2) rodent species richness; and 3) rodent Shannon diversity differed among the study areas and across seasons (Zuur et al. 2009). We calculated rodent abundance by summing all individuals of all species collected from each site in a given trapping bout; and we calculated rodent species richness by summing the number of species collected at each site in a given trapping bout. We calculated Shannon diversity using the vegan package in R. For the models predicting rodent abundance and richness, we used

glmms with a Poisson error distribution, as the data are discrete counts of individuals, while we used a Gamma distribution for the Shannon diversity model (Zuur et al. 2009). For all of these models, we included trapping area and season as fixed effects, and site nested within year as a random effect (Zuur et al. 2009). Following each analysis, we performed pairwise comparisons of least-square means (Tukey HSD) to determine how abundance and richness varied among trapping areas, using the lsmeans package in R (Lenth 2016).

Statistical analysis of socio-environmental predictors of total rodent abundance and individual species abundance

We utilized a multilevel modeling (MLM) approach to determine the relative strength of socio-environmental variables (Table S1.1) as predictors of total rodent abundance and individual species abundance. MLM approaches can be more informative than more traditional methods of assessing variation in assemblage structure (e.g., RDA, CCA, NMDS) by offering greater power and lower sensitivity to collinearity of variance (Jackson et al. 2012). MLM approaches also allow for the simultaneous exploration of drivers of overall rodent abundance as well as species-level abundances within a single model (Jackson et al. 2012). Prior to completing the MLM analysis, we checked all potential predictor variables for co-linearity using correlation analysis (Jackson et al. 2012). We removed the remotely-sensed estimates of grass cover and building cover as they were highly correlated with the proportion of vacant lots on a block (r=0.80, and -0.83, respectively). We also removed the remotely-sensed measure of impervious surface cover, as it was highly correlated with the remotely-sensed measure of tree cover (r=-0.71). After down-selecting the suite of socio-environmental variables for inclusion in the MLM analysis, we standardized all predictor variables to a mean of 0 and a variance of 1. Standardizing variables allows for the direct comparison of coefficients, which are then representative of effect sizes (Jackson et al. 2012). As our dependent variable was a count of total rodent abundance, we compiled a global glmm with a Poisson error distribution that included the suite of socio-environmental predictor variables hypothesized to relate to rodent abundance. This global model included measures of vacant lots, median household income, total human population, remotely-sensed based estimates of tree cover, as well as on-the ground estimates of bare dirt, impervious surfaces, unmaintained buildings, debris/trash, unmaintained vegetation and the richness of tree, shrub, and herbaceous plant species as fixed effects. We also included the interaction terms of vacant lots x unmaintained vegetation, vacant lots x unmaintained buildings, and vacant lots x trash/debris, to represent hypothesized interactions between vacancy and other features of infrastructure loss. As in Jackson et al. (2012), we included species as a random effect in the global model, as well as each fixed effect nested within species as a random effect. We also included the variable year nested within site as a random effect to account for the repeated measurements of rodent abundance at each site. We determined the top-selected model by comparing all combinations of variables, including all single-variable models and a null model. When comparing among models, we always included the fixed effect if the random effect was included in the model (Jackson et al. 2012). We then ranked each model according to AIC (Jackson et al. 2012) and considered the model with the lowest AIC as the top-selected model. In this case the top selected model was

>2 ΔAIC than the next best model, so we did not perform model averaging of coefficients (Burnham and Anderson 2002). All analyses were conducted in R using the lme4 and glmmTMB packages (Bates et al. 2015, Brooks et al. 2017).

To determine that we had properly specified the model and did not have residual kurtosis or spatial autocorrelation, we performed diagnostic tests on randomized quantile residuals that we generated by comparing observed values to simulated observations from 250 runs of the best-fit model (Dunn and Smyth 1996, Hartig 2018). We then checked these models for over/under dispersion using qqplots, and spatial autocorrelation using Moran's I with the R packages DHARMa and ape (Paradis et al. 2004, Hartig et al. 2018). Lastly, to assess how well the best-fit model explained the data, we calculated the conditional and marginal R^2 values for the best-fitting model (Nakagawa and Schielzeth 2013, Johnson 2014) using the sjstats package (Ludecke 2018).

Statistical analysis of socio-environmental predictors of total rodent diversity

Rodent richness varied significantly among study areas (described below), while Shannon diversity was only significantly different between the Lower 9th and Uptown neighborhoods (described below) Thus, we completed a glmm (Poisson) analysis to determine the socio-environmental features that best predict rodent richness only. As with the MLM analysis, we first constructed a global glmm model that included the full suite of socio-environmental variables as fixed effects, and we included year nested within site as a random effect. We selected among the models using AIC and considered the model with the lowest AIC as the top selected model.

Results

Study area characterization

We found evidence of among-year, but not within-year, temporal variability in site characteristics (Figure 1.1). The variables that were collected during multiple seasons (e.g., amount of debris, unmaintained houses, unmaintained vegetation, etc.) did not vary between summer or winter seasons of a given year (repeated measures ANOVA, p>0.05 for all models). Of the variables that were collected yearly, the remote-sensed estimate of urban cover was significantly higher in 2016 relative to 2015 (p=0.04, coef. =0.19). Consistent with this, our on-the-ground measure of unmaintained vegetation (averaged across season) was lower in 2016 relative to 2015 (p=0.003, coef. =-0.06). We also found that the amount of trash (p=0.02, coef. =0.20) as well as the amount of dirt cover (both averaged across season) (p=0.02, coef. =0.02) were higher in 2016 relative to 2015 (Figure 1.1).

After accounting for temporal variation, all the socio-environmental features collected in our study also significantly differed among the focal study areas (Figure 1,1). The Lower 9th Ward neighborhood harbored a significantly lower human population than all of the other study areas, excluding the natural area sites (p<0.05, Tukey HSD, Figure 1.1), while median household income was significantly higher in the Uptown study area relative to all other study locations (p<0.05, Tukey HSD, Figure 1.1). We considered all sites within the natural area as

100% vacant, and thus the natural area had significantly more vacancy relative to all other trapping areas (Figure 1.1). Excluding the natural areas, the focal study blocks in the St. Bernard Parish and Lower 9th Ward trapping areas had a significantly higher proportion of vacant lots relative to all of the other residential trapping areas ($p<0.05$, Tukey HSD, Figure 1.1). Additionally, trapping sites within the Lower 9th Ward also had significantly more unmaintained houses and debris piles relative to sites in all other trapping areas ($p<0.05$, Tukey HSD, Figure 1.1). The proportion of area covered in unmaintained vegetation was similar in both the Lower 9th Ward and natural area sites, though the unmaintained vegetation within the two study areas was significantly higher relative to all other study areas ($p<0.05$, Tukey HSD, Figure 1.1). Tree richness was significantly higher in the natural area sites relative to sites in all other study areas ($p<0.05$, Tukey HSD, Figure 1.1). Shrub species richness was significantly higher in the Uptown study area (mean= 3.0 species) only in comparison to the St. Bernard study area (mean=0.4 species) ($p<0.05$, Tukey HSD, Figure 1.1), while herbaceous species richness was lowest in the Uptown study area (mean=8.5 species) only in relation to the Lower 9th Ward, which registered greater richness than any other study area (mean =14.5 species) ($p<0.05$, Tukey HSD, Figure 1.1).

Spatiotemporal characterization of rodent assemblage structure

We captured 818 rodents from the 48 trapping sites, including individuals of three non-native commensal species (*Rattus rattus* (n=213), *Rattus norvegicus* (n=119), *Mus musculus* (n=461)) as well as two species native to Louisiana (*Sigmodon hispidus* (n=21) and *Oryzomys palustrus* (n=4)) during 2015, 2016, and 2017 (Figure 1.2). We collected both of the native species in the natural area, and one of the native species (*S. hispidus*) in the Lower 9th Ward study area. We captured all of the non-native species in every study area, though not at every trapping site (Figure 1.2). Consistent with this, we found that the composition of the rodent assemblages varied across the study areas (Figure 1.2). Furthermore, we rejected the null hypothesis that there was no spatial relationship in rodent assemblage structure (Mantel's r =0.26, two-tailed p-value <0.001). Rather, more spatially proximate rodent assemblages were more similar than spatially disparate assemblages.

For some species, we found that the probability of occurrence was signicantly related to the occurrence of other species. We found a positive association between *R. norvegicus* and *M. musculus* individuals (p=0.04), whereas we found a negative association between *R. rattus* and the two native rodent species (*R. rattus* - *S. hispidus*: p=0.01; *R. rattus* – *O. palustrus*: p<0.01), as well as between *R. rattus* and *M. musculus* (p<0.01) (Figure 1.4).

We found greater variation in total rodent abundance, richness and diversity among the study areas than over time (Figure 1.3). We captured significantly more rodents in the Lower 9th Ward relative to all other areas ($p<0.05$ for all pairwise comparisons: Lower 9th – Gentilly, coef. = 1.46971; Lower 9th– natural area, coef = 1.41; Lower 9th– St. Bernard, coef. = 2.08; Lower 9th – Uptown, coef. = 2.38; Figure 1.3a). Additionally, we found a statistically significant positive association between rodent abundance and winter season ($p<0.01$, coef. = 0.53488). No association was found with abundance and year (Figure 1.3a). Rodent richness also differed among neighborhoods, with the Lower 9th Ward harboring more richness compared to all other

study areas ($p<0.001$ for all pairwise comparisons: Lower 9 – Gentilly, coef.=0.68197; Lower 9th – natural area, coef.= 0.55192; Lower 9th – St. Bernard, coef.= 1.31; Lower 9th – Uptown, coef.= 1.23)(Figure 1.3b). Additionally, the natural area also harbored a significantly higher rodent richness relative to the Uptown study area (coef. = 0.68, $p<0.05$). We did not find a significant relationship between rodent richness and season or year (Figure 3b). Lastly, Shannon diversity was significantly higher in the Lower 9th Ward study area relative to only the Uptown study area (coef. = 2.44, $p=0.02$). Shannon diversity did not differ significantly among seasons or years (Figure 1.3c).

Socio-environmental predictors of total rodent abundance and individual species abundance

The top selected MLM model for rodent abundance was >2 ΔAIC less than the next best model, and thus is the only model for which results are presented. The marginal R^2 of the rodent assemblage model equaled 0.37 while the conditional R^2 equaled 0.48 (Nakagawa and Schielzeth 2013, Johnson 2014). Lastly, we found no evidence of kurtosis or spatial autocorrelation (one –sample Kolmogorov-Smirnov test, $p>0.05$, $D=0.04$; Moran's I, $p>0.05$).

The fixed effects variables included in the top selected model were: study area, total human population, median household income, remotely-sensed estimates of tree cover, as well as the number of unmaintained buildings, unmaintained vegetation cover, shrub, and herb richness (Table 1.1). The top selected model also included the fixed-effects interaction terms: debris x vacant lots, debris x season, and vacant lots x season (Table 1.1). Of the fixed-effects variables, study area had the strongest influence on total rodent abundance (e.g. natural area coef. = -4.51, $p<0.001$; St. Bernard coef. = -1.16, $p<0.001$; Uptown coef. = -1.13, $p<0.05$). Following study area, the variable with the strongest effect on overall rodent abundance was tree cover (coef.= 1.04, $p=0.03$) followed by total human population (coef.= -0.75, $p<0.001$), the debris x season interaction term (0.38, $p<0.001$), the debris x vacant lot interaction term (coef.=-0.36, $p=0.04$), the vacant lots x season interaction term (coef=0.24, $p<0.001$), and finally the debris term alone (coef.=-0.22, $p=0.03$) (see Table 1.1 for all coefficients and p-values). While the proportion of vacant lots term was not strongly associated with total rodent abundance overall, there was a stronger positive association during winters than in summers (Table 1.1, Figure 1.5). Additionally, we found that areas with higher levels of vacancy (> 50% of lots on a block vacant) exhibited a stronger positive relationship between total rodent abundance and the number of debris piles relative to areas with lower levels of vacancy (< 50% of lots on a block vacant), especially during winters (Table 1.1 fixed effects, Figure 1.6).

Different socio-environmental variables predicted individual species abundances (Table 1.2). The most important predictors of among-species variation in abundance included season, mature canopy cover, and herbaceous species richness (Table 1.1, random effects). For each individual species, season was among the top predictors of abundance, though the direction of the effect varied among species (Table 1.2). The abundance of *Mus musculus* and *Rattus norvegicus* was more strongly associated with winter (i.e., both were more abundant in winter), while *Rattus rattus*, *Oryzomys palustrus,* and *Sigmodon hispidus* were more strongly associated with summer relative to winter (i.e., all were more abundant in summer) (Table 1.2). Abundance of *M. musculus* also was higher in areas with increased levels of vacant lots (coef.

=0.50) and in areas with lower median household income (coef. =-0.36). The abundance of *R. norvegicus* was higher in areas with lower median household income (coef. =-0.52), and in areas with greater unmaintained vegetation (coef. =0.52). The abundance of *R. rattus* was greater in areas with greater tree cover (coef. =0.76) and in areas with greater median household income (coef. =0.50). The abundance of the native species *S. hispidus* was greater in areas with more mature canopy cover (coef. =1.94) and in areas with lower herbaceous species richness (coef. =-1.42). The abundance of the native species *O. palustru*s was greater in areas with more tree cover (coef. =1.82) and in areas with lower household income (coef. =-0.65). It is important to note, however, that this finding is based on a small number of *O. palustrus* individuals (n=5) caught from a single study area (the natural area), where estimates of income were 0 due to the absence of humans.

Socio-environmental predictors of total rodent richness

The top selected model was >2 ΔAIC than the next best model, and thus is the only model for which results are presented. The top selected model predicting rodent richness included the following variables: study area, total human population, and the proportion of vacant lots on a block. The most important predictors of rodent richness were the proportion of vacant lots on a block (coef. =0.74, p=0.02), and study area, with the natural area sites (coef. =-1.03), St. Bernard sites (coef. =-0.91) and the Uptown sites (coef. =-0.56) all having significantly lower richness relative to the Lower 9th Ward study area sites (all p-values <0.05; Table 1.3).

Discussion

There is growing recognition that habitat and resource shifts in counter-urbanizing environments can reshape assemblages by favoring species that serve as hosts for zoonotic pathogens (Rael et al. 2016, Gulachenski et al. 2016, Eskew and Olival 2018). In this study, we sought to assess how rodent assemblages respond to variation in socio-environmental features associated with abandonment and land management (Lewis et al. 2017, Wang et al. 2014). Our findings indicate that rodent abundance and richness vary with counter-urbanization across post-Katrina New Orleans (Figure 1.2, Figure 1.3). In particular, we found that rodent abundance was greatest in residential areas burdened with more vacancy (Table 1.1, Figure 1.3, Figure 1.4) and that feature other conditions associated with counter-urbanization (Table 1.1). Thus, our study offers further evidence that counter-urbanization can result in conditions that are unfavorable to human well-being (Eskew and Olival 2018, Gulachenski et al. 2016).

Though similar to trends that have been observed for other taxa in urbanizing areas, patterns of rodent abundance across New Orleans reflect different underlying conditions. Notably, we found that rodent abundance was significantly lower in non-residential natural areas relative to residential areas that have sustained population losses triggered by Katrina-related flooding (e.g. the Lower 9th Ward study area, Figure 1.3a). Additionally, rodent abundance in more densely populated residential areas was significantly lower relative to residential areas that have experienced population loss (Figure 1.3a). This runs contrary to the prevailing notion that rodent abundance closely mirrors human demography (i.e., the ‘one rodent for every person’ rule of thumb). Evidence of a strong relationship (Figure 1.5) between

vacancy and rodent abundance (where the sites considered fully vacant are in natural areas) is more consistent with trends observed elsewhere indicating that the abundance of commensal fauna (e.g., lizards, birds, butterflies, mammals and several other arthropods) peaks at intermediate levels of urbanization (e.g. Blair 1999, McIntyre 2000, Germaine and Wakeling 2001, Riem et al. 2012). While elevated abundance of some species has been attributed to greater primary productivity in managed green spaces in other cities (Sochat et al. 2006), our findings indicate that unmanaged green spaces resulting from vacancy and abandonment (i.e., green blight) afford the resources and habitat necessary for rodents to become hyper-abundant in New Orleans. This is well illustrated by the finding that rodent abundance not only reflects vacancy, but that it reflects the extent of debris piled in vacant lots, especially in winter when resources may otherwise be limiting (Table 1.1, Figure 1.5) (Masi et al., 2010). Our findings also indicate that areas with more vacant lots provide harborage for a greater number of rodent species (Table 1.3), including rare species. It is thus very likely that the public health outcomes of green blight extend beyond well-recognized concerns such as crime, mental health, and safety (e.g., Branas et al. 2014, Garvin et al. 2013, Bogar and Beyer 2015, Troy et al. 2016, Branas et al. 2018) to include ecological hazards that can come from hyper-abundant pest and pathogen host species (LaDeau et al. 2013).

While important, vacancy was not the sole driver of rodent abundance. Our results indicate that land management acts in conjunction with vacancy to shape rodent assemblages. This is demonstrated with comparisons of rodent abundance and assemblage composition in the Lower 9th Ward and in adjacent areas of St. Bernard Parish. The areas are spatially proximate to one another (Figure 1.2) and exhibit comparable levels of vacancy (Figure 1.1), but they have been subject to contrasting land management policies since Hurricane Katrina. Publicly and privately-owned lots are not managed equivalently in the Lower 9th Ward neighborhood, which falls under the jurisdiction of Orleans Parish (Lewis et al. 2017). While public lots are managed (e.g., regularly mowed and cleared) by the state and municipal government entities, private lot management falls to land owners, which has given rise to a heterogeneous patchwork of maintained and unmaintained areas in the neighborhood (Lewis et al. 2017). In contrast, nearly all vacant lots within St. Bernard Parish are regularly maintained by the Parish, regardless of ownership, resulting in relatively homogeneous vegetation (Lewis et al. 2017). Consistent with this, we found that the average proportion of unmaintained vegetation and the average number of debris piles within trapping areas across the Lower 9th Ward were more than twice as high as in St. Bernard Parish trapping areas (Figure 1.1, Table S1.1). The composition of the rodent assemblage also differed among the adjacent neighborhoods (Figure 1.2), likely reflecting shifts in habitat conditions (Tables 1.2 and 1.3). This is demonstrated by the correlogram of Mantel's r, which demonstrate that similarity among assemblages drops sharply over relatively small distances (Figure S1.1). For example, *R. norvegicus* was abundant and widely distributed across the Lower 9th Ward, but it was rarely encountered in the adjacent St. Bernard neighborhoods. The Parish boundary appears to be a one-way sieve, as all of the species detected in St. Bernard Parish were also found in the Lower 9th Ward. This suggests that maintenance of abandoned areas can be an effective strategy for reducing ecological hazards associated with particular commensal rodents like *R. norvegicus*

(Peterson et al. 2017). This reinforces the often-issued recommendation (Colvin and Jackson 1999, Lambropous et al. 1999, Johnson et al. 2016) that managing public lands and reducing trash is a first line of defense against rodent infestation. It also reiterates the importance of coordination among municipal entities (e.g., trash management, land management, pest control, public health, etc.) to reduce hazards to human well-being (Corrigan 2006).

Management to address public health concerns must account for species-specific responses to socio-environmental factors. Our results indicate that shifts in assemblage structure across New Orleans reflect differences in response to socio-environmental conditions. Using an MLM approach, we were able to elucidate the socio-environmental features related to both total rodent abundance, as well as the abundance of individual species. We found that inclusion of random effect terms- which explain among-species variation in abundance (Nakagawa and Schielzeth 2013, Johnson 2014)- in the final best-fit MLM provided insight into the socio-environmental variables that influence each rodent found in our study area. Season was the most important predictor of among-species variation in abundance (i.e., the variable with the largest effect size, Table 1.1). Not all species appear to be similarly influenced by season. Both *M. musculus* and *R. norvegicus* appear to be more sensitive to seasonality, as both species were more abundant in the winter compared to the summer season. The native species *S. hispidus* and *O. palustrus* also appear to exhibit some sensitivity to seasonality, though we found that both were more abundant in the summer season (Table 1.2). Other factors like tree cover appeared to be stronger predictors of abundance for both native species (Table 1.2). It is important to note, though, that both *S. hispidus* and *O. palustrus* were only rarely captured in our study, and thus further study is warranted to draw more robust inferences about drivers of abundance. We also found that tree cover was the most important predictor of *R. rattus* abundance, suggesting that this species relies less on seasonally variable habitat or resources than its congener. Notably, median household income was among the strongest predictors for all species except *S. hispidus*. We found that *R. norvegicus*, *M. musculus*, and *O. palustrus* were more abundant in low-income areas (Table 1.2), which is consistent with prior work suggesting that elevated rodent abundance in lower-income areas is likely due to infrastructure disinvestment and lower coping capacity of residents (e.g., Easterbrook 2005, Johnson et al. 2016, Rothenburger 2017). Conversely, we found that the abundance of *R. rattus* was greater in higher-income areas, which also have greater tree cover (Table 1.2). This is consistent with the use of trees as habitat (and the use of trees to access elevated habitat like attic spaces in houses) by *R. rattus*, which is an arboreal species (Marsh 1994). As in many cities (e.g. Grove et al. 2014, Schwartz et al. 2015), the prevalence and composition of tree cover varies across New Orleans, with higher-income areas supporting more trees and more native and culturally valuable tree species in particular, relative to lower-income neighborhoods (Lewis et al. 2017). Our findings suggest that native and cultivated trees may be preferred habitat for *R. rattus,* given the positive association between income and abundance of this species. It also indicates that public health risks associated with commensal rodents can transcend income disparities, and that one-size-fits-all approaches to managing risk are likely to prove unsuccessful in cities like New Orleans that harbor a diverse complement of commensal pests.

Intraspecies interactions may also shape rodent assemblage composition across the city of New Orleans. For example, we found that the occurrence of *R. rattus* was negatively associated with both native rodent species as well as *M. musculus*. Some evidence suggests that co-occurrence of *O. palustrus, S. hispidus,* and *R. rattus* may eb limited due to competition (i.e., all exhibit a positive association with increased tree cover). The widespread distribution of *R. rattus*, in comparison to the more limited distribution of the two native species, suggests that *R. rattus* may displace or outcompete the native species across the city, as has been demonstrated elsewhere (e.g. Stokes et al. 2009). Conversely, the negative association between *M. musculus* and *R. rattus* may reflect spatial differences in preferred habitat, but may also similarly indicate direct or indirect competition (Harper and Cabrera 2010), or even predation of *M. musculus* by *R. rattus* (Bridgman et al. 2013). Interestingly*, R. norvegicus* are also thought to inhibit *M. musculus* (Brown et al. 1996, King et al. 1996, Ruscoe et al. 2011), though we found a positive association between these species in New Orleans. This finding is consistent with evidence from other cities that *R. norvegicus* and *M. musculus* demonstrate similar habitat preferences (Cavia et al. 2009). Evidence of a positive association with vacant lots and unmaintained vegetation in low-income areas for both species parallels other findings indicating that areas with decreased infrastructure offer sufficient resources to allow for co-existence (Cavia et al. 2009).

Understanding patterns and drivers of rodent diversity can shed new light on zoonotic pathogen transmission risk, especially in areas like counter-urbanizing landscapes that can be novel human-wildland interfaces (e.g. Keesing et al. 2010). For example, we found evidence of local (i.e., within a block) and regional (i.e., across a study area) species co-occurrence, which raises the possibility that non-host specific pathogens of concern could be transmitted by more than one host in a given area (Figure 1.2). Indeed, the zoonotic parasite *Angiostrongylus* sp. is found to infect both *R. norvegicus* and *R. rattus* in New Orleans (Rael et al. 2018). Rodent abundance and diversity appear to scale together in this system, as both richness and abundance are highest in residential areas with high levels of vacancy, such as the Lower 9th Ward. This commensurate scaling of abundance and diversity is hypothesized to lead to an amplification effect, whereby areas with increased diversity have the highest risk of disease (Mihaljevic et al. 2014). Our results suggest that lower income areas burdened with greater abandonment thus are likely more at risk of zoonotic disease transmission. Though the total risk of zoonotic pathogens may be lower in counter-urbanizing areas like the Lower 9th Ward that support fewer residents, individual-level risk may nonetheless be greater for remaining residents. Moreover, the loss of access to critical infrastructure, like healthcare facilities, only exacerbates this risk in counter-urbanizing areas (Gulachenski et al. 2016, Rael et al. 2016, Eskew and Olival 2018). Thus, further study is warranted to determine whether pathogen loads track rodent diversity. It also would be prudent to assess whether transmission risk reflects species interactions (e.g., competition between *R. norvegicus* and *R. rattus*) that may influence the relative abundance of rodents across human-dominated landscapes.

Conclusions

The observed patterns of rodent abundance and richness illustrate that counter-urbanization can engender and possibly exacerbate environmental justice concerns (Lewis et al. 2017) that may extend well beyond zoonotic disease risk. Our results suggest that lower income areas burdened with greater abandonment are likely more at risk of zoonotic disease transmission. Recent work has also demonstrated, however, that mental health wellness is lower in residents of areas with greater rodent abundance (Germaine and Latkin 2016, Lam et al. 2018), especially in areas that are under-resourced (Germaine and Latkin 2016). Evidence that vacancy is disproportionately concentrated in areas of lower income in cities across the US (Gulachenski et al. 2016) suggests that the conditions found in New Orleans likely occur across many other cities. Importantly, our findings point to the prospects that interventions (e.g., land management, debris removal, etc.) can be executed to address disparities. It has been demonstrated that interventions that reduce blight can serve to reduce real and perceived risk of crime (Branas et al. 201) and improve mental health outcomes (Lam et al 2018). We hypothesize that comparable interventions can similarly reduce concerns associated with rodents, including the risk of pathogen transmission to humans. Given the near global distribution of the three most commonly encountered rodent species (*R. rattus*, *R. norvegicus,* and *M. musculus*) in New Orleans, we expect that interventions are likely to be broadly applicable and of increasing importance with the global progression of counter-urbanization.

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Appendix

Table 1.1. Coefficients and significance of socio-environmental variables included in the best-supported (lowest AIC) model predicting New Orleans rodent community abundance. The magnitude of fixed effect variables indicate the influence of a given variable on overall rodent community abundance, while the magnitude of the random effect variables indicate the influence of a given variable on among-species variation. Statistical significance ($p<0.05$) indicated with *.

Variable	Fixed effects	Random effects
Intercept	-1.55*	0.44
Tree cover	1.04*	0.65*
Total population	-0.75*	NA
Debris	-0.22*	NA
Vacant lots x season	0.25*	NA
Vacant lots x debris	-0.35*	NA
Debris x season	0.38*	NA
Natural study area	-4.51*	NA
St. Bernard study area	-1.16*	NA
Uptown study area	-1.13	NA
Gentilly study area	0.00	NA
Vacant lots	0.25	NA
Season (winter)	0.02	4.10*
Median household income	-0.40	0.37*
Unmaintained buildings	-0.10	0.05*
Unmaintained vegetation cover	0.24	0.07*
Herbaceous species richness	-0.29	0.48*
Shrub species richness	0.13	NA

Table 1.2. Coefficients of random effects included in the best-supported (lowest AIC) model predicting rodent abundance. The top two coefficients (other than season) with the largest effect on the abundance of each rodent species are in bold. Coefficients calculated by adding both fixed and random effects together to account for the mean slope.

Species	Season (summer)	Season (winter)	Tree cover	Med. income	Herb richness	Unm. veg.	Vacant lots	Unm. bldgs.
M. musculus	1.99	2.67	0.28	**-0.36**	0.35	-0.04	**0.50**	-0.33
R. norvegicus	0.93	1.28	0.38	**-0.52**	0.04	**0.52**	0.07	0.10
R. rattus	-0.24	-0.38	**0.76**	**0.50**	-0.12	-0.10	0.00	-0.21
S. hispidus	-1.08	-1.34	**1.94**	-0.88	**-1.42**	0.41	0.36	0.08
O. palustrus	-1.32	-1.65	**1.83**	**-0.65**	-0.30	0.14	0.29	-0.15

Table 1.3. Top predictors of rodent richness across New Orleans study area. Variables included in the top-selected model predicting rodent species richness. Statistical significance ($p<0.05$) indicated with *.

Variable	Estimate	Std. error	P-value
Intercept	0.39	0.18	0.03*
Vacant lots	0.29	0.12	0.01*
Gentilly study area	0.04	0.31	0.91
Natural study area	-1.03	0.27	<0.001*
St. Bernard study area	-0.91	0.36	0.01*
Uptown study area	-0.56	0.29	0.05
Total population	-0.28	0.17	0.10

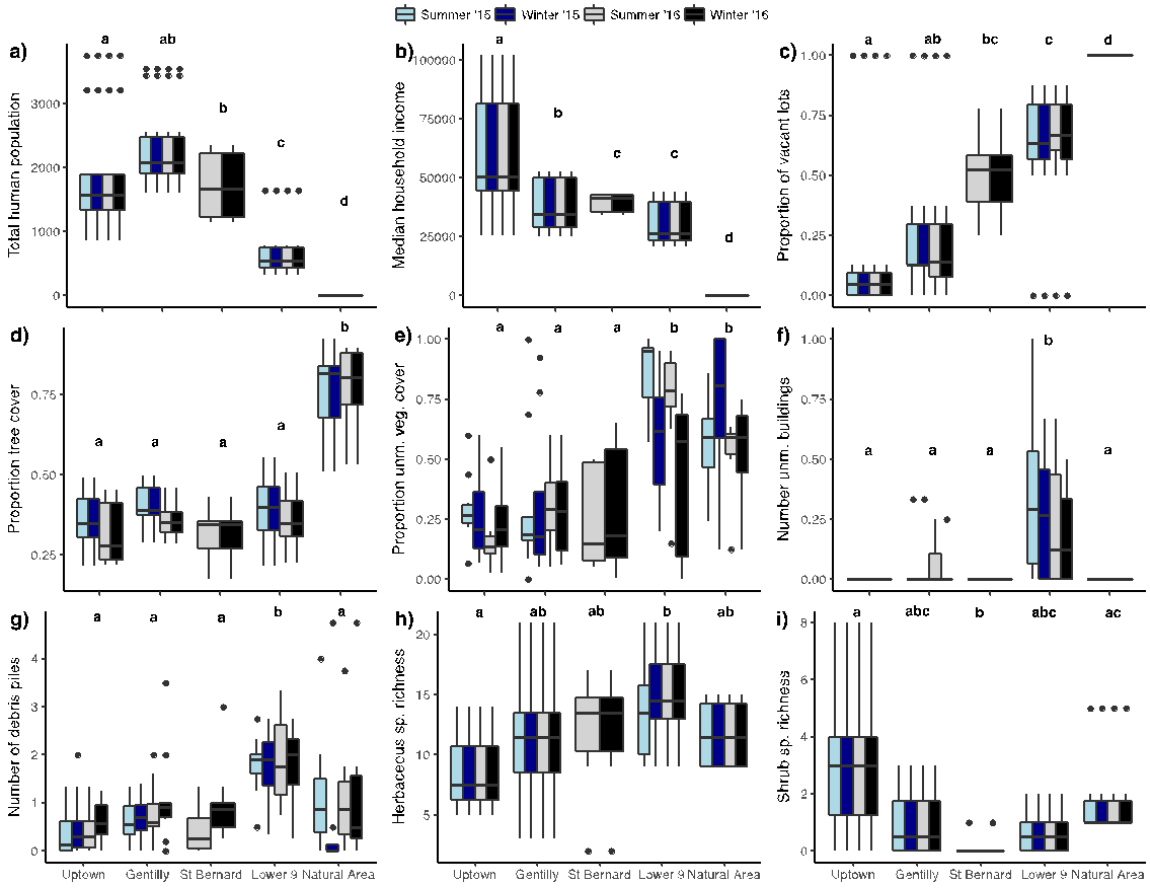

Figure 1.1. Temporal and spatial variation in socio-environmental characteristics across New Orleans. All variables differed significantly across the city. Letter indicate significant differences among the study areas. Some variables exhibited significant inter-annual variation; unmaintained vegetation (e) was significantly lower in 2016 relative to 2015; while the amount of debris (g) was higher in 2016 relative to 2015.

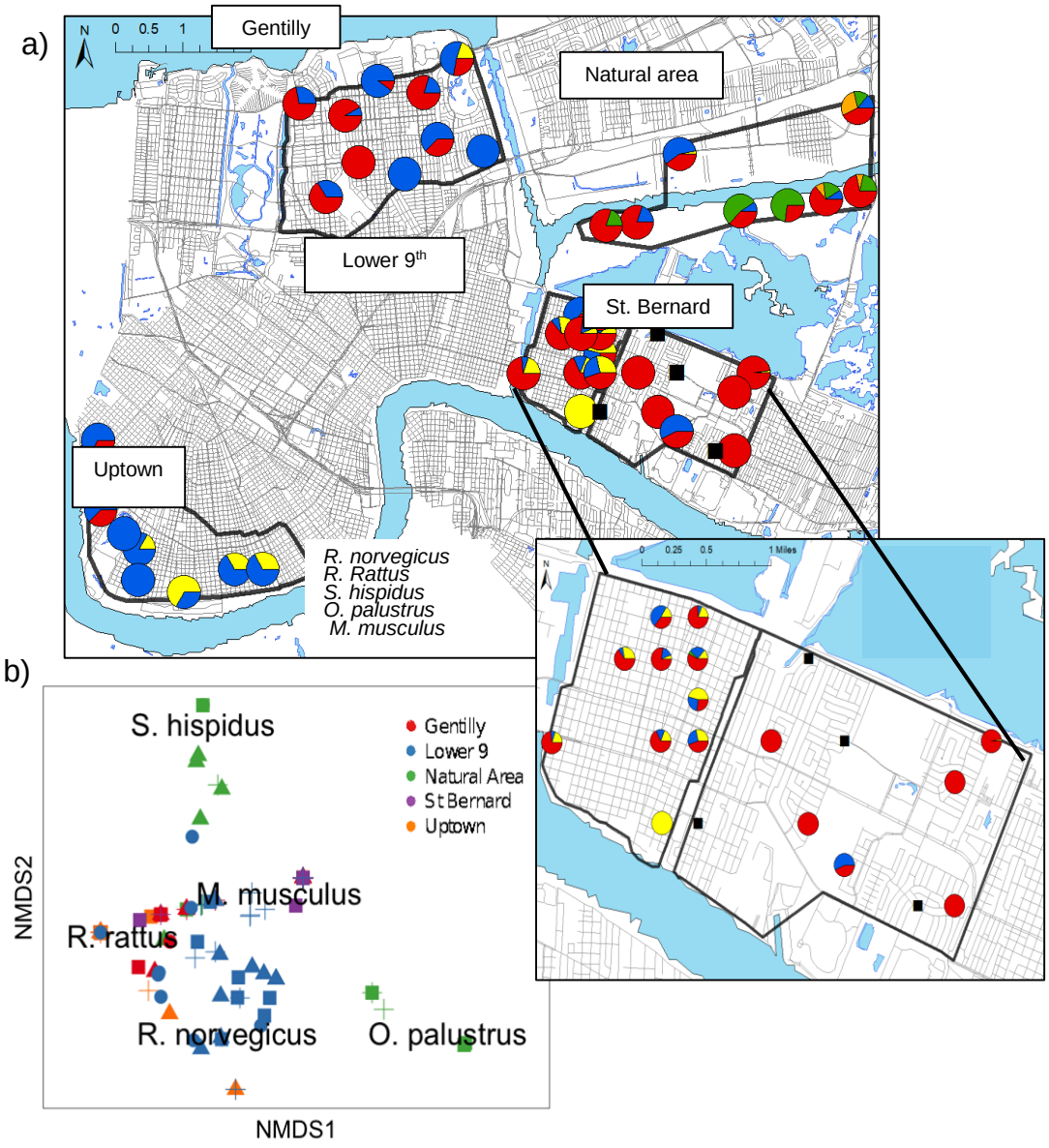

Figure 1.2. Distribution of rodent species across New Orleans. Aggregate estimate of occurrence across New Orleans from trapping conducted from summer 2015 to winter 2016-2017, relative abundance of each species from each trapping location represented via pie charts, with a blow-up of the Lower 9th Ward and St. Bernard Parish study areas (a). Bright green squares represent blocks where we conducted trapping efforts but captured no rodents during any season of trapping. Black outlines correspond to focal study area boundaries. Non-metric dimensional scaling plot of rodent assemblage structure, colored by study area, with symbols representing season of trapping (b).

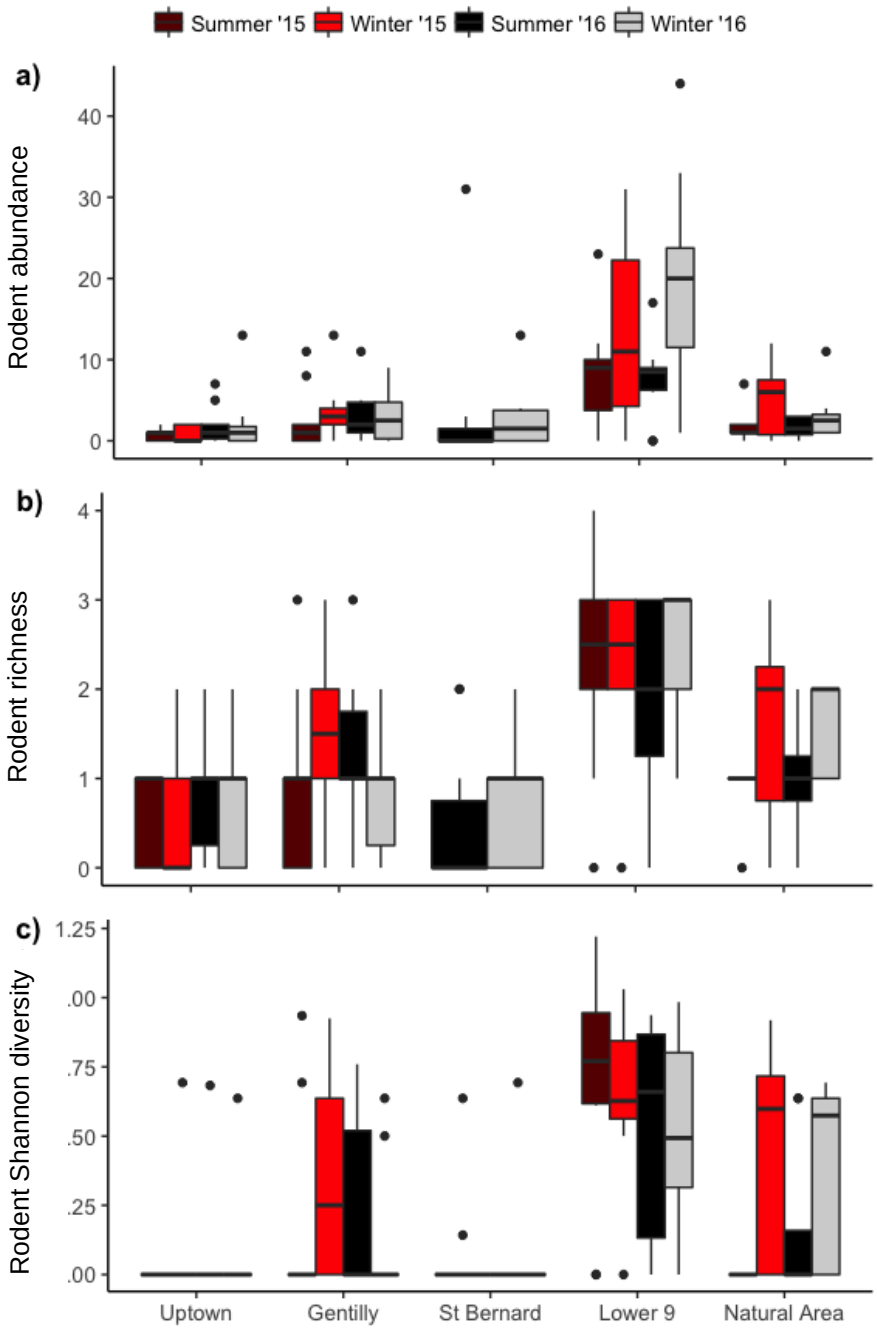

Figure 1.3. Rodent abundance and diversity through time across New Orleans. Total rodent abundance (a); differs significantly across focal study areas, with abundance significantly higher in the Lower 9th Ward relative to all other study areas. No statistically significant relationship was detected between total rodent abundance and season or year. Total rodent richness (b); also differs significantly across study neighborhood, but does not differ significantly across season or year. Shannon diversity (c); differs significantly only between the Lower 9th and Uptown study areas, and there is no significant intra- or inter- annual variation in diversity.

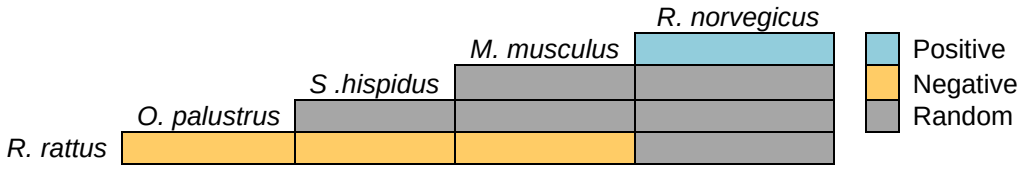

Figure 1.4. Species co-occurrence matrix. Colors represent statistically significant positive or negative association between two species. Matrix includes animals captured in all years.

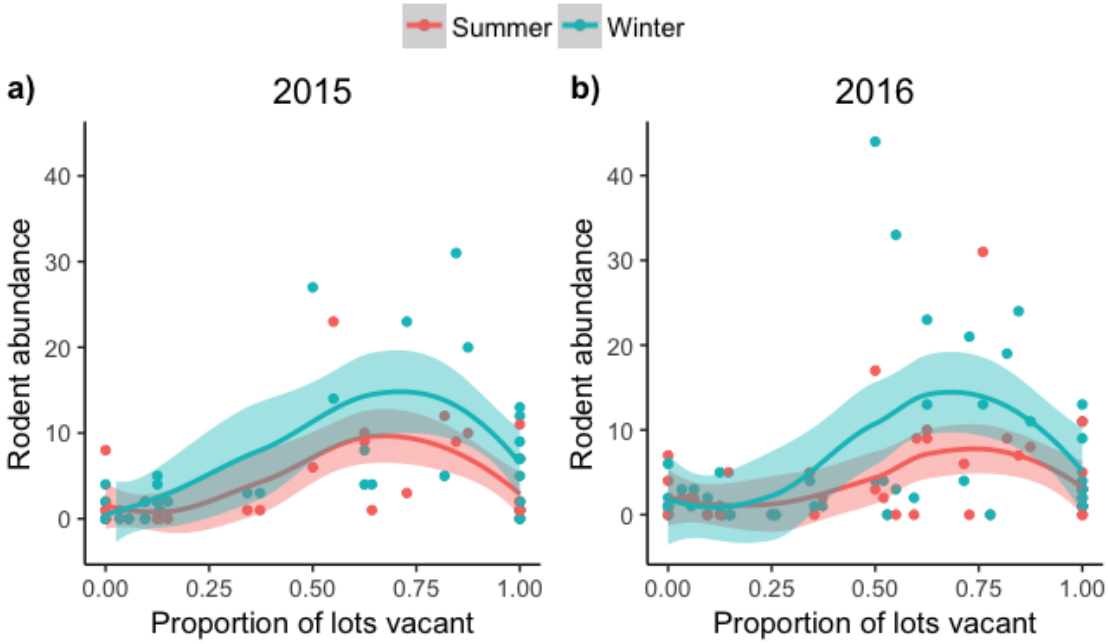

Figure 1.5. The total abundance of rodents relative to the proportion of vacant lots. There is a significant interaction with season, as the relationship is stronger in winter (blue) relative to summer (red) season (p-value=0.03, MLM analysis). Natural areas with no human presence are considered 100% vacant. Lines smoothed with Loess smoothing and 95% confidence intervals to aid visual interpretation.

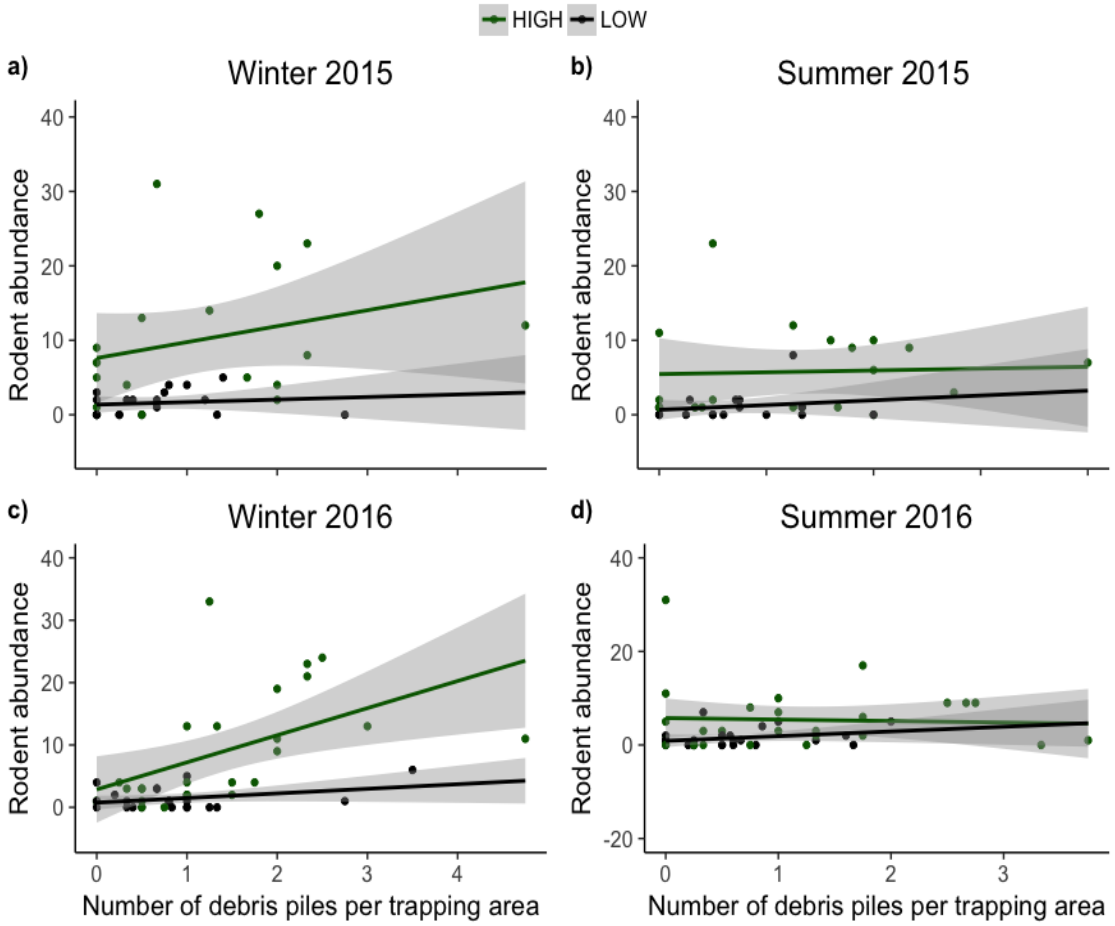

Figure 1.6. The total abundance of rodents is positively related to the amount of debris piles within a trapping area, though the intensity of the effect increases in areas with higher vacancy (green lines) and during winter. High vacancy defined as blocks with ≥50% of lots vacant.

Table S1.1. Supplemental table of study neighborhood socio-environmental profiles.
Mean ± standard deviation of environmental variables averaged across all seasons and all focal trapping blocks within each study neighborhood. Whole block data are variables collected for the entirety of the trapping block, and calculated from satellite imagery. All of the environmental variables hypothesized to relate to rodent abundance differed significantly across study neighborhoods. Superscript letters that are different among rows represent statistically significant differences among study areas $p<0.05$.

	Uptown	Gentilly	Lower 9	St. Bernard	Natural Area
Total Population[1,6]	1862.9[a] ± 872.13	2301.3[ac] ± 653.64	729.2[b] ± 480.53	1696.1[c] ± 492.96	0.00[d] ± 0.00
Median household income[1,6]	61378.7[a] ± 24795.81	38169.7[b] ± 10846.70	30221.7[bc] ± 9338.63	39395[c] ± 3818.58	0.00[d] ± 0.00
Vacant lots (%)[2,6]	14.04[a] ± 29.36	23.50[ab] ± 28.42	62.58[c] ± 24.26	50.93[bc] ± 17.73	1.00[d] ± 0.00
Grass cover (%)[2]	4.17[a] ± 10.25	11.23[a] ± 7.51	24.19[b] ± 9.46	29.02[b] ± 15.52	11.48[a] ± 5.71
Tree cover (%)[2,6]	33.77[a] ± 9.18	37.92[a] ± 6.18	37.64[a] ± 9.21	31.80[a] ± 7.06	79.42[b] ± 12.03
Urban surfaces (%)[2]	30.38[a] ± 10.26	31.73[a] ± 7.35	28.17[a] ± 9.80	37.45[a] ± 12.66	7.65[b] ± 10.10
Buildings (%)[2]	30.87[a] ± 10.14	18.96[b] ± 7.73	10.00[c] ± 5.13	1.60[d] ± 4.92	0.05[d] ± 0.09
Unmaintained vegetation (%)[3,6]	24.77[a] 3.57	30.81[a] 18.44	71.42[b] 20.48	27.89[a] 10.25	59.23[b] 16.89
Unmaintained buildings[4,6]	0.00[a] ± 0.00	0.02[a] ± 0.067	0.25[b] ± 0.27	0.00[a] ± 0.00	0.00[a] ± 0.00
Debris piles[4,6]	0.47[a] ± 0.49	0.78[a] ± 0.67	1.84[b] ± 0.69	0.70[a] ± 0.69	1.05[a] ± 1.40
Cement/asphalt[3]	26.75[a] ± 15.30	11.79[b] ± 9.25	8.89[bc] ± 8.24	2.63[c] ± 4.44	0.00[c] ± 0.00
Bare dirt[3]	24.35[a] 11.00	10.75[b] 8.99	6.18[b] 8.42	4.66[b] 6.4	25.76[a] 27.38
Land cover diversity[2]	0.66[a] ± 0.01	0.69[a] ± 0.02	0.68[a] ± 0.03	0.63[a] ± 0.05	0.36[b] ± 0.15
Tree richness	1.50[a] ± 1.04	0.70[a] ± 0.79	0.80[a] ± 0.76	0.30[a] ± 0.47	2.50[b] ± 1.29
Shrub richness	3.00[a] ± 2.35	1.00[abc] ± 1.12	0.70[abc] ± 0.79	0.40[b] ± 0.20	1.83[ac] ±1.49
Herbaceous richness	8.50[a] ± 2.98	11.80[ab] ± 5.08	14.48[b] ± 3.88	12.10[ab] ± 4.26	11.75[ab] ± 2.86

Table S1.1. Continued.

[1]Data from U.S. 2010 Census

[2]Data calculated from satellite imagery and represents values across entire focal trapping blocks.

[3]Data represent % cover of within-block trapping areas, estimated while conducting trapping efforts.

[4]Number of buildings/debris piles counted within each focal trapping area, divided by the total number of focal trapping areas on a block, estimated while conducting trapping efforts.

[5]Data collected within 0.5-acre circular vegetation plots randomly places within focal trapping blocks.

[6]Variables included in the top-selected MLM predicting rodent abundance.

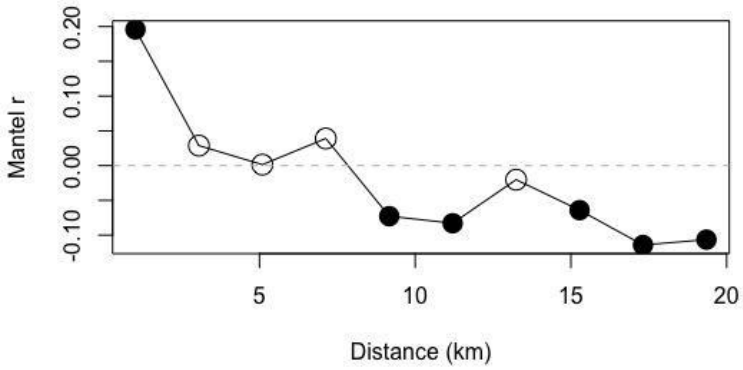

Figure S1.1. Mantel's r of assemblage structure at increasing spatial lag. Open circles represent non-significant Mantel's r values, while closed circles represent significant positive (above 0), or negative (below 0) spatial autocorrelation in rodent assemblage structure.

CHAPTER II
CARRIAGE LOADS AND DIVERSITY OF PATHOGENIC *LEPTOSPIRA* INCREASES WITH RODENT HOST ABUNDANCE AND CO-OCCURRENCE ACROSS POST-KATRINA NEW ORLEANS

A version of this chapter by Anna C. Peterson, Bruno M. Ghersi, Claudia Riegel, Elsio A. Wunder, James E. Childs, and Michael J. Blum is in revision for publication. M. Blum, J. Childs, E. Wunder, C. Riegel, and A. Peterson conceived of the study. B. Ghersi and A. Peterson collected rodent samples with support from C. Riegel. E. Wunder and J. Childs supported laboratory analyses. A. Peterson conducted all analyses and wrote the manuscript. All authors provided edits and comments to the manuscript.

Abstract

Efforts to conserve biodiversity are often motivated by concerns that biodiversity loss contributes to zoonotic disease emergence despite evidence that greater biodiversity can amplify, rather than dilute, disease risk. The relationship between biodiversity and zoonotic disease risk has largely been unexplored in cities, despite growing concerns that elevation of urban biodiversity could collaterally increase disease risk by promoting contact between humans and pathogen hosts. In this study, we tested the hypothesis that diversity tempers disease risk by characterizing pathogenic *Leptospira* infection relative to rodent host diversity and abundance across a gradient of counter-urbanization in New Orleans (LA, USA). We found that *Leptospira* infection loads were higher in urban areas with more rodent species in syntopy relative to areas harboring a single rodent species. Areas where species co-occurred also harbored a greater abundance of hosts, including the most competent hosts, indicating that shifts in overall and relative host abundance influence *Leptospira* infection. Evidence of shared infection among rodent hosts indicates that cross-species transmission of *Leptospira* likely also increases infection at sites with greater syntopy. Additionally, evidence that rodent co-occurrence and abundance and *Leptospira* infection load parallel abandonment suggests that 'greening' from counter-urbanization can elevate zoonotic disease risk within cities, particularly in underserved communities that are burdened with disproportionate concentrations of abandoned and vacant properties.

Introduction

It is often argued that greater biodiversity is beneficial to human well-being because it can help mitigate infectious disease risk, but some evidence suggests otherwise (Salkeld at el. 2013, Wood 2014). A number of studies indicate that biodiversity loss can increase the risk of pathogen and parasite transmission to humans (hereafter referred to 'disease risk') (LoGiudice et al. 2003, Keesing et al. 2006, Johnson et al. 2013, Johnson et al. 2015, Civitello et al. 2015). Diversity can dilute disease risk when, for example, a pathogen is capable of infecting multiple hosts with varying competence (i.e., the ability to maintain or transmit infection), and when increasing diversity predictably reduces the relative abundance of the most competent host(s) (Ostfeld and Keesing 2012, Johnson et al. 2013). It remains unclear, however, whether dilution is a widespread phenomenon, as it has also been shown that greater diversity can amplify disease risk (Randolph and Dobson 2012, Salkeld et al. 2013, Wood et al. 2014). Disease risk can be amplified if, for example, there are more competent hosts present in more diverse

assemblages (Dobson 2004). The likelihood of dilution or amplification can depend on scaling between host diversity and density (Mihaljevic et al. 2014, Faust et al. 2017).

A focus on particular landscapes and pathogens may have collaterally constrained understanding of relationships between diversity and disease risk. Thus far, most empirical studies have assessed conditions in suburban or exurban landscapes where humans come into contact with wildlife across peripheral interfaces. Furthermore, a majority of empirical studies have focused on vector-borne pathogens like West Nile Virus and species of *Borrelia* that cause Lyme disease (LoGuidice et al. 2003, Ezenwa et al. 2006, Swaddle and Calos 2008), or pathogens with complex multi-host life cycles such as amphibian trematodes (Johnson et al. 2013). Studies of conditions in urban landscapes could be particularly informative since human activity is a key risk factor in predictions of zoonotic disease outbreaks (Jones et al. 2008, Patz et al. 2004, Hassell et al. 2017). Furthermore, cities can feature highly tractable gradients of host and vector abundance and diversity (LaDeau et al. 2013, Peterson et al. in review), which can give rise to asymmetries in zoonotic disease risk (LaDeau et al. 2013). Thus, examining conditions in cities could afford fresh perspectives on relationships between biodiversity and disease risk (McKinney 2008, Bradley and Altizer 2006). Similarly, examining a wider variety of pathogens, including those transmitted directly through host contact and the environment, might offer greater understanding of the processes underlying disease risk.

Better understanding of relationships between biodiversity and disease might be gained by examining rodents and rodent-associated pathogens in cities. Urban and peri-urban rodent populations host a number of vector-borne pathogens (e.g., *Bartonella spp.*; Peterson et al. 2017), as well as pathogens that are directly or environmentally transmitted to humans (e.g., *Leptospira spp.*; Faria et al. 2008, *Hantavirus*, Childs et al. 1994, Cross et al. 2014). Rodent abundance and co-occurrence also often vary across urban landscapes (Peterson et al., in review; Cavia et al. 2009), and likewise, pathogen infection can be highly heterogeneous, even on small geographic scales (Himsworth et al. 2013a, 2013b, Firth et al. 2015, Peterson et al. 2017, Rael et al. 2018). Determining the conditions that contribute to rodent-associated disease can potentially yield globally relevant insights, as many rodents found in urban environments have near-cosmopolitan distributions (Lund 1994, Aplin et al. 2011, Puckett et al. 2016). Work on rodents is also globally relevant because close contact and associations with humans coupled with trends in urbanization, make it increasingly likely that rodents will drive future infectious disease risk worldwide (McFarlane et al. 2012).

Thus far, there has been little work done on urban rodent diversity and disease risk. Some studies of *Leptospira* infection, however, offer insight into whether the diversity or abundance (i.e., assemblage structure) of rodents might influence disease risk in cities (Derne et al. 2011, Theuerkauf et al 2013). Zoonotic pathogenic bacteria in the genus *Leptospira* are the causative agents of leptospirosis, which despite being the most common bacterial zoonosis worldwide, remains a 'neglected' disease (Costa et al. 2015a, Picardeau 2015). Serovars of *Leptospira* bacteria infect and colonize the kidneys of a diverse range of mammalian hosts including small and large rodents (i.e., mice and rats), which are primary reservoirs of pathogenic *Leptospira* in urban areas (e.g. Ko et al. 1999, Faria 2008, Costa et a. 2014). Infected rodents exhibit chronic infection, maintain high leptopiral loads in their kidneys, and shed high

loads of bacteria into the environment via urination (Costa et al. 2015b). Susceptible hosts, including humans, most frequently acquire infection through contact with contaminated water or soil (Ko et al. 1999, Guerra 2009). While infection in animal reservoirs is generally asymptotic, leptospirosis can result in a range of symptoms in humans, from mild febrile-illness to debilitating and sometimes life-threatening organ failure. Some work suggests that host diversity reduces leptospiral infection risk. Derne et al. (2011), for example, found a negative correlation between human infection and mammal richness across several Pacific islands. Theuerkauf et al. (2013), on the other hand, found that infection in several rodent hosts increased following the introduction of non-native black rats (*Rattus rattus*) to a Polynesian island, suggesting that a rise in rodent diversity amplified host infection. It is also possible, however, that the observed shifts were due to differences in host competence, differences in host abundance, or differences in *Leptospira* species circulating in co-occurring rodent species. Further study of *Leptospira* infection among co-occurring hosts might thus reveal whether and how disease risk is related to rodent diversity.

The City of New Orleans (Louisiana, USA) presents exceptional conditions for ascertaining and deconstructing relationships between the abundance and co-occurrence of rodent species and risk of *Leptospira* infection. Catastrophic flooding, discriminatory implementation of resettlement and recovery programs, and differences in post-disaster landscape management transformed the city following Hurricane Katrina in 2005 (Lewis et al. 2017). Notably, abandoned and vacant properties have accrued unevenly across the city, resulting in public health concerns that reinforce persistent legacies of sociocultural disparity. Foremost among these are gradients in rodent diversity and abundance that have manifested with abandonment (Rael et al. 2016, Lewis et al. 2017, Peterson et al. in review). While the diversity of rodents present in urban environments may be low relative to that found in natural ecosystems, New Orleans supports several syntopic rat species (Peterson et al. in review), unlike other cities that have been the focus of most work so far done on rodent-associated pathogens (e.g. Himsworth et al. 2013, Firth et al. 2014). Notably, the dilution of disease is also predicted to be strongest in low diversity systems (Johnson et al. 2015), which highlights the value of conducting work on relationships between host richness, abundance, and zoonotic pathogen risk in urban environments. Accordingly, we have tested the hypothesis that diversity tempers disease risk by examining how *Leptospira* infection varies with rodent assemblage structure across the city. We did so by assessing whether (1) *Leptospira* infection prevalence and loads (i.e., host competence) differ among rodent species; and whether (2) host abundance, infection prevalence and loads vary with species co-occurrence. To address the possibility that risk is driven by spill over among co-occurring host species, we also assessed whether (3) the same *Leptospira* species infect different host species, including different species found at the same location. Finally, we assessed whether (4) environmental, rodent assemblage (i.e., richness and abundance), or individual-level host features (e.g., host characteristics and co-infection with other parasites) best predict *Leptospira* infection in rodents across post-Katrina New Orleans.

Methods

Study area and study design

Hurricane Katrina struck the City of New Orleans in August 2005. Considered to be one of the most devastating hurricanes in United States history, the storm surge and consequent levee failures resulted in flooding of over 80% of the urban core of the city, which displaced nearly 90% of the resident population. Resettlement and population recovery since the storm has been heterogeneous across the city; some areas have fully recovered, whereas others remain far below pre-Katrina levels (Fussel et al. 2010, 2014, Lewis et al. 2017). Historically marginalized and low-income communities have become disproportionally burdened by land abandonment, which has largely been driven by prejudiced resettlement programs and differences in municipal land management policies since the storm (Fussel et al. 2010, Gulachenski et al. 2016, Lewis et al. 2017). Previous work also has shown that the overall abundance and richness of rodent species are higher in areas with greater levels of vacancy and other conditions associated with counter-urbanization (Peterson et al. *in review*). For example, *Rattus norvegicus* were more abundant in areas with lower income and more unmaintained vegetation (Peterson et al., *in review*). Similarly, *Mus musculus* were also more abundant in low-income areas with more vacant lots, and while *Rattus rattus* abundance was found to vary with tree cover, it also trended with increasing income (Peterson et al., in review).

In this study, we tested rodents captured across 96 trapping sites for infection with pathogenic *Leptospira*. The trapping sites were located within 10 study areas that align with eight neighborhoods in the urban footprint of New Orleans, a neighborhood in adjacent St. Bernard Parish, and a non-developed ("natural") area located adjacent to New Orleans (Figure 2.6). The 10 focal study areas were selected to capture a gradient of sociodemographic conditions, history of Katrina-related flooding, and property abandonment (Lewis et al. 2017, Peterson et al. in review) (Table 2.4). Within each neighborhood, we selected 8-10 blocks for rodent trapping by placing a 500-m point-line grid generated in ArcGIS over the city, and randomly selecting blocks falling at intersections of the gridlines, as described in Lewis et al. (2017) and Peterson et al. (in review). We similarly selected eight equally-sized trapping sites in the non-residential 'natural area'.

Rodent trapping

Small and large-bodied rodents (i.e., mice and rats, respectively) were captured in the study areas from May 2014 to February 2017. With the exception of the sites in the natural area and study blocks in the French Quarter and St. Bernard Parish, we used live Tomahawk traps to capture rats at each site across a succession of six alternating summer and winter trapping bouts (Summer 2014-Winter 2016/2017; 2.1). We trapped rats in the natural area and French Quarter across a succession of four trapping bouts (Summer 2015-Winter 2016/2017), and we trapped rats in St. Bernard Parish across two successive trapping bouts (Summer 2016-Winter 2016/2017; Table 2.1). We concurrently trapped mice using Sherman traps at 38 sites starting in the summer of 2015 (Table 2.1). Animals were captured and handled following Tulane IACUC approved protocols #0451 and #0460.

During each trapping bout, we set 30 live Tomahawk traps to capture rats in areas with observed or potential rodent activity (e.g. near visible runways, trash, compost, debris piles, etc.) within each trapping block. We set an additional 30 Sherman traps to capture small rodents starting in Summer 2015 at a subset of the study sites (Table 2.1). Outside of the natural area, all of the trapping sites were in residential neighborhoods, except for one site in the Uptown study area and one site in the Lakeview study area, which both were located in public parks. Additionally, one site in the Gentilly study area corresponded to a road median. Selections of trapping locations on residential blocks were dictated by property access. All trapping occurred on the selected study block, but when access was limited, we placed traps on properties that were directly adjacent to the focal trapping block. We set all Tomahawk traps for a minimum of three continuous nights and sustained trapping efforts at each site until the trap rate reached an asymptote (i.e., no individuals were captured). Sherman traps were set for four continuous trapping nights. We set and baited Sherman and Tomahawk traps each afternoon and checked and closed all traps the following morning.

Tissue sampling

On the morning of capture, rodents were transported to the City of New Orleans Mosquito, Termite, Rodent Control Board facilities, where necropsies were conducted following a standard protocol (Tulane IACUC approved protocols #0451 and #0460). We euthanized all rodents using isoflurane anesthesia followed by cardiac puncture. Blood collected from the cardiac puncture was immediately spun down to separate serum from coagulate. We then measured standard weight and length attributes (e.g., full body length (nose to tip of tail), tail length (base to tip of tail), foot and ear length). This allowed us to calculate a mass index by taking the residuals of a linear regression between weight and length measurements following Aryal et al. (2015). We also determined species, sex, and sexual maturity (determined based on visible scrotal testes in male and perforate vagina in females) as well as parity (i.e., the presence of placental scarring/active pregnancy) in females (Aplin et al. 2003). Each individual was given a wound score based on the presence of visible external wounds (0 = no visible wounds to 5 = extensive wounding) (Glass et al. 1988). Each individual was combed for ectoparasites, which were placed in ethanol for later identification. We then collected lung, liver, spleen, kidney, urine, and tail tissue samples from each rodent. We noted infection with parasites such as *Angiostrongylus* sp. (Rael et al. 2018), as well as infection with tapeworm parasites encysted in liver tissue. All tissues, serum and blood coagulates were archived in -80°C freezers. All carcasses were retained and frozen at -20°C.

Study site characterization

We collected on-the-ground estimates of percent cover and other key habitat features during each trapping bout at each location (Peterson et al. *in review*). We first demarcated the trapping site boundaries within a block, which typically aligned with property boundaries. We then visually estimated the proportion of coverage within each trap area corresponding to unmaintained vegetation (categorized as grass taller than 6 inches and bushes <6 inches from the ground), bare dirt (including unpaved areas underneath raised homes), and impervious

surfaces (concrete and asphalt). We also counted the total number of unmaintained buildings (identified as buildings that were missing major structural features such as the roof or windows), as well as the number of discrete trash and debris piles (e.g., food waste, compost, and miscellaneous trash such as tires and construction debris) falling within each designated trapping site. We used Google Earth to determine the proportion of vacant lots on a block by overlaying spatial layers of parcel boundaries for both Orleans and St. Bernard Parishes (htpps://www.gis.nola.gov: https://gis-stbernard.opendata.arcgis.com, respectively), on to Google Earth satellite imagery. This allowed us to count the total number of lots on each block and the number of vacant lots, defined as those that did not contain a home, shed or other man-made structure (e.g., swimming pool) within its boundaries (Peterson et al. *in review*). We considered all lots in trapping locations outside of residential areas as 100% vacant. The availability of true-color historical imagery from multiple time points enabled us to estimate annual variation in vacancy over the course of the study period. Additionally, we intersected the study site boundaries with US Census block group boundaries to derive estimates of sociodemographic attributes, including median household income, and total population size, according to the 2010 US Census (Gotham et al. 2014, Lewis et al. 2017, Peterson et al. *in review*).

Leptospira *screening and sequencing*

In a sterile biosafety hood, we removed exactly 0.2 g of tissue from the cortex of one kidney from each individual captured for DNA extraction and screening for pathogenic *Leptospira*. We extracted DNA from the kidney samples using Qiagen Blood and Tissue kits following the manufacturer protocol, with a final elution volume of 200 µL. We then screened the genomic DNA for pathogenic *Leptospira* using quantitative polymerase chain reaction (qPCR) of the lipL32 gene (Wunder et al. 2016, Stoddard et al. 2009) using an Applied Biosystems 7500 real-time quantitative PCR machine. We first ran all samples in duplicate and screened for presence/absence of pathogenic *Leptospira* DNA, using DNA extracted from an uninfected laboratory Norway rat as a negative control and 2uL of the standard as a positive control. Any sample for which at least one replicate was considered positive was then re-run with a quantitative standard starting at 1 x 10^7 genome equivalens (GEq) DNA copies provided by E. Wunder in a 10-fold serial dilution to determine the quantitative load of *Leptospira* DNA present in each sample. All qPCR reactions were completed using a total reaction volume of 25 µL (12.5 µL master mix, 1.25 µL each of forward and reverse primers, 0.5 µL probe, and 4.5 µL PCR grade H_20). We ran quantitative samples in duplicate, with the final load determined as the average across duplicates. Any plates that did not have a slope of -3.33 to -3.60, an intercept of 40 and an $R^2 > 0.97$ were invalidated and re-analyzed. Additionally, all extracted kidney DNA was run with a qPCR assay of the rodent housekeeping gene glyceraldehyde-3-phophate dehydrogenase (GAPDH) gene (Costa et al. 2015b, Wunder et al. 2016) to serve as an internal qPCR control. None of the samples failed to amplify using GAPDH primers.

To identify pathogenic *Leptospira* to species, we performed a PCR of the *glmU* gene (Thaipadungpanit et al. 2007) using genomic kidney DNA from all individuals that tested positive for *Leptospira* infection in the qPCR screen. We cleaned PCR products with ExoSAP-IT

(Affymetrix, Santa Clara, CA, USA) and completed a final sequencing amplification reaction consisting of 3.75 µL PCR grade H_20, 3.75 µL 5uM $MgCl_2$, 1.0 µL each of 10 mM forward and reverse *glmU* primers and 0.5 µL BigDye terminator (Applied Biosystems, Foster, CA, USA). We cleaned reactions using Sephadex columns prior to electrophoresis on an ABI 3730xl (Applied Biosystems). We aligned trimmed sequences with GenBank archived sequences of the *glmU* gene for all pathogenic *Leptospira*. We then constructed phylogenetic trees with all new and representative archived sequences using Bayesian Inference in MrBayes 3.2.6 (Ronquist et al., 2012). Trees were built using the GTR+G model, and a Markov chain Monte Carlo analysis with four chains running for $8x10^6$ generations. Trees were sampled every 1000 generations and the first 1000 trees were discarded as burn-in. Convergence was determined when final deviation of split frequencies fell below 0.02. We assigned sequences to *Leptospira* species based on relationships recovered in the final tree, with confirmation from BLAST comparisons (Madden 2002).

Statistical analyses of host competence

To assess if the prevalence of pathogenic *Leptospira* infection varied among the different rodent species, we used a generalized linear model with a binomial error distribution and the proportion of infected individuals as the dependent variable (Zuur et al. 2009). To assess if *Leptospira* infection load varied among species, we used a generalized linear model with the natural log transformed quantitative load estimates (in genome equivalents) as the dependent variable. For both models, we included species, season, and a season x species interaction term as independent variables. The models were based on data from all 96 trapping locations. We estimated the least-square means of interaction terms and conducted pairwise analyses with Tukey's p-value correction for multiple comparisons using the emmeans package in R (Lenth 2018, R development core team).

Statistical analyses of assemblage-level patterns of infection

We used generalized linear models and linear models, when appropriate, to determine the relationships between: (1) number of animals captured, (2) proportion of infected individuals, (3) average infection load, and (4) total infection load for each species and the total rodent species richness at a given trapping location in a given trapping season. We completed these analyses using only data collected from locations where we trapped for both small and large bodied rodents. We included the richness variable as a factor, and thus utilized post-hoc Tukey tests to compare abundance, prevalence and load at sites where one, two, three or four species were present. To account for heterogeneity in trapping effort, we included the total number of Sherman or Tomahawk trap nights (i.e., trapping effort) corrected to account for non-target and sprung but empty traps (Beauvais and Buskirk 1999), respectively, as an offset function in all models for each species (Kery 2010). Models that included trapping effort as an offset did not improve upon any of the models investigating abundance, prevalence or load estimates for *M. musculus* (e.g., increase in deviance and AICc values with addition of offset function), and thus we did not include an offset function in these models. However, including

trapping effort for the models comparing *R. rattus* and *R. norvegicus* abundance, prevalence and load reduced deviance and AICc values, and thus we included the offset in these models.

Statistical analyses of host specificity

The availability of locality information for all captured animals allowed us to compare the nature of infection among different species collected from the same trapping location. We used a binomial generalized linear model to quantify how the prevalence of different *Leptospira* spp. varied among rodent host species. The dependent variable was a measure of the proportion of individuals infected with a given species relative to the total of all individuals from which we were able to obtain *Leptospira* sequences, and the independent variables were a rodent species x *Leptospira spp.* interaction term. We used an analysis of variance (ANOVA) of log transformed *Leptospira* load to quantify how infection load with different *Leptospira* spp. varied among rodent hosts by using rodent species x *Leptospira* spp. interaction term as the independent variable. We included data from the full set of 96 trapping locations for these analyses. We conducted post-hoc analyses to compare the least-square means of categorical variables (host species and *Leptospir*a species) using the emmeans package in R, with a Tukey p-value adjustment for multiple comparisons.

Environmental, assemblage, and individual level predictors of infection

We used the data collected from the subset of trapping sites where we trapped for mice and rat species (Table 2.1) to determine the extent to which socio-environmental, rodent assemblage and individual host-level factors predicted the likelihood of pathogenic *Leptospira* infection and infection intensity. We did not include *O. palustrus* individuals in the analysis, as we collected only four individuals of this species across all study areas, and thus did not have sufficient sample sizes to assess predictors of *Leptospira* infection.

We first undertook an analysis of 'presence-absence' likelihood of infection using a generalized linear mixed model with a binomial error distribution. We then undertook an analysis of infection load in *Leptospira*-positive individuals utilizing a linear model on the log transformed load data. For both models, we included site as a random effect to account for repeated measures. Prior to analysis, we standardized all predictor variables to a mean of 0 and standard deviation, and checked all variables for collinearity of variance in the usdm package in R (Naimi et al. 2014) and included only variables which had a variance inflation factor of <3 (Zuur et al. 2010).

We created two global models including the following predictor variables: total rodent abundance (an index calculated as the number of all rodents captured per 100 corrected trap nights, following Beauvais and Buskirk (1999)), species richness, and a suite of socio-environmental variables that either have been found to predict rodent diversity and abundance in New Orleans (Peterson et al. in review) or that have been shown to relate to *Leptospira* infection in other areas (Reis et al. 2008, Hagan et al. 2016). These were: the proportion of vacant lots, the proportion of unmaintained vegetation, the number of trash and debris piles, median household income, the total human population, elevation, and trapping season. We also included the following individual-level variables that have been identified as important

predictors of pathogen infection in other rodent host systems (e.g. Himsworth et al. 2013a, Costa et al. 2015a, Minter et al. 2017, Peterson et al. 2017): species identity, infestation status with ectoparasites, infection status with internal parasites, degree of external wounding, mass index, sex, and sexual maturity.

To determine the top-selected model(s) predicting the likelihood of infection and infection load, we compared models that included all combinations of variables, including global models, single-variable models, and null models. Using the lme4 and glmmTMB packages in R (Bates et al. 2015, Brooks et al. 2017), we ranked each model according to AIC, and considered the model with the lowest AIC as the top-selected model (Burnham and Anderson 2002). We had a single top-selected model (all delta AICc > 2) predicting both likelihood of infection and infection load. To assess the appropriateness of the top-selected model predicting the likelihood of infection, we generated randomized quantile residuals by running 250 simulations of the best-fit model and comparing the observed to simulated values (Dunn and Smyth 1996). We then checked randomized quantile residuals for kurtosis using the one-sample Kolmogorov-Smirnov (KV) test, and for spatial autocorrelation with Moran's I, using the DHARMa and ape packages in R (Hartig 2019, Paradis et al. 2004). We also checked the residuals of the top-selected model predicting infection load for kurtosis using the KV test and for spatial autocorrelation with Moran's I. Both models showed no evidence of over/under-dispersion with the KS test (p-values>0.05). We also did not find evidence of spatial autocorrelation with either model using Moran's I (p>0.05 for both models). We repeated the analyses just with information from locations where we collected more than 30 rodent individuals, which is a large enough sample size to estimate a 5-10% prevalence rate with $\alpha = 0.95$, $\beta = 0.8$ (Sergeant 2016, Alan et al. 2018).

Results

Rodent trapping and Leptospira *infection*

Between 2014 and 2017, we captured a total of 1,472 individuals of five rodent species across 96 trapping areas in New Orleans, including: *R. rattus* (n=628), *M. musculus* (n=484), *R. norvegicus* (n=339), *Sigmodon hispidus* (n=21), and *O. palustrus (n=4)*. One or more individuals in all species but *O. palustrus* tested positive for *Leptospira* infection (Figure 2.1). Across all years, 15% of all captured individuals tested positive for *Leptospir*a infection. Infection varied widely among trapping sites and study areas (Figure 2.1, Table 2.2). When considering individual trap sites, *Leptospira* infection prevalence ranged from 1-100%. However, the number of animals captured in a given trap site also varied widely. Consequently, some estimates of high prevalence reflect small sample sizes. When considering the trapping sites (n = 13) from which we captured ≥30 individuals over the course of the study, prevalence estimates ranged from 6-52% (mean 32.0%).

Host competence

Of the individuals captured, 85 *R. rattus* (13.5%), 158 *M. musculus* (32.6%), 121 *R. norvegicus* (35.7%), and one *S. hispidus* (4.8%) tested positive for infection with pathogenic

Leptospira (Table 2.2). No *O. palustrus* tested positive for *Leptospira* infection. The prevalence of *Leptospira* infection varied among species, with both *R. norvegicus* (coef=1.30, p<0.001), and *M. musculus* (coef=1.07, p<0.001) having significantly higher prevalence of infection relative to *R. rattus* and *S. hispidus*. While infection varied significantly among species, *Leptospira* infection prevalence did not vary by season in aggregate (p>0.05), or for different species (p>0.05 for all interactions) (Figure 2.2).

Infection load differed among host species. When considering only infected individuals, *M. musculus* (mean load = 294,767 GEq) supported significantly higher loads relative to both *R. norvegicus* (mean load = 220,988 GEq; coef. = 1.88, p<0.001) and *R. rattus* (mean load = 22979 GEq, coef. = 4.62, p<0.001). Additionally, *R. norvegicus* had significantly higher loads relative to *R. rattus* (coef. = 2.74, p<0.001). The small number of infected *S. hispidus* (n = 1, load = 28 GEq) prevented comparisons of infection load to other species. Infection load did not vary by season in aggregate (p>0.05) or for different species (Figure 2.2, p>0.05 for all interactions).

Assemblage-level patterns of infection

We captured four species (*M. musculus, R. rattus, R. norvegicus,* and *S. hispidus*) at only one trapping site in one trapping bout, and thus could not draw statistical comparisons of abundance, prevalence or load estimates relative to locations with fewer species. However, we captured three species at 24 trapping sites across four trapping bouts, which afforded opportunities for comparison to sites with one or two species. We captured *R. rattus*, *M. musculus,* and *R. norvegicus* at all but one of the locations harboring three species. The abundance of each species was highest at sites where we captured all three species (*R. rattus*: p<0.01, coef. = 0.63, d.f. = 79; *M. musculus*: p<0.01, coef. = 1.3, d.f. = 83; *R. norvegicus*: p<0.01, coef. = 1.2, d.f. = 39) relative to sites where we captured only two species or where each species was detected in isolation of others (Figure 2.3). The abundance of the native *O. palustrus* and *S. hispidus* did not differ according to species richness (p>0.05, for both species). Infection prevalence did not differ with richness for any species except for *R. rattus*, which exhibited greater prevalence at sites where it co-occurred with two other species relative to sites where it occurred in isolation (p<0.01, coef. = 1.0, d.f. = 79). Both *R. rattus* and *M. musculus* also had higher average infection loads at sites where we captured three species (*R. rattus*: p<0.01, coef. = 3.1; *M. musculus*: p<0.01, coef. = 6.8) relative to sites with one other species and where each species was detected in isolation. In contrast, *R. norvegicus* infection loads did not differ according to species richness (p>0.05). Similarly, the total infection load was greatest in *R. rattus* and *M. musculus* at locations where three species were present (*R. rattus*: p<0.01, coef. = 3.9; *M. musculus*: p<0.01, coef. = 8.1) relative to trapping sites with one or two species present. The total infection load in *R. norvegicus* did not vary with richness (Figure 2.3).

Host specificity

We obtained high-quality sequences from 289 of the 365 (79%) positive individuals captured across the study area. Sequence data were acquired for three species: *R. rattus* (55 individuals), *M. musculus* (130 individuals), and *R. norvegicus* (104 individuals) (Figure 2.4, Figure 2.5).. The recovered sequences aligned with three species of *Leptospira*: *L.*

borgpetersenii (100% coverage, 98-100% similarity), recovered in 42% of infected animals, *L. interrogans* (95-99% coverage, 97-98% similarity), recovered in 35% of infected animals, and *L. kirschneri* (79-100% coverage, 99% similarity). recovered in 24% of infected animals. Infection with *L. borgpetersenii* was significantly more likely relative to infection by the other species (*L. interrogans*, log odds = 4.79, *L. kirschneri,* log odds=-1.34, $p < 0.001$) (Figure 2.4).

We found that rodent host species were consistently infected by different *Leptospira* species (Figure 2.4), though we also found evidence of spillover. The likelihood of *L. interrogans* infection was significantly higher in *R. norvegicus* relative to *R. rattus* (log odds: 2.84, $p < 0.001$), and *M. musculus* (log odds: 5.72, $p<0.01$). We detected *L. interrogans* in only two *M. musculus* individuals, both of which were captured at sites with *L. interrogans*-positive *R. norvegicus* (Figure 2.5). Similarly, the likelihood of infection with *L. borgpetersenii* was significantly higher in both *M. musculus* and *R. rattus* relative to *R. norvegicus* (coef. = 3.62, 3.17, $p<0.01$). All of the *L. borgpetersenii*-positive *R. norvegicus* (n = 5) were captured at sites with either *L. borgpetersenii*-positive *M. musculus* or *R. rattus* (Figure 2.5). Infection with *L. kirschneri* also was significantly lower in *R. norvegicus* (n = 13) than in *M. musculus* (coef. = 1.24, $p<0.01$) (Figure 2.4), and all *L. kirschneri*-positive *R. norvegicus* were captured at locations with *L. kirschneri*-positive *M. musculus,* and/or *R. rattus*. Notably, of the 55 *R. rattus* for which we were able to obtain *Leptospira* sequence data, all but two individuals were captured at locations with *Leptospira*-positive *M. musculus* or *R. norvegicus*. All species of *Leptospira* infecting *R. rattus* at these locations also were detected in *M. musculus* or *R. norvegicus* captured at the same location.

Infection intensity varied significantly within host species ($p<0.01$, df = 2) and by *Leptospir*a species ($p<0.01$, df = 2). Also, there was a significant interaction between host species identity and *Leptospira* species infection intensity ($p<0.01$, df = 4; Figure 2.4).

Socio-environmental, assemblage, and individual level predictors of infection

The top selected model resulting from analyses based on data from sites with >30 animals (Table S2.1) was very similar to the top selected model resulting from the analysis based on the full dataset. Thus, we are only presenting results based on the full dataset. The top-selected model predicting the likelihood of infection across all trapping locations included sociodemographic, environmental, rodent assemblage, and host attribute variables (Table 2.3). The top model (AICc = 956.5) was <2 ΔAICc than the next best model (AICc = 957.7), thus we performed model averaging and present the model-average coefficients. This second-best model included elevation along with the same predictor variables as the top-selected model. The proportion of the total variance explained by the random effect (trapping sites) for the top selected model was 0.04 (s.d.= 0.19). Of the fixed effect variables included in the top selected models, species identity had the largest effect on the likelihood of infection, with *M. musculus* more likely to be infected relative to *R. rattus* (coef. = -1.03). Additionally, animals were more likely to be infected if captured in areas with greater unmaintained vegetation (coef. = 0.46), in areas with more trash and debris piles, in areas of higher median household income (coef. = 0.41), if they were sexually mature (coef. = 0.42), and when they exhibited higher external wound scores (coef. = 0.28). Lastly, infestation with any type of ectoparasite (i.e., fleas, mites,

lice) was included in the top-selected model, with individuals supporting ectoparasite infestation slightly more likely to also be infected with *Leptospira* (coef. = 0.04)

The top selected model predicting infection load resulting from analyses based on data from sites with >30 animals (Table S2.2) was very similar to the top selected model resulting from the analysis based on the full dataset, with the exception that elevation was not included in the top selected model utilizing data from sites with >30 animals captured. The top-selected model utilizing the full dataset (AICc = 1358.2) predicting infection load was >2 ΔAICc than the next best model (AICc = 1360.8) and included one environmental variable alongside rodent assemblage and host attribute variables (Table 2.4) as fixed effects. The proportion of variance explained by the random effect for the full dataset (trapping site) was 0.25 (s.d.= 0.50). Of the fixed effects variables included in the model, species identity had the largest effect on infection load, with *M. musculus* individuals supporting significantly greater loads relative to both *R. norvegius* (-2.39) and *R. rattus* (-5.19). Individuals captured at sites with lower trap success (coef. = -0.94), lower elevation (coef. = -0.45),greater species richness (coef. = 0.46), and those with ectoparasite infestation (coef. = 0.19) had higher infection loads.

Discussion

We found that pathogenic *Leptospira* infection risk increases in areas with greater rodent host abundance and co-occurrence in the City of New Orleans. Consistent with other work (Barragan et al. 2017, Moseley et al. 2018), we found that infection prevalence and average infection loads (i.e., host competence) varied by species. We also found, however, that in two of the three most abundant host species, both the prevalence of *Leptospira* infection and average infection load were significantly higher at trapping sites harboring other host species (Figure 2.3), indicating that co-occurrence amplifies zoonotic disease risk. This finding runs contrary to predicted relationships between *Leptospira* infection and host diversity (Derne et al. 2011), affirming the value of examining relationships between biodiversity and disease risk in cities (Derne et al. 2011). It is worthwhile to note, however, that departures between our findings and those of prior studies (e.g., Derne et al. 2011) might be a reflection of differences in the spatial scale at which conclusions are drawn (Johnson et al. 2015). For example, the processes that likely drive variation in rodent assemblages in New Orleans, such as rodent control efforts or increased resource availability in vacant areas, operate at a smaller spatial scale than those that structure mammalian diversity within and among islands (Derne et al. 2011). Further studies, such as multi-city comparisons, could clarify how local and regional phenomena structure disease risk across urban landscapes.

Identifying the host species that contribute more to the maintenance or spread of a pathogen is central to understanding and managing disease risk (Kilpatrick et al. 2006, Paull et al. 2012, Lloyd-Smith 2005). Our findings indicate that *M. musculus* and *R. norvegicus* are the most highly competent *Leptospira* hosts across the study area. We found that *M. musculus* and *R. norvegicus* exhibited greater infection prevalence relative to other species, and *M. musculus* consistently supported significantly higher infection loads relative to other hosts (Figure 2.2). Interspecific differences in infection may reflect the forms of *Leptospira* present in New Orleans, as it has been previously shown that *R. rattus* support greater infection prevalence

relative to *R. norvegicus* for some *Leptospira* serovars (Hathaway and Blackmore 1981). While competency can be a plastic trait (Gervasi et al. 2015), infection prevalence among hosts collected from widely different systems exhibit similar trends to those observed in New Orleans (Vanasco et al. 2003, Moseley et al. 2018). For example, *M. musculus* exhibited the highest prevalence of *Leptospira* infection, followed closely by *R. norvegicus*, relative to several other native and non-native species in Madagascan small mammal communities (Moseley et al. 2018). Similar to our findings (Figure 2.2), *R. rattus* individuals also exhibited lower overall *Leptospira* infection prevalence relative to *R. norvegicus* and *M. musculus* from Madagascar (Moseley et al. 2018). The observed parallels between New Orleans and elsewhere might reflect differences in habitat use among species, as terrestrial species (i.e., *R. norvegicus* and *M. musculus*) may be more likely to come into contact with contaminated soil or water that are arboreal species like *R. rattus*.

Dilution of disease risk has been observed when the most competent hosts decrease in abundance in areas with increasing species richness (LoGiudice et al. 2003, Johnson et al. 2013). The conditions governing the distribution and prevalence of *Borrelia* (which causes Lyme disease) arguably constitute the best example of this, where the abundance of the most competent hosts, white-footed mice, declines in areas supporting greater host richness. Paired with concurrent increases in less competent hosts, overall risk is diluted in areas supporting greater biodiversity (LoGuidice et al. 2003). We found the opposite trend in New Orleans, where the abundance of highly competent hosts like *M. musculus* is greater at sites harboring more species (Figure 2.3). This pattern could, in part, be explained by differences in resource availability or pest-control activities across the city. The three most common species encountered in New Orleans- *R. rattus*, *R. norvegicus,* and *M. musculus*- are frequently targeted by municipal and privately-funded control efforts (*C. Riegel, personal comm.*), which can influence the structure of urban rodent assemblages (Ruscoe et al. 2011). However, in a prior study (Peterson et al. *in review*) we found that sites supporting more rodents also have higher levels of vacancy and associated features like trash and debris piles. Vacant areas are not often targeted by pest control entities and also can offer greater habitat and resource availability for commensal rodents (Gulachenski et al. 2016, Rael et al. 2016, Eskew and Olival 2018, Peterson et al. *in review*). This parallels evidence that insect vectors can reflect elevated resources in the form of tire trash in low low-income urban landscapes (LaDeau et al. 2013).

Positive relationships between abundance and richness can amplify disease risk because there are more hosts present in more diverse assemblages, particularly when infection is density-dependent and when a pathogen is capable of infecting multiple hosts (Dobson 2004, Mihaljevic et al. 2013). Consistent with this expectation, density-dependent transmission is thought to play the primary role in maintaining *Leptospira* infection in urban rodents (Minter et al. 2018). We also found higher infection prevalence and loads in *M. musculus* and *R. rattus* in areas harboring other host species (Figure 2.3). The observed pattern could be a result of shared infection among co-occurring species. While we found that the prevalence of *Leptospira* species differed among host species (Figure 2.4), infections were not exclusive. This alone is not definitive evidence of spill-over, but we also found that patterns of infection corresponded to host co-occurrence. For example, the only *M. musculus* individuals found with *L. interrogans*

were captured at sites with *L. interrogans*-positive *R. norvegicus* (Figure 2.5). The majority of infected *R. rattus* also were captured in areas where at least one other host species was detected carrying the same *Leptospira* species. While the other infected host species were not always captured in the same trapping bout, *Leptospira* bacteria can persist in water and soil for weeks to months (Andre-Fontaine et al. 2015, Casanovas-Massana et al. 2018), so infection may still be shared among hosts, even if the presence of different hosts varies over time at a given location. Lastly, the likelihood of shared infection is probably greater than what is suggested by our findings. It has been noted, for instance, that Sanger sequencing tends to under-estimate the diversity of *Leptospira* co-infection present within an individual (Moseley et al. 2018). Evidence of shared infection from studies that have utilized methods more capable of detecting co-infection suggests that co-infection can be common (Moseley et al. 2018). If so, then shared infection among host species might be a common mechanism that elicits positive associations between *Leptospira* infection and host diversity.

Identifying individual-level drivers of variation in infection can advance understanding of pathogen transmission and spread (Llyod-Smith et al. 2005) and help identify conditions that lead to infection 'hotspots' (Paull et al. 2012). We found that several individual-level features are important predictors of *Leptospira* infection across rodents captured in New Orleans (Table 2.3, 2.4). Consistent with studies of rats captured in urban Vancouver and Brazilian slums (Himsworth et al. 2013a, Costa et al. 2014), our results indicate that *Leptospira* infection is more likely in individuals with more external wounding (Tables 2.2 & 2.3). Notably, we also found that infestation with ectoparasites was a strong predictor of both the likelihood of *Leptospira* infection and infection load. Considering that *Leptospira* is not transmitted by an ectoparasite vector, this relationship may be a reflection of wounding (i.e., of injuries inflicted by ectoparasites, which may increase the likelihood of *Leptospira* infection) or it may be a result of host immune response (Ezenwa et al. 2010, Ezenwa and Jolles 2011, Nunn et al. 2014). For example, work with laboratory rodents has shown that infection with helminth parasites can induce immunological shifts that facilitate secondary infection with microparasites, so long as the two pathogens do not share resources (Graham 2008). Similar mechanisms may be responsible for the positive association observed between ectoparasite infestation and *Leptospira* infection. This warrants further attention, as interactions among pathogens infecting an individual can scale up to influence pathogen dynamics within a population (Ezenwa et al. 2010, Ezenwa and Jolles 2011, Nunn et al. 2014).

Our findings also indicate that environmental heterogeneity can contribute to differences in *Leptospira* infection across urban landscapes. In New Orleans, rodents collected from sites at lower elevations were more likely to be infected by *Leptospira* and have higher infection loads (Tables 2.2, 2.3). A similar pattern has been observed in the City of Salvador (Bahia, Brazil), where *Leptospira* antibodies have been more frequently detected in humans from households inhabiting low-lying areas (Reis et al. 2008, Hagan et al. 2016). The pattern observed in Salvador is likely due to increased accumulation of water in low-elevation areas, resulting in greater human contact with soil/water interfaces where transmission is thought to occur (Hagan et al. 2016). Contact with soil/water interfaces may also be an important determinant of *Leptospira* transmission to humans in Louisiana, where increased incidence of

leptospirosis has been observed following flooding events (Frawley et al. 2017). In New Orleans, lower-lying areas are at greater risk of flooding from inclement weather and tropical storms (Colton et al. 2008). Thus *Leptospira* bacteria may accumulate in lower elevation areas that collect water, which could increase the likelihood of infection in rodents and humans. This supposition is consistent with public health records showing that there is seasonal variation in diagnoses of leptospirosis in Louisiana, with more instances occurring in warmer, wetter months (Louisiana Leptospirosis Annual Report), as has been reported in many other regions across the globe (e.g. Sanchez-Montes et al. 2015, Benacer et al. 2016, de Wit et al. 2017). The absence of seasonal variation in *Leptospira* infection in our study is not surprising however since, once infected, rodent hosts tend to remain infected over the course of their lifetime (Athanazio et al. 2008). This suggests that observed variation in human infection is likely due to seasonal changes in human activity that relates to contact with soil/water interfaces.

This work underscores how storm damage, discriminatory resettlement policies and municipal differences in landscape management have reinforced socio-environmental disparities since Hurricane Katrina (Lewis et al. 2017). We found that *Leptospira* infection prevalence and infection loads were higher in storm-damaged, predominantly lower-income areas of the city that are home to historically underserved communities. The affected neighborhoods have been disproportionately burdened by abandonment (Gulachenski et al. 2016, Lewis et al. 2017). Though the greening of unmanaged vacant lots has been touted by some as a potential social good, as it can increase urban biodiversity and services like temperature mitigation (e.g., Kattwinkel et al. 2011, Gardiner et al. 2013, Pearsall 2017, Riley et al. 2018), our results offer further support for the counter-argument that 'green blight' can imperil the well-being of affected communities (Troy et al. 2012, Katz et al. 2014, Gulachenski et al. 2016, Rael et al. 2016, Branas et al. 2018, Eskew and Olival 2018). Concerns about human well-being are receiving greater attention by those charged with safe-guarding public health in cities (e.g., Garvin et al. 2013, Bogar and Beyer 2015, Troy et al. 2016, Branas et al. 2018), prompting landscape-scale interventions and initiatives (e.g., Branas et al. 2018) that demonstrate how remediation of 'green blight' can have transformative outcomes. The predictors identified in our study indicate that disease risk can be mitigated by logistically simple approaches, such as regular mowing of overgrown vegetation and clearing debris. Combining land management with targeted pest control campaigns (i.e., focusing on competent hosts like *M. musculus*) may be an especially efficient approach to reducing zoonotic disease risk. It would thus be prudent to investigate how interventions can be mounted to best achieve parity across post-Katrina New Orleans and other cities experiencing counter-urbanization.

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Appendix

Table 2.1. Trapping and socio-environmental conditions in the study areas. Human population values are per block in a given study area.

Neighborhood	# Sites	# Summer/ Winter visits	Trap type(s)	Human population	Median income	Vacancy (%)
Bywater	10	3/3	T	656.1 (±276.4)	41495 (± 10606)	5.3 (±7.7)
French Quarter	8	2/2	T	527.4 (±191.2)	51351 (± 20841)	29.5 (±41.4)
Gentilly	10	3/3	S+T	553.9 (±312.4)	50877 (± 44981)	22.5 (±30.2)
Lakeshore	10	3/3	T	946.6 (±362.0)	112427 (± 28282)	1.3 (±2.2)
Lakeview	10	3/3	T	488.5 (±258.3)	68942 (± 24119)	21.4 (±28.4)
Lower 9th	10	3/3	S+T	117.9 (± 127.5)	27823 (± 10312)	63.3 (±25.2)
Upper 9th	10	3/3	T	353.2 (±261.2)	21382 (± 5234)	33.7 (±20.7)
Uptown	10	3/3	S+T	699.1 (±203.6)	69065 (±59159)	12.7 (±30.7)
Natural Area	8	2/2	S+T	79.0 (146.3)	5363 (± 9930)	100.0 (±0.0)
St. Bernard	10	1/1	S+T	676.7 (±437.9)	42045 (±13911)	49.6 (±18.0)

Table 2.2. Number of animals tested and number of animals infected of each species across all trapping seasons.

Season	*M. musculus (# test, # +)*	*R. norvegicus (# test, # +)*	*R. rattus (# test, # +)*	*S. hispidus (# test, # +)*	*O. palustrus (# test, # +)*
Summer '14	NA[a]	74, 21	104, 11	0,0	0,0
Winter '14	NA[a]	37, 12	75, 6	2, 0	0,0
Summer '15	48, 16	71, 34	150, 27	4, 0	0,0
Winter '15	124, 31	68, 24	101, 15	12, 1	0,0
Summer '16	96, 35	46, 19	125, 13	3, 0	4,0
Winter '16	216, 75	43, 11	73, 13	0, 0	0,0

[a]Did not target capture of *M. musculus* individuals during the year 2014 at any location

Table 2.3. Model-averaged socio-environmental, rodent assemblage, and rodent host attribute variables included in the top-selected model predicting the likelihood of *Leptospira* infection.

Variable	Coefficient	SE	P-value
Intercept	-0.69	0.17	<0.001
S. hispidus	-1.80	1.08	0.09
R. rattus	-1.03	0.25	<0.001
R. norvegicus	-0.34	0.30	0.25
Unmaintained veg.	0.46	0.10	<0.001
Sexual maturity[1]	0.42	0.10	<0.001
Household income	0.41	0.11	<0.001
Wound score	0.28	0.09	<0.005
Debris piles	0.25	0.10	<0.01
Total trap rate	0.17	0.09	0.06
Male	0.08	0.17	0.62
Ectoparasites collected[2]	0.04	0.10	0.68
Elevation	-0.04	0.09	0.66

[1]Sexually mature individuals = 1, juvenile individuals = 0
[2]Ectoparasites detected = 1, not detected = 0

Table 2.4. Socio-environmental, rodent assemblage, and rodent host attribute variables included in the top-selected model predicting *Leptospira* infection load.

Variable	Coefficient	S.E.	P-value
Intercept	**10.5**	**0.66**	**<0.01**
R. norvegicus	**-2.36**	**0.80**	**<0.01**
R. rattus	**-5.15**	**0.69**	**<0.01**
Trap rate	**-0.89**	**0.27**	**<0.01**
Elevation	**-0.44**	**0.23**	**0.05**
Rodent species richness	0.45	0.25	0.07
Male	-0.26	0.44	0.54
Sexual maturity[1]	0.20	0.66	0.38
Wound score	-0.27	0.26	0.30
Ectoparasites collected[2]	0.19	0.56	0.50

[1]Sexually mature individuals = 1, juvenile individuals = 0

[2]Ectoparasites detected = 1, not detected = 0

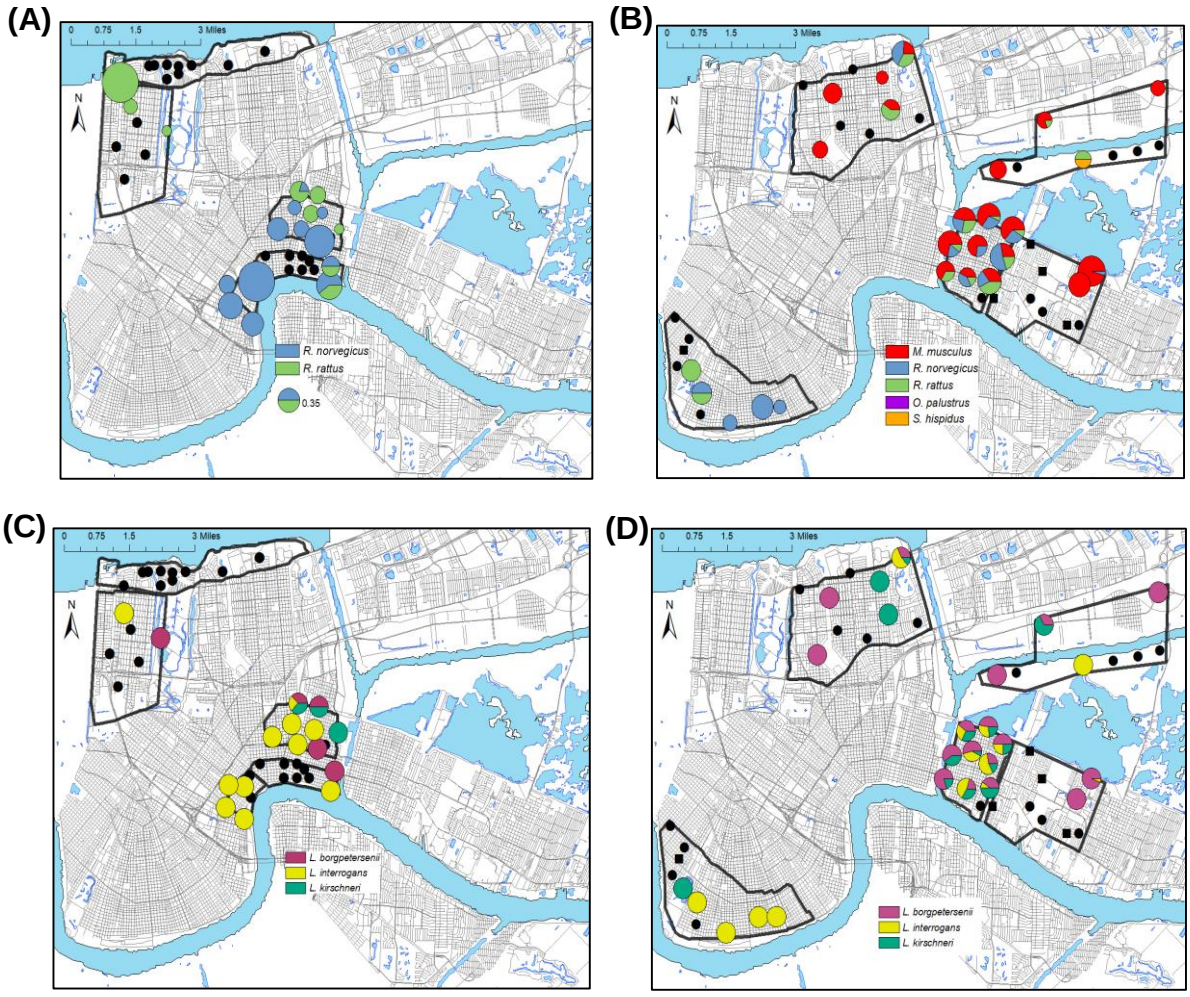

Figure 2.1. (A-B) Leptospira infection in rodent hosts across the study areas; (C-D) presence of Leptospira species across the study areas. (A) The proportion of infected rats, and (B) the proportion of infected rats and mice, corresponds to the size of the circle. (C) Distribution and proportion of *Leptospira* species in rats in the study areas where we only trapped rats, and (D) rats and mice in areas where we trapped both large and small-bodied rodents.

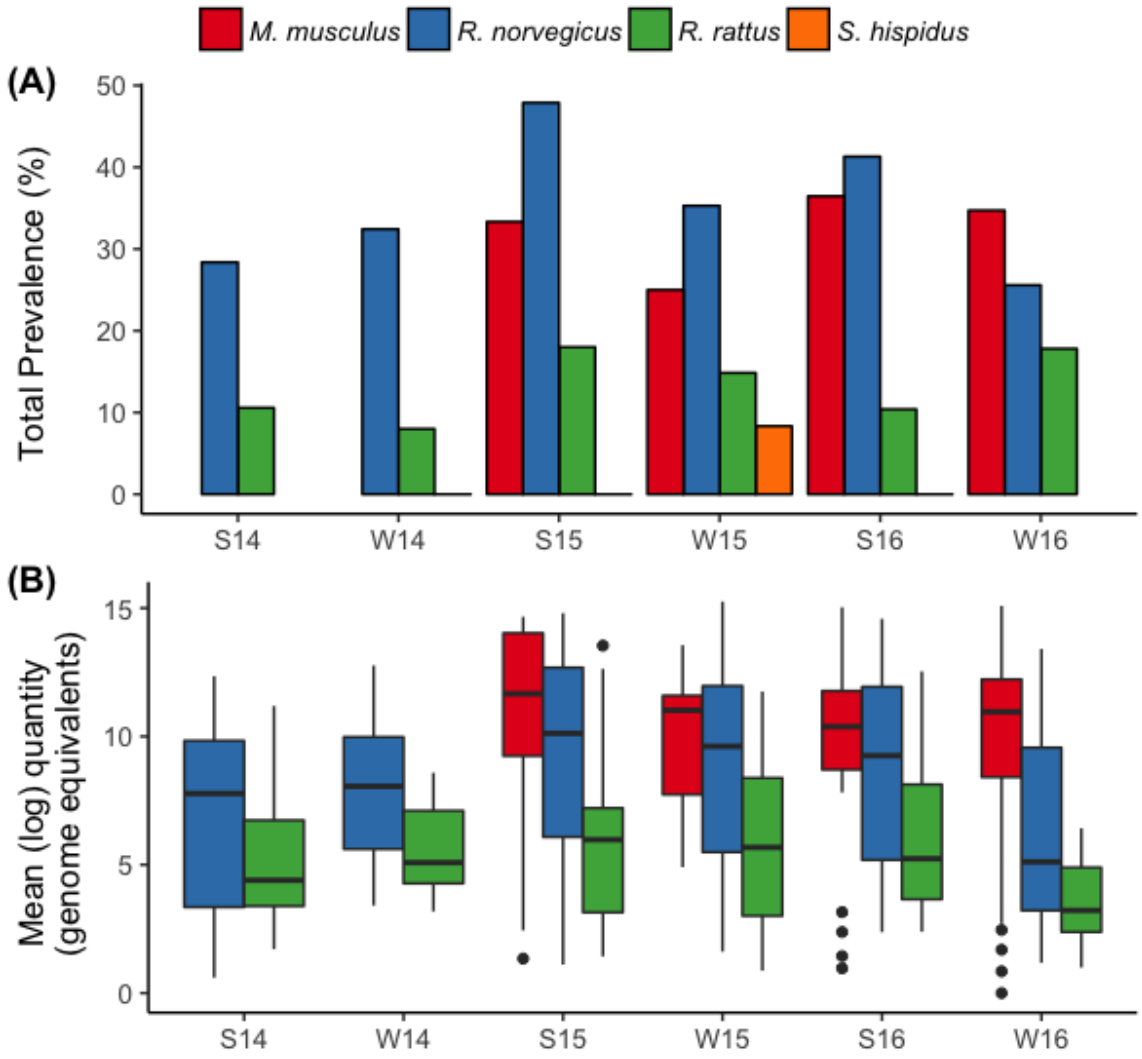

Figure 2.2. (A) Seasonal comparisons of Leptospira infection prevalence in summer (S) and winter trapping seasons (W), and (B) infection load in rodent host species, from the years 2014 (14), 2015 (15), and 2016 (16). Infection prevalence was significantly lower in *R. rattus* individuals relative to *R. norvegicus* and *M. musculus* ($p<0.01$) in all seasons and years, and infection load was significantly higher in *M. musculus* relative to all other species, while *R. norvegicus* carried higher loads relative to *R. rattus* individuals ($p<0.05$). There were no significant seasonal differences for all species in aggregate, or for different species. Sample sizes above bars represent the number of animals tested for pathogenic *Leptospira* infection in each season.

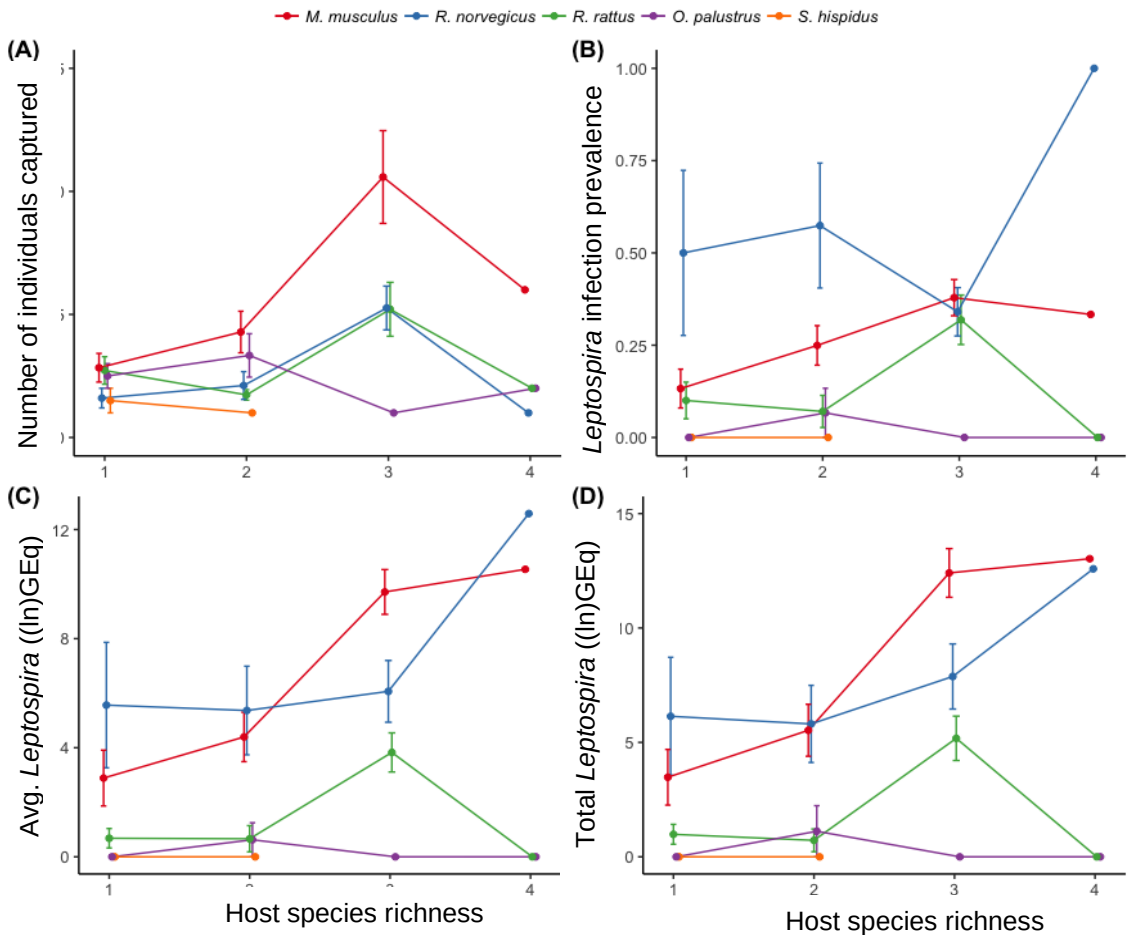

Figure 2.3. (A) Number of individuals captured for each species; (B) proportion of infected individuals of each species; (C) the average of Leptospira load per individual of each species; and (D) the sum of all of the Leptospira contributed by all individuals of a given species, with increasing species richness. Error bars represent standard error. Four species were only captured on one trapping block in one trapping bout, which prevented calculation of standard error.

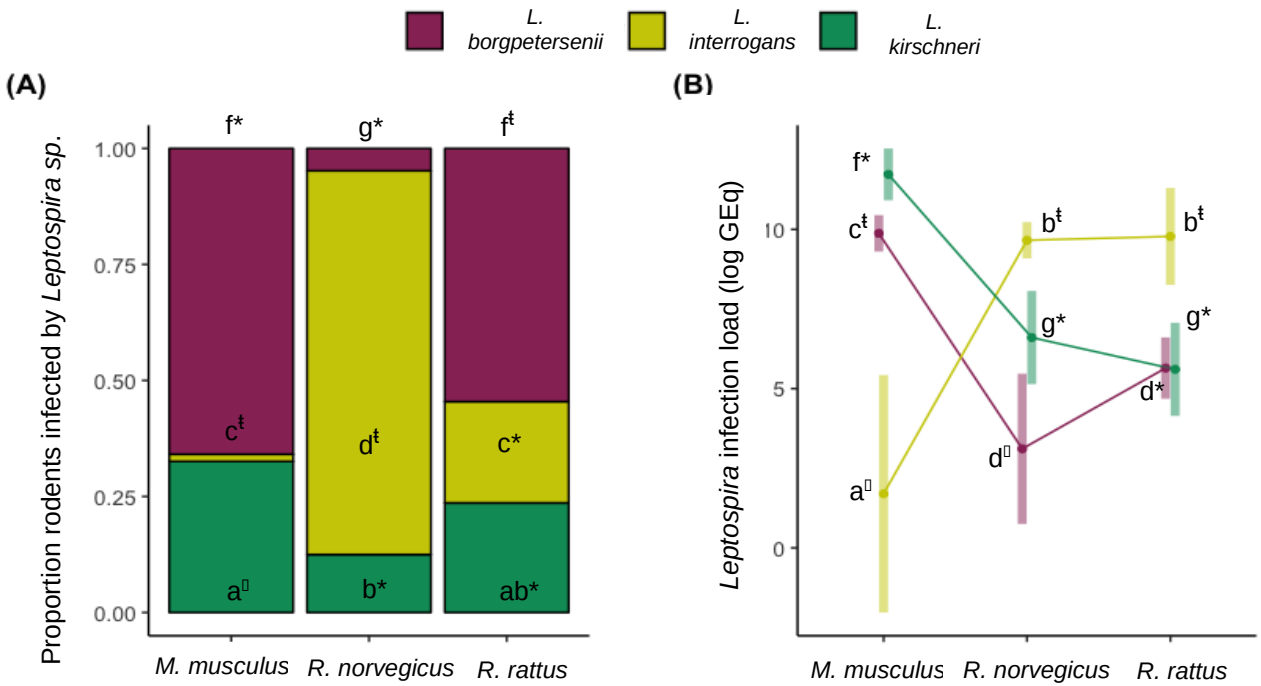

Figure 2.4. (A) Proportion of Leptospira species in infected rodents by host species; (B) average load of different Leptospira species in infected rodents by host species. Lettering denotes statistically significant differences in infection (proportion and load) with the same *Leptospira* species in different host species. Symbols denote statistically significant within-host differences in infection with different *Leptospira* species.

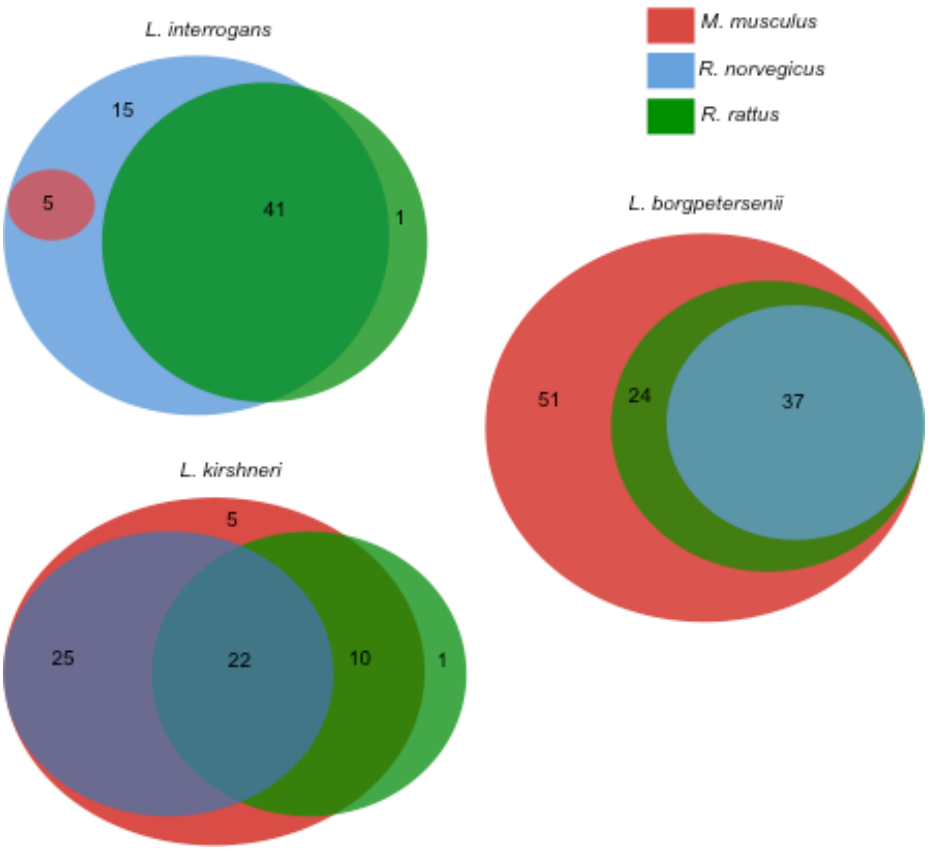

Figure 2.5.Venn diagram of Leptospira overlap among co-occurring host species. Numbers indicate the sum of all *M. musculus* (red), *R. norvegicus* (blue), and *R. rattus* (green) infected with each *Leptospira* species from locations where each species was detected alone (non-overlapping area within circles) or in syntopy with other rodent species (overlapping areas within circles).

Table S2.1. Supplemental table of socio-environmental, rodent assemblage, rodent host attribute variables included in the top-selected model predicting the likelihood of *Leptospira* infection from locations where we captured >30 individuals.

Variable	Coefficient	S.E.	P-value
Intercept	-1.01	0.23	<0.01
R. norvegicus	-0.38	0.32	0.23
R. rattus	-0.95	0.27	<0.01
S. hispidus	-14.49	624.19	0.98
Sex (M)	-0.01	0.18	0.94
Sexually mature[1]	0.99	0.24	<0.01
Wound score	0.30	0.10	<0.01
Unmaintained vegetation	0.29	0.10	<0.01
Median household income	0.25	0.10	0.01
Ectoparasites collected[2]	-0.19	0.23	0.42

[1]Sexually mature individuals = 1, juvenile individuals = 0
[2]Ectoparasites detected = 1, not detected = 0

Table S2.2. Supplemental table of socio-environmental, rodent assemblage, and rodent host attribute variables included in the top-selected model predicting *Leptospira* infection load from locations where we captured >30 individuals.

Variable	Coefficient	S.E.	P-value
Intercept	10.06	0.67	<0.01
R. norvegicus	-2.78	0.81	<0.01
R. rattus	-4.91	0.71	<0.01
Trap rate	-0.95	0.23	<0.01
Ectoparasites collected1	0.59	0.59	0.32
Sexually mature2	0.45	0.68	0.50
Sex (male)	-0.17	0.46	0.71
Wound score	-0.24	0.27	0.38

[1] Ectoparasites detected = 1, not detected = 0
[2] Sexually mature individuals = 1, juvenile individuals = 0

CHAPTER III
RODENT-BORNE *BARTONELLA* INFECTION VARIES ACCORDING TO HOST SPECIES WITHIN AND AMONG CITIES

A version of this chapter has been published by Anna Peterson, Bruno Ghersi, Fernando Alda, Cadhla Firth, Matthew Frye, Ying Bai, Lynn Osikowicz, Claudia Riegel, Ian Lipkin, Michael Kosoy and Michael Blum:

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This article was reformatted from the published version as part of this dissertation. M. Blum, C. Riegel, B. Ghersi, A. Peterson, and M. Kosoy conceived of the study. B. Ghersi and A. Peterson collected New Orleans samples. C. Firth, M. Frye, and I. Lipkin contributed New York samples. Y. Bai, L. Osikowicz, M. Kosoy, and A. Peterson contributed to laboratory culturing and sequencing. F. Alda completed the phylogenetic analyses. A. Peterson conducted all other analyses and wrote the manuscript. All authors provided edits and comments to the manuscript.

Abstract

It is becoming increasingly likely that rodents will drive future disease epidemics with the continued expansion of cities worldwide. Though transmission risk is a growing concern, relatively little is known about pathogens carried by urban rats. Here, we assess whether the diversity and prevalence of *Bartonella* bacteria differ according to the (co)occurrence of rat hosts across New Orleans, LA (NO), where both Norway (*Rattus norvegicus*) and roof rats (*Rattus rattus*) are found, relative to New York City (NYC) which only harbors Norway rats. We detected human pathogenic *Bartonella* species in both NYC and New Orleans rodents. We found that Norway rats in New Orleans harbored a more diverse assemblage of *Bartonella* than Norway rats in NYC and that Norway rats harbored a more diverse and distinct assemblage of *Bartonella* compared to roof rats in New Orleans. Additionally, Norway rats were more likely to be infected with *Bartonella* than roof rats in New Orleans. Flea infestation appears to be an important predictor of *Bartonella* infection in Norway rats across both cities. These findings illustrate that pathogen infections can be heterogeneous in urban rodents and indicate that further study of host species interactions could clarify variation in spillover risk across cities.

Introduction

Zoonotic pathogens are an emerging threat to human health and well-being (Jones et al. 2008), especially in areas where humans and wildlife frequently come in contact (Despommier et al. 2007; Jones et al. 2008; Lloyd-Smith et al. 2009). Rodent-borne pathogen transmission is of particular concern in cities, where rodents can be widely distributed and hyper-abundant (Bradley and Altizer 2007; Rael et al. 2016). Commensal rodents like Norway rats (*Rattus norvegicus*) and roof rats (*Rattus rattus*) can carry bacterial and viral assemblages, including pathogens of concern (Ellis et al. 1999, Himsworth et al. 2013a, b; Firth et al. 2014). With

rodents likely to drive future epidemics as cities continue to expand worldwide (Bordes et al. 2013; Han et al. 2015), determining the diversity and prevalence of rodent-borne pathogens in cities represents a vital step toward understanding how disease risk will progress with global demographic trends.

Many bacteria within the genus *Bartonella* are rodent- borne pathogens of concern (Anderson and Neuman 1997). *Bartonella* are gram-negative bacteria that can infect erythrocytes and endothelial cells in mammals (Anderson and Neuman 1997). At present, over 40 *Bartonella* species have been described, with most having been detected in bats and rodents (Jiyipong et al. 2012). Though *Bartonella* infections are thought to be relatively benign in rodents, several rodent-borne *Bartonella* species cause disease in humans, including febrile illness and endocarditis (Buffet et al. 2013). Humans can indirectly acquire pathogenic *Bartonella* from blood-feeding arthropods such as fleas (Bai et al. 2009; Billeter et al. 2011; Morick et al. 2011; Gutierrez et al. 2015), or through biting or scratching by an infected mammalian host (Tsai et al. 2010; Billeter et al. 2011; Harms and Dehio 2012, Kosoy et al. 2012).

Despite potential public health risks, little work has been done to assess the diversity and prevalence of *Bartonella* in urban rodents. So far, studies have primarily surveyed Norway rats at small geographical scales, such as in a neighborhood within a city (Easterbrook et al. 2007, Gundi et al. 2012; Himsworth et al. 2013a, 2015). Yet infection in rodents appears to be heterogeneous, suggesting that ecological factors like host population size and movement might determine the diversity and prevalence of *Bartonella* in cities (Firth et al. 2014; Himsworth et al. 2015). Thus, it is possible that patterns of *Bartonella* infection may vary across and among cities, especially cities that harbor different rodent assemblages (Kosoy et al. 2015).

In this study, we examined the incidence of *Bartonella* in rats from two cities: New Orleans, Louisiana (NO) and New York City, New York (NYC). Several species of rats, including Norway rats and roof rats, occur in NO (Rael et al. 2016), whereas only Norway rats occur in NYC (Childs et al. 1998). Prior surveys of rats in NO have detected *Bartonella* (Ellis et al. 1999) among a suite of other zoonotic pathogens (Campbell and Little 1988; Cross et al. 2014). A recent survey in NYC also found that *Bartonella* was the most prevalent bacterial agent infecting Norway rats (Firth et al. 2014). We characterized the diversity and distribution of *Bartonella* in NO and NYC to assess whether the prevalence of *Bartonella* differs according to the (co)occurrence of host species within and among cities (Keesing et al. 2006, 2010). This enabled us to identify factors that might influence spillover risk (i.e., transmission from wildlife hosts to humans) and thus provide practical guidance for improving pathogen surveillance programs.

Methods

Sample collection

In NO, we collected a total of 342 rats from May 2014 to March 2015 (Table 3.1) following Tulane University IACUC- approved protocol #0451. A subset of 272 rats was collected during a quantitative population survey across 78 residential city blocks in eight neighborhoods

(Figure 3.1) (Gulachenski et al. 2016; Rael et al. 2016). Each block was visited twice, once during May–August 2014, and a second time during November 2014–February 2015. During each trapping period, we set 30 Tomahawk traps (Tomahawk Live Trap Company, Tomahawk, WI) in areas with potential or evident rodent activity for a minimum of three consecutive nights. Trapping efforts were sustained at each site until no additional rodents were captured. We trapped the remaining 70 rats opportunistically as part of control efforts conducted by the City of New Orleans Mosquito, Termite, Rodent Control Board (NOMTCB) between May 2014 and March 2015. Rats were collected using the same methods reported above, but the number of trapping days varied by location.

We necropsied all NO rats at NOMTRB's facility following a standard protocol. We euthanized NO rats using isoflurane anesthesia followed by cardiac puncture. Blood samples were spun down to separate serum from coagulates. We took standard weight and length measurements and determined the species, sex, sexual maturity, and parity in females. We combed each individual for ectoparasites, which we later identified using standard keys (Furman and Catts 1970). We also collected lung, liver, spleen, kidney, urine, and tail tissue samples, which we archived in - 80°C freezers.

In NYC, we collected 133 Norway rats (R. *norvegicus*) from five locations in midtown and lower Manhattan be- tween September 2012 and June 2013 (Figure 3.1; Table 3.1) (Firth et al. 2014). Trapping sites included high-density housing complexes, a mixed-use indoor public space and an urban park (Firth et al. 2014; Frye et al. 2015). We trapped all rodents using Tomahawk traps that were baited and left open for 7–10 days to allow for acclimation by the rodents, followed by up to 10 nights of trapping. All captured individuals were euthanized with an over-anesthetization of isoflurane according to Columbia University IACUC-approved protocol #AC-AAAE6805. Following euthanasia, we fumigated carcasses with ethyl-acetate combed carcasses over dry ice for ectoparasite collection. Ectoparasites were identified as outlined in Frye et al. (2015). Data on rat weight and sex were obtained, and following a standardized necropsy protocol (described in Firth et al. 2014), serum, liver, spleen, kidney, and heart tissue samples were collected and stored at - 80°C.

Bartonella *screening*

Both NO and NYC samples were screened for *Bartonella* following PCR-based protocols. With the exception of one Norway rat (R. *norvegicus*) and one roof rat (*R. rattus*), we extracted and screened DNA from all NO samples (Table 3.2). We homogenized 10–20 mg of spleen tissue from each NO individual in a bead vial with 100 lL of brain– heart infusion medium (BHI), which was then lysed overnight at 55°C. All DNA extractions were subsequently completed using a QIAxtractor (Qiagen, Valencia, CA) following manufacturer instructions. We used the resulting DNAs in a multiplex qPCR of the tmRNA region to screen for *Bartonella* as well as other pathogens (Bai et al. 2013). We also screened for *Bartonella* through conventional PCR of the ITS (325, 1100) region (Table 3.2) (Diniz et al. 2007). For all individuals that tested positive for *Bartonella* through either method, we confirmed infection by sequencing both strands of the citrate synthase gene *gltA* region using forward and reverse primers BhCS781.p and BhCS1137.n (Norman et al. 1995). Only animals from which we were able to sequence the *gltA*

region were considered positive for *Bartonella* infection (Table 3.2). Similarly, for all NYC rodents, DNA extracted from fecal, liver, serum, and spleen tissue was screened separately for *Bartonella* using a PCR assay targeting the *gltA* region (Table 3.2), which was then sequenced to confirm infection (Firth et al. 2014).

We also screened for *Bartonella* by culturing from blood sampled from all NO rodents (Table 3.2) and by culturing from heart tissue of NYC rodents that tested positive according to *gltA* PCR screening from any tissue (Table 3.2). For NO rodents, we plated 10 uL of blood, while for NYC rodents we homogenized heart tissue in 400 uL of BHI medium and plated 100 uL of the homogenate. All cultures were plated on BHI agar supplemented with 10% rabbit blood and incubated at 5% CO_2 and 35°C for 4 weeks. We checked all plates once weekly to screen for the presence of colonies exhibiting a morphology consistent with *Bartonella* (e.g., round, opaque, white-to-cream in color) as well as the presence of other bacterial colonies. For all instances of putative *Bartonella* growth, a single colony was collected from each plate and placed in glycerol and heated for 10 min at 95°C for lysis and DNA extraction. The lysate was used for PCR and sequencing of a partial region of the *gltA* gene using forward and reverse primers BhCS781.p and BhCS1137.n (Norman et al. 1995). If a plate showed evidence of morphologically dissimilar *Bartonella* colonies, we extracted and sequenced a separate isolate from each *Bartonella* morphotype. NO samples (n = 28) that exhibited overgrowth of putative non-*Bartonella* bacterial contamination were excluded from all analyses.

Phylogenetic analyses

We edited and trimmed all sequences of the *gltA* gene to a 327-bp fragment overlapping the most extensive archive of reference sequence data available in GenBank. In addition to retrieving all available *Bartonella gltA* reference sequences for comparison to NO and NYC isolates, we also retrieved *Rickettsia gltA* sequences to serve as outgroups. We constructed phylogenetic hypotheses using Bayesian Inference in MrBayes 3.2.6 (Ronquist et al. 2012). Using the GTR + G model, we ran two simultaneous Markov chain Monte Carlo analyses with four chains for 4×10^6 generations. Trees were sampled every 1000 generations and the first 1000 trees (25%) were discarded as burn-in. Convergence was established when the final deviation of split frequencies fell below 0.005. All analyses were performed in the CIPRES Science Gateway 3.1 Portal (Miller et al. 2010). Sequences of all variants encountered in NO and NYC were deposited in GenBank (accession numbers MG027916–MG027998).

Species were identified according to percent sequence similarity and coverage of *gltA* amplicons in comparison with archived sequences using the Basic Local Alignment Search Tool for nucleotides (BLAST), and through phylogenetic analysis of sequence variation.

Ecological analyses

We developed generalized linear models (GLMs) with a binomial error distribution to determine the relationship between *Bartonella* infection and attributes of individual rodents. For NO, we ran a single GLM model to determine if species was a significant predictor of

Bartonella detection (i.e., 0 vs. 1) and then ran two separate GLM analyses to determine whether age class, sex, or flea infestation status (i.e., 0 vs. 1) were significantly related to *Bartonella* infection in Norway rats and roof rats (Table 3.2), respectively. Similarly, we ran a GLM analysis to determine whether age class, sex, or flea infestation status was significantly related to *Bartonella* infection in Norway rats from NYC. All individuals for both NYC and NO were placed into an age class (juvenile, subadult, adult) based on body weight (Table 3.1) (Mcguire et al. 2006; King et al. 2011).

We used several approaches to assess the (co)occurrence and distribution of *Bartonella* variants. We first constructed median-joining networks of variants in Network (Fluxus Technology Ltd., http://www.fluxus-en gineering.com) according to screening method, species, and city. Following Firth et al. (2014), we also explored patterns of coinfection within individuals using the Fortran software PAIRS v 1.1, which implements a Bayesian approach to detect non-random associations between pairs of taxa. This was done only for Norway rats, as no roof rats exhibited coinfection with more than one *Bartonella* species. We considered coinfection in individuals from both NO and NYC together, as well as from each city individually. Additionally, we compared *Bartonella* diversity between cities, accounting for variation in the scale and intensity of sampling efforts. Using the package rareNMtests in R (Cayuela et al. 2015; R Core Team 2013), we employed biogeographic and ecological null model comparisons of sample-based rarefaction curves of *Bartonella* variant diversity in NO and NYC. The test of the ecological null model states that models were drawn from a single assemblage, and thus differences in characteristics reflect only sampling effects. The biogeographic null model states that species composition differs between the two assemblages being compared, but share similar species richness and species abundance distributions greater than would be expected from a random sampling from a single assemblage (Cayuela et al. 2015).

Results

Bartonella *infection prevalence*

Collections of rats and fleas differed between cities. We collected three species of rat in NO: hispid cotton rats (*Sigmadon hispidus*; n = 2), Norway rats (R. *norvegicus*, n = 163), and roof rats (*R. rattus*, n = 177). Only Norway rats (n = 133) were collected in NYC (Table 3.1). We detected *Xenopsylla cheopis* and *Ctenocephalides felis* fleas on NO rats, but only *X. cheopis* was detected on NYC rats (Frye et al. 2015) (Table 3.1).

We confirmed *Bartonella* infection in 13.5% of rats from NO and 23% of rats from NYC. *Bartonella* infection was confirmed in 40 Norway rats from NO and in 31 Norway rats from NYC (Table 3.2), though it was only confirmed in 5 roof rats from NO. We did not detect *Bartonella* in either of the NO cotton rats; thus, no further consideration was given to cotton rats in this study. For the NO rats, direct PCR of the ITS region for NO rats identified a greater number of individuals as putatively positive than the number confirmed to be infected through sequencing of the *gltA* region (Table 3.2). We also confirmed infection in a greater number of individuals

through a combination of culture and sequencing than through direct PCR and sequencing in NO (Table 3.2). Direct PCR and sequencing yielded a slightly higher number of confirmed infections in rats from NYC (Table 3.2).

The prevalence of *Bartonella* infection was heterogeneous in both cities. In NO, within-site prevalence ranged from 0 to 97% of individuals infected, with 85% of all *Bartonella* positive individuals captured within a single city block. All *Bartonella* positive Norway rats were captured at two locations, where no roof rats were present. We captured *Bartonella* infected roof rats from five locations. Both roof rats and Norway rats were captured at four of these locations, though none of the Norway rats were *Bartonella* positive at the locations. We detected *Bartonella* positive Norway rats at all five trapping locations in NYC, though within-site prevalence ranged from 10 to 85%.

Species, flea infestation, and age class were significant predictors of *Bartonella* infection. Species identity was a predictor of *Bartonella* infection in NO rats ($P < 0.01$, coef. = - 2.05, d.f. = 329). When considering Norway and roof rats separately, flea infestation was a significant predictor of infection in Norway rats from both NO and NYC, whereas it was not a predictor of infection in roof rats from NO (Table 3.3). *Bartonella* infection corresponded to age class in Norway rats from NYC, with juvenile individuals less likely to harbor *Bartonella* relative to subadults and adults. Both male and female rats had an equal likelihood of infection in both NO and NYC (Table 3.3).

Bartonella *diversity*

There were significant differences in *Bartonella* diversity among host species within the same city and in the same host species between cities (Table 3.2; Figures 3.2, 3.3). Sequences from NO rats aligned with *B. coopersplainsensis* (100% similarity, 100% coverage), *B. rochalimae* (98–99% similarity, 100% coverage), *B. elizabethae* (99–100% similarity, 100% coverage), *B. tribocorum* (99–100% similarity, 99– 100% coverage), and *B. queenslandensis* (100% similarity, 100% coverage). Direct PCR and sequencing recovered variants of *B. rochalimae*, *B. elizabethae*, and *B. tribocorum*, whereas culture and sequencing recovered variants of *B. elizabethae*, *B. tribocorum*, and B*. queenslandensis* (Table 3.2, Figure 3.3) from Norway rats in NO. A variant of *B. coopersplainsensis* was only detected in roof rats from NO via culture and sequencing (Table 3.2, Figure 3.3). Sequences from NYC Norway rats aligned with *B. elizabethae* (100% similarity, 100% coverage) and *B. tribocorum* (99–100% similarity, 99–100% coverage). Provisional identifications of variants agreed with the recovery of aligned sequences in well-supported clades (Figure 3.2).

Results of the rarefaction null model comparisons indicate that *Bartonella* variant richness and species assemblages (as detected through culture) significantly differed between NO and NYC. The ecological null model was rejected ($P < 0.05$), indicating that differences in variant richness observed between NO and NYC are greater than would be expected from a random sampling from a single assemblage (Cayuela et al. 2015). The biogeographical null model also was rejected ($P < 0.05$), further indicating that there are significant differences in variant richness between NO and NYC, regardless of host species composition (Cayuela et al. 2015).

Bartonella *coinfection and co-occurrence*

Norway rats from NO and NYC were infected by more than one species of *Bartonella*, but we did not detect a significant association between any particular species pair (PAIRS analysis, $P > 0.05$). In NO, 20% of Norway rats harbored more than one *Bartonella* species, while only 9% of Norway rats in NYC harbored more than one species. We detected up to three different *Bartonella* species in individuals sampled from NO and up to two different *Bartonella* species in individuals sampled from NYC. Coinfections were observed between all combinations in Norway rats from NO and NYC. Additionally, we detected different *Bartonella* species in spleen versus blood from the same individual from NO.

Discussion

Bartonella *infection*

Public health threats from rodent-borne pathogens are expected to increase with global trends in urbanization (Han et al. 2015). Understanding the prevalence and distribution of rodent-borne bacteria can help mitigate transmission risk and spread of pathogenic species, especially in areas where humans and rodent reservoirs come into frequent contact. Notably, we detected pathogenic species (*B. tribocorum, B. elizabethae, and B. rochalim*ae) in all but one of the Norway rats collect in NO, and in all of the Norway rats from NYC (Daly et al. 1993; Comer et al. 2001; Eremeeva et al. 2007). However, we found that the prevalence of *Bartonella* (including pathogenic species) is highly heterogeneous within and among the two cities. Consistent with patterns of prevalence in other temperate cities such as Vancouver (Himsworth et al. 2015), prevalence of *Bartonella* infection ranged from 0 to 97% among sites in NO and 10–85% among sites in NYC. This suggests that the potential risk of pathogen spillover is likely also asymmetrically distributed across urban landscapes and that there may be localized hot spots of risk in cities.

Though environmental or built features of the urban landscape may govern clustering of infection in rodent populations (i.e., by facilitating or impeding movement), our results indicate that clustering may instead reflect host– ectoparasite interactions. We found that *X. cheopis* flea infestation is a significant predictor of *Bartonella* infection in Norway rats (R. *norvegicus*) in both cities (Table 3.3). The relationship between flea infestation and *Bartonella* infection is evident even at very small spatial scales. In NO, 35 of the 38 Norway rats with detectable flea infestation were collected from a single location where 97% of individuals were also positive for *Bartonella* infection.

Differences in ectoparasite communities may also account for differences found in *Bartonella* infection between co-occurring rat species. We found comparable levels of flea infestation in Norway rats from NO (~24%) and NYC (~30%), whereas flea infestation was much rarer in roof rats (*R. rattus*) from NO (~1%). Similarly, Norway rats were more likely to be infected with *Bartonella* than were roof rats in NO. *Bartonella* infection was rare in roof rats (~3% tested positive), and concordantly, flea infestation was not a significant predictor of *Bartonella* infection in roof rats (Table 3.3). Evidence that roof rats only carry *B. coopersplainsensis* also suggests that the spread of *Bartonella* species differs according to host–

ectoparasite interactions. Neither *X. cheopis* nor *C. felis* develop on their hosts, but rather develop in the nest, in organic debris, or in soil (e.g., Rothschild 1975). Differences in nesting behaviors may provide more (Norway rats) or less (roof rats) hospitable microclimates for flea development, as has been seen in other flea–rodent systems (Krasnov et al. 1997). Behavioral differences related to grooming may also be important (Bordes et al. 2007; Hawlena et al. 2007). Additionally, ectoparasites other than fleas may spread *Bartonella* infection in roof rats, such as rat mites and rat lice (Tsai et al. 2010), which were found on all of the *Bartonella* positive roof rats in NO. Though this inference is consistent with prior surveys that have detected *B. tribocorum* and *B. elizabethae* where *X. cheopis* was more prevalent on roof rats (Morick et al. 2009), further comparisons will be necessary to clarify whether host–ectoparasite interactions mediate transmission of different *Bartonella* species in urban rats, including those known to cause human disease.

Bartonella *diversity*

Results indicate that the diversity of *Bartonella* bacteria differs between hosts within a city and within a host between cities. Norway rats harbor a more diverse and distinct complement of *Bartonella* compared to roof rats in NO, and Norway rats in NO harbor a greater diversity of *Bartonella* than do Norway rats in NYC. Rejection of the biogeographical null model suggests that *Bartonella* diversity may reflect local conditions or historical events (Cayuela et al. 2015), while rejection of the ecological null model suggests that meta-community processes may play a role in structuring *Bartonella* diversity (Cayuela et al. 2015). This is consistent with our inference that *Bartonella* infection varies according to host–ectoparasite interactions. It is also consistent with prior work showing that arthropod vectors influence *Bartonella* diversity in rodent hosts (e.g., Buffet et al. 2013) and that the same host species harbors distinct ectoparasite assemblages in different cities. In NO, we detected *C. felis* and *X. cheopis* on rat hosts, whereas only *X. cheopis* was detected on rats in NYC. While we did not detect *C. felis* on infected rats in NO, experimental infections (Bouhsira et al. 2013) show that *C. felis* can carry *Bartonella*, which suggests that it can promote infection of rat hosts. This hypothesis could be tested by assessing *Bartonella* diversity within arthropod vectors and their associated rodent hosts.

Bartonella *detection*

Despite differences in *Bartonella* variant assemblages, we detected identical *Bartonella* variants in Norway rats in both cities (Figure 3.3). We recovered sequences from NO and NYC within clades of *B. tribocorum* and *B. elizabethae*, which are globally distributed species (Daly et al. 1993; Buffet et al. 2013). We found no overlap of *Bartonella* species or variants in Norway and roof rats in NO, even though nearly all roof rats were collected from locations that also harbored Norway rats (Figure 3.1). This indicates that there is little-to-no transmission of *Bartonella* between Norway and roof rats in NO, which is consistent with evidence from wild sylvatic rodents that co-occurring host species can harbor unique assemblages of *Bartonella* (Kosoy et al. 1997). As with prevalence and diversity, patterns of *Bartonella* infection in urban rats could be attributable to differences in host–ectoparasite interactions.

Our results affirm that more than one method of testing can be necessary to detect and identify all *Bartonella* that may be present in urban rodents. Individual rodents can harbor several *Bartonella* species, which may not reside in the same tissue. Our findings also illustrate that direct PCR may not detect all *Bartonella* species or variants within an individual host or tissue (Harms and Dehio 2012). Culturing also has limitations; though the approach can be useful for detecting and sequencing morphologically dissimilar *Bartonella* isolates collected from a single individual host, some *Bartonella* species can be difficult to cultivate, including known human pathogens like *B. rochalimae* (Gundi et al. 2012). Consistent with this, we only detected *B. rochalimae* through direct PCR (Figures 3.2, 3.3) (Firth et al. 2014).

Public health implications

Urban populations of commensal rats can support diverse and heterogeneous assemblages of *Bartonella*, including pathogenic species of concern. Variation in prevalence and diversity may give rise to hot spots of public health risk— even on very small spatial scales (e.g., we detected six unique *Bartonella* variants, including two species known to cause human disease, on a single city block in NO). Variation in prevalence and diversity may be a common phenomenon, as heterogeneous distributions of *Bartonella* have been detected in other cities (Himsworth et al. 2015). Similar patterns also have been observed with other rodent- borne pathogens such as hantaviruses and Rickettsia (Himsworth et al. 2015). Accordingly, additional cross-city comparisons could help constrain and reduce potential risk by informing disease surveillance programs. Further understanding of host–parasite interactions also could help reduce infection risk. Consideration should be given to factors that foster interaction diversity (Dyer et al. 2010), including conditions like mosaics of abandonment (Gulachenski et al. 2016; Rael et al. 2016) that can yield differences in ectoparasite communities on rodent hosts (Krasnov et al. 2007). Consideration should also be given to landscape management as an approach for reducing infection risk, particularly in cities where rodents are more abundant in disadvantaged neighborhoods (Gulachenski et al. 2016; Rael et al. 2016; Lewis et al. 2017).

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Appendix

Table 3.1. Sex, age categories, and percent of all captured rodent individuals infested with fleas (*C. felis* and *X. cheopis*) from New Orleans, LA (NO) and New York City, NY (NYC).

	Age class			Total infested with *C. felis* (%)	Total infested with *X. cheopis* (%)
	Juvenile	Subadult	Adult		
R. norvegicus (NO)	12 F 7 M	18 F 13 M	53 F 59 M	2	23
R. rattus (NO)	21 F 19 M	62 F 42 M	11 F 21 M	< 1	< 1
S. hispidis (NO)	0 F 0 M	0 F 0 M	1 F 1 M	0	0
R. norvegicus (NYC)	26 F 29 M	16 F 24 M	19 F 19 M	0	30

[a]Number of female rodents captured
[b]Number of male rodents capture

Table 3.2. Numbers of New Orleans (NO) and New York City (NYC) rodents positive for Bartonella infection using direct PCR and culture methods. Specific primers used for screening include *tmRNA* (qPCR), *ITS* (PCR), and *gltA* (PCR and sequencing).

Method	# Rats Tested	# Rats Positive	# *B. tribocorum*(+)	# *B. elizabethae*(+)	# *B. queenslandensis*(+)	# *B. coopersplanensis*(+)	# *B. rochalimae*(+)
R. norvegicus (NO)							
Culture	163	29[a]	19	10	5	0	0
Direct PCR (*tmRNA)*[b]	162	17 [c]					
Direct PCR (*ITS*)[b]	125	40[c]					
Direct PCR (*gltA*[b]	86	19[a]	4	1	0	0	13
R. rattus (NO)							
Culture	177	5[a]	0	0	0	5	0
Direct PCR (tmRNA)[b]	176	3[c]					
Direct PCR (ITS)[b]	79	6[c]					
Direct PCR (gltA)[b]	66	0	0	0	0	0	0
S. hispidus (NO)							
Culture	2	0	0	0	0	0	0
Direct PCR (tmRNA)	2	0	0	0	0	0	0
Direct PCR (ITS)[b,c]	2	0	0	0	0	0	0
Direct PCR (gltA)[b]	0	0	0	0	0	0	0
R. norvegicus (NYC)							
Culture	31	25[a]	26	3	0	0	0
Direct PCR (*tmRNA*)							
Direct PCR (*ITS*)[d]							
Direct PCR (*gltA*)[d]	133	31[a]	24	1	0	0	6

Individuals that were successfully PCR-amplified for tmRNA and ITS were not all necessarily confirmed positive

[a]Confirmed *Bartonella* positive through sequencing of the gltA gene, some individuals infected with more than one *Bartonella* variant.

[b]Direct PCR of spleen tissue.

[c]Successfully amplified (produced a PCR band), considered putatively positive.

[d]Direct PCR of spleen and heart tissue, considered positive if either heart or spleen was positive.

Table 3.3. Predictors of individual level *Bartonella* infection in rats from New Orleans (NO) and New York City (NYC).

Outcome	Predictor	Coefficient	Standard Error	p-value
R. norvegicus (NYC)[a] Infection	**Flea Infestation**	2.23	0.55	**<0.05**
	Age Class (juv.)	-2.65	0.73	**<0.05**
	Sex (M)	0.25	0.51	0.63
R. norvegicus (NO)[b] Infection	**Flea Infestation**	5.40	0.75	**<0.05**
	Age Class (juv.)	-0.56	1.31	0.67
	Sex	-0.95	0.76	0.21
R. rattus (NO)[c] Infection	Flea Infestation	-16.16	10754.01	0.99
	Age Class	-17.41	1788.03	0.99
	Sex	-0.39	0.81	0.64

Statistically significant predictors are in bold.
[a]*d.f.*= 158
[b]*d.f.*= 166
[c]*d.f.* = 132

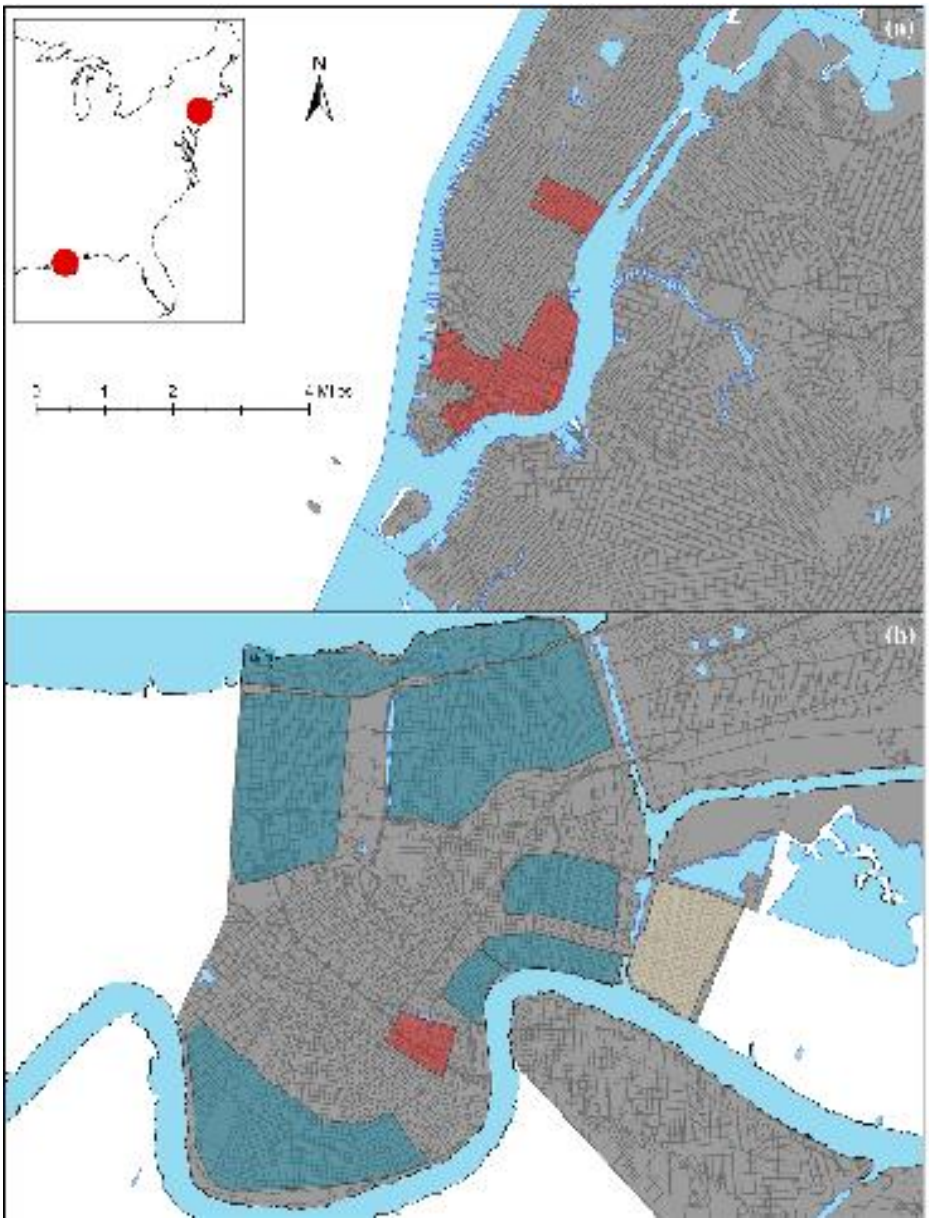

Figure 3.1. Locations of trapping efforts in New York City (a) and New Orleans (b). Neighborhoods in red harbored Norway rats, neighborhoods in blue harbored both Norway rats and roof rats, and neighborhoods in yellow supported Norway rats, roof rats, and hispid cotton rats.

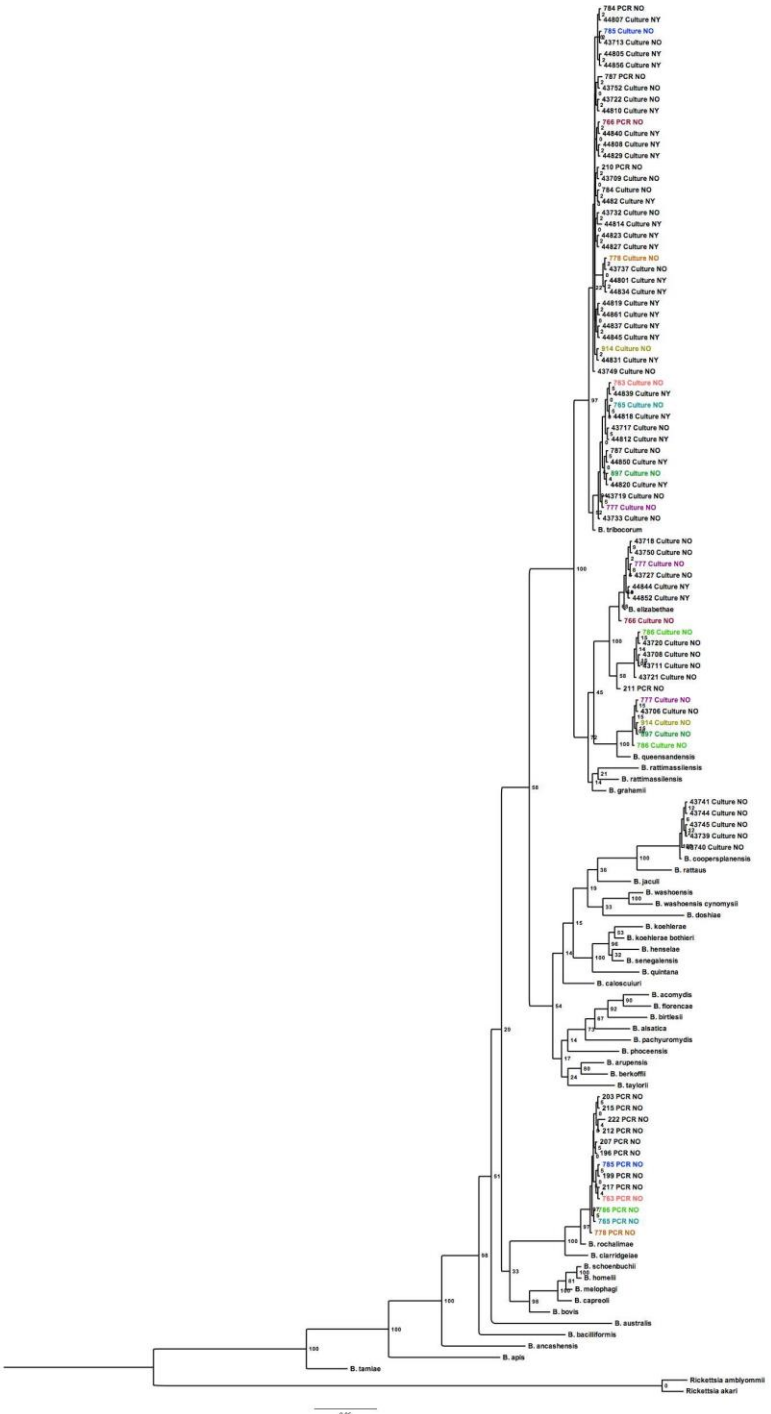

Figure 3.2. Phylogenetic hypothesis of Bartonella from NO and NYC using constructed sequences of the gltA gene and Bayesian inference. Sequences from NO were obtained from culture (blood) and direct PCR (spleen), and sequences from NYC were obtained from culture (heart). Numbers above nodes indicate posterior probabilities. Matching colors represent sequences identified as different Bartonella species obtained from the same individual from NO.

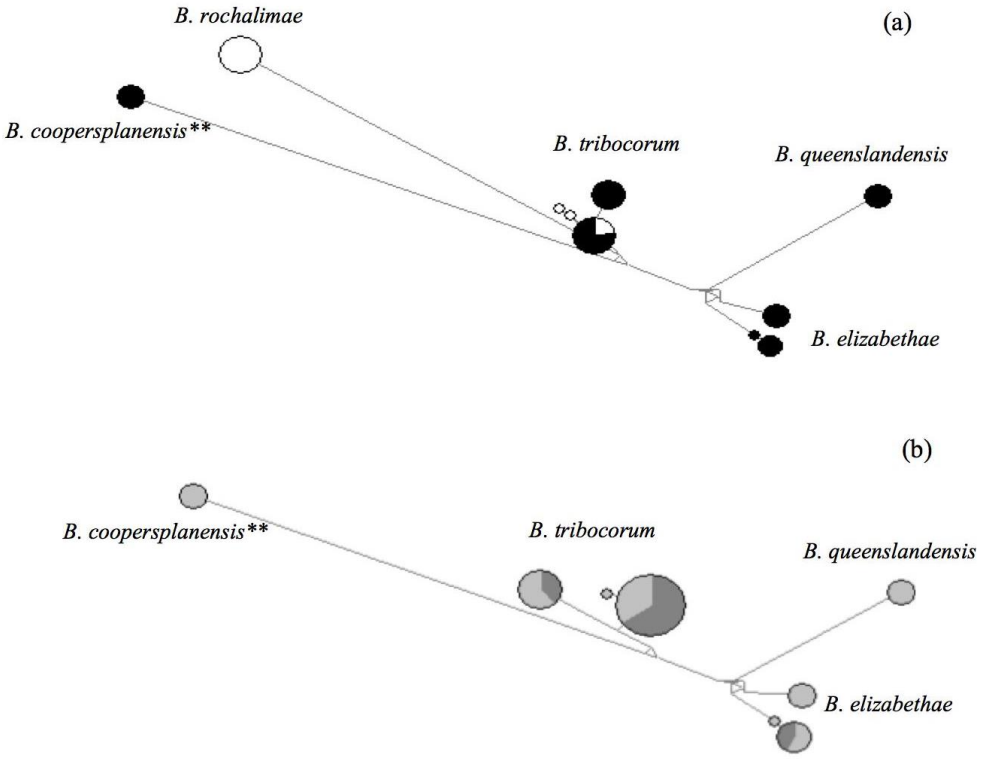

Figure 3.3. Median-joining network of Bartonella gltA variants. Detected in: (a) tissue culture methods (black) versus direct PCR methods (white) from NO rats; (b) NYC (dark gray) versus NO (gray) rats from heart and blood cultures. Size of circles proportional to number of individuals infected. Asterisk indicates Bartonella variants obtained from roof rats in NO.

CHAPTER IV
RODENT VIROME DIVERSITY AND DIFFERENTIATION ACROSS POST-KATRINA NEW ORLEANS

Abstract

Rodents are expected to increasingly influence infectious disease risk due to global trends in urbanization, yet remarkably little is known about pathogen assemblages in urban rodent populations. Viral pathogens, which can elicit global pandemics, have received considerably less attention than other rodent-associated public health concerns. Here we characterize blood-borne viral assemblages (i.e., viromes) of three widespread commensal rodents (*Rattus norvegicus, Rattus rattus*, and *Mus musculus*) across New Orleans (Louisiana, USA). We assessed virome diversity and differentiation according to host species as well as prevailing landscape conditions known to shape rodent assemblage structure. We detected 20+ viruses from unbiased metagenomic analysis of 100+ blood-borne viromes from each host species. We found that host species exhibit distinct virome profiles. Local virus richness (i.e., alpha diversity) also differed among host species. We did not find an association between local virus and host richness, however, suggesting that some transmission cycles are host specific. We also found that spatial differentiation (i.e., beta diversity) differed by host species, though we did not find associations with abandonment, a key factor that influences commensal rodent assemblage structure across the city. Our findings illustrate that further exploration of urban rodent viromes is warranted to better understand drivers of variation and to determine whether specific constituents can serve as indicators of pathogen exposure risk.

Introduction

Pathogen surveillance can help prevent the emergence and spread of zoonotic infectious diseases. A key first step in surveillance is determining the diversity and prevalence of potential pathogens in species of concern. Several recent studies of commensal rodents illustrate the merits of characterizing pathogen assemblages (Meerburg et al. 2009, Himsworth et al. 2013a). For example, targeted surveillance of urban rodent populations has shed new light on the diversity and prevalence of known zoonotic bacterial and viral pathogens of concern, including *Leptospira* (Bharti et al. 2003, Easterbrook et al. 2007, Himsworth et al. 2013b, Peterson et al. in review), *Bartonella* (Himsworth et al. 2015, Peterson et al. 2017), *Rickettsia* (Himsworth et al. 2015), Hantaviruses (Himsworth et al. 2015), and lymphocytic choriomeningitis virus (Childs et al. 1992, Easterbrook et al. 2007). Unbiased metagenomic assays also have begun to provide novel perspectives on pathogens carried by commensal rodent hosts, including identification of previously unknown bacteria and viruses infecting urban populations (Firth et al. 2014, Williams et al. 2018a, Williams et al. 2018b, Wu et al. 2018). Iterative discovery of previously unknown microbiota suggests that further characterization is warranted, especially considering that commensal rodents are predicted to be a leading source of novel zoonotic pathogens in the future (Meerburg et al. 2009, Han et al. 2015).

Viruses have received considerably less attention than other rodent-associated public health concerns. Viruses carried by rodent hosts are increasingly being recognized as potential risk factors, as several of mammalian origin have been responsible for recent pandemics (Morse et al. 2012, Han et al. 2018). Regions of the world with dense human occupancy- including areas

in North America and Europe- also are considered to be global hotpots of rodent-associated viral diversity (Olival et al. 2017), raising concerns about risk of transmission to humans. Concerns about transmission have motivated targeted surveillance of known viral pathogens (e.g., Himsworth et al. 2015) and surveys of viral assemblages (hereafter 'viromes'), but to date, unbiased genomic assessments of rodent viromes have largely been limited to a single host species (Firth et al. 2014, Williams et al. 2018a). This work has nonetheless been quite revealing, illustrating that the composition and richness of viromes can differ according to geography (Williams et al. 2018a). The among-species comparisons so far done (Wu et al. 2018) have also revealed that viromes can differ among hosts, yet little else is known about the factors that govern virome variation, including the possibility that local host co-occurrence shapes virome diversity and differentiation.

Prior studies of urban rodents describing host infection by a single (i.e., target) pathogen offer some perspective on potential drivers of virome variation. Geographic heterogeneity in pathogen infection appears to be the norm in urban rodent populations and assemblages, even on relatively small spatial scales (e.g. Himsworth et al. 2013ab, Peterson et al. 2016, Rothenburger et al. 2017). For instance, work in Vancouver on *Leptospira* infection found that prevalence in *R. norvegicus* populations varied from 0-66% among spatially proximate city blocks (Himsworth et al. 2013b). This finding parallels patterns of variation observed in New Orleans, which appear to be driven by host species diversity and environmental features (e.g., abandonment) that structure rodent assemblages (Peterson et al. in review). Similarly, Hantavirus infection in rodents is related to host assemblage characteristics, with hosts in areas of higher species richness exhibiting lower infection prevalence (Mills 2005, Dizney and Ruedas 2009, Dearing et al. 2015). This work highlights the possibility that host co-occurrence and factors that structure host assemblages may exert influence on virome diversity.

It is well established that host diversity can influence the structure of symbiont communities. For example, positive relationships between host and parasite diversity appear to be the norm (Hechinger and Lafferty 2005, Kimiya et al. 2014), akin to relationships observed between the biodiversity of free-living species and resource availability (Kamiya et al. 2014). Similar mechanisms appear to underlie parallels between these relationships. For example, host heterogeneity, like habitat heterogeneity, influences parasite diversity in amphibian hosts (Johnson et al. 2015). This suggests that host heterogeneity probably exerts similar influence on other symbiont communities, including viromes (Mihaljevic 2012). By extension, factors that influence host heterogeneity likely also influence the structure of symbiont communities. However, it has also been shown that some factors, such as human activity, can decouple relationships between host heterogeneity and the structure of symbiont communities (e.g., host-parasite diversity; Wood et al. 2018). Thus studies of relationships between host diversity-virome diversity in urban landscapes could be particularly informative since pathogen pool diversity and human activity are key risk factors in predictions of zoonotic disease outbreaks (Jones et al. 2008, Patz et al. 2004, Hassell et al. 2017, Hosseini et al. 2017).

Several other macroecological phenomena may also structure rodent-borne viromes (Stephens et al. 2016). For example, distance decay relationships have been detected with some parasite communities (Krasnov et al. 2005, Stephens et al. 2016), where the similarity of

host-dependent communities decreases with increasing spatial separation. While this might suggest that viromes also are structured according to spatial proximity, work on free-living bacteria suggests that distance decay is not universal to all microbial communities (e.g., Fierer and Jackson 2006). Other studies nonetheless indicate that spatial proximity is an important consideration for viromes. For example, an experimental manipulation of plant viromes did not find evidence of spatial structure for the overall assemblage, but did find signatures of aggregation for particular viruses (Kendig et al. 2017). Understanding spatial variation of viromes can provide a stronger basis for assessing risk of transmission to humans.

The City of New Orleans (Louisiana, USA) presents exceptional conditions for assessing whether and why rodent-borne viromes exhibit spatial and host assemblage structure. Unlike many other cities, three cosmopolitan commensal species- *Mus musculus*, *Rattus norvegicus,* and *Rattus rattus*- cohabitate in New Orleans (Peterson et al. in review). The diversity and abundance of rodent species also vary across the city, reflecting a mosaic of habitat conditions that have arisen as a consequence of Hurricane Katrina flooding, discriminatory resettlement policies, and heterogeneous post-disaster land management practices (Lewis et al. 2017, Rael et a. 2016, Peterson et al. in review). For example, a recent assessment of assemblage structure (Peterson et al. in review) found that rodents are more abundant and more diverse in areas burdened with greater levels of abandonment and infrastructure decline. In this study, we undertook an unbiased metagenomic study of blood-borne viromes found in *M. musculus*, *R. norvegicus* and *R. rattus* collected from study areas located across New Orleans. The selected study areas vary in spatial proximity to one another and also in rodent assemblage structure, thus enabling us to determine the extent to which (1) viromes differ according to host species as well as individual-level attributes such as sex and infection status with other known pathogens; (2) viromes differ according to spatial proximity; and the extent to which (3) virome diversity reflects rodent host diversity. Additionally, we tested the hypothesis that areas undergoing de-urbanization harbor more diverse pathogen pools (Eskew and Olival 2018) by determining whether (4) virome diversity varies according to the extent of abandonment within and among study areas.

Methods

Rodent trapping

We examined blood-borne viromes of rodents that were captured for quantitative studies of rodent demography and assemblage structure across New Orleans (Rael et al. 2016, Peterson et al. in review). For this study, we examined animals captured at trapping sites located within nine select areas (Figure 1) corresponding to: seven neighborhoods in the urban footprint of New Orleans, a neighborhood in adjacent St. Bernard Parish, and a nearby non-developed (“natural”) area located within Orleans Parish (Figure 1). The study areas span gradients of sociodemographic conditions, Katrina-related flooding, and property abandonment (Lewis et al. 2017, Peterson et al. in review). Rodents were trapped on eight to ten randomly selected blocks in each neighborhood (Lewis et al. 2017) and eight equally-sized trapping sites in the non-residential ‘natural area’ (Peterson et al. in review). Land use was characterized for

all study areas according to high-resolution satellite imagery supported by plot-based estimates of vegetation and ground cover (Lewis et al. 2017, Peterson et al. in review). Google Earth imagery was used to determine the proportion of vacant lots on each trapping site (Peterson et al. in review), with all lots in trapping locations outside of residential areas considered to be 100% vacant.

All rodents were trapped between May 2014 and February 2017. For this study, we examined rats that were captured across a total of 77 sites using Tomahawk traps (Figure 1). Trapping was conducted across a succession of six alternating summer and winter bouts (Summer 2014-Winter 2016/2017) in the Gentilly, Uptown, Lower 9th, Upper 9th, Bywater, Lakeshore and Lakeview neighborhoods. Rats were trapped in the natural area and French Quarter across a succession of four bouts (Summer 2015-Winter 2016/2017), and a succession of two trapping bouts (Summer 2016-Winter 2016/2017) in St. Bernard Parish. We concurrently captured mice at 25 sites in five study areas starting in the summer of 2015 using Sherman traps (Figure 1). All animals were captured and handled following Tulane IACUC approved protocols #0451 and #0460.

We placed 30 Tomahawk and 30 Sherman live traps (when applicable) at each trapping site. Traps were located at sites according to property access, with all traps put in areas of observed or potential rodent activity. All traps were set and baited in the afternoon and checked and closed each morning. Sherman traps were set for four continuous trapping nights, whereas Tomahawk traps were set for a minimum of three continuous nights, with trapping efforts sustained at each site until the trap rate reached an asymptote (i.e., when no more individuals were captured).

Additional animals were collected through supplemental trapping of rats at one additional site (hereafter referred to as the 'Underpass' site) as part of control efforts conducted by the City of New Orleans Mosquito, Termite, Rodent Control Board (NOMTCB) in the Summer of 2014 (Figure 1). Only Tomahawk traps were set at the site, which were placed in a regular grid for four continuous nights.

Tissue collection

Upon capture, we euthanized and necropsied all animals following a standard protocol (Tulane IACUC approved protocols #0451 and #0460) at the City of New Orleans Mosquito, Termite Rodent Control Board facility. We euthanized all rodents using isoflurane anesthesia followed by cardiac puncture. Blood collected from the cardiac puncture was immediately spun down to separate serum from coagulate. Species, sex, sexual maturity, weight and standardized length measurements were recorded for each animal. We then collected replicate samples of urine as well as lung, liver, kidney, spleen, and tail tissue. All liquid and tissue samples were immediately transferred to a -80°C freezer and stored until later use.

Metagenomic sequencing

We utilized serum samples to characterize the viromes of 482 rodents, consisting of 149 *Mus musculus,* 160 *Rattus norvegicus,* and 173 *Rattus rattus*. Individual samples were combined into 110 pools on the basis of host species and trapping site. Each pool was centrifuged at 5000

rpm for 5 minutes. After centrifugation, 120 µl of supernatant was treated with nuclease enzyme to remove cell free host nucleic acid, which was followed by total nucleic acid (TNA) extraction using QIAamp Viral RNA Mini Kits (Qiagen, Germany) according to the manufacturer's instructions. The TNA from each pool was tagged with a unique barcode during cDNA synthesis and second strand synthesis. The TNA from each pool was then reverse transcribed with Superscript III reverse transcriptase (Thermo Fisher Scientific) and barcoded with a variant of the A0 primer (5'-CGTCAAATCCCTCGGTCAGGNNNNNNN-3') followed by second strand DNA synthesis with Klenow Exo- (New England Biolab). For each barcoding primer variant, the first four nucleotides and the last 11 nucleotides were held constant, whereas a distinct set of 12 intervening nucleotides (underlined above) differentiated each barcode. The tagged nucleic acid was amplified with the pool specific barcode primer, without the random sequence at the 3' end, to obtain a sufficient amount of nucleic acid for Illumina library preparation. The PCR product was size selected for library preparation using 1.3X of Axyprep beads (Axygen Scientific) as per the manufacturer's instruction except that elutions were done with 14 µl of buffer. Samples were included in pools on the basis of equimolar concentration and subjected to A tailing and Illumina adaptor ligation. Adaptor ligated libraries were amplified with Illumina I7 and I5 primers as follows: 98˚C for 30 sec; 10 cycles of 98˚C for 15 sec, 65˚C for 30 sec, 72˚C for 30 sec; 72˚C for 5 minutes; and an extended hold at 10˚C. Libraries were then purified with 0.7X of Axyprep beads (Axygen Scientific) and eluted in 30 µL of buffer. Library size was determined using a High Sensitivity DNA kit on an Agilent BioAnalyzer 2100 instrument (Agilent, Santa Clara, CA) and concentrations were measured using a KAPA Library Quantification Kit (Kapa Biosystems, Woburn, MA)**.** Libraries were then sequenced on a HiSeq™ 4000 platform (Illumina) for 2 × 150 cycles at the Institute for Genomic Medicine, Nationwide Children's Hospital (Columbus, Ohio, USA).

Bioinformatics

FastQ files were demultiplexed based on a sample's pool-specific barcode present within 25 base pairs at the 5' and 3' ends, allowing for a one base pair mismatch in the barcode search using BBDUK (BBTools). The demultiplexed FastQ files were adapter trimmed using cutadapt v1.8.3. This was followed by adapter trimming and generation of quality reports using FastQC software (v0.11.5). To verify our pipeline, we also included 30 different known virus sequences as positive controls in each quality filtered FastQC file. Demultiplexed and Q30-filtered FastQ files were mapped against host genomes (*M. musculus, R. norvegicus*, *R. rattus*) and the Phi X reference genome using Bowtie v2.3.3.1 (http://bowtie-bio.sourceforge.net) to determine the host background and Phi X level percentage. All unaligned reads were then clustered to remove duplicate sequences using CD-HIT v4.6.5 software. The resulting reads were *de novo* assembled using MIRA (v 4.0) assemblers, and contigs and unique singletons were subjected to a homology search using BLASTN [BLAST 2.7.1+] to determine nucleotide similarity, with an e-value cutoff of $1e^{-8}$ against the GenBank nucleotide database. Sequences that exhibited poor or no homology at the nucleotide level were screened by BLASTX against the viral GenBank protein database. All non BLASTN sequences (i.e., contigs and singlets) were also processed for a protein similarity search using DIAMOND v0.9.13.114 against a non-

redundant protein database. All sequences classified as a virus from DIAMOND were again processed using a BLASTX [BLAST 2.7.1+] protein alignment with an e-value cutoff of 0.01 against a non-redundant NCBI database for accurate taxonomic classification. Final reports were generated by combining BLASTn and BLASTx virus classification entries for different pool wise comparisons.

Statistical analyses

We accounted for differences in the numbers of individuals included in each pool by rarefying values recovered for all pools relative to the pools with the lowest numbers of individuals (e.g. Emerson et al. 2013, Weiss et al. 2017), using the vegan package in R (R core team, 2017) to generate a community dataframe (Oksanen et al. 2017) following Heck et al. (1975). We then visualized virome composition according to host species by plotting the first two principal components of $\log_2$ rarefied virus communities (Figure 4.2). We also visualized rarefied virome composition from only the trapping sites for which we obtained data from more than one species (Figure 4.3). We then completed a PERMANOVA (McArdle and Anderson 2001) with 999 permutations to determine the extent to which host species explains the sum of squared variance in rarefied virome composition. We completed a second PERMANOVA utilizing only data from locations with virome data from >1 species to determine whether variance was explained by species, trapping block, and a species x trapping block interaction term.

We also examined whether host attributes relate to virome composition, focusing on host sex and infection with other pathogens and parasites. First, we compared viromes from pools composed of only male animals (n=13, Table 4.1) to viromes from pools composed of only female animals (n=7) with a PERMANOVA. We did this for all species and included a species x sex interaction term. Similarly, we compared pools of individuals that were not infected with *Leptospira* to pools of individuals in which ≥ 50% of individuals in a given pool were infected with *Leptospira*, also with a PERMANOVA. We included a species x *Leptospira* infection interaction term, though we only had sufficient pools to draw these comparisons for *R. norvegicus* and *M. musculus*. Lastly, we performed a PERMANOVA to determine if ectoparasites were related to variation in rodent viromes, using only data from pools in which all individuals supported ectoparasites (n=8) and pools in which all animals were clear of ectoparasite infestation (n=5). The ectoparasite analysis was done only with *R. rattus*, as this was the only species for which we had sufficient numbers of pools to draw comparisons.

We conducted separate Mantel tests for each species to determine whether viromes from hosts collected from geographically proximate locations exhibited greater similarity than those from hosts collected from disparate locations (Figure 4.4). Due to the patchiness in collection locations for some species (i.e., for some species, there were samples obtained from locations that were geographically isolated from all other locations), we restricted these analyses to data from specimens captured across contiguous trapping areas to limit the potential influence of outlier sites and landscape features (e.g., waterways) that can influence population connectivity (Combs et al. 2018). Thus, for *M. musculus* we examined spatial differentiation of viromes from animals captured in the Lower 9th Ward and St. Bernard Parish

neighborhoods. For *R. rattus*, we examined spatial differentiation of viromes from animals captured in the Lakeview, Lakeshore, and the Gentilly neighborhoods. For *R. norvegicus* we examined spatial differentiation of viromes from animals captured in the Lower 9th and Upper 9th Ward neighborhoods (Figure 4.1). We plotted the correlation coefficients between the Bray-Curtis dissimilarity matrix of rarefied viromes and the geodesic distance matrix based on latitude and longitude coordinates representing the centroid of each trapping block, as well as scatterplots of the dissimilarity matrix relative to geodesic distance (Figure 4.4).
We utilized a linear model with post-hoc comparisons with Tukey's p-value correction to determine if standardized rarefied virus richness differed among host species (Figure 4.5), and if virus richness differed according to vacancy. For this analysis, we standardized richness estimates by taking the natural log of the rarefied richness estimate divided by the total number of animals of a given species that were tested. Furthermore, to determine relationships among viral richness and rodent assemblage characteristics (diversity, species co-occurrence), we completed two separate analyses. In the first analysis, we compared the richness of all viruses from all species for which we have virome data that were captured at sites where only one species was detected (n=5) to the richness from all species for which we have virome data from blocks where we detected three species (n=4) with an ANOVA of natural log transformed virome richness estimates that were standardized to account for differences in sample sizes among locations. We standardized richness estimates by dividing the rarefied richness estimate by the total number of animals of a given species from each block for which virome data was available. We could not draw comparisons to sites where only two species were detected (Peterson et al., in review) due to a lack of virome data from those sites. The second set of analyses compared the richness in *Rattus* species from locations where there was one or the other species detected to locations where both species co-occurred, with an ANOVA utilizing the natural log transformed standardized virome richness estimates. We completed a separate ANOVA for each *Rattus* species. We could not draw comparisons to assess how virome diversity varied according to co-occurrence of *Rattus* species with *M. musculus* due to the limited number of the locations with an appropriate complement of species (i.e., *R. rattus* and *M. musculus*, *R. norvegicus* and *M. musculus*, and all three together). Lastly, we determined whether sex, infection with *Leptospira,* or ectoparasite infestation are related to virus richness by completing three separate generalized linear models, each with a poission error distribution.

All statistical analyses were completed in R utilizing the vegan, glmmTMB, ecodist, and multcomp packages (Oksanen et al. 2017, Brooks et al. 2017, Goslee and Urban 2007, Hothorn et al. 2008).

Results

We recovered clear evidence that viromes differed by host species, but mixed support for differences among trapping areas. When considering all host species from all locations included in this study, we found that most of the variation in virome composition was explained by rodent host species identity ($p=0.001$, $R^2=0.50$). We also detected a significant species *x* neighborhood interaction ($p=0.03$, $R^2=0.06$), though this interaction was largely driven by variation of *R. norvegicus* viromes ($p=0.006$, $R^2=0.33$). The PERMANOVA based on data from

trapping blocks from which we had virome data from >1 species showed that only species identity was significantly related to variance in virome composition (p=0.01, R^2=0.42); virome composition did not correspond to the site where an animal was captured (p=0.49), nor was there a significant interaction (p=0.43) between trapping site and species identity (Figure 4.3). Furthermore, we found that sex was not a significant predictor of variation in rodent viromes (p=0.77, R^2=0.02), nor was there a significant interaction with host species (p=0.99, R^2<0.01). Similarly, we found that *Leptospira* infection status was not a significant predictor of variation in rodent viromes (p=0.53, R^2=0.02), and that there was not a significant interaction with host species (p=0.43, R^2=0.02). Ectoparasite infestation status also did not explain a significant proportion of the variation in viromes of *R. rattus* (p=0.28, R^2=0.11).

Relationships between virome dissimilarity and geographic distance varied among host species. We did not find a significant relationship between geographic distance and virome dissimilarity for either *M. musculus* or *R. norvegicus* (*M. musculus*: all p > 0.4, mantel r = -0.01; *R. norvegicus*: all p > 0.1, mantel r = 0.12), but we did find a significant relationship for *R. rattus*. Animals collected from more proximate locations exhibited more similar viromes than those that were geographically farther apart (p-value one = 0.01, mantel r = 0.44), and those farther apart exhibited significantly different virome communities (p-value three = 0.01). The corresponding mantel correlogram indicates that virome similarity emerges over distances of around 2 kilometers whereas virome dissimilarity in *R. rattus* emerges over fairly large distances, with *R. rattus* viromes showing significantly negative correlation at distances >6 kilometers (Figure 4.4).

We found that standardized viral richness significantly differed among host species, with *R. norvegicus* exhibiting significantly higher richness relative to both *M. musculus* (coef.= 0.31, p<0.01) and *R. rattus* (coef.=-0.76, p<0.01). We also found that *M. musculus* supported higher virus richness than *R. rattus* (coef.= 0.45, p<0.01). Notably, the richness of *R. norvegicus* viromes did not differ according to co-occurrence with *R. rattus* (p>0.05; Figure 4.6). Likewise, the richness of *R. rattus* viromes did not differ according to co-occurrence with *R. norvegicus* (p>0.05; Figure 4.6). We also did not recover a significant relationship with virus richness and the level of vacancy on a trapping block (p>0.05), nor with sex, *Leptospira* infection status, or ectoparasite infestation (all p>0.05). Lastly, virome richness at locations with three host species did not significantly differ from virome richness at locations with only one species (ANOVA, p=0.08).

Discussion

In this study, we examined the extent to which blood-borne viromes in three cosmopolitan rodent hosts reflect geography, host species, the diversity of rodent assemblages, and landscape features known to structure rodent assemblages across New Orleans. Our results indicate that rodent viromes are primarily constrained by host identity. We detected little compositional overlap among different rodent host species, even when comparing viromes of the different species captured at the same trapping site. However, virome richness was not higher at locations harboring more than one host species. These results parallel evidence from studies of other rodent-dependent communities, like gut microbiota, that

highlight the importance of host identity (Knowles et al. 2019). Yet, contrary patterns have been observed for single pathogens, such as *Leptospira*, which indicate that infection may be shared among co-occurring rodent species (Peterson et al. in review). Evidence of heterogeneous distributions of known pathogens also indicates that more detailed assessments could shed further light on the factors structuring virome diversity and differentiation.

Infection status with one pathogen or ectoparasite has been shown to relate to infection with secondary rodent associated pathogens, such as *Leptospira* (Peterson et al. in prep) and *Bartonella* (Firth et al. 2014, Peterson et al. 2017). Indeed, evidence of this phenomenon has been found in many animal hosts, where increased diversity of some pathogens, like helminthes, can facilitate invasion by (and thus increase the diversity of) intracellular macroparasites (Nunn et al. 2014). While we found little indication that *Leptospira* infection and ectoparasitism relate to virome composition or virus diversity, we had limited capacity to assess patterns of co-infection, particularly within and among individuals. Similarly, we did not find evidence that other individual-level factors (i.e., sex) influence virome structure, though it has been shown that infection by some rodent-associated pathogens does vary with host attributes (Firth et al. 2014, Peterson et al. 2017, Peterson et al. in prep). This is consistent with prior studies of commensal rodent viral diversity, which found no association between host gender and virome composition (Firth et al. 2014, William et al. 2018). Further work to better understand how individual-level variation may relate to virome composition or diversity is warranted, however, as other individual-level features like age and size can be important drivers of pathogen infection and transmission (Lloyd-Smith et al. 2005 , Paull et al. 2012)

Consistent with prior comparisons of rodent-borne viromes (Wu et al. 2018), we found that *R. norvegicus* harbor the highest diversity of viruses relative to both *M. musculus* and *R. rattus*. Differences among host species could reflect intrinsic variation in the capacity to sustain infections (Cronin 2010, Huang et al. 2013). It is also possible that variation in virome richness reflects ecological differences in infection risk reflecting host-specific habitat use, diet, or social interactions (Faust 2017). The observed differences in virus richness could, however, could be a legacy of past events and conditions that have shaped host demography and distributions across the city. As all three species are non-native to New Orleans, viral richness might differ according to invasion history. Introduced species often support depauperate parasite communities compared to source or native range populations (Torchin et al. 2003), which raises the possibility that differences in richness could reflect time since invasion, founding population size, the number of introductions, and source area(s). Cross-city comparisons structured according to biogeographic reconstructions (e.g., Puckett et al. 2016), could help illustrate the relative influence of historical and contemporary factors on rodent viromes.

Because prior work has shown that rodent host communities are spatially structured across the city of New Orleans (Peterson et al. in review), we also expected to find spatial variation in the composition of blood-borne viromes (Mihaljevic 2012, Nieto-Rabiela et al. 2018) We found that spatial variation differed by host species. While the viromes of *R. rattus* exhibited signatures of being structured by proximity and distance, the viromes of *M. musculus* and *R. norvegicus* did not (Figure 4.4). Though this finding is consistent with prior work on the spatial scale of variation in rodent virus metacommunities (Nieto-Rabiela et al. 2018), elements

of our study design might have limited detection of spatial variation. Because we compiled data from across seasons and years, it is possible that temporal variation reduced signatures of spatial variation in viromes of one or more host species. Studies of single viruses, such as LCMV, in rodent hosts have found that infection varies over time (Tagliapietra 2009), suggesting that further study of temporal and spatial variation could yield better understanding of rodent virome differentiation. This is certainly worth careful consideration, but it is also possible that differences in virome differentiation among hosts reflects variation (or the lack thereof) in infection risk. Evidence that viromes in *R. rattus* became significantly more dissimilar with increasing distance (Figure 4.4) might indicate, for example, that localized transmission of viruses among *R. rattus* individuals is heterogeneous across the city. It is also possible that the observed differences among host species reflects differences in host dispersal, where hosts with lower dispersal ability are expected to exhibit more pronounced patterns of distance decay (Poulin 2003). Features of the urban environment can limit dispersal of *R. norvegicus* (Combs et al. 2018), though smaller rodents, such as white-footed mice, exhibit signatures of even greater dispersal limitation in urban environments (Munshi-South and Kharchenko 2010, Combs et al. 2018). Thus, our findings could suggest that *R. rattus* is more dispersal limited than either *M. musculus* or *R. norvegicus*. Alternatively, it is possible that viromes in some, but not all, rodent hosts are saturated or are approaching saturation within the city; if so, then there would not be consistent patterns of spatial variation or clear associations with host dispersal ability (Poulin 2003).

Prior work on other symbionts (Kimiya et al. 2014) suggests that virome diversity should scale with host diversity, but we did not find evidence of greater virome richness in areas harboring greater rodent richness. This finding also is inconsistent with work on rodent-associated bacterial pathogens (e.g., *Bartonella*, *Leptospira*) indicating that local symbiont diversity varies with local host diversity across New Orleans (Peterson et al. 2017, Peterson et al. in review), as well as studies of particular groups of viruses (e.g., Hantaviruses) indicating that infection declines with host diversity (Mills 2005, Dearing et al. 2015). As with estimates of spatial variation, it is possible that our study design has constrained measures of virome-host diversity relationships. It is also possible, however, that these relationships have been disrupted by human activity. Work on host and parasite diversity suggests that even well established relationships can be weakened or disrupted by human activity (Wood et al. 2018). Further work will be needed to clarify whether patterns observed in other host-symbiont systems also hold for virome and host diversity. For example, cross-habitat assessments (e.g., modified versus natural) that capture comparable variation in host assemblage structure could help illustrate whether and how human activity indirectly exerts influence on the relationship between virome and host diversity.

Contrary to expectation, we did not find evidence that virome diversity corresponded to landscape features known to shape urban rodent diversity in New Orleans. We expected that virome diversity would trend with vacancy, a signature element of counter-urbanization in the city (Gulachenski et al. 2016, Rael et al. 2016, Lewis et al. 2017). In part, this is because a positive relationship has been found between vacancy and rodent diversity (Peterson et al. in review), and between vacancy and *Leptospira* infection across New Orleans (Peterson et al. in

preparation). It also has been hypothesized that counter-urbanizing areas support a greater diversity of host species and a larger and more diverse pool of pathogens, which could translate to elevated disease risk (Eskew and Olival 2018). However, greater diversity of hosts *per se*, may not necessarily equate to greater diversity of pathogens in counter-urbanizing environments or greater disease risk. The 'amplification' effect observed in a prior study of *Leptospira* infection (Peterson et al., in preparation), for example, reflects shifts in rodent abundance and richness, which can independently or conjointly influence symbiont diversity. Some prior work on viral pathogens also indicates that infection may increase with lower host diversity in more modified (i.e., urbanized) environments (Mills 2005, Dearing et al. 2015). It is possible, for example, that disease risk corresponds more to the prevalence of particular constituents of concern, like LCMV, which exhibits a heterogeneous distribution across New Orleans and other cities (Childs et al. 1992). Thus deconstructing observed patterns of host-virome diversity could clarify whether and how counter-urbanization poses a risk to residents and nearby communities

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Appendix

Table 4.1. Locations and information of animals included in each pool.

Neighborhood	Block Code	Species	# Pools	# Indiviuals	Sex (M, F, Mix) # of pool	Proportion *Leptospira* (+)	Proportion Ectoparasite (+)	# Unrarefied reads (/individual)
Bywater	302	R	1	3	0,0,1	0.33	0	26.7
Bywater	Multiple	R	2	7	0,0,2	0.00-0.25	0	9.3
Bywater	Multiple	N	2	6	2,0,0	0.33	0.66	47.8
Bywater	OR92	R	1	2	0,0,1	0.00	0.50	19.0
French Quarter	CNH17	R	1	4	0,0,1	0.00	0.75	21.5
French Quarter	CNH19	N	3	14	0,2,1	0.00-0.6	0.60-1.0	89.9
French Quarter	CNH20	N	2	11	0,0,2	0.20-0.33	1.0	310.4
Gentilly	NO11	M	1	5	0,0,1	0.20	0	1.8
Gentilly	NO122	M	1	4	0,0,1	0.00	0.25	203.5
Gentilly	NO124	R	1	4	0,0,1	0.00	0	3.3
Gentilly	NO127	M	1	5	0,0,1	0.20	0	3.2
Gentilly	Multiple	R	3	13	0,0,3	0.00-0.40	0.50-0.75	60.3
Gentilly	NO128	M	1	6	1,0,0	0.50	0.33	59.3
Gentilly	NO128	N	2	10	0,0,2	0.40-0.80	0.80-1.0	2.7
Gentilly	NO128	R	1	4	0,0,1	0.00	0.75	0.5
Gentilly	OR10	M	1	5	0,0,1	0.20	0.20	4.0
Lakeshore	CNH1	R	1	2	0,0,1	0.00	0.50	223.0
Lakeshore	CNH11	R	1	2	0,0,1	0.00	1.0	50.0
Lakeshore	CNH12	R	1	2	1,0,0	0.00	0.50	92.0
Lakeshore	CNH5	R	2	11	0,0,2	0.00	0.40-0.50	192.5
Lakeshore	CNH6	R	1	3	0,0,1	0.00	0.67	80.0
Lakeshore	NO03	R	1	3	0,0,1	0.00	1.0	27.0
Lakeshore	NO158	R	1	2	0,0,1	0.00	0.50	15.5
Lakeshore	Mixed	R	1	3	0,0,1	0.00	0.33	3.7
Lakeview	Mixed	R	2	10	0,0,2	0.16-0.33	0.33	7.5

Table 4.1. ***Continued***

Neighborhood	Block Code	Species	# Pools	# Indiviuals	#M, #F, #Mix	Proportion *Leptospira* (+)	Proportion ectoparasite (+)	# Unrarefied reads (/individual)
Lakeview	NO42	R	2	14	0,0,2	0.00-0.29	0.57-1.0	9.8
Lower 9	OR1	M	2	9	0,0,2	0.33	0.17-0.67	9.3
Lower 9	OR1	N	2	5	1,0,1	0.00-0.33	0.67	23.8
Lower 9	OR11	M	3	15	0,0,3	0.00-0.83	0.33	16.7
Lower 9	OR11	N	2	12	0,0,2	0.16-0.33	0.67	33.8
Lower 9	OR19	M	1	4	0,0,1	0.50	0.50	65.8
Lower 9	OR19	N	2	9	0,1,1	0.40-0.50	0.75-1.0	420.2
Lower 9	OR19	R	1	3	0,0,1	0.30	1.0	167.0
Lower 9	OR2	N	1	4	0,0,1	0.50	0.75	76.3
Lower 9	OR20	M	1	6	1,0,0	0.17	0.33	5.5
Lower 9	OR21	M	2	13	0,0,2	0.00-0.16	0.14	2.8
Lower 9	OR26	M	3	17	1,0,2	0.00-0.20	0.00-0.50	8.6
Lower 9	OR26	N	1	6	0,0,1	0.17	0.83	167.3
Lower 9	OR26	R	1	4	1,0,0	0.25	0.75	71.5
Lower 9	OR37	M	1	2	0,0,1	0.50	0	2.0
Lower 9	OR37	N	2	12	0,0,2	0.16-0.33	1.0	23.3
Lower 9	OR37	R	1	3	0,0,1	0.00	0.33	9.3
Lower 9	Multiple	R	2	11	0,0,2	0.00-0.40	0.60-0.83	32.8
Lower 9	Multiple	N	2	7	1,0,1	0.20-0.50	0.80-1.0	311.0
Natural Area	NA1	R	2	11	0,0,2	0.00	0.67-1.0	173.9
Natural Area	NA3	M	1	5	0,0,1	0.00	0.20	13.2
Natural Area	NA4	M	1	2	0,0,1	0.00	0.50	3.0
Natural Area	NA5	M	1	6	0,0,1	0.00	0.50	1.2
Natural Area	NA6	M	1	3	0,0,1	0.00	0.33	10.0
Natural Area	NA7	M	1	3	0,0,1	0.00	0.33	6.3
Saint Bernard	OR30	M	1	2	1,0,0	0.50	0.00	8.0
Saint Bernard	OR31	M	1	2	1,0,0	0.00	0.50	1523.0
Saint Bernard	OR41	R	1	4	0,0,1	0.00	0.25	76.8

Table 4.1. ***Continued***

Neighborhood	Block Code	Species	# Pools	# Indiviuals	#M, #F, #Mix	Proportion *Leptospira* (+)	Proportion Ectoparasite (+)	# Unrarefied reads (/individual)
Saint Bernard	OR47	M	4	26	0,0,4	0.60-0.85	0.00	263.6
Saint Bernard	OR52	M	1	2	0,0,1	0.50	0.00	3.5
Underpass	Underpass	N	5	26	1,0,4	0.00	1.0	98.0
Upper 9	536	N	2	11	1,0,1	0.16-0.20	0.50	4.0
Upper 9	Multiple	N	1	5	0,0,1	0.60	0.40	238.2
Upper 9	Multiple	R	2	11	0,0,2	0.00-0.40	0.16-0.60	22.2
Upper 9	NO14	R	1	2	0,0,1	0.00	NA	11.0
Upper 9	OR84	N	1	5	0,0,1	0.40	0.80	107.0
Upper 9	OR89	R	1	3	0,0,1	0.33	0.67	67.0
Upper 9	OR98	N	1	5	0,0,1	0.20	0.60	23.0
Upper 9	OR98	R	1	4	0,0,1	0.00	NA	0.3
Uptown	CNH7	R	1	5	0,0,1	0.20	0	5.6
Uptown	CNH8	R	1	2	1,0,0	0.00	0.50	60.5
Uptown	CNH9	M	1	2	0,0,1	0.00	0	6.5
Uptown	NO166	R	1	2	0,0,1	0.00	1.0	21.5
Uptown	NO54	R	1	5	0,0,1	0.20	0.80	31.4
Uptown	Multiple	N	2	7	0,0,2	0.40-0.50	0.50-0.80	1146.1
Uptown	Multiple	R	2	9	0,0,2	0.00-0.20	0.50-0.60	10.1

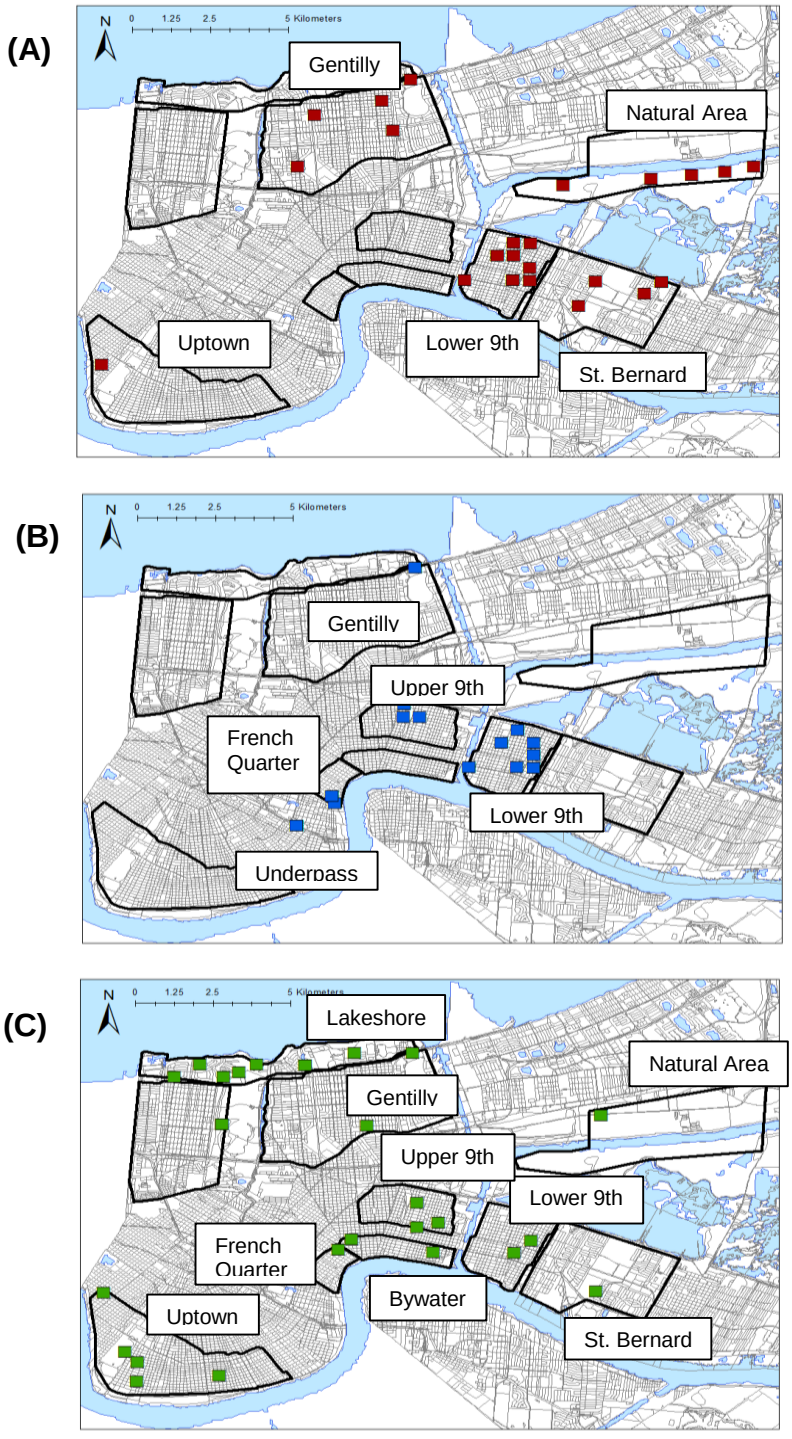

Figure 4.1. Locations of trapping areas and sites from which we obtained virome information from (A) M. musculus; (B) R. norvegicus, and (C) R. rattus. Study neighborhoods are labeled with text.

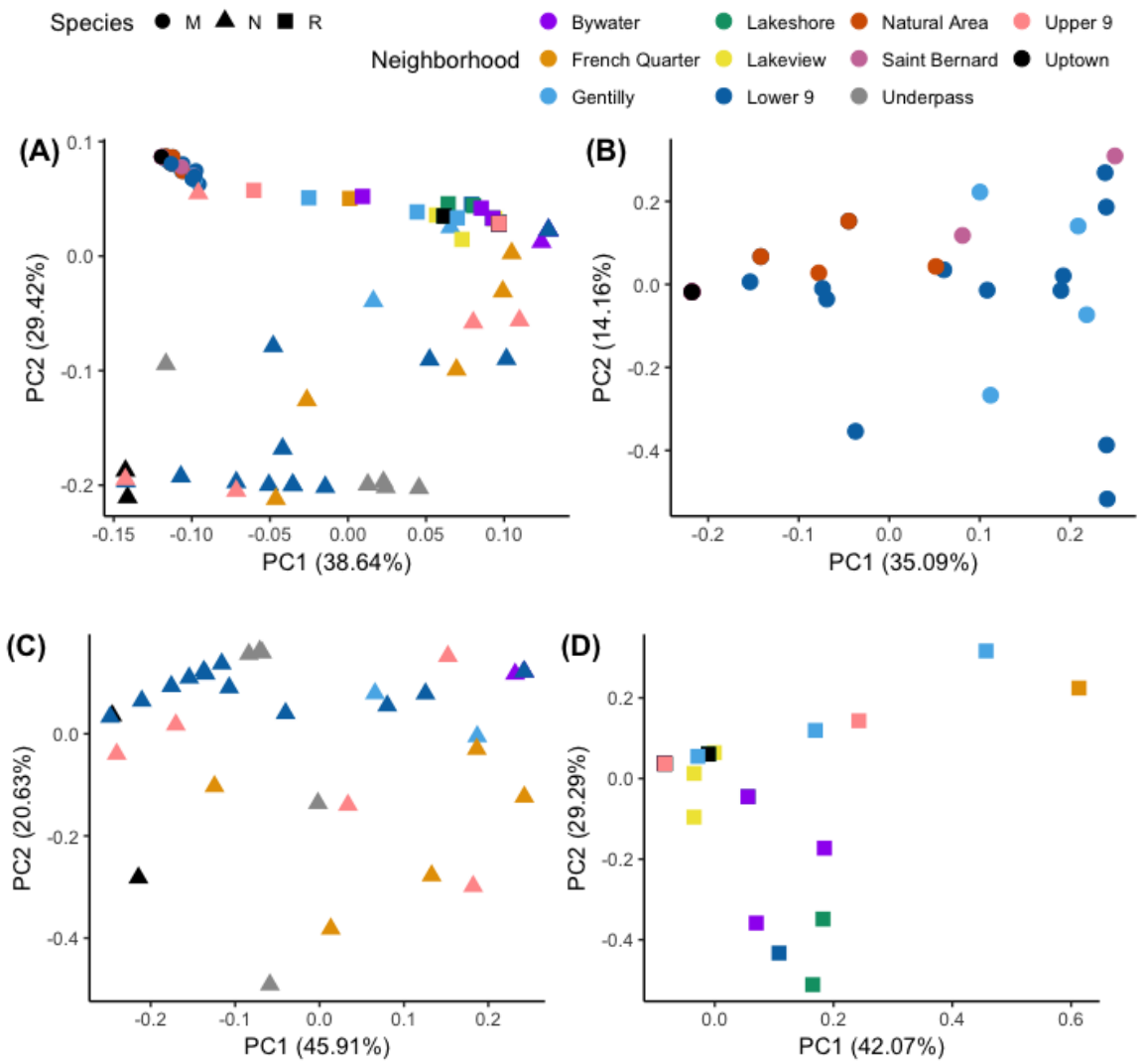

Figure 4.2. PCA plot of log2 transformed rarefied virus communities by species and neighborhood. All species combined (A); viromes of *Mus musculus* (B); viromes of *Rattus norvegicus* (C); viromes of *Rattus rattus* (D).

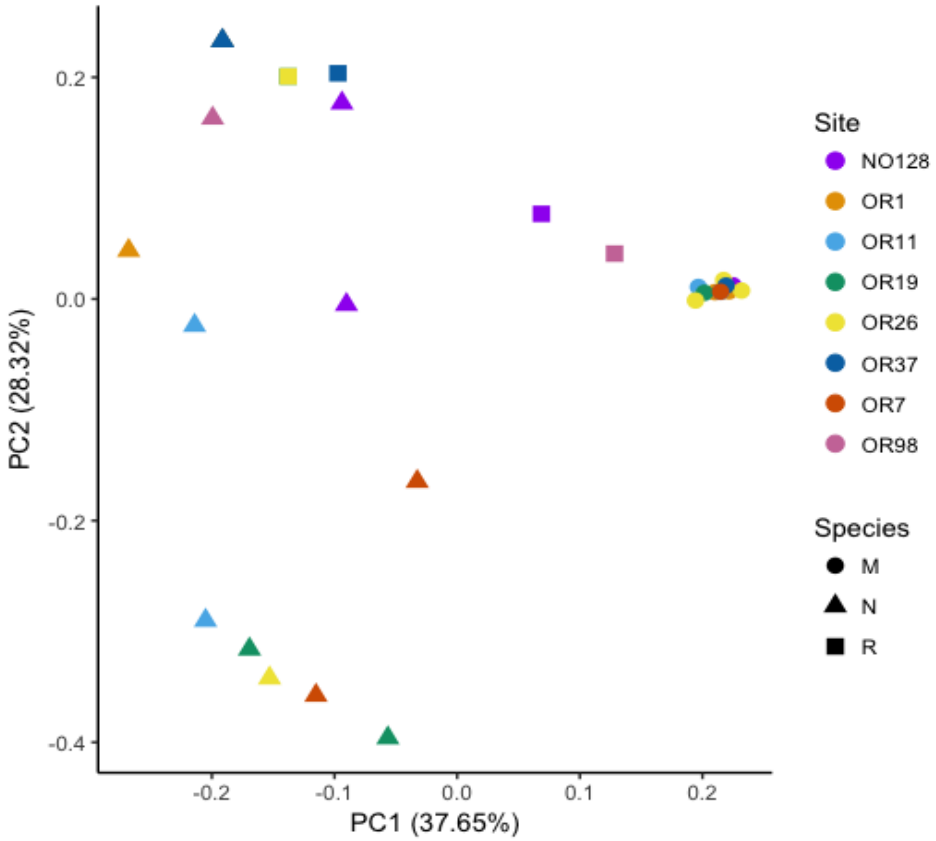

Figure 4.3. PCA plot of log_2 transformed rarefied virus communities from each host species collected on trapping blocks with virome data for >1 species.

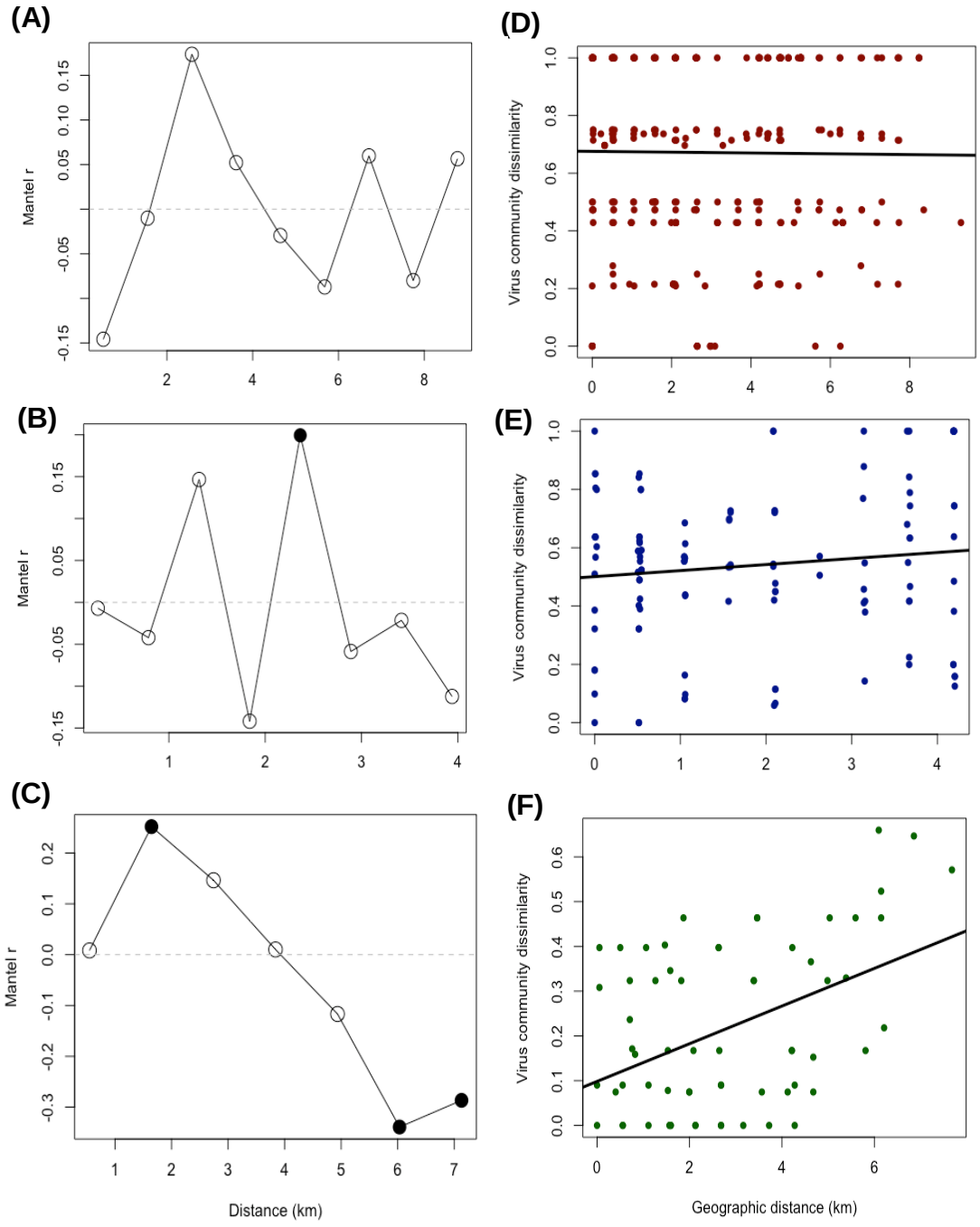

Figure 4.4. Mantel correlograms (A-C) and scatterplots of virome dissimilarity by geographic distance (D-F). Correlations between mantel values from virome dissimilarity and distance matrices (A-C) from trapping sites from contiguous neighborhoods only. Filled-in points represent distances with significantly positive or negative correlation with community composition at a given spatial lag (A-C)

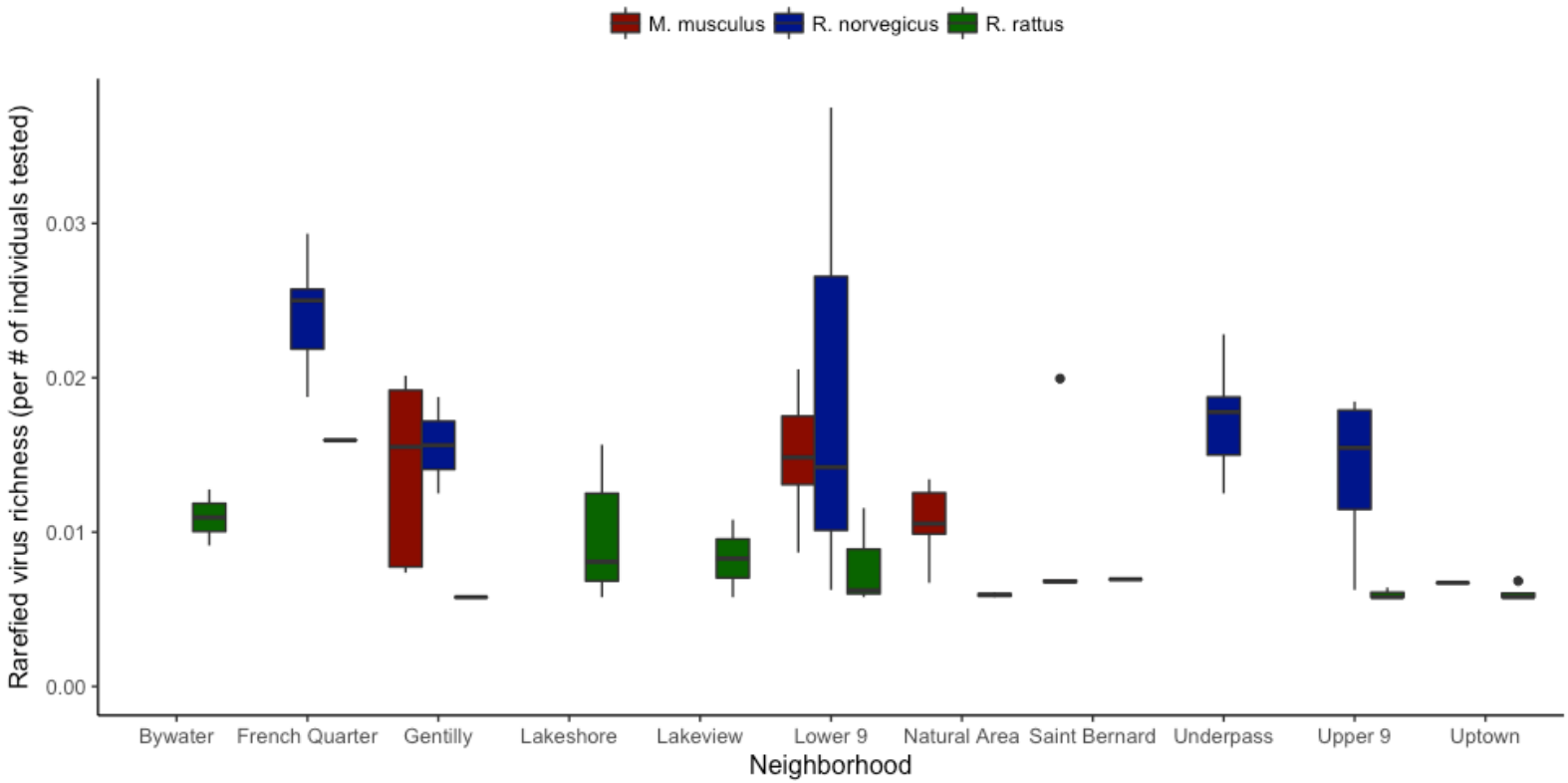

Figure 4.5. Average standardized rarefied virus richness by species and neighborhood.

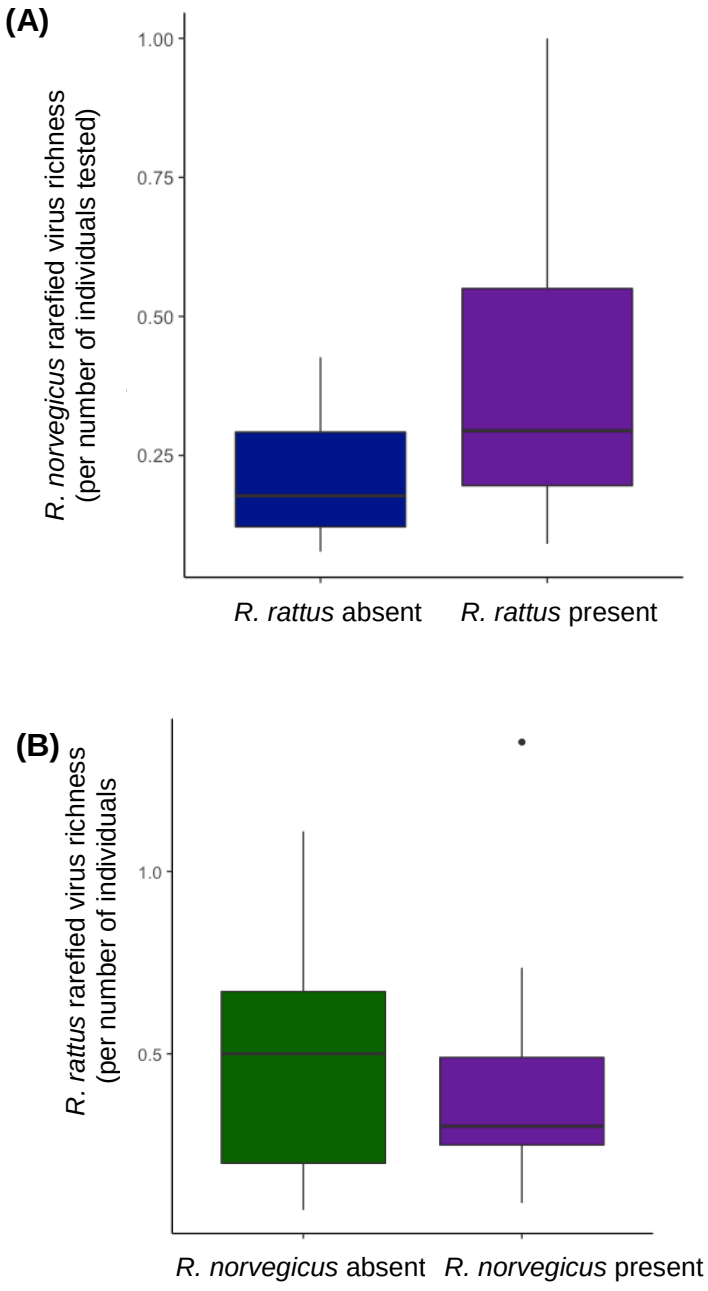

Figure 4.6. Standardized rarefied virus richness in (A) *R. norvegicus* and (B) *R. rattus* from locations where both *Rattus* species were detected, versus locations where each species was detected without the other.

CONCLUSION

Overall, thse findings underscore how storm damage, discriminatory resettlement policies, and municipal differences in landscape management in New Orleans, Louisiana have reinforced socio-environmental disparities since Hurricane Katrina. Urban rodent assemblages reflect the socio-ecological mosaics of abandonment that are present across post-Katrina New Orleans. Though, this work also indicates that management of abandoned areas may potentially mitigate public health concerns.

The risk of some zoonotic pathogens is greatest in areas experiencing counter-urbanization, but pathogen ecology likely influences these patterns. For example, the multi-host pathogen *Leptospira* shows increasing prevalence and infection loads in areas supporting more abundance rodent populations, while *Bartonella* infection, which exhibits a frequency-dependent vector transmission, instead reflects host-ectoparasite interactions, even when comparing across cities. Similarly, virus communities did not show clear associations with counter-urbanization or host diversity. However, virus communities were different among host speces. Suggesting that areas supporting increased host diversity may therefore support an increased total richness of viruses.

The predictors identified in our study indicate that increased risk of rodent pests and some rodent-borne zoonotic pathogens could be mitigated by relatively simple approaches, such as mowing of overgrown vegetation and clearing debris. Combining land management with targeted pest control campaigns may be an especially efficient approach to reducing zoonotic disease risk, particularly from *Leptospira*. Further work is warranted to investigate how interventions can be mounted to best address uneven abandonment and the resulting disparities present across post-Katrina New Orleans and other cities experiencing counter-urbanization.

VITA

Anna Christine Peterson was born in Springville, Utah to Brent and Shirley Peterson as the second of three children. At the age of 7, she, along with her family, moved from Utah to Colorado, where she lived on her families ranch outside of Silt, Colorado until the age of 18. It was here she became fascinated with the natural world, and decided to pursue a career studying ecology. In 2004, she began her undergraduate education at the University of Colorado, Boulder, obtaining a Bachelor's of arts degree in Ecology and Evolutionary Biology in 2008. Her undergraduate honor's thesis work focused on amphibian communities and trematode parasites in Colorado. After several field technician positions and a year spent teaching 7th grade English in Zhengzhou China, she returned to the University of Colorado and obtained a master's of arts degree in Ecology and Evolutionary Biology in 2013. Her master's degree research focused on invasive North American bullfrogs in Colorado and their role as a reservoir for the endemic fungal pathogen *Batrachochytrium dendrobatidis*. Following completion of her master's degree, Anna spent six months in bush Alaska working with the Fish and Wildlife Service as a wilderness fellow. Shortly after, she moved to New Orleans, to begin pursuing her PhD in Ecology and Evolutionary Biology at Tulane University. In 2017, she, and the rest of her lab, transferred from Tulane University to the University of Tennessee, where she completed her dissertation work, presented in this document.