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Applying Ecological Theory to Understand Biological Diversity, Anthropogenic Disturbance, and Disease among Terrestrial Small Mammals in Western Uganda

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Doctor of Philosophy

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B.A., Florida State University, 2003
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An abstract of
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Abstract

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Through the study of disease ecology, we seek to understand the mechanisms driving the complex interactions occurring among parasites, their hosts, and their environment. Ecologists dissect these complex ecological interactions through generalized principles, theories, and hypotheses. In order to understand the applicability and universality of these concepts, they must be applied to investigations conducted in natural systems. The aim of my dissertation was to test the generality of several ecological theories in a complex natural system. These studies were conducted in and around Kibale National Park, which is located in forested western Uganda. I examined terrestrial small mammal assemblages, consisting of members of the Order Rodentia (rodents) and Soricomorpha (shrews). These small mammals were collected from natural habitats experiencing varying intensities of anthropogenic disturbance. Additionally, I investigated the parasites they harbor, specifically poxviruses, *Trypanosoma* spp., *Giardia* spp., *Cryptosporidium* spp, and ectoparasites, which included fleas, lice, ticks, and mites. My work contributes to the field of disease ecology by exploring ideas and theories primarily restricted to less complex study systems. The four studies contained within my dissertation represent the successfully application of several foundational theories and novel ideas to improve our knowledge of how parasites are impacted by habitat disturbance and alterations in host community structure. My findings provide empirical evidence of the value and pitfalls of using generalized principles, theories, and hypotheses to understand host and disease dynamics in a complex natural system.

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CHAPTER 1

Ecological theory and practice at the interface of biological diversity, anthropogenic disturbance, and disease

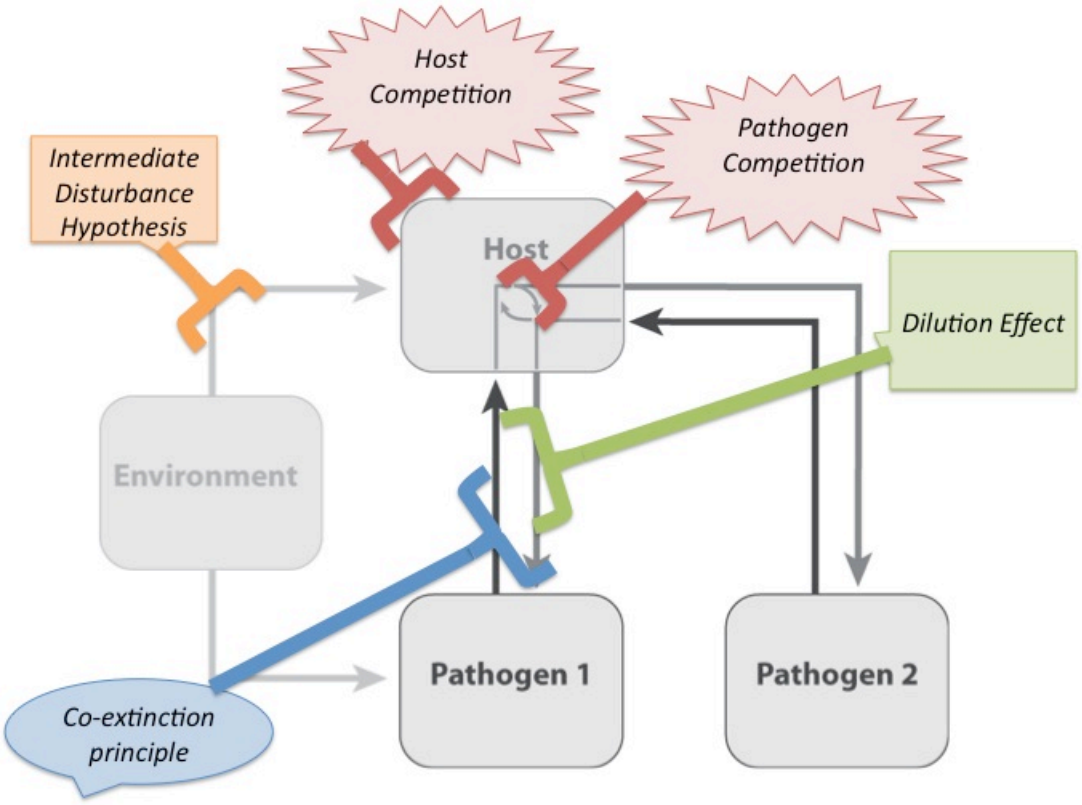
Central to the study of ecology is the quest to understand the mechanisms underlying patterns observed in nature to generate hypotheses, theories, and principles (Lawton 1999; Lange 2005). Ideally, these hypotheses, theories, and principles should be broadly applicable and repeatable in different ecological systems under varying conditions. Conducting controlled experimental studies, both in the laboratory and the field, build upon our knowledge of these ecological mechanisms. Experimental studies often reduce the complexity of natural systems by systematically controlling influential variables. Such controlled environments provide the framework for gaining insights into many of the underlying mechanisms of ecological theory. Ideally, these insights are then examined through observational studies of natural systems to determine the generality of such theoretical concepts in more complex systems (Evans *et al.* 2013)

Disease ecology is the study of interactions and patterns among parasites, hosts, and their habitats in complex environments (Poulin 2007). This approach has improved our understanding of disease occurrence and emergence (Shea & Chesson 2002; Morand & Krasnov 2008; Lloyd-Smith 2013). Emerging and re-emerging infectious diseases are a global health concern (Daszak, Cunningham & Hyatt 2000). The dominant paradigm

for the study of such pathogens (also referred to as parasites) has been reductionist—controlled experiments of single-host, single-pathogen interaction. However, the need for alternate ecologically-driven paradigms to address emerging infectious disease concerns has been increasingly acknowledged (Blaser 2006; Telfer *et al.* 2010; Roche *et al.* 2012). It is widely accepted that interactions between abiotic environmental factors affect disease emergence and disease risk (McMichael, Woodruff & Hales 2006; Pounds *et al.* 2006). Although, less-often investigated biotic factors (e.g. host diversity, habitat disturbance, pathogen-pathogen competition) likely influence disease dynamics, as well, and may provide valuable information for identifying critical control points for improved public health and wildlife conservation (Daszak, Cunningham & Hyatt 2000; Roche *et al.* 2012).

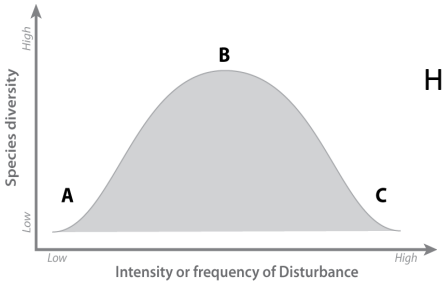
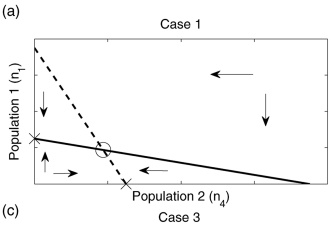
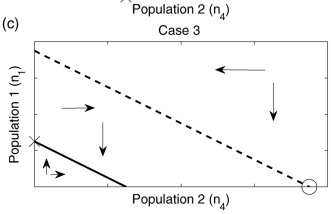
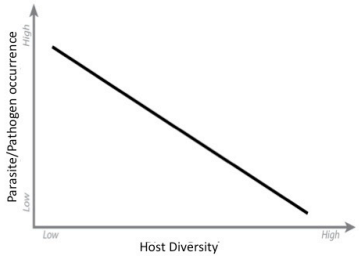
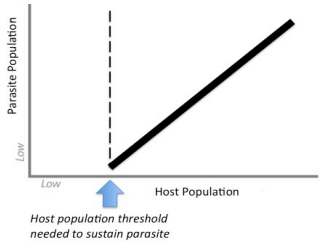
Habitat quality, host community assemblage, host susceptibility/resistance, and pathogen-pathogen interactions are interconnected in complex and dynamic ways (Woolhouse *et al.* 1997; Ostfeld, Keesing & Eviner 2008; Beldomenico & Begon 2010). These factors can act independently, synergistically, and in feedback loops to influence disease dynamics in natural settings (Figure 1.1: see grey-scale components). Consequently, disease dynamics can rarely be explained by examining one component of a complex natural system. Understanding the community components and interactions associated with disease emergence and persistence are expected to provide valuable information for unlocking mechanisms driving disease dynamics in the environment (Dobson 2004; Lafferty 2010; Telfer *et al.* 2010).

Figure 1.1: Complex interactions among the environment, host, and pathogens. Several ecological theories, principles, and hypotheses (shown in color) relate to the interactions among host, pathogens, and their environment (shown in grey). The colored bars indicate the specific ecological interactions these theoretical concepts directly impact. These theories are not mutually exclusive and can be applied synergistically or in conflict to determine disease dynamics.



A greater understanding of these complex interactions among parasites, hosts, and their environments benefits science, but more importantly, it improves conservation and potentially public health outcomes. There are numerous foundational ecological theories, hypotheses, and principles (Table 1.1) that are key to understanding disease dynamics in natural and anthropogenic environments (Figure 1.1: see color components). These theories are rarely mutually exclusive, often act synergistically, and at times may even be contradictory. Progress in disease ecology requires further empirical testing of foundational principles and theories as well as development of additional novel concepts.

Table 1.1: List of relevant ecological concepts, their graphical expression, basic equations.

Theoretical Concept	Components	Graphical representation	Basic equation	Key References
Intermediate Disturbance Hypothesis	**H=Species Diversity** (measured with Shannon index (H) or Simpson (D)) **D= Habitat disturbance** measured along a gradient from least (D=-1) to most disturbed (D=1)		$H = \lvert D \rvert$	Diamond 1989; Koh *et al.* 2004; Dunn *et al.* 2009
Competition/ Competitive exclusion principle	**A and B= competing species** **N= population size of A or B** **r= reproductive rate,** which if unrestricted follows logistic growth **K= carrying capacity** for each population **α and β= competition coefficient,** which functions to convert one species into the other. As the coefficient increases for one species, so does the influence and negative impact on the other species	  *Image from Fujiwara et al. 2011	*Lotka and Volterra model* $dN_A/dt = r_A*N_A*((K_A-N_A-\alpha N_B)/K_A)$ $dN_B/dt = r_B*N_B*((K_B-N_B-\beta N_A)/K_B)$ When KB α > KA, the interspecific effect of species B on species A is greater than the intraspecific competition among members of species A. When KB α < KA, the intraspecific competition effect on KA, is greater than the interspecific competition.	Volterra 1926; Lotka 1932; Gause 1932; de Roode et al. 2004; Blaser 2006; Balmer et al. 2009
Disease-Diversity Patterns (broad application of *dilution effect*)	**H=Host Diversity** (measured with Shannon index (H) or Simpson (D) **P= Pathogen/parasite occurrence/prevalence** among population of host		$P = 1/H$	Ostfeld 1995; Ostfeld & Keesing 2000; Randolph & Dobson 2012;
Co-extinction Principle	**P= Pathogen/parasite occurrence/prevalence** among population of host **M= Host occurrence/prevalence** in environment **x= coextinction coefficant**, x>1 if closely assocaited with host, which is shown graphically with a host population threshold		$P = M/x$ If the pathogen/parasite is closely assocaited with host, the parasite population will reach 0 (extinction) prior to extinction of the host.	Diamond 1989; Koh *et al.* 2004; Dunn *et al.* 2009

1.1 THEORY and ITS ROLE IN DISEASE ECOLOGY

The primary aim of my dissertation was to test the generality of specific ecological theories in a complex natural system. The studies within my dissertation examine how habitat disturbance may affect host-parasite interactions in a complex natural system. The following section outlines the principles, theories, hypotheses, and ideas (all terms are synonymous for our purposes) that form the basis for my work. Table 1.1 provides an overview of these concepts.

Competitive exclusion principle

The *competitive exclusion principle* was first articulated in theory and then examined empirically in the laboratory and field. Although Charles Darwin did not specifically refer to his thoughts and theory as the "exclusion principle," the ideas were eloquently presented (Darwin 1859). Darwin stated, "Competition will be the most severe between those forms which are most nearly related to each other in habitats, constitution, and structure." He elaborated on this observation with examples of invasive species negatively impacting native species. Darwin recognized that an invasion by an invasive species might result in competition fierce enough to cause exclusion, and therefore extinction, of the native species from a habitat (Hardin 1960). The theoretical discussions and recognition of competitive exclusion in nature preceded all mathematical theory and experimental studies. This theoretical framework, masterfully laid out by Darwin, was later applied to mathematical models (Volterra 1926; Lotka 1932), tested in the laboratory (Gause 1932), and then tested in the field (Connell 1961).

Competition occurs when two or more species, which share a limited resource, are both negatively impacted by the presence of the other. Theory suggests that where competing species share an identical ecological niche, one species will dominate and exclude its competitor, leading to local extinction. This process is referred to as competitive exclusion. Evolutionary outcomes of such competition include niche partitioning and character displacement, where competition either drives competing species to use different resources or selectively encourages phenotypic changes to accentuate the largest differences among species (Brown & Wilson 1956). The importance of competition in determining the distribution and abundance of plants and animals has strong historical support. These classic empirical studies of competition and exclusion were conducted in controlled laboratory investigations of microorganisms (Gause, Nastukova & Alpatov 1934) and in natural settings investigating larger organisms, such as warblers (MacArthur 1958) and barnacles (Connell 1961). This theory of competition has been less-often applied to understanding spatial and temporal patterns of microorganisms in natural environments.

Competition plays a major role in defining a species niche and spatial distribution. Abiotic and biotic environmental factors, interspecific interactions, and other ecological factors contribute to restricting a species' distribution. Joseph Grinnell stated that it would be "axiomatic that no two species regularly established in a single fauna have precisely the same niche relationship" (Grinnell 1917). He adopted the term "niche" to describe the causes for the small spatial range of the California Thrasher (Grinnell 1917). The Grinnellian niche was defined generally as the spatial range of a population. Charles Elton elaborated on the definition, declaring a niche as an organism's functional role in

the community, much like “trades or jobs or professions in a human community” (Elton 1927; Begon, Townsend & Harper 2006). Elton stressed the importance of community interactions and food web structure on niche formation. Later, Hutchinson combined both niche concepts into one model accounting for the biotic factors and interspecific interactions (Hutchinson 1957). The amount of limiting similarity between two organisms determines the intensity of interspecific competition and results in either exclusion from the shared niche or establishment of two niches in coexistence (Macarthur & Levins 1967). Niche theory and the *competitive exclusion principle* are closely associated. Intense competition between two species with an identical ecological niche will continue until the better competitor excludes the other. These theories are applicable to both hosts (e.g. warbles and thrashers) and pathogens (e.g. poxviruses) (Lloyd-Smith 2013).

Competition between pathogens is usually indirect and often identified specifically as apparent competition. Although other types of competition between pathogens exist, such as exploitive (Gause, Nastukova & Alpatov 1934) and interference (Connell 1961), I will focus specifically on apparent competition. Apparent competition is an indirect interaction between two competitors through a shared natural enemy. The presence of the shared enemy has a varied negative effect on the two populations of competing organisms. This varied negative effect provides a competitive advantage to one species (Holt 1977; Morris, Lewis & Godfray 2004). Apparent competition between pathogens occurs when the host immune system is considered the *predator*. Apparent competition can be observed within an individual host and at the population level. Immune response to infection can be either innate or adaptive. The adaptive immune

system is responsible for producing antigen-specific defense mechanisms in the form of antibodies. Antibodies can provide long-lasting and protective immunity to the host. This adaptive immune response not only protects the host from future infections from the initial infectious agent, but may also protect the host from antigenically similar pathogens (Lloyd-Smith 2013). An adaptive cross-protective immune response can render an individual host non-consumable to a competing pathogen after infection, recovery, and proper immune response. These pathogen interactions deplete the resource by causing a decrease in the susceptible host population and driving the population's immunity (herd immunity) above the threshold needed to eradicate the competing pathogen (Gani & Leach 2001). Another example of apparent competition occurs within the host during coinfection. When the cell-mediated immune response is activated during coinfection, the immune response might prey upon another coinfected pathogen at an increased rate. This unbalanced predation could be more detrimental to one pathogen than another, leading to exclusion. This has been experimentally explored between two clones of the blood cell parasite *Plasmodium chabaudi* (de Roode *et al.* 2005). Immunocompetent mice infected with a mixed population of clones will immunologically "prey upon" the less-virulent strain giving the virulent strain a competitive advantage in coinfected mice. In the absence of an immune response or "predator," virulent clones have no apparent competitive advantage over avirulent clones (Raberg *et al.* 2006). For apparent competition to ultimately lead to competitive exclusion on the population level, the pathogen (or vaccine) must persist at relatively high prevalence in the population, provide immunity over long periods of time, or result in a chronic infection.

Intermediate disturbance hypothesis

The *intermediate disturbance hypothesis* (which incorporates the *competitive exclusion principle*) was first discussed by E. P. Odum in 1963 and strengthened by J.P. Grime and J.H. Connell (Odum 1963; Grime 1973; Connell 1978) before examination in field or laboratory settings. This hypothesis predicts that species diversity will peak at intermediate levels of habitat disturbance along a gradient of disturbance. This unimodal-peaked relationship between species diversity and habitat disturbance was originally considered to be the product of coexistence of disturbance-tolerant species (or colonizers) and competitively dominant species within the community (Horn 1975; Connell 1978). Following well-articulated theoretical arguments by E.P. Odum, J.P. Grime, and J.H. Connell, this hypothesis was then tested in multiple field experiments, primarily using systems involving sessile marine organisms (Lubchenco 1978; Sousa 1979).

The *intermediate disturbance hypothesis* has evolved since first defined by Joseph Connell in 1978. It has been proposed that coexistence of species at intermediate levels of disturbance should be attributed to a complex of several coexistence-promoting mechanisms and not simply to the reduction of competitive exclusion pressures in the intermediately disturbed habitats (Roxburgh, Shea & Wilson 2004; Shea, Roxburgh & Rauschert 2004). The numerous mechanisms producing the peaked pattern associated with disturbance and diversity include, but are not limited to, alterations in the dynamics of ecosystems. This includes alterations of predation in disturbed habitats (Paine 1966; Chesson 2000), an increase in niche diversification and spatial patchiness (Hutchinson 1957; Bartha, Czaran & Scheuring 1997), and the varied adaptability of different species to disturbance (Connell 1978).

Rarely has the *intermediate disturbance hypothesis* been tested in terrestrial organisms. Therefore, our understanding of its applicability to non-sessile terrestrial communities experiencing anthropogenic disturbance is minimal (Colwell & Fuentes 1975; Fuentes & Jaksic 1988; Ferreira & van Aarde 2000; Granjon *et al.* 2005). A recent study investigating small mammals in the Philippines identified an increase in disturbance-tolerant species driving an overall increase in species richness associated with disturbed habitats (Rickart *et al.* 2011). These results support similar findings in Africa, which identified an increase in small mammal diversity associated with severe anthropogenic disturbance (Ferreira & van Aarde 2000). Overall, however, there are few empirical examples of the *intermediate disturbance hypothesis* being generalized to motile terrestrial systems.

The *intermediate disturbance hypothesis* has become an established ecological theory; however, despite its acceptance, this hypothesis is not consistently supported by empirical evidence (Fox 2013). The unimodal-peaked curve associated with the interaction between diversity and disturbance has been recorded to occur in less than 20% of field studies (Mackey & Currie 2001). The failure to observe this peaked pattern is arguably due to necessary conditions not met in most of these study systems (Fuentes & Jaksic 1988; Mackey & Currie 2001).

Once we have established the impact of habitat disturbance on host diversity and community structure, we can then explore disease dynamics. Habitat disturbance, as described by the *intermediate disturbance hypothesis*, leads to direct alterations of host community structure, followed by an indirect impact on disease dynamics (Figure 1.1).

Host Diversity and Patterns of Infection

It is hypothesized that disease dynamics will be directly altered by changes in host diversity. The most discussed and debated hypothesis related to the ecological link between host diversity and disease is the *dilution effect* (Schmidt & Ostfeld 2001). The *dilution effect* describes how high species diversity may "dilute" the ability of a pathogen-amplifying host and vector to readily transmit a pathogen (Ostfeld & Keesing 2000b; Schmidt & Ostfeld 2001). Thus, when an ecosystem experiences a loss of biodiversity, pathogen prevalence should increase due to increased transmission events among dominant susceptible reservoir species (Schmidt & Ostfeld 2001).

In order for *dilution effect* to be successfully applied to a disease system, several strict requirements need to be met (Ostfeld & Keesing 2000a; Keesing, Holt & Ostfeld 2006). The *dilution effect,* as defined by these requirements, is not as general as often proposed (Carver *et al.* 2011) and rarely explains other vector-borne disease systems (Loss *et al.* 2009). Recently, this hypothesis has been more broadly described as *any* situation where higher biodiversity results in lower disease risk (Salkeld, Padgett & Jones 2013; Young *et al.* 2013). This broader definition of the *dilution effect* has been preferred for its generality to many disease systems (Mills 2005; Allan *et al.* 2009; Clay *et al.* 2009; Young *et al.* 2013).

Disease dynamics are dependent on the idiosyncrasies of pathogen, host, vectors, and habitat (Salkeld, Padgett & Jones 2013). A recent meta-analysis of 16 studies suggests that the relationship between biodiversity and disease risk is not as simple as proposed by the *dilution effect* (Salkeld, Padgett & Jones 2013). This meta-analysis, and other recent studies, provided conflicting evidence that biodiversity has a protective

effect against disease emergence (Randolph & Dobson 2012), broadening discussion regarding the relationship between host diversity and pathogen prevalence (Randolph & Dobson 2012; Young *et al.* 2013).

The idea that disease dynamics may be altered due to changes in host community composition is a primary focus of this dissertation, as opposed to proving or disproving the *dilution effect* in particular. The *dilution effect* is often presented as a method and argument for conserving global biodiversity (Ostfeld & Keesing 2000b), but this may not be a theoretically sound argument in the context of the *intermediate disturbance hypothesis* and our understanding of the impacts of habitat disturbance on host diversity. A recent review noted that investigators often assume that habitat disturbance leads to a loss of diversity and that these two variables can be interchanged in investigations of the *dilution effect* (Salkeld, Padgett & Jones 2013) (Table 1). But, from our understanding of the *intermediate disturbance hypothesis*, there is not necessarily a linear relationship between disturbance and diversity. Thus, following *dilution effect* and *intermediate disturbance hypothesis,* we would expect areas of intermediate disturbance (i.e. high host diversity) to have the lowest occurrence of pathogen prevalence, while the most pristine and most disturbed areas (i.e. lowest host diversity) will harbor the highest prevalence of infection.

It would be prudent for both conservation and public health efforts to find further evidence proving that protecting biodiversity can lead to declines in overall disease occurrence and emergence, but conflicting theories suggests this is not always the case (Hudson, Dobson & Lafferty 2006; Randolph & Dobson 2012). In general, my dissertation examines a variety of disease-diversity relationships observed in natural

systems (not specifically *dilution effect)* in order to improve our understanding of the mechanisms driving or opposing these patterns.

Co-extinction principle

Although reductions of biodiversity have been linked to higher pathogen prevalence (Keesing *et al.* 2010), in some cases, pathogen richness declines with the loss of biodiversity (Lafferty 2012). There are four primary external ecological drivers of extinction that are well accepted and not necessarily mutually exclusive; these include, habitat loss, species invasions, overkill, and co-extinctions or cascade events of extinctions (Diamond 1989). The mechanism driving co-extinction is supported by mathematical models and derived from the theory that extinction of a host leads to an often unnoticed and understudied extinction of their closely-associated parasites (Dunn 2009). Co-extinction is estimated to account for a majority of all global species extinction events (Koh *et al.* 2004; Dobson *et al.* 2008). The poorly recognized co-extinction of parasites with hosts has been further underestimated due to a historical inability to properly classify cryptic species of parasites. More accurate accounts of parasite diversity now occur due to the growing accessibility of molecular methods for phylogenetic classification (Miura *et al.* 2005). But, unfortunately, we are likely identifying these parasites at a slower rate than the rate of parasite extinction (Dobson *et al.* 2008).

The indirect impacts of parasite extinctions on food webs are predicted to cause notable disruptions to ecosystems (Lafferty *et al.* 2008; Dunne *et al.* 2013), as parasites maintain the integrity of the web itself (Dobson 2008). Additionally, parasites with high

host specificity are thought to be at greater risk of extinction than their hosts (Anderson & May 1979; Altizer, Nunn & Lindenfors 2007). Parasites that are sensitive to habitat disturbance may act as valuable sentinels for extinction risk of their hosts (Altizer, Nunn & Lindenfors 2007; Bush, Reed & Maher 2013). Despite the theory's conceptual strength, there are few empirical studies providing evidence of a loss of both host and parasite as a consequence of habitat disturbance (Bush, Reed & Maher 2013).

1.2 EXAMINING the THEORY

Empirical challenges to theories need to occur not only in the laboratory but also in natural field studies. In the words of Joseph H. Connell, "Ecological theory does not establish or show anything about nature. It simply lays out the consequences of certain assumptions. Only a study of nature itself can tell us whether these assumptions and consequences are true ... not to suggest that theory as such is worthless, but to test it" (Connell 1983).

The aim of the next four chapters of my dissertation was to examine the generality of several ecological theories in a complex natural system. Broadly, my work focused on examining theories and ideas aforementioned as they pertained to the impacts of habitat disturbance on host communities and host-parasite interactions. All studies within this dissertation were conducted in and around Kibale National Park, in rural forested Uganda. The host species of interests were terrestrial small mammals, specifically members of the Order Rodentia (rodents) and Soricomorpha (shrews) from habitats experiencing various types and degrees of anthropogenic disturbance (i.e. logging and forest fragmentation). Additionally, parasites associated with these small mammals were

also examined, which included *Trypanosoma* spp., *Giardia* spp., *Cryptosporidium* spp., and ectoparasites (fleas, lice, ticks, and mites).

Chapter 2 builds on historical studies conducted in Kibale National Park that identified a positive association between small mammal diversity and logging intensity (Isabirye-Basuta 1979; Isabirye-Basuta & Kasenene 1987; Struhsaker 1997). In these works, the point of highest diversity (i.e. intermediate disturbance) was not identified but considered to follow the *intermediate disturbance hypothesis*. Chapter 2 examined small mammals along a broader gradient of habitat disturbance than the previous studies examined, providing a more complete understanding of the impacts of habitat disturbance on small mammal diversity and community structure than previously known. This chapter lays the groundwork for studies investigating the impacts of disturbance on host community composition and disease dynamics.

Chapter 3 explored the idea that parasite prevalence is linked to host diversity and species richness (i.e. broad concept of the *dilution effect*). In this chapter, I investigated if parasite prevalence and richness are indeed impacted by alterations in host diversity, host density, and habitat disturbance. Additionally, each individual small mammal's parasite community was explored to determine if an individual host's taxonomic classification (i.e. species) or habitat was a more significant determinant of the parasites present on each individual host.

After examining parasites at the coarse level in Chapter 3, Chapter 4 focused on a finer taxonomic characterization. Trypanosomes are of particular importance because African rodents are known to be infected with native trypanosomes (Adams, Hamilton & Gibson 2010) but also show susceptibility to an invasive trypanosome (*T. lewisi*)

(Dobigny *et al.* 2011). In this chapter, I characterized these parasites to the species level and identified two species of trypanosomes—one native, one invasive. Using a finer scale of phylogenetic examination, anthropogenic habitat disturbance was investigated as a potential threat of co-extinction on native trypanosome parasites and their associated mammalian hosts. Additionally, in Chapter 4, I further investigated the risk of pathogen spillover of an invasive parasite into native host populations.

I explored parasite-host relationships in Chapters 3 and 4 by examining active infections determined either by microscopy or molecular analysis. In Chapter 5, I examined pathogens, specifically poxviruses, by serological techniques (i.e. ELISA and western blot assay). Members of the Poxvirus family within the genus *Orthopoxvirus,* induce long-term adaptive immunity within recovered hosts. This immunity provides cross-protection to all viruses within this genus due to a high degree of cross-immunogenicity within the genus. Members of this viral genus include monkeypox, cowpox, and variola (the causative agent of smallpox). A host's adaptive immune response can act as a competitor against other similar pathogens preventing future infections with competing cross-protective poxviruses. The restricted distribution of monkeypox virus to central Africa may result from such pathogen-pathogen competition. This competition may lead to the exclusion of monkeypox from these neighboring areas or at least prevent its invasion (Lloyd-Smith 2013). In Chapter 5, I explored the idea that the c*ompetitive exclusion principle,* resulting from apparent competition among immunologically cross-protective viruses, may result in the restricted spatial distribution of similar pathogens.

The findings within my dissertation provide empirical evidence supporting several generalized ecological theories and aid in our understanding of disease dynamics in natural settings. My work contributes to the field of disease ecology by exploring ideas and theories primarily restricted to less complex study systems. The four studies I conducted in rural forested Uganda represent the successful application of several foundational theories and novel ideas to improve our knowledge of how parasites are impacted by habitat disturbance and alterations in host community structure.

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CHAPTER 2

Effects of Habitat Disturbance on African Small Mammal Communities

2.1 SUMMARY

Anthropogenic habitat disturbance can radically disrupt the structure of native terrestrial small mammal communities. Habitat disturbance, in the form of logging and fragmentation, may lead to local extinctions of native forest-dwelling species. Additionally, disturbance may also alter the environment in ways that may be more suitable for grassland-associated and invasive or peridomestic species. To improve our understanding of this process, I surveyed terrestrial small mammals along a gradient of habitat disturbance in and around Kibale National Park (KNP) in western Uganda. I collected 327 small mammals from seven distinct habitats including relatively intact forest, forest logged at low intensity, forest logged at high intensity, forest edge, two forest fragments, and human dwellings. In total, 23 terrestrial small mammal species were identified from within and around KNP. Ten of the species observed were previously unreported in this region, including *Dasymys incomtus, Mastomys natalensis, Oenomys hypoxanthus, Praomys misonnei, Scutisorex somereni, Crocidura dolichura, C. fuscomurina, C.* cf. *littoralis, C. maurisca* and *C. olivieri*. I examined species composition among habitats and found small mammal community structure to be sequentially altered along the gradient of habitat disturbance. Additionally, I found highest levels of species richness and diversity in areas that experience moderate levels of

disturbance outside the national park (i.e. forest edge and fragments). High species richness was positively associated with increased coexistence of both grassland and forest-dwelling species. Finally, I compared the species diversity results within the boundaries of KNP from this study to those historically recorded, and I found them to be similarly associated. Anthropogenic disturbance in forested Uganda was associated with alterations in small mammal community composition, creating higher species richness and coexistence in moderately disturbed habitats.

2.2 INTRODUCTION

Habitat disturbance in the form of logging and forest fragmentation may lead to extinction of native forest-dwelling species while simultaneously becoming more suitable for the coexistence of species that prefer grassland and peridomestic environments. Overall, small mammals tend to be more tolerant to anthropogenic disturbance compared to larger mammals, due to their small home ranges, resource utilization, and relatively prolific reproductive rates (Lambert *et al.* 2003; Stenseth *et al.* 2003; Previtali *et al.* 2009). In addition, anthropogenic disturbance can lead to declines or extinctions of competitors and predators of small mammals, which may promote small mammal abundance and species richness in more disturbed habitats (Caro 2001; Goheen *et al.* 2013).

In response to habitat disturbance and fragmentation, small mammal species richness and diversity has been found to increase and peak at intermediate levels of disturbance (Caro 2001; Lambert *et al.* 2003; Pardini 2004). This positive association between species richness and habitat disturbance was initially thought to be the product

of the co-existence of disturbance-tolerant species (or colonizers) and competitively dominant species within intermediately disturbed habitats (Horn 1975; Connell 1978). More recently, the coexistence of species at intermediate levels of disturbance has been attributed to a complex of several coexistence-promoting mechanisms occurring synergistically to produce the unimodal relationship between species diversity and habitat disturbance (Roxburgh, Shea & Wilson 2004; Shea, Roxburgh & Rauschert 2004). These mechanisms include, but are not limited to, alterations in the dynamics of ecosystems, including altered predation (Paine 1966); increased niche diversification and spatial patchiness (Hutchinson 1957; Bartha, Czaran & Scheuring 1997); and varied adaptability of different species to disturbance with a coexistence occurring between disturbance tolerant and intolerant species (Connell 1978).

In order to understand the impact of habitat disturbance on community structure and composition among small mammal communities, I selected a study site, Kibale National Park (KNP) in western Uganda. There is a legacy of investigating floral and faunal diversity in KNP, often across a gradient of habitat disturbance including relatively intact primary forest, as well as areas that have experienced a history of both low and high intensity selective logging. Comparisons of species diversity among these forest compartments have been used to understand the impact of logging on primates (Skorupa 1998; Chapman *et al.* 2000), birds (Dranzoa 1998; Seavy & Apodaca 2002), plants (Sekercioglu 2002), parasites (Gillespie, Chapman & Greiner 2005), and insects (Molleman *et al.* 2006). Although species richness and diversity has been studied among small mammals in areas within KNP, the structural changes in the communities of these species across a gradient of disturbance extending outside the boundaries of KNP has

never been examined. Additionally, mammalian taxonomic identifications using both morphologic and molecular techniques have been minimally explored in this area. Understanding changes in species occurrence across habitats provides information on the mechanisms driving the changes observed in species richness.

Terrestrial small mammal diversity was examined in KNP in the 1970-80s (Isabirye-Basuta 1979; Kasenene 1984; Isabirye-Basuta & Kasenene 1987; Muganga 1989). These studies revealed a positive relationship between habitat disturbance and small mammal diversity when comparing relatively intact forest to areas recovering from variable intensities of selective logging (Struhsaker 1997). These results laid the foundation for future studies to examine a greater range of disturbed habitats and the underlying mechanisms of this pattern of increased species richness and diversity. To improve our understanding of the impact of disturbance on community composition and species richness, I examined small mammal communities across a broad gradient of habitat disturbance. I hypothesized that small mammal communities of similar composition and species richness would be higher in areas of intermediate disturbance due to the introduction and coexistence of grassland forest-dwelling and peridomestic species.

2.3 MATERIALS and METHODS

Study area

KNP is a medium-altitude tropical moist forest of approximately 795km^2 that was once part of contiguous forest from the Rwenzori Mountains in the west to Lake Victoria in the east [0 13’ to 0 41’N, 30’19’ to 30’22’E] (Struhsaker 1997; Chapman & Lambert

2000). Portions of KNP were logged to varied intensities in the 1960s creating compartments composed of relatively intact forest, as well as compartments that experienced either high or low intensity selective logging (Chapman *et al.* 2000). KNP was designated as a protected national park in 1993 and has since been protected by the Uganda Wildlife Authority (Struhsaker 1997).

Study design

KNP and surrounding areas represent a mosaic of habitats that have undergone various types and frequency of habitat disturbance. The sampling sites examined in my study were representative of these varying disturbance histories. The degree and nature of disturbance has been determined for these locations using previously described methods (Gillespie & Chapman 2006; Gillespie & Chapman 2008; Goldberg *et al.* 2008b). I sampled seven habitats, representing different treatments of anthropogenic disturbance (Figure 2.1). Three locations were sampled within the boundaries of KNP—a relatively intact forest compartment known as CC, a compartment that experienced low-intensity logging known as K14, and a compartment that experienced high-intensity logging known as K15. Small mammal communities were historically examined in these three forest compartments (Isabirye-Basuta 1979; Kasenene 1984; Isabirye-Basuta & Kasenene 1987; Muganga 1989; Lwanga 1994; Struhsaker 1997). Additionally, I examined areas outside KNP, which had not previously been documented; these sites included forest edge, two forest fragments, and 31 village homes on or near the western boundary of the park. Fragment 1 was located near the village of Bugembe and was

known as *Bugembe*. Fragment 2 was located near the trading center of the village of Kiko and was known as *Kiko*.

These two forest fragments have experienced different degrees of anthropogenic disturbance. Fragment 1 was 0.66 km^2 in area (Goldberg *et al.* 2008b), which bordered small-scale agriculture, homes, and papyrus swamp and was considered to experience a medium level of disturbance (Gillespie & Chapman 2008; Goldberg *et al.* 2008b). Additionally, Fragment 1 supports populations of red colobus (*Procolobus badius*), red-tailed guenons (*Cercopithecus ascanius*), and black-and-white colobus (*Colobus guereza*), which is indicative of a less disturbed forest fragment (Onderdonk & Chapman 2000). Fragment 2 has experienced more intense habitat loss, greater degradation, and no longer supports primate populations, but is over twice the size of Fragment 1 (1.48 km^2) (Onderdonk & Chapman 2000; Goldberg *et al.* 2008b). Fragment 2 was considered a heavily disturbed fragment (Goldberg *et al.* 2008a) and was surrounded by an industrial tea plantation, traditional trading center, small-scale agriculture, and human dwellings.

I used trapping webs 200 meters in diameter with 12 radii to accurately estimate the small mammal population (Anderson *et al.* 1983). Each radius contained 12 Sherman traps (7.6 x 8.9 x 22.9cm, H.B. Sherman Traps Inc., Tallahassee, FL, USA), with the first four traps set five meters apart and eight distal traps set at 10 meter intervals (Mills *et al.* 1999). The center of the web contained four Sherman traps and one Tomahawk trap (48.3 x 15.2 x 15.2 cm, Tomahawk Live Trap Co., Tomahawk, WI, USA). Additional Tomahawk traps were set 50 meters from the center in each cardinal direction. Each web contained a total of 153 traps and operated for three consecutive nights at each site during the summer of 2009. Since webs were not suitable for human dwellings, 10 traps were set

per dwelling with permission from homeowners. Traps were baited with peanut butter and millet in the evenings. Animals were collected the following morning to prevent trap-associated deaths.

Animal collection

Terrestrial small mammal collection and handling protocols were approved by IACUC committees at both Emory University (#062-2009) and the Centers for Disease Control and Prevention (CDC) (#1768). The Uganda Wildlife Authority and the Uganda National Council for Science and Technology granted permission for animal collection. Additionally, animals from human dwellings were collected with permission from local authorities (Local Chairman) and homeowners.

Terrestrial small mammals were removed from traps and immediately anesthetized with 5% isoflurane vapor to alleviate any stress from capture (Mills *et al.* 1995). While in a deep plane of anesthesia, blood was collected via cardiocentesis and animals were humanly euthanized using either exsanguination or a lethal dose of isoflurane. All animals were photographed and morphometrics were recorded (Nagorsen & Peterson 1980). Tissue samples were collected for molecular identification of species and other collaborative studies. Representative specimens of collected small mammals were decapitated, and the heads were stored in 70% ethanol.

Species identification

Skulls from a subset of animals were prepared using standard procedures (Nagorsen & Peterson 1980). Each skull was identified to species using established

mammalian guides (Delany 1975; Thorn & Kerbis Peterhans 2009) as well as the reference collection at the Field Museum of Natural History (Chicago, Illinois, USA).

Molecular techniques supplemented my photographic and morphological species determinations. DNA was extracted from spleens of *Praomys* spp., *Mus* spp., and *Crocidura* spp. using the DNA EZ1 tissue kit (Qiagen, Valencia, California, USA). The first half of the Cytochrome b gene (480bp) was amplified and sequenced using the forward MVZ05 and reverse 400R primers established in previously published material (Smith & Patton 1991; Peppers & Bradley 2000). Sequencing results were analyzed and compared to reference strains and GenBank sequences. Each species was further classified as primarily associated with forest (i.e. forest-dwelling) or grassland habitats based on the natural history of each species and historical records (Kingdon 1974; Delany 1975; Thorn & Peterhans 2009; Happold & Happold 2013a; Happold & Happold 2013b).

Community structure and species composition

Small mammal community composition was measured using pair-wise beta-diversity measurements, which identified relatedness of community structure between localities. Jaccard dissimilarity (Jost 2006), Bray-Curtis (Bray 1957), Morista-Horn (Horn 1966), and Chao indices (Chao *et al.* 2005) are pair-wise coefficients used for understanding community similarity and dissimilarity across ecological gradients (Faith, Minchin & Belbin 1987). Jaccard and Bray-Curtis (also known as Sørensen) ignore shared absences and only account for shared presence of species as evidence of similarity. Morista-Horn and Chao coefficients are less influenced by sample sizes than other indices (Anderson & Millar 2004). Additionally, the Chao index accounts for the

number of unseen species shared among communities (Chao *et al.* 2005). Habitats with communities of similar species composition will have pair-wise coefficients closer to zero compared to communities experiencing very different species composition, which will be closer to one. All analyses were performed using the R statistical program, *Vegan* package (R Development Core Team, http://www.R-project.org).

To measure levels of coexistence of species with preferences for forest, grassland, and mixed habitats, I first examined the natural history of each species through previously published studies. I measured the proportion of forest-dwelling species (F_p) within each habitat and accounted for those species, which primarily occupy mixed forest-grassland as 50% proportion. To generate a coexistence measurement or index (CoEx), I devised a simple formula [$CoEx = (|F_p - 0.5|)$]. This index (CoEx) indicated the degree of coexistence of species within a habitat. When a habitat experiences equal coexistence, 50% of species being grassland and 50% being forest-dwelling, CoEx will equal 0.05. Similarly, if no coexistence exists in a habitat, CoEx will equal 0. I then used linear regression to explore associations between coexistence and species richness and host diversity.

Abundance, richness, and diversity

Species richness and small mammal population density was measured for each habitat. Small mammal density was calculated as the total number of small mammals collected per location per 100 trap nights. Population density could not be compared in the village home habitats because collection methods differed from all other trapping locations. Observed species richness was equal to the total number of species identified

in the sampled habitat. Population density of small mammals was expected to vary among communities; therefore, I rarefied species richness to account for the varied sample sizes (Hurlbert 1971; Heck, Belle & Simberloff 1975) to further examine species richness while controlling for the biases associated with uneven sample sizes. The relationship between species richness and coexistence among habitats (CoEx) was further investigated using linear regression.

The Shannon-Weiner index (H) was used to measure small mammal diversity and took into account both species abundance and richness (Shannon 1949). Evenness of species composition was measured using Pielou's evenness index (J) (Pommerening 2002). Using linear regression, the results of small mammal diversity and evenness from this study were compared to those results previously reported in historical small mammal studies in KNP (Struhsaker 1997). Additionally, I used linear regression to investigate the relationship between the H generated in my dataset and rarefied species richness results to examine relationships between these measurements of diversity.

2.4 RESULTS

From May to July 2009, 327 terrestrial small mammals were collected from within and around KNP (Figure 2.1). These mammals represented 23 species, including 10 species previously not recorded in the historical ecological studies within KNP (Table 2.1) (Isabirye-Basuta & Kasenene 1987; Struhsaker 1997) or identified in other reference guides (Delany 1975; Thorn & Kerbis Peterhans 2009; Happold & Happold 2013a; Happold & Happold 2013b). Representative skull samples (n=137) were catalogued at the Field Museum (#210384-210540). Species previously unrecorded in and around KNP

included: *Dasymys incomtus, Mastomys natalensis, Oenomys hypoxanthus, Praomys misonnei, Scutisorex somereni, Crocidura dolichura, C. fuscomurina, C.* cf. *littoralis, C. maurisca,* and *C. olivieri* (Table 2.1). With the exception of *Mastomys natalensis* and *Praomys misonnei*, the other eight species were only associated with either the forest edge or areas outside KNP (Table 2.1).

Using both morphometric and molecular techniques, I was able to identify all specimens with the exception of two individuals. I identified a single *Crocidura* sp. from the papyrus swamp within Fragment 1 that morphologically most closely resembled *C. littoralis*. Similarly, I collected an individual morphologically determined to be *Dendromus mystacalis,* but according to molecular results (Voelker, unpub data), this specimen belongs to an undescribed species. For the moment, I refer to this specimen (FMNH 210399) as *Dendromus* c.f. *mystacalis*.

Community structure and species composition

I classified 9 species as primarily forest-dwelling species, 9 as primarily grassland, and 5 as capable of living in either habitat (Table 2.2) (Kingdon 1974; Delany 1975; Thorn & Kerbis Peterhans 2009; Happold & Happold 2013a; Happold & Happold 2013b). Comparing all habitats, the forest fragments experienced the highest level of coexistence of forest-dwelling and grassland species (CoEx=0.40 in Fragment 1 and CoEx=0.43 in Fragment 2). All areas within KNP experienced low coexistence (CoEx<0.07) (Table 2.1). The proportion of forest-dwelling species significantly declined in the communities with higher levels of habitat disturbance (rho=0.96, p=0.003) (Figure

2.2). In contrast, peridomestic species were positively associated with increased habitat disturbance (rho=0.87, p=0.010) (Figure 2.2).

Beta-diversity measurements were used to understand how similar the compositions of small mammal communities were across the habitat disturbance gradient controlling for sampling effort (Figure 2.2). These indices were also used to understand the relationship and coexistence of forest dwelling species and colonizing species. All pair-wise comparisons showed that forest compartments had small mammal communities more similar to each other than to other habitats. In general, the small mammal community of Fragment 1 was more similar to communities within KNP and the forest edge (Morista-Horn <0.5) than to communities of more extremely disturbed habitats of Fragment 2 and village homes (Morista-Horn >0.6). Small mammal communities associated with human dwellings were the most distinct of all the communities examined (Morista-Horn 0.88 - 0.94). Assessing all pair-wise coefficients, the order of communities based on their relatedness to each other was determined to be 1) CC, 2) K14, 3) K15, 4) Edge, 5) Fragment 1, 6) Fragment 2, and 7) Village homes (Figure 2.2).

Seven shrew species were collected and all were associated with areas outside KNP (Table 2.1), although five were only represented by one specimen. Previous studies of small mammals in KNP identified all shrews as members of genus *Crocidura* and did not identify the species collected. Shrews in my study were identified as *C. nigrofusca, C. fuscomurina, C.maurisca C. dolichura, C. olivieri*, and one individual was determined to be *C.* cf. *littoralis*. One *Scutisorex somereni* was captured in Fragment 2. I did not find any shrews within the boundaries of KNP, although they are known to exist and have been identified in previous studies (Struhsaker 1997). My work further differentiated

members of the genus *Crocidura* associated with KNP and confirms that these species occur outside the boundaries of KNP as well.

Density, richness, and diversity

Rarified species richness was measured for each habitat and peaked species richness was found within moderately disturbed habitats (Figure 2.3). Correlations between habitat disturbance and rarefied species richness were examined using Spearman's rank order and no associations were identified. I examined habitats with similar species compositions (Morista-Horn value < 0.5, Figure 2.2) and found a statistically significant positive relationship between rarefied species richness and disturbance (rho=1, p=0.017). Small mammal population density was measured and correlations between density and habitat disturbance of all habitats and only those of similar composition were not found to be significant. Using linear regression, I evaluated the relationship between rarefied species richness and coexistence index (CoEx), and I found a positive relationship (R^2= 0.725, p=0.015) (Figure 2.4).

Habitat disturbance was not associated with small mammal species evenness (Pielou's J) or diversity (Shannon's H). Using linear regression, I compared my findings to historical measurements of evenness and diversity from areas within KNP. Evenness observed in this study does not agree with previously published findings that associated habitat disturbance with decreases in evenness (Isabirye-Basuta & Kasenene 1987) (Table 2.1). Diversity (H) measured in my study was closely associated with diversity measurements found in historical studies, but the results are difficult to interpret because of the limited sample sites (R^2 = 1, p=0.001). I compared the Shannon H generated in

this study to the measured rarefied species richness using linear regression and found concordance (R^2= 0.747, p= 0.008).

2.5 DISCUSSION

As human population growth further encroaches on wildlife habitats, the impact of anthropogenic habitat disturbance on mammalian populations is of increasing concern (Ceballos & Brown 1995; Ceballos *et al.* 2005). Areas surrounding KNP can be characterized as countryside landscape—composed of small-scale agriculture, forest fragments, and fallow land (Daily, Ehrlich & Sanchez-Azofeifa 2001). Globally, countryside landscapes support high species diversity compared to both intensive agricultural landscapes (Karp *et al.* 2012) and environmentally protected areas (Caro 2001). Forest fragments within the countryside landscape near KNP are at the nexus of human-wildlife interactions and experience frequent anthropogenic disturbance. Previous studies often found these fragmented habitats unstable and insufficient to maintain populations of wildlife, such as endangered primates (Onderdonk & Chapman 2000). I have shown that forest fragments have higher levels of small mammal species richness and diversity compared to more pristine areas within KNP. The disturbed forest fragments (specifically Fragment 1) had similar species composition to relatively undisturbed areas within KNP but experienced higher levels of species richness. Despite Fragment 1's relatively small size, Fragment 1's small mammal community was more similar to KNP than to Fragment 2. Fragment 2 was confirmed by multiple beta-diversity measurements to have unique species composition. Identifying the habitat associated with the greatest small mammal diversity and species richness is important for encouraging

individuals and governing institutions to maintain the integrity of these fragmented forest habitats and the species that they harbor. These results suggest that regardless of size, relatively small fragments can support high levels of small mammal species richness.

In western Uganda, high species richness was associated with coexistence between forest-dwelling species, grassland species, and invasive/peridomestic species. This finding supports theories suggesting intermediate levels of disturbance allow for niche diversification and reduction of competition and predators that leads to an increase in coexistence. This coexistence involves small mammal species of various natural history strategies occupying the same habitat.

My work provides evidence that high diversity of small mammals may exist in areas outside protected wildlife refuges and therefore are not typically sampled in small mammal surveys. In cataloging biodiversity, it is critical that mammalogists survey a broad range of habitat quality. Using this technique, I have expanded our knowledge of small mammal diversity in KNP by 10 species collected from the edge of the national park and in fragmented habitats. These 10 species may have been overlooked in previous studies because of the history of trapping only within forested and protected habitats associated with KNP. I encourage others to examine biodiversity outside the traditional protected areas, when possible, in order to gain a broader understanding of species diversity and richness.

A number of small mammal species were relatively abundant and were closely associated with habitats outside the boundaries of KNP. These included *Lophuromys aquilus, Mus gratus*, and *Crocidura* spp. In the most disturbed habitats (i.e. village homes), the invasive peridomestic species *Rattus rattus* dominated and represented 68%

of animals collected. *Rattus rattus* was more commonly associated with human dwellings than any other habitats in and around KNP but appears to have only minimally invaded areas outside of human dwellings. Historically, this rodent was found in areas within KNP (Struhsaker 1997). The absence of *R. rattus* from KNP, in my study, may provide evidence of forest regeneration post-logging.

The most abundant small mammal species within the protected borders of KNP were *Praomys jacksoni*, *P. misonnei*, and *Hylomyscus stella*—all considered forest-dwelling species. Similar to historical studies in KNP, *P. jacksoni* was found both within and outside KNP and occasionally within human dwellings (Isabirye-Basuta & Kasenene 1987). In contrast, *P. misonnei* was only found within forest compartments and closely associated with habitats within KNP in comparison to outside KNP (Table 2.1). Comparing *P. jacksoni* and *P. misonnei* abundance across disturbed habitats indicates that *P. jacksoni* is more disturbance-tolerant. These results provide evidence that *P. misonnei* may be at higher risk of local extinction as a consequence of anthropogenic disturbance. Previous studies at KNP identified *P. jacksoni* as the only member of the *Praomys* genus identified (Isabirye-Basuta & Kasenene 1987), which is primarily because *P. misonnei* was not identified until 1987 (Happold & Happold 2013b). My findings indicate that both *P. jacksoni* and *P. misonnei* inhabit KNP and it is likely that *P. misonnei* has been mistaken for *P. jacksoni* in previous studies due to its cryptic nature.

Lophuromys aquilus was the dominant species in Fragment 2, representing 62% of individuals within this habitat. This species was collected at lower frequencies from the heavily logged area within KNP, the Edge, Fragment 1, and human dwellings. Although known to occur in KNP, *L. aquilus* was found at high densities in more

disturbed habitats. This species is at higher risk of infection with *Giardia* spp. and population increases may pose a risk to amplification of this potentially zoonotic parasite (Salzer *et al.* 2014).

Five species were specifically associated with forest fragments and not found in other habitats. These species included: *C.* cf. *littoralis*, *D.* cf. *mystacalis, Gerbilliscus kempi ruwenzorii, Lemniscomys striatus,* and *Oenomys hypoxanthus.* Interestingly, *G. kempi ruwenzorii, L. striatus,* and *D.* cf. *mystacalis,* are commonly associated with grassland habitats and indicate the coexistence of both forest-dwelling and grassland species in these areas (Delany 1975). *Gerbilliscus kempi ruwenzorii* was only collected in the forest fragments. Although I found *D.* cf. *mystacalis* and *L. striatus* only inhabiting forest fragments, they have been observed in KNP in previous studies (Struhsaker 1997). The increase in species richness, specifically in Fragment 1, was likely impacted by the presence of these diverse species.

In KNP, the habitats hosting the most diverse and species-rich communities included heavily logged forest, forest edge, and a forest fragment. All of these disturbed habitats experienced coexistence of disturbance tolerant species (e.g. *M. grata* and *L. aquilus*), forest-dwelling species (e.g. *P. jacksoni* and *H. stella*), and grassland species (e.g. *D. mystacalis, G. kempi ruwenzorii*, and *O. hypoxanthus*). This coexistence supports niche diversification as a mechanism driving diversity. Our knowledge of the habitat preferences and natural history of these animals support my hypothesis that elevated species diversity and richness in moderately disturbed habitats was due, in part, to habitat diversification allowing the introgression of grassland species and coexistence of disturbance tolerant species.

Alterations in rodent population density, diversity, and richness in habitats at the nexus of wildlife-human interactions have been linked to increased risk of zoonotic rodent-borne disease. If high levels of diversity in a habitat were protective in reducing rodent-borne disease, the Forest Fragments would be the habitat of least disease in this study. My previous investigations in KNP (Salzer *et al.* 2014), showed that this is not the case. There must be other contributing mechanisms, other than species diversity, driving the disease dynamics in natural systems (Keesing, Holt & Ostfeld 2006; Randolph & Dobson 2012).

Edge effects and source-sink dynamics are theoretical mechanisms that may contribute to the observed diversity-disturbance relationship (Murcia 1995; Chesson 2000). Edge effects include physical, functional, and biotic differences between neighboring habitat types. Habitat convergence and overlap at the point (or edge) where two habitats meet has been associated with increased diversity. In western Uganda, I hypothesize that edge effect occurs at both the forest edge of KNP and within forest fragments, because both habitats are experiencing coexistence among grassland and forest-dwelling species. Edge effect and source-sink dynamics are not mutually exclusive and likely work synergistically, resulting in the observed patterns associated with disturbance and diversity.

Source-sink dynamics are suggested as an alternate mechanism for species diversity patterns in forest fragments (Pulliam 1988). Although the fragments examined in this study were once a contiguous forest block, they are geographically isolated from KNP, limiting opportunities for source-sink dynamics to play a role in maintaining high species diversity. Thus, due to the geographic isolation and demographically healthy

small mammal populations of the fragments in this study, it is likely that source-sink dynamics are not the primary mechanism for the observed diversity in forest fragments.

Human dwellings were dominated by the invasive peridomestic rodent, *Rattus rattus*, but were also found to harbor a few individuals of species more typically associated with forest habitats, such as *H. stella*. It is likely that proximity of human dwellings to KNP and forest fragments influenced the small mammal community within these dwellings. Homes may serve as a sink for forest species, while also maintaining invasive peridomestic roof rats. Further studies investigating small mammal communities within homes from areas more distant from forest habitats are needed to better understand the dynamics of such peridomestic communities. Although source-sink dynamics likely play a minor role in conserving diversity within forest fragments, small mammal communities associated with human dwellings are more likely to be influenced by source-sink dynamics.

My results paralleled the diversity patterns observed within KNP in previous studies despite variability in methodology, seasonality, and taxonomic determination (Struhsaker 1997). In general, the association between disturbance and diversity in this study followed a unimodal-peaked curve. Although not statistically significant, this peaked pattern is expected, following the intermediate disturbance hypothesis (Connell 1978). Had my study not included a broad gradient of disturbance, I would have likely observed the positive linear relationship observed in previous studies followed by a negative relationship in the most disturbed areas. I recognize that this data is limited and reflects one trapping season (dry season). I cannot make assumptions on the static nature of the pattern I found in this study or assume that communities are stable across various

seasons and years. Among studies within KNP, there are inconsistent findings in small mammal abundance and richness (Isabirye-Basuta & Kasenene 1987; Muganga 1989; Lwanga 1994; Struhsaker 1997). Further investigations will be needed to investigate the temporal variability of these ecological patterns since this study is limited to a single field season.

The relationship between diversity and disturbance should be examined within the context of the generation times of the organisms of interest (Padisak 1994; Wilson 1994; Roxburgh, Shea & Wilson 2004). The level of habitat disturbance supporting the highest level of diversity is expected to differ between r- and K-selected organisms (MacArthur & Wilson 1967), since K-selected species are generally less resistant to disturbance compared to r-selected species (Padisak 1994). Thus, the r-selected life histories of rodents and shrews predict that their diversity should increase with disturbance compared to K-selected taxa, such as primates. This relationship was supported by my findings and likely contributes to the small mammal diversity peak in habitats experiencing lower primate diversity.

The rarefied species richness measurements and community structure measurements also provide strong evidence that forest fragment habitats around KNP are not equally diverse and are composed of different communities of small mammals. The forest fragments in this study differed in size, surrounding habitats, and anthropogenic disturbance legacy. Although I only investigated two forest fragments, the patterns of diversity and species richness I observed are consistent with other studies investigating disturbance/diversity dynamics (Caro 2001; Pardini 2004). Like most forest fragments around KNP, both fragments studied were associated with wet bottomlands. I concluded

that the difference in the small mammal diversity observed between these fragments is likely due to differences in mesic conditions and neighboring habitats (Ceballos & Brown 1995; Newmark 1995).

My findings indicate that the higher species richness observed in selectively logged and fragmented forests were due to both greater niche diversification and higher coexistence of grassland species as well as disturbance tolerant and/or peridomestic species. Terrestrial small mammal communities in rural Uganda appear to be highly adaptable to a certain level of anthropogenic disturbance. In areas of high human-wildlife interaction, small mammal diversity peaks in mixed habitats. The identification of high species richness in anthropogenically-disturbed habitats is especially useful as conservation biologists strive to identify effective methods to conserve global species diversity.

2.6 FIGURES

Figure 2.1: Map of Kibale National Park and surrounding areas.
Specific locations of trapping webs (200m in diameter) are identified as: **CC**-relatively intact pristine forest, **K14**- lightly logged forest, **K15**-heavily logged forest, **Edge**-forest edge that overlaps agriculture fields, **Fragment 1**- located near the village of Bugembe and surrounded by small scale agriculture and human dwellings, **Fragment 2-** located near the village of Kiko and surround by small-scale agriculture, a trading center, and monoculture in the form of tea plantations. Small mammals were also collected from human dwellings located in areas around KNP and the forest fragments.

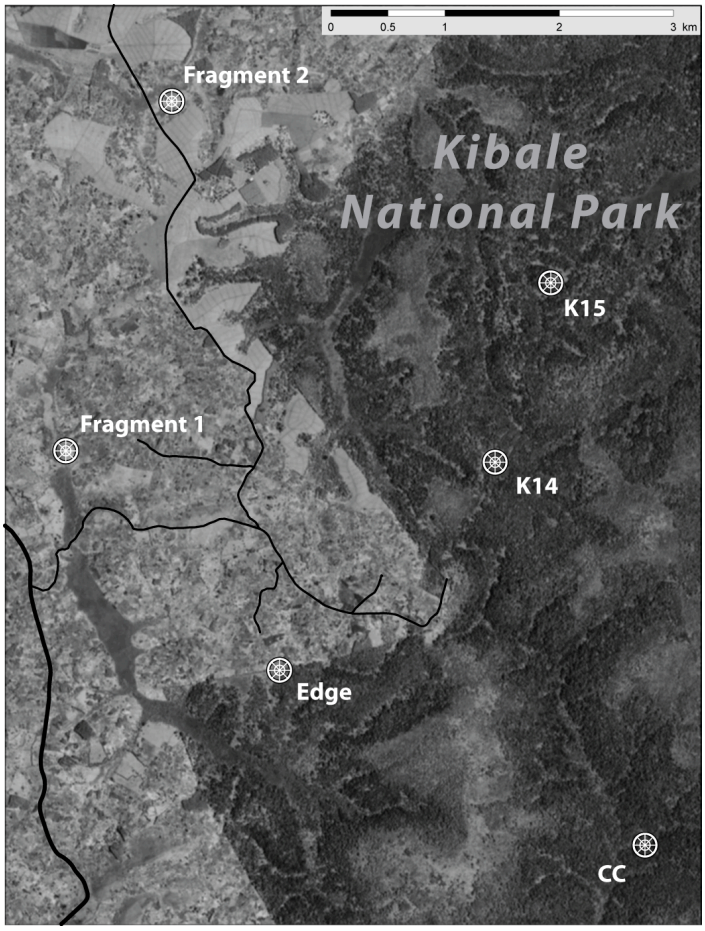


Figure 2.2: **Comparison of the composition of small mammal communities in and around Kibale National Park**.
The graph demonstrates the proportion of forest-dwelling and grassland species, as well as peridomestic species. Heat maps provide pair-wise comparisons of community similarity using four indices where lower values (dark grey) indicate higher similarity.

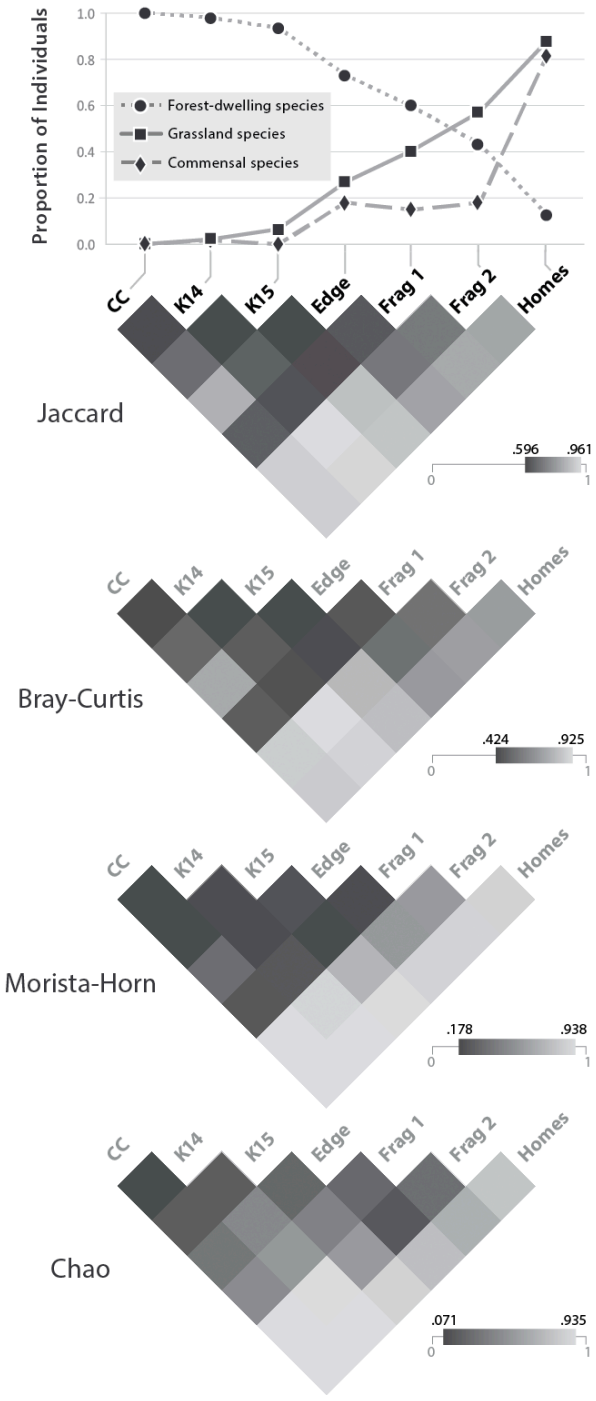


Figure 2.3: Rarefied species richness of small mammals.
Rarefied species richness (and standard error) was measured for each of the seven habitats sampled. Habitats are listed from least to most disturbed on the x-axis. Species richness increased in areas within KNP experiencing increased habitat disturbance. Fragment 1 had the highest level of species richness**.**

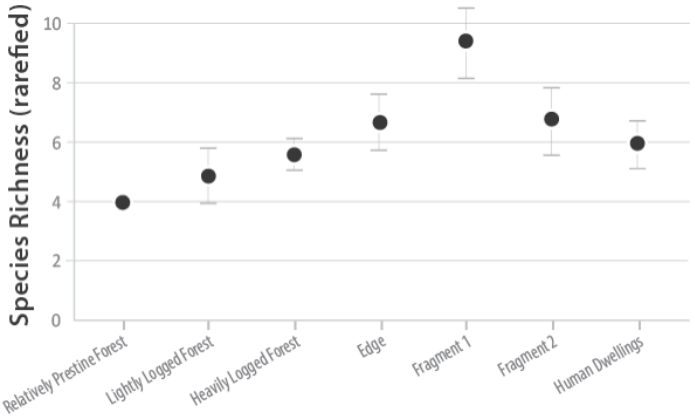
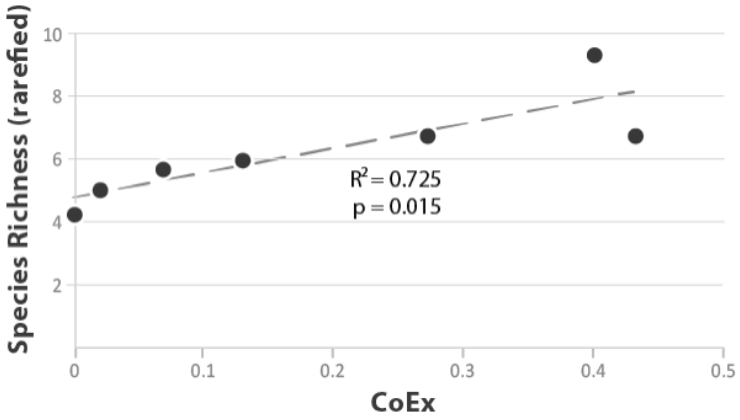


Figure 2.4: Relationship between species richness and coexistance.
Coexistance was measured using the CoEx index which is defined as the absolute value of the proportion of forest-dwelling species (F_p) minus 0.5. A CoEx value of 0.5 indicates there are equal proportions of forest-dwelling and grassland species present in a habitat and a value of 0 indicates a habitat is either dominated by grassland or forest-dwelling species. Relationships were measured using linear regression.



2.7 TABLES

Table 2.1: Small mammals collected in and around Kibale National Park.

Species[Reference]	Inside KNP				Outside KNP			
	CC	K14	K15	Edge	Frag 1	Frag 2	Village	**Total**
Crocidura dolichura [a]	0	0	0	1	0	0	0	1
Crocidura fuscomurina [a]	0	0	0	1	0	0	0	1
Crocidura cf. *littoralis* [a]	0	0	0	0	1	0	0	1
Crocidura maurisca[a]	0	0	0	0	1	0	0	1
Crocidura nigrofusca [b]	0	0	0	2	0	1	0	3
Crocidura olivieri [a]	0	0	0	0	1	1	4	6
Scutisorex somereni [a]	0	0	0	0	0	1	0	1
Dendromus cf. *mystacalis* [b]	0	0	0	0	1	0	0	1
Lophuromys aquilus [c]	0	1	3	4	5	31	3	47
Gerbilliscus kempi ruwenzorii[c]	0	0	0	0	5	2	0	7
Dasymys incomtus [a]	0	0	0	1	0	0	0	1
Hybomys lunaris [c]	0	1	3	0	1	0	0	5
Hylomyscus stella [c]	11	14	13	1	8	0	1	48
Lemniscomys striatus [c]	0	0	0	0	3	0	0	3
Malacomys longipes [c]	1	2	0	0	0	0	0	3
Mastomys natalensis [a]	0	1	0	0	0	0	0	1
Mus bufo [d]	0	0	2	0	0	0	0	2
Mus grata [d]	0	0	0	6	6	7	5	24
Mus triton [d]	0	0	0	0	0	0	4	4
Oenomys hypoxanthus [a]	0	0	0	0	1	2	0	3
Praomys jacksoni [c]	11	29	16	23	14	4	3	100
Praomys misonnei [a]	4	18	1	0	0	0	0	23
Rattus rattus [c]	0	0	0	0	0	1	40	41
Total	27	66	38	39	47	50	60	327
Rarefied Species Richness (se)	4.00 (0)	4.88 (0.95)	5.59 (0.55)	6.67 (0.92)	9.35 (1.16)	6.71 (1.09)	5.92 (0.80)	
Number of species (observed richness)	4	7	6	8	12	9	7	23
Number of trap nights	918	918	459	459	918	918	na	4590
Density/trap success	0.029	0.072	0.083	0.085	0.051	0.054	na	
Proportion of forest-dwelling species	1.00	0.98	0.93	0.73	0.60	0.43	0.13	
Proportion of peridomestic	0.00	0.02	0.00	0.18	0.15	0.18	0.82	
Coexistence Index (CoEx)	0.00	0.02	0.07	0.27	0.40	0.43	0.13	
Diversity (Shannon H')	1.14	1.34	1.38	1.36	2.07	1.34	1.21	
Historicallv recorded Diversitv[1]	0.938	1.49	1.59	-	-	-	-	
Diversity (Simpson index)	0.64	0.69	0.69	0.61	0.84	0.58	0.53	
Eveness (J)	0.82	0.69	0.77	0.65	0.83	0.61	0.62	
Historically evenness (J)	0.391	0.551	0.618	-	-	-	-	

[a] Species not previously identified in Kibale National Park (based on published literature).
[b] Thorn and Kerbis Peterhans 2009.
[c] Isabirye-Basuta and Kasenene 1987.
[d] Struhsaker 1997. This comprehensive text discusses that there are likely several species of *Mus* spp. present in KNP.
[e] Historical data was collected from relatively intact forest compartment K30, which is similar in disturbance to CC.

Table 2.2: Classification of species as forest-dwelling, grassland, and/or peridomestic. Each species was coded (i.e. 1,0, or 0.5) to generate the proportion of forest-dwelling species per habitat (F_p) and a Coexistence index measurement (CoEx). Species were also identified as either peridomestic or non-peridomestic.

Species	Forest=1	Grassland=0	Both=0.5	Peridomestic=1	NonPeridomestic=0	Brief Description	References
Crocidura dolichura	*1*				*0*	primarily a forest species	Thorn and Kerbis Peterhans 2009, Happold and Happold 2013a
Crocidura fuscomurina	*0.5*				*1*	Recorded to have wide habitat tolerance and can live in modrately arid habitats and may enter domestic dwellings	N.J.Dippenaar and R. M. Baxter 2013
Crocidura cf. *littoralis*	*1*				*0*	forest and stream edges	JCKP pers obs, Happold and Happold 2013a.
Crocidura maurisca	*1*				*0*	forest, stream edges, and swamps	Happold and Happold 2013a
Crocidura nigrofusca	*0.5*				*0*	mixed habitats including forest , swamps, forest-savannah mosiacs, secondary forest, and forest patches	Happold and Happold 2013a
Crocidura olivieri	*0.5*				*1*	mixed habitats including savannahand rainforest, flood-plain grassland, swamp, farmland, and human dwellings	Happold and Happold 2013a
Dasymys incomtus	*0*				*0*	primarily reed beds, long grass close to water, and marshes	Happold and Happold 2013b
Dendromus cf. *mystacalis*	*0*				*0*	occur in grass, herbage, and bush of savanna	Delany 1975
Gerbilliscus kempi ruwenzorii	*0*				*0*	grassland, savannahs, and forest edge	Delany 1975
Hybomys lunaris	*1*				*0*	all forest with preference for low lying muddy flats and streams	Delany 1975, JCKP pers obs
Hylomyscus stella	*1*				*0*	forest- considered a climbing forest species	Delany 1975
Lemniscomys striatus	*0*				*0*	grassland, savanna, and cultivated land	Delany 1975
Lophuromys aquilus	*0.5*				*0*	mixed habitats includig both forest and grassland, specifically moist situations in scrub and forest	Delany 1975, JCKP pers obs
Malacomys longipes	*0*				*1*	mostly grassland and agricultural plots but not proper forest-considered commensal	Delany 1975, JCKP pers obs
Mastomys natalensis	*0*				*0*	forest, specifically low lying forest and along streams	Delany 1975, JCKP pers obs
Mus bufo	*0.5*				*0*	grassland and savanah- considered invasive in western Uganda mixed habitats including moist montane forest, edge of swamp, and shrub	Delany 1975
Mus grata	*0*				*1*	occurs in grassland, heath, and scrub	Delany 1975
Mus triton	*0*				*0*	forest clearings and arable areas where the vegetation is thick and moist, including swamps	Delany 1975, JCKP pers obs
Oenomys hypoxanthus	*0*				*0*	all types of forest	Delany 1975
Praomys jacksoni	*1*				*0*	when found in open, usually associated with human dwellings and food storage- considered invasive	JCKP pers obs, Van der Straeten and Dieterlen 1987, Happold and Happold 2013b
Praomys misonnei	*1*				*0*	all types of forest	JCKP pers obs
Rattus rattus	*0*				*1*	forest, usually along saturated forest floor and stream edges	Delany 1975
Scutisorex somereni	*1*				*0*		JCKP pers obs

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CHAPTER 3

Effects of Anthropogenic and Demographic Factors on Patterns of Parasitism in African Small Mammal Communities

3.1 SUMMARY

Habitat disturbance often results in alterations in structure of small mammal communities. Additionally, the parasites harbored by these small mammals may be impacted by environmental changes or indirectly influenced by changes in available hosts. To improve our understanding of this interplay, I examined patterns of parasitism in small mammal communities from a variety of habitats in forested Uganda. Small mammals were collected from areas experiencing variable habitat disturbance, host density, and species richness. My analysis focused on the three most abundant rodent species, *Lophuromys aquilus*, *Praomys jacksoni*, and *Hylomyscus stella,* and a diverse group of parasites they harbor. The impact of various habitat and host community factors on parasite prevalence was examined using linear regression and Spearman's rank order correlation. I further investigated the parasite communities associated with each individual using correspondence analysis. I determined that although these results are preliminary, parasite prevalence and richness may be occasionally influenced by community and habitat factors, but taxonomy is a driving force in influencing the parasite community harbored by an individual host. Ultimately, applying general principles

across a broad range of disturbance levels and diverse host communities needs to be approached with caution in complex communities.

3.2 INTRODUCTION

Habitat quality, host community assemblage, host susceptibility/resistance, and pathogen-pathogen interactions are interconnected in complex and dynamic ways (Woolhouse *et al.* 1997; Ostfeld, Keesing & Eviner 2008; Beldomenico & Begon 2010; Johnson *et al.* 2013). Disease dynamics can rarely be explained by examining one component of a complex natural system, although general patterns are often sought. Understanding the host community components and interactions associated with disease emergence and persistence may provide valuable information for unlocking mechanisms driving disease dynamics in the natural environment (Dobson 2004; Lafferty 2010; Telfer *et al.* 2010).

Anthropogenic disturbance and subsequent loss of both biodiversity and community structure have been associated with increases in disease emergence (Keesing *et al.* 2010; Roche *et al.* 2012), as well as a reduction in disease occurrence (Lafferty 2012; Bush, Reed & Maher 2013; Young *et al.* 2013). There is evidence supporting both positive and negative general linear relationships among disease and factors, such as diversity, density, and relative abundance (Randolph & Dobson 2012). Additionally, empirical evidence is mounting that a host species' relative abundance and population density are also linearly associated with parasite prevalence and/or parasite richness (Arneberg 2002; Froeschke *et al.* 2013). Regardless of shape or direction and the indirect

or direct influences, anthropogenic disturbance can affect disease dynamics in natural systems (Randolph & Dobson 2012; Salkeld, Padgett & Jones 2013).

To improve our understanding of the general patterns between host and parasite communities in relation to anthropogenic disturbance, I investigated parasite dynamics in terrestrial small mammal communities in and around Kibale National Park (KNP), western Uganda. Specifically, I focused on three common forest dwelling rodent species: *Lophuromys aquilus*, *Praomys jacksoni*, and *Hylomyscus stella* (Delany 1975; Struhsaker 1997). I examined parasites of these host species and investigated correlations between parasite prevalence and habitat disturbance, host population density, and host species richness. I further evaluated the parasite communities harbored by each of these host species. These small mammals (or hosts) were collected from habitats experiencing variable intensities of habitat disturbance. The specific parasites investigated in my study included: gastrointestinal protozoans (*Giardia* spp. and *Cryptosporidium* spp.), blood-borne parasites (*Trypanosoma* spp.), and ectoparasites from the taxonomic orders Ixodida (ticks), Acarina (mites), Siphonaptera (fleas), and Phthiraptera (lice). I investigated broad patterns pertaining to the relationships among parasite prevalence and community structure in habitats that vary by species richness, host density, and disturbance intensity.

3.3 MATERIALS and METHODS

Study area

KNP is a mid-elevation tropical moist forest located in the foothills of the Rwenzori Mountains in western Uganda [0 13’ to 0 41’N, 30’19’ to 30’22’E] (Struhsaker 1997; Chapman & Lambert 2000; Hartter 2009). The park and surrounding areas

represent a mosaic of habitats that have undergone various types and frequency of habitat disturbance (Hartter 2009). Portions of KNP were logged at varied intensities in the 1960s, resulting in a series of contiguous forest compartments of lightly logged, heavily logged, and unlogged status within KNP (Struhsaker 1997). Over the last four decades, KNP and the surrounding forest fragments have supported research on the influence of habitat disturbance on a variety of forest dwelling species (Kasenene 1984; Isabirye-Basuta & Kasenene 1987; Lwanga 1994; Dranzoa 1998; Chapman *et al.* 2000; Seavy & Apodaca 2002; Gillespie & Chapman 2008; Hartter *et al.* 2011). The sampling sites represent a broad gradient of anthropogenic disturbance in the region, including, from least to most disturbed: 1) relatively pristine forest (known as CC), 2) low-intensity selectively logged forest (known as K14), 3) high-intensity selectively logged forest (known as K15), 4) forest-agricultural interface (known as forest edge), 5) forest fragments (referred to as Fragment 1 and 2), and 6) human settlements (Figure 2.1). Previous studies within KNP (Chapman *et al.* 2000) and these forest fragments (Gillespie & Chapman 2006) have extensively evaluated the gradient of habitat disturbance occurring in the areas studied in this investigation. These previous studies allow my specific study sites to be categorically placed along a gradient of disturbance (Gillespie, Chapman & Greiner 2005; Gillespie & Chapman 2006; Gillespie & Chapman 2008).

Animal collection

Trapping webs were used in all habitats except within village homes to accurately estimate the abundance, population density, and structure of the small mammal community within each habitat (Anderson *et al.* 1983). Each web was 200 meters in

diameter with 12 radii each containing 12 Sherman traps (3 x 3.5 x 9", H.B. Sherman Traps, Inc, Tallahassee FL, USA), with the first four of these traps set at five meter intervals and the eight distal traps were set at 10 meter intervals (Mills *et al.* 1999). The center of the web contained 4 Sherman traps and 1 Tomahawk trap (19 x 6 x 6", Tomahawk Live Trap Co., Tomahawk, WI, USA). An additional four Tomahawk traps were each set 50 meters from the center in the cardinal directions. In total, 153 traps were used in each web. Trapping webs were operated for three consecutive nights on each trapping occasion for a total of 5049 trap nights at 7 sites. All sites were sampled twice except the forest edge and heavily logged forest sites, which were sampled once. Traps were baited in evenings and animals were collected at sunrise the following morning to prevent trap-associated deaths. All traps were baited consistently with peanut butter and millet. All trapping was conducted in the dry season between May and July of 2009. Sites that had repeated sampling had, at minimum, a 6-week rest period between sampling efforts.

Terrestrial small mammal collection was approved by the Uganda Wildlife Authority, the Uganda National Council for Science and Technology, local authorities, and homeowners at trap sites. Animal handling protocols were approved by Institutional Animal Care and Use Committees (IACUC) from Emory University (#062-2009) and the Centers for Disease Control and Prevention (CDC) (#1768).

Standard field methods for small mammal handling, necropsy, and tissue collection techniques were followed (Mills *et al.* 1995). Necropsies were performed and tissue samples were collected for molecular identification of mammalian species and *Trypanosoma* spp. Feces were collected from the descending colon and preserved in

10% buffered formalin for detection of *Giardia* spp. and *Cryptosporidium* spp. Skulls from a subset of small mammals sampled (n=137) were prepared using standard procedures and identified to species using established mammalian guides (Delany 1975; Nagorsen & Peterson 1980; Thorn & Kerbis Peterhans 2009). Specimens were catalogued at the Field Museum in Chicago, Illinois, USA (Reference #210384-210540). Molecular identification using Cytochrome b gene analysis was necessary for species indistinguishable by morphometrics (i.e. *P. jacksoni* and *P. misonnei*) (Peppers, Carroll & Bradley 2002).

Immediately following humane euthanasia, each small mammal was placed in an individual plastic bag. Plastic bags containing euthanized small mammals were then opened and contents were placed in a clean white plastic tub (approximately 8x40x30cm) (Bush 2009). Each individual and the contents of their plastic bag were processed within the tub for easy visualization and collection of ectoparasites. Ectoparasites were dislodged and collected by vigorous brushing of euthanized small mammals. Attached ectoparasites (i.e. ixodid ticks) were collected by parting the fur of each individual animal with forceps and visually inspecting the skin. Small mammals were processed until no additional ectoparasites were collected or for a maximum of 20 minutes, in the case of heavily infested animals. All ectoparasites were placed into 70% ethanol.

Parasite detection

All parasites were identified to the level of Order. DNA was extracted from spleen tissue (stored at -80C) of all animals collected (n=327) using the DNA EZ1 tissue kit (Qiagen, Hilden, Germany). To detect *Trypanosoma* spp. DNA, I amplified the

highly variable region of the 18S ribosomal RNA gene using previously-described nested PCR methods (Noyes *et al.* 1999) and Platinum Taq polymerase (Invitrogen, Grand Island, New York, USA). I used external primers, TRY927F and TRY927R and internal primers, SSU561F and SSU561R, to confirm trypanosome-positive individuals (Noyes *et al.* 2002).

Formalin-preserved feces were screened for *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts using the MERIFLUOR immuno-flourescent assay (Meridian BioScience Inc. Cincinnati, Ohio, USA). Fecal samples were concentrated into fecal pellets and resuspended in 1g/mL concentrations and scored for presence/absence (Salzer *et al.* 2007).

All ectoparasites were shipped to Emory University in Atlanta, Georgia, USA where they were examined by light microscopy. Ectoparasites from each animal were quantified and categorized according to taxonomic order: Ixodida (ticks), Acarina (mites), Siphonaptera (fleas), or Phthiraptera (lice). Identification was performed using established reference guides (Lane 1993). Only motile stages were identified for each ectoparasite, including larval, nymphal, and adult stages for ticks and mites; nymphal and adult stages for lice; and adult stage for fleas.

Data analysis

Relative small mammal population density for each habitat was calculated and measured by trapping success divided by trapping effort for each habitat. Additionally, relative species abundance was measured by the number of total *L. aquilus*, *H. stella*, and *P. jacksoni* divided by the total animals collected at each site. Using individual-based

rarefaction, species richness was estimated for each habitat. A rarefaction curve was generated to determine adequate sampling effort and species richness to correct for varied sample sized across habitats.

Parasite point prevalence (referred to as prevalence) was calculated for each habitat and the most abundant rodent species (*L. aquilus, H. stella,* and *P. jacksoni*). Correlations between parasite prevalence and species richness, small mammal population density, and relative species abundance were measured using linear regression models with prevalence of each parasite as outcome variable. Additionally, correlations between habitat disturbance and parasite prevalence were evaluated using Spearman's rank order correlation, because habitat disturbance is a qualitative and ordinal variable. Statistical analyses were performed using the stats package in R version 3.0.1 (R Core Team 2013).

To determine if parasite assemblages were more influenced by habitat or taxonomic category of host (i.e. species), I considered each individual small mammal collected as a patch or community of parasites. Each patch was associated with parasite presence/absence in addition to a variable considered "parasite free" to account for small mammals free of parasites. Using constrained correspondence analysis (a.k.a. canonical correspondence analysis) (CCA), I investigated the influence of the taxonomic classification of each individual and the habitat the individual was from in order to understand what serves as the best predictor for the parasites of each individual animal captured. CCA was performed for habitat while conditioning for species and vice versa. The results of each CCA were analyzed using an ANOVA-like permutation test for constrained correspondence analysis to assess the significance of constraints. All analyses

were performed using the Vegan package on the R statistical platform (version 3.0.1) (R Core Team 2013) following established methods (Bellier 2012).

3.4 RESULTS

From May to July 2009, 327 small terrestrial mammals were collected in and around KNP. Small mammal density, species richness (rarefied with standard error), and relative abundance of all three host species were calculated for each habitat (Table 3.1). Mammals collected represented 23 species and parasites varied in prevalence across habitat and host species (Table 3.1). All parasites examined were found to be harbored by *P. jacksoni* and *L. aquilus*, while *H. stella* harbored all but *cryptosporidium*.

Linear regression was used to investigate parasite prevalence among the various species, and several patterns were identified (Table 3.2). I identified a significant positive association between the overall small mammal density of a community and the prevalence of trypanosomes (R^2= 0.586, p=0.047) and lice (R^2= 0.781, p=0.012) among *P. jacksoni*. Additionally, there was a positive association among the relative abundance of *H. stella* and the prevalence of trypanosomes (R^2= 0.708, p=0.011) and mites (R^2= 0.717, p=0.010). Therefore, the more dominant *H. stella* becomes in the community of small mammals, the more individual *H. stella* are harboring trypanosomes and mites. Parasite prevalence among *L. aquilus* was more closely associated with small mammal species richness with an association with flea infestation (R^2=0.755, p=0.016) and trypanosome (R^2=0.759, p=0.014). Relationships between habitat disturbance and parasite prevalence were examined using Spearman's rank order correlation, and a negative association was found between habitat disturbance and trypanosome prevalence

among *H. stella* (r_s=-0.880, p=0.021). A Bonferroni correction was not applied to these analyses because of the increased criticism of its validity in ecological cases and the increased risk of a Type II error (Krasnov *et al.* 2008), but I cautiously interpret the data in consideration of Type I error.

Correspondence analysis was performed to examine the parasite communities (presence/ absence) harbored by each individual. Using the parasite communities associated with each individual within the populations of *P. jacksoni*, *L. aquilus*, and *H. stella,* I investigated the influence of habitat and/or taxonomic classification on parasite community. I found that a host's parasite community was more closely associated to other individuals of that same host species than to other hosts within their same habitat but of various species. When species was controlled and habitat type was examined, no significant associations were identified (p=0.784) (Figure 3.1A). Alternatively, when habitat was controlled, species was significantly clustered ($p < 0.0001$) (Figure 3.1B).

3.5 DISCUSSION

Generalized laws and theories, which are broadly applicable and repeatable under various conditions, are used to understand ecological interactions (Lawton 1999; Lange 2005; Poulin 2007). I investigated a broad range of habitat types that harbor diverse communities of host species and parasites. I focused this study on a single point in time and identified relatively few patterns of parasitism among host species. In general, I did not identify universal relationships or patterns. Despite the preliminary nature of my study, this work does provide additional empirical evidence for the importance of understanding host taxonomy and parasite specificity (Froeschke *et al.* 2013).

Several patterns among the different species did emerge in this study, although at this point I can only speculate as to the dynamic drivers. Among the *P. jacksoni* population in forested Uganda, I found an association between trypanosomes and lice prevalence with increases in small mammal density. Both lice and trypanosomes are moderately host-specific, usually infecting multiple hosts of restricted phylogenies or taxonomic classifications (Dobigny *et al.* 2011; Froeschke *et al.* 2013). Therefore, the association I found between parasite prevalence and total small mammal density (as opposed to relative abundance of *P. jacksoni*) may be associated with spill-over of trypanosomes and lice from other dominant host species. This pattern may also be indicative of a decline in host resistance in competitive environments (Johnson *et al.* 2013). Trypanosome prevalence among *H. stella* was associated with increases in relative abundance of *H. stella,* which may indicate *H. stella* as a potential primary host for trypanosomes. *Lophuromys aquilus* experienced a positive association between trypanosomes and overall small mammal richness. This finding may compete with hypotheses (i.e. *dilution effect*) that predict an increase in host species richness leads to declines in host-specific parasite prevalence. This relationship between trypanosomes among *L. aquilus* and overall small mammal richness may also be explained by spill-over if the trypanosomes are less host-specific and have the ability to infect a wide range of hosts. The association between mite prevalence on *H. stella* and relative abundance of *H. stella* is not a surprising association. Mites are recognized as being predominately host-specific, and I would suspect their infestation rate to increase as available hosts increase in abundance (Fain 1994).

My study highlights the importance of taxonomic classification of host species to understand their parasite communities, with environmental factors being a secondary influence on a host's parasite community. The results support recent findings that relationships between biodiversity and pathogen transmission are idiosyncratic and highly dependent on the host species and parasites studied (Salkeld, Padgett & Jones 2013). It is possible that general patterns between parasite prevalence and host dynamics/environment would emerge in simpler community assemblages of small mammals experiencing less extensive habitat disturbance. Additionally, there are possible factors and interactions unaccounted for in this work, which may be more influential on parasite prevalence than those examined. Such factors in more disturbed habitats could likely include pathogen spill-over from human and domestic animal sources, the influence of invasive species (and their parasites), and alterations in individual host susceptibility to parasites (Daszak, Cunningham & Hyatt 2000; Torchin *et al.* 2003; Dobson *et al.* 2008). General linear patterns associated with parasitism among different host communities are likely more easily identified when these communities examined maintain a certain level of shared species and experience a more gradual gradient of habitat disturbance. To more accurately investigate relationships between host diversity and parasite prevalence/richness in KNP, I would need to have a larger sampling effort with substantial replications over multiple sites and seasons. The sampling in this study was from seven distinct habitats experiencing distinct small mammal communities with an absence of paired samplings. Replicate sites would need to first be identified and then sampled at various time points to more accurately answer questions related to the dilution effect and amplification of parasites in forested Uganda.

The coarseness of the parasite data limited my ability to identify the host species primarily responsible for the maintenance of specific parasites within these communities and parasite-host specificity. Future studies could provide further taxonomic characterizations of these parasites to species level. This finer examination of parasites, particularly the ectoparasites, would certainly identify even greater parasite richness and identify parasite-host dynamics specific to KNP (Alvarado-Otegui *et al.* 2012; Salyer *et al.* 2012). Despite the coarseness of the data and the innate limitations, host taxonomy is still significant in determining the parasites an individual host harbors. Further identification of parasite species will only strengthen this finding. Additionally, given recent identification of viral anti-bodies circulating in small mammals around KNP, future work using serologic assays would broaden our knowledge of disease life history for each individual small mammal (Salzer *et al.* 2013).

Ecosystems are complex, with an intricate network of hosts and parasites. General patterns would be expected to be less common as complexity increases in these natural systems. In the absence of a larger sampling effort, this impact of habitat disturbance, host population density, and species richness in western Ugandan small mammal communities appear less influential in determining disease dynamics among these hosts. My data provides empirical evidence that within the small mammal communities in western Uganda, very few patterns emerged to explain these ecological drivers of parasite prevalence. However, I did find strong associations between individual small mammal taxonomy and the community of parasites they harbor. This study provides further evidence that the parasite communities found in the mosaic

landscape in western Uganda are strongly influenced by the taxonomy of the dominant host species and the natural history of the parasites they harbor.

3.6 FIGURES

Figure 3.1—A and B: Examining associations between habitat, host species, and parasite communities.

Figure 3.1A) Correspondence analysis of habitats associated with KNP using parasite communities within each habitat harbored by the most abundant rodent species—*P. jacksoni*, L. aquilus, and *H. stella*. The seven habitats are shown in the ordination plot represent a gradient of habitat disturbance (least to most disturbed is listed from top to bottom in the figure key) with each habitat represented by a specific color indicated in the figure key and each point an individual. Some points are superimposed on each other. The correspondence analysis determined parasite communities on each individual small mammal were not associated with the habitat the host occupied.

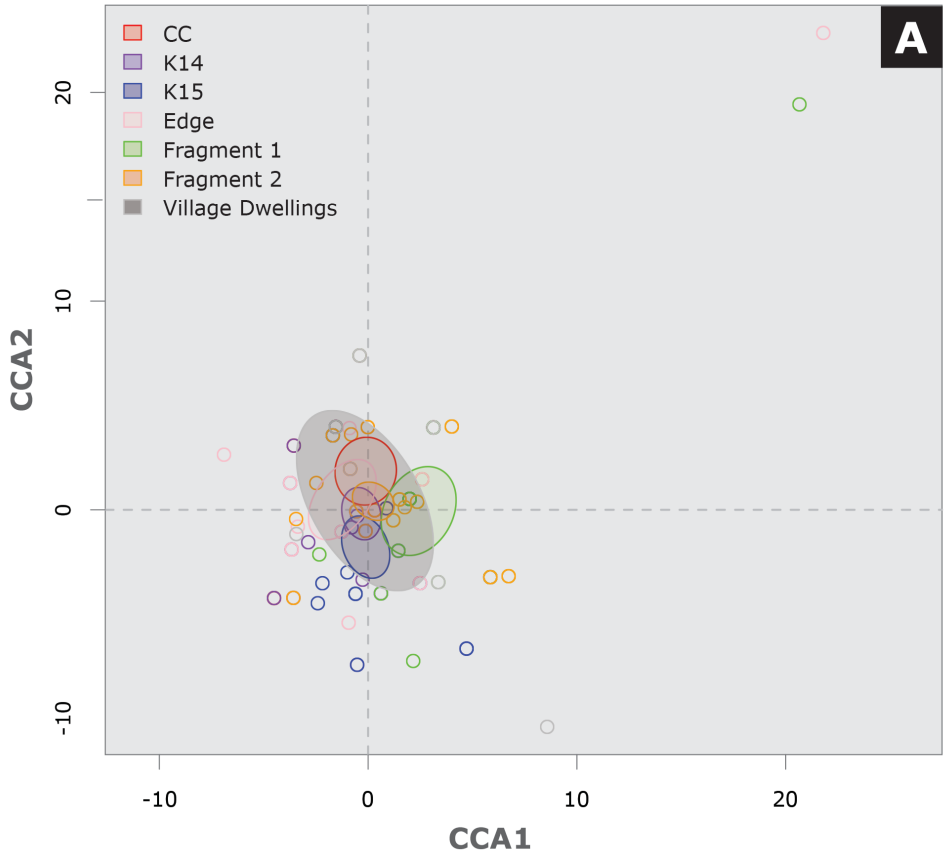
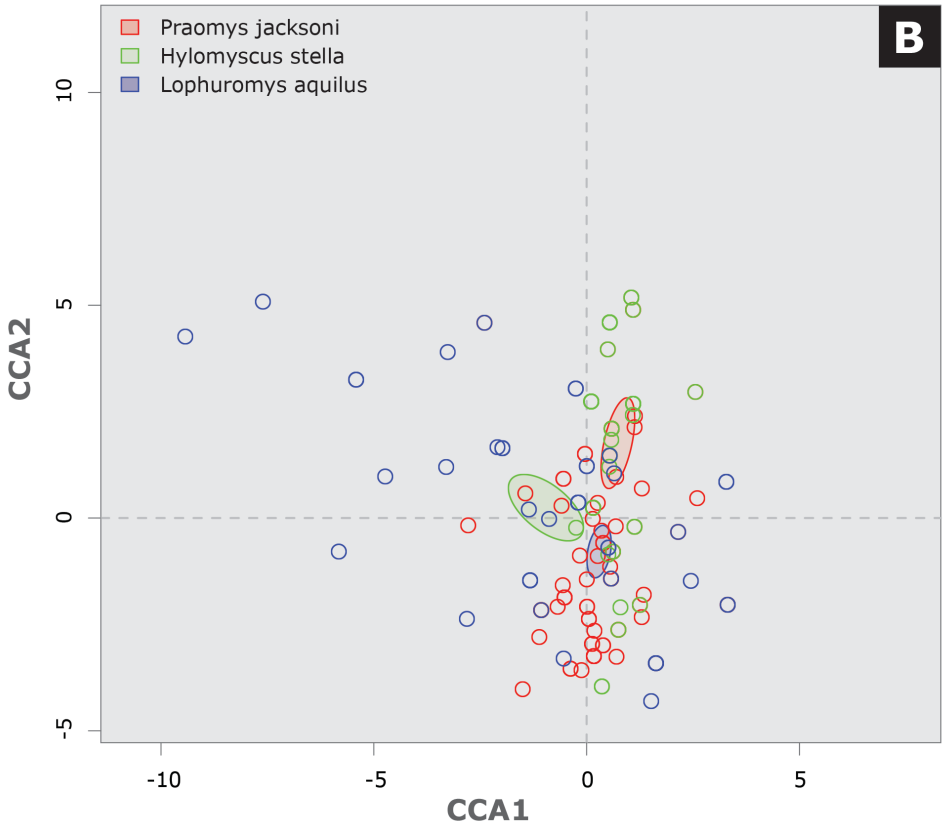


Figure 3.1B) Correspondence analysis of the parasite communities harbored by the three most abundant rodent hosts —*P. jacksoni*, L. aquilus, and *H. stella* sampled from all habitats. Each species is represented by a specific color indicated in the figure key and each point represents an individual. Ellipses represent 95% confidence interval of the species centroid, and non-overlapping ellipses are interpreted as significant differences between the species at α=0.05. Some points are superimposed on each other. All three rodent species show distinct parasite community differences. The correspondence analysis determined parasite communities were significantly associated with taxonomic classification of their host species (p=0.0001).



3.7 TABLES

Table 3.1: Hosts and parasites collected from various habitats in western Uganda.

Habitat	Species	Species Richness (standard error)	Trapping effort	Density/trap success	Total number collected	Relative abundance	*Trypanosoma*	*Giardia*	*Cryptosporidium*	Ticks	Mites	Fleas	Lice
Prestine forest (CC)	*Hylomyscus stella*				11	41%	2	0	0	6	11	0	0
	Malacomys longipes				1	4%	0	0	0	0	1	0	0
	Praomys jacksoni				11	41%	1	0	0	4	11	3	0
	Praomys misonnei				4	15%	1	0	0	0	4	0	2
	Total in habitat	**4.00** (0.00)	**918**	**0.029**	**27**		**4**	**0**	**0**	**10**	**27**	**3**	**2**
Lightly logged forest (K14)	*Hybomys lunaris*				1	2%	0	0	0	0	1	0	0
	Hylomyscus stella				14	21%	2	0	0	8	14	4	1
	Lophuromys aquilus				1	2%	0	0	0	0	1	0	1
	Malacomys longipes				2	3%	0	0	0	0	2	1	1
	Mastomys natalensis				1	2%	0	0	0	1	1	1	0
	Praomys jacksoni				29	44%	11	2	0	8	29	9	11
	Praomys misonnei				18	27%	1	0	0	4	18	5	6
	Total in habitat	**4.88** (0.95)	**918**	**0.072**	**66**		**14**	**2**	**0**	**21**	**66**	**20**	**20**
Heavily logged forest (K15)	*Hybomys lunaris*				3	8%	2	0	0	1	3	1	2
	Hylomyscus stella				13	34%	2	0	0	4	13	5	1
	Lophuromys aquilus				3	8%	0	3	1	0	1	0	1
	Mus bufo				2	5%	0	0	0	0	2	1	0
	Praomys jacksoni				16	42%	7	0	0	5	16	9	11
	Praomys misonnei				1	3%	0	0	0	0	1	0	1
	Total in habitat	**5.59** (0.55)	**459**	**0.0828**	**38**		**11**	**3**	**1**	**10**	**36**	**16**	**16**
Forest edge	*Crocidura dolichura*				1	3%	0	0	0	0	1	0	0
	Crocidura fuscomurina				1	3%	0	0	0	0	0	0	0
	Crocidura nigrofusca				2	5%	0	0	0	2	1	2	1
	Dasymys incomtus				1	3%	0	0	0	0	1	0	0
	Hylomyscus stella				1	3%	0	0	0	1	1	0	0
	Lophuromys aquilus				4	10%	0	2	1	3	4	1	3
	Mus grata				6	15%	0	0	0	2	4	0	0
	Praomys jacksoni				23	59%	12	1	0	9	21	5	14
	Total in habitat	**6.67** (0.92)	**459**	**0.085**	**39**		**12**	**3**	**1**	**17**	**33**	**8**	**18**
Fragment 1 (Bugembe)	*Crocidura littoralis*				1	2%	0	0	0	1	0	1	0
	Crocidura maurisca				1	2%	0	0	0	1	1	0	0
	Crocidura olivieri				1	2%	0	0	0	0	1	1	0
	Dendromus mystacalis				1	2%	0	0	0	0	1	0	0
	Gerbilliscus kempi ruwenzorii				5	11%	0	0	0	1	5	1	2
	Hybomys lunaris				1	2%	0	0	0	0	0	0	0
	Hylomyscus stella				8	17%	0	0	0	2	7	1	0
	Lemniscomys striatus				3	6%	3	0	0	1	3	2	0
	Lophuromys aquilus				5	11%	1	2	0	1	5	3	2
	Mus grata				6	13%	0	1	0	1	6	2	2
	Oenomys hypoxanthus				1	2%	0	0	0	0	1	0	0
	Praomys jacksoni				14	30%	1	0	0	2	13	6	3
	Total in habitat	**9.35** (1.16)	**918**	**0.0512**	**47**		**5**	**3**	**0**	**10**	**43**	**17**	**9**
Fragment 2 (Kiko)	*Crocidura nigrofusca*				1	2%	0	0	0	1	0	0	1
	Crocidura olivieri				1	2%	0	0	0	1	0	1	0
	Gerbilliscus kempi ruwenzorii				2	4%	0	0	0	2	2	1	1
	Lophuromys aquilus				31	62%	0	21	3	15	30	6	18
	Mus grata				7	14%	0	0	0	5	6	1	3
	Oenomys hypoxanthus				2	4%	0	0	0	2	1	0	2
	Praomys jacksoni				4	8%	0	0	0	1	4	2	2
	Rattus rattus				1	2%	0	0	0	0	0	0	0
	Scutisorex somereni				1	2%	0	0	0	1	0	1	0
	Total in habitat	**6.71** (1.09)	**918**	**0.0545**	**50**		**0**	**21**	**3**	**28**	**43**	**12**	**27**
Village homes	*Crocidura olivieri*				4	7%	0	0	0	0	0	1	3
	Hylomyscus stella				1	2%	0	0	0	1	1	0	0
	Lophuromys aquilus				3	5%	0	2	1	2	3	1	3
	Mus grata				5	8%	0	0	0	3	3	0	0
	Mus triton				4	7%	3	0	0	2	3	0	3
	Praomys jacksoni				3	5%	0	0	0	1	2	1	0
	Rattus rattus				40	67%	11	0	1	2	4	11	3
	Total in habitat	**5.92** (0.80)	**na**	**na**	**60**		**14**	**2**	**2**	**11**	**16**	**14**	**12**
	TOTAL		**4590**		**327**		**60**	**34**	**7**	**107**	**264**	**90**	**104**

Table 3.2: Relationships between parasite prevalence and environmental factors.
Linear regression was used to investigate relationships between parasite prevalence and small mammal density, species richness, and relative species abundance of the three most abundant rodent species (*P. jacksoni, L. aquilus*, and *H. stella*). Additionally, correlation between habitat disturbance and parasite prevalence among these three host species was examined using Spearman's rank order correlation test. Non-significant associations are indicated as "ns."

Parasite	Total Small Mammal Density			Relative Species Abundance			Species Richness			Habitat Disturbance		
	P. jacksoni	*L. aquilus*	*H. stella*	*P. jacksoni*	*L. aquilus*	*H. stella*	*P. jacksoni*	*L. aquilus*	*H. stella*	*P. jacksoni*	*L. aquilus*	*H. stella*
Trypanosoma spp.	R^2=0.586 p= 0.047	ns	ns	ns	ns	R^2=0.708 p= 0.011	ns	R^2= 0.759 p=0.016	ns	ns	ns	r_s=-0.880 p=0.021
Giardia spp.	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cryptosporidium spp.	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Ticks	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mites	ns	ns	ns	ns	ns	R^2=0.717 p= 0.010	na	ns	ns	ns	ns	ns
Fleas	ns	ns	ns	ns	ns	ns	ns	R^2=0.755 p=0.016	ns	ns	ns	ns
Lice	R^2= 0.781 p=0.012	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

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CHAPTER 4

Effects of Anthropogenic Disturbance on Native and Invasive Trypanosomes of Rodents in Forested Uganda

4.1 SUMMARY

Habitat disturbance and anthropogenic change is globally associated with extinctions and invasive species introductions. Less understood is the impact of environmental changes on the parasites harbored by endangered, extinct, and introduced species. To improve our understanding of the impacts of anthropogenic disturbance on such host-parasite interactions, I investigated an invasive small mammal trypanosome (*Trypanosoma lewisi*) in Uganda. I screened 348 individuals, representing 26 species, across a gradient of habitat disturbance in rural forested Uganda. Using both microscopy and PCR, I identified 18% of individuals (all from the order Rodentia) as positive for trypanosomes. Further phylogenetic analyses revealed two trypanosomes circulating in these wild rodent populations, *T. lewisi* and *T. varani*. *Trypanosoma lewisi,* was found in seven rodent species both native and invasive, while *T. varani* was identified in only three native forest species. The lack of *T. varani* in more disturbed habitats is likely due to the absence or decline of competent hosts and suggests it is a natural parasite of forest-dwelling rodents. These findings provide evidence that anthropogenic disturbance may lead to spillover of an invasive parasite (*T. lewisi*) harbored by non-native hosts into

native species and lead to local co-extinction of a native parasite (*T. varani)* and associated native forest-dwelling hosts.

4.2 INTRODUCTION

As habitats experience anthropogenic disturbance, small mammal community structure is altered. Changes in community structure may also affect the prevalence of parasites that infect small mammal hosts (Keesing, Holt & Ostfeld 2006; Randolph & Dobson 2012; Young *et al.* 2013). In some cases, such changes have resulted in losses of host populations and subsequently the parasites they harbor (Koh *et al.* 2004; Altizer, Nunn & Lindenfors 2007; Dunn *et al.* 2009; Bush, Reed & Maher 2013). As ecological theory predicts, the rates of spread and occurrence of parasites of these reduced populations should decline as the host population decreases in size and density (Anderson & May 1979; May & Anderson 1991). Alternatively, anthropogenic changes may also lead to introductions of invasive, disturbance-tolerant species, and their associated parasites (Verneau *et al.* 2011). Habitat loss has been linked to a decline in large predators, leading to a subsequent increase in both rodent populations and the diseases they harbor (Young *et al.* 2014). Disease dynamics are considered to be dependent on the idiosyncrasies of pathogen, host, vector, and habitat (Salkeld, Padgett & Jones 2013). A native pathogen may be driven to extinction in disturbed habitats where opportunistic and generalist species are less competent hosts (Pedersen *et al.* 2007), while an invasive and/or more generalist pathogen will thrive in this same environment.

Trypanosomes are flagellated protozoan blood-borne parasites that are known to infect humans, wildlife, and domestic animals. There are at least 43 recorded

trypanosome species infecting 125 rodent species throughout the world (Hoare 1972). Despite the large number of trypanosomes associated with wild rodents, their infection dynamics, molecular characteristics, zoonotic potential, and pathogenicity in their natural hosts have received little attention (Sato *et al.* 2003).

Our knowledge of the diversity of species within the genus *Trypanosoma,* as well as the host-specificity of these parasites, is a growing field of study (e.g. (Hamilton, Gibson & Stevens 2007; Averis *et al.* 2009). *Trypanosoma lewisi* is closely associated with peridomestic rodents of the genus *Rattus* (e.g. *Rattus rattus,* and *R. norvegicus*) (Hoare 1972). Rattus spp. have origins in Asia but now occupy areas outside their historical range due to human migration and importation (Carleton & Musser 2005). The global spread of *T. lewisi* has mirrored that of its host, which explains this parasite's broad global distribution (Pinto *et al.* 2006; Wyatt *et al.* 2008; Adams, Hamilton & Gibson 2010). Although other rodents are susceptible to *T. lewisi*, the occurrence of this parasite is often associated with pathogen spillover from *Rattus* spp. to native species (Wyatt *et al.* 2008; Dobigny *et al.* 2011; Milocco *et al.* 2013). Native trypanosomes are known to infect native small mammals, but they do not appear to occupy as broad of a host range as the invasive parasite *T. lewisi* (Dobigny et al. 2011).

I hypothesized that the small mammals of western Uganda, like other areas of Africa, will be infected with the invasive parasite *T. lewisi* as a consequence of pathogen spillover and species invasion in disturbed habitats. Additionally, if other native species of trypanosomes are identified, these parasites are hypothesized to be more closely associated with native small mammals and less disturbed habitats.

I investigated small mammal communities across a gradient of anthropogenic disturbance in western Uganda. I screened for trypanosomes using both microscopy and molecular techniques to determine parasite prevalence. Further molecular characterization, to identify the trypanosome species within the genus *Trypanosoma,* was conducted to understand the impacts of habitat disturbance on host-parasite dynamics within this natural system.

4.3 MATERIALS and METHODS

Study area

Kibale National Park (KNP) is a 795km^2 mid-elevation tropical moist forest located in the foothills of the Rwenzori Mountains in western Uganda [0 13' to 0 41'N, 30'19' to 30'22'E] (Hartter 2009). Portions of KNP were logged at varied intensities in the 1960s resulting in a series of contiguous forest compartments recovering from various logging intensities (Chapman *et al.* 2000). KNP was designated as a national park in 1993, and is now protected by the Uganda Wildlife Authority (Struhsaker 1997).

The sampling sites examined in my study were representative of various levels of anthropogenic disturbance in the region, including: 1) relatively intact primary forest, 2) low-intensity logged forest, 3) high-intensity logged forest, 4) forest-agricultural edge, 5) forest fragments (referred to as Fragment 1 and 2), and 6) human settlements (i.e. homes, trading-centers, and gardens), associated with villages outside KNP. The degree and nature of disturbance has been determined for these locations using methods previously described (Gillespie & Chapman 2006). Fragment 2 has experienced a higher intensity of habitat loss and degradation, including loss of all primate species by 2005 (Goldberg *et*

al. 2008; Chapman *et al.* 2012). In contrast, Fragment 1 has experienced relatively less habitat degradation and still supports populations of wild primates (Goldberg *et al.* 2008; Chapman *et al.* 2012). Previous studies within KNP (Chapman *et al.* 2000) and these forest fragments (Gillespie & Chapman 2006; Chapman *et al.* 2007; Goldberg *et al.* 2008a) have extensively evaluated the gradient of habitat disturbance occurring in the areas studied in this investigation. These previous studies allow the specific study sites in my study to be categorically placed along a gradient of disturbance (Gillespie, Chapman & Greiner 2005; Gillespie & Chapman 2006; Gillespie & Chapman 2008).

Animal collection and identification

Animal collection was approved by the Uganda Wildlife Authority, the Uganda National Council for Science and Technology, local authorities (Local Chairman), and homeowners, as well as IACUC committees from both Emory University (#062-2009) and the Centers for Disease Control and Prevention (CDC) (#1768).

Animals from KNP and forest fragments were collected using trapping webs measuring 200 meters in diameter with 12 radii as described previously (Mills *et al.* 1999 and destails in Chapter 2). In total, 153 traps were used in each web. Additionally, I used pitfall traps at two forested sites within the forest but only 1 individual animal was collected via this method. Sampling was conducted within human dwellings where homeowners were interested in pest removal. All traps were consistently baited in the evenings with a millet and peanut butter mixture and were collected at sunrise the following morning to prevent inadvertent death due to exposure.

Animals were removed from traps, immediately anesthetized with 5% isoflurane inhalation, and blood was collected from each animal via cardiocentesis. Animals were humanely euthanized using exsanguination and a lethal dose of isoflurane. Animals were photographed, and morphometric data were recorded. Necropsies were performed on each animal, and spleen samples were collected for molecular identification of both mammalian species and trypanosome species. Skulls and blood smears were retained from a subset of animals. Molecular identification was necessary for individuals indistinguishable by external morphology (i.e. *Praomys jacksoni* and *P. misonnei*). These cryptic small mammal species were molecularly confirmed using PCR amplification and sequencing of the first half of the Cytochrome b gene (480bp) (Peppers, Carroll & Bradley 2002).

Trypanosome detection and differentiation

Initially, I screened a subset of animals (n=103) for the presence of trypanosomes using microscopy. Two thin smear slides were made from blood collected during cardiocentisis from each animal and fixed using 100% methanol. Slides were stained in a 10% giemsa solution for 20 minutes. Blood smears were examined using standard light microscopy with phase contrast on a compound light microscope using high magnification (1000x oil immersion) inspection. Approximately 100 fields of view were observed for each slide. Presence or absence notation was determined for *Trypanosoma* spp. by inspecting for trypomastigotes in the blood smear.

I further screened for the presence of *Trypanosoma* spp. in DNA retained from spleen tissue of all animals collected (n=348). DNA was extracted using EZ1DNA tissue

kit (Qiagen, Valencia, California, USA) from frozen spleens. To detect and identify trypanosomes in DNA extracted from small mammal spleens, a region of the 18S ribosomal RNA gene was used (Pinto *et al.* 2012). The 18S rRNA gene fragment was amplified following previously-described nested PCR methods using external primers, TRY927F and TRY927R, and for the internal primers, SSU561F and SSU561R (Noyes *et al.* 2002).

The positive PCR products were purified with ExoSAP-IT (Affymetrix Inc., Santa Clara, California, USA) and then sequenced using the internal primers SSU561F and SSU561R with the ABI Big Dye chemistry on an ABI 3730xl DNA Analyzer automatic sequencer (Applied Biosystems, Inc., Foster City, California, USA). For molecular identification of the trypanosomes, my research team followed the phylogenetic approach implemented by Pinto et al. (2012) and Cottontail et al. (2014) to align the obtained short sequences of the 18S rRNA with a more comprehensive set of longer sequences and then concatenated it with another phylogenetically informative gene (Cottontail *et al.* 2014). An alignment of the new sequences was constructed, using the Geneious Alignment tool using default parameters in the software Geneious (Drummond *et al.* 2010), then reduced the matrix to contain only unique sequences. The newly generated data was then combined with the datasets for the 18S rRNA and gGAPDH genes (Hamilton, Gibson & Stevens 2007), using the Consensus Align tool in Geneious. The alignment was checked and corrected manually. A maximum likelihood tree was conducted using RAxML (Stamatakis, Ludwig & Meier 2005), and node support was estimated by 1,000 bootstrap pseudo replicates. To corroborate the identifications of the trypanosomes detected in this study, delimitation analysis Poisson Tree Processes (PTP) was performed (Zhang *et al.*

2013), with the maximum likelihood tree using 500000 MCMC generations and a thinning of 100.

Data Analysis

The most abundant small mammal species were identified as either associated with areas within KNP (less disturbed) or outside KNP (more disturbed habitats) using Fisher's exact test. Correlations among trypanosome prevalence and habitat disturbance, small mammal density, and host species abundance (of the most abundant species) were investigated using Spearman's rank order correlation. Small mammal density for each habitat was calculated and measured by trapping success divided by trapping effort for each habitat (omitting human settlements from this analysis). Additionally, relative species abundance was measured by the number of members of a single species divided by the total animals collected from a given site (all habitats were included in this analysis). Analysis focused on species that were found to be susceptible to trypanosomes (>5% infected) and abundance (total collected n > 20), which included *Praomys jacksoni*, *P. misonnei*, *Hylomyscus stella*, and *R. rattus*. Statistical analyses were performed using the stats package on the R version 3.0.1 (R Core Team 2013).

4.4 RESULTS

Animal collection and identification

From May through July 2009, 348 individual small terrestrial mammals were collected in and around KNP, representing 26 species (Table 4.1) from 7 distinct habitat

types. Representative skull samples were identified and catalogued at the Field Museum in Chicago, Illinois (#210384-210540)

Praomys misonnei and *Hylomyscus stella* were more closely associated with forested habitats within KNP, compared to other small mammal species. Adult members of the genus *Praomys* (e.g. *P. jacksoni* and *P. misonnei*) were identified using cranio-dental features and confirmed molecularly. These two species co-occurred within the forested habitats of KNP, but only *P. jacksoni* was found in areas outside KNP (Figure 4.1). The association between *P. misonnei* and areas within KNP was significant when compared to *P. jacksoni* (Fisher's exact test, $p< 0.001$). *Praomys misonnei* appeared to be more susceptible to habitat disturbance compared to *P. jacksoni*, which occur in forest fragments and human dwellings. *Hylomyscus stella* was one of the most abundant species collected during this study and was commonly found within forested habitats. Although *H. stella* was associated with some disturbed habitats (Figure 4.1), it was strongly associated with habitats within KNP (Fisher's exact test, $p= 0.002$).

Human dwellings, areas within homes, surrounding gardens, trading centers, and the research station were dominated by *R. rattus*. This species was more closely associated with this highly disturbed environment than all other habitats. Of all the *R. rattus* collected in this study (n=44), all but one individual was found within human dwellings.

Trypanosome detection and differentiation

Using light microscopy, 18 of the subset of blood smears (n=103) were positive for trypanosomes. All 348 animals were screened for trypanosome DNA using the 18S

rRNA gene PCR assay. Sixty-three individuals, all rodents, representing 17.5% of the total collected specimens, were positive by PCR. The microscopy results and molecular results were congruent, confirming the presence of DNA represents active trypanosome infections. All shrews screened (n=14) were negative for trypanosomes. The phylogenetic analysis of the newly generated 18S rRNA sequences, integrated with comprehensive dataset of 18S rRNA and gGAPDH genes, coupled with the PTP species delimitation analysis, revealed that 50 individuals were positive for *T. lewisi* and 13 individuals were positive for *T. varani*, a parasite belonging to the lizard-hosted clade of trypanosomes (Table 4.1). Standard PCR was performed, which did not allow for the quantification of infection within each individual host. The prevalence of both of these parasites varied among habitats and host species (Figure 4.2 and Table 4.1). Trypanosomes were found in all habitats except Fragment 2 (Figure 4.2A).

The novel parasite *T. varani,* was restricted to protected areas within KNP, which included the relatively unlogged forest, forest edge, and areas with a legacy of high and low intensity logging (Figure 4.2A). *Trypanosome varani* prevalence was negatively correlated with increase in habitat disturbance (rho= -0.964, $p< 0.001$). This parasite was only found in three native, forest-dwelling rodent species: *P. jacksoni*, *P. misonnei*, and *H. stella* (Table 4.1 and Figure 4.2B) (Delany 1975). Subsequently, the prevalence of *T. varani* was positively associated with relative abundance of both *P. misonnei* (rho= 0.900, p= 0.006), and *H. stella* (rho= 0.778, p=0.039), but no correlation was found with the relative abundance of *P. jacksoni*. *Trypanosoma varani* was more closely associated with forest habitats in contrast to *T. lewisi,* which was found in a variety of habitats (Fisher's exact test, $p<0.001$) (Figure 4.2A).

Trypanosoma lewisi infections were equally abundant in areas within and outside KNP (Figure 4.2). *Trypanosoma lewisi* was identified in 7 rodent species. *Praomys jacksoni* was the only host species of both *T. varani* (n=5) and *T. lewisi* (n=25) (Table 4.1). The additional 6 species infected with *T. lewisi* included *R. rattus*, *Lophuromys aquilus*, *Lemniscomys striatus*, *Mus triton*, *Hybomys lunaris*, *and Graphiurus murinus* (Table 4.1). No correlation was found between the prevalence of *T. lewisi* and relative abundance of host species (i.e. *P. jacksoni* and *R. rattus).* The prevalence of *T. lewisi* among all small mammals was found to be positively associated with total small mammal density (rho= 0.841, p= 0.039). Unlike the prevalence of *T. varani*, *T. lewisi* prevalence was not associated with an increase or decrease in habitat disturbance.

4.5 DISCUSSION

In western Uganda, areas of extreme habitat disturbance have low relative abundance of native forest-dwelling small mammals while simultaneously experiencing a higher abundance of invasive species. I found that many native forest-dwelling species associated with KNP are found to be positive for a newly identified trypanosome (*T. varani*), but are free of this trypanosome in more disturbed habitats outside the forest. Interestingly, although this parasite has the ability to infect the disturbance-tolerant species *P. jacksoni*, the parasite is absent from populations that occur outside KNP. This suggests possible alterations in the presence or abundance of a necessary vector and/or competent mammalian host or possibly competitive exclusion of *T. varani* by *T. lewisi*. Additionally, the occurrence of *P. misonnei* in forested habitats appears to play a key role in the maintenance of *T. varani* within KNP. The impacts of habitat disturbance may

synergistically drive local extinction of the host and subsequently a local extinction of the parasite.

It is hypothesized that disease dynamics will be directly altered by changes in habitat disturbance and, subsequently, host diversity. The most discussed and debated hypothesis related to this ecological link is the *dilution effect* (Schmidt & Ostfeld 2001). The *dilution effect* describes how high species diversity may "dilute" the ability of a pathogen-amplifying host and vector to readily transmit disease (Ostfeld & Keesing 2000; Schmidt & Ostfeld 2001). Following this hypothesis, when an ecosystem experiences a loss of biodiversity, pathogen prevalence will be assumed to increase due to increased transmission events among dominant susceptible reservoir species (Schmidt & Ostfeld 2001). But, this may not be the case when the reservoir host is negatively impacted by disturbance and these changes in species composition lead to its local extinction.

My study does not examine the *dilution effect* because, unlike other studies examining *dilution effect*, this study area does not experience a linear relationship between disturbance and diversity (specifically small mammal diversity). Interestingly, the least disturbed habitats within KNP, which are experiencing the highest proportion of native forest species, are experiencing similar small mammal diversity as the village home communities, although vastly dissimilar small mammal communities (Salzer *et al.* 2014). A larger sampling effort would be needed to further examine the impact of host diversity in the absence of habitat disturbance in and around KNP. A recent meta-analysis of 16 studies of various disease systems suggests that the relationship between biodiversity and risk of disease is not as simple as proposed in the original models of

dilution effect (Salkeld, Padgett & Jones 2013). This meta-analysis and other recent studies provide conflicting evidence concerning the broader idea that biodiversity regularly protects against increases in disease occurrence (Randolph & Dobson 2012). These conflicting ideas create a much larger discussion of the true and complex relationship between host diversity and pathogen prevalence (Young *et al.* 2013).

Recently, a single *Mastomys natalensis* was found in Niger to be infected with a trypanosome species considered a sister taxa of *T. varani* (Dobigny *et al.* 2011). Although not molecularly confirmed, previous studies in Kenya have identified two phenotypically different trypanosome species infecting *P. jacksoni* (Wanyonyi *et al.* 2011). Additional phylogenic analysis will need to be conducted in order to understand how related the *T. varani* identified in my study is to other historical and newly identified African trypanosomes.

Trypanosoma lewisi was found in seven rodent species, while the newly identified *T. varani* was identified in only three rodent species. Previous studies found *T. lewisi* almost exclusively associated with *R. rattus* and rarely associated with other native African forest species (Dobigny *et al.* 2011). In contrast to these previous studies, I found seven other native rodent species infected with *T. lewisi*. These findings broaden knowledge of the host-specificity of *T. lewisi* and rodent trypanosomes in general. These results are congruent with recent results identifying *Rattus* spp. and other peridomestic species as reservoirs for *T. lewisi* in Asia (Pumhom *et al.* 2013). Additionally, my work parallels recent studies demonstrating that rodent trypanosomes have broad host ranges and are widespread among competent small mammals but experience variable distributions across landscapes (Averis *et al.* 2009; Dobigny *et al.* 2011; Milocco *et al.*

2013). It may be possible for co-infection to occur between these two species of parasites, although I found no evidence of co-infection in my study. Additional studies will need to be conducted to properly identify host competency and co-infection among small mammals in this area.

Praomys jacksoni appears to play a primary role in maintaining *T. lewisi* in western Uganda and specifically in forest habitats where *R. rattus* are absent (Figure 4.3C). No correlation was found between the prevalence of T. *lewisi* and relative abundance of its host species (i.e. *P. jacksoni* and *R. rattus),* but I did find a positive association between *T. lewisi* and total small mammal density. This association was expected of such an invasive and generalist parasite as *T. lewisi,* because this parasite's presence should be more dependent on general rodent density and less dependent on the occurrence of a specific host. Within human dwellings, *R. rattus* is likely the primary rodent host of *T. lewisi* in western Uganda, which follows patterns seen on a global scale (Dobigny *et al.* 2011). Unlike the prevalence of *T. varani,* which was found to be sensitive to habitat disturbance, *T. lewisi* prevalence was not associated with an increase or decrease in habitat disturbance.

In my study, trypanosomes were found to infect rodents in all habitats examined, except one forest fragment (Fragment 2). This fragment was located near the trading center of the village of Kiko. The absence of trypanosome infections from all small mammals trapped in Fragment 2 may be a result of the limited small mammal diversity in this habitat, which is dominated by *L. aquilus*, a species that experiences lower prevalence of *T. lewisi* compared to *P. jacksoni, H. stella*, and *R. rattus*. In Fragment 1

11% of the small mammals captured were positive for *T. lewisi*, but *T. varini* was not identified in this fragment.

Rattus rattus in western Uganda is the dominant rodent species to invade and persist in areas of extreme disturbance, especially within human dwellings. Although *T. lewisi* was likely introduced by *R. rattus*, it is now maintained in western Uganda by other native rodent populations that exist outside human dwellings, such as *L. striatus*, *P. jacksoni, and M. triton.* My study identified *T. lewisi* occurring in habitats free of *Rattus* spp. populations. A spillover event of *T. lewisi* occurred some point prior to May 2009 and has resulted in an endemic invasive parasite, *T. lewisi,* that is now more closely associated with areas of higher small mammal density than of relative abundance of *R. rattus*. Additional temporal studies are needed to understand the invasion potential of this parasite in and around KNP. The impact and pathogenicity of this parasite on native African rodents is unknown; however, in England, field voles infected with *T. microti* have decreased red blood cell counts (Beldomenico *et al.* 2009). This physiological change was considered significant in reducing longevity and/or fitness due to increased susceptibility to other infections. Future laboratory studies are needed to understand the impact of trypanosomes on rodent health.

Multiple genera of fleas serve as vectors for *T. lewisi*. This broad vector range likely aids spillover of *T. lewisi* into native host species (Molyneux 1969), as was observed in this study. As vector-trypanosome associations were beyond the scope of this study, I can only speculate as to how the behavior of the unknown vector may impact parasitism among rodent populations. Thus, I cannot rule out that the pattern of rodent

species infected with trypanosomes observed in my study was more a product of a vector's host-preference than parasite-host compatibility.

Although *T. lewisi* is primarily associated with rodent infections, human cases of disease have been reported (Verma *et al.* 2011). Despite equivocal data, fleabites may allow transmission of *T. lewisi* to humans. Rare human infections with *T. lewisi* have been reported and most occurred in infants or immunosuppressed adults (Truc *et al.* 2013). The high prevalence of *T. lewisi* in rodents captured from human dwellings during this study may pose a previously unidentified public health risk, particularly in western Uganda, where a proportion of individuals are immunosuppressed by HIV-AIDS. The impact of *T. varani,* on both rodent and human health, is unknown.

Previous work examining small mammals in KNP found that host taxonomy and natural history as more influential in parasite occurrence in a habitat than any ecological factor examined (Salzer *et al.* 2014). This previous work was conducted on a subset of the samples examined in this current study and investigated a large variety of parasites to a crude level. My current study supports this previously-identified relationship between host natural history and parasite dynamics while using a finer scale of parasite identification. These findings strengthen the belief that simple patterns are not easily found in complex communities.

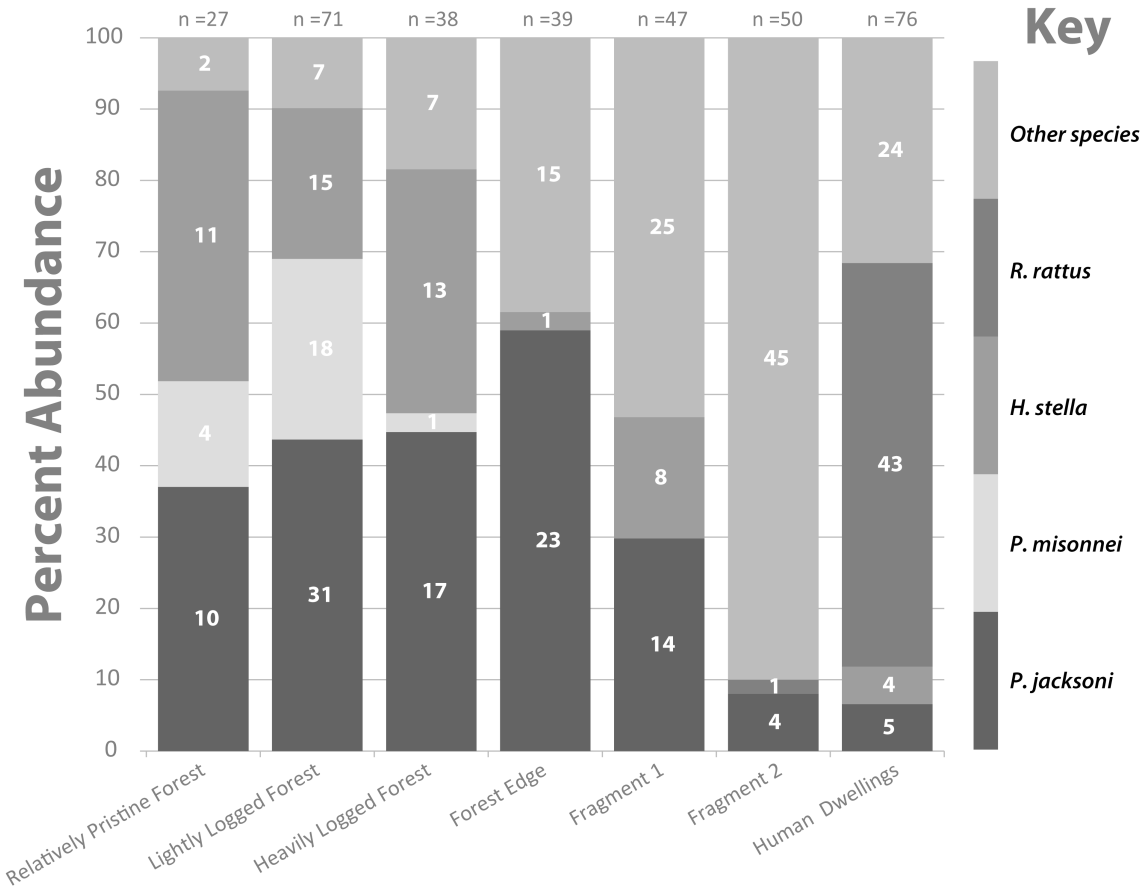
There is growing evidence that parasites play a primary role in food web structure and maintenance of diversity within ecosystems (Dunne *et al.* 2013), and parasite extinctions may have an overlooked negative impact on the health of larger free-living species (Dobson *et al.* 2008). The results of my study suggest that habitat disturbance in western Uganda has led to local extinctions of *P. misonnei* and subsequently a loss of its

novel trypanosome, *T. varani.* Although this novel trypanosome is found to infect other, more disturbance-tolerant host species (i.e. *H. stella* and P. *jacksoni*), the presence of this parasite is highly associated with the presence of *P. misonnei*. This suggests *P. misonnei* or another unidentified forest dwelling species plays a significant role in maintenance of this parasite within forest habitats. My work provides evidence of local co-extinction of host and parasite linked with a disruption in ecosystem structure, although the mechanisms of causation are impossible to identify within the context of the current study (Lafferty 2012).

My work provides empirical evidence that the loss of species from our global environment comes with a potential undetected loss of their native parasites. In the effort to catalogue and conserve biodiversity, future studies need to molecularly identify parasites to further understand human impact on their mammalian hosts.

4.6 FIGURES:

Figure 4.1: Relative abundance of small mammals across various habitats. Relative abundance of *Praomys jacksoni, P. misonnei, Hylomyscus stella*, *Rattus rattus,* and all other individuals among the various habitats in and around Kibale National Park, Uganda. Total sample size for each habitat is given on the top of each habitat and counts of individuals per species are given within bars representing the species.



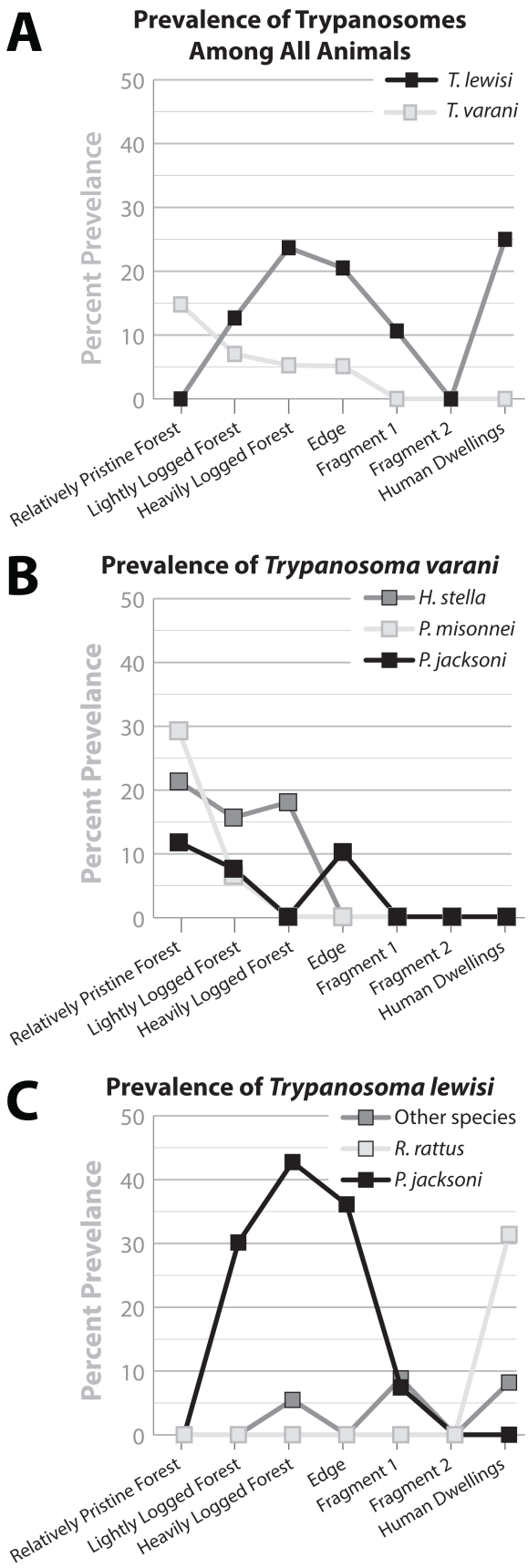


Figure 4.2: **Prevalence of *Trypanosoma varani* and *T. lewisi*.** Graph A, B, and C show prevalence of rodent-borne trypanosomes along a gradient of habitat disturbance in and around Kibale National Park, Uganda. Habitats are listed from least to most disturbed (left to right) on the x-axis. Prevalence of *Trypanosoma lewisi* and *T. varani* among all animals are shown in Figure 3A. Figure 3B presents prevalence of *T. varani* for the most abundant infected rodent species: *Praomys jacksoni*, *P. misonnei*, and *Hylomyscus stella*. Figure 3C presents prevalence of *T. lewisi* among the most abundant infected rodent species: *Rattus rattus* and *P. jacksoni*.

4.7 TABLES

Table 4.1: Prevalence of trypanosomes among host species.
In total, 348 small mammals, representing 26 species, were collected from in and around Kibale National Park in western Uganda for this study. Each individual was tested for both *Trypanosoma lewisi* and *T. varani*. Prevalence of trypanosome infection varied among host species.

		Number infected (% prevalence)	
Species	Number tested	*T. lewisi*	*T.* cf. *varani*
Arvicanthus niloticus	1	*0*	*0*
Crocidura dolichura	*1*	0	0
Crocidura fuscomurina	*1*	0	0
Crocidura cf. *littoralis*	*1*	0	0
Crocidura maurisca	*1*	0	0
Crocidura nigrofusca	*3*	0	0
Crocidura olivieri	*6*	0	0
Dasymys incomtus	*1*	0	0
Dendromus cf. *mystacalis*	*1*	0	0
Gerbilliscus kempi ruwenzorii	*7*	0	0
Grammomys sp.	*1*	0	0
Graphiurus murinus	*1*	1 (100)	0
Hybomys lunaris	*5*	2 (40.0)	0
Hylomyscus stella	*52*	0	6 (12.0)
Lemniscomys striatus	*3*	3 (100)	0
Lophuromys aquilus	*48*	1 (2.0)	0
Malacomys longipes	*4*	0	0
Mastomys natalensis	*1*	0	0
Mus bufo	*2*	0	0
Mus grata	*27*	0	0
Mus triton	*6*	5 (83.0)	0
Oenomys hypoxanthus	*3*	0	0
Praomys jacksoni	*104*	25 (24.0)	5 (4.8)
Praomys misonnei	*23*	0	2 (9.0)
Rattus rattus	*44*	13 (30.0)	0
Scutisorex somereni	*1*	0	0
Total	348	50 (14.4)	13 (4.0)

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CHAPTER 5

Serologic Evidence for Circulating Orthopoxviruses in Peridomestic Rodents from Rural Uganda

5.1 SUMMARY

The prevalence of orthopoxviruses (OPXV) among wildlife, including monkeypox virus (MPXV), remains largely unknown. Outbreaks of human monkeypox in central Africa have been associated with hunting, butchering, and consuming infected forest animals, primarily rodents and primates. Monkeypox cases have not been reported in east Africa, where human contact with wildlife is more limited. Whether this lack of human disease is due to the absence of MPXV in rodents is unknown. However, testing of wildlife beyond the known geographic distribution of human cases of monkeypox has rarely been conducted, limiting our knowledge of the natural distribution of MPXV and other OPXV. To improve our understanding of the natural distribution of OPXV in Africa and related risks to public health, I conducted a serosurvey of peridomestic rodents (*Rattus rattus*) in and around human dwellings in Kabarole District, Uganda. I tested for OPXV antibody in areas free of human monkeypox. Sera from 41% of the *R. rattus* sampled reacted to OPXV specific proteins from multiple purified OPXV, but did not react by ELISA. I conclude that an OPXV or a similar poxvirus is circulating among wild rodents in Uganda. With the known geographic range of OPXV in rodents now increased, factors that dictate OPXV prevalence and disease will be identified.

5.2 INTRODUCTION

Orthopoxvirus is a genus within the viral family *Poxviridae*. Orthopoxviruses (OPXV) include several pathogens of human health concern such as monkeypox, cowpox, and variola (causative agent of smallpox) (Damon 2006). Orthopoxviruses were originally defined by their similar immunologic properties. Once an individual is infected and develops a humoral immune response to an OPXV infection, anti-OPXV immunoglobulin G (IgG) will be present in this individual's serum. In addition to providing cross-protection, these antibodies provide serologic evidence of a previous exposure to an OPXV (Karem *et al.* 2005).

Since the eradication of smallpox and the discontinuation of vaccination programs in 1979 (Breman & Henderson 1998), monkeypox has emerged as a threat to human health. In endemic zones of central and west Africa, mortality associated with monkeypox virus infections reaches 10% (Heymann, Szczeniowski & Esteves 1998). Although monkeypox occurs throughout the Democratic Republic of Congo (DRC), no human cases have been reported across the eastern political border shared between DRC and Uganda (Levine *et al.* 2007). This restricted spatial distribution could be due to several factors, including the absence of viable rodent reservoirs, environmental conditions, or cultural norms resulting in lower human contact with potential wildlife reservoirs beyond the zone of monkeypox endemism (Levine *et al.* 2007).

The roof rat (*Rattus rattus*) is considered a highly invasive, opportunistic species, thought to have been first introduced to Uganda in 1910 (Hopkins 1949; Delany 1975). Roof rats are known to transmit zoonotic pathogens such as *Yersinia pestis* (Eisen *et al.* 2008), *Leishmania* spp. (Quinnell & Courtenay 2009), *Cowpox virus* (Martina *et al.*

2006), and hantaviruses (Cueto *et al.* 2008). In western Uganda roof rats are usually found in areas associated with human dwellings (Delany 1975). It is common for people in western Uganda to find rodents in food storage areas and fecal contamination on food for human consumption (T. R. Gillespie, personal communication). Because roof rats typically have an overlapping habitat with humans, their infection status can provide evidence of zoonotic disease risk in the human population.

Although the rodent reservoir species is not known for many OPXVs, members of this viral genus infect a broad range of rodent hosts (Damon 2006). Despite much effort, MPXV has only been isolated once from the wild (Khodakevich, Jezek & Kinzanzka 1986); therefore, serosurveys are valuable for evaluating OPXV circulation among wildlife in the absence of viral isolation. I hypothesized that the evaluation of serum from roof rats for the presence of OPXV IgG would provide evidence of potential zoonotic disease risk at the interface of human-wildlife interactions.

5.3 MATERIALS and METHODS

Acquisition of animal samples

From May to July 2008, 44 roof rats were live-trapped inside and within close proximity of traditional dwellings near Kibale National Park in the Kabarole District of Uganda (0°13′ – 0° 41′ N and 30°19′ – 30° 32′ E; Figure 5.1) using Sherman live-capture traps (3 x 3.5 x 9; H. B. Sherman Traps, Inc., Tallahassee, Florida, USA). I targeted areas with high human population densities, such as villages and trading centers. Traps were baited with various types of local human food and set overnight. In the morning, traps were picked up and transported a short distance to a field laboratory. Rats were removed

from traps and anesthetized with 5% Isoflurane. While the rats were in a deep plane of anesthesia, 2 ml of blood was collected from each via cardiocentitis followed by humane euthanasia. Each rat was photographed and standard measurements were recorded and used for species confirmation (Delany 1975). Samples of the lung, liver, spleen, and any apparent skin lesions were collected postmortem. All samples were shipped on dry ice to the Centers for Disease Control and Prevention, Atlanta, Georgia, USA. All procedures and animal collection were approved by the University of Illinois Urbana-Champaign institutional animal care and use committee (#06037).

Polymerase Chain Reaction (PCR) Analysis

DNA was extracted from spleen tissue from all 44 rats and from skin lesions of two rats using the DNA EZ1 tissue kit (Qiagen, Valencia, California, USA). All DNA samples were screened for poxvirus DNA using GC content-based pan-pox universal PCR assay (Li *et al.* 2010). These assays should detect all known viruses within the subfamily *Chordopoxviridae* except members of the genus *Avipoxvirus*. In addition, DNA extracted from skin lesions was analyzed using more specific RT-PCR assays to identify OPXV genus-specific DNA (Li *et al.* 2007).

Enzyme-linked immunosorbent assay (ELISA)

Serum from each rodent was assessed for IgG antibodies to OPXV using a modification of a previously published ELISA (Karem *et al.* 2006; Hutson *et al.* 2009; Keckler *et al.* 2011). Specifically, I tested each sample against *vaccinia virus*, a member of the OPXV genus. The ELISA plates were prepared with crude *vaccinia virus* (DryVax

strain) with two modifications made from the published assay (Keckler et al., 2011). The first modification was that each serum sample was run at a 1:100 dilution in quads (four times each run) to ensure quality of results and reduce false-positive results due to variability among individual wells. The second modification was the use of a *R. rattus*-specific peroxidase-labeled affinity purified antibody, Goat-anti-Rat IgG at a 1:1000 dilution in blocking buffer (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Maryland, USA). The final optical density (OD) values were obtained by averaging the OD values from the four wells containing *vaccinia virus* minus the average OD and 2 standard deviations of the OD values from the corresponding lysate wells (Keckler *et al.* 2011).

Western blot (WB) assay

A WB assay was performed to identify specific OPXV protein profiles in the captured animals. Specific viruses included: Cowpox-Brighton (CPXV-BR), Cowpox-Sweden (CPXV-SW), Monkeypox-ROC2005 (MPXV), Vaccinia-DryVax (VACV), Camelpox (CMLV), and Taterapox (TATV). Each virus was propagated in BSC-40 cells. Crude virus was purified using a 36% sucrose cushion and standard poxvirus purification protocols (Smallwood *et al.* 2010). BSC-40 cell lysate proteins were included in each assay as a control to test for nonspecific binding of antibodies to cellular debris potentially remaining in semipurified viral stock. In addition to using crude BSC-40 lysate protein, BSC-40 cells were mock-purified using identical methods designed for poxvirus purification and tested against serum samples to insure adequate purification. Purified viral proteins (15 μg) from CPXV-BR, CPXV-SW, MPXV, VACV, CMLV, and

TATV and crude BSC-40 lysate were incubated with Laemmli buffer containing 5% 2-mercaptoethanol and boiled for 5 min. Each sample was separated by 4-20% gradient polyacrylamide gel electrophoresis (Ready Gel Tris-HCL) and proteins were transferred to polyvinylidene difluoride (PVDF) membranes (BioRad, Hercules, California, USA). Molecular weight standards were run on each gel (Precision Plus Kaleidoscope 1Kb, BioRad). After the protein transfer, PVDF membranes were blocked for 2 hr using 200 mg of dry milk in 100 ml of PBS and 0.1% Tween (PBST). Membranes were then washed with PBST three times each for 10 min and membranes were probed with rodent serum at a dilution of 1:1000 in blocking buffer at 4 C overnight. Next, membranes were washed with PBST three times each for 10 min and exposed to Goat-anti-Rat secondary antibodies, alkaline phosphatase (AP) conjugate IgG at a dilution of 1:3000 -1:6000 in blocking buffer for 1 hr (Promega, Madison, Wisconsin, USA), followed by three washings as described above. Immunoreactive interactions between rodent serum antibodies and OPXV proteins were identified using autoradiography detection (Immun-Star AP substrate, Biorad). Immunodominant bands present on each blot were recorded and the molecular weights in kilodaltons (kDa) of each of these bands were determined from a linear regression on the log scale of the molecular weight standards for each blot (Turner & Baxby 1979). Any viral protein band that matched in size to those found in the BSC40 lysate were considered negative. Antiserum from rodents with confirmed exposure to MPXV and from rodents with no known OPXV exposure were used as positive and negative controls respectively.

5.4 RESULTS

PCR and ELISA

Extracted DNA from spleens (*n*=44) and skin lesion samples (from 2 of the 44 rodents) were negative for poxvirus DNA using Pan-Pox Universal PCR assays (data not shown) (Li *et al.* 2007). The DNA from skin lesions was also negative for OPXV DNA using RT-PCR. Thus, all animals were negative for current infection with a poxvirus at the time of euthanasia. All samples had ELISA OD values <0.01 and were considered antibody-negative by ELISA (data not shown).

Western blot assay for anti-OPXV antibodies

Antiserum collected from a MPXV-infected prairie dog was used as a positive control and detected multiple viral proteins from all OPXVs tested (Figure 5.2, panel A). Although many bands are present, a select few were consistently more immunodominant (darker) than others. This positive control antiserum detected an immunodominant doublet in the range of 30-39 kDa from all purified OPXVs surveyed. This homologous doublet is consistent with two immune-dominant proteins characterized in mature OPXV virions: the 32-kDa D8 envelop protein and the 39-kDa A4 core protein (sizes given are in accordance to VACV) (Demkowicz, Maa & Esteban 1992). MPXV-infected prairie dog serum also detected a high molecular weight band consistently immunodominant in lanes containing VACV and MPXV proteins and often present but less dominant against other OPXVs. This third immunodominant homologous protein was 62 kDa in VACV and is consistent with the molecular weight of A10, a well-characterized viral core protein, and support findings in previous VACV studies (Demkowicz, Maa & Esteban

1992). Serum from uninfected prairie dogs (Fig. 5.2, panel B) produced no such reaction, suggesting that the bands detected in the positive control here were due to bona fide reactions between anti-OPXV antibodies and viral antigens. The mock-purified lysate antigen did not produce any reactive bands on the membranes when tested against the controls and sample serum. Therefore I concluded that the purified virus used in this study was of very high quality, with no detectable cell lysate material in the viral stocks.

The A10, A4, and D8 proteins exhibit profound immunodominant characteristics on WB using serum from humans inoculated with VACV and testing this serum against VACV proteins (Demkowicz, Maa & Esteban 1992) and this pattern is identical to WB patterns against VACV and MPXV found using serum from both prairie dogs (Figure 5.2, panel A) and humans recovered from monkeypox (Dubois & Slifka 2008). Thus, the ability of antiserum to detect these bands is considered as an indicator of a past OPXV infection. This method for defining positive protein banding patterns of OPXV has been used and accepted in previous studies (Azwai *et al.* 1996), and is more specific and informative than simply identifying positive samples as any sample with nonspecific reaction to viral proteins (Goldberg *et al.* 2008). Thus, I defined a serum sample from wild rodents as positive for OPXV antibodies if the serum detected the immunodominant doublet in a majority of the immunoblotted denatured OPXV. Since the A10 (62 kDa in VACV) protein is only immunodominant in a select few OPXVs (specifically MPXV and VACV) I noted the immunodominant bands present, but did not require this immunodominant band to be present for a blot to be considered positive.

Using the guidelines described above to define a sample as positive for anti-OPXV antibodies, I identified 18 of the 44 wild *R. rattus* samples as having IgG

antibodies reactive to the 39-kD (putative A4) and 32-kD (putative D8) proteins. Panels C and D (Figure 5.2) are examples of wild rodent antisera from two *R. rattus* that were considered positive with immunodominant bands against A4 and D8 proteins (39 and 32 kDa in VACV). In addition, these blots are examples of immunodominant reaction to A10 protein measuring 62 kDa in VACV. Panel E and F (Figure 5.2) are two examples of wild rodent antisera considered negative for anti-OPXV antibodies due to a lack of immunodominant doublet bands between 32 and 39 kDa on majority of OPXV proteins.

I concluded that 40.9% (18 of 44) of the roof rats sampled in this study had previous exposure to an OPXV or a related virus within the *Poxviridae*. Of the 18 positive samples, 15 (34.1% of rats tested) also reacted with a homolog 62-kDa band that is representative of protein A10 (Figure 5.2, panel C and D) (Demkowicz, Maa & Esteban 1992; Demkowicz, Maa & Esteban 1992; Dubois & Slifka 2008). Although the banding patterns of these blots appear similar to patterns expected from MPXV and VACV exposed individuals, I cannot definitively say these viruses are responsible for the antibody-positive results in this sample set.

5.5 DISCUSSION

I identified anti-OPXV IgG antibodies in serum from peridomestic roof rats in rural western Uganda—an area with no previous reports of OPXV in rodents and no reports of MPXV in humans. Despite the substantial field effort that has been made in central Africa to identify the reservoir of MPXV, a viral isolate has only been identified in one wild animal (Khodakevich, Jezek & Kinzanzka 1986). In addition to the historical difficulties identifying viral isolates from wildlife, small sample size precluded us from

isolating a specific OPXV from these wild rodents; however, my confirmation of OPXV antibodies in this system highlights the need for ongoing research at this interface.

I also observed that the OPXV WB assay was a more sensitive diagnostic than ELISA when screening serum samples from roof rats for antibodies to specific OPXVs. This sensitivity difference could be because ELISA methods utilize whole virus particles and therefore test the ability of IgG antibodies to bind to mainly membrane proteins, while WB assays use denatured proteins consisting of membrane and core proteins and test the ability of IgG antibodies to bind to both membrane and core proteins. My results are consistent with historical studies which conclude that two of the three most immunodominant proteins are core proteins, specifically A4 and A10; therefore IgG would have the ability to bind to these proteins more readily using WB assays as opposed to ELISA (Moss 2007). Another potential explanation for the difference in sensitivity between these two assays is that the antiserum reaction seen in these samples may be due to exposure to an unknown non-OPXV member of the *Poxviridae* with similar conserved core proteins as those in OPXVs but variation in the less conserved membrane proteins.

Although my WB assay results do not identify the specific OPXV responsible for the immunologic reaction seen in this study, I can conclude that the rats have been infected and mounted an immune response to a member of the *Orthopoxvirus* genus or an unknown relative in the *Poxviridae* family. Currently, MPXV is the only known zoonotic OPXV in sub-Saharan Africa. Historically, monkeypox outbreaks have occurred in the Congo basin where handling of infected animal tissue is thought to be the main source of infection with human-to-human transmission occurring less frequently (Khodakevich *et al.* 1987; Jezek *et al.* 1988). Areas where previous outbreaks of monkeypox have

occurred consist primarily of a population of people that rely on hunting and bushmeat as a primary food source (Khodakevich, Jezek & Messinger 1988). The people of western Uganda rely mainly on subsistence farming and domestic cattle, goats, and sheep (Struhsaker 1997). This difference in primary food sources and subsequent wildlife contact indicates a critical control point at which the people of western Ugandan are possibly not exposed to zoonotic pathogens transmitted through tissue handling to the same level as individuals in DRC. Therefore the human population of western Uganda may be at lower risk for transmission of MPXV or novel zoonotic OPXVs.

My results theoretically suggested that the restricted spatial distribution of monkeypox in its native range in Africa could possibly be due to pathogen competition. This apparent competition is thought to occur between MPXV and another antigenically similar but possibly less virulent Orthopoxviruses in regions neighboring monkeypox endemic zones (such as Uganda). This competition could possibly lead to the exclusion of monkeypox from these neighboring areas or at least prevent emergence. There is currently a lack of evidence supporting the idea that *Rattus* sp. are susceptible to monkeypox infections (Marennikova & Seluhina 1976), but we do know the host range for monkeypox is very broad (Guarner *et al.* 2004). We also know *Rattus* sp. are host to other OPXV (Martina *et al.* 2006). If indeed *Rattus* sp. are susceptible to monkeypox virus infections, the peridomestic rodent population in western Uganda could already be protected against an introduction of the deadly zoonotic pathogen. The transmission rate of MPXV is unknown but hypothesized to be low. If indeed the rodent population is naturally immunized from an introduction of MPXV, the human population could also be protected. Historically, human infections of MPXV are most often associated with rodent

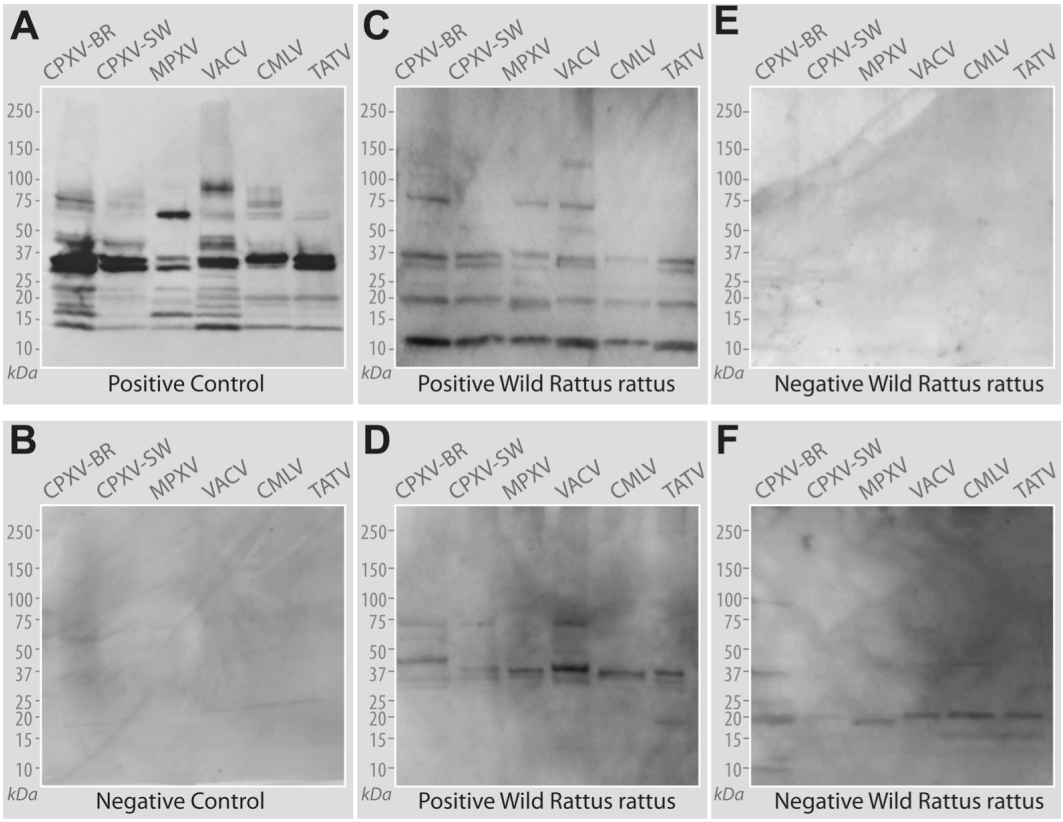
contact; therefore, a MPXV immune rodent population could contribute to the control the disease in the human population. Game-theory models have shown that strains with suboptimal reproductive rate and virulence level are expected to die-out while the excluder strain is the pathogen that optimizes these basic rates (Bremermann & Thieme 1989). In considering the case of MPXV and other competing OPXVs, if the transmission rate is high and the virulence is lower in the competing pathogen, we could expect to see the restrict spatial distribution of OPXVs in Central Africa.

My results expand the known geographic range of poxviruses in wild rodent populations. Future work examining factors contributing to the absence of poxvirus infection in Ugandan human populations despite geographic overlap with infected small mammals may help us understand how endemic African OPXVs have remained contained and may help us determine future poxvirus-related risks to human health.

5.6 FIGURES

Figure 5.1: Map of Uganda. Roof rats (*Rattus rattus*) were collected in and around human dwellings near Kibale National Park in Uganda to test for antibodies against orthopoxviruses such as monkeypox virus. Although monkeypox occurs throughout the Democratic Republic of Congo (DRC), no human cases have been reported across the eastern political border between DRC and Uganda.

Figure 5.2: Orthopoxvirus Western Blot assay results. Specific purified viruses used for the orthopoxvirus (OPXV) western blot assay were; Cowpox-Brighton (CPXV-BR), Cowpox-Sweden (CPXV-SW), Monkeypox-ROC2005 (MPXV), Vaccinia-DryVax (VACV), Camelpox (CMLV), and Taterapox (TATV). Shown are blots are of serum from A) a MPXV-infected prairie dog (positive control), B) an uninfected prairie dog (negative control), C&D) two individual roof rats (*Rattus rattus)* representing positive serum samples for OPXV reactivity, E&F) two roof rats considered negative for OPXV reactivity.



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CHAPTER 6

Strengths, Limitations, and Conclusions

As human population growth further encroaches on wildlife habitats, the impact of anthropogenic habitat disturbance on mammalian populations is of increasing concern (Ceballos & Brown 1995; Ceballos *et al.* 2005). Areas in rural western Uganda are not immune to these anthropogenic changes (Gillespie & Chapman 2008). Specifically, forest fragments within the mosaic landscape near Kibale National Park are at the nexus of human-wildlife interactions. Additionally, alterations in the natural dynamics between reservoir hosts and the pathogens they harbor could lead to increased rates of disease emergence (Daszak, Cunningham & Hyatt 2000) as well as parasite and host extinctions (Altizer, Nunn & Lindenfors 2007).

The data, the results, the analyses, and their interpretations within this dissertation contribute to our understanding of relationships among habitat disturbance, diversity, and disease occurrence impacting small mammals in western Uganda. This dissertation reports several scientific findings, some which are further supported by empirical evidence. Additionally, this dissertation has important limitations. Despite these limitations, I have presented new ideas and posed questions whose answers will require further scientific exploration into the community dynamics driving changes in disease dynamics among small mammal in rural western Uganda.

6.1 KEY FINDINGS and STRENGTHS

Disturbed habitats harbor high levels of small mammal species richness

My dissertation research found that moderately to severely disturbed habitats harbored a relatively rich community of small mammals and parasites (Salzer *et al.* 2014). These were the same habitats considered to experience higher human-wildlife interactions (Goldberg *et al.* 2008). These habitats were previously considered inadequate environments to host other forest dwelling species, such as wild primates (Chapman *et al.* 2012). Nevertheless, due to the notably high small mammal species richness revealed by my dissertation research, these habitats may be of particular interest for future natural history studies focused on cataloging biodiversity in western Uganda. Additionally, results described in this dissertation provide empirical evidence to support the need for conservation of forest fragments in western Uganda.

Host taxonomy associated with disease dynamics

Ecological factors are known to impact disease dynamics among communities of hosts (Johnson *et al.* 2013), but host taxonomy may be a more reliable determinate of disease dynamics (Salzer *et al.* 2014). Despite claims of a protective effect of biodiversity on disease (Keesing, Holt & Ostfeld 2006), habitat disturbance and diversity in western Uganda may not influence disease dynamics as much as the idiosyncratic relationship of small mammal host and parasite natural history.

Community of hosts and a community of parasites

Studies of parasites in natural systems tend to focus either on multiple parasites infecting a single host species (Froeschke *et al.* 2013) or on a single parasite species infecting multiple hosts (Essbauer *et al.* 2009). The level of detail of both host and parasite taxonomy and the complexity of interactions examined in my dissertation allowed for a more thorough assessment of the convoluted influences of host community and habitat variables on parasite community dynamics. My work examined a diverse community of small mammals and a complex group of parasites, therefore breaking the mold of a standard host-parasite study model that has more traditionally been used in disease ecology studies of natural systems (Lafferty 2010).

Previously unreported and unknown species

By identifying each small mammal captured to species level of taxonomic classification, I increased the number of known reported small mammal species associated with Kibale National Park from 13 to 23 (Struhsaker 1997). This identification was conducted using molecular techniques combined with traditional morphometric methods (Nagorsen & Peterson 1980; Peppers, Carroll & Bradley 2002; Thorn & Kerbis Peterhans 2009). Without the combination of traditional and modern methods, several cryptic species may not have been identified and therefore would have continued to be unreported in western Uganda. Additionally, two individuals collected during this research are now considered to be potentially new species. Future research into the details of their morphology, natural history, and phylogenic classification is already underway by mammalogists specializing in Ugandan small mammals (J. Kerbis Peterhans pers. comm.).

Findings support historical research

Historical studies in Kibale National Park were conducted in the 1970s and 1980s (Isabirye-Basuta 1979; Isabirye-Basuta & Kasenene 1987) and were the first to identify an increase in small mammal diversity associated with areas of intense logging. My dissertation confirms the patterns associated with diversity and habitat disturbance observed by Dr. Isabirye-Basuta and Dr. Kasenene over 30 years ago. These congruent results between the recent and historical studies provide evidence that Kibale National Park and surrounding areas may serve as a reliable study site for future investigations of the mechanisms impacting the relationship among habitat disturbance, small mammal communities, and the parasites they harbor.

Small mammal parasite presence congruent with wild primate studies

Wild primates have primarily been the focus of studies investigating the impacts of habitat disturbance on wildlife disease in western Uganda. My research focused on parasites among rodents and shrews in western Uganda and revealed interesting patterns of parasite prevalence from areas experiencing varying levels of habitat disturbance. The patterns of parasite prevalence observed in my study, specifically gastrointestinal protozoans, are very similar to parasite findings in wild primates occupying the same habitats (Salzer *et al.* 2007). These findings serve as evidence of the potential for small mammals to serve as sentinels for parasite prevalence and disease risk among primates inhabiting the same habitats. This valuable finding suggests a non-invasive method for

identifying risk of disease in endangered primates and warrants further investigation alongside future wild primate disease studies.

6.2 LIMITATIONS

Despite the strengths and numerous findings within my dissertation, my data and analyses had significant limitations. Many of these limitations are due to the inherent shortcomings of the methods and study design related to the field collection. Replication of sampling in both time and space was severely limited. This shortcoming would have been reduced by increasing the number of sites sampled within each habitat category, repeatedly sampling over time at each site, quantifying disturbance as a continuous variable, and collecting animals across multiple years and seasons. Because little was known concerning small mammals and their parasites in western Uganda prior to this study, I focused on collecting small mammals over a shorter period of time and investigating each host and parasite sample in great detail.

Lack of seasonal variation in sampling

Small mammal population dynamics vary seasonally in Kibale National Park (Struhsaker 1997). The time allotted to animal collection for this research limited the sampling effort to the dry season in the summer of 2009. Therefore the results should be interpreted as a single season and time point, and they ought not be extrapolated to other seasons and years. Although I would anticipate capturing similar small mammal species during other seasons, the relative abundance and density of these species are likely to vary throughout the year (Isabirye-Basuta & Kasenene 1987). Future work investigating

changes in population dynamics of hosts and parasites across multiple seasons are needed to understand the results of my dissertation in the context of seasonal variability.

Lack of replication within habitat types

Six different habitat types were sampled with a single trapping location representing each habitat type. This lack of replication of habitats was an innate bias in the assessment of each type of disturbance and prevented statistical analysis among habitats experiencing similar disturbance. In the original study design, I intended to replicate the sampling conducted in the forest fragments, by sampling two fragments. I found these two fragments to contain communities more dissimilar to each other than to other sites. Due to the high level of dissimilarity, these two fragments were analyzed as separate independent habitat types. Future studies will need to be conducted at various locations within each habitat category to measure the variability among sites within each habitat category and properly exclude environmental variables (e.g. elevation, moisture, ecotones) impacting small mammal communities.

Small number of species found across multiple habitats

The total small mammal species richness found in this study was higher than anticipated based on historical studies (Isabirye-Basuta & Kasenene 1987) and records (Delany 1975). While the unprecedented high species richness found in this study provided valuable contributions to our knowledge of the diversity of small mammals of western Uganda, it had an unintended consequence of reducing the overall sample size for each species collected. Additionally, the sample size collected for this study is

relatively small when compared to other natural history studies (Hice & Velazco 2012). Species composition varied greatly among sites and only three rodent species were found in a majority of the habitats, causing reduction in my statistical capability to analyze associations across multiple habitats controlling for species taxonomy (Salzer *et al.* 2014). This shortcoming could be solved in future studies by an increased sampling effort across multiple seasons, which would strengthen the associations seen between hosts and their parasites across habitats.

Quantification of habitat disturbance

Habitat disturbance was quantified as an ordinal variable based on long-term studies conducted by others (Chapman *et al.* 2000; Gillespie, Chapman & Greiner 2005; Gillespie & Chapman 2006). Several statistical restrictions emerged during my analysis of the data because habitat disturbance was measured as an ordinal variable. More detailed analyses investigating the associations with habitat disturbance could have been conducted on my dataset if disturbance had been quantified as continuous variables (e.g. percent open canopy, tree stump counts). Additionally, if disturbance had specifically been measured at the level of trapping webs, as opposed to forest compartment, these finer scale results would have improved the strength of the analyses (e.g. tree stump count within 200 meter trapping web area). This level of investigation of habitat disturbance may have detected additional idiosyncratic relationships among hosts and parasites not found within the current analysis.

Crude classification of ectoparasites

Small mammals were identified to the taxonomic level of species, while the community of parasites was not analyzed to the same level of taxonomic classification. The preservation of the ectoparasites samples in the field was performed for morphometric identification and did not allow for molecular analysis. Despite this shortcoming, my results confirmed that the crude level of taxonomic classification of parasites used in Chapter 2 was adequate to find patterns associated with host-parasite relationships. But, the identification of parasites to a finer level of taxonomic classification may have increased my power to identify specific host-parasite relationships within this complex study system.

6.3 FUTURE DIRECTION and NEXT STEPS

This dissertation has answered many questions, but it has also laid the foundation for several exciting new avenues of future exploration for the next generation of research of small mammals in western Uganda.

My research identified specific areas and habitat types in western Uganda that were associated with high levels of small mammal species richness. Additionally, species richness was associated with coexistence of grassland and forest-dwelling species. But, the mechanisms driving changes in species richness and coexistence cannot be definitively identified with the data collected thus far. Future studies in western Uganda would need to more extensively examine the habitat variables and possibly include manipulated field experiments to understand the ecological mechanisms underlying changes in species richness and coexistence.

I identified parasites associated with small mammals that may be of greatest ecological interests in western Uganda (Salzer *et al.* 2014). Further molecular analysis to determine parasite taxonomic classification is needed to understand the host specificity of these parasites and their zoonotic potential. Although some parasites examined in this study were identified to the species level (i.e. trypanosomes), others were preserved in formalin, making molecular analysis impossible. This missing information would possibly unlock the true idiosyncratic relationships and changes in disease dynamics among different habitats and hosts. Now that the parasites of greatest ecological interests have been examined, this lays the foundation for more detailed ecological disease studies of small mammals in the future.

Throughout this dissertation I have speculated on how the results of rodent-borne diseases investigated in this project could indicate a risk to human health. But to truly understand the risk of small mammal communities to human health, parasites that pose a known human health risk need to be examined, as does the associated human population. Newly acquired knowledge of small mammal species occupying areas of human-wildlife interface in western Uganda can further identify parasites of greatest interests in future surveillance studies of both small mammals and humans. Given the specific species of small mammals I collected in peridomestic environments and previous knowledge of these species serving as reservoirs for disease in other areas, a more detailed list of zoonotic parasites of greatest concern to humans can be generated. These zoonotic parasites would include (but not be limited to) leptospires, lassa virus, hantaviruses, rickettsial agents, and lyssaviruses.

In conclusion, the reaction of an ecosystem to habitat disturbance can create simultaneous changes in the abundance of hosts and parasites. In addition, a complex feedback loop exists among the hosts' community structure and pathogen occurrence and interactions. Certain species can be the primary reservoir host of a multi-host parasite. These host species may be disproportionally responsible for maintaining a particular pathogen within a community of hosts (Dobson 2004). The population and community status of these reservoir hosts can determine the invasion and extinction potential of a parasite (Cross *et al.* 2007). Accounting for all the variability that exists in a given field study system is not currently feasible. Despite this hurdle, exploring the generality of broad concepts is still beneficial to understanding underlying mechanisms. I believe my research revealed valuable patterns among disturbance, diversity, and disease of small mammals, while also laying a foundation for future ecological studies of small mammals in western Uganda.

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