## INFECTIOUS DISEASES IN NATURAL SONGBIRD POPULATIONS ALONG A GRADIENT OF URBANIZATION

by

CATHERINE A. BRADLEY

(Under the Direction of Sonia Altizer)

#### ABSTRACT

Natural environments are increasingly encroached upon by urban development. In the United States, over 80% of the human population now lives in metropolitan habitats. This demographic shift is accompanied by drastic and often permanent changes to the habitat within and around cities, with consequences for wildlife populations. Research has shown that urbanization dramatically alters the composition of wildlife communities, leading to overall loss of species richness and an increased abundance of urban-adapted species. Although urbanization probably lowers the abundance of many wildlife parasites, for some, transmission could increase due to changes in host community composition, greater vector abundance, or increased host susceptibility and recruitment rates. In this doctoral thesis, I investigated associations between wild songbird communities and their pathogens at sites representing an urban gradient in northcentral Georgia (U.S.A.) to identify patterns of infection and to investigate potential mechanisms underlying these patterns. Major findings include: (1) antibody prevalence of West Nile Virus (WNV) in songbirds increased with greater measures of urban land use, (2) WNV antibody prevalence is negatively associated with avian community diversity and positively associated with avian abundance and a composite measure of community competence, (3) avian malaria

infections were common in Northern Cardinals (*Cardinalis cardinalis*), though prevalence was higher in juveniles, and (4) *Salmonella enterica* serovar Muenchen strains were detected in songbirds sampled at one non-urban site, and these were genetically similar to strains previously identified in human outbreaks. In general, results of this dissertation have implications for predicting how wildlife pathogens will respond to future increases in urbanization. Further studies to investigate the mechanisms by which multi-host pathogens respond to urbanization, including reductions in host species diversity and changes in host condition and susceptibility, could aid in future conservation efforts and may facilitate the development of effective preventative measures against human disease.

INDEX WORDS: urbanization, wildlife disease, multi-host pathogen, species diversity, dilution effect, West Nile Virus, Avian Malaria, *Salmonella enterica*, Northern Cardinal (*Cardinalis cardinalis*)

### INFECTIOUS DISEASES IN NATURAL SONGBIRD POPULATIONS ALONG A GRADIENT OF URBANIZATION

by

#### CATHERINE A. BRADLEY

B.S., Tulane University, 2000

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial

Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

© 2009

Catherine A. Bradley

All Rights Reserved

# INFECTIOUS DISEASES IN NATURAL SONGBIRD POPULATIONS ALONG A

#### GRADIENT OF URBANIZATION

by

#### CATHERINE A. BRADLEY

Major Professor:

Sonia Altizer

Committee:

Steven B. Castleberry John M. Drake Gary D. Grossman David E. Stallknecht

Electronic Version Approved:

Maureen Grasso Dean of the Graduate School The University of Georgia December 2009

#### ACKNOWLEDGEMENTS

I would like to thank Sonia Altizer for her guidance in the development of this project and contribution to the resulting manuscripts. I also thank Andy Davis, Samantha Gibbs, Susan Sanchez, and John Maurer who contributed to this work in various ways, from providing training to conducting laboratory analyses of samples. For their work and dedication to this research, I thank those that assisted in the field and in the lab: Laura Gold, Adrienne Tecza, Olivia Wright, Kai Kincheloe, and Ben Stoner-Duncan. My advisory committee, composed of Steven Castleberry, John Drake, Gary Grossman, and David Stallknecht, provided helpful feedback and encouragement during project development. Finally, I would not have been able to complete this work without the welcome of many private homeowners and institutions. I'm very appreciative of their interest, curiosity, and early morning coffee.

#### TABLE OF CONTENTS

			Page
ACI	KNO	WLEDGEMENTS	iv
CH	APTE	R	
	1	INTRODUCTION	1
	2	LITERATURE REVIEW: URBANIZATION AND THE ECOLOGY OF	
		WILDLIFE DISEASES	6
		Abstract	7
		Introduction	7
		Wildlife communities in urban environments	9
		Resource provisioning, host contact rates and susceptibility to infection	10
		Pathogen exposure, pollution, and stress	15
		Changes mediated by climate and seasonality	16
		Consequences for wildlife conservation and public health	18
		Challenges for future research	19
		Concluding remarks	21
		Acknowledgements	21
		Box 1. The scope and study of urbanization	22
		Box 2. Red squirrel-gray squirrel paramyxovirus in the UK	24
		Box 3. Exotic hosts, translocations, and commerce	26

3	URBAN LAND USE PREDICTS WEST NILE VIRUS EXPOSURE IN				
	SONGBIRDS	27			
	Abstract				
	Introduction				
	Methods and Materials	31			
	Results	41			
	Discussion	46			
	Acknowledgements	50			
4	URBANIZATION, HOST COMMUNITY COMPOSITION AND WEST NILE				
	VIRUS EXPOSURE IN SONGBIRDS	51			
	Abstract	52			
	Introduction	53			
	Methods and Materials	56			
	Results	63			
	Discussion	71			
	Acknowledgements	76			
5	JUVENILE NORTHERN CARDINAL (CARDINALIS CARDINALIS) COND	ITION			
	AND THE PREVALENCE OF AVIAN MALARIA IN ADULTS: PATTERNS				
	ACROSS AN URBAN GRADIENT	78			
	Abstract	79			
	Introduction	79			
	Methods and Materials	83			
	Results	90			

#### UNDANT AND LICE DEDICTO WEGT NILE VIDUG EVDOCUDE DI 2

Discussion	93
Acknowledgements	97
6 SALMONELLA SURVEILLANCE IN SONGBIRDS FROM NORTHERN	
GEORGIA, USA REVEALS S. MUENCHEN ISOLATES THAT ARE SIMILAR	
TO THOSE OF HUMANS	98
Abstract	99
Introduction	99
Methods and Materials	101
Results	103
Discussion	107
Acknowledgements	111
7 CONCLUSIONS	112
REFERENCES	117

#### CHAPTER 1

#### INTRODUCTION

Worldwide, and for the first time in human history, a majority of people now live in urban environments (United Nations Department of Economic and Social Affairs 2007). Furthermore, the urban landscape characteristic of many metropolitan areas, dense urban centers encircled by broad areas of sprawl, has precipitated a fast, and often permanent, conversion of land from field or forest to residential and commercial uses. Concomitant with land conversion, urbanization is associated with the loss of biodiversity across a broad range of taxa and the development of an urban wildlife community composed of a relatively few, abundant, and highly adaptable species (Luniak 2004; Chase and Walsh 2006; Olden et al. 2006; McDonald et al. 2008). A better understanding of the characteristics of urban-adapted species (Luniak 2004) and the mechanisms that may drive species loss in urban environments, such as resource competition and altered trophic interactions (Faeth et al. 2004; Shochat et al. 2006), is necessary to determine the potential impacts urban wildlife may have on humans and ways to promote urban biodiversity in this increasingly urbanized world.

In addition to the mechanisms mentioned above, the presence and abundance of species in an urban environment can also be influenced by infectious disease. Theoretical models have shown that the presence of multi-host pathogens can result in the local extirpation of more susceptible host species (Dobson 2004; de Castro and Bolker 2005). The influence of urban landscapes on wildlife infectious disease dynamics is a significant question for both conservation

efforts and public health considerations. Increasing global travel and commerce associated with metropolitan areas creates opportunities for novel contacts between humans and wildlife (Karesh et al. 2005) and these movements have been responsible for the introduction of pathogens with significant impacts to human, wildlife, and plant populations, such as West Nile Virus (Marra et al. 2004), SARS-associated coronavirus (Xu et al. 2004) and *Phytophthora ramorum*, the causative agent of sudden oak death (Rizzo and Garbelotto 2003). As human-wildlife interactions increase both with respect to novel associations and in the intensity of contacts, the emergence and reemergence of significant zoonotic infections could become more significance for global human health (Daszak et al. 2000; Patz et al. 2004).

In the second chapter of this dissertation, Sonia Altizer and I review the potential impacts of urbanization on wildlife infectious disease. We describe the underlying mechanisms driving these alterations, highlight recent studies from across animal taxa, and suggest research areas and methodologies to direct future work. Several factors are identified as likely to influence the epizootiology of wildlife host-pathogen systems in urban areas including host contact rates, host and vector population densities, host stress and tolerance to infection, host community composition, and indirect effects mediated by interspecific interactions such as competition for resources. The mode of transmission, whether the pathogen of interest is a macroparasite or a microparasite, whether the pathogen is host-specific, and the ecology of the vector are among many factors that will determine how the prevalence and impacts of infection will vary as a result of intensified urban land use. In this chapter, we also suggest that urban planning represents a potential tool for altering habitats to lower disease risks for both humans and wildlife hosts, and this represents an avenue for future collaboration among urban planners, veterinary biologists and wildlife ecologists.

The majority of my dissertation work focused on field data collected from songbirds across a network of sites representing a continuum of low-to-high urbanization in north Georgia, USA. This work was conducted across three years, and the overall goal was to determine how pathogen prevalence in wild songbird varied among sites with differing degrees of urbanization and to explore three possible drivers that may underlie the observed patterns: host community composition, variation in host tolerance to infection, and host abundance. In Chapter 3, I show that the prevalence of antibodies to West Nile Virus (WNV) in wild songbird populations increased with greater measures of urbanization, and also found that this might be partly due to lowered host condition or recovery in the least urban sites. Using existing land use maps, ground-truthing procedures, and recent census information, I quantitatively characterized the area at each site composed of various land use types and used this information to develop a measure of urbanization for each site. With this measure as the predictor variable, I looked at the association between urban land use and WNV antibody prevalence in songbirds across age, gender, and a suite of ecological characters such as nesting type and primary food resource. I found that WNV antibody prevalence increased with increasing urban land use and that this pattern was stronger in adult birds than in juvenile birds. Furthermore, I found that the antibody prevalence in Northern Cardinals (*Cardinalis cardinalis*), a ubiquitous and urban-adapted species, was greater than in all other songbirds sampled and suggest that this species may be an important amplifying host in the enzootic cycle of WNV in the region studied here.

Chapter 4 focuses on the relationship between increasing WNV antibody prevalence in wild songbird populations and homogenization of the avian community associated with increasing urban land use. Using previously published information on host competence for a subset of avian species (Komar et al. 2003; Komar et al. 2005) and abundance data obtained

from point counts, I calculated a measure of community competence which represents the functional diversity of the avian community with respect WNV transmissibility. This measure and passerine abundance were both strongly associated with one measure of urban land use, the proportion of forested area that is core area, and with WNV antibody prevalence in the songbird community. The results of this study suggest that species identity and the role of individual species, rather than overall host species diversity *per se*, is key to understanding the variation in WNV prevalence across a landscape.

Chapter 5 focuses on Northern Cardinals as the single most abundant bird species captured across the entire urban-rural gradient. Here, I examine how host condition and measures of stress vary across sites in relation to measures of urbanization. Specifically, I looked at a series of condition measures in the juveniles birds, including a measure of body weight to size, heterophil-to-lymphocyte ratios, red feather coloration, and feather growth rates. I found that juveniles in urban areas had higher H:L ratios, redder feather coloration, and decreased feather growth rates. This may indicate that individuals in urban areas are stressed and have fewer resources (Luniak 2004). I also show that avian malaria infections are extremely common in this species, and for the subset of adult birds, infection was less common in urban areas.

Finally, in Chapter 6 I examine the prevalence of a directly-transmitted bacterial pathogen, *Salmonella enterica*, in songbirds across the same sites sampled for previous chapters. Results showed a low prevalence of infection (<2%) during this non-outbreak year, and the identification of a single serovar, *Salmonella enterica* serovar Muenchen, in three individuals sampled at one site. Isolates from the wild birds were closely related to isolates made from humans based on records at the CDC, and thus could point to the potential for wild songbird

surveillance as a tool for identifying circulating strains of *Salmonella* responsible for human disease.

#### CHAPTER 2

# LITERATURE REVIEW: URBANIZATION AND THE ECOLOGY OF WILDLIFE DISEASES

Bradley, C.A. and S. Altizer. 2007. *Trends in Ecology and Evolution*. 22:95-102. Reprinted here with permission of publisher.

#### Abstract

Urbanization is intensifying worldwide, with two-thirds of the human population expected to reside in cities within 30 years. The role of cities in human infectious disease is well established, but less is known about how urban landscapes influence wildlife–pathogen interactions. Here, we draw on recent advances in wildlife epidemiology to consider how environmental changes linked with urbanization can alter the biology of hosts, pathogens and vectors. Although urbanization reduces the abundance of many wildlife parasites, transmission can, in some cases, increase among urban-adapted hosts, with effects on rarer wildlife or those living beyond city limits. Continued rapid urbanization, together with risks posed by multi-host pathogens for humans and vulnerable wildlife populations, emphasize the need for future research on wildlife diseases in urban landscapes.

#### Introduction

Urbanization is increasing at a global scale, with ecological consequences that extend beyond city boundaries (Box 1). Defined as growth in the area and numbers of people inhabiting cities, urbanization generates landscapes dominated by built-up structures for human use (Grimm et al. 2000; Shochat et al. 2006). Most studies of the ecological impact of urbanization focus on patterns of biodiversity loss, with declines in species richness from rural areas towards the urban core documented across multiple taxonomic groups (Marzluff 2001; McKinney 2002). More recently, ecologists have begun to explore the mechanisms by which urbanization affects biodiversity, including processes related to resource competition, altered trophic interactions and disease (Faeth et al. 2005; Shochat et al. 2006).

An increasing number of studies point to links between human activity and the emergence of wildlife diseases (Daszak et al. 2000; Lafferty and Gerber 2002: Patz et al. 2004), yet only a few address how wildlife–pathogen interactions respond to urban land use. However, urbanization can influence shifts in host ranges and densities (Prange et al. 2003), interspecific interactions (Faeth et al. 2005) and contamination of the environment with pathogens (Coyner et al. 2002). For example, across 176 foraging sites for wading birds in coastal Florida, a highly pathogenic nematode occurred only at sites disturbed by stream engineering and nutrient fluxes (Coyner et al. 2002). Many of the above processes have been studied in the context of agricultural land use or forest edge habitats, and are relevant to pathogen spread in urbanized landscapes.

Here, we identify key hypotheses concerning the role of urbanization in the transmission and impacts of infectious diseases in wildlife populations. To capture a range of mechanisms and changes in their intensity, we consider patterns that occur across the urban–rural gradient (Box 1), in some cases focusing on wildlife species that inhabit both the urban core and surrounding suburban and rural areas. Understanding the ecology of wildlife pathogens in urban environments will become increasingly important for managing disease risks to wildlife and, in some cases, humans. Indeed, most pathogens are capable of infecting multiple host species (Cleaveland et al. 2001; Woolhouse et al. 2001), and some pose serious threats to human health and already vulnerable wildlife populations (Woolhouse and Gowtage-Sequeria 2005). Finally, we emphasize several priorities for future research, including identifying those pathogens for which urbanization is likely to have the greatest impact.

#### Wildlife communities in urban environments

Urbanization dramatically alters the composition of wildlife communities, leading to biodiversity loss (McKinney 2002; Pauchard et al. 2006) and increases in the abundance of species that thrive in urban areas. Indeed, one recent study exploring the patterns and causes of 'biotic homogenization' found a negative relationship among human population size, urban land use and species richness across all major taxonomic groups in North America (Olden et al. 2006). This effect is due, in large part, to simplified habitat structures (Shochat et al. 2006), increased resource availability (Marzluff 2001) and altered trophic interactions (Faeth et al. 2005).

Many animal species disappear from cities altogether, occur at low abundance, or are restricted to parks, forest fragments and other less intensely used areas. Because most wildlife parasites (especially those restricted to one or a few host species) will also be missing from urban centers, an important consideration is how relevant urbanization is to the ecology of wildlife diseases. The answer involves at least three crucial processes (Table 2.1). First, for diseases such as toxoplasmosis or rabies, which affect urban-adapted wildlife species, infection dynamics can change across a gradient of habitats, in some cases leading to increased prevalence in urban or suburban environments. Second, rarer wildlife species, such as many wood warblers or flying squirrels and other small rodents, which persist within city parks or surrounding natural areas, can be affected negatively by pathogens maintained in urban-adapted hosts (Box 2). The increased dominance of a few key host species, and conditions that favor interspecific contact rates, could cause declines of rarer wildlife through competition mediated by multi-host parasites (Woolhouse et al. 2001; de Castro and Bolker 2005). Third, reduced biodiversity of urban wildlife can influence the transmission of some vector-borne diseases through a process termed the 'dilution effect' (Schmidt and Ostfeld 2001; LoGuidice et al. 2003; Keesing et al. 2006).

Here, high host species richness can lower parasite transmission if vectors feed on multiple host species varying in competence with respect to contracting, amplifying and transmitting the pathogen. The reverse situation could occur in urbanized areas if low host diversity increases the proportional abundance of key reservoir hosts.

Lyme disease, caused by the bacterium *Borrelia burgdorferi*, is the best studied example of the dilution effect. This pathogen is transmitted by *Ixodes scapularis* ticks that feed on a large number of mammal species. Studies in suburban environments of northeastern USA, characterized by high forest-edge and low mammalian biodiversity, indicate that a greater proportional abundance of the most competent reservoir for *B. burgdorferi* ( the white-footed mouse, *Peromyscus leucopus*) is linked to increased infection prevalence in ticks, mice and humans (Allan et al. 2003; LoGuidice et al. 2003). Reduced host species diversity probably has a similar role in other wildlife pathogens (Table 2.1) such as West Nile virus (WNV) (Ezenwa et al. 2006), although it is also possible that low host diversity in the extreme urban core could exclude competent reservoir hosts or vectors (Kilpatrick et al. 2006a). Further studies are needed to assess the general importance of the dilution effect and its significance for pathogens in urbanized areas.

#### Resource provisioning, host contact rates and susceptibility to infection

Many urban-adapted species occur at much higher densities in urban and suburban environments than in less-disturbed areas (Marzluff 2001). Abundant resources not prone to seasonal fluctuations, either through accidental (e.g. household waste) or intentional (e.g. bird feeders) provisioning, support these populations. Such high population densities can elevate contact rates

Host	Pathogen <sup>a</sup>	Locality	Effects on host or parasite biology	Refs			
Biodiversity loss and the dilution effect							
White footed	Borrelia	Northeast N.	Forest fragmentation, often near suburbs, linked with	(Allan et al. 2003;			
mouse	burgdorferi (Lyme	America	greater densities of infected ticks and white-footed mice;	LoGuidice et al.			
(Peromyscus	disease)		can result from loss of predators and less-competent	2003)			
leucopus)			hosts				
Passeriformes and	WNV	North America	Seroprevalence in wild songbirds higher in areas densely	(Ezenwa et al. 2006;			
other vertebrate			populated by humans; non-passerine bird diversity	Gibbs et al. 2006b)			
hosts			associated with lower infection rates in mosquitoes and				
			humans				
Resource provision	ning and contact rate	s within urban-a	dapted species				
Raccoon	Baylisascaris	Northeast USA	Higher raccoon abundance and birth rates in urban-	(Prange et al. 2003;			
(Procyon lotor)	procyonis (raccoon		suburban areas; clumped resources increase within-	Wright and			
	roundworm); other		species contact rates, leading to higher parasite richness	Gompper 2005)			
	endoparasites		and increased B. procyonis prevalence				
House finch	Mycoplasma	East N.	Hosts more abundant in regions of high human	(Dhondt et al. 2005)			
(Carpodacus	gallisepticum	America	population density; aggregation at bird feeding stations				
mexicanus)	(mycoplasmal		could increase contact rates and pathogen transmission				
	conjunctivitis)						
Red fox (Vulpes	Echinococcus	European cities	Shifts in dietary behavior and lack of suitable	(Deplazes et al.			
vulpes)	multilocularis		intermediate hosts reduces prevalence in foxes inhabiting	2004; Fischer et al.			
	(tapeworm)		urban centers; risk to humans could increase owing to	2005)			
			encounters with urban-dwelling foxes				
Mule deer	CWD	CO, USA	Disease more prevalent in highly developed and	(Farnsworth et al.			
(Odocoileus			residential areas, possibly owing to host crowding and	2005)			
hemionus)			aggregated food resources				
Interactions with reservoir hosts in or surrounding urbanized habitats							
Gray fox	CPV	San Francisco,	Greater seroprevalence in wild canids captured in urban	(Riley et al. 2004)			
(Urocyon		CA, USA.	zone surrounding park; could be caused by direct or				
<i>cinereoargenteus</i> ) indirect contact with domesticated dogs							
Allegheny	B. procyonis	East. N.	Declines in woodrat from fatal B. procyonis infections	(LoGuidice 2003)			
woodrat		America	linked with exposure to raccoon feces; exposure could				

Table 2.1. Examples and mechanisms illustrating effects of urbanization on the ecology of wildlife–parasite interactions

(Neotoma magister)			increase at the periphery of urban-suburban development		
Red squirrel (Sciurus vulgaris)	Squirrel paramyxovirus	UK	Non-native gray squirrels introduced highly lethal virus; food provisioning in urban–suburban environments could increase squirrel contact rates and influence pathogen-	(Rushton et al. 2000; Tompkins et al. 2002)	
Environmental cor	tominanta haat stra	a and augoantih	mediated declines		
Environmental cor		s, and susceptio		0.011 ( 1.0000)	
Southern sea otter	Toxoplasma gondu	West coast of	Infections higher in areas of maximum freshwater runoff	(Miller et al. 2002)	
(Enhydra lutris	(meningoencephalitiUSA		associated with regions of high human density or		
nereis)	c disease)		activity; probably owing to exposure to cat feces via sewage contamination		
Great tit (Parus	Stress biomarkers	Southwest	Measure of oxidative stress affected by air pollution	(Isaksson et al.	
major)	(no specific pathogen)	Sweden	increased from rural–urban locations; greater stress and reduced plumage condition could be associated with susceptibility to disease	2005)	
European	Acute stress	Munich,	City-born hosts showed reduced acute stress response	(Partecke et al.	
blackbird ( <i>Turdus merula</i> )	response (no specific pathogen)	Germany	relative to forest-born conspecifics; indicates that species capable of evolutionary adaptation might thrive in urban environments and could be less affected by infectious diseases	2006)	

<sup>a</sup>Abbreviations: CPV, canine parvovirus; CWD, chronic wasting disease;

within and among wildlife species, and favor the transmission of parasites spread by direct contact or oral–fecal routes (Table 2.1). In response to resource provisioning, increased birth rates among urban-adapted species could provide further opportunities for parasite transmission by increasing the abundance of susceptible juvenile hosts (e.g., Prange et al. 2003).

The spatial distribution of resources also influences host aggregation and contact patterns. One study of macroparasite infections in wild raccoons (*Procyon lotor*) showed that experimentally clumping resources resulted in elevated host densities and increased prevalence of the raccoon roundworm (*Baylisascaris procyonis*), particularly among juvenile animals (Wright and Gompper 2005). This approach also provided evidence for greater parasite species richness per individual host under the clumped resource treatment, supporting a direct effect of aggregated resource distributions on within-species contact rates and parasite transmission.

Concentrated resources also influence host migration into urban landscapes and amongspecies contact rates, including contact between humans and wildlife hosts. One example is provided by the increasing number of red foxes (*Vulpes vulpes*) in European cities (Figure 2.1) (Deplazes et al. 2004). Red foxes are the primary sylvatic reservoir of *Echinococcus multilocularis*, a tapeworm that causes liver disease in humans. The parasite is transmitted to intermediate hosts (typically rodents) by the ingestion of eggs deposited in fox feces. Although human cases are widely restricted to rural areas in northern Europe, where outdoor work and pet ownership are significant risk factors (Gottstein 2001), migrations of infected foxes into urban areas might elevate human infection risk in cities. Current evidence suggests that there is little impact to humans living in urban areas; however, because *E. multilocularis* has a long incubation period (decades in some patients), long-term surveillance will be required to assess shifts in human infections associated with fox movements. The red fox–*E. multilocularis* system further

emphasizes that multiple mechanistic drivers associated with urbanization can influence patterns of infectious disease risk. One recent study showed that, although fox populations are increasing in Zurich, Switzerland, the prevalence of *E. multilocularis* within those populations is not (Fischer et al. 2005). Transmission of *E. multilocularis* could decline if foxes consume fewer intermediate hosts (as prey) in favor of human-generated resources in cities.



Figure 2.1. Many wildlife species can be attracted to urban centers, potentially bringing infectious disease with them. The European red fox, the major sylvatic host for *Echinococcus multilocularis*, has become increasingly urban-adapted in cities such as Zurich and Geneva, Switzerland. Many factors, including primary and intermediate host densities, the parasite life cycle, and host dietary behaviors influence the prevalence of infection in urban centers, and infection risks for domestic animals and humans. Reproduced with permission from Peter Arnold, Inc.

Resource provisioning in urban environments also affects host susceptibility and responses to both existing and introduced pathogens, mediated through effects on physical condition and immune defenses. For example, animals that are malnourished owing to low protein intake can become immunosuppressed, shedding more parasite eggs in feces and suffering higher rates of mortality following infection (Ezenwa 2004). Thus, although elevated food resources for urban-adapted species could increase contact rates and pathogen transmission, supplemental feeding might also improve host condition, increase immunity to infection and decrease pathogen impacts on host survival and reproduction.

#### Pathogen exposure, pollution, and stress

Cities serve as significant hubs of pathogen introductions (Box 3) and as sources of infection for wildlife that exist at the periphery of urban centers (Riley et al. 2004; Suzan and Ceballos 2005). For example, infectious oocytes of the protozoan parasite *Toxoplasma gondii*, the causative agent of toxoplasmosis, are shed in the fecal material of domesticated and wild felids. This pathogen also causes infections in other mammals associated with both urban landscapes and more natural habitats (e.g., Dubey et al. 2004). For example, Toxoplasma infections have been linked to southern sea otter (*Enhydra lutris nereis*) mortalities off the coast of California, USA (Miller et al. 2002). Infection rates in otter populations were three times higher in areas of maximum freshwater runoff along the Californian shoreline, most of which were associated with regions of high human density. This association is probably a result of water contamination by cat feces and parasite amplification by benthic filter feeders that comprise a major component of sea otter diets (Miller et al. 2002).

Changes in stress and pollution along the urban–rural gradient could also affect host susceptibility to infectious diseases (Table 2.1). Among vertebrate animals, chronic stress can lower resistance to infection and intensify the harmful effects of pathogens through effects on the host immune system (McCabe et al. 2000; Padgett and Glaser 2003), mediated, in part, by glucocorticoid hormones, such as cortisol. Increased interspecific competition in urban environments (Ruiz et al. 2005) has been linked to chronically elevated stress indices in wild bird populations, although further research is needed to evaluate associations among urbanization, immunity and stress in wildlife populations.

Furthermore, some heavy metal and pesticide pollutants become concentrated in the environment around developed areas and can become detrimental to vertebrate immune function (Bernier et al. 1995; Krzystyniak et al. 1995). A five-year field study of two amphibian species, the marine toad (*Bufo marinus*) and whistling frog (*Eleutherodactylus johnstonei*), found high levels of copper, cadmium and a byproduct of DDT decomposition in tissues samples, and further documented a decrease in B cell-mediated immunity and an increase in helminth infections (Linzey et al. 2003). More generally, however, the effects of environmental pollutants on host resistance to infectious diseases in wild animals are not well understood, and this area of research will become increasingly important as air, soil and water pollutants continue to accumulate around areas of human activity.

#### Changes mediated by climate and seasonality

Research conducted in major metropolitan centers such as Tel Aviv, Israel and Phoenix, AZ, USA indicates that urban microclimates are typically warmer than outlying areas (Saaroni et al. 2000; Baker et al. 2002). The urban heat island results from the increased retention of solar heat

by impervious surfaces, radiant heat trapped by smog and a lack of shade vegetation. Several vector-borne diseases might respond to temperature changes associated with urbanization, particularly as these areas can also provide irrigated regions necessary for vector reproduction (Shochat et al. 2006). Specifically, more moderate winters and the dampened seasonality of urban centers could increase the survival, breeding success and activity of arthropod vectors that are essential for the transmission of many pathogens. In Stockholm, Sweden, for example, such conditions have lengthened the period of activity in the tick *Ixodes ricinus*, coinciding with an elevated incidence of tick-borne encephalitis in rodents and humans (Lindgren and Gustafson 2001). However, other factors could also have a role in this pattern, including human outdoor activity and higher rodent host densities; few studies have examined heat island effects on the ecology of vector-borne diseases.

Reduced seasonality in urban areas could also affect the persistence of parasite transmission stages in the environment (Louis et al. 2005) and could favor the migration into cities of animals that serve as reservoir hosts for pathogens affecting humans (Parris and Hazell 2005). Reduced seasonality can also increase population growth rates of some wildlife hosts by lengthening their breeding season, an effect that has been demonstrated for dark-eyed juncos (*Junco hymenalis*) inhabiting an urban site in California (Yeh and Price 2004). In such cases, a longer recruitment period could increase the probability of epidemics for pathogens that depend on susceptible hosts produced via new births (Altizer et al. 2006). By contrast, milder winter climates might reduce the individual-level impacts of infectious disease, especially if infected animals in harsher seasonal climates frequently die of secondary causes, such as exposure or starvation. Thus, similar to the effects of food provisioning, changes in the urban microclimate have multiple and, in some cases, opposite effects on pathogens affecting wildlife hosts.

#### **Consequences for wildlife conservation and public health**

Although habitat loss and overexploitation are widely recognized as major causes of wildlife population declines, infectious diseases have become increasingly significant to animal conservation (Daszak et al. 2000; Lafferty and Gerber 2002). Beyond the direct impacts of urbanization on biodiversity, epidemiological processes altered by urban habitats can generate further challenges for wildlife populations. Of particular importance are multi-host pathogens that affect animals living at low population densities through interactions with other host species (Box 2; Table 2.1). For example, Cooper's hawks (*Accipiter cooperii*) nesting in urban areas experienced more than double the nest failure rate of hawks nesting in the suburbs. Most nestling mortalities were caused by trichomoniasis, a protozoan disease that can be transmitted through feeding on infected pigeons and doves (Boal and Mannan 1999). Efforts targeted at lowering transmission among urban-adapted species (such as vaccination, treatment with anti-parasitic drugs or reducing supplemental food resources) could therefore limit pathogen transmission to less-abundant wildlife hosts.

Many wildlife species are absent from urban centers, and species that survive well in cities generally do not warrant conservation concern. However, several examples in Table 2.1 emphasize that processes occurring in cities and suburbs influence remnant wildlife populations within cities and can reach beyond the city limits. In at least one case, environmental contamination with *B. procyonis* by infected raccoons is directly linked to population declines in an endangered host species, the Allegheny woodrat (*Neotoma magister*) (LoGuidice 2003). Decreasing risks to wildlife for this and other pathogens might require limiting the build up of environmental pathogen pollution, or reducing the population densities of reservoir hosts and tracking their movements from urban to more rural areas.

A better understanding of processes that impact wildlife–pathogen dynamics in urban landscapes should also point towards new approaches for limiting the risk of human exposure to zoonotic diseases. For example, information about population densities, contact rates and movements of skunks and raccoons have been used to predict urban sites at high risk for rabies outbreaks (Broadfoot et al. 2001). Similar information for foxes harboring *E. multilocularis* can suggest strategies for concentrating urban disease control efforts, including baited vaccines or chemotherapy targeted at urban foxes (e.g., Hegglin et al. 2003) and limiting resource accumulation where animals might congregate near human dwellings.

Finally, urban planning represents a potential tool for altering habitats to reducing disease risks for both humans and wildlife hosts. Efforts to decrease impervious surface coverage, such as urban reforestation projects, could lower the potential for heat island effects on host reproduction, vector breeding and pathogen transmission. Because high wildlife biodiversity might reduce the net transmission of some multi-host vector-borne pathogens (Keesing et al. 2006), increasing native vegetation and creating habitat corridors to facilitate reintroductions could also reduce pathogen prevalence and limit the risk of human exposure. We are not aware of any such strategies in use in urban settings to mitigate disease risk, but this represents an avenue for future collaboration among urban planners, veterinary biologists and wildlife ecologists.

#### **Challenges for future research**

As human populations continue to migrate into cities and urban areas expand (Box 1), managing disease threats for humans and wildlife will depend on future research at the interface of two rapidly growing disciplines: urban ecosystem studies and infectious disease ecology.

Surveillance programs targeted towards zoonotic agents would improve the ability of scientists to detect new agents entering cities and to document infections of pre-existing pathogens in novel host species. Also needed are studies that identify host–pathogen systems restricted to wildlife for which urbanization has significant impacts. Often overlooked for zoonotic diseases, these pathogens can have important consequences for animal species already threatened by other factors (de Castro and Bolker 2005; Woolhouse and Gowtage-Sequeria 2005).

Perhaps most importantly, experimental and modeling approaches are needed to move beyond associational patterns and to tease apart the complex mechanisms by which urbanization affects hosts, pathogens and vectors. For example, to what degree do air pollution, noise and other environmental stressors influence wildlife susceptibility to infection, and are these effects counterbalanced by increased food resources or milder winter climates in cities? How does host immunity interact with physical landscape characteristics that alter host contact rates? In terms of vector-borne diseases, better information is needed regarding how biting arthropods are influenced by the heat island effect, distribution of breeding sites and shifts in host-species availability across urban–rural gradients.

To address these and other questions, manipulative field studies will become increasingly important for investigating wildlife–pathogen interactions in urban environments. Within the past decade, two cities in the USA were added to the Long-Term Ecological Research site network (http://www.lternet.edu/; funded by the National Science Foundation): Baltimore, MD and Phoenix, AZ (Grimm et al. 2000). Together with urban research sites in Europe, Asia and Latin America, these cities can serve as grounds for testing hypotheses concerning the effects of host species diversity, food provisioning and environmental variables on the spread of wildlife diseases (Allan et al. 2003; Kilpatrick et al. 2006a). In turn, studies replicated across multiple

locations can begin to explore how factors such as wildlife community composition, pollution and urban microclimates are affected by human population density, socioeconomic variables and land-use patterns.

#### **Concluding remarks**

Our goal here was to identify key hypotheses concerning how wildlife-pathogen interactions will respond to urbanization and to highlight several examples that best illustrate these processes. Unlike other land-use changes that can influence wildlife disease emergence (e.g. forest fragmentation or agricultural intensification), the extreme changes that accompany urbanization probably cause declines or losses of most wildlife species and their associated parasites. However, the debate over positive versus negative effects of urbanization on the prevalence and impacts of wildlife diseases is likely to intensify as more research is published. Better understanding of the types of wildlife pathogens that do persist in urbanized areas, and mechanisms that cause increases in prevalence or impacts, can point to new strategies for limiting the risk of human and wildlife exposure in urban centers, and will improve our understanding of the ecological drivers behind spatial variation in pathogen occurrence.

#### Acknowledgements

We thank A. Davis, J. de Roode, E. Lindsey, A. Pedersen, P. Rohani, H. Wearing, S. White, and four anonymous referees for helpful comments on the article. This work was supported, in part, by a University of Georgia graduate fellowship to C.A.B. and a research grant from the Southeastern Center for Emerging Biological Threats (Atlanta, GA, USA).

#### Box 1. The scope and study of urbanization

Over 65% of the human population worldwide will reside in cities by 2025 and those areas will double in land coverage over the same period (United Nations Department of Economic and Social Affairs 2003). Most of this shift will occur in the developing world (Figure 2.2), where the urban population is expected to grow to four billion by 2030 (United Nations Department of Economic and Social Affairs 2003). Urban population growth and movement into cities closely mirrors social and economic reforms, such that shifts in human populations worldwide from rural to urban areas often reflect changes in employment opportunities, access to education and healthcare, and environmental degradation outside of cities.



Figure 2.2. The urban sprawl of Rio de Janeiro, Brazil.

The spread of urban centers results in rapid and dramatic landscape-level changes that are relatively permanent over ecologically significant time periods. Thus, urbanization signifies a drastic form of land conversion typified by dense human habitation, transportation, industry and associated infrastructure. In addition to greater human population density, changes that occur along urban–rural gradients include the loss of biota and natural habitat, increased densities of roads, buildings and other impervious surfaces, and microclimatic shifts (e.g. heat island effects).

Urban ecologists examine the interactions and feedbacks between human activities and ecological systems in urban landscapes (Grimm et al. 2000; Shochat et al. 2006). One major challenge involves the need to categorize land-use types consistently based on quantifiable patterns that are relevant to ecological processes (Theobald 2004). Often, urbanized areas are classified subjectively using terms such as urban, suburban, exurban and rural (see Theobald 2004 for discussion). From a quantitative perspective, human population density and impervious surface coverage are commonly used to quantify the degree of urbanization and facilitate amongsite comparisons. Census data on socioeconomic conditions at study sites can provide additional information relevant to underlying ecosystem processes. For example, higher family incomes and older buildings were strong predictors of plant species diversity in the Phoenix metropolitan area in Arizona, USA (Hope et al. 2003). This association is probably related to higher priority placed on gardening and landscaping among families with higher incomes, and was also a significant predictor of wildlife biodiversity in some taxonomic groups.

An important consideration in urban landscape studies is the appropriate spatial scale for analysis. Conducting analyses at multiple scales is essential for detecting the relative importance of key landscape variables. In the case of infectious disease risk, host abundance and diversity, vector activity and pathogen survival in the environment will probably be affected by urban

landscape measures at different spatial scales (Ostfeld et al. 2005); these scaling patterns will further depend on host dispersal abilities, host and vector habitat preferences, and the mode of parasite transmission.

#### Box 2. Red squirrel-gray squirrel paramyxovirus in the UK

The populations of red squirrels (*Sciurus vulgaris*) (Figure 2.3a) throughout the UK have declined following the introduction of gray squirrels (*Sciurus carolinensis*) (Figure 2.3b), a species endemic to North America (Wauters and Grunell 1999). Although reductions in red squirrel populations can result from direct competition for resources between the two species (Wauters and Grunell 1999), gray squirrels also introduced a squirrel paramyxovirus that is highly lethal to red squirrels (but causes no discernible pathology in gray squirrels; Tompkins et al. 2002).

Results from a model developed by Tompkins et al. (2003) support the conclusion that observed red squirrel declines are consistent with both direct and indirect competition with gray squirrels (Figure 2.3c). Using a stochastic individual-based model and applying this model to real landscapes, Rushton et al. (2000) explored the role of the paramyxovirus virus in red squirrel population reductions. The results indicate that apparent competition between red and gray squirrels led to local loss of red squirrels under a large range of parameter values and assuming low levels (10%) of infection in gray squirrels. The population-level effects of the pathogen were most strongly influenced by interspecific encounter rates (Figure 2.3d), suggesting that increased contact between infected and healthy individuals could raise the risk of disease-mediated population loss. Such encounter rates could increase around urban areas if food provisioning leads to greater contacts within and between host species. The introduction of this urban-adapted

animal species and the subsequent spread of paramyxovirus in red squirrels illustrates the potential synergistic effects of urbanization and pathogen-mediated competition on the abundance of more vulnerable wildlife hosts.



Figure 2.3. Red squirrels (a) versus gray squirrels (b) in the UK. (c) The number of 5-km grid squares occupied by gray (solid line) and red (dashed line) squirrels between 1960 and 1982 in Norfolk, UK (i) Observed data adapted from Reynolds (1985). (ii) Model predictions incorporating direct and indirect interspecies competition. (iii) Model predictions assuming only direct competition. Model results shown in (ii) more closely replicate the field data both in timing and amplitude, providing evidence for the role of infectious disease in the population dynamics of both species. Model simulations (d) indicate that the persistence of red squirrels declines with increasing encounter rates between squirrel individuals and the rate of infection given an encounter occurs. Reproduced with permission from Tompkins et al. (2003) (b) and Rushton et al. (2000) (c).

#### Box 3. Exotic hosts, translocations, and commerce

As centers of transportation and trade, cities can function as 'first points of entry' for novel pathogens and, in some cases, might provide new opportunities for cross-species transmission. Anthropogenic introductions have expanded the geographical ranges of pathogens such as WNV in humans and wildlife (Marra et al. 2004), amphibian chytridiomycosis (Rachowicz et al. 2005) and *Phytophthora ramorum*, the causative agent of sudden oak death (Rizzo and Garbelotto 2003). Among wildlife populations, the 1999 introduction of WNV into New York City was particularly significant. The strain isolated from this outbreak belongs to the more virulent 1a clade of WNV and most closely resembles virus circulating in Israel, suggesting that human activity facilitated the movement of this agent into North America (Lanciotti et al. 2002). Once established around New York City, the virus spread rapidly across the continent and has caused >2800 cases of human disease and tens of thousands wild bird mortalities (Marra et al. 2004; Peterson and Hayes 2004).

Cities also bring wildlife and their products in close proximity to humans and domesticated animals through live animal markets and the wildlife trade (Karesh et al. 2005). Examples of disease emergence events linked with the wildlife trade and live animal markets, including SARS-associated coronavirus (Xu et al. 2004), emphasize that as global commerce and travel increase, documenting pathogen introductions and the contacts among of novel host species will become a more pressing concern in public health management. As such, periodic surveillance centered on cities with large human populations and commerce in wildlife products could represent a useful approach for detecting novel transmission events.

#### CHAPTER 3

#### URBAN LAND USE PREDICTS WEST NILE VIRUS EXPOSURE IN SONGBIRDS

Bradley, C.A. S.E.J. Gibbs, and S. Altizer. 2008. *Ecological Applications*. 18:1083-1092. Reprinted here with permission of publisher.
### Abstract

Urbanization is a widespread phenomenon that will likely influence the prevalence and impact of wildlife pathogens, with implications for wildlife management and public health policies towards zoonotic pathogens. In this study, wild songbird populations were sampled at 14 sites along an urban-rural gradient in the greater metropolitan Atlanta (Georgia, USA) area and tested for antibodies to West Nile virus (WNV). The level of urbanization among sites was quantitatively assessed using a Principle Component Analysis of key land use characteristics. A total of 499 individual birds were tested during the spring and summer over three years (2004-2006). Antibody prevalence of WNV increased from rural to urban sites and this trend was stronger among adult birds relative to juveniles. Furthermore, antibody prevalence among Northern Cardinals (*Cardinalis cardinalis*) was significantly higher than in other songbird species along the urban gradient. Findings reported here indicate that ecological factors associated with urbanization can influence infection patterns of this vector-borne viral disease, with likely mechanisms including changes in host species diversity and the tolerance or recovery of infected animals.

## Introduction

Increasing urbanization, characterized by drastically altered landscapes and dense human populations, is a worldwide phenomenon. Fully two-thirds of the world's population, or 4.9 billion people, are expected to reside in cities by 2030 (U.N. World Prospects 2005 Revision). Studies exploring the influence of urban landscapes on wildlife ecology demonstrate lowered biodiversity and shifting community assemblages (Olden et al. 2006, McKinney 2002), changes

in interspecific competition, individual stress and reproduction, and altered trophic interactions in these urbanized areas (Faeth et al. 2005, Partecke et al. 2006, Shochat et al. 2006).

Urbanization can also affect the dynamics of infectious diseases in wildlife, with several recent articles pointing towards potential underlying mechanisms such as altered host contact rates, changes in vector ecology, or factors that affect host susceptibility to infection (Patz et al. 2004, Bradley and Altizer 2007). For example, recent work by Farnsworth et al. (2005) showed that Chronic Wasting Disease is significantly more prevalent in mule deer (*Odocoileus hemionus*) populations inhabiting developed areas than those in natural areas. This is potentially due to increased contact with the infective agent, or due to higher rates of intraspecific contact as a result of habitat loss. In another example, Cooper's hawks (*Accipiter cooperii*) nesting in urban areas demonstrated more than double the nest failure rate of hawks residing in more rural environments; a likely cause was trichomoniasis, which was observed more commonly in the urban-dwelling hawks (Boal and Mannan 1999).

West Nile virus (WNV; Flaviridae; Flavivirus) is a vector-borne zoonotic virus maintained in avian hosts and principally transmitted by mosquito species in the Culex genus (Peterson et al. 2004). After the initial introduction of WNV to North America in New York City in 1999, the virus rapidly spread and reached Georgia by the summer of 2001 (Petersen and Hayes 2004). The virus has been associated with thousands of avian mortalities since its initial introduction to North America with significant impacts to highly susceptible species. For example, Caffrey et al. (2005) reported an estimated 72% decline in an American crow (*Corvus brachyrhynchos*) population after the first year of WNV exposure; similarly, four greater sage-grouse (*Centrocercus urophasianus*) populations experienced a 25% reduction in late-summer survival upon the arrival of WNV (Naugle et al. 2004). Such high mortality rates are likely due

to the virulence of the WNV strain introduced into North America coupled with a lack of immunologic resistance that might have been provided by previous exposure to other closely related flaviviruses (Peterson et al. 2004, Brault et al. 2004).

Mechanisms that cause variation in WNV prevalence are not well understood, but recent work indicates that changes in host community composition can influence patterns of viral transmission. Specifically, high host species diversity can lower the transmission of some vectorborne diseases if less competent reservoir hosts dilute pathogen transmission between vectors and highly competent hosts (a mechanism termed the 'dilution effect;' Ostfeld and Keesing 2000; Allen et al. 2003; Ezenwa et al. 2006). Passeriformes, an order that includes most songbirds, represent highly competent hosts for WNV (Komar et al. 2003b). However, the ability to contract, amplify and transmit the virus varies greatly among bird species (Komar et al. 2003b, Marra et al. 2004, Gibbs et al. 2006a). If habitat changes associated with urbanization function to lower host species diversity and also increase the relative abundance of key hosts, then WNV transmission could be higher than expected at urban sites.

Vector feeding preferences will also affect the dynamics of this multi-host arbovirus as demonstrated by Kilpatrick et al. (2006b), who showed that American Robins (*Turdus migratorius*), relatively uncommon in their sample population, accounted for a large fraction of mosquito blood meals in the Washington D.C. area. Shifts in vector population dynamics associated with increasing breeding sites or warmer microclimates in urban areas could increase exposure to West Nile virus among birds and humans inhabiting urban environments (Epstein 2001; Campbell et al. 2002).

As a third mechanism, resource provisioning in urban environments (e.g., bird feeders and fruiting plants in residential areas) could improve avian host condition or immune defenses,

facilitating host survival following infection (Bradley and Altizer 2007). Thus, the observed frequency of exposed and recovered birds could increase with greater urbanization not because of differential viral transmission, but owing to differential host recovery or tolerance of infection.

In this study, songbird populations were sampled along an urban-rural gradient to evaluate how West Nile virus antibody prevalence in natural avian communities co-varied with urban land use in Atlanta (Georgia, USA), a rapidly growing metropolitan area. Urban sprawl in this area is associated with the net loss of 133 acres of forest each day (American Forests 2001), and a recent state-wide survey of wild songbirds in Georgia demonstrated a weak positive association between WNV antibody prevalence in songbirds and urban/suburban land use on a broad spatial scale (Gibbs et al. 2006b). We also investigated the role of host age, nest type, diet, and taxonomic family in explaining variation in WNV antibody prevalence. Finally, a subset of analyses focused on patterns of antibody prevalence and body condition across the urban-rural gradient in Northern Cardinals (C. cardinalis), an abundant species in the southeastern USA. Northern Cardinals have been shown to be competent hosts of WNV in studies of captive birds, and display significant tolerance to the infection as evidenced by the high seroprevalence rates observed in wild populations (Komar et al. 2005; Gibbs et al. 2006a).

### **Methods and Materials**

# Site selection and characterization

Between April and August of 2004-2006, wild songbirds were captured and sampled at 14 sites in and around metropolitan Atlanta (Figure 3.1). Sites were chosen to reflect variation in land use (residential, commercial, or recreational), human presence (e.g., residential areas or nature preserves), and distance from the city's center (Table 3.1). Selection was also based on land



Figure 3.1: Map showing the geographic location of the 14 study sites and the location of the sample area within Georgia. 132km separate the two most distant sites and the minimum distance between sites was 1 km.

Site ID	Urban Score	Category	Site Description
*1	1.419	U	Dense urban residential area
2	1.391	U	Forest fragment located inside dense urban area
*3	1.203	U	Border of central city park and downtown residential neighborhood
4	0.983	U	Emory University campus annex with low use
5	0.540	U	Emory University main campus
6	0.481	U	Forest fragment in a residential neighborhood with little active management
7	0.205	U	Urban neighborhood and park mix
*8	-0.400	NU	Relict farmland preserved by county park department; low density residential
9	-0.708	NU	Suburban residential neighborhood
*10	-0.711	NU	Forest fragment preserved by local climbers as a bouldering site
11	-0.963	NU	Relict farmland preserved by county park department
12	-0.998	NU	Habitat set aside for songbirds within managed park
13	-1.219	NU	Rural, low density; farm and residence mix
*14	-1.223	NU	Rural, low density; farm and residence mix

Table 3.1. Quantitative measures of urbanization (Urban Score, described in Methods) and habitat description for the sampling locations around Atlanta, Georgia, USA.

U=Urban, NU=Non-Urban; \*Omitted in the analyses restricted to sites with > 30 samples.

owner permission, accessibility, and the presence of woody vegetation to facilitate the capture of birds using mist nets. Birds were captured within a 50m x 50m area in the center of each site, and adjacent sites were separated by a minimum of 1 km.

The degree of urbanization at each site was evaluated using a 44-class land use map of Georgia with 30m x 30m resolution developed by NARSAL (the Natural Resources Spatial Analysis Laboratory, UGA, 1998). Using spatial analyst in ArcMap 8.3 (ESRI; Redlands, CA, U.S.A.) and V\_Late (Vector-based Landscape Analysis Tools Extension available at: www.geo.sbg.aac.at/larg/vlate.htm), the coverage area of each land use class was calculated at a radial distance of 500m from the center of the sampling area (Figure 3.2a). From these data, four variables were extracted: impervious (i.e. road or building) surface coverage (m<sup>2</sup>), total forested area (m<sup>2</sup>), number of forest patches, and total core forest area given a 10m buffer edge (m<sup>2</sup>). Total forest area was divided by the number of forest patches to obtain an average measure of 'forest patch size.'

To compare urbanization measures derived from the NARSAL land use map with those from finer resolution aerial images, we obtained digitized orthophoto quarter-quadrangles (DOQQs; Figure 3.2b) compiled in 1999 and available through the Georgia GIS Data Clearinghouse (www.gis1.state.ga.us). At the same 500m radius, land use objects were digitized using a GIS database in ArcInfo 8.3 (ESRI; Redlands, CA, U.S.A.). For this classification, building, road, forest canopy, yard, water, and pasture were delineated (Figure 3.2c). Each map was ground-truthed by recording the geographic coordinates of land use boundaries using a hand-held GPS unit (Magellan Pro-Tracker; Santa Clara, CA, U.S.A.) to account for any digitizing errors or recent development that would not be observed on the DOQQ. As previously described, V\_Late was used to obtain three variables: impervious surface coverage (m<sup>2</sup>),





Figure 3.2. The digital orthophoto quarter-quad (DOQQ) for site 8 (a) and maps extracted from two sources of land use information: the Georgia land use map (b) and the digitized, ground-truthed DOQQ (c). Colors correspond to land use designations: red-building or road; orange- urban forest; light green-park/agriculture; dark green-forest; light blue-clearcut or outcrops; dark blue-water.

b.

total core forest area (m<sup>2</sup>), and average forest patch size. Digitizing and ground-truthing of DOQQs provides detailed information but is very time consuming. Therefore, we compiled DOQQ data from 7 of the 14 sampling sites (4, 6, 7, 8, 10, 11 and 13) chosen to represent a range of high to low urbanization, and used these data to examine correspondence with measures derived from the NARSAL land use map.

Finally, human population density was estimated for each site using 2000 census data available at the finest-scale level of the census block-group. Here, it was assumed that the human population was evenly distributed throughout the block group and we calculated the proportion of each block group comprising a site using ArcMap. This proportion was then multiplied by the population in the census block. Because most sites contained portions of 2-3 census block groups, population estimates were summed to provide a single estimate of human population density for each sample site.

#### Field sampling and data collection

Wild birds were sampled 3-6 times per year at each site during 2004, 2005 and 2006. We captured animals during the breeding season (Apr – Aug) and sampled each location for 2-3 consecutive days every 6-8 weeks. Using 6 and 9 m long, 30mm mesh mist nets (Avinet; Dryden, New York, U.S.A.) open from dawn until late morning, a total of 802 birds were trapped; blood samples from 534 individuals were collected across all sites and years (Table 3.2).

Species identity, age and sex were determined following Pyle (1997); age was assigned as juvenile (hatch-year) or adult (after-hatch-year) based on plumage, gape and skull ossification; we also examined adults for the presence of a brood patch or cloacal protuberance (indicative of breeding status). For each individual, body mass to the nearest 0.1g and length of the right tarsus to the nearest 0.1 mm were measured. Two categorical measures of condition were also noted.

		Site number																	
Species	Scientific Name	Family	Nest	Diet	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Sum
Northern Cardinal	Cardinalis cardinalis	Cardinalidae	В	S	14 <sup>1</sup>	15 <sup>7</sup>	3 <sup>1</sup>	27 <sup>3</sup>	<b>8</b> <sup>1</sup>	19 <sup>7</sup>	19 <sup>4</sup>	1	14 <sup>2</sup>	2 <sup>1</sup>	$20^{1}$	6	23 <sup>4</sup>	2 <sup>1</sup>	173
Carolina Wren	Thryothorus ludovicianus	Troglodytidae	В	Ι		18 <sup>6</sup>	4	7	5	18 <sup>6</sup>	7	5 <sup>1</sup>	11 <sup>1</sup>	3	11 <sup>1</sup>	3	3	2	97
Tufted Titmouse	Baeolophus bicolor	Paridae	С	S	1	2		6 <sup>1</sup>	2	1	9	1	11			$1^{1}$	7	6 <sup>2</sup>	47
Gray Catbird	Dumetella carolinensis	Mimidae	В	Ι		19 <sup>1</sup>	1	4 <sup>2</sup>	6	4 <sup>1</sup>						8			42
Eastern Towhee	Pipilo erythrophthalmus	Emberizidae	G	S		4		2	3	2 <sup>1</sup>	5 <sup>1</sup>		4	1	2 <sup>1</sup>	1			24
American Robin	Turdus migratorius	Turdidae	Т	Ι		3 <sup>2</sup>	$1^{1}$	6 <sup>2</sup>		3 <sup>1</sup>	1		4 <sup>1</sup>		2				20
House Finch	Carpodacus mexicanus	Fringillidae	С	S				$1^{1}$					2			1	11		15
Song Sparrow	Melospiza melodia	Emberizidae	В	S		2		3	6				2						13
Blue Jay	Cyanocitta cristata	Corvidae	Т	Ι				2			3		3		1	1			10
Blue Grosbeak	Guiraca caerulea	Cardinalidae	В	Ι								$1^1$			3	4			8
Downy Woodpec- ker	Picoides pubescens	Picidae	С	Ι				1	4							3 <sup>1</sup>			8
Northern Mocking- bird	Mimus polyglottos	Mimidae	В	S			3 <sup>1</sup>	5 <sup>1</sup>											8
Brown Thrasher	Toxostoma rufum	Mimidae	G	Ι		2			1 <sup>1</sup>							3	1		7
Common Yellow- throat	Geothlypis trichas	Parulidae	G	Ι								1				3			4
Indigo Bunting	Passerina cyanea	Cardinalidae	В	Ι												4 <sup>1</sup>			4
Eastern Phoebe	Sayornis phoebe	Tyrannidae	Т	Ι						1		1				1			3
Chipping Sparrow	Spizella passerina	Emberizidae	Т	S									2						2
Field Sparrow	Spizella pusilla	Emberizidae	G	Ι												2			2
Red-	Melanerpes carolinus	Picidae	С	Ι						2									2

Table 3.2. Summary of the field sampling effort in terms of number of individuals captured by species and site across the 3-year study period.

bellied Woodpec-																			
ker Wood Thrush	Hylocichla mustelina	Turdidae	В	Ι		1				1									2
v enow- breasted Chat	Icteria virens	Parulidae	В	Ι												2			2
American Goldfinch	Carduelis tristis	Fringillidae	В	S												1			1
Brown- headed Cowbird	Molothrus ater	Icteridae		S												1			1
Eastern Meadow- lark	Sturnella magna	Icteridae	G	Ι												1			1
Eastern Wood Pewee	Contopus virens	Tyrannidae	Т	Ι												1			1
Great- crested Flycatch-	Myiarchus crinitus	Tyrannidae	С	Ι													1		1
er Mourning Dove	Zenaida macroura	Columbidae	Т	S		16	3	- 10	2	- 14	5		4	-1	3	3	1	2	1
Total					151	6610	123	6410	354	5110	44°	104	53*	61	393	47°	47*	$10^{3}$	499'

Sites are arranged from most to least urban as determined by the PCA-urban score measure (see text). Species are arranged by their abundance in the sample population. The total number of individuals sampled and the number of WNV antibody positive birds (as superscripts) at each site are presented. Nest location: B-brush, C-cavity, G-ground, T-tree. Diet: I-insect, S-seed

The pectoral muscle development around the carina (or breastbone) was scored following Gosler (1991) as: 1-severely sunken pectoral muscle; 2-sunken pectoral muscle; 3- pectoral muscle even with the carina; or 4-pectoral muscle development beyond the carina. The amount of visible subcutaneous fat in the furculum was similarly scored as: 0-no fat visible; 1-furculum one-third full; 2-furculum one-third to two-thirds full; 3-furculum full; or 4-fat bulging from the furculum, following Hartup et al.(2001). Data on species nest location (ground, brush, cavity, or tree canopy), and primary diet (seed or insect) were later recorded based on species accounts from the Birds of North America periodical series (American Ornithological Union, 1992-2002). Northern Cardinals were banded using U.S. Fish and Wildlife Service metal bands; all other species were color-marked to track recaptures.

From birds weighing over 10g, 50-100 µL of blood was collected by ulnar (wing) venipuncture. Blood samples were maintained at ambient temperature for at least ten minutes and were then kept cool until returning to the lab. The samples were then centrifuged at 10,000g for 10 minutes and both serum and erythrocytes were stored at -70°C until the WNV antibody assay was performed. Assays to detect antibodies to WNV were conducted using an epitope-blocking ELISA developed by Blitvich et al. (2003). The assay employs the flavivirus-specific MAb6B6C-1 and the WNV-specific monoclonal antibody (MAb)3.112G to distinguish WNV from other flaviviruses including St. Louis encephalitis. An inhibition value of greater than or equal to 30% was considered to indicate the presence of viral antibodies. All tests were repeated and samples too small to perform replications were excluded from the analyses. Previous work demonstrated that the assay was valid across a wide range of avian taxa and results were in good agreement with those from plaque-reduction neutralization tests (PRNT) (Blitvich et al. 2003). In

one study involving Rock Pigeons (Columba livia), ELISA results detected circulating antibodies at least 45 weeks post-infection (Gibbs et al. 2005).

### Analysis of land use variables

All land use data were transformed using the z-score ( $(x - x_{\mu})/x_{s.d.}$ ; Gotelli and Ellison 2004) to place measures on the same proportional scale prior to analysis. A principle component analysis (PCA) was conducted using JMP 4.0.4 (SAS Institute; Cary, NC, U.S.A.) to derive a composite measure of urbanization for each site (Table 3.1). The results from all unique four-, three-, and two-way combinations of land use variables (impervious surface coverage, total core forest area, and average forest patch size) and human population density were evaluated. Because average forest patch size is the ratio of total forested area and number of forest patches, we did not include these latter two variables separately in the analysis. The PCA with the highest variable loadings, highest percent of variance described, and best fit to the seroprevalence data was retained. Hereafter, this variable is referred to as the 'urban score.' Principle component analyses were performed separately for the Georgia land use map and the DOQQ-derived data, and we used Spearman's correlation to compare urban score variables obtained from these two approaches.

### Analysis of antibody prevalence, host condition and urbanization

We used generalized linear models (glm) with binomial errors in R2.2.0 (available at: www.rproject.org) to investigate the association between antibody prevalence and urban score; the full model included host age, year and month of sampling, urban score and all relevant two- and three-way interactions as explanatory variables. The minimum adequate model was obtained by removing non-significant terms starting with the highest-order interactions and model comparison was performed based upon p-values and Akaike's Information Criterion (following

Crawley 2002). Two separate sets of analyses were conducted, first using data from all 14 locations, and second using data from 9 sites where greater than 30 individual birds had been tested (sites removed: 1, 3, 8, 10, and 14). Taxonomic (species and family) and ecological (nest type and primary diet) associations with antibody prevalence were tested separately using an analysis of deviance with binomial errors, treating site as a categorical variable (urban or non-urban; Table 3.1). Only species with 10 or more (American Robin, Carolina Wren (*Thryothorus ludovicianus*), Eastern Towhee (*Pipilo erythrophthalmus*), Tufted Titmouse (*Baeolophus bicolor*), Gray Catbird (*Dumetella carolinensis*), House Finch (*Carpodacus mexicanus*), and Northern Cardinal), and families with 15 or more (Cardinalidae, Emberizidae, Mimidae, Paridae, Troglodytidae, Turdidae) individuals sampled were included in the taxonomic comparisons. All models were checked for overdispersion and models where quasibinomial distributions were required are noted in the results (Crawley 2005). West Nile Virus antibody proportions reported in the text were compared using a binomial proportions test with 95% CI.

Because Northern Cardinals were well represented in the data set and accounted for over half of the seropositive samples from all birds (described in Results text), we conducted a final set of analysis focused on antibody prevalence, body condition, and urban land use for this species. To develop a composite measure of individual body condition, a PCA was performed using the ratio of body mass to tarsus length, pectoral muscle development score, and subcutaneous fat score. The first principal component was retained as a measure of individual condition. An analysis of covariance in JMP4.0.4 was used to examine the association between individual condition in Northern Cardinals, host age, exposure to WNV (presence of antibodies as a fixed factor), urban land use (as a continuous covariate), and all two- and three-way interactions. Model simplification was performed as described above.

### Results

### Land use characterization

Urban score values were derived from a PCA of two variables, human population density and average forest patch size, explaining 85.2% of the variation in land use measures between sites. Individual variable loadings were: human population density 0.707 and average forest patch size -0.707. Therefore, a high urban score represents a site characterized by high human population density and low average forest patch size (Table 3.1). Urban scores obtained from analysis of DOQQ-derived data were highly correlated to those from the Georgia land use map (Spearman's R=0.93, p=0.007), supporting use of the NARSAL Georgia land use data for further analyses. *WNV antibody prevalence* 

From the 802 birds captured throughout the course of the study, 23 individuals (2.9%) were recaptured and only data from the first sample obtained is included in this study. Of the 534 samples collected for testing, assay results were obtained from 499 samples. The remaining 35 samples were excluded either because they were too small to perform replicate tests or results were inconclusive. A total of 73 samples (14.6%) were positive for antibodies to WNV (see Supplemental Materials, Table S1). Among the 14 study sites, WNV antibody prevalence ranged between 6.3% (N=43) and 30.8% (N=52). There was no significant difference in WNV antibody prevalence by month or year of sampling. However, WNV antibody prevalence was higher among adult birds (18.3%) relative to juveniles (10.8%;  $\chi^2$ =7.0429, p=0.008). Of adult birds, Northern Cardinals represented 37.0% of the total sample population (N<sub>adult</sub>=257) and were the only species sampled across all 14 sites. Antibodies to WNV were detected in 18.8% of all sampled Northern Cardinals (N=170), and 27.4% of all adult Northern Cardinals (N=95). Adult

Northern Cardinals accounted for 55.3% of all seropositive samples obtained from adult birds (N=47).

### WNV antibody prevalence and urban land use

Model simplification showed that urban score and host age were strong predictors of seroprevalence, but the 2-way interaction between these factors was not significant (Urban score:  $\chi^2$ =43.994, d.f.=1, p=0.006, Age:  $\chi^2$ =36.512, d.f.=1, p=0.006). Because WNV antibody prevalence significantly differed between age groups, the strength of the association between urban score and WNV antibody prevalence was examined separately for each age class. West Nile virus antibody prevalence in adult birds increased with higher urbanization ( $\chi^2$ =14.306, d.f.=1, p=0.003, R<sup>2</sup>=0.77), but no relationship was detected between urban score and antibody prevalence in juveniles ( $\chi^2$ =21.4596, d.f.=1, p=0.251, R<sup>2</sup>=0.89). When the statistical model was repeated using only data from sites with > 30 samples, p-values and fit of the regression lines were similar for the adult population, but improved considerably for juveniles ( $\chi^2$ =7.2738, d.f.=1, p=0.005, R<sup>2</sup>=0.72 for adults and  $\chi^2$ =8.2910, d.f.=1, p=0.064, R<sup>2</sup>=0.76 for juveniles, Figure 3.3).

Sites were categorized as urban (positive urban score) and non-urban (negative urban score) to compare observed patterns in WNV antibody prevalence by host species, taxonomic family, nest location, and diet. Overall, antibody prevalence in urban sites (18.5%) was higher than at non-urban sites (9.6%;  $\chi^2$ =6.832; d.f.=1, p=0.009). Each species with 10 or more sampled individuals and each family with 15 or more sampled individuals (see Methods) were compared to all others combined to test for taxonomic associations with WNV antibody prevalence. Northern Cardinals showed significantly higher seroprevalence than all other species combined ( $\chi^2$ =4.763; d.f.=1, p=0.03) and, similarly, Cardinalidae differed from all other families combined ( $\chi^2$ =5.049; d.f.=1, p=0.0046). No other comparisons of WNV

a) Juvenile Birds







Figure 3.3: Relationship between urban score and antibody prevalence of West Nile virus infections in songbird populations by age. To linearize the proportional data, antibody prevalence is presented as the logit transformation of seropositives (= Ln(seropositive/seronegative)) and values were standardized to zero. In both graphs closed circles indicate sites where > 30 individuals were sampled and open circles indicate sites that were removed from a subset of the analyses due to low sample sizes. Regression lines are derived from the estimated slope and intercept values in the glm using the restricted sample set. a) Slope = 0.502; R<sup>2</sup>=0.76 for juveniles; b) Slope = 0.642; R<sup>2</sup>=0.72 for adults.

antibody prevalence among different species or families were statistically significant. The slope and strength of the relationship between urban score and antibody prevalence for adult Cardinals was similar to the relationship observed among all other bird species (Figure 3.4;  $\chi^2$ =5.767, d.f.=1, p=0.016 for Northern Cardinals;  $\chi^2$ =5.147, d.f.=1, p=0.023 for all other species; models run using the quasibinomial distribution to account for overdispersion). Finally, neither nest location nor primary diet explained variation in WNV antibody prevalence across all sites or within site categories.



Figure 3.4: Comparison of West Nile virus prevalence in adult Northern Cardinals and adults of all other songbirds represented by 10 or more samples (see Methods text). Prevalence data were transformed and standardized as described in Figure 2. Site 1 was removed from the analysis because no adult Northern Cardinals were tested at this site. Northern Cardinals (closed circles and black regression line) had significantly higher seroprevalence than other bird species (grey diamonds and a dashed regression line), and this difference was observed at all but two sites. The overall relationship between WNV antibody prevalence and urban score was similar for Northern Cardinals (Slope = 0.603; R<sup>2</sup>=0.28) and all other bird species (Slope = 0.574; R<sup>2</sup>=0.35).

The association between WNV antibody prevalence, urban land use and individual body condition was examined in Northern Cardinals using data from the nine sites where >30 individual birds were sampled. The first principal component from a factor analysis of the ratio

of body mass to tarsus length and the subcutaneous fat score explained 62.6% of the totalvariance. Birds with high condition scores had more subcutaneous fat and weighed more relative to their body size than birds with negative scores (component coefficients were subcutaneous fat =-0.707 and mass:tarsus length =0.707). Because visual inspection of the data suggested a non-linear relationship between urban score and the condition of Northern Cardinals sampled at each site (Figure 3.5; condition was greatest at sites of intermediate urbanization) we included both urban score and urban score<sup>2</sup> in the full model, together with antibody status, age, and all relevant interactions. Model simplification provided no support for antibody status, age, or two-way interaction effects on condition, but showed a significant relationship between host body condition and the squared term for urban score (F  $_{2,140} = 3.27$ , p=0.014).



Figure 3.5: Relationship between urban score and a composite measure of body condition in Northern Cardinals at the study sites where >30 individuals were sampled. Open circles represent individual condition values and closed circles indicate the average condition of individuals sampled at each site. The regression line shown is derived from the average condition values and fitted to the following relationship: Slope-urban score =0.123; Slope-urban score<sup>2</sup> = -0.344, R<sup>2</sup> =0.41.

### Discussion

The prevalence of antibodies against West Nile virus in wild songbird populations increased with greater measures of urbanization across locations sampled around Atlanta, Georgia USA. Among adult birds, seroprevalence was nearly 2.5 times higher at urban sites as compared to non-urban sites. This association was not significantly affected by month or year of sampling, although adult birds were more likely to have WNV antibodies than juveniles. The effect of host age probably resulted from the limited sampling period each year, as many juvenile birds were sampled before the end of the peak transmission period. Moreover, because we tested for antibodies to West Nile virus rather than current infection, and because antibodies to WNV can be long-lasting (Gibbs et al. 2005), greater antibody prevalence among adult birds would also be expected due to longer exposure times.

Northern Cardinals, the most commonly sampled host species, had higher WNV antibody prevalence than all other species combined. This is consistent with previous studies conducted in the southeastern USA (Godsey et al. 2005; Komar et al. 2005; Gibbs et al. 2006a). The Northern Cardinal's ubiquitous occurrence along the urban-rural gradient and high abundance points to their utility as a surveillance species, as suggested in Gibbs et al. (2006a). The role of Northern Cardinals in WNV epidemiology, however, is not well understood. High WNV antibody prevalence rates observed across several studies indicate that cardinals may tolerate infections with WNV more successfully than other avian species (e.g. Caffrey et al. 2005). Komar et al. (2003, 2005) concluded that both Northern Cardinals and House Sparrows (Passer domesticus) were important amplifying hosts in southern Louisiana based on species abundances, exposure rates and a competence index derived from experimental infections (the product of susceptibility, infectiousness, and the duration of infectiousness). Moreover, Apperson et al. (2002) observed

that American Robins, Northern Cardinals and Northern Mockingbirds (Mimus polyglottus) accounted for a high proportion of mosquito blood meals around Queens, NY (16%, 13%, and 13% respectively). In contrast, Kilpatrick et al. 2006b found that Northern Cardinals were poorly represented in mosquito blood meals around the Washington, D.C. area. Collectively, these studies suggest that the role of Northern Cardinals in WNV transmission could vary over space and time, and point to the need for more data on the contribution of different bird species to WNV transmission.

The positive association between WNV antibody prevalence and urban land use observed in this study could arise from several mechanisms. First, several previous studies of North American metropolitan areas demonstrate declines in avian species diversity with urban land use (Green and Baker 2003) combined with a dominance of non-native and anthropophilic species (Hennings and Edge 2003; Crooks et al. 2004). Because viral amplification and transmission are known to vary among avian species (Komar et al. 2003), host communities characterized by high species richness could dilute the influence of highly competent hosts. This process, termed the 'dilution effect,' has been proposed as a major cause of variation in Lyme disease occurrence in response to suburban land use in the northeastern USA (Schmidt and Ostfeld 2001; LoGuidice et al. 2003; Keesing et al. 2006); its significance for other pathogens in urbanized areas, however, remains unknown (Bradley and Altizer 2007). Ezenwa et al. (2006) found evidence for a dilution effect in West Nile virus transmission by linking non-passerine avian species richness to reduced infection levels in mosquito vectors and fewer human cases in Louisiana, USA. If host species diversity affected patterns observed in the present study, we would expect to find lower species diversity and greater dominance of Northern Cardinals with increasing urbanization. Thus,

examining measures of host diversity in conjunction with WNV antibody prevalence in avian species represents an important goal for future work.

Changes in vector ecology with increasing urbanization could also affect viral exposure among wild songbirds. Breeding in man-made water containment systems (like borrow pits, wastewater treatment plants and sewers), Culex spp. are well adapted to human-dominated environments, and an increased abundance of mosquitoes could lead to higher avian seroprevalence in urban environments. Indeed, this has been suggested as a likely cause behind urban foci in recent WNV outbreaks in humans in the USA (Epstein 2001; Campbell et al. 2002). Warmer urban microclimates could also favor higher rates of virus replication within the vector and more efficient transmission to susceptible hosts (Reisen et al. 2006).

Finally, habitat changes associated with urban landscapes could affect a host's tolerance to WNV infections. Although urbanization can increase stress levels and reduce immunocompetence in some host species, abundant and consistent food resources available to urban-adapted wildlife may improve host recovery or survival following infection (Bradley and Altizer 2007). Because this study examined only WNV antibody prevalence, it is not possible to exclude the possibility that recovery, rather than exposure, varies with the intensity of urban development. Importantly, we observed significant variation in individual Northern Cardinal condition along the urban gradient, with greatest measures of body condition at sites with intermediate levels of urbanization. Because these sites are primarily suburban or residential, a likely explanation is that supplemental food sources (in the form of bird feeders and fruiting vegetation) are also highest at these sites, leading to increased foraging success and continuous access to food throughout the seasons. If high nutrition or reduced energy expenditure during foraging increases the body condition of birds at these sites, their tolerance to infection and

survival following exposure might increase. To the authors' knowledge, there are no published studies that report on the impact of nutritional supplementation to WNV tolerance and recovery in wild birds. With respect to this field study, increased body condition among Northern Cardinals at sites with intermediate urbanization might partially explain the pattern of low WNV antibody prevalence at the least urbanized sampling locations.

We found no evidence of yearly changes in average WNV antibody prevalence, counter to Gibbs et al. (2006a) who demonstrated increasing WNV seroprevalence in wild avian hosts from 2000 to 2004 throughout the state of Georgia. This is not surprising, however, because data reported in Gibbs et al. (2006a) spanned the period of virus introduction (with human cases starting in Atlanta in 2001). Increasing prevalence of WNV antibodies in the primary hosts was observed during the establishment phase of the pathogen, whereas samples in the current study were collected several years after viral introduction.

Finally, our analysis of land use characteristics indicate that the composite measure of urbanization derived from a previously developed map of Georgia was an accurate and efficient manner of land use characterization. In comparison to the more labor-intensive method of digitizing and ground-truthing orthophotographs, it appears that the coarser-scale land use map was an accurate reflection of land use at the 500m scale. Such data could then be applied over larger geographic areas to create predictive risk assessment maps of WNV antibody prevalence in wild songbird hosts.

The impact of urban landscapes on infectious disease dynamics within wildlife hosts is significant for wildlife management and public health policies (Bradley and Altizer 2007). With respect to multi-host generalist pathogens, the presence of more competent reservoir hosts (and factors that increase their tolerance to infection) can contribute to the extirpation of vulnerable

host species that suffer high mortality rates following infection (Woolhouse et al. 2001; Naugle et al. 2004; deCastro and Bolker 2005). Because the majority of emerging human infectious diseases are zoonotic (Taylor et al. 2001), determining how urban landscapes influence wildlife infectious disease will become increasingly important for predicting human disease risks as well. Our study represents an important step towards understanding the dynamics of WNV at a regional scale in a rapidly-growing metropolitan area by demonstrating that WNV antibody prevalence in the avian community was strongly associated with urbanization. Further studies to identify the mechanisms driving this pattern are critical for understanding the dynamics of this and other complex multi-host infectious diseases.

### Acknowledgements

We thank Jaap de Roode and David Stallknecht for helpful discussions and comments on earlier drafts of the manuscript, and Andy Davis, Ben Stoner-Duncan, Laura Gold, Adrienne Tecza, and Olivia Wright for field support. We also thank the private and public landowners who allowed access to the field sites. This work was supported by a seed grant from the Southeastern Center for Emerging Biological Threats (SECEBT) and graduate research fellowships from Emory University and the University of Georgia to CAB. Animal protocols described herein were approved Animal Care and Use Committees at Emory University (2004-05; IACUC No. 183-2004) and the University of Georgia (2005-06; AUP No. A2006-10051). Banding permits were granted by the US Bird Banding Laboratory (#23141) and the Georgia Department of Natural Resources (permit No. 29-WMB-02-176).

# CHAPTER 4

# URBANIZATION, HOST COMMUNITY COMPOSITION, AND WEST NILE VIRUS EXPOSURE IN SONGBIRDS

Bradley, C.A., S.E.J. Gibbs, and S. Altizer. Submitted to Oecologia, June 2, 2009.

### Abstract

Previous work has shown that the level of exposure to West Nile virus (WNV), a mosquito-borne generalist pathogen infecting passerine birds, increases with levels of urban land use. One explanation for this pattern is that high avian community diversity in less urban areas reduces WNV transmission through a mechanism termed the 'dilution effect,' whereby host species with low reservoir competence account for a high proportion of vector blood meals. Here, we examine the association between urbanization, avian community composition and WNV antibody prevalence in wild songbirds sampled across replicate sites representing an urban-rural gradient in northern Georgia (USA). Urbanization was quantified at three spatial scales using GIS-derived landscape variables, and avian abundance and diversity at each site were estimated based on point counts. Results showed that WNV antibody prevalence increased with greater measures of urbanization, and was most strongly associated with reduced core forest area measured at the largest (1km radius) spatial scale. Of the avian community indices examined here, total passerine abundance and a composite measure of functional diversity based on the ability of host species to amplify WNV were the strongest indicators of WNV exposure across sampling locations. Importantly, avian community diversity increased and host community competence decreased with greater core forest area at each site. Finally, because the effects of land use variables on WNV antibody prevalence were stronger than any single avian community measure examined here, our results support the idea that urbanization influences multiple factors, including vector abundance, that determine WNV transmission, and point to a role for the conservation of core forest habitat as a management tool in reducing WNV risk.

### Introduction

Understanding how the composition and diversity of ecological communities affect the prevalence and transmission of multi-host pathogens is an area of growing concern in infectious disease ecology (Begon and Bowers 1994; Dobson 2004; Keesing et al. 2006). This attention is prompted in part by recognition that species diversity in natural ecosystems is declining at both global and local scales (e.g., Hilton-Taylor et al. 2000), often due to habitat destruction and other land use changes. One proposed mechanism linking decreased host species diversity with greater pathogen transmission is the dilution effect (e.g., Ostfeld and Keesing 2000; Allan et al. 2003; Keesing et al. 2006), whereby high host diversity reduces the number or proportion of contacts between competent hosts. This process requires that host species vary in their ability to amplify and transmit infectious agents (i.e. competence), and predicts that following the addition of less competent host species less able to transmit the infection (reviewed in Begon 2008). In this way, the addition of multiple host species serves to dilute the transmission process overall and lower pathogen prevalence in the host community (Keesing et al. 2006).

The dilution effect and predictions involving host species diversity effects on pathogen transmission have been examined most thoroughly for Lyme disease, a tick-borne bacterial disease caused by Borrelia burdorferi (Ostfeld and Keesing 2000; Loguidice et al. 2003). Studies in suburban environments of northeastern USA indicate that forest fragmentation and a greater proportional abundance of the most competent reservoir for B. burgdorferi (the white-footed mouse, Peromyscus leucopus) are associated with increased infection prevalence in ticks, mice and humans (Allan et al. 2003; Loguidice et al. 2003). Reduced host species diversity probably has a similar role in other vector-borne wildlife disease systems (e.g., Telfer et al. 2005; Ezenwa

et al. 2006; Dobson et al. 2006), although studies that control for host density and the numerical dominance of competent host species are needed. Another important concern is that in many cases, variation in host diversity or community composition is caused by land use changes that affect other aspects of host, pathogen or vector biology (e.g., Allan et al. 2003, Ezenwa et al. 2007, Bradley and Altizer 2007). Thus, changes in pathogen prevalence with host community composition could be caused by or confounded with other potential mechanisms.

Here, we examine how exposure to West Nile Virus (WNV), a vector-borne multi-host pathogen transmitted by mosquitoes in the Culex genus (Peterson et al. 2004), varies with avian community composition along an urban-rural gradient. Passerine birds and a handful of other species are highly competent hosts of WNV, although competence does vary among individual bird species (Komar et al. 2003; Marra et al. 2004; Gibbs et al. 2006a). Declines in avian species richness, although not necessarily total avian abundance, are strongly linked to intense urban development (McKinney 2002, Pauchard et al. 2006), with bird species sensitive to the loss of natural habitat replaced by urbanphilic species that can nest and forage in human-developed landscapes. At least two previous studies have shown that songbird exposure to WNV is greater in urban as compared to rural landscapes (Gibbs et al. 2006b; Bradley et al. 2008), although mechanisms that cause this association are unknown. If urban bird communities are dominated by highly competent hosts, then mosquitoes in urban environments have a higher probability of contacting amplifying host species. In support of this idea, recent work by Ezenwa et al. (2006, 2007) in southeastern Louisiana showed that WNV infection in mosquito vectors was lower at sites dominated by wetlands, and these same sites had a high diversity and abundance of nonpasserine birds. Other recent work showed that the prevalence of WNV infection in mosquito vectors and in humans increased with decreasing bird species diversity at both local (Allan et al.

2008) and large spatial scales (Swaddle and Calos 2008), suggesting that changes in viral transmission at the level of bird communities can translate to changes in human cases of infection.

On the other hand, using DNA sequence data from mosquito blood meals Kilpatrick et al. (2006b) indicated that a single uncommon bird species, American robins, accounted for the majority of WNV infected mosquitoes around Washington, DC (USA), suggesting that mosquito encounter rates with vertebrate hosts do not correspond to their proportional abundances. Another recent study (Loss et al. 2009) showed no effect of increasing avian species richness per se on WNV transmission in Chicago, IL (USA.). Collectively, these results indicate that conventional measures of biodiversity (i.e., species richness and evenness) might not capture well the effects of host community composition on infectious disease transmission, a point underscored by a recent comprehensive study of tick infection prevalence with Lyme disease in New York (LoGuidice et al. 2008).

In this study, songbirds were captured and sampled for antibodies to WNV at eighteen sites in the Piedmont region of Georgia, USA, including sites at two urban centers. In addition to examining which land use measures best predicted WNV exposure (and at what spatial scale), we quantified avian abundance and richness at each site to investigate two potential mechanisms accounting for observed patterns. First, we examined whether avian diversity (as measured by species richness and two composite measures of diversity) was correlated with WNV exposure. Following Allan et al. (2008), we also tested total passerine abundance and developed an index of host community competence based on the observed abundance of nine common bird species and their competence as amplifying hosts for WNV (Komar et al. 2003; Komar et al. 2005); we then examined associations between this variable, urbanization and WNV antibody prevalence.

In each case, we compared the fit of models based on avian community measures with those based on land use measures to identify factors that best explain the variation in WNV exposure observed across a land use gradient.

### **Methods and Materials**

### Site selection and characterization

Between April and August of 2006, wild songbirds were captured and sampled at 18 sites in the Piedmont region of Georgia, USA, including the Atlanta and Athens metropolitan areas (Figure 4.1). Birds were captured within a 50m x 50m area at the center of each site, adjacent sites were separated by a minimum of 5 km, and the entire study area encompassed approximately 2500 km<sup>2</sup>. Sites were chosen to include a range of land use types (residential, commercial, or recreational) and human impacts (e.g., built-up areas or nature reserves). The history of land use was also determined at each site to ensure that no recent, significant land use change had occurred. Site selection was also based on land owner permission and the presence of woody vegetation to facilitate the capture of birds using mist nets.

The degree of urbanization at each site was evaluated using a 44-class land use map of Georgia with 30m x 30m resolution developed by NARSAL (the Natural Resources Spatial Analysis Laboratory, UGA, 1998). Previous work has shown that data obtained from land use maps used here are highly correlated with finer-scale measures derived from digitized and ground-truthed aerial photographs (Bradley et al. 2008). Four land use variables (impervious (i.e. road or building) surface area (m<sup>2</sup>), total forested area (m<sup>2</sup>), number of forest patches, and core forest area (m<sup>2</sup>; based on subtracting a 10m buffer edge from each forest patch) and human population density were determined at three spatial scales (100m, 500m, and 1000m) using the



Figure 4.1 The geographic location of the 16 study sites used to test the associations between WNV antibody prevalence, land use, and attributes of the avian community. Two additional sites, shown as hollowed points, were excluded from the analyses due to low sample size. Counties are labeled and areas designated as cities are grayed. Clarke County is entirely shaded because it is a consolidated city-county encompassing the city of Athens, Georgia.

methods detailed in Bradley et al. 2008. These spatial scales were chosen to encompass the range sizes of most resident birds during the breeding season and are consistent with the spatial scales examined in other studies of bird community richness and diversity (Hennings and Egde 2003; Crook et al. 2004; Fernańdez-Juricic et al. 2004). We estimated the average forest patch size per

site based on the total forested area divided by the number of forest patches, and quantified the proportion of core forest based on core forest area divided by total forest area.

# Songbird sampling and serum analysis

Wild birds were sampled on three separate dates at each location during the breeding season, once each in April, June, and August. Using 6 and 9 m long, 30mm mesh mist nets (Avinet; Dryden, New York, USA) open from dawn until late morning, a total of 808 birds were trapped across the entire study duration. Species identity, age and sex were determined following Pyle (1997); age was assigned as juvenile (hatch-year) or adult (after-hatch-year) based on plumage, gape and skull ossification. We also confirmed adult status based on the presence of a brood patch or cloacal protuberance (indicative of breeding activity). Northern Cardinals were banded using U.S. Fish and Wildlife Service metal bands; all other species were color-marked or tail feather-clipped to track recaptures.

From 356 individuals, 50-100  $\mu$ L of blood was collected by ulnar (wing) venipuncture. Individuals weighing less than 15 grams (e.g. House Wrens (Troglodytes aedon), Carolina Chickadees (Poecile carolinensis), and Brown-headed Nuthatches (Sitta pusilla)) were not sampled because an adequate amount of blood for testing could not be acquired consistently.

Blood samples were handled and stored as described in Bradley et al. 2008 and an epitope-blocking ELISA was conducted to detect the presence of WNV antibodies (Blitvich et al. 2003). Previous work demonstrated that the results of the assay are robust across avian taxa and agree with those from plaque-reduction neutralization tests (PRNT) (Blitvich et al. 2003). Samples that tested positive for antibodies to flaviviruses but not for WNV-specific antibodies were treated as negative samples in the analyses. All tests were repeated and samples too small to perform replications were excluded from the analyses.

### Avian species diversity and community competence

The abundance of individual bird species at each site was recorded following the protocols of Fraterrigo and Wiens (2005). Each site was visited twice during the breeding season, once in May and once in June, when weather conditions were suitable (no precipitation or winds). The two visits were made at different times: early morning (05:30-07:30) and late morning (08:00-10:00), and the scheduling assignment was made such that half the site visits each month were in the early morning and the other half were in the late morning. Bird presence was determined based on visual and auditory cues, and we recorded data at ten points (each with a 50m radius) centered on the spot where mist nets were placed for trapping. Each observation point was separated by at least 150m from all other points and observations at each point lasted 5 minutes. Abundance data was summed across the two visitation dates for each site. We recorded the presence of all bird species with a total of 67 species recorded across all study sites examined.

Avian survey data were used to measure host abundance, diversity and community composition in four ways. First, we calculated total passerine abundance by summing the number of passerines observed across the two survey periods, and measured avian species richness as the total number of bird species observed over the two survey periods at each site.

Second, we used Shannon's diversity index, given as 
$$H' = -\sum_{i=1}^{s} p_i * Ln(p_i)$$
, as a combined  
measure of species diversity and evenness, where s=number of species and  $p_i$  is the proportional  
abundance of species i. Third, we followed the protocols of Stoner and Joern (2004) and  
Fraterrigo and Weins (2005) to derive a composite value of avian community composition using  
a Principal Component Analysis (PCA), hereafter termed 'PC-diversity.' Here, the total number  
of each species observed over the two survey periods was included in the analysis. The resultant

composite value, based on the covariance matrix, is a measure of species richness and evenness where high PC-diversity values represent populations characterized by a high number of species, with abundances evenly distributed between species, and low PC-diversity values represent populations characterized by a few species, where one or more are over-represented.

Finally, using species-specific competence information based on experimental infections by Komar et al. (2003) and (2005), we derived a composite index to more directly examine the influence of amplifying hosts, hereafter called 'community competence.' Species–specific competence has been previously quantified as the product of (i) the proportion of birds that become infected upon exposure, (ii) the proportion of exposed vectors that become infectious each day, and (iii) the duration of infectious viremia (Komar et al. 2003). Because the absolute and relative abundance of a species may not be correlated across sites, host community competence was calculated in two ways, hereafter referred to as numerical vs. proportional community competence. First, we multiplied the numerical abundance of each bird species by its

competence score based on the following expression:  $CC = \sum_{i=1}^{s} A_i * CI_i$  where A is the abundance of species i and CI<sub>i</sub> is the competence index of species i for West Nile virus. Second, we calculated the same measure using the proportional abundance of species i. In the latter calculation, we included count data for all 67 bird species observed across the sampling locations and assumed that the competence of species without experimental infection data was zero. Competence data were available for nine common songbird species that were well represented in our field data (comprising between 17 and 34% of the total bird abundance observed across sites during diversity surveys and 54% of samples tested for WNV): Northern Cardinal (Cardinalis cardinalis), House Sparrow (Passer domesticus), Blue Jay (Cyanocitta cristata), Common

Grackle (Quiscalus quiscula), House Finch (Carpodacus mexicanus), American Crow (Corvus brachyrhynchos), American Robin (Turdus migratorius), Red-winged Blackbird (Agelaius phoeniceus), and Northern Mockingbird (Mimus polyglottos). Although competence data are not comprehensive, species making up this index occur across the urban-rural gradient and a high proportional abundance of these species tends to be associated with low-diversity assemblages (Blair 1999; Marzluff 2001, Crooks et al. 2004).

# Statistical analysis

We used the logit transformation (Ln( antibody positive/ antibody negative)) to linearize antibody prevalence data for each site prior to analyses, and treated site as the unit of observation for statistical analyses. All predictor variables were tested for normality prior to analysis using the Shapiro-Wilk test for normality and data transformations were performed prior to model testing. Analyses were conducted using R version 2.1.1 (R Development Core Team 2005). We used Akaike's information criterion adjusted for small samples sizes (AICc) to evaluate how well the models explained variation in the data. For each analysis, we calculated adjusted-R<sup>2</sup> for the minimum AICc model and competing models. Final models were examined for constancy of variance and normality of errors.

After performing initial tests to examine associations between WNV antibody prevalence, host age and species identity, we investigated the relationship between avian community composition and WNV in three ways, First, we examined the association between land use measures and WNV antibody prevalence by comparing the fit of models based on four variables (impervious surface area, average forest patch area, proportionate core forest area and human population density) measured at each spatial scale (100m, 500m and 1000m radius). At each radius, we examined the following full model: WNV Antibody Prevalence = Intercept +

Impervious Surface + Core Forest Area + Forest Patch Size + Human Density and performed model simplification following Crawley (2007). To mitigate for multiple tests of land use influences on WNV antibody prevalence, we used a significance level of  $\alpha$ =0.0167 based on a Bonferroni approximation of  $\alpha$ =0.05/3 tests.

Second, we examined the relationship between measures of avian community composition and WNV antibody prevalence. Specifically, we tested whether seroprevalence was associated with (i) measures of avian diversity and abundance at each site [Full model: WNV Antibody Prevalence = Intercept + Species Richness + Shannon's Index + PC-diversity + Passerine Abundance] and (ii) composite indices of avian community competence [Full model: WNV Antibody Prevalence= Intercept + Numerical Community Competence + Proportional Community Competence]. As in the previous set of models, we considered  $\alpha$ =0.025 (0.5/2 tests) an appropriate level of significance for the two models testing the association between characteristics of the avian community and WNV antibody prevalence. We also tested for a negative relationship between community competence and measures of avian diversity, as would be predicted by the dilution effect [Full model: Community Competence= Intercept + Richness + Shannon's Index + PC-diversity + Passerine Abundance].

Third, for those avian community measures most strongly associated with WNV antibody prevalence, we performed a final analysis to examine their relationship with a subset of land use measures. As before, model simplification was performed based on AICc, and we report significance tests and adjusted- $R^2$  for the minimal models.

In addition to using Bonferroni adjustments when establishing appropriate levels of significance, when testing predictions involving (i) antibody prevalence and avian community composition, (ii) community competence and measures of avian diversity and (iii) avian

community composition and land use, for which we had a priori directional expectations, we used directed tests rather than one-tailed tests, as these enable detection of patterns that are opposite to predictions while retaining much of the statistical power of one-tailed tests (Rice and Gaines 1994). We followed the guidelines in Rice and Gaines (1994) by setting  $\gamma/\alpha$  to 0.8, giving values of  $\gamma$ =0.04 and  $\delta$ =0.01, and report p-values for directional tests accordingly.

### Results

### General results

From the 354 blood samples tested, WNV antibody presence or absence could be determined for 312 individual birds. Average WNV antibody prevalence was 9.9% and ranged from 0-32% among sampling locations. Two sites were excluded from further analyses because serum samples producing consistent results were available for < 10 individuals across the entire study period. Excluding these data, overall antibody prevalence for the remaining 16 sites was 10.1% (N = 296 samples). Among individuals for which age could be determined (N = 246), antibody prevalence was 9.7% for adults (N = 195) whereas none of the 51 samples obtained from juvenile birds were positive. Because the effect of age was only marginally significant ( $\chi^2$ =4.105, df = 1, p=0.04), and because the proportions of juvenile, adult and unknown-age birds were similar across sampling locations, we included data from all individuals (irrespective of age) in subsequent analyses. In addition, data collected throughout the entire sampling period were combined as there was no significant difference in antibody prevalence among the three sampling periods (April, June, and August;  $\chi^2$ =2.855, df = 2, p=0.24).

Northern Cardinals accounted for 34.3% (N=107) of the tested serum samples, represented 14.1% (N=427) of the birds observed in point counts, and were significantly more
likely to test positive for antibodies to WNV than all other bird species combined

(seroprevalence=17.8%,  $\chi^2$ =9.841, p=0.002). American Robins accounted for 3.2 % (N=10) of serum samples and represented 7.4% (N=225) of birds observed in the point counts. Although American Robin antibody prevalence was high (20%), this level was not significantly different from that of all other bird species combined ( $\chi^2$ =0.174, p=0.68), possibly owing to their small sample size in this data set. Table 4.1 summarizes the relative abundance of the 10 most common bird species caught, sampled, and observed in this study.

#### WNV antibody prevalence and land use measures

The landscape surrounding each sampling location ranged from highly urbanized (high density residential areas within 1km of downtown Atlanta) to rural sites dominated by a mix of forest and pasture land. At each spatial scale, correlation analysis showed that land use measures were highly interrelated, with the strongest positive correlation between the proportion of core forest and average forest patch size at 1000m (r = 0.86, p <0.001), and the strongest negative correlation between proportion core forest and impervious surface area at 1000m (r = -0.73, p <0.001). We tested the relationship between individual predictors (impervious surface, core forest area, average forest patch size and human density) and WNV antibody prevalence, and repeated this analysis at each spatial scale (100m, 500m, and 1000m). At a spatial scale of 1000m, results showed that WNV antibody prevalencedecreased significantly with an increase in the proportion of core forest (Table 4.2; Figure 4.2a); this minimal model had the lowest AICc score and highest adjusted R<sup>2</sup> of any model at the three spatial scales.

Table 4.1. Most abundant bird species recorded and captured across study sites. Summary variables are shown for total number captured via mist nets across all sites, % of all serum samples attributed to each species, the total number recorded based on field survey data, and the % of the total avian community represented by each species.

Species	Number captured	% serum	Total abundance	% of total
		samples		community
Northern Cardinal	161	34.3	427	14.1
(Cardinalis				
cardinalis)				
House Finch	81	13.5	149	4.9
(Carpodacus				
mexicanus)				
Tufted Titmouse	81	12.2	232	7.6
(Baeolophus				
bicolour)				
Carolina Wren	50	7.4	200	6.6
(Thryothorus				
ludovicianus)				
Song Sparrow	39	4.2	14	0.5
(Melospiza				
melodia)				
American Robin	16	3.2	225	7.4
(Turdus				
migratorius)				
Brown Thrasher	14	2.9	88	2.9
(Toxostoma				
rufum)				
Eastern Towhee	12	1.3	98	3.2
(Pipilo				
erythrophthalmus)				
Gray Catbird	11	2.9	84	2.8
(Dumatella				
carolinensis)				
Blue Jay	11	2.6	183	6.0
(Cyanocitta				
cristata)				

\*93 Carolina Chickadees (*Poecile carolinensis*) and 33 American Goldfinches (*Carduelis tristis*), comprising 6.7% and 1.3%, respectively, of the total community observed during counts, were captured in addition to the above, but no serum samples were collected because a sufficient amount of blood to run the ELISA could not be obtained consistently.

Table 4.2. Associations between WNV antibody prevalence and land use variables. Akaike's information criterion adjusted for small samples sizes (AICc) and adjusted R<sup>2</sup> are shown for all models, and the regression coefficient ( $\beta$ ) and p-values are shown only for the minimum adequate models. Separate analyses were performed at each spatial scale, and significance levels were adjusted for multiple tests (assuming  $\alpha = 0.0167$ ). Significant p-values and significant variables are bolded. Human density, average forest patch size, and impervious surface data were log-transformed, and proportion core forest area was arcsin-square root transformed prior to analysis. IMP=impervious surface, CORE=core forest area, PATC=average forest patch size and DENS=human density.

Spatial Scale	Model	AICc	adjusted R <sup>2</sup>	β	p-value
1000 meters	IMP+CORE+PATC+DEN S	49.97	0.25		
	-IMP	44.72	0.31		
	-PATC	40.46	0.36		
	-DENS	37.28	0.38	-4.63	0.006
500 meters	IMP+CORE+PATC+DEN S	52.50	0.12		
	-PATC	47.17	0.19		
	-IMP	42.88	0.25		
	-CORE	39.91	0.27	0.29	0.022
100 meters	IMP+CORE+PATC+DEN S	51.91	0.15		
	-PATC	46.58	0.22		
	-IMP	42.29	0.28		
	-CORE	38.78	0.32	0.30	0.013



Figure 4.2 Relationship between (a) antibody prevalence of West Nile virus infections in songbird populations and core forest area at 1000m, (b) West Nile virus antibody prevalence in songbird populations and a measure of numerical community competence, and (c) avian diversity (based on PC-diversity) and numerical community competence.

#### Avian community composition and WNV antibody prevalence

We tested four measures of avian community diversity obtained from the point count surveys: species richness, Shannon's index, PC-diversity, and total passerine abundance against WNV antibody prevalence. Only total passerine abundance was retained in the final model (Table 4.3), and showed a significant positive relationship with antibody prevalence. However, correlation analysis showed that PC-diversity and passerine abundance were highly negatively correlated ( $R^2$ =0.78). When the model was repeated without passerine abundance, the relationship between PC-diversity and WNV antibody prevalence was negative, but non-significant ( $\beta$ =-0.416, AICc= 42.106, p= 0.067). We also tested a model examining the association between WNV antibody prevalence and community competence, measured both numerically and proportionally. Numerical community competence was a significant and positive predictor of WNV antibody prevalence (Table 4.3; Figure 4.2b), whereas proportional competence was not retained in the final model.

An assumption of the 'dilution effect' is that host community competence will be negatively associated with host species diversity. To test this idea, numerical community competence was regressed against the four measures of avian diversity described above. PCdiversity was retained as a highly significant variable in the final model, and was strongly negatively associated with numerical community competence ( $\beta$ = -0.43, AICc= 4.72, p<0.0001; Figure 4.2c). Table 4.3. Associations between WNV antibody prevalence and measures of avian diversity and community composition. Akaike's information criterion adjusted for small samples sizes (AICc) and adjusted  $R^2$  are shown for all models. The regression coefficient ( $\beta$ ) and p-values, adjusted for directional tests, are shown only for the minimum adequate models. Significance levels were adjusted for multiple tests (assuming  $\alpha = 0.025$ ). Significant p-values and significant variables are bolded. Richness and the abundance measures were square root-transformed, proportional community competence was arcsin-square root transformed and numerical community competence and numerical community competence and numerical community competence as described in Methods text.

Model		AICc	adjusted R <sup>2</sup>	β	p-value
Diversity and	richness+ Shannon's D+ PC-diversity+ passerine abundance	53.43	0.06		
Abundance	-PC-diversity	48.09	0.14		
	-richness	43.74	0.21		
	-Shannon's D	40.60	0.24	0.26	0.019
Community Competence	proportional CC + numerical CC	44.07	0.19		
(CC)	-proportional CC	40.71	0.24	1.023	0.021

#### Avian community composition and land use

Finally, we examined the association between urban land use and avian community characteristics across sites included in the WNV analyses. Specifically, we tested the association between avian community measures that were the strongest predictors of WNV antibody prevalence (PC-diversity, passerine abundance and numerical community competence) and land use measures that were retained in the final models of antibody prevalence (human population density (100m), human population density (500m), and proportionate core forest (1000m)). For all three avian community measures, proportionate core forest at 1000m was the strongest land use predictor (Table 4.4); this variable was positively associated with avian community diversity and negatively associated with total passerine abundance and WNV community competence.

Figure 4.3 summarizes the key directional effects and levels of support provided by our study, based on the final results of minimum adequate models.

Table 4.4. Associations between land use variables and (i) avian diversity, measured as PCdiversity as explained in Methods text (ii) total passerine abundance based on point count data, and (iii) numerical community competence, as described in Methods text. Akaike's information criterion adjusted for small samples sizes (AICc) and adjusted R<sup>2</sup> are shown for all models. The regression coefficient ( $\beta$ ) and p-values, adjusted for directional tests, are shown only for the minimum adequate models. Significant p-values and significant variables are bolded. Data transformations were performed as in previous analyses (see legend for Tables 2 and 3). CORE=core forest area and DENS=human population density.

PC diversity						
i C-diversity	AICc	adjusted $R^2$ $\beta$		p-value		
DENS_100+ DENS_500+ CORE_1000	49.88	0.25				
-DENS_100	45.79	0.30				
-DENS_500	42.29	0.34	4.98	0.006		
Dessering about day as						
Passenne abundance	AICc	adjusted R <sup>2</sup>	β	p-value		
DENS_100+ DENS_500+ CORE_1000	68.21	0.33				
-DENS_500	64.56	0.35				
-DENS_100	61.26	0.38	-9.79	0.004		
Numerical community						
competence	AICc	adjusted R <sup>2</sup>	β	p-value		
DENS_100+ DENS_500+ CORE_1000	20.96	0.43				
-DENS_100	16.60	0.47				
-DENS_500	13.57	0.49	-2.69	0.001		



Figure 4.3 Diagram illustrating key a priori hypotheses (bolded +/-) for the relationships between land use (as described by proportionate core forest at a 1000m scale), measures of the avian community, and the prevalence of West Nile virus. Regression coefficients and p-values for significant relationships tested are given in parentheses. \*The association between PC-diversity and WNV antibody prevalence was non-significant and was not retained in the minimum adequate model.

#### Discussion

Results shown here are consistent with recent work demonstrating that West Nile Virus antibody prevalence in wild songbirds in northern Georgia increases with increasing urbanization (Gibbs et al. 2006b, Bradley et al. 2008). Our results further indicate that this effect could be influenced by changes in the community composition of avian hosts, consistent with other recent local and large-scale analyses in N. America (e.g, Ezenwa et al. 2006, 2007; Allan et al. 2008; Swaddle and Calos 2008). Measures of urbanization examined in this study, namely human population density, impervious surface coverage, average forest patch size and the proportion of core forest area, collectively represent the level of human-mediated disturbance at a site and the degree of

forest fragmentation. Testing antibody prevalence against each variable at three spatial scales showed that increasing proportion of core forest area at the largest (1000m radius) scale best predicts decreases in WNV exposure in wild songbirds sampled across sites.

Past work on forest fragmentation and avian community ecology has shown that core forest area is a landscape feature that defines the species composition of a site (Blair et al. 1996; Crooks et al. 2004) and determines habitat suitability for a large number of passerine (and nonpasserine) bird species. In urban environments and surrounding suburban areas across eastern North America, core forest has declined rapidly over the past several decades, with a concomitant decline in bird species such as warblers and thrushes that require core forest habitat and respond poorly to human-mediated disturbance (Fernández-Juricic 2004; Fraterrigo and Wiens 2005). At the opposite extreme, Crooks et al. (2004) found that the abundance of exotic species (European Starlings, House Sparrows, and Rock Doves) was lowest in core forest habitats and highest in urban areas. It is also important to note that other species such as Northern Cardinals, Tufted Titmice, American Robins and Carolina Wrens can tolerate a range of disturbance levels and were captured across all locations in the current study.

To better account for the observed negative relationship between WNV antibody prevalence and core forest area, we examined a composite variable (community competence) that quantified the abundance of bird species that are competent amplifying hosts for WNV. Consistent with a recent study published by Allan at al (2008), we found that this measure of the numerical abundance of hosts and their efficacy in viral amplification and transmission is a highly significant predictor of WNV antibody prevalence. Thus, because avian hosts vary greatly in their competence for WNV infection and transmission, the composition of bird species at any given site could strongly determine overall levels of WNV transmission. Transmission patterns

as indicated by antibody prevalence among wild birds could ultimately predict human exposure to infection, as demonstrated by Allen et al. (2008) using data on mosquito infection rates at small spatial scales, and human cases at larger spatial scales.

Results reported here and from a handful of other studies focused on WNV in N. America (Ezenwa et al. 2006, 2007, Allen et al. 2008; Swaddle and Carlos 2008) are consistent with predictions from the dilution effect, namely that the presence of host species that are poor amplifying hosts can lower the transmission and prevalence of multi-host pathogens (Ostfeld and Keesing 2000; Keesing et al. 2006). In contrast to the studies by Ezenwa et al. (2006, 2007) that showed that the species richness of non-passerine birds, but not passerines, was negatively associated with mosquito infection rates, our study suggests that the abundance and composition of passerine communities plays a key role as well. This is probably because previous studies in wetland environments captured a larger number of non-passerine species (including ducks, geese, herons and egrets), whereas in the forested areas surrounding urban sites in North Georgia, passerine species predominated, accounting for >87% of bird species recorded in our point count surveys.

In this study, the effects of urbanization on WNV antibody prevalence were stronger than effects of avian community measures on antibody prevalence, indicating that urbanization could affect other mechanisms that are also important for WNV transmission. Previous work has shown that the dominant vector for WNV in the southeastern U.S., Culex quinquefasciatus, is an anthrophilic mosquito species, found in conjunction with high human population densities (Reisen and Brault 2007). Increased vector abundances could therefore increase transmission of WNV in urban habitats. However, dense C. quiquefasciatus populations are very localized and typically occur at wastewater spillways, drainage canals, or other stagnant, nutrient-rich water

sources (Calhoun et al. 2007). Efforts were made to choose sites for this study that were not adjacent to, and not likely to be influenced by, such habitats. Although total mosquito abundance and mosquito infection rates were not tested directly in this study, human population density, a strong predictor of C. quinquefasciatus abundance (Campbell et al. 2002), was positively associated with WNV antibody prevalence at the smallest spatial scale (100m), suggesting that C. quinquefasciatus abundances could present an additional underlying mechanism in the observed WNV antibody prevalence patterns.

A second factor that could contribute to positive associations between WNV antibody prevalence and urbanization is a higher rate of avian host recovery following infection among birds sampled in urban habitats. In other words, if avian case fatality rates are lower in urban environments, then a higher fraction of these birds will show antibodies to past infections (e.g., Bradley and Altizer 2007, Bradley et al. 2008). Such a relationship could be caused by two possible factors: first, studies show that urban centers retain heat more efficiently than outlying areas, leading to longer warm seasons, more temperate cold seasons, and warmer nights (Saaroni et al. 2000, Baker et al. 2002). A milder climate could decrease physiological stress and improve the rate of recovery in infected individuals. Additionally, many sampling locations in this study were in close proximity to supplemental food sources (in the form of bird feeders and fruiting vegetation); these concentrated food sources could be more common in urban areas, leading to high nutritional status or reduced energy expenditure during foraging. Thus, in conjunction with a favorable urban microclimate, reliable access to concentrated food sources could improve tolerance to or recovery from WNV among infected birds. Because this study examined antibody titer rather than current infection status, differential recovery rates of infected individuals among sites cannot be discounted as a potential mechanism contributing to the observed pattern.

Unlike other studies (Kilpatrick et al. 2006b, Savage et al. 2007, Hamer et al. 2009), we found no evidence that the presence of American Robins, a species that is relatively competent in terms of its ability to amplify virus and on which Culex mosquitoes preferentially feed, was associated with greater WNV antibody prevalence. However, it is important to note that this species accounted for a very low percentage of total birds captured (3.2%, N = 10) and recorded in point counts (7.4%, N = 225) across sites sampled here. In addition, we found no support for a correlation between WNV antibody prevalence and the abundance of Northern Cardinals, a species common to all sites examined that shows higher-than-average antibody prevalence (Gibbs et al. 2006a; Bradley et al. 2008; Loss et al. 2009), Therefore, although the presence and abundance of a small number of key avian hosts may be significant drivers of WNV transmission in some geographic locations, those trends might be difficult to detect using field abundance data such as those examined here, especially if mosquitoes do not contact bird species in relation to their proportional abundance in the avian community.

In summary, our results suggest that core forest area, passerine abundance, and avian community competence were the most important predictors of WNV antibody prevalence across the urban-rural gradient examined in this study. We acknowledge that establishing causality falls beyond the scope of work described here, in part owing to a limited number of sampling locations and a lack of data on host recovery and vector abundance / infection rates. Nevertheless, patterns shown here are consistent with recent studies pointing to a role for avian community composition in West Nile virus infections in humans and mosquito vectors (e.g., Ezenwa et al. 2006, 2007; Allan et al. 2008; Swaddle and Calos 2008). Our results are also consistent with recent studies showing no or only weak relationships between traditional measures of biodiversity (i.e., species richness) and vector infection rates for WNV (Loss et al.

2009) and Lyme disease (LoGiudice et al. 2008), underscoring that the identity of key host species in a community should be included in measures of community competence. Importantly, this calls for expanded data on vertebrate reservoir competence, as data on experimental infections are available for a relatively small number of hosts. These data will allow researchers to develop a more comprehensive measure of community competence and evaluate more rigorous hypotheses of single hosts or host species groups that have unusually strong influence on WNV transmission.

Finally, although the loss of species diversity in urban landscapes is well documented for birds and other wildlife groups (McKinney 2002, Pauchard et al. 2006, Olden et al. 2006), reasons beyond the aesthetic value of species diversity to aggressively manage against this trend are scant (but see Dobson et al. 2006). From one perspective, results of this study indicate that a 10% increase in core forest habitat could reduce WNV antibody prevalence in the avian community by more than 5%. Urban green spaces are increasingly incorporated into urban development plans, but these designs are typically limited to open parks with little tree canopy to support forest birds, or to smaller forest patches that exclude many rarer species. Urban planning to increase core forest habitat, such as reforesting riparian corridors to link smaller forest patches, will likely alter the composition of avian communities found there and, consequently, decrease the prevalence of WNV or other multi-host pathogens.

#### Acknowledgements

We thank Andy Davis, Ben Stoner-Duncan, Laura Gold, Adrienne Tecza, and Olivia Wright for field support, and Becky Bartel, Patrick Stephens, and Daniel Streicker for comments on earlier drafts of the manuscript. We also thank the private and public landowners who allowed access to

the field sites. This work was supported by a seed grant from the Southeastern Center for Emerging Biological Threats (SECEBT) and a graduate research fellowship from the University of Georgia to CAB. Animal protocols described herein were approved by the University of Georgia (2005-06; AUP No. A2006-10051). Banding permits were granted by the US Bird Banding Laboratory (#23141) and the Georgia Department of Natural Resources (permit No. 29-WMB-02-176).

## CHAPTER 5

# JUVENILE NORTHERN CARDINAL (*CARDINALIS CARDINALIS*) CONDITION AND THE PREVALENCE OF AVIAN MALARIA IN ADULTS: PATTERNS ACROSS AN URBAN GRADIENT

Bradley, C.A. To be submitted to Animal Ecology.

#### Abstract

Northern Cardinals (*Cardinalis cardinalis*) are an abundant and ubiquitous bird species found throughout the eastern United States. The species thrives in urban environments and is often found at high densities in metropolitan areas. However, these high densities can lead to increased intra-specific competition for territories and resources that may have indirect effects on individual condition and response to infections. In this study, Northern Cardinal populations were sampled at eighteen sites representing a gradient of urban land use in north-central Georgia (USA). The overall prevalence of avian malaria was 68%, with 87% of juveniles infected. Among adults, infection prevalence was significantly lower in urban areas compared to non-urban areas. Infected juveniles in more urbanized habitats had high heterophil-to-lymphocyte (H:L) ratios and increased plumage coloration. Furthermore, increasing H:L ratios, indicative of stress, were negatively correlated with feather growth rates, a signal of individual nutritional condition. The results of this study indicate that reduced condition in infected juvenile Northern Cardinals in urban environments may reduce individual tolerance to infection. This may be one explanation for the lowered prevalence of infection observed in adult birds.

#### Introduction

Avian communities have been significantly impacted by increases in urbanization. Generally, urban areas tend to support reduced species richness owing to a loss of native species, and a greater abundance of a small number of urban-adapted species (Melles et al. 2003; Crooks et al. 2004; Lepczyk et al. 2008). For animal species that thrive in urban environments, the unique attributes of those environments may significantly influence individual condition. Ruiz et al. (2002) showed that rural-dwelling Rufous-collared sparrows (*Zonotrichia capensis*) captured at

rural sites had lower blood glucose concentrations, higher body weight, and lower heterophil-tolymphocyte ratios than those living in cities, all indicative of better condition in the rural birds. Furthermore, when these birds were taken into captivity, the haematological parameters measured were no longer different from those of urban birds after two weeks, indicating that the stress response was inducible. However, blackbirds from both urban and rural sites that were raised under laboratory conditions showed lower levels of the stress hormone corticosterone for birds from urban sites, suggesting a genetic adaptation in this species to living in cities (Partecke et al. 2006).

As with stress, nutritional status is also likely to differ between urban and rural habitat types. Artificial sources, such as bird feeders, provide nutrient-rich foods and reduce seasonal fluctuations in the abundance of food resources (reviewed in Chase and Walsh 2004). In addition to availability, resource acquisition also differs in urban areas. Shochat et al. (2004) demonstrated that bird foraging behaviour at urban sites, as measured by the amount of food left over after a foraging event, reflected increased competition among birds over perceived predation risk (Shochat 2004).

Both the stress and nutritional status of an animal could affect its response to infectious disease. Chronic stress can reduce resistance to infection and increase the morbidity and mortality associated with infections (McCabe et al. 2000; Padgett and Glaser 2003) through the interference of glucocorticoid hormones, such as corticosterone, and catecholamines, such as epinephrine, in immune cell signalling. Klasing (1998) reviews the mechanisms associating nutritional status and immune response to infection in poultry, including nutrient roles in immune development *in ovo* and direct involvement in leukocyte regulation. For example, vitamin A mediates immune cell differentiation during development (Woods et al. 1995) and

chicks born to hens with vitamin A deficiencies have been shown to have lowered resistance to infections (Sklan et al. 1994; Friedman and Sklan 1997).

The Northern Cardinal (Cardinalis cardinalis) is one species that thrives in urban areas throughout its range in the eastern United States. Several studies have examined condition measures in Northern Cardinals, primarily the intensity of carotenoid-based plumage coloration. Red coloration in male Northern Cardinals is associated with higher quality territories, earlier breeding mates, and, as a consequence, increased reproductive success (Wolfenbarger 1999). Jawor et al. (2004) found that the redness of underwing feathers in females correlated with body size, body condition, early breeding, and improved reproductive success. Carotenoids, which play a role in the immune response by reducing the oxidative stress that is a by-product of cellmediated immunity (Bendich 1989; von Shantz et al. 1999), must be obtained from food (Goodwin 1950). The underlying cause of individual variation in coloration is not well understood, nor is the potential trade-off between immune function and coloration. However, carotenoid coloration is clearly affected by food availability. For example, following individuals across three years, Linville and Breitwisch (1997) found that after a severe winter reduced food availability, male Northern Cardinal plumage declined significantly and rebounded the following year with improved food availability. Navara and Hill (2003) also showed that diet significantly influenced coloration in American Goldfinches (Carduelis tristis) under laboratory conditions, but there was no evidence of a trade-off of immune function and coloration mediated by diet. Work by others has shown that carotenoid-based plumage is associated with increased heterophil-to-lymphocyte ratios and indicative of increased stress levels (Figuerola et al. 1999; Maney et al. 2008). Maney et al. (2008) suggest that this association may be a result of increased dominance behaviors and competitive ability in more brightly coloured individuals.

In this study, I examined the condition of juvenile Northern Cardinals with avian malaria infections across the urban gradient to identify a potential differential response to infection. Avian malaria is caused by protozoan parasites in the genera *Plasmodium* and *Haemoproteus*. Molecular analysis has shown that *Plasmodium* is paraphelytic to *Haemoproteus* and that there is a high diversity in parasite lineages across avian host species, with a high degree of host switching among lineages (Bensch et al. 2000; Ricklefs and Fallon 2002; Perez-Tris et al. 2005). The primary infection is associated with most morbidity and mortality because the pre-patent period is short and parasitemia reaches it maximum level (van Riper et al. 1986; Valkiūnas 2005). Fitness costs associated with infection are often not observed in field and laboratory studies (Woodworth et al. 2005; Valkiūnas et al. 2006; Bensch et al. 2007). However, Møller and Nielsen (2007) in a comparative analysis across avian prey species, found that those species with a higher prevalence of infection with protozoan parasites (*Plasmodium*, *Haemoproteus*, Leucocytozoan, and Trypanosoma genera) had a higher risk of predation from European Sparrowhawks (Accipiter nisus) and Eurasian Goshawks (Accipiter gentilis). In another example, van Oers et al. (2009) found in a mark-recapture study that Seychelles Warblers (Acrocephalus sechellensis) infected with avian malaria as juveniles had lower survival than uninfected juveniles. Consistent with Sol et al. 2003, they also found that avian malaria prevalence decreased with age (though see Ricklefs et al. 2005).

There were three aims to the study presented here. First, the age-related prevalence of infection with avian malaria was determined in Northern Cardinals captured at eighteen sites characterized by a gradient of urban land use in north-central Georgia (USA). Second, the prevalence of infection across the study sites was related to measures of urbanization to determine how avian malaria prevalence varies with the degree of urbanization. Finally,

variables associated with host health and condition (body size, leukocyte profiles, plumage coloration, and feather growth rates) in infected juvenile Northern Cardinals were examined across sites to assess the relative health of individuals fledged in environments with varying levels human disturbance. The results of these analyses are used to make predictions on the relative roles of recovery and disease-induced mortality to explain the pattern of infection in adults across sites.

#### **Methods and Materials**

#### Field Sampling

Eighteen sites in the Piedmont region of Georgia (USA) were chosen for this study to reflect a diversity of land use types (residential, agricultural, business, recreational) and a range of distances from two major metropolitan areas in the region, Atlanta and Athens (Figure 1). Adjacent sites were separated by a minimum of 5 km and the entire study area encompassed approximately 2500 km<sup>2</sup>. Northern Cardinals were sampled three times at each site between April and August of 2006: once each in the early, mid, and late breeding season. Birds were captured using mist nets between the hours of 06:00 to 12:00 following protocols described in Bradley et al. (2008). Each individual was aged (HY-hatch year or AHY- after hatch year) by plumage and gape characteristics and sexed based on sexually dimorphic characteristics and breeding status (Pyle 1997).



Figure 5.1. The geographic location of the 18 sites used in this study. Counties are labelled and urban areas, as designed by the 2004 U.S. Census, are shaded.

Weight (in grams), wing chord length and tarsus length (in millimeters), and molt status (feathers emerging at the time of sampling) were recorded. Pectoral muscle development around the carina (or breastbone) was scored following Gosler (1991) as: 1-severely-pectoral muscle; 2-sunken pectoral muscle; 3- pectoral muscle even with the carina; or 4-pectoral muscle development beyond the carina. The amount of visible subcutaneous fat in the furculum was similarly scored as: 0-no fat visible; 1-furculum one-third full; 2-furculum one-third to two-thirds full; 3-furculum full; or 4-fat bulging from the furculum, following Hartup et al.(2001). When

birds were not molting, the outermost pair of retrices (tail feathers) were taken and stored in glassine envelopes for plumage coloration and growth analysis (described below).

Using ulnar (wing) venipuncture with a 26-gauge needle, 50-100ul of blood was collected in heparinized microhematocrit tubes. Blood samples were maintained at ambient temperature for at least ten minutes and were then kept cool until returning to the lab. All blood samples were centrifuged at 10,000g for 10 minutes and both serum and erythrocytes were stored in separate cryovials at -70°C. For leukocyte profiles, two blood smears were prepared in the field using the two slide wedge technique (Walberg 2001), air dried, and fixed in methanol. Each individual Northern Cardinal was banded using U.S. Fish and Wildlife Service bands prior to release.

#### Identifying Malarial Infections

Because blood smears have been found to be an insensitive method for the identification of low levels of malarial parasitemia (< 40 parasites/µl of blood; Bruce and Day 2002), more recently developed PCR techniques (Jarvi et al. 2002; Waldenström et al. 2004; however, see Valkiūnas et al. 2006) were used to improve the likelihood of detecting infections. In this study, malaria parasites (*Plasmodium* spp. and *Haemoproteus* spp., Perez-Tris et al. 2005) were identified using the nested PCR protocol developed by Waldenström et al. (2004) which amplifies a 478-bp fragment of the mitochondrial cytochrome-b gene. Following DNA extraction from a 5-10µl sample of erythrocytes using the Qiagen DNEasy Blood and Tissue Kit (Valencia, CA, USA), two rounds of PCR were performed. The first round used the primer set HaemNF (5'-GGGCAGGGACGTAG TCAGC-3') and HaemNR2 (5'-AGAGGTGTGCATATCTATCTAC-3'). Reactions were run in 25µl volumes consisting of 12.5µl GoTaq Master Mix (ProMega; Madison, WI), 1µl of each primer, 2 µl of extraction product and 8.5µl of ddH20. The second

round of PCR was also run in 25µl volumes (12.5µl GoTaq, 1µl of each primer, 1µl of PCR product, 9.5µl of ddH20) using the primer set HaemF (5'-ATGGTGCTTTCGATATATG CATG-3') and HaemR2 (5'-GCATTATCTGGATGTGATAATGGT-3'). Each round of PCR was run with 48 samples, including a negative and positive control. PCR products were separated on 2% agarose gels and visualized with CyberSafe DNA gel stain under UV light. Bands observed in the expected region of 478bp were used to identify samples containing malarial parasites.

#### Body Condition, Plumage Coloration and Feather Growth

Body condition was assessed for each individual by regressing body weight on tarsus length and using the residual of this regression as a relative measure of individual condition (Brown 1996). Regressions were performed separately for each age class (HY: slope=0.951, R<sup>2</sup>=0.20,  $F_{(32,1)}$ =8.233, p=0.007 and AHY: slope=0.842, R<sup>2</sup>=0.122,  $F_{(110,1)}$ =15.338, p<0.001).

Plumage coloration was based on digital analysis of the outermost pair of tail feathers. Feathers were scanned on an EPSON Perfection 4180 Photo scanner following color calibration to the Adobe 1998 colorspace and using VueScan 8.4.43 scanning software (Hamrick Software; Phoenix, AZ). Scans were made at 600dpi resolution and RGB values were encoded at 24bpp (bytes per pixel). Using the image processing program ImageMagick (open source software available at www.imagemagick.org), the red value (between 0 and 255) of each pixel in the feather image was retained and an average of these values was calculated. Specifically, feathers with high values are characterized as a more vibrant red than those feathers with low values which were greyer. Red values ranged from 96.79-130.33, with an average of 523,545 pixels evaluated in each sample. The mean and variance of feather growth were quantified for the right outermost retrix following the protocols outlined in Grubb (1989) and Jawor et al. (2004). First, the full length of the feather was measured to the nearest 0.01mm using digital calipers. To account for varying lengths, the point two-thirds from the proximal end of the feather was used as the midpoint. From this midpoint, 5 growth bars above and 6 growth bars below were measured from the proximal end of the feather. The distance between each successive growth bar, and the mean and variance of the 10 distances, were then calculated for each sample. Each growth bar represents 24 hours of growth (Brodin 1993) and the rate of growth is an index of nutritional status (Grubb 1991). Greater mean values indicate that feathers were produced at a faster rate, and higher variances indicate inconsistency in the rate of feather growth.

#### Heterophil to Lymphocyte Ratio

The ratio of heterophils to lymphocytes (H:L ratio) was determined for each individual for which blood smears were made. This ratio has been shown to increase with higher levels of stress and infection (reviewed in Davis et al. 2008). Smears were stained with Giemsa and viewed at 1000x power under oil immersion to identify leukocytes (Campbell 1995). For each slide, every heterophil and lymphocyte were counted until 100 total cells were observed or 150 fields of view had been examined, as described in Maney et al. (2008). The number of heterophils were divided by the number of lymphocytes to determine the H:L ratio for each individual.

#### Site Characteristics

The level of urbanization and avian abundance at each site was quantified using two measures identified in previous studies (Bradley et al. 2008; Bradley et al. *in review*) that best explained levels of exposure among wild songbirds to another vector-borne disease (West Nile virus). To estimate urbanization, the proportion of the forested area at each site that consists of core forest

was measured, defined as area interior to a 10m buffer around each forest patch, using a 44-class land use map of Georgia developed by NARSAL (the Natural Resources Spatial Analysis Laboratory, UGA, 1998). Previous work has shown that the land use variables determined from this map are strongly correlated with those derived from ground-truthed aerial photographs (Bradley et al. 2008) and indicate that none of the sites have undergone significant development in the past decade. Total forest area (m<sup>2</sup>) and core forest area (m<sup>2</sup>) were evaluated for each site at a radial distance of 1000m from the sampling location using the methods described in Bradley et al. (2008). Proportion core forest was then calculated by dividing the core area by the total area. This parameter ranged from 0.238 to 0.584 (mean=0.376, SE=0.027) for the 18 sites used in this study. Previous studies showed that this measure is highly negatively correlated with human population density and impervious surface area (Bradley et al. 2008, *in review*) and represents an inverse measure of the degree of urbanization.

Northern Cardinal abundance and the total abundance of all passerine species at each site were also measured using point counts as described in Fraterrigo and Wiens (2005) and Bradley et al. (*in review*). Briefly, each site was surveyed twice, once in the early morning and once in late morning. At each occasion, all birds seen and heard during a 5 minute period at each of 10 point locations were recorded. Point locations were approximately 150m apart and randomly determined, but centered on the location where bird capture took place. The total abundance of passerine birds ranged from 62 to 211 across sites (mean=146, SE=10.58), and Northern Cardinal abundance ranged from 8 to 45 (mean=24, SE=2.42). Past work (Bradley et al. *in review*) showed that total passerine abundance was strongly negatively correlated with measures of avian community diversity (e.g., species richness, Simpson's index), and was positively correlated with site urbanization. This finding is consistent with other studies. For example,

Crooks et al. (2004) found that in southern California, areas with high core forest consisted of many passerine species and low overall abundance, and areas with low core forest consisted of few species at high abundances. In this study, passerine abundance decreased with increasing proportion core forest (slope=-1.908, R<sup>2</sup>=0.45,  $F_{(16,1)}$ =12.959, p=0.0002) as did Northern Cardinal abundance (slope=-1.836, R<sup>2</sup>=0.22,  $F_{(16,1)}$ =4.534, p=0.049) (Figure 2).



Figure 5.2 The relationship between proportion core forest (arcsine-square root transformed) and avian abundance (log transformed). Passerine abundance is depicted in black and Northern Cardinal abundance is depicted in gray. Hollow points represent those sites used in the analyses relating condition measures to measures of urban land use (restricted to hatch year or HY birds).

#### Statistical Analysis

Analyses were performed using R2.2.0 (open source software available at www.r-project.org). Both H:L ratios and the proportion of core forest were arcsine-square root transformed prior to

analysis, and measures of passerine and Northern Cardinal abundance were log10 transformed to

normalize the error variance. First, the association between malarial infection and host age was tested with logistic regression, with capture site as a random variable. Second, to examine the association between malaria prevalence and site characteristics (core forest area, passerine abundance, and site category (urban or non-urban) based on core forest area) for each age separately, linear regression was used treating each site as the unit of observation and using the logit transformation (Ln( antibody positive/ antibody negative)) to linearize antibody prevalence data for each site prior to analyses. Third, the association between individual condition measures in juveniles (body condition, H:L ratio, plumage coloration and feather growth), sex, and site characteristics, where the individual is the unit of observation and capture site is a random variable, was examined (Hurlbert 1984; Crawley 2007). Bivariate correlations between each pair of condition measures in infected juvenile birds were also examined and repeated for each sex independently. Model simplification was performed using AIC adjusted for small sample sizes (AICc) following Crawley (2007). Wherever appropriate, the minimum adequate models are reported and the associated test statistic, R-squared, and p-value are given for the final model and significant predictors.

#### Results

#### Malaria Infections

A total of 161 Northern Cardinals were sampled, ranging from 2 to 20 individuals per site. For the 130 individuals where blood samples were analyzed, 68% were positive for avian malaria parasites. A higher percentage of juvenile (HY) birds tested positive for avian malaria (87%, N = 30) as compared to adult (AHY) birds (61%, N = 100), and this effect of age was significant (zvalue=2.537, p=0.011). Of the four HY birds testing negative for malaria parasites, three were

from the same site and this site was characterized as highly urban (proportion core forest=0.24 and passerine abundance=211).

Though not significantly related to passerine abundance (p=0.29) or core forest area (p=0.14), the prevalence of malaria in AHY birds was significantly greater in low urban sites when sites were treated as categories based on proportion core forest (z-value=2.069, p=0.04; non-urban prevalence=69.5%, urban prevalence=48.8%). The prevalence of malaria infection in HY birds did not differ significantly across sites (passerine abundance: p=0.56; core forest area: p=0.66, site category: p=0.75; non-urban prevalence=94%, urban prevalence=79%).

#### Juvenile Condition Parameters

Tests of associations between condition parameters and urban land use across the study sites were restricted to juvenile birds infected with malaria parasites. This restriction limited the analyses to nine of the study sites, but these remain representative of the urban gradient with respect to proportion core forest and passerine abundance (Figure 2).

Increased plumage coloration was significantly associated with increased passerine abundance but not with sex [MAM: plumage coloration=passerine abundance +random=1|site; slope=8.36,  $F_{(6,1)}$ =13.218,  $R^2$ =0.29, p=0.011; Figure 3a]. H:L ratios were not associated with passerine abundance but significantly increased with declining proportion core forest [full model: H:L ratio=proportion core forest + random=1|site; slope=-0.522,  $F_{(6,1)}$ =20.855,  $R^2$ =0.26, p=0.004; Figure 3b]. Neither the average nor the variance of feather growth rates were significantly related to either measure of urban development. The residual of the regression of weight on tarsus length was also not significantly related to either urban measure.

When each sex was considered separately, several condition measures were strongly correlated with one another (Table 1). Body size was positively correlated with plumage

coloration and average feather growth in females. Average feather growth rate was negatively correlated with H:L ratios for both males and females. Plumage coloration and H:L ratios were positively correlated for males, but not for females



Figure 5.3. a) The relationship between passerine abundance (log transformed) and plumage coloration for hatch year (HY) birds with malarial infections. b) The relationship between proportion core forest (arcsine-square root transformed) and the heterophil:lymphocyte (H:L) ratio (arcsine-square root transformed) for hatch-year (HY) birds with malarial infections.

	Body size		Plumage Coloration		H:L Ratio		Average Feather Growth	
	male	female	male	female	male	female	male	female
Body size			-0.15	0.37*	0.02	-0.02	-0.10	0.25*
Plumage Coloration					0.49*	-0.11*	< 0.01	0.06
H:L Ratio							-0.14*	-0.50*
Average Feather Growth								
Glowin								

Table 5.1. Correlations between condition measures for male and female juvenile Northern Cardinals with avian malaria infections. \* Significant at the  $\alpha$ = 0.05 level.

#### Discussion

The results of this study demonstrate high infection rates with avian malaria among Northern Cardinals sampled in North Georgia, with the majority of individuals harboring parasites based on PCR analysis. Although fewer hatch year birds were sampled as compared to adults, juveniles were significantly more likely to harbor infections. This result is consistent with previous work by van Oers et al. (2009) who found a similar relationship with age and *Haemoproteus* infections in the Seychelles Warbler (*Acrocephaeus sechellensis*). Using markrecapture and re-sighting methods, these researchers also showed that both parasite-mediated mortality and resistance to infection played a role in this pattern, similar to the conclusions of Sol et al. (2003). Specifically, infected juveniles had lower survival than uninfected juveniles, but of those infected juveniles that did survive to be recaptured, prevalence also declined.

The mechanism behind the association between age and infection was not directly examined here, but data on the condition of infected juveniles along a gradient of urbanization suggest that individuals in urban environments have higher stress levels and lower nutritional health than those residing in rural areas. Specifically, the heterophil-to-lymphocyte ratio increased with increasing urban land use and this ratio was negatively associated with average feather growth rate in both male and female juveniles. This may contribute to a higher level of parasite-mediated mortality in urban birds and explain the lowered prevalence of infection observed in adult birds at urban sites.

Other studies have found an association between habitat types and feather growth rates in wild avian populations. Stratford and Stouffer (2001) showed that two species of birds typically found in the core forest area (Wedge-billed Woodpeckers (*Glyphorynchus spirurus*) and Whitecrowned Manakins (*Pipra pipra*)) had significantly lower feather growth rates when residing in small forest fragments compared to the growth rate in individuals in contiguous forest. Average feather growth rates also differed in Loggerhead Strikes (*Lanius ludovicianus*) captured in four different habitat types (Grubb and Yosef 1994). Specifically, birds in pasture habitats had the highest growth rates while those in citrus fields had the lowest. As one possible explanation for the observed pattern, Grubb and Yosef (1994) suggest that management strategies in citrus fields, including the use of pesticides and herbicides, could affect the condition of strikes residing there.

Hill and Montgomerie (1994) and Senar et al. (2003) both found associations between plumage coloration and feather growth rates in Great Tits (*Parus major*) and House Finches (*Carpodacus mexicanus*), respectively; however, this association was not observed here in juvenile Northern Cardinals. Carotenoid-based plumage coloration did increase in urban areas. Two, non-exclusive mechanisms could be driving this association: an increased abundance of carotenoid-rich resources in urban areas (the "foraging hypothesis"; Hill 1992) or an increased competitive advantage to color deposition in urban areas (the "condition hypothesis"; Hudon 1994). Though the results here do not support a link between nutritional status and plumage coloration, a strong association between coloration and elevated H:L ratios was observed in male

juveniles, consistent with the hypothesis of increased competition (Figuerola et al. 1999; Maney et al. 2008).

If carotenoids are not limiting in most natural environments and plumage coloration is primarily an expression of fitness, bright coloration may contribute to increased stress in urban areas. One potential source of stress may be increased competition in crowded habitats. Luniak (2004), reviewing the characteristics of species that are thriving in urban areas, found that several species of birds have increased abundances and reduced territory sizes in urban environments which can be associated with increased intra-specific competition. This study did not look at territory size, but did observe an increase in Northern Cardinal abundance concomitant with increasing urban development as measured by declining proportion core forest.

Several characteristics of urban environments could support a high abundance of Northern Cardinals despite the presence of potential stressors. Urban environments do present habitats with abundant resources (Jokimäki et al. 2002) and moderated seasonality (Arnfield 2003). Such shifts have been associated with the early onset of breeding condition in the spring, increased number of clutches produced, and improved juvenile survival in a number of urbanadapted avian species (reviewed in Boutin 1990; Shochat et al. 2006). For example, Bowman et al. (1998) found that Florida Scrub-Jays (*Aphelocoma coerulenscens*) in suburban environments laid eggs earlier in the season and had larger clutches than those residing in more natural habitats. Following this work, Schoech and Bowman (2001) showed that female scrub-jays residing in a suburban area had higher plasma protein levels prior to the commencement of the breeding season than those in a natural habitat and attribute this to the quality and availability of food resources in more urbanized habitats.

One issue that could affect the results of this study is the unknown identity of the malaria parasite species and lineages infecting each individual. In one study, Ricklefs et al. (2005) identified 34 different lineages in an avian community observed over three years in southern Missouri (USA) and co-infections of up to 14 different lineages in a single host individual. Malaria parasites are a very diverse group (Bensch et al. 2000; Ricklefs and Fallon 2002) and infections resulting from different parasite species can produce varying levels of disease in host species. As one example, Zehtindjiev et al. (2008) showed that the pre-patent period, maximum level of parasitemia, and host immune response resulting from the experimental infection of two different malarial parasites (*Plasmodium ashfordi* and *P. relictum*) in the Great Reed Warbler (*Acrocephalus arundinaceus*) differed significantly. Because the study described here did not identify the malaria parasite lineages in infected individuals, it is possible that the pathogenicity of the malaria lineages examined here differed across the study sites and that this, rather than differences in mortality associated with stress, explains the observed pattern of prevalence between urban and non-urban sites.

Consistent with previous work, this study presents evidence of a lowered prevalence of malaria parasites in Northern Cardinals with age. Observing that the prevalence of malaria in urban-dwelling, adult Northern Cardinals is less than in adults residing in more natural habitats, the data further indicates that increased stress, as measured by H:L ratios, feather growth rates, and greater investment in plumage coloration, may be associated with a decline in resistance to infection.

#### Acknowledgements

I thank Sonia Altizer for comments on earlier drafts and suggestions for improvement to the manuscript, and Ben Stoner-Duncan for field and laboratory support. I also thank the private and public landowners who allowed access to the field sites. Andy Davis provided guidance with avian leukocyte measures, feather analysis and field protocols. This work was supported by a seed grant from the Southeastern Center for Emerging Biological Threats (SECEBT), an American Ornithological Union research grant, and a graduate research fellowship from the University of Georgia. Animal protocols described herein were approved by the University of Georgia (2005-06; AUP No. A2006-10051). Banding permits were granted by the US Bird Banding Laboratory (#23141) and the Georgia Department of Natural Resources (permit No. 29

### CHAPTER 6

## SALMONELLA SURVEILLANCE IN SONGBIRDS FROM NORTHERN GEORGIA, USA REVEALS S. MUENCHEN ISOLATES THAT ARE SIMILAR TO THOSE OF HUMANS

Bradley, C.A., S. Altizer, S. Sanchez, Y. Cheng, M.D. Lee, L.K. Theobald, P. Gerner-Smidt, J. Castleman, S. Valeika, and J. J. Maurer. To be submitted to *Emerging Infectious Diseases*.

#### Abstract

Between April and August of 2006, 247 wild songbirds across 21 sites in Northern Georgia, USA, were sampled for *Salmonella* isolates. *S. enterica* Muenchen was isolated from a Brown Thrasher (*Toxostoma rufum*), Northern Cardinal (*Cardinalis cardinalis*), and Carolina Wren (*Thryothorus ludovicianus*) captured at the same location. The carriage of *Salmonella* in songbirds was 1.2%. The pulsed-field gel electrophoresis (PFGE) profiles of the songbird isolates were unrelated to *S. Muenchen* from other animal species, but two of them were similar to patient isolates present in the PulseNet USA database. Human cases of *S.* Muenchen cluster geographically in the South Atlantic states, overlapping the geographic range of the bird species positive for *S.* Muenchen. We suggest that *Salmonella* surveillance in songbird populations can reveal potential sources of pathogens that pose risks for human health.

#### Introduction

In a 2006, *Salmonella* was the second leading cause of foodborne outbreaks in the United States; accounting for 112 outbreaks and 3,252 laboratory-confirmed cases (CDCP 2006). A single commodity could be identified and linked to a foodborne outbreak in only 20% of all events. In addition to foodborne transmission, environmental exposures have been linked to sporadic human cases of gastroenteritis associated with *Salmonella* (Kapperud *et al.* 1998; Jones *et al.* 2006; Ashbolt and Kirk 2006; Denno *et al.* 2009), *Escherichia coli* O157 (Michel *et al.* 1999; Innocent *et al.* 2005), and *Campylobacter* (Ethelberg *et al.* 2005; Green *et al.* 2006). Specifically, exposure to reptiles, amphibians, having well water, and swimming or playing in fresh or saltwater was associated with increased risk of *Salmonella* infection in children and infants
(Jones *et al.* 2006; Denno *et al.* 2009). In two outbreaks, illnesses were directly attributed to the handling of *Salmonella* infected wild birds or wild bird feces (Thornley *et al.* 2003; Smith *et al.* 2005).

Salmonella enterica has been recovered from numerous free-ranging avian species, including passerine, psittacine, and gallinaceous birds, free-ranging and captive waterfowl, and raptors (Craven *et al.* 2000; Hudson *et al.* 2000; Refsum *et al.* 2002; Hernandez *et al.* 2003; Thornley *et al.* 2003). Cases of salmonellosis in free-ranging birds, particularly in passerines, have been reported in the eastern and midwestern United States and several other countries since 1957, when this disease was first documented in wild birds (Hudson and Tudor 1957). Recent outbreaks of salmonellosis in North America and Scandinavia have resulted in widespread mortality in passerine populations (Kapperud *et al.* 1998; Daoust *et al.* 2000; Refsum *et al.* 2002). These epizootic outbreaks often involve the spread of a single *S.* Typhimurium strain through the songbird population (Kapperud *et al.* 1998; Daoust *et al.* 2000; Hudson *et al.* 2000; Refsum *et al.* 2002). These *Salmonella* outbreaks in wild birds often occur in the winter months and may be due to the increased host contact through feeder stations and environmental stressors such as lower temperatures (Tizard 2004).

Bird watching is a popular past time in the United States with 55.5 million Americans, spending on average 115 day observing songbirds at their home feeders (U.S. Fish and Wildlife Service 2006) and spend \$3.5 billion/year on bird seed alone (U.S. Fish and Wildlife Service 2000). These activities may pose a risk of *Salmonella* transmission to humans. However, little is known about the prevalence and distribution of endemic *Salmonella* species in free-ranging passerines (Hall and Saito 2008). We examined *Salmonella* prevalence in healthy songbirds, passerine species colonized and distribution of *S. enterica* serovars and the possible link to

human illnesses either as a reservoirs, amplifiers, or sentinels of environmental contamination. Describing the distribution of infections by species, time, and location provides the foundation for understanding the epidemiological and ecologic role of songbirds in *Salmonella* transmission.

### **Materials and Methods**

We obtained samples for *Salmonella* isolation from wild passerines captured at 21 locations surrounding metropolitan Atlanta and Athens, GA, USA between April and August 2006 (Figure 6.1). The entire study area encompassed approximately 2500 km<sup>2</sup> with adjacent sites separated by a minimum of 5 km. The landscape surrounding each site ranged from small forest patches in urban areas to rural sites surrounded by forest and pasture (Bradley *et al.* 2008). Every site was visited three times, once each in early, middle and late summer. Birds were captured in mist nets between 06:30 and 12:00 on sampling days and were held in paper lunch bags for up to 30 minutes. Fecal samples and cloacal swabs were collected from each individual; we also recorded age, sex, species and morphological data. Birds were released shortly after samples were obtained. Each Bird was handled according to international welfare standards. The animal's health was observed during the capture process. We also observed and recorded the passerine, species and numbers congregating at each field site.

Fecal samples were immediately placed into selenite broth (Silliker *et al.* 1964) and transported to the Athens Diagnostic Lab (University of Georgia, College of Veterinary Medicine). Following 18 h enrichment at 42°C, a loopful of the selenite broth was streaked for isolation onto XLT4 and BGN plates (Hajna and Damon 1956) and incubated overnight at 37°C. PCR was also used to screen enrichments for *Salmonella* as follows. DNA template was



Figure 6.1. Geographic locations and county names for 21 collection sites in northern Georgia, USA where songbirds were caught and tested for *Salmonella*. Sandy Creek Nature Center (Athens, GA) where the *Salmonella*-positive songbirds were identified is illustrated as an open circle.

prepared from a 200-µl aliquot of selenite enrichment using a MOBIO soil DNA extraction kit (MOBIO, Carlsbad, CA, USA). Real-time PCR was then used to identify *Salmonella* positive selenite enrichments (Daum *et al.* 2002). All culture negative samples were subjected to a secondary enrichment and a second PCR screen. There was 100% agreement between culture and PCR results.

Suspect *Salmonella* colonies from either XLT4 or BGN plates were picked and streaked again for isolation on sheep blood (5%), tryptic soy agar plates. Final identification was determined based on biochemical results for triple-sugar iron and motility-indole-ornithine media and whole cell agglutination test using *Salmonella*-specific, poly A-I Vi antiserum (Fisher

Scientific, Pittsburgh, PA USA) (Farmer and Kelly 1991). *Salmonella* isolates were submitted to the National Veterinary Surveillance Laboratory (Ames, IA, USA) for serotyping. Pulsed-field gel electrophoresis (PFGE) was used to determine the genetic-relatedness (Hunter *et al.* 2005; Koort *et al.* 2002; CDCP 2009) of *Salmonella* songbird isolates to each other and to other isolates of the same serovar obtained from other animal species in Georgia.

Statistical analyses were performed in Stata 9.2 (College Station, TX). A two-sample proportion test was used to compare the proportion of captured birds belonging to a species with the proportion of observed birds belonging to a species. Adjustments for multiple comparisons were not performed, nor appropriate (Rothman 1990).

## Results

## Salmonella enterica in healthy songbirds present in Northern Georgia

A total of 247 samples were obtained from individual songbirds (n=1 to 22 per site) representing 31 passerine species. The most commonly sampled species included House Finches (*Carpodacus mexicanus*), Northern Cardinals, Carolina Chickadees (*Poecile carolinensis*), Tufted Titmice (*Baeolophus bicolor*) and Carolina Wrens (Table 6.1). For 2006, these bird species ranked among the top 25 songbirds sighted at bird feeders in Athens, GA (Great American Backyard Bird Count: http://gbbc.birdsource.org/gbbcApps/results).

For most species, the proportion captured in the sample population was similar to the proportion amongst all observed birds at each field site. In eleven species, the difference in captured proportion vs. overall proportion was statistically significant, however meaningful differences were only observed for Goldfinches, Chickadees, and House Finches. There were no differences in the sampled vs. observed proportions for our three Salmonella positive birds (Brown Thrasher, Northern Cardinal and Carolina Wren) (Table 6.1).

Species	Birds Captured (% of Sample) N=247	Birds Observed (% of Total) N=2616	p- value <sup>*</sup>
American Goldfinch	13 (5.26%)	53 (2.03%)	0.001
American Robin	6 (2.43%)	238 (9.10%)	0.000
Black-throated Blue Warbler	1 (0.40%)	2 (0.08%)	0.127
Blue Jay	5 (2.02%)	209 (7.99%)	0.001
<b>Brown Thrasher</b>	5 (2.02%)	92 (3.52%)	0.215
Brown-headed Cowbird	3 (1.21%)	26 (0.99%)	0.741
Brown-headed Nuthatch	3 (1.21%)	63 (2.41%)	0.232
Carolina Chickadee	33 (13.36%)	223 (8.52%)	0.011
Carolina Wren	19 (7.69%)	213 (8.14%)	0.804
Chipping Sparrow	6 (2.43%)	43 (1.64%)	0.363
Eastern Towhee	4 (1.62%)	104 (3.98%)	0.063
Eastern Tufted Titmouse	20 (8.10%)	242 (9.25%)	0.548
Field Sparrow	1 (0.40%)	17 (0.65%)	0.641
Gray Catbird	6 (2.43%)	85 (3.25%)	0.482
Hairy Woodpecker	1 (0.40%)	4 (0.15%)	0.365
Hooded Warbler	2 (0.81%)	6 (0.23%)	0.099
House Finch	49 (19.84%)	162 (6.19%)	0.000
House Wren	4 (1.62%)	17 (0.65%)	0.088
Indigo Bunting	4 (1.62%)	12 (0.46%)	0.019
Kentucky Warbler	1 (0.40%)	6 (0.23%)	0.593
Mourning Dove	4 (1.62%)	99 (3.78%)	0.081
Northern Cardinal	35 (14.17%)	444 (16.97%)	0.259
Northern Mockingbird	1 (0.40%)	138 (5.28%)	0.001
Purple Finch	1 (0.40%)	3 (0.11%)	0.243
Red-bellied Woodpecker	1 (0.40%)	56 (2.14%)	0.062
Red-breasted Grosbeak	1 (0.40%)	2 (0.08%)	0.127
Slate-colored Junco	1 (0.40%)	1 (0.04%)	0.037
Song Sparrow	7 (2.83%)	16 (0.61%)	0.000
Summer Tanager	1 (0.40%)	1 (0.04%)	0.037
White-breasted Nuthatch	7 (2.83%)	37 (1.41%)	0.083
White-throated Sparrow	2 (0.81%)	2 (0.08%)	0.003
Totals	247 (100%)	<b>2616 (100%)</b> **	

Table 6.1. Distribution of bird species tested for Salmonella

\* This is the p-value for the test of the hypothesis that the proportion of some species in the sample equals the proportion of that species amongst all of the birds observed. \*\*The remaining 20.6% are birds from species that were not captured, and are not included in this

table.

*Salmonella* was isolated from three songbirds, a Brown Thrasher, Northern Cardinal, and Carolina Wren, and identified as *S. enterica* serovar Muenchen (UGA1, UGA2, and UGA3, respectively). These positive cases represented 1.2% of the sampled population (Table 6.1). Notably, all three birds were captured at Sandy Creek Nature Center in Clarke County, GA (Figure 6.1) (sample prevalence= 16.7%; n=18). The positive Brown Thrasher sample was collected on April 24, 2006, and the other two positive samples were collected on July 6, 2006.

Sandy Creek Nature Center had the fourth highest number of samples collected there (n = 18). This location did not differ significantly from the average of other sites with respect to overall avian abundance (p=0.20) or avian species richness (p=0.67) based on point count surveys of bird species present at each location. Although this site was in moderate proximity to several livestock and poultry rearing operations (U.S. Department of Agriculture 2007), we were unable to perform a quantitative comparison at a site-level owing to data available at the county resolution.

#### Salmonella Muenchen isolated from songbirds are similar to human isolates

We compared the songbird *Salmonella* PFGE patterns to themselves and *S. Muenchen* from other animal sources. Two of the three *Salmonella* songbird isolates produced similar PFGE patterns to cluster together (87.5% similarity) when comparing DNA fingerprints generated with restriction enzymes, *Xba* I and *Bln* I (Figure 6.2), but distinctly different from *S.* Muenchen isolated from a dog and horse isolated the same year.

In a search of the CDC PulseNet database, we identified six *Salmonella* Muenchen isolates from humans with *Xba*I PFGE patterns similar to the songbird isolates UGA 2 and UGA 3, differing by 2-3 bands (Figure 6.3). *Salmonella* Muenchen isolate UGA1 exhibited very limited similarity in PFGE patterns to the other isolates including the two songbird *Salmonella* in our study, and failed to cluster with any human *S*. Muenchen isolates in the CDC PulseNet database. This *Salmonella* isolate was serotyped a second time and still typed out as *S*. Muenchen.

Combined	PFGE-X bal	PFGE-Bini	-				
			Көу	Ser oty pe	Origin	source	Year
<u></u>		11111	UGAsm2	Muenchen	GA	Northeim Cardinial	Jul. 2005
<u></u>			UGAsm3	Muenchen	GA	Carolina Wiren	Jul: 20.05
[			UGA sm6	Muenchen	GA	Golden Retriever	May 2008
· · · · · · · · · · · · · · · · · · ·			UGAsm5	Muenchen	SC	Arab horse	Oct. 2007
			UGAsm1	Muenchen	GA	Brown Thrasher	Apr. 2006

Figure 6.2. Cluster analysis of *S*. Muenchen, from animal sources, pulsed-field gel electrophoresis (PFGE) patterns generated with the restriction enzyme *Xba*I and *Bln*I. A Tiff image of *S*. Muenchen PFGE patterns were compared using DNA pattern recognition software, BioNumerics (Applied Maths, Inc., Austin, Texas). Level of similarity was calculated using the band-based Dice similarity coefficient, and clustering of samples was performed using the unweighted pair-group method with arithmetic averaging (UPGMA). *Salmonella* isolates UGA1-3 were from wild birds, UGA5 and UGA6 from Arab horse and Golden Retriever, respectively.



Figure 6.3. Cluster analysis of human and songbird *S*. Muenchen pulsed-field gel electrophoresis (PFGE) patterns generated with the restriction enzyme *Xba*I. A Tiff image of *S*. Muenchen *Xba*I-generated PFGE patterns for songbird isolates, illustrated in Figure 2, were compared to human isolates in CDC Pulsenet database using DNA pattern recognition software, BioNumerics (Applied Maths, Inc., Austin, Texas). Level of similarity was calculated using the band-based Dice similarity coefficient, and clustering of samples was performed using the unweighted pair-group method with arithmetic averaging (UPGMA). *Salmonella* isolates UGA2 and UGA3 were from wild birds, UGA5 and UGA6 from Arab horse and Golden Retriever, respectively. All other isolates, JJ6X01.xxxx were from humans.

# Discussion

Beyond previous studies of epizootic outbreaks of salmonellosis in wild birds (Daoust *et al.* 2000; Hudson *et al.* 2000; Tizard 2004; Hall and Saito 2008), there have been few studies addressing the percentage of the sampled, healthy bird population carrying *Salmonella* (Morishita *et al.* 1999; Refsum *et al.* 2003; Kobayashi *et al.* 2007). The low proportion (1.2%) of

healthy songbirds, captured in north Georgia, with *Salmonella* is similar to percentages of 2-7% reported in other studies (Morishita *et al.* 1999; Refsum *et al.* 2003; Kobayashi *et al.* 2007). However, certain bird species appear to be more likely colonized with *Salmonella* than others, with infections more commonly reported from passerines, gulls and pigeons than other wildlife species (Refsum *et al.* 2002). As we recovered so few *Salmonella* positive birds, our results are unable to identify a correlation between species type and *Salmonella* carrier state.

The Northern Cardinal, Carolina Wren and Brown Thrasher have geographic ranges that cover much of the eastern United States (Figure 6.4), although the Brown Thrashers are restricted to the southeastern region in the winter. All three species vary in diet with Northern Cardinals primarily seed eaters, Carolina Wrens primarily insectivores, and Brown Thrashers omnivores. All are primarily ground foragers and this behavior may increase the risk of exposure to *Salmonella*. However, samples collected from other ground foraging passerine species, such as a Dark-Eyed Junco (*Junco hyemalis*) at the same site, were negative for *Salmonella*.



Figure 6.4. Population density and songbird species distribution across the United States as determined from volunteer observer reports for the Brown Thrasher, Carolina Wren and Northern Cardinal, 2006. (Source-The Great Backyard Bird Count, Laboratory of Ornithology, Cornell University: <u>http://gbbc.birdsource.org/gbbcApps/maproom</u>)

Our findings of *S*. Muenchen from healthy songbirds captured during the spring-summer of 2006 stands in contrast to studies of periodic songbird epizootics, where the predominant isolate is a *S. enterica* Typhimurium strain responsible for significant morbidity and mortality in affected populations (Kobayashi *et al.* 2007; Hall and Saito 2008). In human infections, *S.* Muenchen was the 8<sup>th</sup> most commonly reported *S. enterica* serovar in 2006 (CDCP 2006). With the exception of two foodborne outbreaks in 1999 (CDCP 1999; Proctor *et al.* 2001), the distribution of *S*. Muenchen cases reported to the CDC is greatest in the South Atlantic states, South Carolina, Georgia and Alabama. Additionally, numerous cases have been reported from California and Texas (Figure 6.5) (years 1999-2006). The distribution of *S*. Muenchen cases in humans in the eastern U.S. also overlaps with the highest population densities and distribution of the three birds species positive for *Salmonella* in this study (Figure 4). The PFGE patterns for two of our songbird isolates were very similar to human isolates, differing by only 2-3 bands.



Figure 6.5. Distribution of human *S*. Muenchen isolates across the United States, 1999-2006. Data were compiled from the CDC PulseNet USA database for years 1999-2006.

In summary, this study indicates that *Salmonella* is present in wild songbirds in northern Georgia, and that these birds are infected with strains that may cause human infection. Despite its small size, our study provides a guide for characterizing habitat types and wildlife species that are associated with the occurrence of specific Salmonella serovars. Our results are useful for generating specific hypotheses regarding the role of wild birds in *Salmonella* epidemiology. For example, one hypothesis is that ground-feeding species such as those described in our study, and those that frequent flat surfaces on or surrounding bird feeders could be more frequently exposed to fecal material containing *Salmonella* bacteria. In addition, birds sampled during the winter months or at bird feeding stations could show higher prevalence of Salmonella infections owing to increased contact with fecal droppings when large flocks aggregate at feeders (e.g., Pennycott et al. 2002). Although sites in close proximity to agricultural areas and livestock rearing facilities were not sampled here, we also expect that birds captured near such areas could be more frequently exposed to Salmonella. Surveillance of wild songbird populations for Salmonella during non-outbreak periods can indicate the degree to which specific bacterial isolates are transmitted and maintained in wild bird populations, and the potential for these wild bird Salmonella strains to serve as a reservoir of human infection. However, it should be stressed that the songbirds themselves may not be directly involved in transmission of S. Muenchen to humans, but rather sentinels of environmental contamination. In a survey of Little River watershed in southern Georgia, April 2005-April 2006, Salmonella was frequently isolated from one of six sites, with S. Muenchen 2<sup>nd</sup> most frequently identified Salmonella serovar from this watershed (Haley et al. 2009). Sandy Creek Nature Center consists of 225 acres of woodlands and wetlands, including several ponds and the Sandy Creek, which runs through the park.

Sampling water, creek and pond sediment for *Salmonella* may reveal the environmental source for *S*. Muenchen in songbirds caught at the Sandy Creek Nature Center site.

## Acknowledgments

We thank B. Stoner-Duncan and A. Davis for assistance with field collection of samples. This work was supported by an Ecology of Infectious Diseases (EID) seed grant from the University of Georgia, a grant from the Southeastern Center for Emerging Biologic Threats (SECEBT) and a graduate research fellowship from the University of Georgia to CAB. Animal protocols described herein were approved by the University of Georgia (2005-06; AUP No. A2006-10051). Banding permits were granted by the US Bird Banding Laboratory (#23141) and the Georgia Department of Natural Resources (permit No. 29-WMB-02-176).

## CHAPTER 7

### CONCLUSIONS

A better understanding of how urban landscapes affect the persistence or prevalence of infectious diseases is necessary to promote wildlife conservation and public health. Here, three avianpathogen associations were studied to examine the patterns and mechanistic links between observed prevalence and urban land use. First, the antibody prevalence of the generalist arbovirus West Nile Virus (WNV) and its relationship to host community composition was examined. Second, age-related patterns in avian malaria infections in Northern Cardinals and juvenile condition in infected individuals were explored to determine how condition varies across urban landscapes. Third, the directly transmitted enterobacteria *Salmonella* was surveyed across sites to determine endemic prevalence in wild songbird populations.

The prevalence of WNV antibodies in wild songbird populations increased with greater levels of ubanization in north-central Georgia (USA). The pattern was consistent across the years studied (2004-2006) and was most evident in adult birds. This latter observation was expected as newly fledged juveniles had had limited exposure to the virus and less time to seroconvert prior to sampling. Northern Cardinals had high antibody prevalence across all sites sampled compared to other bird species. Previous work conducted by others showed that cardinals are competent hosts for the virus (Komar et al. 2005) and a significant component of mosquito blood meals (Apperson 2002), suggesting that this species may be a useful surveillance species in the region studied here and may be an important host in the maintenance of and prevalence of WNV in the avian community.

One potential mechanism to explain the observed pattern in WNV antibody prevalence, the loss of avian species that are less competent hosts of WNV (concomitant with the overall lost of biodiversity in urban landscapes), was examined. Specifically, reduced host encounter rates between vectors and competent host species is one mechanism under which the 'dilution effect' (reviewed in Keesing et al. 2006) may act to reduce the overall prevalence of infection in a host community. In this study, the proportion of core forest area at a 1000m scale was the most significant land use predictor of WNV exposure. In addition, we and others have found this measure to be a strong indicator of the bird species present in a community (Crooks et al. 2004). Furthermore, host community competence (based on numerical abundance) and total passerine abundance were both strong indicators of WNV antibody prevalence consistent with the expectations of the dilution effect. Recent studies showed that these measures were also found to be important indicators of human WNV risk at local and regional scales in other areas of the U.S. (St. Louis, MO, Allan et al. 2008; northeastern U.S., Swaddle and Calos 2008).

Our results also showed that core forest area was a stronger predictor of WNV antibody prevalence than any measure of host community composition, suggesting that other factors associated with urban land use are likely to be important mechanistic links between urbanized landscapes and WNV. First, vector abundance, primarily *Culex quinquefasciatus*, is known to be associated with human-dominated landscapes (Calhoun et al. 2007; Reisen and Brault 2007) and is probably a significant reason for increased WNV antibody prevalence in urban environments. Second, it can not be ruled out that increased host recovery in urban environments is a component of the observed pattern. Luniak (2004) points out that predator release, improved

resource availability, and reduced seasonality for urban-adapted species may improve recovery to infection and, thus, the observed higher rates of antibody prevalence in urban populations.

Analyses focused on Northern Cardinals showed significant age-related and site-related patterns of avian malaria, and a high overall prevalence of this vector-borne protozoan parasite. Specifically, juveniles were more likely to be infected with malaria parasites than adult birds and, of adult birds sampled, prevalence was lower at urban sites. A separate set of analyses focused on the condition of infected juvenile birds across sites to investigate how condition covaried with measures of urbanization. Host condition in these individuals was assessed by measuring a variety of parameters associated with immunological response, stress, and nutritional health. Furthermore, condition measures in juveniles are likely to be a reliable indicator of the influence of habitat on individual condition, as juveniles recently fledged have been fed from resources foraged nearby and exposed to the conditions characteristic of their habitats. Heterophil-to lymphocyte (H:L) ratios, an indicator of stress, increased with increasing urbanization and average tail feather growth, though not directly related to measures of urban land use, decreased with increasing H:L ratios. Both results are indicative of nutritional stress in urban areas; however, plumage coloration also increased with increasing urban land use and was associated with higher H:L ratios in males. As shown in other research (e.g. Jawor and Breitwisch 2004), coloration in this species is a true indicator of many male qualities associated with increased reproductive potential including territory quality and foraging ability. Indeed, in urban environments where Northern Cardinals are in higher abundance than in non-urban areas, there may be significant intraspecific competition that influences investment in plumage coloration.

A final study showed that the prevalence of *Salmonella* was generally very low among wild songbirds across all sites sampled. Furthermore, all positive samples (N=3) were isolated from birds capture at one site. Because that site did not differ significantly from all other sites sampled with respect to avian abundance or species richness, tests of the influence of host density and contact rates associated with aggregation at feeding stations could not be conducted. However, the three positive samples were all found to be of the same serovar, *S. enterica* serovar Muenchen, and to cluster in a phylogenetic analysis with strains obtained from humans infected with Muenchen. Though this study does not indicate that songbirds are a wildlife reservoir for Muenchen, it does indicate that songbird surveillance may point to environmental contamination with *Salmonella* strains of zoonotic potential during non-epidemic periods.

The work presented here examines how disease risk and pathogen prevalence are influenced by habitats that differ with respect to of urban land use. Many questions remain that would require other field, modelling or experimental studies of specific host-pathogenenvironment interactions. For example, data collected here could be used to parameterize a model assessing the relative importance of host community competence, vector abundance, and vector feeding preference to the overall prevalence of WNV infection in the avian community. Under experimental conditions, mixed species models could be used in a manner similar to Johnson et al. (2008) to examine the roles of host density, host diversity, and other mechanisms associated with multi-host-pathogen systems hypothesized under the 'dilution effect' in influencing the transmission and pathogenicity of WNV. Experimental manipulation could also be utilized to determine specific links between characteristics of urban landscapes, such as altered food resources, and host condition. Data from MAPS (Monitoring Avian Productivity and

Survival, Institute for Bird Populations; http://www.birdpop.org/maps.htm) activities indicates that site fidelity in Northern Cardinals would make such a study feasible.

Urbanization is likely to intensify in the future and this process is certain to have major effects on the wildlife populations living both in cities and in habitats buffering urban/ suburban environments. Associated with this, disease dynamics within wildlife species, among hosts, and between wildlife and humans are also likely to be altered. Studying how patterns of disease change with increasing urban land use and the underlying mechanisms mediating these changes will be an important direction for future research both for conservation concerns and for public health considerations. I hope the work presented here provides a meaningful contribution to this field and precipitates the development of future work.

### REFERENCES

- Allan B.F., F. Keesing, and R.S. Ostfeld. 2003. Effect of forest fragmentation on Lyme disease risk. Conservation Biology 17:267-272.
- Allan B.F., B. Langerhans, W.A. Ryberg, W.J. Landesman, N.W. Griffin, R.S. Katz, B.J. Oberle, M.R. Schutzenhofer, K.N. Smyth, A. de St. Maurice, L. Clark, K.R. Crooks, D.E. Hernandez, R.G. McLean, R.S. Ostfeld, and J.M. Chase. 2009. Ecological correlates of risk and incidence of West Nile virus in the United States. Oecologia 158:699-708.
- Altizer, S., A. Dobson, P. Hosseini, P. Hudson, M. Pasqual, and P. Rohani. 2006. Seasonality and population dynamics: infectious diseases as case studies. Ecology Letters 9:467–484.
- American Forests. 2001. Urban ecosystems analysis: Atlanta metro area. American Forest Report August 2001:1-8.
- Apperson C.S., B.A. Harrison, T.R. Unnasch, H.K. Hassan, W.S. Irby, H.M. Savage, S.E. Aspen, D.W. Watson, L.M. Rueda, B.R. Engber, and R.S. Nasci. 2002. Host feeding habits of Culex and other mosquitoes (Diptera: Culicidae) in the Borough of Queens in New York City, with characters and techniques for identification of Culex mosquitoes. Journal of Medical Entomology 39:777–785.
- Arnfield, A.J. 2003. Two decades of urban climate research: a review of turbulence, exchanges of energy and water, and the urban heat island. International Journal of Climatology 23: 1-26.
- Ashbolt R. and M.D. Kirk. 2006. Salmonella Mississippi infections in Tasmania: the role of native Australian animals and untreated drinking water. Epidemiology and Infection 134:1257-1265.
- Baker L., A.J. Brazel, N. Selover, C. Martin, N. McIntyre, F.R. Steiner, A. Nelson, and L. Musacchio. 2002. Urbanization and warming of Phoenix (Arizona, U.S.A.): impacts, feedbacks and mitigation. Urban Ecology 6:183–203.
- Begon M. and R.G. Bowers. 1994. Host-host-pathogen models and microbial pest-control: the effect of host self-regulation. Journal of Theoretical Biology 169:275–287.
- Begon, M. 2008. Effects of host diversity on disease dynamics. In: R. Ostfeld, F. Keesing, and V.T. Eviner. Infectious Disease Ecology. Princeton University Press, Princeton, NJ.

- Beissinger, S.R. and D.R. Osborne. 1982. Effects of urbanization on avian community organization. The Condor 84:75-83.
- Bendich, A. 1989. Carotenoids and the immune response. Journal of Nutrition 119:112-115.
- Bensch, S., M. Stjernman, D. Hasselquist, Ö. Östman, B. Hansson, H. Westerdahl, and R.T. Pinheiro. 2000. Host specificity in avian blood parasites: a study of Plasmodium and Haemoproteus mitochondrial DNA amplified from birds. Proceedings of the Royal Society of London B, Biological Sciences 267:1583-1589.
- Bensch, S., J. Waldenström, N. Jonzén, H. Westerdahl, B. Hansson, D. Sejberg, and D. Hasselquist. 2007. Temporal dynamics and diversity of avian malaria parasites in a single host species. Journal of Animal Ecology 76: 112-122.
- Bernier, J., P. Brousseau, K. Krzystyniak, H. Tryphonas, and M. Fournier. 1995. Immunotoxicity of heavy metals in relation to Great Lakes. Environmental Health Perspectives 103:23–34.
- Blair, R.B. 1996. Land use and avian species diversity along an urban gradient. Ecological Applications 6:506–519.
- Blitvich B.J., N.L. Marlenee, R.A. Hall, C.H. Calisher, R.A. Bowen, J.T. Roehrig, N. Komar, S.A. Langevin, and B.J. Beaty. 2003. Epitope-blocking enzyme-linked immunosorbent assays for the detection of serum antibodies to West Nile virus in multiple avian species. Journal of Clinical Microbiology 41:1041–1047.
- Boal, C.W. and R.W. Mannan. 1999. Comparative breeding ecology of Cooper's hawks in urban and exurban areas of southeastern Arizona. Journal of Wildlife Management 63:77–84.
- Boutin, S. 1990. Food supplementation experiments with terrestrial vertebrates: patterns, problems, and the future. Canadian Journal of Zoology 68:203-220.
- Bowman, R., N. Hamel, L.A. Riopelle, and S.P. Rowe. 1998. Demography and habitat characteristics of Florida Scrub Jays at the Avon Park Air Force Range: Final report 1994-1998. Final report to U.S. Department of defense, MacDill Air Force Base, Tampa, FL.
- Bradley C.A. and S. Altizer. 2007 Urbanization and the ecology of wildlife diseases. Trends in Ecology and Evolution 22:95-102.
- Bradley C.A., S.E.J. Gibbs, and S. Altizer. 2008. Urban land use predicts West Nile virus exposure in songbirds. Ecological Applications 18:1083-1092.

- Bradley, C.A., S.E.J. Gibbs, and S. Altizer. In review. Urbanization, host community composition and West Nile virus exposure in songbirds.
- Brault, A.C., S.A. Langevin, R.A. Bowen, N.A. Panella, B.J. Biggerstaff, B.R. Miller, and N. Komar. 2004. Differential virulence of West Nile strains for American Crows. Emerging Infectious Diseases 10:2161-2168.
- Broadfoot, J.D., R.C. Rosatte, and D.T. O'Leary. 2001. Raccoon and skunk population models for urban disease control planning in Ontario, Canada. Ecological Applications 11:295–303.
- Brodin, A. 1993. Radio-ptilochronology: Tracing radioactively labelled food in feathers. Ornis Scandinavica 24:167-173.
- Brown, M. 1996. Assessing body condition in birds. In: E.D. Ketterson and V. Nolan. Current Ornithology, Vol. 13. Plenum Press, New York, NY.
- Bruce, M.C. and K.P. Day. 2002. Cross-species regulation of malaria parasitaemia in the human host. Current Opinion in Microbiology 15:431-437.
- Caffrey, C., S.C.R. Smith, and T.J. Weston. 2005. West Nile virus devastates an American Crow population. The Condor 107:128-132.
- Calhoun L.M., M. Avery, L. Jones, K. Gunarto, R. King, J. Roberts, and T.R. Burkot. 2007. Combined sewage overflows (CSO) are major urban breeding sites for Culex quinquefasciatus in Atlanta, Georgia. American Journal of Tropical Medicine and Hygiene 77:478-484.
- Campbell, T.W. 1995. Avian Hematology and Cytology, 2nd Edition. Iowa University Press, Ames, IA.
- Campbell G.L., A.A. Marfin, R.S. Lanciotti, and D.J. Gubler. 2002.. West Nile virus. Lancet Infectious Diseases 2:519–529.
- Centers for Disease Control and Prevention. 1999. Outbreak of Salmonella serovar Muenchen infections associated with unpasteurized orange juice- United States and Canada, June 1999. Morbidity and Mortality Weekly Report 48:582-585.
- Centers for Disease Control and Prevention. PHLIS surveillance data. Salmonella annual summary 2006. Centers for Disease Control and Prevention. Atlanta, Ga. [Online.] [Cited 2009 May 8] Available from http://www.cdc.gov/ncidod/dbmd/phlisdata/Salmonella.htm. 2007.
- Centers for Disease Control and Prevention. 2009. Surveillance for foodborne disease outbreaks- United States, 2006. Morbidity and Mortality Weekly Report 58:609-615.

- Centers for Disease Control and Prevention. PulseNet Protocol. Available from http://www.cdc.gov/pulsenet/ protocols/ecoli\_salmonella\_shigella\_protocols.pdf.
- Chace, J.F. and J.J. Walsh. 2006. Urban effects on native avifauna: a review. Landscape and Urban Planning 74:46-69.
- Cleaveland, S., M.K. Laurenson, and L.H. Taylor. 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philosophical Transactions of the Royal Society of London:B 356:991–999.
- Coyner, D.F., M.G. Spalding, and D.J. Forrester. 2002. Epizootiology of Eustrongylides ingotus in Florida: distribution, density, and natural infections in intermediate hosts. Journal of Wildlife Diseases 38:483–499.
- Craven S.E., N.J. Stern, E. Line, J.S. Bailey, N.A. Cox, and P. Fedorka-Cray. 2000. Determination of the incidence of Salmonella spp., Campylobacter jejuni, and Clostridium perfringens in wild birds near broiler chicken houses by sampling intestinal droppings. Avian Diseases 44:715-720.
- Crawley, M.J. 2002. Statistical Computing: An Introduction to Data Analysis using S-PLUS. John Wiley, West Sussex, England.
- Crawley, M.J. 2005. Statistics: An Introduction using R. John Wiley, West Sussex, England.
- Crawley, M.J. 2007. The R Book. John Wiley, West Sussex, England.
- Crooks K.R., A.V. Suarez, and D.T. Bolger. 2004. Avian assemblages along a gradient of urbanization in a highly fragmented landscape. Biological Conservation 115:451-462.
- Daoust P.Y., D.G. Busby, L. Ferns, J. Goltz, S. Burney, C. Poppe, and H. Whitney. 2000. Salmonellosis in songbirds in the Canadian Atlantic provinces during the winter-summer 1997-98. Canadian Veterinary Journal 41:54-59.
- Daszak, P., A.A. Cunningham, and A.D. Hyatt. 2000. Emerging infectious diseases of wildlife: threats to biodiversity and human health. Science 287:443–449.
- Daum L.T., W.J. Barnes, J.C. McAvin, M.S. Neidert, L.A. Cooper, W.B. Huff, L. Gaul, W.S. Riggins, S. Morris, A. Salmen, and K.L. Lohman. 2002. Real-time PCR detection of salmonella in suspect foods from a gastroenteritis outbreak in Kerr county, Texas. Journal of Clinical Microbiology 40:3050-3052.
- Davis, A.K., D.L. Maney, and J.C. Maerz. 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Functional Ecology 22:760-772.

- de Castro, F. and B. Bolker. 2005. Mechanisms of disease-induced extinction. Ecology Letters 8:117–126.
- Denno D.M., W.E. Keene, C.M. Hutter, J.K. Koepsell, M. Patnode, D. Flodin-Hursh, L.K. Stewart, J.S. Duchin, L. Rasmussen, R. Jones, and P.I. Tarr. 2009. Tri-county comprehensive assessment of risk factors for sporadic reportable bacterial enteric infection in children. Journal of Infectious Diseases 199:467-476.
- Deplazes, P., D. Hegglin, S. Gloor, and T. Romig. 2004. Wilderness in the city: the urbanization of Echinococcus multilocularis. Trends in Parasitology 20:77–84.
- Dhondt, A.A., S. Altizer, E.G. Cooch, A.K. Davis, A. Dobson, M.J.L. Driscoll, B.K. Hartup,
  D.M. Hawley, W.M. Hochachka, P.R. Hosseini, C.S. Jennelle, G.V. Kollias, D.H. Ley,
  E.C.H. Swarthout, and K.V. Sydenstricker. 2005. Dynamics of a novel pathogen in an
  avian host: Mycoplasmal conjunctivitis in house finches. Acta Tropica 94:77–93.
- Dobson, A. 2004. Population dynamics of pathogens with multiple host species. American Naturalist 164:S64–S78.
- Dobson, A., I. Cattadori, R.D. Holt, R.S. Ostfeld, F. Keesing, K. Krichbaum, J.R. Rohr, S.E. Perkins, and P.J. Hudson. 2006. Sacred cows and sympathetic squirrels: the importance of biological diversity to human health. PLoS Medicine 3:e231.
- Dubey, J.P., D.H. Graham, R.W. De Young, E. Dahl, M.L. Eberhard, E.K. Nace, K. Won, H. Bishop, G. Punkosdy, C. Sreekumar, M.C.B. Vianna, S.K. Shen, O.C.H. Kwok, J.A. Sumners, S. Demarais, J.G. Humphreys, and T. Lehmann. 2004. Molecular and biologic characteristics of Toxoplasma gondii isolates from wildlife in the United States. Journal of Parasitology 90:67–71.
- Epstein, P.R. 2001. West Nile virus and the climate. Journal of Urban Health 78:367-371.
- Ethelberg, S., J. Simonsen, P. Gerner-Smidt, K.E.P. Olsen, K. Molbak. 2005. Spatial distribution and registry-based case control analysis of Campylobacter infections in Denmark, 1991-2001. American Journal of Epidemiology 162:1008-15.
- Ezenwa, V.O. 2004. Interactions among host diet, nutritional status and gastrointestinal parasite infection in wild bovids. International Journal of Parasitology 34: 535–542.
- Ezenwa, V.O., M.S. Godsey, R.J. King, and S.C. Guptill. 2006. Avian diversity and West Nile virus: testing associations between biodiversity and infectious disease risk. Proceedings of the Royal Society of London: Biological Sciences 273:109-117.
- Ezenwa, V.O., L.E. Milheim, M.F. Coffey, M.S. Godsey, R.J. King, and S.C. Guptill. 2007. Land cover variation and West Nile virus prevalence: patterns, processes, and implications for disease control. Vector-Borne and Zoonotic Diseases 7:173-180.

- Faeth, S.H., P.S. Warren, E. Shochat, and W.A. Marussich. 2005. Trophic dynamics in urban communities. Bioscience 55: 399-407.
- Farmer III, J.J. and M.T. Kelly. 1991. Enterobacteriaceae. In: A. Balows, W. J. Hausler Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy. Manual of Clinical Microbiology, 5th Edition. ASM Press, Washington, D.C.
- Farnsworth, M.L., L.L. Wolfe, N.T. Hobbs, K.P. Burnham, E.S. Williams, D.M. Theobald, M.M. Conner, and M.W. Miller. 2005. Human land use influences Chronic Wasting Disease prevalence in Mule Deer. Ecological Applications 15:119-126.
- Fernańdez-Juricic, E., R. Vaca, and N. Schroeder. 2004. Spatial and temporal responses of forest birds to human approaches in a protected area and implications for two management strategies. Biological Conservation 117:407-416.
- Figuerola, J., E. Munoz, R. Gutierrez, and D. Ferrer. 1999. Blood parasites, leucocytes, and plumage brightness in the Cirl Bunting, Emberiza cirlus. Functional Ecology 13:594-601.
- Fischer, C., L.A. Reperant, J.M. Weber, D. Hegglin, P. Deplazes. 2005. Echinococcus multilocularis infections of rural, residential and urban foxes (Vulpes vulpes) in the canton of Geneva, Switzerland. Parasite 12:339–346.
- Fraterrigo, J.M. and J.A. Wiens. 2005. Bird communities of the Colorado Rocky Mountains along a gradient of exurban development. Landscape and Urban Planning 71:263-275.
- Friedman, A. and D. Sklan. 1997. Effects of retinoids on immune responses in birds. World's Poultry Science Journal 53:186–195.
- Gibbs S.E.J., D.M. Hoffman, L.M. Stark, N.L. Marlenee, B.J. Blitvich, B.J. Beaty, and D.E. Stallknecht. 2005. Persistence of antibodies to West Nile virus in naturally infected Rock Pigeons (Columba livia). Clinical and Diagnostic Laboratory Immunology 12:665–667.
- Gibbs, S.E.J., A.B. Allison, M.J. Yabsley, D.G. Mead, B.R. Wilcox, and D.E. Stallknecht. 2006a. West Nile antibodies in avian species of Georgia, USA: 2000–2004. Vector-borne and Zoonotic Diseases 6:57–72.
- Gibbs, S.E.J., M.C. Wimberly, M. Madden, J. Masour, M.J. Yabsley, and D.E. Stallknecht.
   2006b. Factors affecting the geographic distribution of West Nile virus in Georgia, USA:
   2002–2004. Vector-borne and Zoonotic Diseases 6:73–82.
- Godsey Jr., M.S., M.S. Blackmore, N.A. Panella, K. Burkhalter, K. Gottfried, L.A. Halsey, R. Rutledge, S.A. Langevin, R. Gates, K.M. Lamonte, A. Lambert, R.S. Lanciotti, C.G.M. Blackmore, T. Loyless, L. Stark, R. Oliveri, L. Conti, and N. Komar. 2001. West Nile virus epizootiology in the Southeastern United States. 2001. Vector-borne and Zoonotic Diseases 5:82-89.

- Goodwin, T.W. 1950. Carotenoids and reproduction. Biological Reviews of the Cambridge Philosophical Society 25:391-413.
- Gosler, A.G. 1991. On the use of greater covert moult and pectoral muscle on measures of condition in passerines with data for the Great Tit Parus major. Bird Study 38:1-9.
- Gotelli, N.J. and A.M. Ellison. 2004. A primer of ecological statistics. Sinauer Associates, Sunderland, Massachusetts.
- Gottstein, B. 2001. Is high prevalence of Echinococcus multilocularis in wild and domestic animals associated with disease incidence in humans? Emerging Infectious Diseases 7:408–412.
- Green, D.M. and M.G. Baker. 2003. Urbanization impacts on habitat and bird communities in a Sonoran desert ecosystem. Landscape and Urban Planning 63:225–239.
- Green, C.G., D.O. Krause, and J.L. Wylie. 2006. Spatial analysis of Campylobacter infection in the Canadian province of Manitoba. International Journal of Health Geographics 5:2.
- Grimm, N.B., J.M. Grove, S.T.A. Pickett, and M.J. Grove. 2000. Integrated approaches to long-term studies of urban ecological systems. Bioscience 50:571–584.
- Grubb Jr., T.C. 1989. Ptilochronology: feather growth bars as indicators of nutritional status. The Auk 106: 314-320.
- Grubb Jr. T. C. 1991. A deficient diet narrows growth bars on induced feathers. The Auk 108:725-727.
- Grubb Jr., T.C. and R. Yosef. 1994. Habitat-specific nutritional condition in Loggerhead Shrikes (Lanius ludovicianus): Evidence from ptilochronology. The Auk 111: 756-775.
- Hajna, A. and S. Damon. 1956. New enrichment and plating media for the isolation of Salmonella and Shigella organisms. Applied Microbiology 4:341-345.
- Haley, B.J., D.J. Cole, and E.K. Lipp. 2009. Distribution, diversity, and seasonality of waterborne salmonellae in a rural watershed. Applied Environmental Microbiology 75:1248-1255.
- Hall, A.J. and E.K. Saito. 2008. Avian wildlife mortality events due to salmonellosis in the United States, 1985-2004. Journal of Wildlife Diseases 44:585-593.
- Hamer, G.L., U.D. Kitron, T.L. Goldberg, J.D. Brawn, S.R. Loss, M.O. Ruiz, D.B. Hayes, and E.D. Walker. 2009. Host selection by Culex pipiens mosquitoes and West Nile virus amplification. American Journal of Tropical Medicine and Hygiene 80:268-278.

- Hartup, B.K., J.M. Bickal, A.A. Dhondt, D.H. Ley, and G.V. Kollias. 2001. Dynamics of conjunctivitis and Mycoplasma gallisepticum infections in House Finches. Auk 118:327-333.
- Hegglin, D., P.I. Ward, and P. Deplazes. 2003. Anthelmintic baiting of foxes against urban contaminations with Echinococcus multilocularis. Emerging Infectious Diseases 9:1266– 1272.
- Hennings, L.A. and W.D. Edge. 2003. Riparian bird community structure in Portland, Oregon: habitat, urbanization, and spatial scale patterns. The Condor 105:288-302.
- Hernandez, J., J. Bonnedahl, J. Waldenström, H. Palmgren, B. Olsen. 2003. Salmonella in birds migrating through Sweden. Emerging Infectious Diseases 9:753-755.
- Hill, G.E. 1992. Proximate basis of variation in carotenoid pigmentation in male House Finches. The Auk 109:1-12.
- Hill, G.E. and R. Montgomerie. 1994. Plumage coloration signals nutritional condition in the House Finch. Proceedings of the Royal Society of London: Biological Sciences 258:47-52.
- Hilton-Taylor, C. 2000. 2000 IUCN red list of threatened species. IUCN, Gland, Switzerland and Cambridge, UK.
- Hope, D. C. Gries, W. Zhu, W. Fagan, C.L. Redman, N.B. Grimm, A.L. Nelson, C. Martin, and A. Kinzig. 2003. Socioeconomics drive urban plant diversity. Proceedings of the National Academy of Sciences 100:8788–8792.
- Hudon, J. 1994. Showiness, carotenoids, and captivity: a comment on Hill (1992). The Auk 111:218-221.
- Hudson, C.B. and D.C. Tudor. 1957. Salmonella typhimurium infection in feral birds. Cornell Vet. 47:394–395.
- Hudson, C.R., C. Quist, M.D. Lee, K. Keyes, S.V. Dodson, C. Morales, S. Sanchez, D.G. White, and J.J. Maurer. 2000. Genetic relatedness of Salmonella isolates from nondomestic birds in Southeastern United States. Journal of Clinical Microbiology 38:1860-1865.
- Hunter, S.B., P. Vauterin, M.A. Lambert-Fair, M.S. Van Duyne, K. Kubota, Graves L, D. Wrigley, T. Barrett, and E. Ribot. 2005. Establishment of a universal size standard strain for use with the PulseNet standardized pulsed-field gel electrophoresis protocols: converting the national databases to the new size standard. Journal of Clinical Microbiology 43:1045-1050.

- Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54:187-211.
- Innocent, G.T., D.J. Mellor, S.A. McEwen, W.J. Reilly, J. Smallwood, M.E. Locking, D.J. Shaw, P. Michel, D.J. Taylor, W.B. Steele, G.J. Gunn, H.E. Ternent, M.E.J. Woolhouse, S.W.J. Reid. 2005. Spatial and temporal epidemiology of sporadic human cases of Escherichia coli O157 in Scotland, 1996-1999. Epidemiology and Infection 133:1033-1041.
- Isaksson, C., J. Örnborg, E. Stephensen, and S. Andersson. 2005. Plasma glutathione and carotenoid coloration as potential biomarkers of environmental stress in Great Tits. EcoHealth 2:138–146.
- Jarvi, S.I., J.J. Schultz, and C.T. Atkinson. 2002. PCR diagnostics underestimate the prevalence of avian malaria (Plasmodium relictum) in experimentally-infected passerines. Journal of Parasitology 88:153-158.
- Jawor, J.M., N. Gray, S.M. Beall, and R. Breitwisch. 2004. Multiple ornaments correlate with aspects of condition and behaviour in female northern cardinals, Cardinalis cardinalis. Animal Behaviour 67:875-882.
- Jokimäki, J., P. Clergeau, and M.-L. Kaisanlahti-Jokimäki. 2002. Winter bird communities in urban habitats: a comparative study between central and northern Europe. Journal of Biogeography 29:69-79.
- Johnson, P.T.J., R.B. Hartson, D.J. Larson, and D.R. Sutherland. 2008. Diversity and disease: community structure drives parasite transmission and host fitness. Ecology Letters 11:1017-1026.
- Jones, T.F., L.A. Ingram, K.E. Fullerton, R. Marcus, B.J. Anderson, P.V. McCarthy, D. Vugia, B. Shiferaw, N. Haubert, S. Wedel, and F.J. Angulo. 2006. A case-control study of the epidemiology of sporadic Salmonella infection in infants. Pediatrics 118:2380-2387.
- Kapperud, G., H. Stenwig, and J. Lassen. 1998. Epidemiology of Salmonella typhimurium O:4-12 infection in Norway. American Journal of Epidemiology 147:774-782.
- Karesh, W.B., R.A. Cook, E.L. Bennett, and J. Newcomb. 2005. Wildlife trade and global disease emergence. Emerging Infectious Disease 11:1000–1002.
- Keesing, F., R.D. Holt, and R.S. Ostfeld. 2006. Effects of species diversity on disease risk. Ecology Letters 9:485-498.
- Kilpatrick, A.M., P. Daszak, M.J. Jones, P.P. Marra, and L.D. Kramer. 2006a. Host heterogeneity dominates West Nile virus transmission. Proceedings of the Royal Society of London: Biological Sciences 273:2327-2333.

- Kilpatrick, A.M., L.D. Kramer, M.J. Jones, P.P. Marra, and P. Daszak. 2006b. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. PLoS Biology 4:e82.
- Klasing, K.C. 1998. Nutritional modulation of resistance to infectious diseases. Poultry Science 77:1119-1125.
- Kobayashi, H., M. Kanazaki, Y. Shimizu, H. Nakajima, M.M. Khatun, E. Hata, and M. Kubo. 2007. Salmonella isolates from cloacal swabs and footpads of wild birds in the immediate environment of Tokyo Bay. Journal of Veterinary Medical Science 69:309-311.
- Komar, N. 2003. West Nile virus: epidemiology and ecology in North America. Advances in Virus Research 61:185-234.
- Komar, N., S. Langevin, S. Hinten, N. Nemeth, E. Edwards, D. Hettler, B. Davis, R. Bowen, and M. Bunning. 2003. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerging Infectious Diseases 9:311-322.
- Komar, N., N.A. Panella, S.A. Langevin, A.C. Brault, M. Amador, E. Edwards, and J.C. Owen. 2005. Avian hosts for West Nile virus in St. Tammany Parish, Louisiana, 2002. American Journal of Tropical Medicine and Hygiene 73:1031–1037.
- Koort, J., S. Lukinmaa, M. Rantala, E. Unkila, and A. Siitonen. 2002. Technical improvement to prevent DNA degradation of enteric pathogens in pulsed-field gel electrophoresis. Journal of Clinical Microbiology 40:3497-3498.
- Krzystyniak, K., H. Tryphonas, and M. Fournier. 1995. Approaches to the evaluation of chemical-induced immunotoxicity. Environmental Health Perspectives 103:17–22.
- Lafferty, K.D. and L.R. Gerber. 2002. Good medicine for conservation biology: the intersection of epidemiology and conservation theory. Conservation Biology 16:593–604.
- Lanciotti, R.S., G.D. Ebel, V. Deubel, A.J. Kerst, S. Murri, R. Meyer, M. Bowen, N. McKinney, W.E. Morrill, M.B. Crabtree, L.D. Kramer, and J.T. Roehrig. 2002. Complete genome sequences and phylogenetic analysis of West Nile virus strains isolated from the United States, Europe, and the Middle East. Virology 298:96–105.
- Lepczyk, C.A., C.H. Flather, V.C. Radeloff, A.M. Pidgeon, R.B. Hammer, and J. Liu. 2008. Human impacts on regional avian diversity and abundance. Conservation Biology 22:405-416.
- Lindgren, E. and R. Gustafson. 2001. Tick-borne encephalitis in Sweden and climate change. Lancet 358:16–18.

- Linville, S.U. and R. Breitwisch. 1997. Carotenoid availability and plumage coloration in a wild population of northern cardinals. The Auk 114:796-800.
- Linzey, D., J. Burroughs, L. Hudson, M. Marini, J. Robertson, J. Bacon, M. Nagarkatti, and P. Nagarkatti. 2003. Role of environmental pollutants on immune functions, parasitic infections and limb malformations in marine toads and whistling frogs from Bermuda. International Journal of Environmental Health Research 13:125–148.
- LoGuidice, K. 2003. Trophically transmitted parasites and the conservation of small populations: raccoon roundworms and the imperiled Allegheny woodrat. Conservation Biology 17:258–266.
- LoGiudice, K., R.S. Ostfeld, K.A. Schmidt, and F. Keesing. 2003. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. Proceedings of the National Academies of Sciences 100:567-571.
- Loss, S.R., G.L. Hamer, E.D. Walker, M.O. Ruiz, T.L. Goldberg, U.D. Kitron, and J.D. Brawn. 2009. Avian host community structure and prevalence of West Nile virus in Chicago, Illinois. Oecologia 159:415-424.
- Louis, V.R., I.A. Gillespie, S.J. O'Brien, E. Russek-Cohen, A.D. Pearson, and R.R. Colwell. 2005. Temperature-driven Campylobacter seasonality in England and Wales. Applied and Environmental Microbiology 71:85–92.
- Luniak, M. 2004. Synurbization-adaptation of animal wildlife to urban development. In: W.W. Shaw, L.K. Harris, and L. van Druff. Proceedings 4th International Urban Wildlife Symposium. University of Arizona Press, Tucson, AZ.
- Maney, D.L., A.K. Davis, C.T. Goode, A. Reid, and C. Showalter. 2008. Carotenoid-based plumage coloration predicts leukocyte parameters during the breeding season in Northern Cardinals (Cardinalis cardinalis). Ethology 114:369-380.
- Marra, P.P., S. Griffing, C. Caffery, A.M. Kilpatrick, R. McLean, C. Brand, E. Saito, A.P. Dupuis, L. Kramer, and R. Novak. 2004. West Nile virus and wildlife. BioScience 54:393–402.
- Marzluff, J.M. 2001. Worldwide urbanization and its effects on birds. In: J.M. Marzluff, R. Bowman, and R. Donnelly. Avian Ecology and Conservation in an Urbanizing World. Kluwer Academic Publishers, New York, NY.
- McCabe, P., J.F. Sheridan, J.M. Weiss, J.P. Kaplan, B.H. Natelson, and W.P. Pare. 2000. Animal models of disease. Physiology and Behavior 68:501–507.
- McDonald, R., P. Karieva, and R. Forman. 2008. The implications of current and future urbanization for global protected areas. Biological Conservation 141:1695-1703.

McKinney, M.L. 2002. Urbanization, biodiversity, and conservation. Bioscience 52:883-890.

- Melles, S., S. Glenn, and K. Martin. 2003. Urban bird diversity and landscape complexity: species-environment associations along a multiscale habitat gradient. Ecology and Society 7:5.
- Michel, P., J.B. Wilson, S.W. Martin, R.C. Clarke, S.A. McEwen, and C.L. Gyles. 1999. Temporal and geographical distributions of reported cases of Escherichia coli O157:H7 infection in Ontario. Epidemiology and Infection 122:193-200.
- Miller, M.A., I.A. Gardner, C.Kreuder, D.M. Paradies, K.R. Worcester, D.A. Jessup, E. Dodd, M.D. Harris, J.A. Ames, A.E. Packham, and P.A. Conrad. 2002. Coastal freshwater runoff is a risk factor for Toxoplasma gondii infection of southern sea otters (Enhydra lutris nereis). International Journal of Parasitology 32:997–1006.
- Molaei, G., T.G. Andreadis, P.M. Armstrong, J.F. Anderson, and C.R. Vossbrinck. 2006. Host feeding patterns of Culex mosquitoes and West Nile virus transmission, northeastern United States. Emerging Infectious Diseases 12:468-474.
- Møller, A.P. and J.T. Nielsen. 2007. Malaria and risk of predation: a comparative study of birds. Ecology 88:871-881.
- Morishita, T.Y., P.P. Aye, E.C. Ley, and B.S. Harr. 1999. Survey of pathogens and blood parasites in free-living passerines. Avian Diseases 43:549-552.
- Naugle, D.E., C.L. Aldridge, B.L. Walker, T.E. Cornish, B.J. Moynahan, M.J. Holloran, K. Brown, G.D. Johnson, E.T. Schmidtmann, R.T. Mayer, C.Y. Kato, M.R. Matchett, T.J. Christiansen, W.E. Cook, T. Creekmore, R.D. Falise, E.T. Rinkes, and M.S. Boyce. 2004. West Nile virus: pending crisis for greater sage-grouse. Ecology Letters 7:704-713.
- Navara, K.J. and G.E. Hill. 2003. Dietary carotenoid pigments and immune function in a songbird with extensive carotenoid-based plumage. Behavioural Ecology 14: 909-916.
- Olden, J.D., N.L.R. Poff, and M.L. McKinney. 2006. Forecasting faunal and floral homogenization associated with human population geography in North America. Biological Conservation 127:261–271.
- Ostfeld, R.S. and F. Keesing. 2000. The function of biodiversity in the ecology of vector-borne zoonotic diseases. Canadian Journal of Zoology 78:2061-2078.
- Ostfeld, R.S., G.E. Glass, and F. Keesing. 2005. Spatial epidemiology: a emerging (or reemerging) discipline. Trends in Ecology and Evolution 20:328–336.
- Padgett, D. and R. Glaser. 2003. How stress influences the immune response. Trends in Immunology 24: 444–448.

- Parris, K.M. and D.L. Hazell. 2005. Biotic effects of climate change in urban environments: the case of the grey-headed flying-fox (Pteropus poliocephalus) in Melbourne, Australia. Biological Conservation 124: 267–276.
- Partecke, J., I. Schwabl, and E. Gwinner. 2006. Stress and the city: urbanization and its effects on the stress physiology in European blackbirds. Ecology 87:1945-1952.
- Patz, J.A., P. Daszak, G.M. Tabor, A.A. Aguirre, M. Pearl, J. Epstein, N.D. Wolfe, A.M. Kilpatrick, J. Foufopoulos, D. Molyneux, D.J. Bradley, and Members of the Working Group on Land Use Change and Disease Emergence. 2004. Unhealthy landscapes: policy recommendations on land use change and infectious disease emergence. Environmental Health Perspectives 112:1092-1098.
- Pauchard, A., M. Aguayo, E. Peña, and R. Urrutia. 2006. Multiple effects of urbanization on the biodiversity of developing countries: The case of a fast-growing metropolitan area (Concepción, Chile). Biological Conservation 127:272-281.
- Pennycott, T.W., A. Park, R.N. Cinderey, H.A. Mather, and G. Foster. 2002. Salmonella enterica subspecies enterica serotype Typhimurium and Escherichia coli O86 in wild birds at two garden sites in southwest Scotland. Veterinary Records 151:563-567.
- Perez-Tris, J., D. Hasselquist, O. Hellgren, A. Krizanauskieve, J. Waldenström, and S. Bensch. What are malaria parasites? Trends in Parasitology 21:209-211.
- Peterson, A.T., N. Komar, O. Komar, A. Navarro-Sigüenza, M.B. Robbins, and E. Martinez-Meyer. 2004. West Nile virus in the New World: potential impacts on bird species. Bird Conservation International 14:215-232.
- Peterson, L.R. and E.B. Hayes. 2004. Westward ho? The spread of West Nile virus. New England Journal of Medicine 351:2257–2259.
- Prange, S., S.D. Gehrt, and E.P. Wiggers. 2003. Demographic factors contributing to high raccoon densities in urban landscapes. Journal of Wildlife Management 67:324–333.
- Proctor, M.E., M. Hamacher, M.L. Tortorello, J.R. Archer, and J.P. Davis. 2001. Multistate outbreak of Salmonella serovar Muenchen infections associated with alfafa sprouts grown from seeds pretreated with calcium hypochlorite. Journal of Clinical Microbiology 39:3461-3465.
- Pyle, P. 1997. Identification Guide to North American birds. Part I: Columbidae to Ploceidae. Slate Creek Press, Bolinas, CA.

- Rachowicz, L.J., J.-M. Hero, R.A. Alford, J.W. Taylor, J.A.T. Morgan, V.T. Vredenburg, J.P. Collins, and C.J. Briggs. 2005. The novel and endemic pathogen hypotheses: competing explanations for the origin of emerging infectious diseases of wildlife. Conservation Biology 19:1441–1448.
- Refsum, T., E. Heir, G. Kapperud, T. Vardund, and G. Holstad. 2002. Molecular epidemiology of Salmonella enterica serovar typhimurium isolates determined by pulsed-field gel electrophoresis: comparison of isolates from avian wildlife, domestic animals, and the environment in Norway. Applied Environmental Microbiology 68:5600-5606.
- Refsum, T., T. Vioren, K. Handeland, G. Kapperud, and G. Holstad. 2003. Epidemiologic and pathologic aspects of Salmonella Typhimurium infection in passerine birds in Norway. Journal of Wildlife Diseases 39:64-72.
- Reisen, W.K., Y. Fang, and V.M. Martinez. 2006. Effects of temperature on the transmission of West Nile Virus by Culex tarsalis (Diptera: Culicidae). Journal of Medical Entomology 43:309–317.
- Rice, W.R. and S.D. Gaines. 1994. 'Heads I win, tails you lose': testing directional alternative hypotheses in ecological and evolutionary research. Trends in Ecology and Evolution 9:235–237.
- Ricklefs, R.E. and S.M. Fallon. 2002. Diversification and host switching in avian malaria parasites. Proceedings of the Royal Society of London B, Biological Sciences 269:885-892.
- Ricklefs, R.E., B.L. Swanson, S.M. Fallon, A. Martínez-Abraín, A. Scheuerlein, J. Gray, and S.C. Latta. 2005. Community relationships of avian malaria parasites in southern Missouri. Ecological Monographs 75:543-559.
- Riley, S.P.D., J. Foley, and B. Chomel. 2004. Exposure to feline and canine pathogens in bobcats and grey foxes in urban and rural zones of a national park in California. Journal of Wildlife Diseases 40:11–22.
- Rizzo, D.M. and M. Garbelotto. 2003. Sudden oak death: endangering California and Oregon forest ecosystems. Frontiers in Ecology and the Environment 1:197–204.
- Rothman, K.J. 1990. No adjustments are needed for multiple comparisons. Epidemiology 1:43-46.
- Ruiz, G., M. Rosenmann, F.F. Novoa, and P. Sabat. 2005. Hematological parameters and stress index in rufous-collared sparrows dwelling in urban environments. Condor 104:162–166.

- Rushton, S.P., P.W.W. Lurz, J. Gurnell, and R. Fuller. 2000. Modeling the spatial dynamics of parapoxvirus disease in red and grey squirrels: a possible cause of the decline in the red squirrel in the United Kingdom? Journal of Applied Ecology 37:1–18.
- Saaroni, H., E. Ben-Dor, A. Bitan, and O. Potchter. 2000. Spatial distribution and microscale characteristics of the urban heat island in Tel-Aviv. Israel. Landscape and Urban Planning 48:1–18.
- Savage, H.M., D. Aggarwal, C.S. Apperson, C.R. Katholi, E. Gordon, H.K. Hassan, M. Anderson, D. Charnetzky, L. McMillen, E.A. Unnasch, and T.R. Unnasch. 2007. Host choice and West Nile virus infection rates in blood-fed mosquitoes, incuding members of the Culex pipiens complex, from Memphis and Shelby County, Tennessee, 2002-2003. Vector-Borne and Zoonotic Diseases 7:365-386.
- Schmidt, K.A. and R.S. Ostfeld. 2001. Biodiversity and the dilution effect in disease ecology. Ecology 82:609-619.
- Schoech, S.J. and R. Bowman. 2001. Variation in the timing of breeding between suburban and wildland Florida Scrub jays: do physiologic measures reflect different environments? In: J.M. Marzluff, R. Bowman, and R. Donnelly. Avian Ecology and Conservation in an Urbanization World. Springer Academic Press, Berlin, Germany.
- Senar, J.C., J. Figuerola, and J. Domènech. 2003. Plumage coloration and nutritional condition in the great tit Parus major: the role of carotenoids and melanins differ. Naturwissenschaften 90:234-237.
- Shochat, E. 2004. Credit or debit? Resource inputs changes population dynamics of city slicker birds. Oikos 106:622-626.
- Shochat, E., S. Lerman, M. Katti, and D. Lewis. 2004. Linking optimal foraging behaviour to bird community structure in an urban-desert landscape: Field experiments with artificial food patches. American Naturalist 164:232-243.
- Shochat, E., P.S. Warren, S.H. Faeth, N.E. McIntyre, and D. Hope. 2006. From patterns to emerging processes in mechanistic urban ecology. Trends in Ecology and Evolution 21:186-191.
- Silliker J.H., R.H. Deibel, and P.T. Fagan. 1964. Enhancing effect of feces on isolation of salmonellae from selenite broth. Applied Microbiology 12:100-105.
- Sklan, D., D. Melamed, and A. Friedman. 1994. The effect of varying levels of dietary vitamin A on immune response in the chick. Poultry Science 73:843–847.

- Smith, K.E., F. Anderson, C. Medus, F. Leano, and J. Adams. 2005. Outbreaks of salmonellosis at elementary schools associated with dissection of owl pellets. Vector-borne and Zoonotic Diseases 5:133-136.
- Sol, D., R. Jovani, and J. Torres. 2003. Parasite mediated mortality and host immune response explain age-related differences in blood parasitism in birds. Oecologia 135:542-547.
- Stoner, K.J.L. and A. Joern. 2004. Landscape vs. local habitat scale influences to insect communities from tallgrass prairie remnants. Ecological Applications 14:1306-1320.
- Stratford, J.A. and P.C. Stouffer. 2001. Reduced feather growth rates of two common birds inhabiting Central Amazonian forest fragments. Conservation Biology 15:721-728.
- Suzan, G. and G. Ceballos. 2005. The role of feral mammals on wildlife infectious disease prevalence in two nature reserves within Mexico city. Journal of Zoo and Wildlife Medicine 36:479–484.
- Swaddle, J.P. and S.E. Calos. 2008. Increased avian diversity is associated with lower incidence of human West Nile infection: observation of the dilution effect. PLos ONE 3:e2488.
- Taylor, L.H., S.M. Latham, and M.E.J. Woolhouse. 2001. Risk factors for human disease emergence. Philosophical Transactions of the Royal Society of London B 356:983-989.
- Telfer, S., K.J. Bown, R. Sekules, M. Begon, T. Hayden, and R. Birtles. 2005. Disruption of a host-parasite system following the introduction of an exotic host species. Parasitology 130:661-668.
- Theobald, D.M. 2004. Placing exurban land-use change in a human modification framework. Frontiers in Ecology and the Environment 2:139–144.
- Thornley, C.N., G.C. Simmons, M.L. Callaghan, C.M. Nicol, M.G. Baker, K.S. Gilmore, and N.K.G. Garrett. 2003. First incursion of Salmonella enterica serotype Typhimurium DT160 into New Zealand. Emerging Infectious Diseases 9:493-495.
- Tizard, I. 2004. Salmonellosis in wild birds. Seminars in Avian and Exotic Pet Medicine 13:50-66.
- Tompkins, D.M., A.W. Sainsbury, P. Nettleton, D. Buxton, and J. Gurnell. 2002. Parapoxvirus causes a deleterious disease of red squirrels associated with UK population declines. Proceedings of the Royal Society of London B 269:529–533.
- Tompkins, D.M., A.R. White, and M. Boots. 2003. Ecological replacement of native red squirrels by invasive greys driven by disease. Ecology Letters 6:189–196.

- U.S. Department of Agriculture. The 2007 Census of Agriculture. [cited 14 Aug 2009]. Available from http://www.agcensus.usda.gov/Publications/2007.
- U.S. Fish and Wildlife Service. 2000. The bottom line: how healthy bird populations contribute to a healthy economy. [cited 2009 Aug 18]. Available from http://library.fws.gov/Pubs/mbd\_bottom\_line2.pdf.
- U.S. Fish and Wildlife Service. 2006 national survey of fishing, hunting, and wildlife associated recreation. [cited 2009 Jul 14]. Available from http://library.fws.gov/pubs/nat\_survey\_survey2006\_final.pdf.
- United Nations Department of Economic and Social Affairs. 2005. World Population Prospects: 2005 Revision. United Nations, New York, New York.
- United Nations Department of Economic and Social Affairs. 2007. World Population Prospects: 2007 Revision. United Nations, New York, New York.
- Valkiūnas, G. 2005. Avian Malaria Parasites and Other Haemosporidia. CRC Press, Boca Raton, Florida.
- Valkiūnas, G., S. Bensch, T.A. Iezhova, A. Križanauskienė, O. Hellgren, and C.V. Bolshakov. 2006. Nested cytochrome *b* polymerase chain reaction diagnostics underestimate mixed infections of avian blood haemosporidian parasites: microscopy is still essential. Journal of Parasitology 92: 418-422.
- van Oers, K., D.S. Richardson, S.A. Sæther, and J. Komdeur. 2009. Reduced blood parasite prevalence with age in the Seychelles Warbler: selective mortality of suppression of infection? Journal of Ornithology DOI 10.1007/s10336-009-0427-x
- van Riper, C., III, S.G. van Riper, M.L. Goff, and M. Laird. 1986. Epizootiology and ecological significance of malaria in Hawaiian land birds. Ecological Monographs 56:327-344.
- von Shantz, T., S. Bensch, M. Grahn, D. Hasselquist, and H. Wittzell. 1999. Good genes, oxidative stress, and condition-dependent sexual signals. Proceedings of the Royal Society of London B: Biological Sciences 226:1-12.
- Walberg, J. 2001. White blood cell counting techniques in birds. Seminars in Avian and Exotic Pet Medicine 10:72-76.
- Waldenström, J., S. Bensch, D. Hasselquist, and Ö. Östman. 2004. A new nested polymerase chain reaction method very efficient in detecting Plasmodium and Haemoproteus infections from avian blood. Journal of Parasitology 90:191-194.
- Wauters, L.A. and J. Grunell. 1999. The mechanism of replacement of red by grey squirrels: a test of the interference competition hypothesis. Ethology 105:1053–1071.

- Wolfenbarger, L.L. 1999. Red coloration of male northern cardinals correlates with mate quality and territory quality. Behavioural Ecology 10:80-90.
- Woods, C., C. Domenget, F. Solari, O. Gandrillon, E. Lazarides, and P. Jurdic. 1995. Antagonistic role of vitamin D3 and retinoic acid on the differentiation of chickenhematopoietic macrophages into osteoclast precursor cells. Bradley\_dissertationEndocrinology 136:85–95.
- Woodworth, B.L., C.T. Atkinson, D.A. LaPointe, P.J. Hart, C.S. Spiegel, E.J. Tweed, C. Henneman, J. LeBrun, T. Denette, R. DeMots, K.L. Kozar, D. Triglia, D. Lease, A. Gregor, T. Smith, and D. Duffy. 2005. Host population persistence in the face of introduced vector-borne diseases: Hawaii amakihi and avian malaria. Proceedings of the National Academies of Sciences 102:1531-1536.
- Woolhouse, M.E.J., L.H. Taylor, and D.T. Haydon. 2001. Population biology of multihost pathogens. Science 292:1109-1112.
- Woolhouse, M.E.J. and S. Gowtage-Sequeria. 2005. Host range and emerging and reemerging pathogens. Emerging Infectious Diseases 11:1842–1847.
- Wright, A.N. and M.E. Gompper. 2005. Altered parasite assemblages in raccoons in response to manipulated resource availability. Oecologia 144:148–156.
- Xu, R.-H., J.-F. He, M.R. Evans, G.-W. Peng, H.E. Field, D.-W. Yu, C.-K. Lee, H.-M. Luo, W.-S. Lin, P. Lin, L.-H. Li, W.-J. Liang, J.-Y. Lin, and A. Schnur. 2004. Epidemiologic clues to SARS origin in China. Emerging Infectious Diseases 10:1030–1037.
- Yeh, P.J. and T.D. Price. 2004. Adaptive phenotypic plasticity and the successful colonization of a novel environment. American Naturalist 164:531–542.
- Zehtindjiev, P., M. Ilievas, H. Westerdahl, B. Hansson, G. Valkiūnas, and S. Bensch. 2008. Dynamics of parasitemia of malaria parasites in a naturally and an experimentally infected migratory songbird, the great reed warbler Acrocephalus arundinaceus. Experimental Parasitology 119:99-110.