

EFFECTS OF ANTHROPOGENIC LAND USE CHANGE  
ON THE ECOLOGY OF THE CHAGAS DISEASE AGENT *Trypanosoma cruzi*

by

NICOLE LYNN GOTTDENKER

(Under the direction of C. Ronald Carroll)

ABSTRACT

Anthropogenic landscape change is associated with increased infectious disease transmission in terrestrial and aquatic ecosystems. Understanding pathogen responses to environmental disturbance is necessary to prevent and control disease emergence in wildlife, domestic animals and humans. The objective of this study is to evaluate how deforestation impacts transmission of the vector-borne parasite *Trypanosoma cruzi*, agent of Chagas disease in humans. *T. cruzi* cycles between triatomine bug vectors and wild mammals, domestic mammals, and humans, and is a significant cause of morbidity and mortality in Latin America. Recent studies suggest human encroachment on forest ecosystems causes increased risk of *T. cruzi* transmission to humans. Key hypotheses of this study are: 1) vector abundance increases in deforested habitats due to an increased contact with opportunistic wild mammals and domestic mammals 2) *T. cruzi* vector infection increases in deforested areas because of increased contact with competent disease reservoirs 3) *T. cruzi* vector infection is lower in intact forests because of higher species diversity and contact with highly competent disease reservoirs (a 'dilution' effect). This study took place in protected forests and deforested landscapes surrounding the Panama Canal, Panama. The triatomine bug *Rhodnius pallescens*, the principal vector of

Chagas disease in Panama, was collected (N=1186) from its primary habitat, the palm *Attalea butyraceae*, in five different habitat types reflecting a gradient of anthropogenic disturbance. Collected *R. pallescens* (N=641) were tested for infection with *T. cruzi* and a congeneric parasite *T. rangeli* by a duplex PCR (polymerase chain reaction) assay. In order to evaluate potential effects of species diversity and composition on vector infection with *T. cruzi*, vector blood meals were identified by PCR amplification of the vertebrate 12S rRNA gene.

Results show that deforestation and forest fragmentation are associated with increased vector abundance and vector infection prevalence with *Trypanosoma cruzi*. Contrary to hypothesis 3, there was a positive association between blood meal species diversity and *T. cruzi* infection prevalence. On the other hand, blood meal species composition appears to be an important driver of *T. cruzi* transmission in the deforested landscape. These results suggest that anthropogenic land use change can drive increased *R. pallescens* abundance and *T. cruzi* vector infection prevalence.

INDEX WORDS: land use change, forest fragmentation, deforestation, disease ecology, multihost pathogen, vector-borne disease, *Trypanosoma cruzi*, *Rhodnius pallescens*, wildlife, zoonoses

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NICOLE LYNN GOTTDENKER

B.A., Colgate University, 1989

D.V.M., Tuskegee University, 2003

M.S., University of Florida, 1996

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of The University of Georgia in Partial Fulfillment  
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Approved:

Major Professor: C. Ronald Carroll

Committee: Sonia Altizer  
David Peterson  
Rick Tarleton  
Uriel Kitron

Electronic Version Approved:

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

TO ROCKET, MATTHEW, MOTHER DOROTHY  
GRANDMA OLGA, AUNT TANYA, AND STEPHANIE

I LOVE YOU ALL

IN MEMORIAM: MY BELOVED FATHER LESTER GOTTDENKER,  
AUNT MARY, AND DOG HAMLET

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## CHAPTER 1

# WHAT DO KISSING BUGS, PALMS, AND MAMMALS HAVE IN COMMON? *Trypanosoma cruzi* AS A MODEL SYSTEM FOR STUDYING THE EFFECTS OF ANTHROPOGENIC LAND USE CHANGE ON DISEASE TRANSMISSION

### 1.1 GENERAL RESEARCH QUESTIONS

Human activity dominates ecosystems at multiple scales, transforming the architecture of landscapes (Forman, 1995), biogeochemical cycles (Tilman, 1999), and plant and animal species diversity and community composition. Understanding relationships between ecosystem degradation and infectious disease emergence in wildlife and humans is one of the most critical environmental issues of the 21st century (Daszak and Ball, 2001; Foley et al., 2005). Anthropogenic landscape disturbance is linked to infectious disease emergence in humans (Foley et al., 2005; Patz et al., 2004; Sutherst, 2004; Eyles et al., 2003), wildlife (McCallum and Dobson, 2002; Daszak and Ball, 2001), and aquatic ecosystems (Harvell et al., 2002). Emergence of infectious disease in wildlife populations is an international issue, related to global climate change (Harvell et al., 2002), economic globalization, environmental resource sustainability, and human health. For instance, the recent spread of West Nile Virus throughout the United States and the threat of global avian influenza pandemics attest to this fact. Justifiably, the study of the anthropogenic footprint on disease transmission has garnered interest in the scientific community. Chapter 2 reviews the ecology, epidemiology, and medical literature in order to evaluate our current scientific understanding (empirical and theoretical) of how anthropogenic land use change impacts disease transmission, and to suggest future research avenues.

Accordingly, my research interests address linkages between wildlife, human health, and ecosystem integrity. Questions that motivate my pursuit of a doctoral degree in ecology include: What role does anthropogenic land use change in ecosystems play on the dynamics and emergence of infectious disease in wild animal populations? Is biodiversity good for your health because of decreased disease transmission in areas of greater species diversity? What rules govern disease persistence of multihost pathogens, in particular those that are vector-borne? What are effects of infectious diseases on population, community, and ecosystem structure and function? How can we minimize public health risks of infectious diseases resulting from human induced landscape change?

Tackling these research problems requires integration of knowledge about disease processes and effects on multiple scales, from molecular, cellular and individual levels to populations, communities, and ecosystems. Advancements in development of ecological and epidemiological theory and well designed empirical studies are key to understanding and predicting how anthropogenic land use change impacts pathogen transmission. The goal of this research project is to analyze effects of anthropogenic landscape change on the ecology of the zoonotic protozoan parasite *Trypanosoma cruzi*.

## 1.2 *Trypanosoma cruzi* A MODEL SYSTEM

*Trypanosoma cruzi* is the agent of Chagas disease in humans. *T. cruzi* is transmitted only in the Americas and ranges from the southern United States to Argentina (Moncayo, 2003). Approximately 100 million inhabitants of Latin America are at risk for *T. cruzi* infections and over 10 million are infected with this parasite, a significant cause of heart failure and other debilitating lesions (Moncayo, 2003). The life cycle of *T. cruzi* is complex (Fig 1.1), cycling between invertebrate vectors, members of the hemipteran family Reduviidae, and many species of wild and domestic mammal reservoir hosts (Miles et al., 2003). When a *T. cruzi*-infected vector takes a blood meal on a host, it defecates, and the mammalian host becomes infected by scratching *T. cruzi* laden fecal material into the bite wound (Fig 1.1).

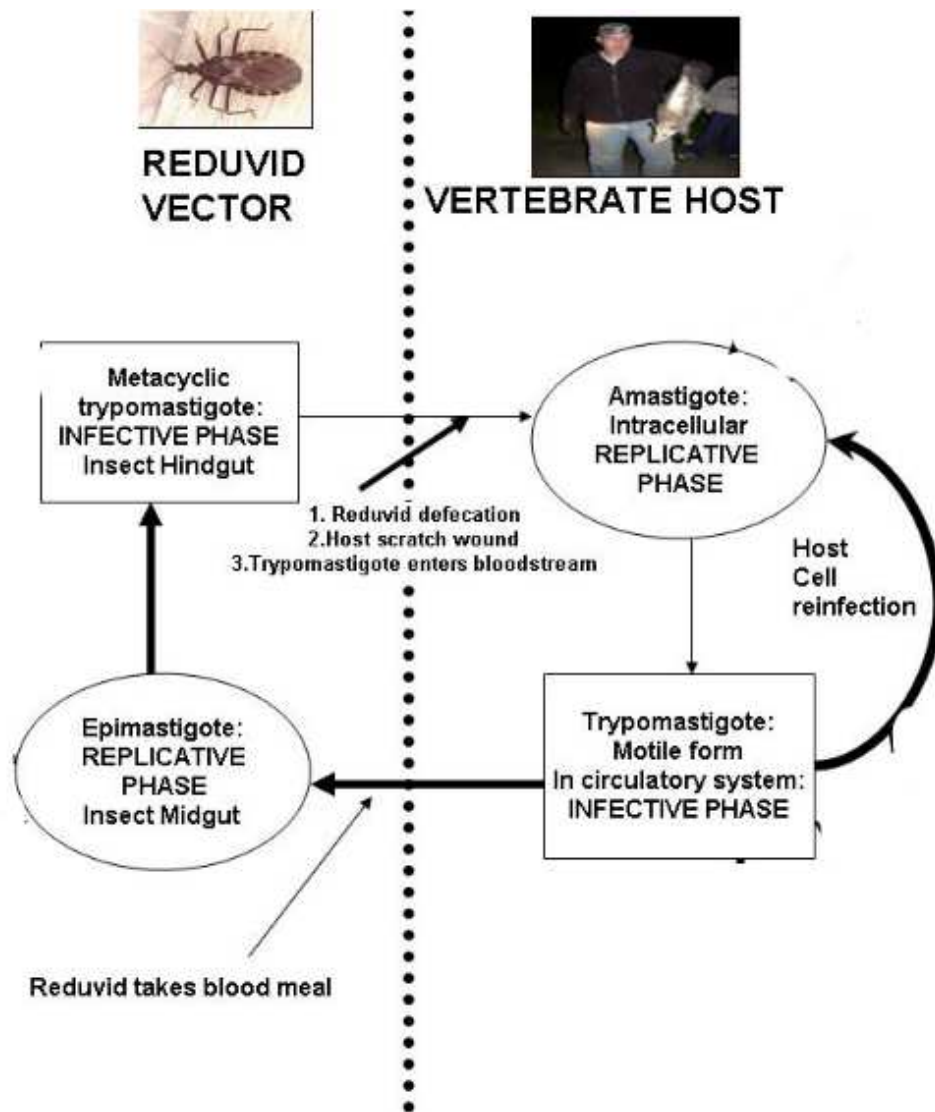


Figure 1.1: General life cycle of *Trypanosoma cruzi*

*T. cruzi* is ideal for studying links between environmental integrity and human health because transmission patterns are sylvatic, associated with wild mammal reservoirs and reduviid vectors, and domestic, linked to humans, domestic mammal reservoirs, and domiciliary-adapted reduviids (Coura et al., 1999). *T. cruzi* is also a tractable organism for field and laboratory studies because its genome has been sequenced (El-Sayed et al., 2005), and genetic markers exist for *T. cruzi* strain typing and identification. The biology of *T.*



*cruzi* vectors is also well studied and key reservoir hosts of the parasite have been identified in most regions. More important, it is hypothesized that human encroachment on forest ecosystems in some areas causes increased risk of *T. cruzi* transmission from sylvatic niches to humans. (Walter, 2003; Teixeira et al., 2001a; Coura et al., 1999, 1994). My proposed doctoral research thus fills a gap in our knowledge of how anthropogenic processes such as forest fragmentation relate to alterations in the ecology and risk of human transmission of a vector-borne disease.

The Panama Canal Watershed, lying at 9°N latitude, contains protected moist tropical forests, such as Soberania National Park, and a mosaic of disturbed forests surrounded by matrices of agricultural and periurban development (Ibanez et al., 2002). This area has undergone a vast degree of deforestation in the past century, with over 75% of deforested land due to pasture extension into forest (Ibanez et al., 2002). In Panama, *Rhodnius pallescens* is a major vector of *T. cruzi* infections in humans and animals (Vasquez et al. 2004). In this region, *T. cruzi* cycles between *R. pallescens* and many species of wild mammals. The common opossum *Didelphis marsupialis* is believed to be an important host (Vasquez et al., 2004; Jansen et al., 1997). *R. pallescens* is closely associated with the palm *Attalea butyraceae* in Panama, and opossums are preferred vector blood sources (Christensen and Devasquez, 1981; Whitlaw and Chaniotis, 1978).

The main hypothesis in this study is that deforestation causes an increase in vector abundance and vector infection prevalence with *T. cruzi*. The abundance of *R. pallescens* is positively correlated with both vertebrate host availability (Cecere et al., 1997) and available microhabitat. In forest islands and fragments in the Panama Canal zone, both forest fragmentation and hunting leads to decreased overall plant species diversity and increased dominance of the *Attalea* palm (Wright and Duber, 2001). Chapter 3 evaluates relationships between land use change, and *R. pallescens* abundance, and population parameters by collection of bugs across a gradient of deforestation and forest fragmentation.

Chapter 4 investigates effects of deforestation on vector infection with *T. cruzi*. Potential mechanisms regarding why land use change should result in increased *T. cruzi* infection prevalence and vector abundance include alterations in host diversity, community structure, and abundance. For example, forest fragmentation causes changes in animal community composition (Terborgh et al., 2001) that has the potential to affect disease dynamics in a landscape. Declines of large predators such as puma and jaguar in Panamanian forests resulting from forest fragmentation and poaching (Wright and Duber, 2001) may allow for loss of direct and indirect negative feedback on competent mammal host populations, such as the common opossum. In accord, elevated densities of the opossum *Didelphis marsupialis* have been observed in small tropical forest islands in Gatun Lake, Panama (Boyett et al., 2000; Adler, 1998), tropical moist forest fragments in Southern Mexico (Estrada and Merritt, 1994), and fragmented Brazilian Atlantic rainforests (Dafonseca and Robinson, 1990). Because opossums are highly competent reservoir hosts for *T. cruzi*, then fragmentation-induced mesopredator release may cause an increase in opossum number, resulting in increased risk of transmission of *T. cruzi* to *R. pallescens* vectors.

In contrast to deforested habitats, contiguous, mature forests are also expected to have higher vertebrate biodiversity. The ability of biodiverse vertebrate communities to act as a buffer for the prevalence of vectors infected with a particular pathogen is known as the dilution effect (LoGiudice et al., 2003; Ostfeld and LoGiudice, 2003; Schmidt and Ostfeld, 2001). Intact forests are expected to have a greater number of alternative vertebrate species for *R. pallescens* vector blood meals. Non-mammalian taxa, such as reptiles and birds, are not competent hosts for *T. cruzi*. Thus, unfragmented forests should contain a lower proportion of *T. cruzi* infected vectors. In contrast, fragmented areas, with lower biodiversity and presumptively higher populations of opportunistic mammals, should have higher proportions of *R. pallescens* infected with *T. cruzi*, thus increasing human disease risk. In order to test this hypothesis, I identified blood meal species composition from

vectors collected in contiguous and deforested sites (Chapter 4) and compared the blood meal diversity and species composition to habitat type and vector infection.

## CHAPTER 2

# REVIEW AND ECOLOGICAL APPRAISAL OF ANTHROPOGENIC LAND USE CHANGE AND INFECTIOUS DISEASE

### 2.1 INTRODUCTION

#### 2.1.1 THE HUMAN FOOTPRINT AND INFECTIOUS DISEASE

Throughout the history of human civilization, there has been local and regional scale alteration by human settlements, including animal domestication, predation on wild animals, agriculture (crops, animal grazing), prescribed burning, natural resource extraction (mining, logging), infrastructure development (dams, road building) and urbanization (Goudie, 2000; Dale et al., 2000; Burney and Flannery, 2005). Arguably, few areas across the earth have been untouched or unaltered by humans in the past few thousand years (Goudie, 2000; Burney and Flannery, 2005). It is well-established that areas of mature tropical forest in parts of Central America were previously areas of dense human settlement and agricultural activities (Whitmore, 1992). A river in the northern Amazon estuary, believed to have been formed by a natural process, was actually created over the course of a few decades by human land use practices (Raffles, 2002).

In the last century, the earth's surface has undergone unprecedented large-scale anthropogenic landscape transformation. Humans have converted over 40% of the earth's land surface for agricultural activities such as croplands and pasture (Sterling and Ducharme, 2008; Vitousek et al., 1997). Some biomes have been nearly completely transformed by human activities, with over 90% of the earth's grasslands transformed primarily for livestock grazing (Sterling and Ducharme, 2008). Clearly, anthropogenic land

use change and its influence are ubiquitous across terrestrial and aquatic ecosystems (Foley et al., 2005).

Primary drivers of anthropogenic land use change are of complex socioeconomic, political, and cultural origins (Nelson et al., 2006). These drivers result in land alterations in the form of urbanization/suburbanization, forest cutting/deforestation, forest or other habitat fragmentation, the construction of corridors/infrastructure development (railroad, road, power lines), agricultural development, reforestation, hydrological alteration (dams, irrigation, canal construction) (Forman, 1995; Foley et al., 2005). Continued large-scale land use change by deforestation, forest fragmentation, industrialization, rangeland expansion, biofuel and crop agricultural development have detrimental impacts on ecological integrity and biodiversity (Laurance et al., 2007; Tilman, 1999).

Negative ecological consequences of land use change on ecosystems and biomes are numerous, and include animal and plant habitat loss and/or fragmentation, alteration of plant and animal diversity, species composition and spatial distribution, animal behavioral changes, disruption of food web structure and function, alteration of terrestrial and aquatic biogeochemical cycles, hydrologic alterations, ecosystem property shifts (e.g. desertification), and the introduction of non-native invasive species, including parasites (Foley et al., 2005; Tilman, 1999; Matson et al., 1997). Additional consequences of anthropogenic land use change include pollution of aquatic and terrestrial systems as well as local, regional, global climate change (Carpenter et al., 1998). Ecological consequences of land use change manifest themselves at multiple scales, from individuals, populations, communities and ecosystems to regional and global levels.

An important, yet until recently, overlooked issue relating to anthropogenic land use change is its impact on infectious disease transmission in humans and animals. Habitat fragmentation, degradation, alteration, and/or transformation arising from anthropogenic land use change have the potential to modify host-parasite relationships within ecosystems (Foley et al., 2005; Lafferty, 2009; Dobson et al., 2006*b,a*; Pongsiri and Roman, 2007).

Indeed, land alteration has been linked to emergence, transmission, and persistence of infectious disease in humans, domestic animals, and wildlife in terrestrial and aquatic systems (Molyneux, 2003; Vora, 2008; Gratz, 1999; Daszak and Cunningham, 2000; Patz et al., 2004, 2000; Pongsiri and Roman, 2007; Wilson, 1995).

In the past 30 or so years, there has been a surge in scientific interest in the role that anthropogenic landscape disturbance plays on the ecology and emergence of infectious disease in humans, domestic animals, and wildlife (Fig 2.1). International meetings, such as those sponsored by the National Council for Science and the Environment in the United States, the International Society for Ecology and Health, and research programs of the Environmental Protection Agency (Pongsiri and Roman, 2007) as well as the Millenium Ecosystem Assessment (Carpenter and Folke, 2006) incorporate relationships between anthropogenic ecosystem changes and infectious disease emergence into their agendas.

### 2.1.2 THE NEED FOR AN EPIDEMIOLOGIC PARADIGM SHIFT TO UNDERSTAND RELATIONSHIPS BETWEEN LAND USE AND INFECTIOUS DISEASE

Defining and predicting how land use change affects disease transmission requires changing the traditional conceptual epidemiological framework in which host/vector disease agent, and environment interact with each other, yet exist independently, to one in which the host/vector and disease agent are embedded within the context of their environment (Fig 2.2), as neither hosts nor disease agents can exist independently from their environment. There are many potential mechanisms by which ecological consequences of anthropogenic land use change can alter infectious disease transmission in humans and animals (Plowright et al., 2008; Sutherst, 2004; Harrus and Baneth, 2005). These human-induced ecological changes can alter niches of pathogens, disease reservoirs and vectors, creating environmental conditions that allow greater pathogen, host, or vector survival, productivity, or persistence. Ecosystem alterations can induce changes in spatial aggregation or behavior of hosts and/or vectors (e.g. increased vector biting rates, intra

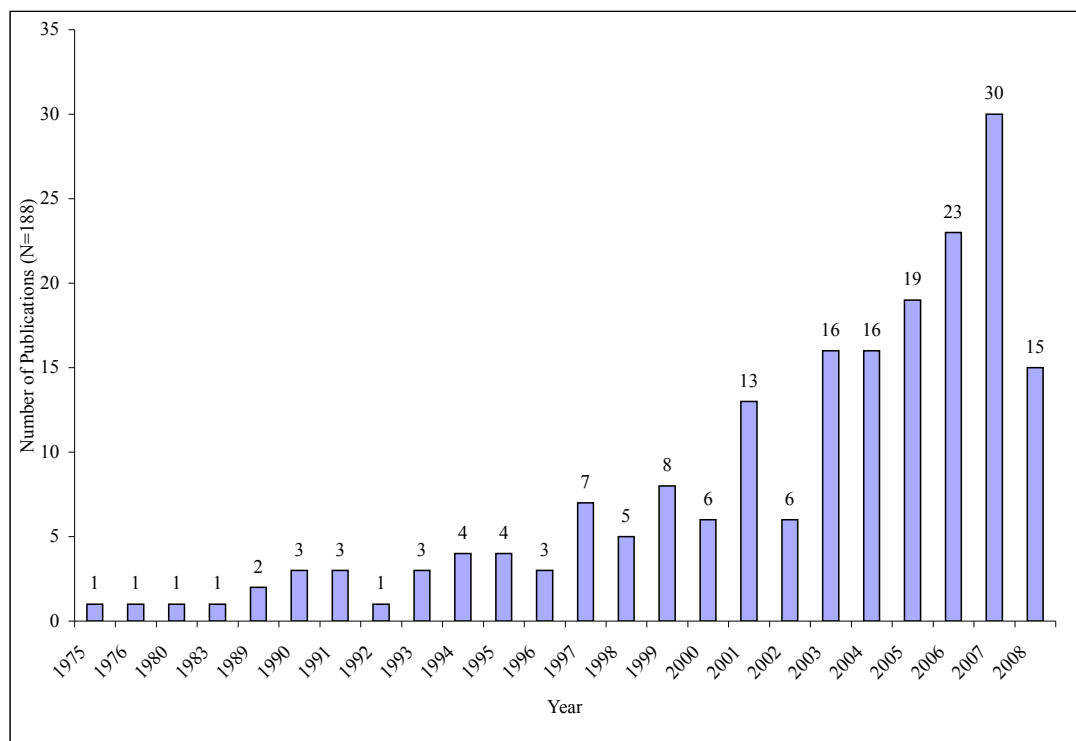


Figure 2.1: Number of scientific publications per year relating to anthropogenic land use change and infectious disease emergence

and interspecific host contact), and population changes in hosts and/or other vectors (Sutherst, 2004; Bradley and Altizer, 2007). Altered spatial distribution or movement of disease reservoirs/vectors can change contact rates and disease transmission between hosts (Gubler, 1998). Biodiversity loss and/or changes in host community structure may cause increased or decreased transmission of generalist pathogens (Keesing et al., 2006). Human induced changes in trophic structure may lead to the loss or introduction of intermediate hosts of a particular parasite, resulting in either increased or decreased pathogen

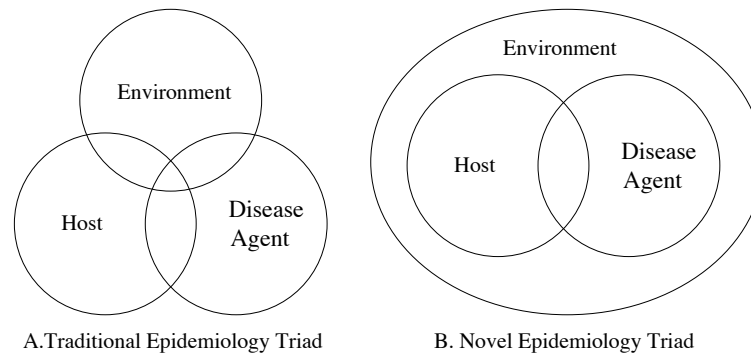


Figure 2.2: A. Traditional epidemiology triad, where the environment is conceptualized as interacting with, but distinct from the host and pathogen or vector. B. A more ecologically realistic triad for evaluating effects of land use change on disease transmission, where the environment is the context in which all interactions between hosts and pathogens take place.

transmission (Romig et al., 2006; Hudson et al., 2006). Land use change may also increase the potential for spillover of infectious disease from a reservoir host into a highly susceptible host (Power and Mitchell, 2004). Additionally, humans can introduce novel pathogens, disease reservoirs and vectors into naive environments, with land use change facilitating their spread (Tompkins et al., 2002; Crowl et al., 2008).

Accordingly, this article reviews scientific literature that investigates relationships between land use change and infectious disease. This review synthesizes current and past land use change-disease research in the framework of the following questions: What patterns exist in published scientific studies addressing land use change in relation to infectious disease transmission? What types of land use alterations are highly associated with changes in



infectious disease transmission? Are there particular regions or ecosystem types and/or human land use (anthropogenic) biomes associated with alterations in pathogen/parasite transmission? Can we predict which systems are more vulnerable to increased disease transmission and which parasites of human, wildlife, and domestic animal health importance may likely increase with human land use change being a proximate driver? Also discussed are mechanisms by which anthropogenic land use change affects host-parasite relationships, disease transmission, and evolution based on ecological principles and theory. This study provides a critical appraisal of the scientific literature and provides suggestions for future avenues of research.

## 2.2 TRENDS AND PATTERNS IN LAND USE CHANGE-INFECTIOUS DISEASE LITERATURE

A literature search using the following search term combinations was conducted in ISI Web of Science and PubMed: (land use change OR land use OR deforest\* OR forest fragment\* OR habitat fragment\* OR habitat change OR habitat loss) AND (disease OR parasite\* OR parasites OR parasitism). Additional articles relating to land use change and disease were also compiled from bibliographic references of articles gathered from search engines. Actual articles evaluated were less than the number of hits resulting from literature searches because after reviewing the article content, some articles were not directly relevant. Studies were classified as observational, experimental, and review or ‘concept’ papers. The following information from each observational and experimental study was tabulated: general disease/pathogen name, species, type of pathogen (virus, bacteria, fungus, protozoa, helminth, other), host specificity (multihost or single-host pathogen), transmission type (direct, vector-borne, or trophic), vector type (if vector-borne), mechanism of disease change (if specified), study results, continent/world region, climate, country, study areas within country, and ecosystem type. Regarding transmission type, direct refers to a disease transmitted by direct contact with other animals or infective materials and trophic refers to transmission by ingestion of a prey item or food chain based

transmission. Studies were also classified according to the type of land use that affected disease transmission; agricultural development (cropland, irrigation, mixed cropland-grazing), forest alteration (deforestation, forest degradation, forest extraction), and urbanization. Studies were limited to vertebrate infectious diseases, although invertebrates may have been important as part of the transmission cycles.

From a total of 191 scientific articles evaluated relating to anthropogenic land use change and disease transmission, 66(34.6%) were literature reviews or 'concept' papers, and 125(65.4%) were longitudinal or cross-sectional observational studies. Most review/concept articles were general in nature and discussed effects of land use change on many pathogens, others focused on vector borne diseases, wildlife diseases, and some on particular pathogen groups or species. Four (2.1%) papers reviewed were experimental studies where some aspect of land use was altered.

Clearly, there is increased global scientific interest in regarding the impact of anthropogenic land transformation on infectious disease in humans and wildlife (Fig 2.1). However, the proportion of review or 'concept' papers regarding this theme remain relatively large (34.6% of papers reviewed) compared to original studies of land use change-disease relationships. This large number of review papers has a tendency towards redundancy, but there are articles that provide useful reference information for relationships between land use change and vector borne disease (Sutherst, 2004; Vora, 2008; Gratz, 1999), including malaria (Guerra et al., 2006; Yasuoka and Levins, 2007) and leishmaniasis (Wijeyaratne et al., 1994; Rotureau, 2006). To their credit, the review papers span a variety of different journals in medicine, epidemiology, parasitology, and ecology, promoting awareness of the need for a greater understanding of potential effects of land use change on disease transmission in humans and wildlife.

Of the 125 observational/experimental studies evaluated, 30.4 % (N=38) occurred in Africa, 24.8%(N=31) in North America/Central America, 22.4%(N= 28) in Eurasia, 18.4% (N=23) in South America, and 4.0% (N=5) in Oceania. The majority of studies (56.8%,

N=71) took place in tropical regions, 24.8%, (N=31) in temperate regions, and 13.6%(N=17) in subtropical regions, with a small number of studies taking place across a combination of tropical and subtropical (3.2%, N=4), and subtropical and temperate (1.6%, N=2) regions, respectively. Accordingly, the risk of infectious disease emergence in humans appears to be higher in lower latitudes (Jones et al., 2008), and tropical regions typically exhibit the highest rates of land use-land cover changes. Some studies suggest that deforestation decreases malaria risk in Asia and increases malaria risk in Africa and the American tropics (Guerra et al., 2006; Walsh et al., 1993). However, Guerra et al. (Guerra et al., 2006), using an analysis of deforestation rate and population at risk for malaria, rank some Southeast Asian countries above African and American countries in terms of the potential magnitude of malarial risk, because they have the highest densities of people living adjacent to closed forests where malaria is present. These large scale studies appear to be useful to assess overall trends relating to deforestation and disease transmission, but the fine spatial grain at which malaria transmission exists and the ecological complexities inherent in variations in transmission (e.g. shifts in particular vector species in different localities and their relative malaria competence pre and post disturbance relative to human behaviors), suggest that a mechanistic understanding of how malaria responds to deforestation at local and regional scales is important in predicting disease-deforestation risks.

The majority of studies (60%) concerned multi-host pathogens, 28.0% (N=35) focused on single-host pathogens, and 8.8% (N=11) on a combination of single and multihost pathogens (Fig 2.3). This observation likely reflects the fact that most animal pathogens can infect more than one host (Woolhouse et al., 2001). Over 60% of the pathogens in the studies reviewed (Table 1) are zoonoses, in accord with observations that 61% of infectious pathogens (Taylor et al., 2001), and 60% of emerging infectious diseases are zoonotic (Jones et al., 2008). A large proportion of the diseases affecting humans in this review

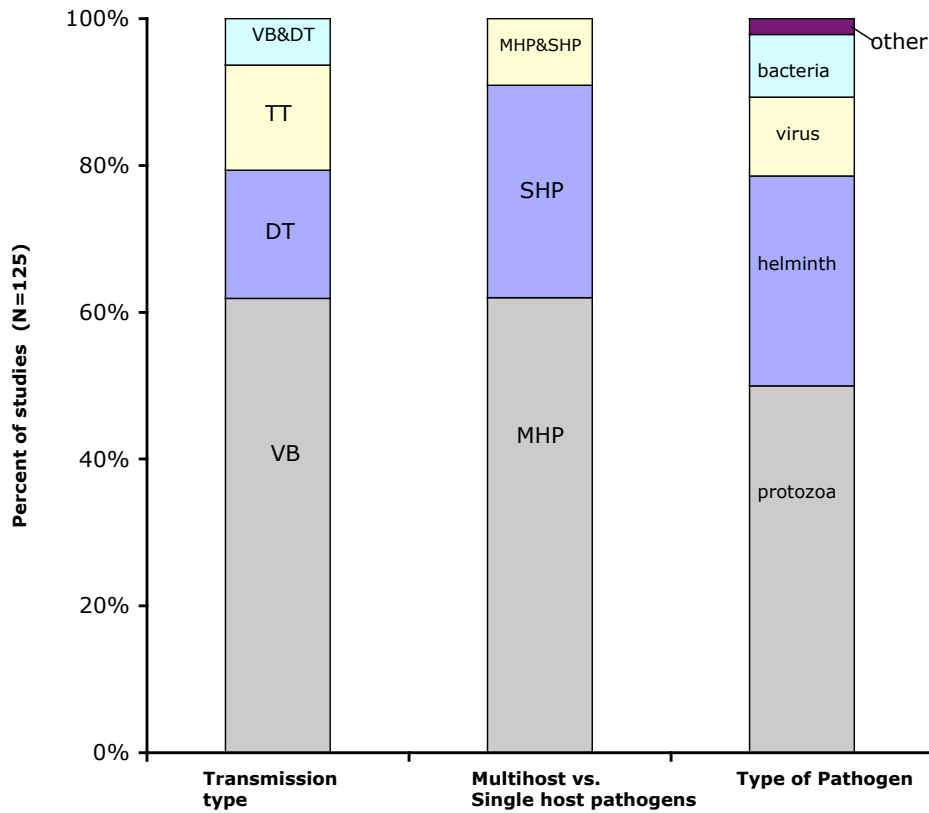


Figure 2.3: Types of transmission, Host range, and pathogen type in observational/experimental studies reviewed. Transmission type: VB vector borne, DT direct, TT trophic transmission, Host range: MPH multihost pathogen, SHP single host pathogen,

(approximately 80%) are also within the World Health Organization's list of neglected tropical diseases ([www.who.int/zoonoses/diseases/en](http://www.who.int/zoonoses/diseases/en)).

Vector-borne and trophically transmitted diseases comprise the majority (76.0%, N=95) of the observational and experimental studies relating to land use change and infectious disease (Fig 2.3). Landscape changes can often alter vector and intermediate host reproduction and survival due to changes in microclimate (e.g. light, moisture),

architecture (e.g. places for resting, larval development) and nutritional resources (e.g. eutrophication, increase in available hosts).

The primary focus of most articles (82.4%, N=103) was on diseases of human concern (either human specific or zoonoses). In total, 67 (53.6%) articles study zoonoses or potential zoonoses, and 65.0% of the diseases of primarily human concern are zoonoses or potential zoonoses. Thirty-two studies (25.6%) focus on pathogens of wildlife and/or domestic animal importance. The observational studies on human diseases or zoonoses included nineteen different pathogens that are considered to be neglected tropical diseases of humans by the World Health Organization. Seventy (56.0%) articles studied protozoan pathogens, 40 (32.0%) helminths, 15 (12.0%) virus, 12 (9.6%) bacteria, and single articles focused on fungal infection (chromomycosis) and a prion disease (Fig 2.3). The pathogens that were most commonly studied were human malaria (N=32), leishmaniasis (N=14), schistosomiasis (N=8), and Lyme disease (N=5). It is possible that protozoan parasites are particularly prone to local land use changes, because changes in ecological interactions that take place can alter vector and reservoir host abundance to a greater variation and extent than directly transmitted disease. The Appendix summarizes diseases studied, transmission type, land use and anthropogenic biome associations, and pathogen type.

Most reports (91.2%, N=114) associate land use change with a general increase in infectious disease prevalence, vector abundance and/or transmission. However, in 9 cases (7.4%), anthropogenic change was associated with a decrease in pathogen prevalence, incidence, or transmission. Two studies (1.6%) describe complex responses to land use change, such as an increase in the malaria vector due to rice irrigation, but a lower childhood prevalence associated with a host feeding shift by the vector in irrigated areas with cattle raising (Mutero et al., 2004).

### 2.3 GEOGRAPHICAL CONSIDERATIONS, ANTHROPOGENIC BIOMES, AND DISEASE

For observational and experimental studies, Holdridge Life-Zone and Anthropogenic biome(s) (Ellis and Ramankutty, 2008) were recorded by mapping study areas/sites relative to ©Google Earth maps of Holdridge biomes ([http://geonetwork3.fao.org/ows/1006\\_ISO\\_19115:2003/19139](http://geonetwork3.fao.org/ows/1006_ISO_19115:2003/19139)) and anthropogenic biomes (Ellis and Ramankutty, 2008). Anthropogenic biomes are a recently developed classification of the world's ecosystems relative to the predominant human land use and ecological landscape features. There are a total of 21 anthropogenic biomes that are divided into five general land use types; 1) Urban (urban, dense settlements), 2) Villages (rice villages, irrigated villages, cropped and pastoral villages, rainfed villages, rainfed mosaic villages), 3) Croplands (residential irrigated croplands, residential rainfed croplands, populated irrigated croplands, populated rainfed croplands, remote croplands), 4) Forested (populated forests, remote forests), and 5) Wildlands (wild forest, sparse trees, barren). Mosaic in the biome descriptions under village and cropland classification refers to villages or croplands mixed with trees. Urban settlements were lumped into one category, thus 20 anthropogenic biomes were evaluated. Information on the net primary productivity of each anthropogenic biome was acquired from Ellis and Ramankutty (2008). For analyses of relationships between transmission type, type of pathogen, and anthropogenic biome, six major groups of anthropogenic biomes were used and the type of anthropogenic biome or biomes present in each study was recorded. Data compiled from the literature search was analyzed using R version 2.7.1 (R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>).

Figure 2.5 shows types of land use change (e.g. forest fragmentation, agricultural development) that were associated with alterations of disease transmission in the articles reviewed. There was no evidence of a significant dependent relationship between agricultural development, urbanization, or forest fragmentation/deforestation and

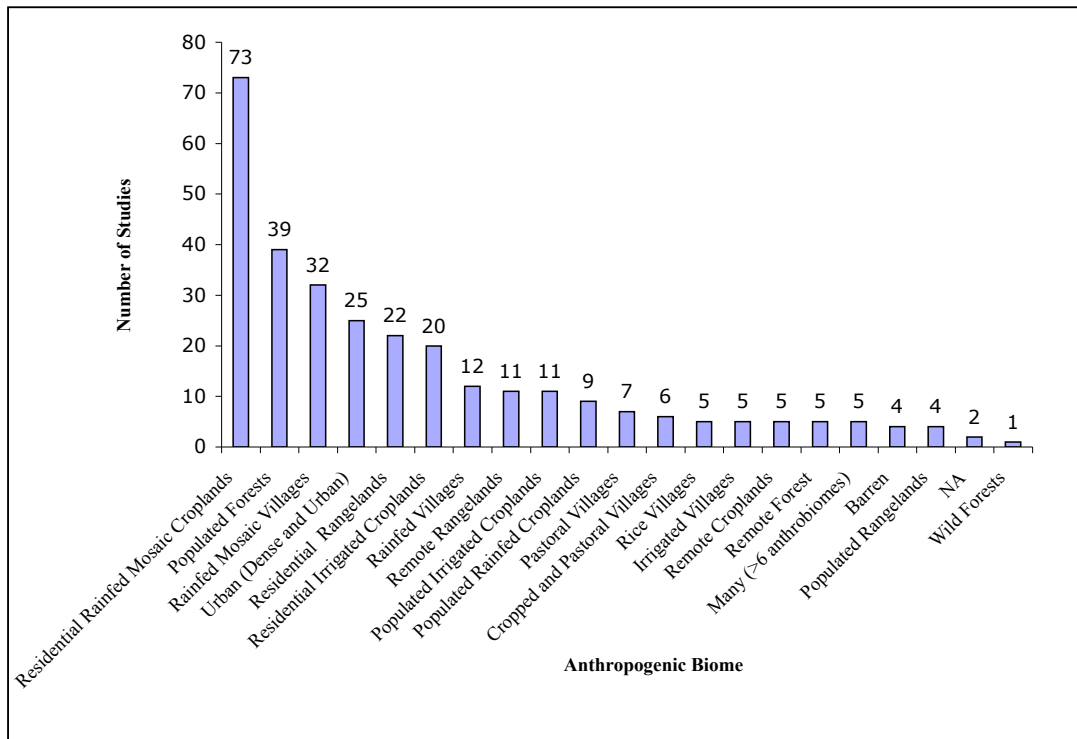


Figure 2.4: Number of scientific publications taking place in respective anthropogenic biomes based on Ellis and Ramankutty (2008).

transmission type (vector-borne, direct, trophic) or pathogen type (e.g. virus, helminth, bacteria) for observational and experimental studies (Fisher's exact test). There was a dependent relationship between pathogen species and deforestation (Fisher's exact test,  $p=.01$ ).

The observational studies took place in all of the anthropogenic biomes described by Ellis and Ramankutty(2008) (Fig 2. 4). Many studies encompassed areas that included more than one anthropogenic biome. Most disease studies focused on disease processes in

croplands (N=85), followed by villages (N=83), urban landscapes (N=27), primarily populated forests (N=37), rangelands (N=29), and few in wild areas (N=5). The three highest ranking anthropogenic biomes in terms of number of observational/experimental disease studies that included them (residential rainfed mosaic croplands, populated forests, and rainfed mosaic villages), were also the top three ranked anthropogenic biomes regarding net primary productivity (gm-2) (640-residential rainfed mosaic croplands, 680-populated forests, and 750-rainfed mosaic villages), respectively (Ellis and Ramankutty, 2008). A marginally significant dependent relationship existed between rangelands and pathogen species (Fisher's exact test,  $p=0.03$ ). There was no evidence of association between any other anthropogenic biome type and transmission type, pathogen type, nor pathogen species (Fisher's exact test  $p > .05$ ).

Anthropogenic land use features such as residential rainfed mosaic croplands, a typical feature of the 'pastoral landscape', appear to be a focal landscape type for infectious disease emergence and transmission. These rainfed croplands often have altered wildlife host communities, with a high diversity and abundance of opportunistic species that can be important disease reservoirs. Heterogeneity present in the mosaic cropland may allow for greater vector survival as well. It is difficult to discern, however, which land use change type is associated with the highest risk of disease emergence. Most studies of urbanization-related disease (90%, 18/20) concerned vector-borne or trophically-transmitted parasites.

There was a weak, yet significant positive relationship between the mean net primary productivity of each anthropogenic biome (data from Ellis and Ramankutty 2008) and the number of observational studies within each anthropogenic biome ( $r^2=.30$ ,  $p=.01$ )(Fig 2.6). This observation may be an artifact of the fact that many people settle in high productivity areas (Ellis and Ramankutty, 2008). Because they tend to be places that are transformed preferentially by humans for agricultural, pastoral, or forestry uses, areas of high net primary productivity may be where most land use change related diseases emerge.



Additionally, areas of high primary productivity may be key sites for parasite/pathogen, reservoir, or vector production, transmission, and diversity.

## 2.4 DRIVERS AND MECHANISMS

### 2.4.1 LAND USE CHANGE DOES NOT ALWAYS RESULT IN INCREASED DISEASE TRANSMISSION

Although the majority of papers demonstrate an increase in disease transmission as a result of anthropogenic disturbance, a handful of studies (N=9) document declines in infectious disease prevalence or transmission with landscape transformation. Over a twenty year period in the Argentinean chaco, a decline in the prevalence of *Trypanosoma cruzi*, agent of Chagas disease, infection in wildlife is attributed to the long term negative effects of deforestation on mammalian reservoir abundance and continued vector control campaigns (Ceballos et al., 2006). In the case of Puumala virus in Belgium, bank voles (primary reservoirs of the disease) in larger forest areas tended to have a higher prevalence than smaller patches in fragmented forest landscapes (Tersago et al., 2008), and remote forest areas exhibit a higher human infection risk (Linard et al., 2007). Long-term spray campaigns and agricultural development have also been associated with declines of visceral leishmania prevalence in Central State, Sudan (Elhassan et al., 1995). Shade-loving simuliid vector declines in a deforested area of Tanzania are also associated with a decrease in onchocerciasis cases in humans (Muro and Raybould, 1990). Complex responses to anthropogenic land use change are observed in the case when rice irrigation was related to an increase in mosquito vector abundance, yet a lower childhood malaria prevalence, presumptively due to zooprophyllactic effect of cattle resulting from changes in mosquito feeding preference from humans to the cow, a non-competent host (Mutero et al., 2004). Shifts in vector host species caused by deforestation has also been associated with decreased onchocerciasis transmission due to an increase in non-anthropophilic simuliid vectors in deforested areas in Uganda (Fischer et al., 1997). Irrigation-related increases in

wetland salinity in Pakistan are believed to indirectly cause a decline in malaria prevalence by causing a shift in the dominant mosquito vector from *Anopheles culifacies*, a highly competent vector of malaria, to *A. stephensi*, believed to be a less competent malaria vector (Klinkenberg et al., 2004). These cases of land use change-related declines in disease transmission are important to recognize, reminding conservation-related scientists, who often assert that anthropogenic change (e.g. biodiversity loss) results in increased pathogen transmission (Dobson et al., 2006a), to remain objective when evaluating how land use change impacts disease transmission.

#### 2.4.2 LAND USE CHANGE AND DISEASE: MANY ASSOCIATIONS, UNKNOWN MECHANISMS

Within this literature, there are an ever-expanding number of experimental and observational studies that associate increased or decreased parasite/pathogen prevalence with anthropogenic land disturbance. Many studies compare disease prevalence or vector abundance in areas pre and post disturbance or areas that have already undergone land use change to relatively undisturbed sites. These studies discuss potential reasons of *why* effects are observed, but do not provide evidence of *how* habitat alteration results in changes in disease transmission (Estrada-Pena, 2006; Gomez et al., 2008; Kawa and Sabroza, 2002). There is a negative relationship between the degree forest fragmentation and nymph infection prevalence with *Borellia burgdorferi*, the Lyme disease agent (Allan et al., 2003) but the actual ecological mechanism that leads to this observation is not fully understood. There is strong evidence that deforestation in the Peruvian Amazon is positively correlated with anopheline mosquito biting rates on humans (Vittor et al., 2006), but why this occurs has not yet been elucidated. There are numerous studies that provide strong evidence of increases in red colobus monkey parasite prevalence and/or abundance in relation to deforestation and forest fragmentation in Uganda (Salzer et al., 2007; Chapman et al., 2005; Gillespie and Chapman, 2008, 2007, 2006; Gillespie et al., 2005; Gillespie and Chapman, 2004). Unfortunately, there is little understanding of why some

parasites tend to be more prevalent in fragmented or disturbed forest habitats, such as alterations in primate spatial structuring and behavior, dietary and nutritional changes that may make animals more susceptible to infection, changes in age structure of primate populations, and immunologic stress-related alterations among individuals.

However, a few studies provide experimental, empirical, or analytical, evidence for mechanisms by which land use change alters infectious disease prevalence or transmission. Most of these studies relate to mosquito disease vectors. A meta-analysis by Yasuoka and Levins (2007) demonstrates that anophelene species exhibiting heliophilia, a factor that may shorten time to maturity in some species of mosquitos, are associated with higher mosquito densities in areas of deforestation and agricultural development. Experimental studies can be particularly useful in understanding mechanisms by which land use can alter disease transmission. For example, Leisnham et al (2006), by comparing larval growth and adult mosquito numbers in forest, pasture, and urban habitat, note that urban and suburban habitats have increased mosquito densities compared to forested sites due to maximum daily and average daily temperatures, likely promoting rapid development and survival (Leisnham et al., 2006).

Another reason for potential malaria vector increase in recently deforested areas is the increase in fallen epiphytes that provide a key habitat for mosquito larval development (Yanoviak et al., 2006). In experimental studies of *An. gambiae* (malaria vector) in Kenya, mosquitos in farmland habitats had a shorter development time and higher pupation rate than mosquitos in forests and natural wetland habitats (Munga et al., 2006). Plant community change causes malaria vector species shifts in Belize wetlands, leading to increase in *A. vestipennis* in a response to growth of the aquatic plant *Typha*, that often grows in areas of high eutrophication from sugar cane expansion (Grieco et al., 2006). Experimental studies show that these mosquito vectors preferentially oviposit in experimental mixtures of low concentration volatiles typically released by *Typha* (Rejmnkov et al., 2005). Understanding and providing evidence as to how land use change

causes disease emergence can be difficult due to indirect effects. In order to advance understanding of the processes by which land use change influences disease transmission, future studies should investigate multiple mechanisms by well-designed experimental and observational studies, and incorporation of statistical and mathematical modeling techniques that test multiple hypotheses of disease emergence in ‘disturbed’ systems. Once mechanisms of how land use change can impact disease transmission are understood, they may be useful for disease prevention or control strategies.

#### 2.4.3 LOSS OF ECOSYSTEM INTEGRITY, COMMUNITY CHANGE, AND PATHOGEN EMERGENCE

As a result of a variety of anthropogenically-driven landscape changes, there are population, community, ecosystem, and landscape alterations that can change patterns of disease transmission and persistence. In many systems, parasites are a component of ecological integrity, and loss of parasite diversity and decline of parasite prevalence, particularly of trophically-transmitted parasites, may indicate a loss of ecosystem integrity. For instance, wetland land restoration actually increases trematode parasite diversity and abundance as compared to the disturbed system (Huspeni and Lafferty, 2004). Host loss might also cause a decline or loss of a particular parasite species due to loss of an intermediate host. For example, red foxes in urban ecosystems of Switzerland display lower prevalence of trophically-transmitted cestodes as compared to rural areas attributed to a decline in a rodent intermediate host in urban areas (Reperant et al., 2007). Changes in host diversity can influence disease transmission. Decreased avian diversity and increased avian infection competence is associated with increased human West Nile Virus prevalence (Allan et al., 2009). On the other hand, increases in the abundance of poor hosts can decrease disease transmission. Experimental studies demonstrate that *Schistosoma mansoni* infection in host snails and cercarial production is lower in the presence of non-host snail species (Johnson et al., 2009).

At the landscape scale, land use change-related outputs from systems external to the one of focus may affect parasite transmission in neighboring or ‘downstream’ ecosystems: e.g. eutrophication in stream, lake, pond, estuarine, reef ecosystems. Converting forest to pasture may impact the abundance and species composition of amphibian parasites because subsequent water quality changes (e.g. eutrophication, higher pH) often enhance resources for intermediate hosts (e.g. snails, mosquitoes) involved in parasite life cycles (Johnson and Chase, 2004; Johnson et al., 2007b). Aquatic amphibians may be particularly prone to experiencing increases in parasitism in agricultural habitats due to the positive relationship between many aquatic intermediate hosts and water quality changes associated with agricultural land use (McKenzie, 2007). Eutrophication experiments in mesocosms demonstrate an increase in snail intermediate hosts for the frog trematode *Ribeiroia ondatrae* and increased parasite infection intensity per snail (Johnson et al., 2007a). Eutrophication also leads to changes in snail host species composition, causing dominance of a species that is the main first intermediate host for *Ribeiroia* (Johnson et al., 2004). Runoff from agricultural and residential areas is believed to contain the multihost pathogen *Toxoplasma gondii* oocysts from wild and domestic felid feces. These oocysts may be taken up by clams in aquatic systems, ingested by otters, and lead to emergence of toxoplasmosis in sea otters (Miller et al., 2002, 2008).

#### 2.4.4 NON-EQUILIBRIUM EFFECTS OF LAND USE CHANGE AND INFECTIOUS DISEASE TRANSMISSION

Anthropogenic land use change is a dynamic process. Plant and animal community structure and food web relationships may be abruptly disrupted following anthropogenic disturbance, and continue to reorganize until a new equilibrium state is reached (Fig 2.7). Infectious disease transmission, vector and/or reservoir abundance, diversity, spatial distribution, and infection risk may vary at different times and spatial extents post disturbance. In frontier Amazonia, there is an increase in malaria transmission during the

initial deforestation-settlement phase, and eventual decline in malaria infection risk as agriculture and infrastructure develops (de Castro et al., 2006). This research emphasizes the importance of long term monitoring strategies post land use change, and awareness of how disease dynamics can change relative to ecosystem state.

#### 2.4.5 SPATIAL AND GEOGRAPHICAL DRIVERS OF DISEASE TRANSMISSION

Spatial and other geographic factors may also contribute to alteration in disease transmission relative to land use change. Increased hantavirus prevalence in rodents in Panama is also associated with flat topography, as where human agricultural overlaps with rodent reservoirs (Suzan et al., 2008). Ecotones may be particularly important areas of spatial overlap of disease reservoirs or facilitate interspecific or inter-population intraspecific contact between hosts and/or vectors (Despommier et al 2006). Agricultural activity at the edge of Ugandan forest fragments incites crop raiding by primates, allowing for increased exchange between human and primate pathogens (Goldberg et al., 2008; Chapman et al., 2006). Suburban development may also increase spatial overlap and contact between deer, potentially facilitating the transmission of chronic wasting disease (Farnsworth et al., 2005). Land use change may also alter landscape architecture in three-dimensional space. This change in architecture can result in increased or decreased vector or host microhabitat or areas of spatial overlap. High human population densities (urbanization, suburbanization) and agricultural activities may provide supplemental resources that allow for increased contact between opportunistic species such as raccoons, thus elevating transmission risk of rabies (Jones et al., 2003; Anthony et al., 1990), macroparasites (Wright and Gompper, 2005), and distemper.

#### 2.4.6 THEORETICAL CONSIDERATIONS: METAPOPOPULATIONS, SOURCES AND SINKS, MULTI-HOST PATHOGENS

Although not addressed in most of the studies reviewed, ecological theory (metapopulation, multi-host pathogen transmission, systems theory) can provide useful hypothesis-testing frameworks for understanding and predicting ways that human land use impact infectious disease transmission and persistence. Land use change may alter spatial relationships between pathogens, hosts, and/or vectors, and metapopulation theory is useful in terms of understanding and predicting how disease may persist in these transformed landscapes (McCallum and Dobson, 2002). Measuring connectivity and subsequent host or vector immigration between habitat patches may be very important in predicting the long-term persistence or fade-out of infectious disease in fragmented or degraded habitats (Hess, 1996; McCallum and Dobson, 2002; Keeling et al., 2003). However, in the literature, there are few empirical studies that explicitly espouse a metapopulation approach to evaluating land use change and disease transmission. Source-sink theoretical approaches to modeling infectious disease may also apply to pathogens, as some host species that maintain and transmit infection can be considered ‘sources’, whereas other hosts that may be infected yet cannot maintain the infection nor transmit the pathogen may be considered ‘sinks’ (Chaves et al., 2007). At another hierarchical level, there may be source and sink habitats in terms of areas where transmission can and cannot be maintained.

Ecosystem changes may also alter the proportion of source and sink habitats of disease transmission. Loss of biodiversity is a common consequence of habitat fragmentation and ecosystem change. Because most pathogens infect more than one host (Woolhouse et al., 2001), theoretical models of multihost pathogen transmission and maintenance apply to predictions of land use change effects on infectious disease. These multihost models evaluate how gains and losses of particular species can lead to increased or decreased transmission (Dobson, 2004). In order to predict how changes in species diversity will affect disease transmission, it is important to identify the dominant transmission type (density or

frequency-dependent), and relative competence of hosts and vectors that maintain and transmit infection (Chaves et al., 2007; Fenton, 2005). For two-host pathogens, understanding relative transmission rates between and within species, the relative susceptibility of hosts, within-host pathogen persistence, and the relative exposure of hosts to disease, is important in order to predict the type of disease event (e.g. spillover versus a true multihost parasite), and to plan disease control strategies, such as culling of the main reservoir host (Fenton, 2005). Lyme disease and West Nile Virus studies support a ‘dilution effect’, because losses of biodiversity such as those that occur with habitat fragmentation lead to an increase in disease prevalence in vectors and/or hosts (Keesing et al., 2006). In cases where there are numerous competent reservoirs, however, it may be difficult to predict if removing or vaccinating a reservoir host will control an infectious disease (Chaves et al., 2007). At even broader scales, ecological theory is useful as a hypothetical framework to test infectious disease effects on populations that may be stressed by ecosystem change. Schmalhausen’s law asserts that organisms existing at environmental boundaries of their tolerance range are highly sensitive to disturbance, such as infectious disease (Chaves et al., 2008). Accordingly, Chaves et al (2008) show that leishmania case rates increase in variability as human populations become socioeconomically marginalized in a landscape undergoing a high rate of deforestation.

#### 2.4.7 PATHOGEN EVOLUTION AND HOST SHIFTS IN A CHANGING LANDSCAPE

Another important, yet virtually unexplored question in theory and practice, is how infectious diseases may evolve in response to anthropogenic landscape transformation (Mouchet and Carnevale, 1997). How rapidly are infectious diseases expected to evolve or switch hosts in novel environments? Will direct or indirect selective pressures on infectious disease caused by land use change lead to an increase in pathogen virulence? Changes in host resource availability (i.e., the parasite’s environment), a likely occurrence in many altered ecosystems, may lead to changes in parasite virulence. Parasite-infected



nutritionally supplemented mosquitoes transmitted parasitic oocysts (parasite offspring) that were more virulent than oocysts of resource deprived mosquitoes (Tseng, 2006). Fragmentation of populations that have a highly structured social relationship where avirulent strains have evolved, may select for an alternate stability of a highly virulent strain (Boots and Bowers, 2004). Land use change may also facilitate conditions of cross-species disease transmission of a multihost parasite, or emergence of a pathogen in a novel host by repeated contacts with a ‘traditional’ host (Dennehy et al., 2006). In Malaysia, agricultural expansion, deforestation, and large-scale swine production facilities adjacent to mango orchards frequented by high densities of fruit bats, the reservoir of Nipah virus, are believed to be a driving factors in the emergence of Nipah virus in pigs and spillover into humans (Field et al., 2007; Epstein et al., 2006; Chua et al., 2002). Empirical research that test theories of how landscape transformation can alter pathogen virulence and host shifts is important to incorporate into disease monitoring strategies in rapidly changing landscapes.

#### 2.4.8 SOCIOECONOMIC AND POLITICAL CONSIDERATIONS

Often ignored by ecological theory, socioeconomic and political factors and other ecological factors, such as climate change, interact with land use change to impact infectious disease transmission and persistence in humans and other animals. Furthermore, when evaluating cases of disease transmission relative to infectious disease transmission or emergence, it is important to pursue a holistic approach by evaluating other environmental and social causes of disease transmission in humans and wildlife, such as climate change, poverty, and behavioral changes. Cases of human leishmaniasis in Teresina, Brazil are associated with areas with breakdown of garbage collection services in underserved urban areas (Costa et al., 2005). As deforested areas of the Amazon frontier become settled, there may be more effective social and political responses to malaria control efforts, leading to an overall decline in cases (de Castro et al., 2006). Linard et al (2007) also evaluate landscape

structure, reservoir host dynamics and socioeconomic factors that affect human infection risk for Puumala virus )(Linard et al., 2007). Urban poverty and race as well as ecological variables are associated with West Nile Virus cases in an urban site in Tennessee (Ozdenerol et al., 2008). El Niño Southern Oscillation (ENSO) climactic variability, poverty (social exclusion) and deforestation interact to affect the risk of American Cutaneous leishmaniasis in Costa Rica (Chaves et al., 2008). In recent years, the re-emergence of Yellow fever in humans and howler monkeys in South America may be influenced by interactions between vaccination breaks and effects of forest fragmentation on monkey densities, mosquito populations, and contact with human populations. Clearly, anthropogenic land use change interacts in complex ways with socioeconomic, cultural, and other large scale environmental factors to affect disease transmission, and holistic approaches are necessary for predicting and preventing disease emergence in these complex landscapes.

This paper reviews evidence that anthropogenic land use change can directly and indirectly influence disease transmission in humans, wildlife, and to a lesser extent, domestic animals. There remains, however, a great deal of uncertainty relative to how anthropogenic disturbances impact the transmission and persistence of infectious disease. Future research programs should espouse ecological theory and principles, ecosystem science, and ecological and evolutionary mechanisms in order to understand, predict, and prevent land use change-related disease emergence.

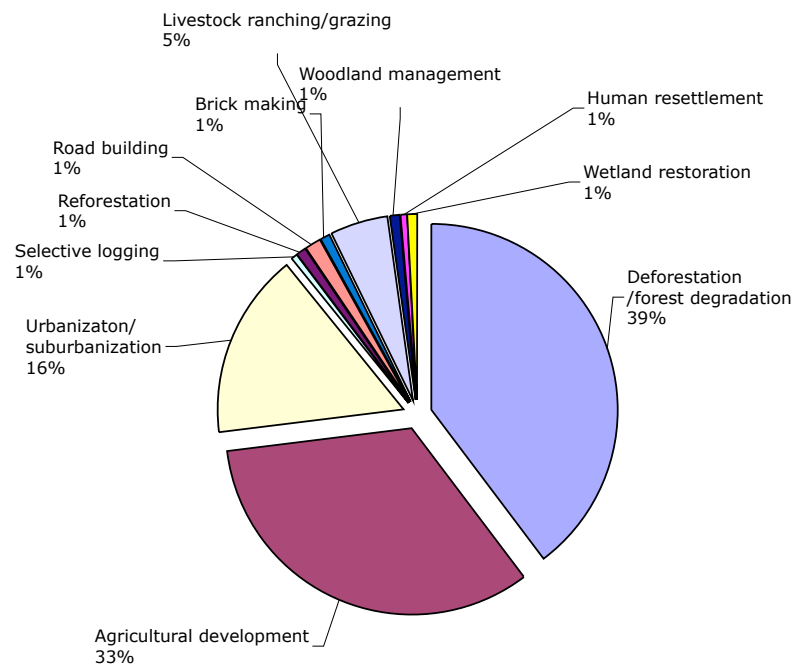


Figure 2.5: Types of land use associated with changes in disease transmission (N=125 studies).

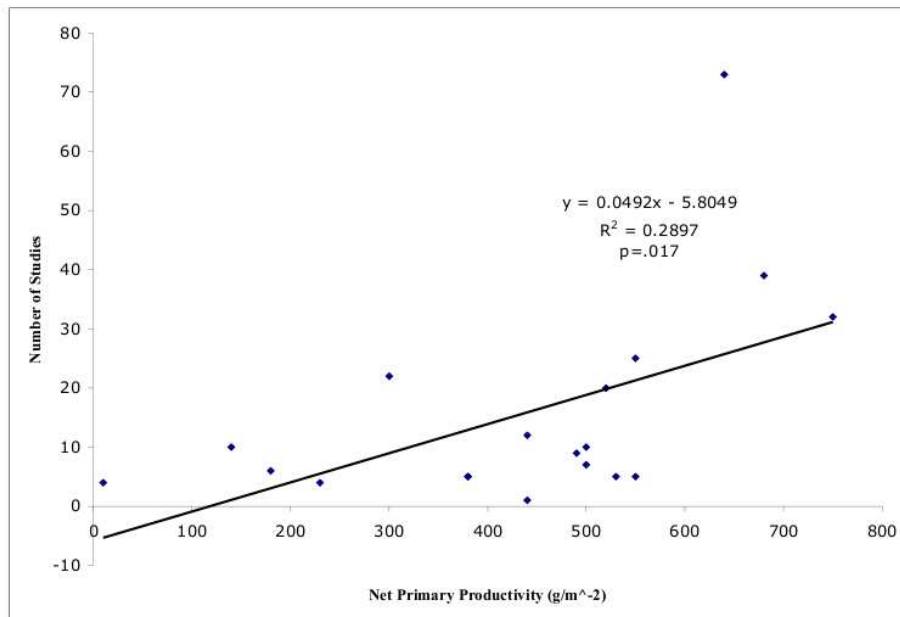


Figure 2.6: Relationship between number of studies and net primary productivity of anthropogenic biomes based on data from Ellis and Ramankutty(2008).

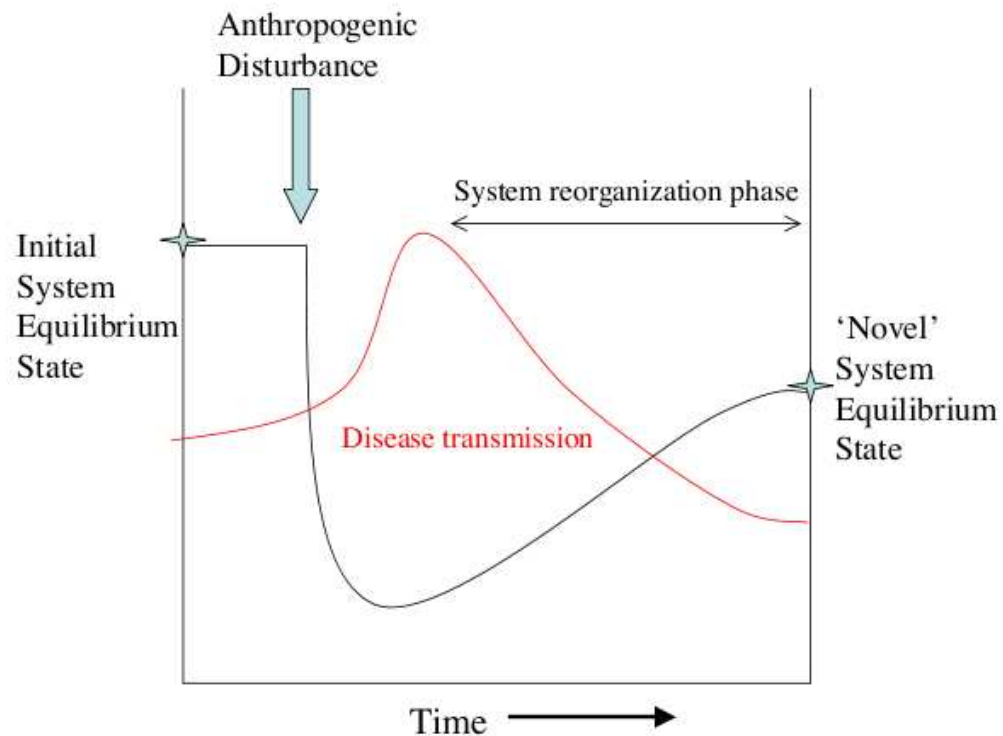


Figure 2.7: Conceptual model for potential relationships between disease transmission and anthropogenic disturbance. Disease transmission may increase shortly after an abrupt change in system state, such as deforestation, as host and/or vector communities change). During a transient reorganizing process, such as ecological succession, further changes in animal and plant community structure and diversity may lead to a decline in disease transmission and prevalence to another enzootic equilibrium (higher or lower than the initial conditions) as the ecosystem attains a novel equilibrium state. Disease prevalence may also decline as a result of human intervention in the 'reorganized' system, such as improved health care, or disease may increase from initial conditions if conditions of health inequities or social marginalization do not improve.

## CHAPTER 3

# ANTHROPOGENIC LAND USE CHANGE IS ASSOCIATED WITH INCREASED ABUNDANCE OF THE CHAGAS DISEASE VECTOR *RHODNIUS PALLESCENS* IN A RURAL LANDSCAPE OF PANAMA

### 3.1 INTRODUCTION

Anthropogenic land use and land cover change (LULCC), manifested by factors such as deforestation, agricultural development, and urbanization, influences the distribution, abundance, and behavior of disease vectors.(Patz et al., 2000; Sutherst, 2004). There is a growing body of evidence that land use change can lead to an increase in vector abundance, and in many cases, increased risk of disease transmission (Walsh et al., 1993; Gratz, 1999; Yasuoka and Levins, 2007). In the northeastern Peruvian Amazon, the malaria vector *Anopheles darlingi* human biting rate in deforested areas near recent road development was much greater than in forested areas (Vittor et al., 2006). Deforestation and agricultural development in the Peruvian Amazon has also been associated with increased mosquito abundance due to an increase in standing water held by plants (e.g. bromeliads and pineapple)(Yanoviak et al., 2006). Many species of anopheline mosquito malaria vectors can increase in abundance in response to increased sunlight availability, a common occurrence in deforested areas. In African highland agricultural sites, increased malarial vector survival rate and juvenile survival rate was observed (Minakawa et al., 2006). Forest fragmentation has been associated with increased tick vector abundance and prevalence of the Lyme disease agent *Borellia burgdorferi* in tick vectors (Allan et al., 2003; Brownstein et al., 2005). Land use disturbance in Israel has been associated with increased

abundance of the cutaneous leishmaniasis vector *Phlebotomus papatasi* and increased infection prevalence of *Leishmania major* in rodent hosts (Wasserberg G, 2003).

In this study, we examine relationships between anthropogenic land use change (deforestation, forest fragmentation, and agricultural development) and population characteristics of the triatomine bug *Rhodnius pallescens* in Panama. *R. pallescens* is considered to be the primary vector for *Trypanosoma cruzi* infections of humans and animals in Panama, some areas of Costa Rica, and Columbia (Whitlaw and Chaniotis, 1978; Christensen and Devasquez, 1981; Zeledon et al., 2001, 2006). *T. cruzi*, the agent of Chagas disease, is a major cause of heart disease and general morbidity among humans in Latin America, with estimates of 8-15 million infected people (Tarleton et al., 2007; Gurtler et al., 2008). It is well-established that palms, in particular the royal palm *Attalea butyraceae*, provide the breeding habitat of *R. pallescens* (Abad-Franch and Monteiro, 2005). The royal palm *A. butyraceae* is ubiquitous throughout lowland tropical forest landscapes in Panama, from intact forests to deforested areas. High densities of *A. butyraceae* within forests are also commonly associated with anthropogenic disturbance, such as hunting of seed predators of these palms and past agricultural activity (Wright and Duber, 2001). The intimate *R. pallescens*-palm relationship is ideal to evaluate how land use may affect vector abundance across a gradient of land use disturbance.

Human settlement and agricultural production throughout the twentieth century has transformed the once highly-forested rural landscape outside of protected forests in the Panama Canal Watershed into a land mosaic dominated by cattle pasture, lesser amounts of cropland, human settlement, and forest fragments (Figure 1) (Condit et al., 2001; Ibanez et al., 2002). This recent deforestation and agricultural development may increase human contact with vector-borne parasites such as the Chagas disease agent *Trypanosoma cruzi* by increasing vector invasion and re-invasion into domiciles from sylvatic foci, *A. butyraceae* palms (Calzada et al., 2006). Specifically, we investigate how *R. pallescens* relative

abundance, age structure, and body condition change in relation to anthropogenic land use and habitat type across a gradient of deforestation in the area of the Panama Canal.

## 3.2 METHODS

### 3.2.1 STUDY AREA, HABITAT TYPES, STUDY SITES

. The study area included deforested rural landscapes and contiguously forested protected areas flanking the Panama Canal (Fig 3.1). This area is classified as lowland tropical moist forest (Holdridge, 1967). Five different habitat types were sampled for *R. pallescens*: contiguous late secondary forest, early secondary forest fragments, mid secondary forest remnants or fragments, cattle pasture, and peridomiciliary areas. Contiguous late secondary forest sites were located in a protected national park adjacent to the Panama Canal. These sites have a known land use history and forest age (approximately 75-100 years old)(Ibanez et al., 2002). Early secondary forest fragments were areas of abandoned pasture or cropland undergoing forest succession. These sites were approximately 5-30 years old, and most trees within the early secondary sites did not exceed 10 meters in height. There was also a predominance of lianas in most of these early secondary forest fragments. Mid secondary forest remnants or fragments were forest patches remaining after large-scale deforestation of late secondary or mature forest. Most of these mid-secondary patches were highly disturbed, as most of the economically valuable adult trees were previously harvested from these sites, and the forest floor of most of these forest patches were heavily trampled by cattle. Peridomiciliary areas consisted of home gardens or yards located within 100 meters of a human dwelling. The gardens and yards surrounding domiciles were highly variable, some with well-manicured lawns and others with tall grass or located near a forest patch. Seven replicate sites were chosen from each of the five habitat types, comprising total of 35 sites were sampled for *R. pallescens* in different habitats along the western and eastern border of the Panama Canal Area (Fig 3.1). Individual sites were at least 200 meters apart from each other, based on an estimated maximum flight distance for *Rhodnius sp.*, with the



majority of sites located greater than 1 km apart from each other. Replicate sites from identical habitat types were located at least 600 m apart from one another. Study sites were spread over an area of approximately 600 km<sup>2</sup>. Palms were sampled once from each site during the wet season, between May 2007 and December 2007, to control for possible effects of season on *R. pallescens* abundance. Sites from multiple habitat types were sampled within each month, and an attempt was made to spread the sampling of different habitat types evenly across the wet season.

### 3.2.2 PALM SAMPLING FOR *R. PALLESCENS*

At each location, a total of five palms were sampled for the relative density of *R. pallescens*; an adult *Attalea butyraceae* and the four nearest accessible adult *A. butyraceae* palms. The initial palm was selected by choosing the nearest palm to a random direction and distance less than 20m from the observer. The height at the top of the crown base, number and ripeness of fruit racemes, and presence of animal (bird and/or mammal nests or resting sites) were also recorded for each palm.

Three baited traps (modified from a method described by Abad-Franch2005) (Noireau et al., 1999; Abad-Franch et al., 2000; Abad-Franch and Monteiro, 2005) were placed within the crown of each palm, left for twenty four hours, and checked for *R. pallescens* the following day. Palm crowns were accessed with a 20 foot ladder or by climbing the palm tree with a rope and harness tree climbing technique modified for palms. The modification of traps included a food and a hydration source that were provided for mice within the traps, and traps were covered with a small sheet of plastic to provide protection from direct sun and rain.

After collecting the baited traps, the palm crowns were searched for 10 minutes for bugs by a skilled individual. The number and stage (first through fifth stage nymphs, adult) of *R. pallescens* was recorded for each tree. We also recorded the sex of adult bugs, weight (mg), length (mm), and nutritional status of bugs (fourth and fifth stage nymphs and adults) by

evaluating abdominal distension and the presence and location of a blood meal within the digestive tract. Bugs were assigned the following body condition scores based on evaluation of degree of abdominal distension and blood meal present in the gut: 1. engorged, 2. good-some abdominal distension blood in fore, mid and hindgut, 3. moderate- no abdominal distension, moderate amount of blood in midgut, hindgut, and rectal ampulla, 4, thin- thin abdomen, starved or small amount of blood present hindgut or rectal ampulla.

### 3.2.3 EVALUATION OF TRAPPING EFFICIENCY

In order to evaluate the efficiency of our trapping method, we performed our described trapping method (mouse-baited traps with direct search) on eight palm trees separated from the sites that were included in the sampling scheme and then chopped down and fully dissected the palm tree and carefully searched for *R. pallescens* that were not caught in the traps. Specifically, we dissected the full base of the palm crown to actively search for bugs. The number of bugs captured by trap and search was then compared to the palm dissection by linear regression analysis.

### 3.2.4 SATELLITE IMAGERY FOR NORMALIZED DIFFERENCE VEGETATION INDEX

A normalized difference vegetation index (NDVI), aspect, and altitude were measured for each site by analysis of 2002 satellite image data of Panama in ArcGIS versions 9.2 and 9.3. A relatively cloud free image of the study area (the best image that could be found) was downloaded from 2002 Landsat 7 satellite imagery from the US Geological Service (USGS), and reflectance data was collected from the image after geometric rectification and atmospheric correction.

### 3.2.5 DATA ANALYSIS

Parametric and non-parametric statistical analyses were performed in R 2.7.1 ( 2004-2008 the R Foundation or Statistical Computing, <http://www.R-project.org>. First, we used

analysis of variance (ANOVA) to compare the number of *R. palleescens* captured in different habitat types. Data were then square root transformed to normalize error variances. Because of the hierarchical nature of the data (bug samples taken from 5 individual palms nested within a site, and site replicates nested within each habitat type), multilevel linear mixed effects models were also used to analyze bug abundance and age using the lmer4 package for R (Bates 2007). To test the effect of habitat type on bug abundance, we used a Laird-Ware formulation of a multilevel linear mixed effects (LME) model with a fixed effect (Habitat), two nested levels of random effects (individual palm nested within site), and quasipoisson errors. The Laplace approximation formula was used to fit the model. Habitat type was considered a fixed effect in this model, because it was an overall invariant classification scheme. Linear mixed models with quasipoisson errors were also used to compare the number of bugs captured within each age class between habitat types (fixed effects=habitat type, random effects= site).

NDVI was calculated from the acquired satellite image reflectance data using Erdas Imagine 9.0 software. Higher NDVI values often represent increase in density of leafy green vegetation, making it suitable measurement for forest cover and/or plant growth (<http://earthobservatory.nasa.gov/Features/MeasuringVegetation>). The resolution used for calculating NDVI for each site was 90m.

A Mantel test was used to investigate relationships between distance between sites and bug abundance.

### 3.3 RESULTS

#### 3.3.1 TRAP EFFICIENCY

There was a significant positive linear relationship between the number of bugs caught with the trap/direct search method and the total number of bugs caught by complete tree dissection with the trap/direct search method (N=8 palms, adjusted  $r^2 = 0.461$ ,  $p=.0383$ ).

There was also a positive, yet non-significant linear relationship between the number of

bugs caught by trap-direct search and the number of bugs caught during tree dissection. Both findings indicate that trapping methodology used in this study was an adequate representation of relative bug abundance. On average, 28% (range=0-43%) of the total number of bugs found were caught with the baited traps only.

### 3.3.2 PALM INFESTATION WITH *R. pallescens*

Across all habitat types, we recovered *R. pallescens* from 80.0% of palms (140/175). The percentage of palms infested with *R. pallescens* was high throughout anthropogenically disturbed habitats, ranging from 77.1% in peridomiciliary palms to 91.4% in mid secondary forest fragments, with the lowest proportion of infested palms in contiguous forests (57.1%) (Table 3.1). There was significant dependence between habitat type and the number of palms in which *R. pallescens* was present (Pearson's chi-squared test,  $\chi^2 = 16.786$ ,  $df = 4$ ,  $p = 0.002$ ). The proportion of palms infested with *R. pallescens* in mid-secondary and early secondary fragmented forests combined (N=70 palms examined) was significantly greater than the number of palms infested with *R. pallescens* in contiguous forests (N=35 palms examined) (2 sample test for equality of proportions with continuity corrections,  $\chi^2 = 11.698$ ,  $df = 1$ ,  $p = 0.0006$ ).

### 3.3.3 *R. pallescens* RELATIVE ABUNDANCE AND DISTRIBUTION

The mean number of bugs captured per site differed significantly between habitat types (F=3.9349,  $df=4$ ,  $p=.011$ ) (Fig 3.3). The mean number of bugs captured in mid-secondary and early secondary forest fragments was significantly higher than the number of bugs captured in contiguous forests (Tukeys test, contiguous forest < early secondary forest fragments < mid secondary forest fragments,  $0.016 < p < .023$ ).

A linear mixed effects model (Table 3.2) applied to the data (poisson errors) for the relationship between bug abundance and habitat type demonstrates significant differences between the number of bugs captured in contiguous forests as compared to early secondary

forest fragments (fixed effects,  $p < 0.001$ ), mid secondary forest fragments (fixed effects,  $p < 0.001$ ) and cattle pasture (fixed effects,  $p = 0.001$ ). There was a slight negative, yet significant ( $p = 0.022$ ) relationship between NDVI and estimated bug abundance via the linear mixed effects model (Table 3.3). There were no significant relationships between slope aspect, altitude, and bug abundance across sites

There was no significant spatial autocorrelation in bug abundance between collection sites ( $n = 35$  sites, Mantel test,  $p = 0.21$ ).

### 3.3.4 NYMPH/ADULT RATIO

In all habitat types, the frequency of nymphs was greater than that of adults (Pearson's  $\chi^2 = 10.4291$ ,  $df = 4$ ,  $p\text{-value} = 0.03$ ). However, there was no apparent significant difference between the nymph/adult ratio and habitat type (fixed effects, minimum  $p = 0.30$ ). The colonization index (number of palms with nymphs/number of palms with *R. pallescens*) was high throughout all habitat types ranging from .90 in contiguous forests to 1.00 in cattle pastures.

### 3.3.5 SEX RATIO OF ADULT BUGS

Overall, there was no significant difference in the relative number of male and female bugs captured across all habitat types ( $\chi^2 = 0.280$ ,  $df = 1$ ,  $p = 0.60$ ). There was no evidence of dependence between *R. pallescens* sex ratio and habitat type ( $\chi^2 = 5.27$ ,  $df = 4$ ,  $p = 0.26$ ).

### 3.3.6 AGE CLASS STRUCTURE OF CAPTURED BUGS.

There was significant dependence between the the number of bugs captured in each age class and habitat type (Pearson's  $\chi^2 = 59$ ,  $df = 20$ ,  $p = 1.008 \times 10^{-5}$ ).

Figure 3.4 summarizes the habitat specific age structure data. Early secondary and mid secondary forest fragments had significantly greater estimated mean numbers of adult bugs (significant result interpreted as a  $t$  value  $> 2.0$ ) in early secondary forest fragments ( $t =$

Table 3.1: *Rhodnius pallescens* palm infestation rates, abundance, density, and population characteristics

Variable	Contiguous	Peridomicile	Early Secondary	Mid Secondary	Pasture	Total
%Positive <sup>a</sup>	57.1% (20/35)	77.1% (27/35)	85.7% (30/35)	91.4% (32/35)	88.6% (31/35)	80% (140/175)
Total No. of Bugs Captured/site ( $\pm 1SE$ )	72 8.3 $\pm$ 5.9	201 28.7 $\pm$ 27.3	299 42.7 $\pm$ 20.1	346 49.4 $\pm$ 40.1	267 38.1 $\pm$ 24.4	1186 33.4 $\pm$ 28.4
Overall density per habitat type <sup>b</sup>	1.66 5.74	8.54	9.86	7.63	6.78	
Mean density per palm <sup>c</sup>	3.05 $\pm$ 2.80 (N=20)	7.44 $\pm$ 9.63 (N=27)	9.97 $\pm$ 8.65 (N=30)	10.81 $\pm$ 12.55 (N=32)	8.61 $\pm$ 8.25 (N=31)	8.47 $\pm$ 9.49 (N=140)
No. of bugs/ trap night <sup>d</sup>	.56 (104) <sup>e</sup>	1.91 (105)	2.85 (105)	3.29 (105)	2.53 (105)	2.3 (524)
Total Adults captured	12	48	65	68	35	228
Total Nymphs captured	46	153	234	277	232	942
Total Nymph/Adult Ratio	3.83	3.19	3.62	4.07	6.63	4.12
Colonization Index (No. Palms with Nymphs/No. palms with Triatomines)	.90(17/19)	.93(25/27)	.90(27/30)	.94(30/32)	1.00(31/31)	.94(130/139)
Weight/length ratio (mg/mm) of N4,N5 and adults	5.10 $\pm$ 2.32	2.77 $\pm$ .42	3.91 $\pm$ 2.24	3.64 $\pm$ 2.45	4.08 $\pm$ 1.56	3.97 $\pm$ 2.2

a b c d e

<sup>a</sup>% = (number of palms with bugs/total number of palms examined)<sup>b</sup>overall density/habitat type= number of bugs captured/number of bugs sampled)<sup>c</sup>Mean density/palm=No. of bugs captured/ infested palm<sup>d</sup>calculation of trap nights incuded 50 minutes of direct searching/palm<sup>e</sup>1 trap predated by a mammal

Table 3.2: Results of general linear mixed model evaluating relationship between the number of *R. pallescens* captured and habitat type. Habitat=fixed effect, site nested within individual palm=random effects, family=poisson(loglink), fit using Laplace

Effects	Estimate	Standard Error	z-value	p value
Fixed Effects				
Early secondary fragment	1.5739	0.4553	3.46	0.00055 ***
Mid secondary remnant	1.6244	0.4550	3.57	0.00036 ***
Cattle Pasture	1.4639	0.4557	3.21	0.00132 **
Peridomicile	0.8632	0.4635	1.86	0.06257
Random effects				
	Variance	St. Deviation		
Palm.Number:Site	1.050	1.02		
AIC=580	BIC=540	log likelihood=-252	deviance=504	

Table 3.3: Results of general linear mixed model examining the relationship between normalized differential vegetation index (NDVI) and the total number of *R. pallescens* captured per site (175 palms sampled across 35 sites).

Effects	Estimate	Standard Error	z-value	p
Fixed Effects				
Intercept	2.43760	0.56292	4.33	1.5x10-5 ***
ndvi	-0.00777	0.00340	-2.28	0.022*
Random effects				
	Variance	St. Deviation		
Palm.Number:Site (Intercept)	1.63	1.28		
AIC=549	BIC=559	log likelihood=-272	deviance=543	

2.15), and mid-secondary fragments, ( $t = 2.06$ ) as compared to contiguous forests. The number of fifth stage nymphs captured in mid secondary forests was significantly greater than the numbers of bug captured in contiguous forests ( $t = 2.254$ ). The estimated mean number of fourth stage nymphs captured throughout anthropogenic landscapes was significantly greater in mid secondary ( $t = 3.02$ ), early secondary forest fragments ( $t=2.67$ ), pastures ( $t=2.1$ ), and peridomiciliary areas ( $t=2.67$ ) was significantly greater than bug captures in contiguous forests. The estimated mean number of third stage nymphs captured in mid-secondary fragments ( $t=2.23$ ), early secondary fragments ( $t=2.20$ ), and pasture ( $t=2.13$ ) was significantly greater than in contiguous forests. The mean number of first and second stage nymphs captured did not differ significantly across habitat types.

### 3.3.7 BODY CONDITION

There was a dependent relationship between habitat type and qualitative measurements of body condition (Fisher's exact test,  $p=1 \times 10^{-5}$ ). The mean weight/length ratio of adult and nymph (fourth to fifth stage nymphs) *R. pallescens* captured in continuous forests was, on average, higher than bugs captured in fragmented and deforested sites (Table 3.1 ). However, there was no significant difference between habitat types and weight/length ratio of adults and nymphs combined (Kruskal-Wallis test  $H=8.2, df=4, p=.08$ ), adults (Kruskal-Wallis  $H = 8.2, df = 4, p\text{-value} = 0.08$ ), and nymphs (Kruskal-Wallis  $H = 8.0, df = 4, p\text{-value} = 0.09$ ). Qualitative analysis of bugs does suggest that there were more fed bugs in contiguous forest sites as compared to human-dominated landscapes, as approximately 60% of bugs in contiguous forests were in good condition to engorged (Figure 3.5 ) There was no significant difference between the mean weight/length ratio measurements between males ( $4.78 \pm 1.54, N=87$ ) and females. ( $4.9 \pm 1.54, N=94$ ). However, the mean weight/length ratio of females (mean female weight-length ratio= $4.9 \pm 1.54, N=94$  examined) was slightly greater than that of males).



### 3.4 DISCUSSION

It is well established that *Attalea butyraceae* palms are the primary ecotope for *Rhodnius pallezens* throughout their range in Central America and Northern South America (Whitlaw and Chaniotis, 1978; Abad-Franch et al., 2008). However, less is understood regarding how *R. pallezens* abundance varies relative to land use/anthropogenic disturbance surrounding their palm microhabitats. We present evidence that anthropogenic activity, namely forest fragmentation and conversion of land into cattle pasture, increases presence and abundance of *Rhodnius pallezens* in *Attalea butyraceae* palms.

The percentage of palms infested with *Rhodnius pallezens* within and among each habitat type was relatively high (range 57.1%-91.4%) compared to other studies of *Rhodnius* palm infestations (Bar and Wisnivesky-Colli, 2001). In Ecuador, *Rhodnius ecuatoriensis* infestation of tagua palms *Phytelephas aequatorialis* was 23% of 110 palms examined. Similar to this study, there was a higher percentage of *Rhodnius ecuatoriensis* palm infestation of tagua palms *Phytelephas aequatorialis* from deforested landscapes in Ecuador. Unlike the present study, where there was a very high palm infestation with *R. pallezens* in forest fragments, there was no evidence of infestation of tagua palms by *R. ecuatoriensis* in forest remnants in Ecuador (Abad-Franch and Monteiro, 2005). *Rhodnius neglectus* infestation rates in *Mauritia* palms in Brazil ranged between 18.6% (23) and 37% (Gurgel-Goncalves et al., 2003). In a previous study of *R. pallezens* infestation of *Attalea butyraceae* palms (N=30) examined from an urban and rural area of Panama, the proportion of infested palms from urban, rural, and forest areas were 100%, 80% and 90%, respectively (Jabin, 2001). Similar to the present study, high palm infestation rates with *Triatoma sordida* (96.2%) were observed in Northeastern Argentina (Bar and Wisnivesky-Colli, 2001). Factors associated with increased triatomine abundance and infestation in palm trees include a large amount of decaying organic matter and vegetative growth at the base of the palm crown and presence of animal nests in the palm such as

bird nests Christensen and Devasquez (1981); D'Alessandro et al. (1984); Bar and Wisnivesky-Colli (2001); Abad-Franch and Monteiro (2005).

In the present study, habitat type and a related measure of forest/vegetation cover, the normalized difference vegetation index(ndvi), influences *R. pallescens* abundance in palm trees, as there was a significantly greater number of bugs captured in anthropogenically-dominated habitats compared to contiguous late secondary forests. Additionally, there was a negative relationship between NDVI and vector abundance (Table 3.3), with the highest NDVI values located in contiguous forest sites. Similarly, in studies of *Rhodnius neglectus* in the palm *Mauritia flexuosa*, there was increased abundance of bugs in palm trees in rural areas versus sylvatic environments (Gurgel-Goncalves et al., 2004). In fragmented and deforested landscapes, the highest *R. pallescens* abundance was in mid-secondary and early-secondary forest fragments, with lower bug abundances in cattle pasture and peridomiciliary areas. The lower mean abundance in peridomiciliary sites may be caused by a greater range of total bug abundance per site due to the diverse nature of the peridomiciliary sites. There was also a variation in the way that each domiciliary site was maintained (well-manicured lawns, adjacent to cattle pasture, and some sites near forest patches) and in the quality of houses and presence of domestic animals (from a few dogs and chickens to a large number of domestic fowl and dogs) surrounding homes in peridomiciliary areas.

According to the data, it is evident that forest fragments support a relatively high abundance of *Rhodnius pallescens*. Mid-secondary forest fragments are scattered throughout the pastoral landscapes in the Panama canal area. These forest patches are primarily riparian remnants surrounded by a matrix of cattle pasture, agriculture, rural dwellings, or dwellings at the suburban/rural interface. Early secondary forest fragments originate from regenerating pasture, but may have some large woody tree species and high densities of palms. Forest fragments may provide hiding places from potential predators, water resources, and protection from temperature extremes. Additionally, fruiting trees,

such as palms, within these forest patches may provide important nutritional sources for many vertebrate species. Forest fragments may serve as key resource patches for insectivorous birds and mammals (Terborgh et al., 2001). Forest species that can live in edge habitats can invade fragments, thus increasing local population densities of animals in forest fragments (Gascon et al., 1999).

Indeed, forest fragments often support relatively high mammal densities due to alterations of trophic structure via mesopredator release (an increased in medium-sized generalist omnivores in the deforested matrix and forest fragments in part due to loss of top predators)(Goodrich and Buskirk, 1995), and supplemental resources (crops, human food waste) provided by human activities within the fragmented landscape. Some animals that respond positively to forest fragmentation may not be commonly hunted for food, and can maintain populations in deforested landscapes. Forest fragments may also be used as an additional refuge for animals that may be dispersing or moving in an agricultural matrix, as well as for permanent residents of these fragments (Laurance et al., 2008). Palm trees within forest fragments may also provide key hiding and nesting sites for arboreal mammals and other animals. Therefore, vertebrate habitat and increased vertebrate palm occupancy in forest fragments can provide a relatively stable and abundant food resource for *Rhodnius pallescens*, leading to the relatively high abundance of *R. pallescens* in forest fragments. Environmental effects, such as altered microclimate, may also lead to higher numbers of *R. pallescens* inhabiting forest fragments and pasture. In tropical forest fragments, edge effects such as lowered humidity can extend 100 m into the fragment (Laurance et al., 2008), influencing the abundance of *R. pallescens* that may benefit from the lower humidity versus excess humidity. High humidity is associated with increased mortality of *Rhodnius prolixus* due to the fungal pathogen *Beauveria bassania* (Luz and Fargues, 1999; Fargues and Luz, 2000). Excess humidity, present in contiguous forest environments, may increase mortality and lower vector abundance.

Additionally, food web structure disruption (potential loss of natural enemies, predators and pathogens of *R. pallelescens*) may support a higher vector abundance in deforested and fragmented landscapes. It is also possible that in there is a loss of ecological trophic complexity in deforested landscapes that lead to a loss of an intact suite of predators, pathogens, and specialized parasitoids from the ecosystem (Teixeira et al., 2001a). There is currently no data available on the response of parasitoids and pathogens of *Rhodnius pallelescens* in response to ecological disturbance, but it is an important avenue for future investigation.

Mid-secondary forest fragments exhibit a greater abundance of fourth stage, fifth stage, and adult nymphs as compared to contiguous forests, and adults, fourth, and third stage nymph abundances are greater in early secondary fragments as compared to contiguous forests. Mid (N3) and late-stage (N4, N5) nymphal survival and adult survivorship is relatively high in forest fragments. implying that they may serve as a population source for surrounding deforested areas, perhaps due to vertebrate blood meal colonization or habitual occupancy in a particular palm within fragments. Compared with other habitat types, the nymph/adult ratio is lowest in cattle pastures. This observation could be due to emigration of adults from pastures, as there are less hosts to feed from in space and time, with adults emigrating from pastures at a high rate to areas with a more constant or reliable food supply or lowered adult survivorship in pastures. The high colonization index (number of palms with nymphs/number of palms with *R. pallelescens* present) is the highest of all habitat types also implies that many adult bugs may reproduce in pastures, yet do not reside for an extended period of time within them, possibly due to higher mortality of adults in cattle pasture (sink habitat), or emigration to higher quality sites (forest fragments, peridomicile). Cattle pastures may thus act as population sinks for *R. pallelescens* at a landscape scale. Alternatively, bugs may use palms in cattle pasture as stopover sites during migration to more suitable habitat patches.

Although it was not statistically significant, *R. palleescens* in contiguous forests tended to be in better physical condition as compared to bugs in fragmented landscapes. Because starved triatomine adults can have a higher dispersal probability or potential than fed bugs (Ceballos et al., 2005), dispersal rates may be higher in fragmented landscapes with hungry adults, and palms with a larger proportion of fed bugs in deforested areas may have a higher probability of entering human dwellings in search of a blood meal.

Study results suggest that anthropogenic landscapes lead to a higher abundance of *R. palleescens*, and that forest fragments may be sources for *R. palleescens* at a landscape scale. It is unknown if these habitat-related effects vary seasonally. Season, climate, and relative humidity can influence dispersal of triatomines (Vazquez-Prokopec et al., 2006). However, samples were only collected during the wet season. It is possible that *R. palleescens* populations decline during the dry season in deforested habitats due to excess sunlight, temperature extremes, and low humidity. In our study, differences in microhabitats (temperature and humidity) within the palm crown were not measured. This study does also does not capture long-term trends in bug abundance, body condition, and age structure that may be caused by climate-related (e.g. El Niño effects) fluctuations in host population abundance. Age structure data is useful for modeling population dynamics of *R. palleescens* at a local and landscape scale. Unfortunately, eggs were not searched for in the *Attalea* palms in this study.

Palm trees are considered to be a risk factor for Chagas disease in neotropical landscapes (Romana et al., 1999; Teixeira et al., 2001a; Abad-Franch et al., 2008). Deforestation is also hypothesized to increase contact between Chagas disease vectors and humans (Romana et al., 1999; Abad-Franch et al., 2008). Despite a higher abundance and evidence that forest fragments may be a local source for *R. palleescens*, removing *Attalea* trees in forest fragments is not a recommended strategy. Cutting palm trees in forest remnants could potentially change the focus blood meal sources to peridomiciliary areas as wild mammals may shift their usage of palms to peridomiciliary or pasture areas. Removal of wild host

species in anthropogenically dominated landscapes is also not a recommended strategy either, as it may cause a shift in bug feeding preferences, human domiciliary invasion, and increase in Chagas disease risk to domestic animals and humans. It is important to emphasize that a higher abundance of *R. pallescens* in a habitat does not necessarily translate to human Chagas disease risk, because socioeconomic and human behavioral factors are important to consider when evaluating disease risk to humans, as emphasized by studies of leishmaniasis in Costa Rica (Chaves et al., 2008) and lyme disease incidence in relation to forest fragmentation in Connecticut (Brownstein et al., 2005).

There is a need for further long term surveillance of *R. pallescens* populations inhabiting a variation of habitat/landscape types in deforested and forested areas in Panama.

Understanding how long term variation in climate and rainfall interacts with habitat associated abundance is very important to being able to predict and identify further localized hotspots of bug abundance and potential *T. cruzi* transmission. Because of seasonal variations, global warming trends, and continued anthropogenic forest destruction, long-term monitoring of different habitat types, at a landscape scale, for triatomine vectors is needed throughout their range. This data will be important to prediction of the potential infestation of human communities and reinfestation of human dwellings from sites adjacent to areas of triatomine control programs. Such long-term integrated monitoring and control strategy has been employed for *Triatoma infestans* populations in some rural areas of the Argentinean Chaco, and it has proven to be an effective strategy for reduction of Chagas disease vectors and risk to local inhabitants (Cardinal et al., 2008).

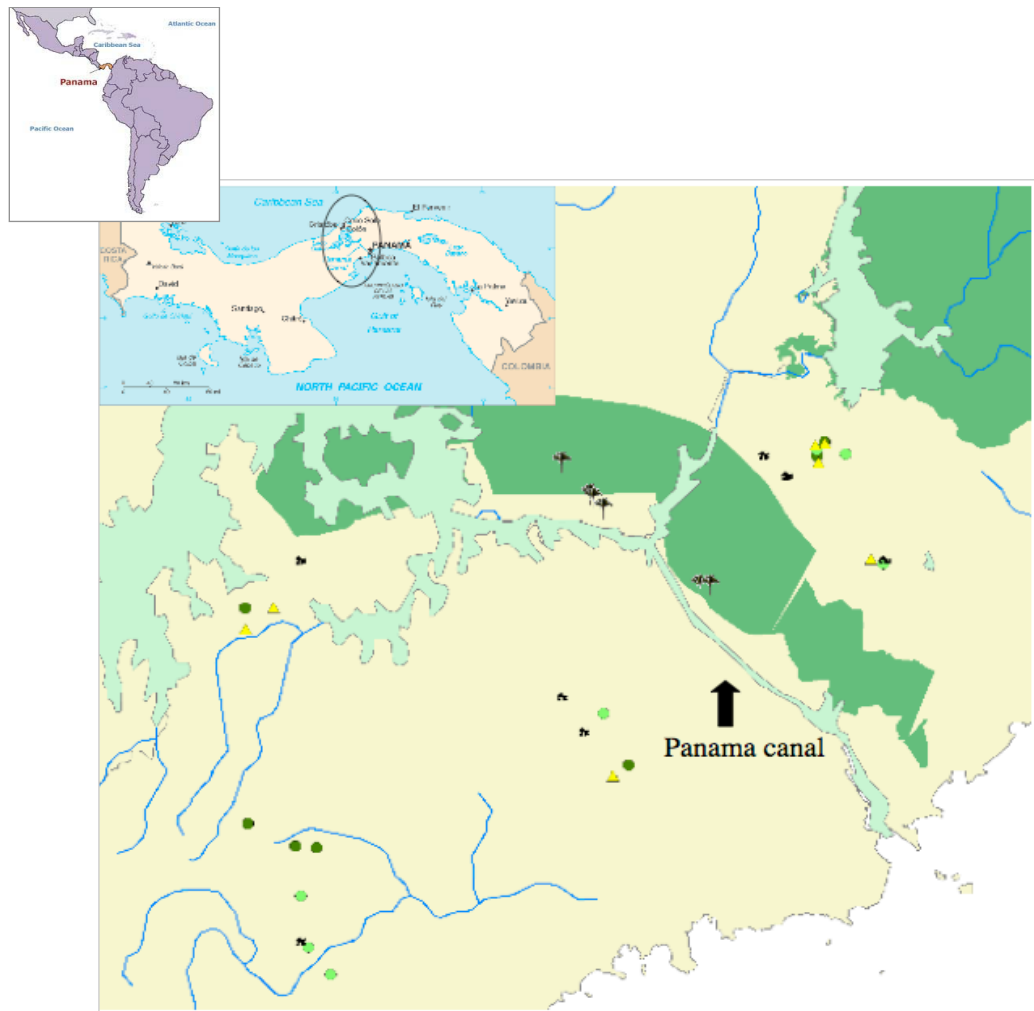


Figure 3.1: Area of the Panama canal. Approximate location of study sites and habitat type shown by the following icons: black palm tree- late secondary contiguous forest, dark green circle- mid secondary forest remnant, light green circle- early secondary forest fragment, yellow triangle- cattle pasture, black house- peridomiciliary area.



Figure 3.2: Field methods. Capture of *R. pallescens* in palms (left). Adult and nymphs of *R. pallescens* captured with a baited sticky trap (right).



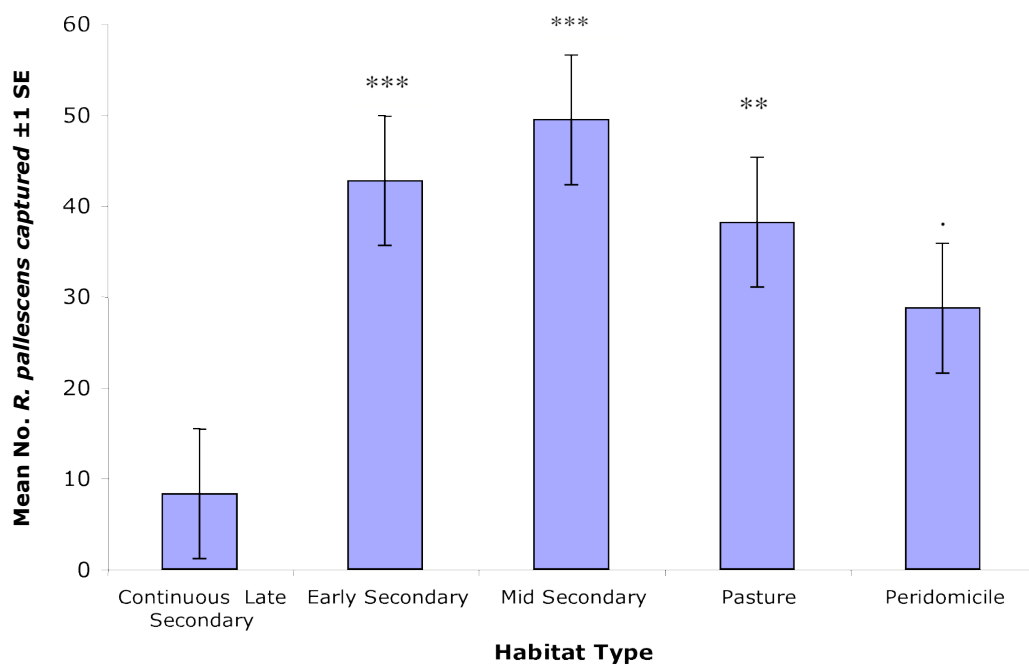


Figure 3.3: Mean number of *Rhodnius pallescens* captured per site across different habitat types (N=7 sites per habitat type) in the Panama Canal Area (Significance codes: \*\*\*0.001 \*\*0.01 \*0.05 ·0.1)

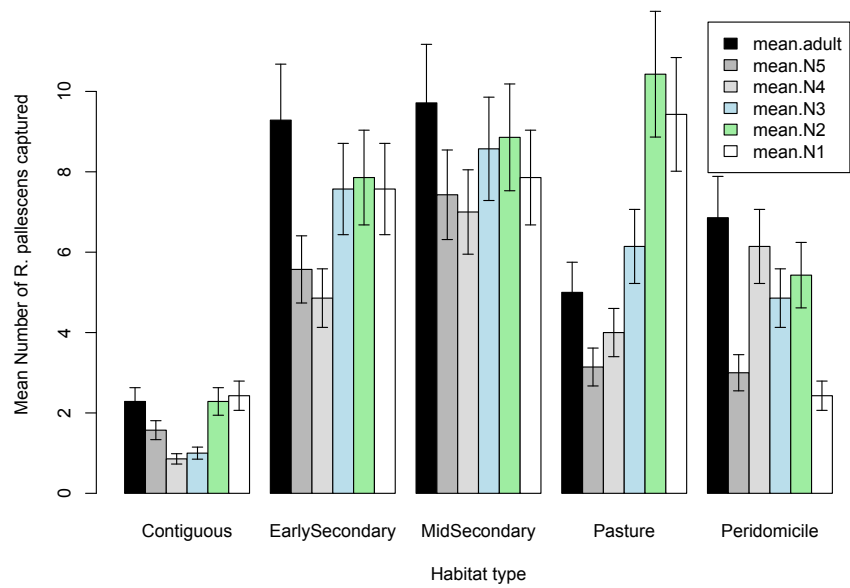


Figure 3.4: Age class structure of *R. pallescens* captured across different habitat types (Error bars: 95% confidence intervals estimate by bootstrapping)

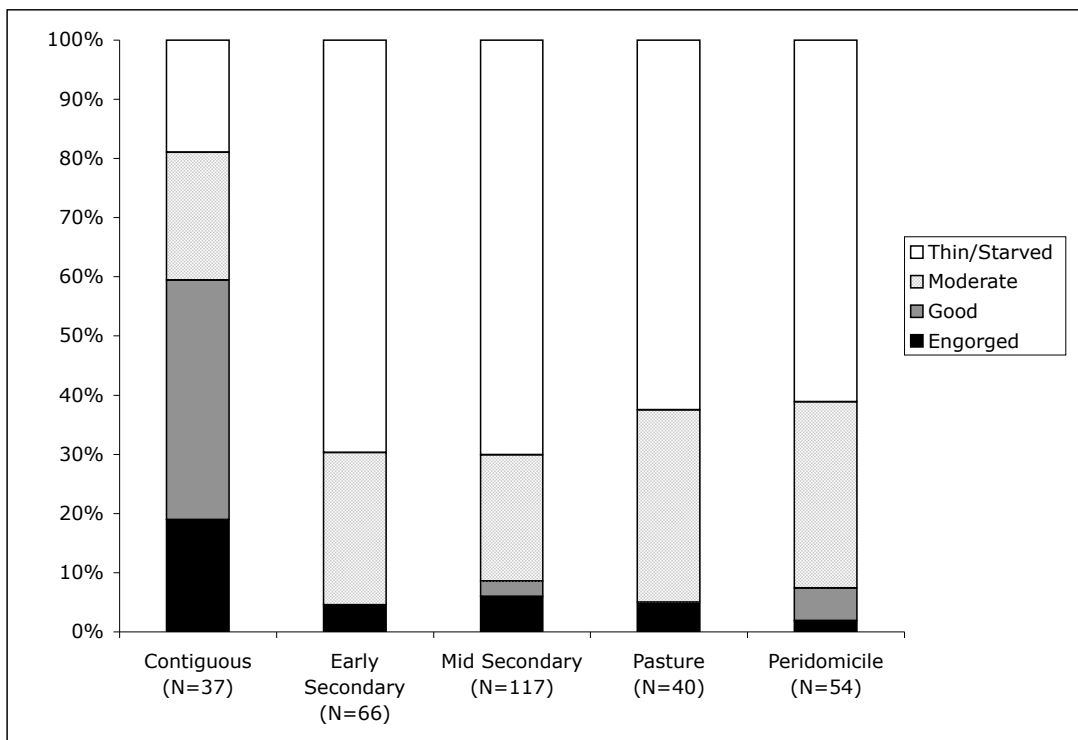


Figure 3.5: Qualitative body condition indices of *R. pallescens* captured in different habitat types.

## CHAPTER 4

### BEYOND THE DILUTION EFFECT: EVALUATING DRIVERS OF *TRYPANOSOMA CRUZI* TRANSMISSION IN A DEFORESTED LANDSCAPE

#### 4.1 INTRODUCTION

The majority of infectious diseases in plants and animals are transmitted between multiple hosts (Woolhouse 2001). Anthropogenic land use change (e.g. deforestation, forest fragmentation, agricultural development, urbanization) has the potential to increase or decrease transmission of multihost pathogens by a variety of potential drivers, such as biodiversity loss and change in host community composition. Biodiversity loss has been linked to an increase in transmission and prevalence of infectious diseases in humans, domestic animals, and wildlife. The ‘dilution effect’ hypothesis (Keesing et al., 2006) states that there is an inverse relationship between species diversity and infectious disease risk. In theory, the ‘dilution effect’ is supported under the following conditions: variation in the competence between different host species to transmit an infectious agent with greater within than between species disease transmission, dominance of the most competent reservoir in cases of species diversity loss, frequency-dependent transmission (e.g. vector-borne disease), or density dependent transmission where an adding an additional host species decreases numbers of the primary reservoir host (Keesing et al., 2006; Rudolf and Antonovics, 2005; Dobson, 2004; Holt et al., 2003; Holt and Pickering, 1985). Other mechanisms by which adding species decreases disease transmission include diminishing encounters between a key host species and/or a vector, decreased efficiency of transmission with an additional host, increase in mortality of susceptible hosts, increased recovery rates of infected hosts, and decline in the number of susceptible hosts (Keesing et al., 2006).

Diversity disease relationships can also be difficult to predict in cases where host competence is similar or unknown (Chaves and Pascual, 2007; Dobson, 2004). In Ireland, where bank voles were introduced into communities with wood mice, the main reservoir for the tick-borne pathogen *Bartonella*, infection prevalence in wood mice declined as densities of bank voles increased, supporting a dilution effect (Telfer et al., 2005; Schmidt and Ostfeld, 2001).

Evidence supporting the ‘dilution’ effect in natural systems is scarce. Empirically based models support the hypothesis that tick nymph infection prevalence with the Lyme disease agent *Borellia burgdorferi* decreases with increasing host diversity (Schmidt and Ostfeld, 2001). Epidemics of a multihost fungal pathogen in *Daphnia sp.* communities were less pronounced in years when less susceptible hosts dominated these communities (Hall et al., 2009). West Nile Virus prevalence in human spillover hosts and mosquito vectors declined as avian diversity increased, and was higher as avian community reservoir competence increased (Allan et al., 2009). Furthermore, the composition of host communities may be important in evaluating diversity disease relationships in anthropogenically altered landscapes. For instance, mosquito and human West Nile Virus infection prevalence declined with an increase in non-passerine species richness, but not passerine species richness (Ezenwa et al., 2006).

Alterations in host biodiversity or community structure are not the only drivers influencing transmission and persistence of multihost pathogens. Many pathogens coexist with a variety of different pathogen strains and species. In ‘natural’ (non-experimental) systems, coinfection of hosts and/or vectors with other multihost pathogens is very common (Graham, 2008), and these coinfections can interact with and affect parasite transmission and virulence (Seabloom et al., 2009; Rohani et al., 2003). In some cases, host coinfection with two different pathogens can facilitate parasitemia or within host pathogenicity, which may increase disease transmission, as in the case of coinfections of HIV and other parasites. Experimental coinfections of mice with an arthritogenic and non-arthritogenic *Borellia*

strain develop a more severe arthritis and increased numbers of the arthritogenic *B. burgorferi* strain as compared to single infections with the arthritogenic strain (Hovius et al., 2007). In other cases, there may be protective cross-immunity between coinfecting species, which may decrease transmission of a second parasite species. Acute infection with *Toxoplasma gondii* inhibits severe lesion development on coinfection with *Leishmania major*, due to an overwhelming Th1 response and prevention of Th2 immune response commonly associated with leishmania pathogenicity (Santiago et al., 1999). Generalist vectors can facilitate pathogen coinfection within and among host species (Seabloom et al., 2009).

In addition to within and between host factors influencing coinfection, environmental change (climate, habitat change) also has the potential to influence patterns of pathogen coinfection in hosts and vectors. If there is a competitive relationship between two pathogens, species loss of a key reservoir host for one parasite may result in an overall increase in transmission and prevalence of the second pathogen species among the altered host community. Changes in host resources as a result of land use change may immunocompromise hosts, resulting in increased pathogen coinfection, or transmission that may threaten human, domestic animal, or wildlife health. However, few studies evaluate influences of anthropogenic environmental change on pathogen coinfection in host communities, and how this feeds back on infection dynamics of each pathogen.

The objective of this study is to evaluate relationships between anthropogenic land use change and transmission of the multihost zoonotic parasite *Trypanosoma cruzi*, in Panama. We evaluate how habitat related changes in blood meal species diversity, species composition, and coinfection with the congeneric multihost parasite *Trypanosoma rangeli* interact with *Trypanosoma cruzi* infection in vectors. *Trypanosoma cruzi* is a vector-borne kinetoplastid protozoan parasite that is the agent of Chagas disease in humans.

Throughout Latin America, infection with *Trypanosoma cruzi* causes significant morbidity and mortality, with estimates of 10 to over 15 million people infected with the parasite

(Reithinger et al., 2009). Clinical symptoms vary from asymptomatic to fatal complications, such as congestive heart failure, present in approximately 30 percent of infected individuals (Gurtler et al., 2007) *T. cruzi* is transmitted between a number of different mammalian reservoir hosts (with over 100 potential host species identified) by hematophagous vectors in the family Reduviidae.

In the area of the Panama Canal, *T. cruzi* poses a significant risk to human health and is primarily transmitted by the vector *Rhodnius pallescens*. *R. pallescens* is associated primarily with ‘sylvatic’ habitats (as opposed to domestic areas such as houses), and lives and reproduces primarily within the ‘royal’ palm tree, *Attalea butyraceae*. Studies estimate *T. cruzi* human infection prevalence in this area between 2.9% and 23% and high peridomestic and domestic vector infection prevalence with *T. cruzi* (Calzada et al., 2006; Saldana et al., 2005). The land surrounding protected areas of the Panama canal has undergone a high degree of deforestation in the past 40 years, converting contiguous old growth and late secondary growth forests to a landscape of small patches of riparian forest remnants surrounded by a sea of cattle pasture, agricultural development, and human settlement, with small patches of regenerating forest from abandoned pasture. Within this disturbed habitat remain high, yet patchy, palm densities. *A. butyraceae* densities are often increased in habitats with a high degree of human disturbance, including hunting and agricultural development (Wright and Duber, 2001). In domestic and peridomestic habitats, the opportunistic mesopredator *Didelphis marsupialis*, the common opossum, is believed to be a key reservoir for *T. cruzi* infection in Panama. In this region, coinfections of *Trypanosoma rangeli* and *T. cruzi* are commonly found in *R. pallescens* in peridomestic and domestic habitats, with an estimated *T. rangeli* infection prevalence of 6.8% in children (Saldana et al., 2005). In fact, *T. cruzi* and *T. rangeli* are commonly found circulating sympatrically with one another in Central and South America (de Moraes et al., 2008). We test the following hypotheses: 1) If deforestation and forest fragmentation increases *T. cruzi* transmission, then *T. cruzi* vector infection prevalence should increase in deforested

and fragmented forest landscapes as compared to contiguous forests 2) If higher host diversity is negatively related to *T. cruzi* infection (a dilution effect), then *T. cruzi* vector infection prevalence should be lowest in contiguous forests, where there is a presumed higher vertebrate diversity, and higher in deforested sites (cattle pasture and peridomiciliary areas) and forest fragments. 3) Anthropogenic land use change alters the relative infection of *T. cruzi* and *T. rangeli* within vectors because of a change in mammalian host composition and possible alteration of competitive effects between the two pathogens.

## 4.2 METHODS

### 4.2.1 STUDY SITE, STUDY DESIGN CAPTURE METHODS FOR BUGS

This study took place in protected areas (contiguous forest sites) and human-dominated landscapes with a low (contiguous forest) to high level of human disturbance to the east and west of the Panama Canal. The study area included deforested rural landscapes and contiguously forested protected areas flanking the Panama Canal (Figure 3.1) and encompassed an area of over 600km<sup>2</sup>. This area is classified as lowland tropical moist forest (Holdridge 1967). Five different habitat types were sampled for *R. pallelescens*: contiguous late secondary forest, early secondary forest fragments, mid secondary forest remnants or fragments, cattle pasture, and peridomiciliary areas. Contiguous late secondary forest sites were located in a protected national park adjacent to the Panama Canal. These sites have a known land use history and forest age (approximately 75-100 years old) (Ibanez et al., 2002). Early secondary forest fragments were areas of abandoned pasture or cropland undergoing forest succession. These sites were approximately 30 to 50 years old, and most trees within the early secondary sites did not exceed 10 meters in height. There was also a predominance of lianas in most of these early secondary forest fragments. Mid secondary forest remnants or fragments were forest patches remaining after large-scale deforestation of late secondary or mature forest. Most of these mid-secondary patches were highly



disturbed, as most of the economically valuable adult trees were previously harvested from these sites, and the forest floor of most of these forest patches were heavily trampled by cattle. Peridomiciliary areas consisted of home gardens or yards located within 100 meters of a human dwelling. The gardens and yards surrounding domiciles were highly variable, some with well-manicured lawns, others with tall grass or located near a forest patch, and some sites with a large number of domestic animals (domestic fowl, dogs).

Seven replicate sites were chosen from each of the five habitat types, comprising total of 35 sites were sampled for *R. pallescens* to the west and east of the along the western and eastern border of the Panama Canal Area (Figure 4.1). Individual sites were at least 200 meters apart from each other, based on an estimated maximum flight distance estimated for *Rhodnius* sp., with the majority of sites located greater than 1 km apart from each other. Replicate sites from identical habitat types were located at least 600 m apart from one another. Study sites were spread over an area of approximately 600 km<sup>2</sup>. Palms were sampled once from each site during the wet season, between May 2007 and December 2007, to control for possible effects of season on *R. pallescens* abundance. Sites from multiple habitat types were sampled within each month, and an attempt was made to spread the sampling of different habitat types evenly across the wet season.

Within each site, a total of five palms were sampled; an adult *Attalea butyraceae* and the four nearest accessible adult *A. butyraceae* palms. The initial palm was selected by choosing the nearest palm to a random direction and distance less than 20m from the observer. The height at the top of the crown base, number and ripeness of fruit racemes, and presence of animal (bird and/or mammal nests or resting sites) were also recorded for each palm. Three baited traps (modified from a method described by Abad-Franch 2005 (Abad-Franch and Monteiro, 2005; Abad-Franch et al., 2000; Noireau et al., 2002)) were placed within the crown of each palm, left for twenty four hours, and checked for *R. pallescens* the following day. After collecting the baited traps, the palm crowns were searched for 10 minutes for bugs by a skilled individual. Palm crowns were accessed with a

20 foot ladder or by climbing the palm tree with a rope and harness tree climbing technique modified for palms. The modification of traps included a food and a hydration source that were provided for mice within the traps, and traps were covered with a small sheet of plastic to provide protection from direct sun and rain.

#### 4.2.2 TRIATOMINE PREPARATION AND MICROSCOPIC ANALYSIS

*R. pallescens* captured from each tree were classified according to stage, weighed, and measured. Only fourth and fifth stage nymphs, and adults were weighed and measured. Using sterile scissors, triatomines were macerated in 500  $\mu$ l of 0.01 Molar, 7.6 pH phosphate buffered saline (PBS). The macerated triatomines were centrifuged at 15,000 G for 10 minutes. The pellet containing portions of exoskeleton and internal organs of *R. pallescens* was then resuspended with a small sterile wooden dowel and subsequently centrifuged at 400 x for five minutes. The supernatant was collected and centrifuged at 15,000G and the pellet containing fragments of exoskeleton was frozen at -20C. The collected supernatant was spun for a final time at 15,000G for 20 minutes. The supernatant with soluble proteins from this spin was then frozen at -20C (Pineda et al., 2008; Calzada et al., 2006). The precipitate was suspended in 200  $\mu$ l of PBS and 5  $\mu$ l of this suspension was evaluated microscopically for the presence of trypanosomes. The rest of this suspended precipitate was frozen at -20C until DNA extraction was performed. DNA was extracted from this suspension using a commercial kit (Promega, Madison, WI).

#### 4.2.3 DUPLEX POLYMERASE CHAIN REACTION FOR THE DETECTION OF *T. cruzi* AND *T. rangeli*

A duplex polymerase chain reaction was performed for the detection of *T. cruzi* and *T. rangeli* using an assay targeted to the 189 base pair telomeric junction of *T. cruzi* and a subtelomeric region of *T. rangeli* developed by Chiurillo et al. (2003) (Chiurillo et al., 2003). The primers used for *T. cruzi* detection were T189Fw2 (5'

-CCAACGCTCCGGGAAAAC-3') and Tc189Rv3 (5' -GCGTCTTCTCAGTATGGACTT-3' ). For *T. rangeli* detection, primers were targeted to a conserved subtelomeric region (The primers were used for *T. rangeli* detection were TrF3 (5' -CCCCATACAAAACACCCTT-3 ) and TrR8 (5'-TGGAATGACGGTGC GGCGAC-3' ). PCR amplifications were performed using a 25  $\mu$ l reaction modified from (Humair et al. 2005) with 5  $\mu$ l of template DNA added to 20  $\mu$ l of a pcr master mix containing 1.25U of Taq Hot Start (Fermentas, Glen Burnie, Maryland), 25mM of MgCl<sub>2</sub>, .2mM dNTP (Qiagen), .8  $\mu$  Moles of forward primer, .8  $\mu$  Moles reverse primer, 2.5  $\mu$ l of PCR Buffer (PCR machine and denature renaturation amplification cycles. PCR products (5  $\mu$ l) were mixed with loading dye and electrophoresed on a 1.5% agarose gel stained with ethidium bromide and evaluated by ultraviolet light for the presence of bands of a length specific for *T. cruzi* (100bp) and *T. rangeli* (170 bp). Positive and negative controls were run for each reaction.

#### 4.2.4 BLOOD MEAL ANALYSIS.

In order to identify the vertebrate species present in insect bloodmeals, extracted DNA from triatomines was used in a pcr assay adapted from Humair et al. 2007 that amplifies the 12S mitochondrial rRNA gene of vertebrates. This assay is able to detect a single blood meal species in the vector and cannot detect dual blood meals. The primers used to amplify the approximately 145 bp fragment of the 12S rRNA gene were 12S-6F (5–CAAAC TGGGATTAGATACC–3) and 12S-9R (5–AGAACAGGCTCCTCTAG–3). Primers were obtained from Integrated DNA Technology services, USA. A 25  $\mu$ l reaction was prepared for PCR amplification with 3.0mM MgCL<sub>2</sub> (Fermentas), 0.2mM dNTPs (Qiagen), 0.8M of each primer, of Taq buffer, and 2.35U of TaqDNA polymerase (Fermentas). 5ul of triatomine DNA template was added to each sample. Positive and negative controls were run for each reaction. PCR reaction conditions were as follows: touchdown - initial denaturation 3minutes at 94C, burst cycle 20 seconds at 94C, 30

seconds at 60C, and 30 seconds at 72 C. 40 cycles of the following were then performed, with the annealing temperature being lowered by 1C until reaching 52C, : 20 seconds at 94C, 30 seconds at 52C, and 30 seconds at 72C). There was a final extension step of 7 minutes at 72 C. After the reactions, 1  $\mu$ l of pcr product was mixed with 5 $\mu$ l of loading dye and run on a 2% agarose gel that was stained with ethidium bromide in order to detect if the reaction was able to amplify vertebrate DNA in the bug. PCR products were stored at -20 until the final pre-sequencing purification step. PCR products were then purified with a high throughput adaptation of gel extraction followed by vacuum manifold pcr product purification using a QIAquick 96 PCR Purification Kit(Qiagen, Valencia, CA 91355). Procedure: 10  $\mu$ l of pcr product was mixed with 2  $\mu$ l of loading dye and run on a 2% agarose gel stained with ethidium bromide. The bands comprising the putative approximately 145 bp segment of the vertebrate rRNA gene were excised from the gel and gel slices were weighed. 3 volumes of buffer QG were added to one volume of gel (e.g. 210  $\mu$ l of buffer for a 70 $\mu$ l gel in a 96 well block). Gel slices in QG buffer were incubated at 50C until they completely dissolved in the buffer. One gel volume of isopropanol was then added to the buffer and mixed. The solubilized gel in buffer QG/isopropanol mix was added to the Quiaquick plate and vacuum was applied until all of the sample was drawn through. An additional 1ml of QG buffer was added to each of the wells and drawn through the filter with a vacuum. 1ml of Buffer PE was then added to each of the wells and vacuum was applied. This step was repeated. After adding Buffer PE to the plates for a second time, the vacuum was left on for an additional 10 minutes to dry the membrane filters in each of the wells. The edges of the top plate of the vacuum manifold and the bottom of the Qiaquick 96 plate were blotted with a paper towel to remove remaining buffer PD. Subsequently, 60  $\mu$ l of nanopure water was added to each of the wells and vacuumed into a collection plate or .5ml tubes. Samples were then tested for purity and DNA concentration with a Nanodrop spectrophotometer and sent for sequencing to the University of Georgia Bioinformatics laboratory. Sequences were identified to genus and

species by performing a nucleotide BLAST (Basic Local Alignment Search Tool) using the Nucleotide collection (nr/nt) database and comparison to the top hits.

#### 4.2.5 STATISTICAL ANALYSES

For all analyses, we used RCRAN version R 2.7.1 GUI 1.25 (5166). R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL

<http://www.R-project.org>. Fisher's chitest was used to measure dependence between Order and species blood meal identification and habitat type. The package used for general linear mixed models was the lme4 package (Pinheiro and Bates) for linear mixed effects models.

#### EVALUATING RELATIONSHIPS BETWEEN TRYPANOSOME INFECTION AND HABITAT TYPE

A general linear mixed model with quasibinomial errors to correct for overdispersion was used to evaluate the relationship between the proportion of trypanosome- infected bugs and habitat type (fixed effects=habitat type, random effects=Site nested within habitat type).

#### EVALUATION OF COINFECTION WITH *T. cruzi* AND *T. rangeli* ACROSS HABITAT TYPES

In order to assess if the probability of vector co-infection with *T. cruzi* and *T. rangeli* was greater than expected due to chance, an index of co-infection was calculated (Ginsberg 2009) as:

$$I_c = \frac{(O - E)}{N} 100 \quad (4.1)$$

O is the observed number of coinfections, N is the total number of infected bugs and E is the expected number of coinfections due to chance, represented by the equation :

$$E = \frac{(a + b)(a + c)}{(a + b + c + d)} \quad (4.2)$$

a is the number of coinfecting bugs, b is the number of bugs infected only with *T. cruzi*, c is the number of bugs infected only with *T. rangeli*, and d is the number of bugs not infected.

When  $I_c$  is positive, the number of coinfections is greater than expected due to chance, and when  $I_c$  is negative, then the number of coinfections is less than expected due to chance.

#### BLOOD MEAL DIVERSITY RELATIONSHIPS WITH HABITAT, AND *T. cruzi* INFECTION PREVALENCE

The host species diversity of blood meals identified by molecular analysis was identified for a respective site (sites with single blood meals identified were not discarded from this analysis). For each site, the number of different blood meal species was recorded as host species richness. In order to account for the relative proportion of each species blood meal identified for each site in relation to diversity, the Shannon Wiener and Simpson's diversity indices were calculated. The Shannon Wiener index (H) was calculated as follows:

$$H = - \sum_{i=1}^S p_i \ln p_i \quad (4.3)$$

where S is the number of different blood meal species,  $n_i$  is the number of individual blood meals of species<sub>*i*</sub>(abundance), and  $p_i$  is the relative abundance of each species<sub>*i*</sub> (number of blood meals of species<sub>*i*</sub> divided by the total number of all individual blood meals). The inverse Simpson's index (1/D) was also calculated for each site as follows:

$$1/D = \frac{N(N-1)}{\sum_{i=1}^S n_i(n_i-1)} \quad (4.4)$$

where N is the total number of blood meals and  $n_i$  is the number of blood meals identified as species<sub>*i*</sub>. The higher inverse Simpson's index indicates a higher diversity. General linear models with binomial errors and quasibinomial errors (to correct for overdispersion) were used to evaluate relationships between blood meal species number, indices of diversity (Shannon-Weiner and inverse Simpson's index), and the proportion of *T. cruzi* infected bugs. For these diversity disease evaluations, the proportion of infected vectors was evaluated at the site level.

RELATIONSHIPS OF A MAMMALIAN HOST LIFE HISTORY PARAMETER,  $R_m$ , TO THE PROPORTION OF *T. cruzi* INFECTED VECTORS

I hypothesize that as the intrinsic rate of increase  $r_m$ , (the maximum per capita rate of population increase) of mammalian hosts increases, so will the transmission of *T. cruzi* and the corresponding proportion of *T. cruzi* infected vectors, mainly due to increased rates of production of susceptible hosts. A composite  $r_m$  score was estimated for each study site by adding together the  $r_m$  values for the blood meal species present at a site divided by the number of different species identified at that site (compiled from the literature). For example, if species A and B were identified at a site, the composite  $r_m$  value was  $(r_{m\text{species A}} + r_{m\text{species B}})/2$ . Relationships between the proportion of *T. cruzi* infected vectors and the composite  $r_m$  value were investigated by a general linear model (binomial errors).

### 4.3 RESULTS

#### 4.3.1 *T. cruzi* INFECTION AND HABITAT TYPE

Overall, 74.3 %, (478/643) of vectors were infected with *T. cruzi*. The overall proportion of vectors infected with *T. rangeli* was 32.8% (211/643). Figure 4.1 summarizes the proportion of infected *T. cruzi* and *T. rangeli* in each habitat type. According to a linear mixed effects model, the estimated number of *T. cruzi* infected bugs is significantly greater in mid secondary ( $p=.005$ ) and peridomiciliary sites ( $p=.007$ ) as compared to contiguous forests. The proportion of *T. rangeli* infected vectors was significantly lower in early secondary ( $p=.03$ ), peridomiciliary ( $p=.03$ ), and pasture habitats ( $p=.001$ ) as compared to contiguous forests (Table 4.1). Developmental stage of vectors was positively related to the proportion of *T. cruzi* infected vectors (data not shown).

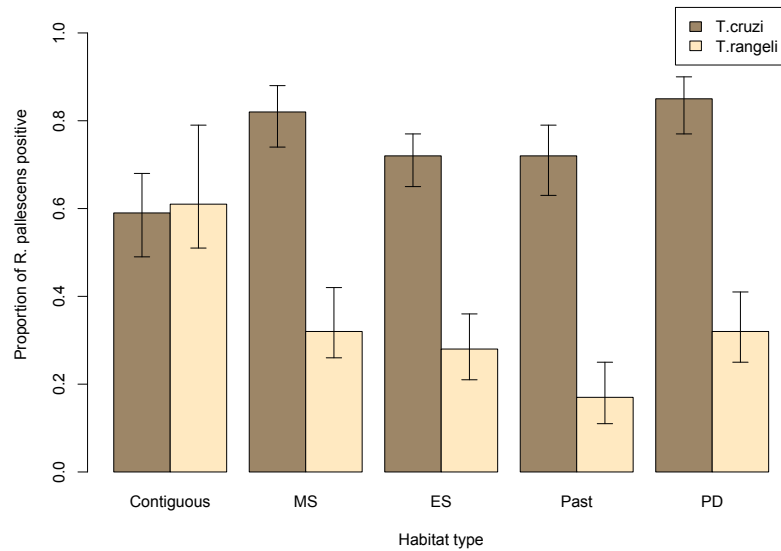


Figure 4.1: *Trypanosoma cruzi* and *Trypanosoma rangeli* vector infection prevalence across habitat types. Contiguous: contiguous forest, MS: mid secondary forest remnant, ES: early secondary forest fragment, Past: cattle pasture, PD: peridomicile.

Table 4.1: Relationship between the proportion of *T. cruzi* and *T. rangeli* infected *R. pallescens* and habitat type (general linear mixed model, fixed effect=Habitat, random effects=Site)

<i>Trypanosoma cruzi</i>				
Habitat type	Estimate	(Std. Error)	z value	P
(Intercept)	0.252	( 0.313)	0.805	0.4207
Early secondary fragments	0.575	(0.472 )	1.219	0.2228
Mid secondary remnants	1.472	(0.524)	2.808	0.0050 **
Peridomiciliary	1.401	(0.520)	2.693	0.0071 **
Pasture	0.571	( 0.479)	1.192	0.2333
<i>Trypanosoma rangeli</i>				
Habitat type	Estimate	(Std. Error)	z value	P
(Intercept)	-0.00839	(.32093)	-0.03	0.9791
Early secondary fragments	-1.02475	( 0.48744)	-2.10	0.0355 *
Mid secondary remnants	-0.85492	(0.50996)	-1.68	0.0937 .
Peridomiciliary	-1.13803	(0.52282)	-2.18	0.0295 *
Pasture	-1.76210	( 0.51980)	-3.39	0.0007 ***

Signif. codes: 0 \*\*\* 0.001 \*\* 0.01 \* 0.05 . 0.1



#### 4.3.2 *T. cruzi* AND *T. rangeli* COINFECTION AND HABITAT TYPE

The proportion of *R. pallescens* that were singly infected with *T. cruzi* were significantly greater throughout deforested habitat types (early secondary mid secondary forest remnant, cattle pasture, and peridomiciliary areas) as compared to contiguous forests, whereas the opposite trend was seen with bugs that were singly infected with *T. rangeli*. Singly-infected *T. rangeli* vectors was markedly lower throughout deforested landscapes, with the lowest proportion of singly-infected *T. rangeli* in mid-secondary and pasture habitats. The proportion of co-infected bugs was similar throughout all habitat types excluding pasture (Figure 4.2). The proportion of bugs co-infected with *T. cruzi* and *T. rangeli* was greater than expected due to chance (positive coinfection index), given the number of singly infected and non-infected bugs, in all habitats excluding pasture. Co-infection indices for bugs from different habitats were as follows; contiguous forest (8.48), mid-secondary forest remnants (4.30), early secondary forests (11.0), pasture (-8.75), and peridomiciliary areas (20.6).

#### 4.3.3 BLOOD MEAL DIVERSITY AND SPECIES COMPOSITION

Blood meals were successfully sequenced from amplification of the 12SrRNA gene fragment in 259 out of 643 bugs (40.3%) tested. The overwhelming majority of blood meals, 88.6% (N=230) were mammalian, the only competent host for *T. cruzi*. Lesser numbers of blood meals were identified from 6.9% (N=18) avian, 3.1% (N=8) reptilian, and .8% (N=4) amphibian origin, respectively. At the order level, Xenarthrans (sloths and tamandua) dominated blood meals, comprising 40.5% (N=105) of the blood meals sequenced, followed by primates (12.7%, N=33), marsupials (12.4%, N=32), ariodactyls (swine and cows) (8.5%, N=22), and carnivores (6.7%, N=16). Table 4.2 summarizes the composition of the blood meals identified from *R. pallescens* at the order level. A total of 42 species were identified by molecular methods (Table 4.3). *Choloepus hoffmanni*, the two-toed sloth dominated the blood meals, comprising 31.0% (N=82) of the blood meals sequenced from the vectors,

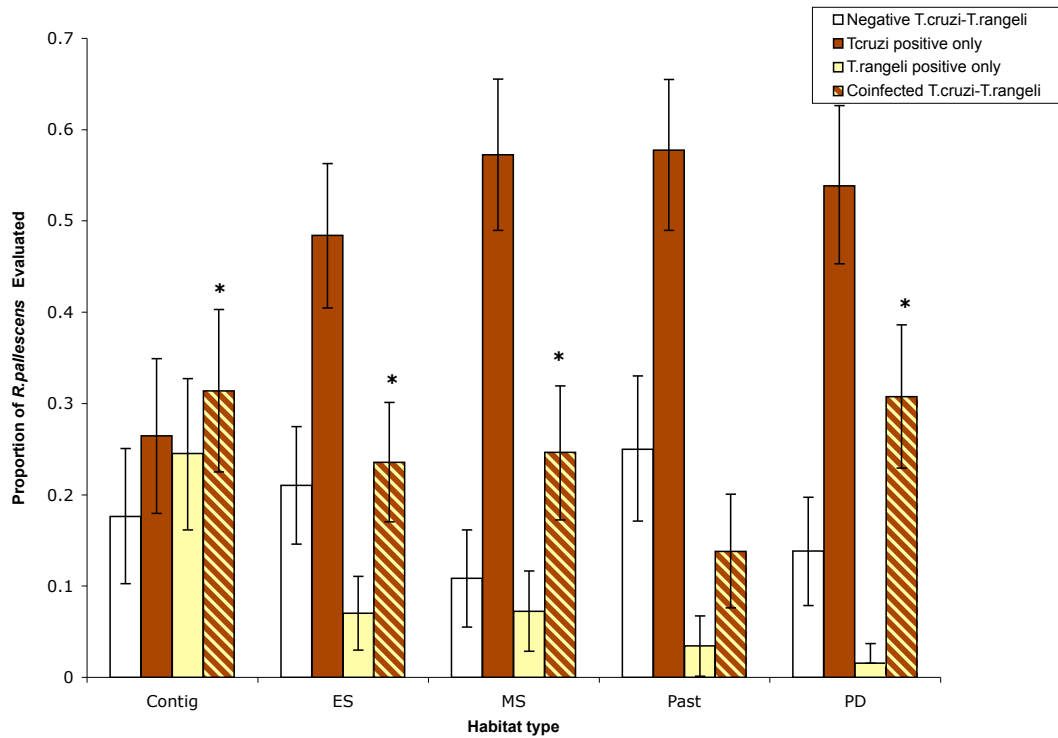


Figure 4.2: *Trypanosoma cruzi* and *Trypanosoma rangeli* vector co-infection and single infection across different habitat types. Stars \* show habitats where vector coinfection is higher than expected due to chance. Contiguous: contiguous forest, MS: mid secondary forest remnant, ES: early secondary forest fragment, Past: cattle pasture, PD: peridomicile.

followed by white-faced capuchins *Cebus albifrons* (8.5%,N=22), tamanduas *Tamandua tetradactyla* (8.5%, N=22), cows *Bos taurus* (6.95% (N=18), the four-eyed opossum *Metachirus nudicaudatus* (6.56%, N=17), the common opossum *Didelphis marsupialis* (4.25%, N=11), howler monkeys *Alouatta palliata*, domestic dogs *Canis familiaris* (3.1%,N=8), and kinkajou *Potos flavus* (1.93%,N=5).

Table 4.2: Class and Order of Blood Meals Identified in *R. pallescens*

Class	Order	No. Blood Meals	% of Total
Mammalia	Xenarthra	105	40.5
	Primata	33	12.7
	Marsupialia	32	12.4
	Artiodactyla	22	8.5
	Carnivora	16	6.2
	Rodentia	11	4.2
	Chiroptera	10	3.9
Aves	Galliformes	11	4.2
	Passeriformes	3	1.2
	Unidentified	2	0.8
	Ciconiiformes	1	0.4
	Falconiformes	1	0.4
Reptilia	Squamata	8	3.1
Amphibia	Caudata	4	1.5
	Total	259	

Data show differences in the species composition as well as the relative proportion of each order present in blood meals across habitat types. There was a significant association between order and Habitat type (Fisher's exact test  $\chi^2$ ,  $p=.002$ ) and species identification and habitat type (Fisher's exact test  $\chi^2$ ,  $p=.003$ ). Figure 4.3 shows the relative proportion of blood meals classified by class and order in each habitat type. Xenarthrans comprise the highest proportion of blood meals in all habitats (between 44-54% of blood meals) except for peridomiciliary areas, where marsupial blood meals are ranked first, making up 28% (N=14) of blood meals, with Xenarthrans making up 14% (N=7) of blood meals. The composition of Xenarthran species differed with varying degrees of anthropogenic disturbance. In contiguous forest sites, tamanduas *Tamandua tetradactyla* made up 39.4%

(N=13) and *Choloepus hoffmani*, two-toed sloths, comprised 60.6% (N=20) of Xenarthran blood meals. However, in mid-secondary, early secondary, and pasture sites, the number of tamandua blood meals decreased, making up 5.3%, 6.3%, and 7.0% of Xenarthran blood meals, respectively, with two-toed sloths comprising over 90% of the Xenarthran blood meals in these sites. In peridomestic sites, only two-toed sloths, and no tamanduas were detected in blood meals. Additionally, primates *Cebus apella* and *Alouatta palliata* blood meal isolations were highest in contiguous forests, comprising 28% of blood meals in this habitat type, and between 10-20% of blood meals in the deforested landscape habitats. Domestic animals identified in peridomestic habitats (N=number of blood meals identified) included cows (N=3), swine (N=2), domestic dogs (N=1), turkey (N=1), and peacock (N=1). Domestic animal blood meals identified in cattle pasture included cows, chicken, turkey, swine, and domestic dog. In early secondary forest fragments and mid secondary forest remnants, domestic animals that *R. pallescens* fed from were cow, domestic dog, and chicken (Table 4.3).

According to the results of a negative binomial GLM, the estimated blood meal species number per site was significantly greater in early secondary fragments, mid secondary fragments, and peridomestic sites as compared to contiguous forests. Similar findings were observed for Shannon-Wiener and inverse Simpson's diversity indices.

#### 4.3.4 BLOOD MEAL DIVERSITY, SPECIES COMPOSITION, AND VECTOR INFECTION PREVALENCE

There was a significant positive relationship between estimated *T. cruzi* vector infection prevalence at each site, species number, and common diversity indices (Shannon-Weiner Index and Simpson's Diversity Index) (Fig 4.4). There was a slight negative, but non-significant relationship between the estimated proportion of infected *T. rangeli* vectors and indices of diversity (glm with binomial errors, data not shown). Results (Table 4.5) suggest that species composition has significant relationships to vector infection, with

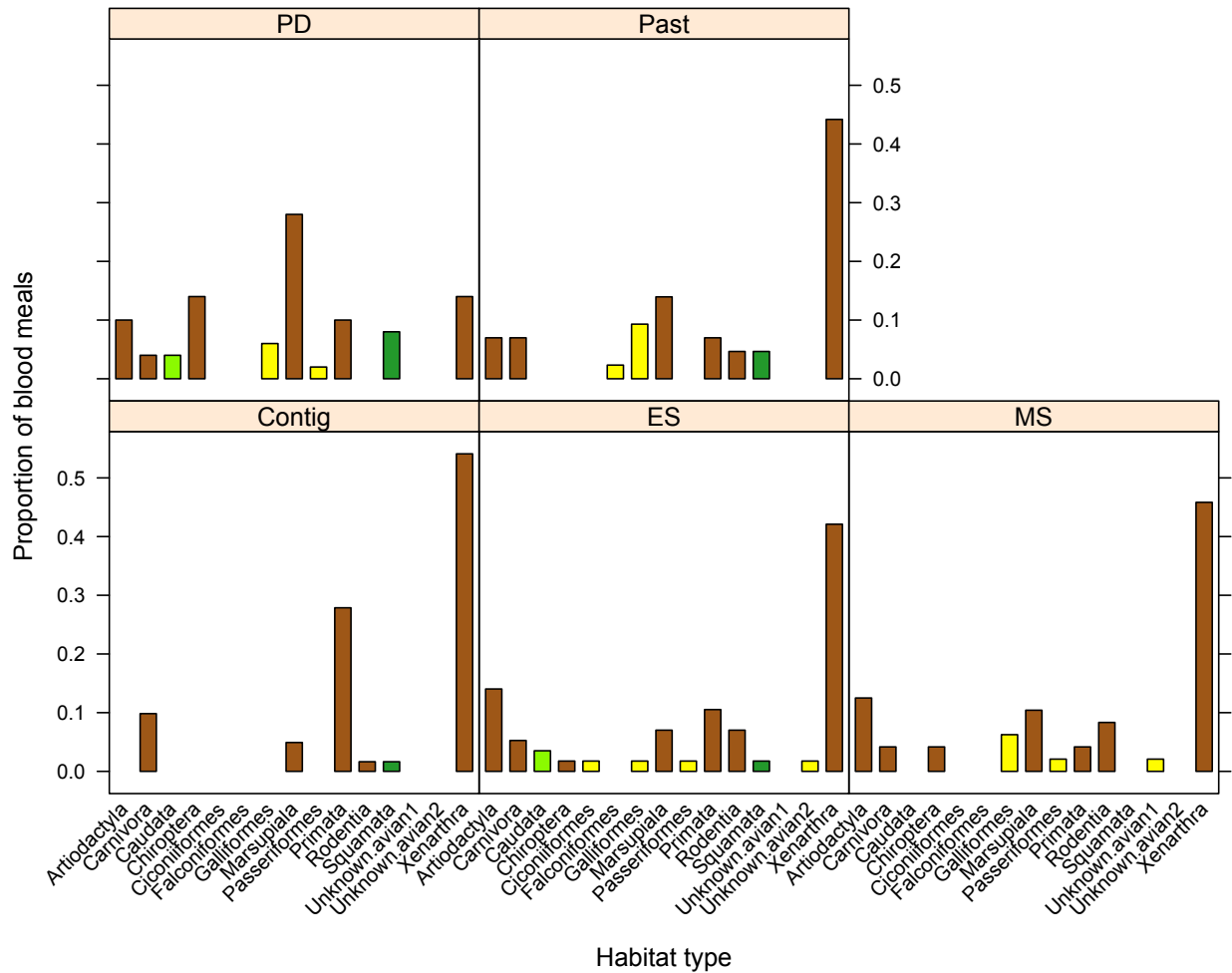


Figure 4.3: Blood meals identified according to Order and Habitat type. Bar colors, brown: mammals, yellow: birds, dark green: reptiles, light green: amphibians

opossum blood meal availability positively and significantly ( $p=.007$ ) associated with the proportion of infected vectors. Tamandua blood meal availability has a slight negative relationship with *T. cruzi* vector infection. On the other hand, there is a positive relationship between Tamandua blood meal availability and the proportion of *T. rangeli*-infected vectors. This relationship is statistically significant analyzed by a general linear model (quasibinomial errors) with tamandua blood meal availability as the only dependent variable ( $t= 2.26$ ,  $p=0.031$ ), but drops to marginally significant when primate and marsupial blood meal availability are included in the model.

There was a significant positive relationship between between the  $r_m$  composite score and the proportion of infected vectors ( $p=.0028$ ) (Table 4.4).

#### 4.4 DISCUSSION

There was a high proportion of *T. cruzi* infected vectors across the landscape. This study shows similarly high infection indices in peridomestic sites to those previously reported in this region of Panama ( $>80\%$ ) (Calzada et al., 2006; Pineda et al., 2008). From other studies, reported *T. cruzi* infection levels in triatomines vary widely, from absent to over 60%. Many factors may account for this large variation, as ecosystem types vary widely across reported studies for triatomine infection. Season may affect relative abundances of vector as well as the host community. Also, triatomines in sites with low prevalence may be feeding from a suite of less competent hosts, because the species composition of hosts varies geographically. The infection indices reported in peridomestic sites in Panama are particularly high. Although contiguous forests had the lowest mean infection prevalence with *T. cruzi*, vector infection was still high compared to many reports (Feliciangeli et al., 2004; Kjos et al., 2009; Gurgel-Goncalves et al., 2003; Sarquis et al., 2004; Bar and Wisnivesky-Colli, 2001; Barrett et al., 1979; Valente et al., 2009; Whitlaw and Chaniotis, 1978; Pizarro and Stevens, 2008; Calzada et al., 2006).

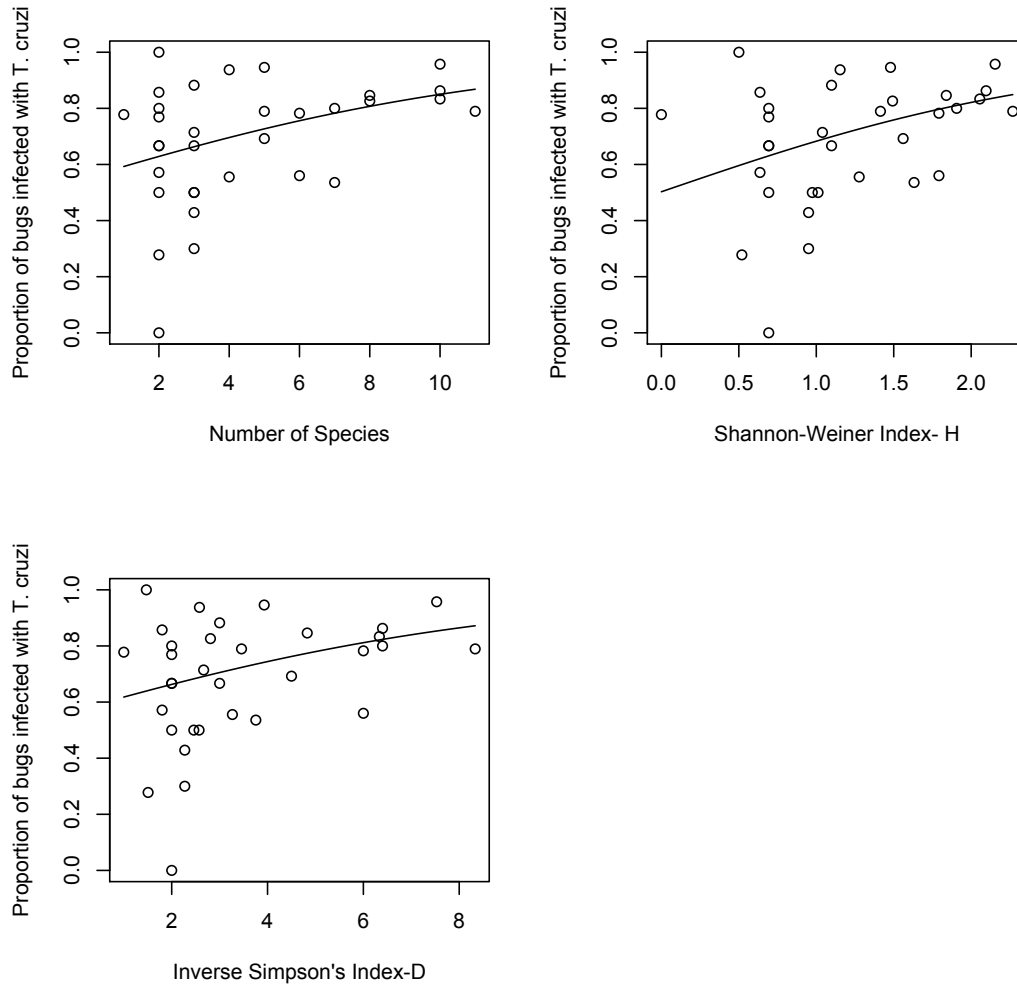


Figure 4.4: Relationships between the proportion of *T. cruzi* infected *Rhodnius pallescens* per site and number of blood meal species identified (upper left), Shannon-Wiener  $-H'$  (upper right), and Inverse Simpson's Diversity Index  $-D$

Comparing different habitat types, contiguous late secondary forests had a significantly lower proportion of *T. cruzi* infected vectors than peridomiliary and mid secondary forests. Potential mechanisms for the higher vector infection prevalence evaluated in disturbed habitats in this study are the potential competitive interaction with the congeneric parasite *T. rangeli*, and/or a change in blood meal species composition and diversity across habitat types. While infection with *T. cruzi* increased in disturbed/deforested habitats, the proportion of vectors infected with *T. rangeli* declined, with a significantly lower proportion of *T. rangeli* infected vectors in deforested sites.

Single infections of vectors with *T. rangeli* are markedly lower in deforested and fragmented habitats, while single infections with *T. cruzi* increase in disturbed habitats. There is the potential that cross immunity to one species of trypanosome in the vertebrate host may lead to lower infection rates with the second congeneric parasite within the host, due to an immunologic response to the first parasite. Laboratory mice injected with epimastigotes of *T. rangeli* developed a lower parasitemia upon challenge with a virulent *T. cruzi* strain, decreased severity of disease outcomes, low parasitemia levels and 100% survival of all mice (Basso et al., 2008; Palau et al., 2003). Domestic dogs vaccinated with *T. rangeli* and subsequently challenged with *T. cruzi* had less parasitemia and lower rate of infection to bugs (Basso et al., 2007). The fact that contiguous forests and mid secondary forests have similar infection prevalence with *T. rangeli* suggests that there may be similarities in the host community composition in mid secondary forest remnants and contiguous forests. Greater levels of coinfection than expected due to chance in all habitat types except for pasture suggests that there is no direct negative interaction between the two parasites within *R. pallezensis*, and that many mammalian hosts are coinfecting. For example, coinfection with *T. cruzi* and *T. rangeli* is often the case in Neotropical wildlife, with coinfections reported in a wide range of mammal species, including golden lion tamarins (Maia da Silva et al., 2008), sloths, tamandua, and opossums (Miles et al., 1983; Christensen and Devasquez, 1981; Yeo et al., 2005). In the Panama Canal area, vertebrate



and vector coinfections with *T. cruzi* and *T. rangeli* have been reported. In all habitats, there may be enough cocirculating *T. rangeli* so that the exposure rates to both parasite species appear to remain relatively high, negating any immunoprophylactic effect of *T. rangeli*, as hosts may become coinfecting simultaneously with *T. cruzi* and *T. rangeli*. However, there may be indirect negative consequences of *T. rangeli* infection on *T. cruzi* transmission, particularly in contiguous forests where *T. rangeli* infection is high. While *T. cruzi* is believed to be relatively innocuous in *Rhodnius*, infecting only the gut with superficial association with the midgut wall (Kollien and Schaub, 2000; Kollien et al., 1998), *T. rangeli* can develop in many different tissues, including the salivary glands and infect the hemolymph (Watkins, 1971a). Additionally, *T. rangeli*, can cause significant muscular, tracheal, and nervous pathology in *Rhodnius* hosts, as well as interfering with symbiote development in the insect gut. These symbiotes are important to insect growth, and trypanosome induced interference with them can lead to moulting and egg deformities (Vallejo et al., 2009; Kollien et al., 1998; Eichler and Schaub, 2002; Watkins, 1971a, 1971b). Additionally, *T. rangeli* can make the vector move more slowly, and also interfere with feeding efficiency by attacking the salivary glands, impeding digestion. Specifically, *T. rangeli* infection can cause the vector to probe multiple times for a blood meal, and reduce ingestion of blood (Schaub, 2006). The impacts of *T. rangeli* infection on reduced feeding efficiency and vector development can cause reduced vector productivity and fitness at a population level. Therefore, the high *T. rangeli* infections in contiguous forests may contribute to the lower numbers of vectors observed (Gottdenker, unpublished thesis data, chapter 2), and perhaps lead to reduced *T. cruzi* transmission. It is possible that high infection rates with *T. rangeli* act to regulate vector populations, and a moderate decline of *T. rangeli* in deforested landscapes may cause ecological release, leading to increased reproductive success of *R. pallenscens* with increased *T. cruzi* transmission.

Host blood meal diversity and community composition may also contribute to the patterns of increased *T. cruzi* vector infections in deforested landscapes. Indeed, the high degree of

*T. cruzi* vector infection prevalence across deforested landscapes may be a function of host diversity and community structure. Across all habitat types, a high diversity of vector blood meals exist, with 42 different species identified. The number of species identified is likely an underestimation, as the molecular test was unable to distinguish between some species, such as the two toed sloth (*Choloepus hoffmanni* and *Choloepus didactylus*). The vast majority of blood meals (88.6%) were identified from competent hosts, mammals, as compared to birds, reptiles, and amphibians that cannot transmit *T. cruzi* nor *T. rangeli*. The predominance of feeding from mammalian hosts may be an important explanation for the relatively high vector infection prevalence of trypanosomes throughout the landscape. In Panama, Pineda et al. 2008 encountered a predominance of mammal blood meals from peridomestic and domestic sites. Most blood meals were from wild mammals. In early secondary fragments, mid secondary forest remnants, and peridomiciliary sites, the relative predicted number of species blood meals that *Rhodnius pallescens* fed upon was higher than in contiguous forests. Domestic animal blood meals were only detected in disturbed habitats (forest fragments, cattle pasture, and peridomiciliary areas). As an order, Xenarthrans (sloths and tamanduas) comprised the majority of *Rhodnius pallescens* blood meals identified across all habitats. Overall, sloths (*Choloepus hoffmanni*), dominated blood meals in all habitat types. Sloths may be an attractive blood meal for *R. pallescens*, and good hosts for trypanosomes and bug populations. In addition to resting in palm crowns, the sloth's slow metabolism and relatively slow movements may prevent them from rapidly removing feeding bugs by grooming or scratching, allowing bugs to feed, defecate on the host, and maintain *T. cruzi* transmission. Although most blood meals identified were arboreal or scansorial species, a few terrestrial species, such as dog, pig, and cow, were identified. Although most terrestrial species blood meals were identified from adults bugs, they were also identified in third, fourth, and fifth stage nymphs, suggesting that nymphs may descend to the ground near palm trees to feed, or that nymphs can secondarily feed from engorged adults who fed from terrestrial species and returned to palm trees to rest.

There was a significant positive relationship between measurements of species number per site and host and estimated vector infection prevalence with *T. cruzi* (Fig 4.4). Estimated species number was also greater in early secondary fragments, mid secondary forest remnants, and peridomiliary areas as compared to contiguous forests. Although there was a negative association between *T. rangeli* vector infection prevalence and host diversity indices (species number, Shannon-Wiener index, Simpson's index, and inverse Simpson's index), the relationship was not statistically significant with the binomial logistic regression model. The positive relationship between host diversity and vector infection prevalence, as well as positive diversity habitat relationships in deforested sites suggests that anthropogenic land use change (forest fragmentation, human settlement) can cause an increase in *T. cruzi* infection prevalence, and that there is a potential parasite amplification in the system in deforested landscapes.

In theory, increased host diversity may lead to an amplification of parasite prevalence when hosts exhibit similar competence as a disease reservoir, when the density of vectors is dependent on host density or abundance, and the increase in vector population size overcomes the negative effect of feeding from hosts that are poor infection reservoirs (Keesing et al., 2006; Dobson, 2004). In this *T. cruzi* - *Rhodnius pallescens* system, it is possible that *R. pallescens* depends upon hosts such as the sloth and the opossum. The increase in vector populations may also be high enough in deforested landscapes to overcome negative effects of feeding from less competent hosts.

Host composition may also play an important role in driving infection patterns in landscapes (LoGiudice et al., 2008). For example, because *Avena fatua* is a very good infection reservoir for barley yellow dwarf virus, and can lead to increased disease prevalence in diverse plant communities relative to a species that is a less competent reservoir (Power and Mitchell, 2004). In terms of West Nile Virus infection risk, community structure can play an important transmission role. In one study, passerine birds had no significant relationship with West Nile Virus prevalence, whereas non-passerine bird

diversity was negatively related with West Nile Virus infection in humans and mosquitos (Ezenwa et al., 2006). Additionally, changes in blood meal/host community composition across different habitat types are likely contributors to increased *T. cruzi* vector infection prevalence in observed in deforested landscapes. There were significant associations between species and order of blood meal, habitat type, and *T. cruzi* infection prevalence. Clearly, host communities change across habitats, with a marked increase in opossum blood meals in peridomestic sites. Sloths remain the top ranking blood meal across most habitat types, with the exception of peridomiciliary areas, where marsupials (*Didelphis* and *Metachirus* dominate. Because marsupials are believed to be a particularly competent reservoir for *T. cruzi* infections (Carreira et al., 2001), they may play an important role in driving the *T. cruzi* vector infection prevalence up in peridomiciliary sites. Similarly, small wild mammals in fragmented forests of Brazil had a higher *T. cruzi* seroprevalence as compared to contiguous forests (Vaz et al., 2007). The number and prevalence of tamandua blood meals also increase in contiguous forest sites and are positively associated with *T. rangeli* vector infection. Because tamandua blood meals markedly increase in contiguous forests, where the highest *T. rangeli* vector infection prevalence is, they may play an important role in driving up *T. rangeli* infection in contiguous forest sites. It is likely that species such as marsupials, sloths, and tamandua, play important roles in trypanosome transmission across the Panamanian landscape.

This study demonstrates that host life history strategies may play an important role in driving vector infection prevalence, as sites with higher composite host intrinsic rate of increase are associated with a higher proportion of *T. cruzi* infected vectors. For example, large, relatively long-lived species (e.g. primates) may not be expected to be as important to long term *T. cruzi* transmission as compared to a shorter lived species with a higher intrinsic rate of increase. Long lived species are also generally less productive, and may develop immunity to trypanosome infection, decreasing the probability of being a source of vector borne transmission to susceptible individuals. In contiguous forest habitats, the

mean composite host intrinsic rate of increase was lower than in deforested sites, as was vector infection prevalence with *T. cruzi*, further suggesting that the life history strategies of hosts, and host composition, are an important factor influencing vector infection prevalence. Typically, circulating parasitemias after reinfection with *T. cruzi* after the course of initial infection are reduced as compared to the initial infection due to acquired immunity (Machado et al., 2001; Bustamante et al., 2002). However, in short-lived, relatively smaller sized individuals such as the opossum, infective adults sharing a nest with juveniles may transmit the disease rapidly to vectors, which can then transmit the parasite to susceptible offspring, helping maintain *T. cruzi* at a population level. Alternatively, it is possible that Xenarthrans and marsupials have a higher tolerance to trypanosome infection, making them particularly competent disease reservoirs. For example, *Didelphis* are commonly coinfecting by many types of protozoan parasites, such as *Sarcocystis* and *Besnoitia* (Elsheikha et al., 2003), and may be able to tolerate and transmit protozoan infection with greater facility than other mammal hosts. A greater understanding of the relative competence of different Neotropical mammal species to *T. cruzi* infection is critical to predicting trypanosome infection dynamics and disease risk across the Neotropics.

In summary, there is no evidence for a dilution effect occurring relative to *T. cruzi* transmission in contiguous forest habitats as compared to deforested landscapes. Results suggest that vector infection prevalence increases with habitat disturbance as well as increasing host blood meal diversity, implying that an amplification effect occurs in fragmented habitats. However, there were limitations inherent in this study. Because it was a cross sectional study, samples were taken only during the wet season, and transmission dynamics may change as a function of seasonality and long term environmental drivers (e.g. climate change). Additionally, host populations were not sampled for trypanosome infections. Further developments need to be made on molecular techniques for blood meal analysis, as the molecular test done in this study did not detect multiple blood meals in

individual vectors. Additionally, the relative reservoir competence for different mammalian hosts identified is unknown and requires future studies (both modeling, observational disease surveys, and experimental infections with trypanosomes and xenodiagnostics) to bring definitive insight into the role that host community structure plays in the transmission dynamics of *T. cruzi* in deforested landscapes.

Table 4.3: Species of blood meals identified in *R. pallescens*

Class	Order	Species	No. of Blood Meals	% of Total
Amphibia	Caudata	<i>Plethodontidae sp</i>	4	1.54
Aves	Aves unknown1	<i>Avian sp 1</i>	1	0.39
Aves	Aves unknown2	<i>Avian sp 2</i>	1	0.39
Aves	Falconiformes	<i>Cathartes aura</i>	1	0.39
Aves	Galliformes	<i>Gallus gallus</i>	4	1.54
Aves	Galliformes	<i>Meleagris gallopavo</i>	3	1.16
Aves	Galliformes	<i>Ortalis vetula</i>	3	1.16
Aves	Galliformes	<i>Pavo cristatus</i>	1	0.39
Aves	Passeriformes	<i>Cranioleuca sp</i>	1	0.39
Aves	Passeriformes	<i>Piranga sp</i>	1	0.39
Aves	Passeriformes	<i>Turdus philomelus</i>	1	0.39
Mammal	Artiodactyla	<i>Bos taurus</i>	18	6.95
Mammal	Artiodactyla	<i>Sus scrofa</i>	4	1.54
Mammal	Carnivora	<i>Canis familiaris</i>	8	3.09
Mammal	Carnivora	<i>Conepatus semistriatus</i>	1	0.39
Mammal	Carnivora	<i>Mustela sp</i>	2	0.77
Mammal	Carnivora	<i>Potos flavus</i>	5	1.93
Mammal	Chiroptera	<i>Carollia sp</i>	1	0.39
Mammal	Chiroptera	<i>Lonchophylla handleyi</i>	1	0.39
Mammal	Chiroptera	<i>Mollosidae sp</i>	1	0.39
Mammal	Chiroptera	<i>Myotis elegans</i>	2	0.77
Mammal	Chiroptera	<i>Nyctanassa violaceae</i>	1	0.39
Mammal	Chiroptera	<i>Phyllostomatidae sp</i>	1	0.39
Mammal	Chiroptera	<i>Pteronotus gymnonotus</i>	2	0.77
Mammal	Chiroptera	<i>Pteronotus personatus</i>	1	0.39
Mammal	Chiroptera	<i>Saccopteryx leptura</i>	1	0.39
Mammal	Marsupialia	<i>Didelphis marsupialis</i>	11	4.25
Mammal	Marsupialia	<i>Marmosa sp</i>	1	0.39
Mammal	Marsupialia	<i>Metachirus nudicaudatus</i>	17	6.56
Mammal	Marsupialia	<i>Philander opossum</i>	3	1.16
Mammal	Primata	<i>Alouatta palliata</i>	11	4.25
Mammal	Primata	<i>Cebus albifrons</i>	22	8.49
Mammal	Rodentia	<i>Coendou bicolor</i>	4	1.54
Mammal	Rodentia	<i>Heteromyidae sp</i>	1	0.39
Mammal	Rodentia	<i>Mus musculus</i>	3	1.16
Mammal	Rodentia	<i>Sciurus sp</i>	3	1.16
Mammal	Xenarthra	<i>Choloepus hoffmanni</i>	82	31.66
Mammal	Xenarthra	<i>Cyclopes didactylus</i>	1	0.39
Mammal	Xenarthra	<i>Tamandua tetradactyla</i>	22	8.49
Reptilia	Squamata	<i>Lepidodactylus</i>	1	0.39
Reptilia	Squamata	<i>Mabuya sp</i>	3	1.16
Reptilia	Squamata	<i>Sphaerodactylus sp</i>	4	1.54
TOTAL			259	100.00

Table 4.4: Relationships between Blood Meal Species Number identified in *R. pallescens* and Habitat Type(glm, negative binomial errors)

Habitat	Estimate	(SE)	z value	P
(Intercept)	0.934	( 0.199)	4.70	$2.6x10^{-6}$ ***
Early secondary fragments	0.770	(0.277)	2.78	0.00548 **
Mid secondary remnants	0.898	(0.300)	2.99	0.00278 **
Peridomiciliary areas	0.953	(0.280)	3.40	0.00067 ***
Cattle pasture	0.487	(0.283)	1.72	0.08531

Significance codes: 0 \*\*\* , 0.001\*\* 0.01 \* 0.05 . 0.1 , 1

Table 4.5: Predicted effects of tamandua, opossum, and primate blood meal availability on *T. cruzi* infected vectors (general linear model, quasibinomial errors).

Blood meal species	Estimate	(SE)	z value	P
(Intercept)	0.832	0.286	2.90	0.007 **
opossum	2.708	1.229	2.20	0.036 *
primate	0.357	1.079	0.33	0.743
tamandua	-1.088	1.017	-1.07	0.294

Significance codes: 0.001\*\* 0.01 \* 0.05 . 0.1 , 1

Availability interpreted as proportion of blood meals identified by the above species at each site

Table 4.6: Relationship between predicted vector infection prevalence and  $r_m$  composite score at each site (general linear model, quasibinomial errors)

Parameter	Estimate	(SE)	t value	P
Intercept	0.381	0.257	1.48	0.1484
rmaxcomp	0.529	0.162	3.27	0.0028 **

Significance codes: 0 \*\*\* , 0.001\*\* 0.01 \* 0.05 . 0.1 , 1



## CHAPTER 5

### CONCLUSION AND FUTURE DIRECTIONS

#### 5.0.1 SUMMARY OF FINDINGS

This study shows that deforestation and forest fragmentation are associated with an increase in *Rhodnius pallescens* abundance and vector infection with *Trypanosoma cruzi*. Species composition and host diversity appear to affect vector infection prevalence. However, *T. cruzi* vector infection prevalence increases as host blood meal diversity increases, suggesting that a dilution effect is not an important mechanism in regulating *T. cruzi* transmission. Rather, host community composition appears to be an important factor driving vector infection. Sloths are a predominant blood meal for *R. pallescens* across all habitat types except for peridomiliary areas. This suggests that they are important hosts for the maintenance of vector populations as well as trypanosome infection. Opossums are significantly and positively associated with vector infection. Interestingly, mammalian life history appears to impact vector infection prevalence, with a trend towards higher vector infection in sites exhibiting more *r* selected mammal hosts. Not surprisingly, the composite  $r_m$  score was lowest in contiguous forests and higher in habitats across the deforested landscape.

Although there was no evidence of direct competition between *T. cruzi* and *T. rangeli* as a potential regulator of *T. cruzi* infection prevalence, *T. rangeli* may be instrumental in explaining why the bug abundance is lower in contiguous forests as compared to deforested sites, as it can be entomopathogenic as well as decrease feeding efficiency of *R. pallescens*. Additionally, the presence of tamanduas might be an important factor in maintaining *T. rangeli* vector infection in deforested landscapes.

### 5.0.2 MANAGEMENT IMPLICATIONS: THE NEED FOR LONG TERM MONITORING OF MULTIHOST PARASITES AND PATHOGENS AT THE LANDSCAPE SCALE

This research provides a mere snapshot into how deforestation may impact the transmission of a multihost pathogen, as it took place over the course of only one year. Additionally, trypanosome infection prevalence in wild mammals was not determined in the study areas. Doing so requires large scale, intensive trapping efforts focused on arboreal and scansorial mammals. Long term monitoring of wildlife and vector populations across a gradient of deforestation is essential in order to understand how climate change, land use change, other parasites and pathogens, and *T. cruzi* interact. Better methods of blood detection in triatomine vectors, particularly ones that can detect multiple blood meals in a variety of hosts should be developed so that vector/host interactions can be better understood. Vector dispersal patterns across deforested landscapes are also important to understand how *T. cruzi* infection is maintained. Environmental monitoring of microhabitats will also be important to model and understand vector population dynamics. Manipulation experiments (e.g. removal of palm trees adjacent to houses, trapping and removal of wild mammals adjacent to homes, creating landscapes that encourage natural predators and pathogens of triatomine bugs) can also provide solutions for the chronic problem of sylvatic reinfestation of houses by vectors such as *R. pallenscens*. However, in my opinion, because *T. cruzi* is so ubiquitous in the environment, the most effective way to control the majority of infection in humans would be to ensure that housing is adequate enough to prevent vector infestation. This would require a complete overhaul of the social, political and economic environment at regional and global scales. In the meantime, current research in vaccine development, biological vector control, public health education, and peridomiliary habitat manipulation are crucial in the fight against this devastating disease.

### 5.0.3 FUTURE DIRECTIONS: CONCEPTUALIZING VECTOR-BORNE MULTIHOST PATHOGEN SYSTEMS

Conceptual and mathematical models provide a better understanding of multihost pathogen dynamics and evolution in complex systems, guide empirical research, and allow the development of effective disease control strategies. In the future, I would like to explore the following:

#### SYSTEMS MODELS OF VECTOR BORNE MULTIHOST PATHOGEN TRANSMISSION

These models help us to understand how indirect effects at an ecosystem and community level (e.g. interactions at one trophic level impacting host-parasite relationships at another) can impact pathogen transmission and persistence.

#### MODELING THE ADAPTIVE IMMUNE LANDSCAPES RELATIVE TO MULTIHOST PATHOGENS AT POPULATION AND COMMUNITY LEVELS

This research may provide insight into virulence evolution and how pathogens emerge or spill over in novel hosts.

#### HIERARCHICAL SOURCE-SINK APPROACHES TO MODELING THE DYNAMICS OF VECTOR-BORNE DISEASE

For spatially structured diseases, habitat patches provide resources for the vector, and can be source habitats, allowing for vector population increase when there are adequate resources, and sink habitats in the absence or dispersal of a host blood meal or decline of habitat quality. At another hierarchical level, these habitat patches may be sources for disease ( $R_0 > 1$ ) when highly competent hosts are in contact with the vector. Alternatively, these patches may be disease sinks ( $R_0 < 1$ ) when vectors contact non competent or immune hosts. Movement of vectors between patches can transform a disease sink habitat in to a source, but this source may not persist if the habitat is a vector population sink. I would

like to use this hierarchical source sink framework to explore how host community structure and vector populations allow for disease persistence and extinction at a landscape scale.

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## APPENDIX

### INFECTIOUS DISEASES ASSOCIATED WITH ANTHROPOGENIC LAND USE CHANGE



Disease	Pathogen name	Pathogen type	Multi or Single Host		Main host	Land Use Type	Region	Climate	Anthropogenic biome	Transmission Type	Reference
			Pathogen type	Host							
						anthropogenic land use and land use (general), agricultural development					Cecchi et al. (2008); De La Rocque et al. (2001); Dagnogo et al. (1997)
African trypanosomiasis	<i>Trypanosoma brucei</i> , other <i>Trypanosoma spp.</i>	protozoa	MHP		mammals	agricultural development	Africa	Tropical	residential rainfed mosaic croplands, remote rangelands, residential rainfed	VB	Wagner et al. (2008)
Buruli ulcer	<i>Mycobacterium ulcerans</i>	bacteria	MHP		human	agricultural development	Africa	Tropical	populated forests, populated rangelands, residential rangelands	direct	
Campylobacter	<i>Campylobacter spp.</i>	bacteria	MHP		human/other	agricultural development	Oceania	Temperate	residential rainfed mosaic croplands, populated forests, residential	direct	Eyles et al. (2006)
Chagas disease	<i>Trypanosoma cruzi</i>	protozoa	MHP		mammals	forest fragmentation, deforestation, agricultural development	S. America	Tropical, Subtropical	remote rangelands, remote rangelands, populated forests, remote croplands	VB	Vaz et al. (2007); Ceballos et al. (2006); Briceno-Leon (2007); Teixeira et al. (2001b)
Chagas and surra	<i>Trypanosoma cruzi</i> and <i>Trypanosoma evansi</i>	protozoa	MHP		mammals	cattle ranching	S. America	Tropical	remote rangelands, residential rangelands, remote forests, wild forests	VB	Herrera et al. (2007)
Echinococcosis	<i>Echinococcus multilocularis</i>	cestode	MHP		rodent/carnivore	deforestation	Eurasia	Temperate	residential rangelands, cropped and pastoral village, residential rangelands, cropped and pastoral village, residential rainfed mosaic croplands, rainfed mosaic villages, urban	trophic	Craig et al. (2000); Giraudoux et al. (2003); Reperant et al. (2007)

Disease	Pathogen name	Pathogen type	Multi or Single Host		Land Use Type	Region	Climate	Anthropogenic biome	Transmission Type	Reference
			Pathogen	Host						
"New World" leishmaniasis	<i>Leishmania mexicana</i>	protozoa	MHP	human	deforestation, urbanization, agricultural development, land resettlement	S. America and N. America(C. and Sub-America)	tropical	pastoral villages, residential rangelands, populated forests, residential rainfed mosaic croplands, residential mosaic villages, urban, rainfed mosaic villages, residential irrigated croplands	VB	Salomon et al. (2006); Ready et al. (1983); Costa et al. (2005); Mouchet et al. (1994); Salomon et al. (2001); Chaves et al. (2008); Guerra et al. (2006); Kawa and Sabroza (2002)
	<i>Leishmania braziliensis</i>									
	<i>Leishmania chagasi</i>									
	<i>Leishmania panamensis</i>									
	<i>Leishmania donovani (cutaneous)</i>									
"Old World" leishmaniasis	leishmania (zoonotic cutaneous ) and viscera, Leishmania major, L. turanica, L. gerbilli	protozoa	MHP	human	agricultural development, woodland pastoral, agricultural development-deforestation and irrigation	Africa and Eurasia	Tropical, Subtropical, and Temperate	barren, remote rangelands, residential rangelands, populated rangelands	VB	Ashford et al. (1976); Thomson (1999); Elhassan et al. (1995); Eliseev et al. (1991); Wasserberg G (2003)
leptospirosis	<i>Leptospira canisrovar pomona</i> , <i>Leptospira spp.</i>	bacteria	MHP	mammals	agricultural development-irrigation	S. America, N. America	Subtropical and Temperate	populated irrigated croplands, urban, populated irrigated croplands	direct	Barcellos et al. (2003); Ghneim et al. (2007)
Lyme disease	<i>Borrelia burgdorferi</i>	bacteria	MHP	mammal (other vertebrates as well)	suburbanization, urbanization, forest fragmentation,	N. America, Eurasia	Temperate	urban, rainfed villages, rainfed mosaic villages, residential rainfed mosaic croplands, residential irrigated croplands	VB	Allan et al. (2003); Glass et al. (1994); Wilder and Meikle (2004); Brownstein et al. (2005); Linard et al. (2007)

Disease	Pathogen name	Pathogen type	Multi or Single Host	Main host	Land Use Type	Region	Climate	Anthropogenic biome	Transmission Type	Reference
										Grieco2006, Castro2006, Conn2002, Vasconcelos2006, Antonio-Nkondjio2005, Kebede2005, Klinkenberg2008, Singh1996, Nanda2000, Singh1999, Krishnamoorthy2005, Stoops2008, Lee1980, Zhou2005, Afrane2007, Afrane2005, Mutero2004, Carlson2004, Jacob2003, Klinkenberg2004, Ataka2001, Yanoviak2006, Vittor2006, Klinkenberg2004, Ramasamy1992, Amerasinghe1991, Sattler2005, Rosenbergl990, LindbladKA2000, Munga2006, Leonardo2005, Vanwambeke2007
					agricultural development (including irrigation and orchard), deforestation, construction, social factors, rice plantation, human settlement, brick making, road building, wetland development, urbanization	N. America/C. America, S. America, Africa, Eurasia, Oceania, Africa	Tropical	residential rainfed mosaic croplands, populated rainfed croplands, populated forests, residential rangelands, remote rangelands, populated forests, urban, irrigated villages, rice villages, residential villages, rainfed villages, irrigated villages, cropped and pastoral villages, rice villages, residential irrigated croplands, rainfed mosaic villages		
malaria	<i>Plasmodium falciparum</i> <i>Plasmodium vivax</i> , <i>Plasmodium ovale</i> , <i>Plasmodium malariae</i>	protozoa	SHP	human						

Disease	Pathogen name	Pathogen type	Multi or Single Host		Land Use Type	Region	Climate	Anthropogenic biome	Transmission Type	Reference
			Pathogen	Host						
dengue	denguevirus (with malaria in article)	protozoa/virus	SHP	human	agricultural development	Eurasia	Tropical	residential irrigated croplands, populated irrigated croplands, rainfed villages, remote croplands	VB	Vanwambeke et al. (2007)
onchocerciasis	<i>Onchocera volvularis</i>	nematode	SHP/MHP	human	deforestation, human settlement (immigration)	Africa	Tropical	rainfed villages, rainfed mosaic villages, residential croplands, populated forests, residential rangeland	VB	Wilson et al. (2002); Traore et al. (1997); Muro and Mziray (1990); Fischer et al. (1997)
	<i>Oesophagostomum sp.</i>									
	<i>Cryptosporidium</i>									
	<i>Giardia</i>									
	nematodes									
	<i>Strongyloides fulleborni</i>									
	<i>Strongyloides stercoralis</i>									
	<i>Oesophagostomum sp.</i> , an unidentified strongyle,									
	<i>Trichuris sp.</i> ,									
	<i>Ascaris sp.</i> ,									
	<i>Colobenterobius sp.</i> , one cestode ( <i>Bertiella sp.</i> ),									
primate endoparasitism	protozoans ( <i>Entamoeba spp.</i> , <i>Giardia sp.</i> )	protozoa, helminth, cestode	MHP	primate-colobus, red-tailed guenons	forest fragmentation, selective logging	Africa	Tropical	residential rainfed mosaic croplands, populated forests,	direct and trophic	Gillespie and Chapman (2006); Salzer et al. (2007); Gillespie et al. (2005); Gillespie and Chapman (2008, 2007)
primate endoparasitism	<i>Giardia sp.</i> , pinworm, <i>helminth</i> , <i>coccidia</i> , <i>trematode</i> , <i>strongyle</i>	fluke, roundworm, protozoa	SHP/MHP	howler monkey	forest fragmentation	N. America/C. America	Tropical	populated forests, remote forests, residential rainfed mosaic croplands	direct and trophic	Vitazkova and Wade (2006); Trejo-Macias et al. (2007)

Disease	Pathogen name	Pathogen type	Multi or Single Host		Main host	Land Use Type	Region	Climate	Anthropogenic biome	Transmission Type	Reference
			Pathogen type	Host							
Hantavirus	hantavirus	virus	MHP		deer mice, Z. breviceauda	habitat fragmentation, forest fragmentation, agricultural development, deforestation	N. America/C. Tem- America	perate	residential rainfed mosaic croplands, remote croplands, populated rainfed croplands	direct	Langlois et al. (2001); Suzan et al. (2006); Ruedas et al. (2004)
malaria and schistosomiasis	plasmodium and schistosoma	protozoa and trematode	SHP and MHP		human	agricultural development	Eurasia	Tropical	residential rainfed mosaic croplands, populated forests, urban, rainfed mosaic villages, populated forests, residential irrigated croplands	VB and trophic	Leonardo et al. (2005)
malaria vector	general	ns	general		vertebrates	deforestation, urbanization, pastoralization	Oceania	Temperate	populated forests, residential rainfed mosaic croplands, urban, pastoral villages, cropped and pastoral villages, remote rangelands, barren, populated rainfed croplands, residential rainfed croplands, residential rangelands, residential rainfed mosaic croplands, residential irrigated croplands, populated forests, rainfed villages	VB	Leishnam et al. (2006)
schistosomiasis	<i>Shistosoma mansoni</i>	trematode	MHP		human	deforestation, urbanization, agricultural development (irrigation, rice), deforestation, dam building, land reclamation, human land use-general	Africa	Tropical and Sub-tropical	residential rainfed mosaic croplands, residential irrigated croplands, populated forests, rainfed mosaic villages	trophic	Njiokou et al. (2004); el Sayed et al. (1995); Yapi et al. (2005); Muigai et al. (1989); Umeh et al. (2001); de Clercq et al. (2000); Matthys et al. (2007)
	<i>Schistosoma hematobium</i>	trematode	MHP		human	deforestation, urbanization, agricultural development (irrigation, rice), deforestation, dam building, land reclamation, human land use-general	Africa	Tropical and Sub-tropical	residential rainfed mosaic croplands, residential irrigated croplands, populated forests, rainfed mosaic villages	trophic	Njiokou et al. (2004); el Sayed et al. (1995); Yapi et al. (2005); Muigai et al. (1989); Umeh et al. (2001); de Clercq et al. (2000); Matthys et al. (2007)

Disease	Pathogen name	Pathogen type	Multi or Single Host	Main host	Land Use Type	Region	Climate	Anthropogenic biome	Transmission Type	Reference
schistosomiasis	<i>Schistosoma japonicum</i>	trematode	MHP	human	agricultural development-rice and irrigation, deforestation	Eurasia	Subtropical, Temperate	residential rainfed mosaic croplands, populated forests, residential rangelands, populated rainfed croplands, rice villages, irrigated villages, rainfed villages	trophic	Spear et al. (2004); Yang et al. (2005); Leonardo et al. (2005)
schistosomiasis and hookworm	schistosoma and ancylostoma	trematode and nematode	MHP and SHP (nematode)	human	urban farming-agricultural	Africa	Tropical	rainfed mosaic villages, urban	trophic and DT	Matthys et al. (2007) Ozdenrol et al. (2008); Gibbs et al. (2006a); Gomez et al. (2008); Gibbs et al. (2006b); Bradley et al. (2008)
West Nile Virus	arbovirus-Flavivirus	virus	MHP	vertebrates	urbanization, suburbanization, deforestation,	N. America	Temperate, Subtropical	residential rainfed mosaic croplands, populated forests, residential irrigated croplands	VB	
Venezuelan equine encephalitis	encephalitis virus	virus	MHP	mammals, birds	deforestation	S. America	Tropical	populated inland croplands, residential rainfed mosaic croplands, residential rangelands, pastoral villages	VB	Barrera et al. (2001)
tuberculosis-bovis	mycobacterium bovis	bacteria	MHP	vertebrates-wild boar this study, bovine	woodland management	Eurasia	Temperate	residential rainfed mosaic croplands, rainfed mosaic villages, populated forests	direct	Vicente et al. (2007)
amphibian trematodiasis-Ribeiroia	<i>Ribeiroia ondatrae</i>	trematode	MHP	amphibian larvae, snail, bird	aquatic eutrophication	N. America	Temperate	NA	trophic	Johnson et al. (2007a)

Disease	Pathogen name	Pathogen type	Multi or Single Host	Main host	Land Use Type	Region	Climate	Anthropogenic biome	Transmission Type	Reference
amphibian trematodiasis and nematodiasis	trematodes and nematodes, different species	trematode, nematode	MHP and SHP	amphibian host- frog intermediate Rana sylvatica, duck definitive host frog and snail, amphibian, other intermediate host, snail, canid, Hyla versicolor gray tree frog	deforestation and agricultural development	N. America/C. America	Tropical	residential rainfed mosaic croplands, residential rangelands	trophic, direct	McKenzie (2007)
amphibian trematodiasis-echinostomiasis	<i>Echinostoma trivolvis</i>	trematode	MHP		aquatic eutrophication, urbanization, agricultural development	N. America	Temperate	residential rainfed mosaic croplands, rainfed mosaic villages, urban, rainfed mosaic villages, populated forests, residential irrigated croplands	trophic	Belden (2006); Skelly et al. (2007); Koprivnikar et al. (2006); King (2007)
trematode communities-general	trematode	trematode	MHP	avian, snail	restoration of saltland marshes	N. America	Temperate	residential rainfed croplands, urban	trophic	Huspeni and Lafferty (2004)
meningococcal meningitis	group A meningococci	bacteria	SHP	human	desertification, loss of vegetation cover	Africa	Tropical and Sub-tropical	many-trans African study	direct	Molesworth et al. (2003)
mermithid nematodes	mermithidae	nematode	MHP	spider	habitat fragmentation	N. America	Tropical	residential rainfed mosaic croplands, populated forests	direct?	Vandergast and Roderick (2003)

Disease	Pathogen name	Pathogen type	Multi or Single Host		Main host	Land Use Type	Region	Climate	Anthropogenic biome	Transmission Type	Reference
			Pathogen	Host							
mosquito-borne disease general	various		SHP and MHP		agricultural development, rice cultivation and irrigation, deforestation, agricultural development, agricultural development-irrigation	S. America, Eurasia	Subtropical and Tropical	rainfed mosaic villages, populated forests, residential rainfed mosaic croplands, rainfed mosaic villages, residential rainfed mosaic croplands, populated forests, cropped and pastoral villages, residential irrigated croplands, populated irrigated croplands	VB		Forattini et al. (1993); Amerasinghe and Ariyasena (1990); Forattini and Massad (1998); Mukhtar et al. (2003)
Chromoblastomycosis	<i>Cladophialophora carrionii</i>	fungus	MHP		human	Africa	Subtropical	populated rangelands, remote rangelands	direct		Esterre et al. (1997)
<i>Ribeiroia ondatrac</i>	<i>Ribeiroia ondatrac</i>	trematode	MHP		amphibian larvae, snail, bird	N. America	Temperate	NA	trophic		Johnson et al. (2007a)
amphibian parasites	trematodes and nematodes, different species	trematode, nematode	MHP and SHP		and agricultural development	N. America/C. America	Tropical	residential rainfed mosaic croplands, residential rangelands	trophic, direct		McKenzie (2007)



Disease	Pathogen name	Pathogen type	Multi or Single Host		Main host	Land Use Type	Region	Climate	Anthropogenic biome	Transmission Type	Reference
			Pathogen type	Host							
bobwhite intestinal helminth	<i>Raillietina spp.</i> , <i>Aproctella stoddardi</i> , <i>Cheilosporira spinosa</i> , <i>Cyrnea colini</i> , <i>Dispharynx nasuta</i> , <i>Heterakis isolonche</i> , <i>Tetrameres pattersoni</i> , <i>Trichostrongylus tenuis</i>	helminth (tape-worm and round-worm)	MHP and SHP		Bobwhite quail	agricultural development	N. America	Subtropical	residential rainfed mosaic, populated, irrigated croplands, residential irrigated croplands	direct and trophic	Davidson et al. (1991)
	Chromoblastomycosis	<i>Cladophialophora carrionii</i>	fungus	MHP	human and important host	deforestation	Africa	Subtropical	residential rainfed mosaic croplands, populated forests, residential rangelands, populated rangelands, remote rangelands	direct	Esterre et al. (1997)
Chronic wasting disease	Chronic wasting disease	prion	MHP		deer(mule, white-tailed) multiple species wild and domestic animals, such as cattle, goats, sheep and hares	suburban development	N. America	Temperate	populated irrigated croplands, urban, residential rangelands	direct	Farnsworth et al. (2005)
Crimean-congo hemorrhagic fever	Nairovirus-Bunyaviridae	virus	MHP			fragmentation of agricultural land between forests and shrubs	Eurasia	Temperate	many residential rainfed mosaic croplands, populated forests, remote rangeland	VB	Estrada-Pena et al. (2007)
Cryptosporidiosis	<i>Cryptosporidium spp</i>	protozoa	MHP		cattle, other mammals	agricultural development	N. America	Temperate	residential rainfed mosaic croplands, populated forests, remote rangeland	direct	Ziegler et al. (2007)

Disease	Pathogen name	Pathogen type	Multi or Single Host		Land Use Type	Region	Climate	Anthropogenic biome	Transmission Type	Reference
			Pathogen type	Host						
dengue and malaria	plasmidium denguevirus	protozoa virus	SHP	human	agricultural development	Eurasia	Tropical	residential irrigated croplands, populated irrigated croplands, rainfed croplands, remote croplands	VB	Vanwambeke et al. (2007)
diarrheal pathogens	giardia, <i>E. coli</i> , rotaviruses	bacteria, viruses, protozoa	SHP/MHP	human	road building	S. America	Tropical	residential rainfed mosaic croplands, populated forests	direct	Eisenberg et al. (2007)
Ehrlichiosis and Anaplasmosis	<i>Ehrlichia chaffeensis</i> and <i>Anaplasma phagocytophilum</i>		MHP	deer reservoir	forest fragmentation	N. America	Subtropical and Temperate	many	VB	Wimberly et al. (2008)
Highly Pathogenic Avian Influenza	influenza (orthomyxovirus)	virus	MHP	avian	agricultural development forest	Eurasia	Tropical	rice villages, irrigated villages	direct	Gilbert et al. (2007)
Puumala virus	puumalavirus	virus	MHP	bank vole	fragmentation, deforestation	Eurasia	Temperate	rainfed mosaic villages	direct	Tersago et al. (2008); Linard et al. (2007)
rabbit intestinal cestodiasis/nematodiasis	<i>Trichostrongylus retortaeformis</i> and the cestode <i>Mosgovoyia pectinata</i>	nematode and cestode	SHP	Lepus timidus/rabbit	healthland to forestry stands	Eurasia	Temperate	residential irrigated croplands, populated irrigated croplands, remote forests	direct and trophic	Hulbert and Boag (2001)
Rabies reptile hemoparasites	lyssavirus	virus	MHP	mammals-raccoons	deforestation	N. America	Temperate	urban, residential rainfed mosaic croplands, populated rainfed croplands, irrigated croplands, cropped and pastoral villages, pastoral villages	direct	Jones et al. (2003)
	hemogregarine	protozoa	NS	reptile(lizard)	forest degradation	Eurasia	Temperate	residential rainfed mosaic croplands	VB	Amo et al. (2007)

Disease	Pathogen name	Pathogen type	Multi or Single Host		Main host	Land Use Type	Region	Climate	Anthropogenic biome	Transmission Type	Reference
			Pathogen type	Single Host							
Toxoplasmosis	<i>Toxoplasma gondii</i>	protozoa and trematode	MHP and SHP		vertebrate	agricultural development, urbanization, runoff	N. America	Subtropical	residential rainfed mosaic croplands, populated irrigated croplands, residential croplands, residential rangelands, urban	trophic	Miller et al. (2008)
schistosomiasis and hookworm	schistosoma and ancylostoma	nematode	(nematode)		human	urban farming-agricultural	Africa	Tropical	rainfed mosaic villages, urban	trophic and DT	Matthys et al. (2007)