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Land-use change and zoonotic disease risk:
Dynamics at the human-animal interface

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Author's declaration

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Abstract

Emerging infectious diseases are a significant threat to global public health and biosecurity. The majority (~60%) are zoonoses, which are infectious diseases that are transmitted from animals to humans, with most of these (>70%) caused by pathogens with a wildlife origin. EID events caused by wildlife pathogens are increasing in impact and through time – more than half of the most recent EIDs (1990-2000) had a wildlife origin. Bats are hosts to some of the highest profile emerging zoonoses, including Ebola, Nipah, and Hendra viruses, and severe acute respiratory syndrome (SARS). In recent years, circumstances such as land-use change, human incursion into tropical forests and wildlife trade for food have resulted in the spillover of several zoonotic viruses. In the last two decades, viruses from the *Coronaviridae* family have caused two large-scale pandemics (i.e. SARS and Middle East respiratory syndrome), and just this year a novel coronavirus was detected in China (nCoV-2019) linked to the wildlife trade. At the time of writing, nCoV-2019 had spread to over 20 countries with over 4,500 confirmed cases. Thus, understanding the factors that increase disease emergence risk will be critical in developing strategies that can help minimize and manage the risk of future EIDs.

This thesis is a targeted study emphasizing rigorous and systematic sampling methodology to address the ecological and social factors that may drive zoonotic disease emergence due to land-use change, specifically habitat fragmentation. Habitat fragmentation could modify risk of cross-species transmission ("spillover") by perturbing the dynamics of pathogens in wildlife hosts and/or by bringing novel host-pathogen pairs (including humans) into unprecendented contact. Here, I evaluate how habitat fragmentation affects 1) patterns of host diversity, 2) corresponding patterns of viral diversity, and 3) patterns of human occupancy, and behaviors that may influence contact rates with wildlife in dynamic landscapes. This research integrates theory and field data spanning the disciplines of ecology, virology, biology and public health.

Keywords: viral diversity, zoonoses, land-use change, bat diversity, habitat fragmentation, human-animal contact, emerging infectious diseases

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1 Introduction

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1 Introduction

During the past decade, tropical forests have continued their long-term reduction in extent (*1, 2*), largely due to human activities such as agricultural expansion, deforestation, and conversion of natural habitats to other land uses (*1*). These types of land-use change have created highly fragmented landscapes and perturbed biotic systems with direct and indirect impacts on human and wildlife populations (*3, 4*). Land-use change is a clear threat to global biodiversity and ecosystem services (*5*). It is also considered a key driver of emerging infectious diseases (EIDs) (*6, 7*), which are defined as those that are increasing in incidence or geographic range; those characterized as newly evolved strains of pathogens, and novel pathogens that have entered human populations for the first time (*8*). A systematic quantitative review from the Australian continent suggests that around one-fifth of past EID events was linked to some form of land-use change (*9*). Further, although most human EIDS are zoonotic, (*10, 11*) and increasing in frequency (*8*), current understanding of the relationships between host diversity, disease risk, and land-use change is still in its infancy. These relationships are likely complex, and other mechanisms that can influence disease risk, such as human ecology (e.g. human activity, behaviour, and occupancy), must also be considered (*6, 12*).

1.1 Links between land-use change, biodiversity and disease

During the last half-century, human activities have rapidly transformed much of the Earth's natural systems (*13*). Significant changes in land-use are ongoing, particularly in tropical forest countries that are rapidly developing (*14*). It is estimated that annual forest loss has remained

steady between 2001 and 2015 at approximately 5 Mha (*1*). Much of this forest loss can be attributed to growing global demand for food and natural resources (*15, 16*). In extent, the most significant form of land-use change is the expansion of crop and pastoral lands, which continue to have serious negative long-term consequences for the preservation of global biodiversity (*17*), as agricultural expansion has largely come at the expense of intact forests (*14*). In fact, in the 30 years following 1950, more land was converted to cropland than during the 150 years between 1700 and 1850. Today, more than one quarter of Earth's terrestrial surface has been converted to agricultural systems (*3*).

With the exception of the last remaining expanses of tropical forest in the Amazon and Congo basins, most tropical landscapes now typically comprise a mixture of human-modified landscapes that can include remnants of old-growth and secondary-growth forests, logged forests, agroforestry systems, agricultural land and plantations, and urban areas (*18*). In many tropical countries, these landscapes are comprised of small, irregularly shaped patches that are highly prone to habitat-edge effects (*19, 20*). For example, in Brazil, more than 80% of remaining Atlantic Forest exists in small fragments (<50 ha) that are isolated from each other and are comprised of secondary-growth forests in early to medium stages of succession (*21*).

1.2 Effects of fragmentation on hosts

Habitat fragmentation and loss has been described as the single greatest threat to global biodiversity (*22*). Habitat fragmentation is the landscape-level process by which habitat loss results in the alteration of previously large, continuous habitat into spatially separate, smaller patches surrounded by a matrix of altered habitat (*23*). Though recent studies have confirmed the

importance of fragment area, shape and isolation in predicting species responses (*24-26*), growing evidence suggests that the composition and structure of the matrix is also important in determining how species are distributed in fragmented landscapes.

Research suggests that the decline and extinction of vertebrate populations due to fragmentation can be variously attributed to habitat change (e.g. loss, degradation, edge effects, isolation)(*27, 28*), altered species interactions (predation, parasitism)(*29*), changed behaviour (e.g. edge avoidance, disrupted dispersal), and introduced species or pathogens (*30*), as well as stochastic threats (e.g. temperature, weather) associated with small population size (*31*). Further, landscape-level studies have demonstrated that, across taxa, species responses to fragmentation and disturbance are often species-specific, sensitive to spatial and temporal scales and are strongly moulded by the characteristics of the prevailing landscape matrix (*32*). Gibson et al. (2011) found that mammals tended to be less sensitive to disturbances compared to other taxonomic groups such as birds, and in several cases actually benefitted from disturbances. This discrepancy, largely attributable to higher mammal abundance in certain disturbance types, may be explained by the high level of tolerance to degraded forests displayed by some small mammals, particularly rodents and bats. Yet, a recent metanalysis spanning multiple biomes and scales, five continents, and 35 years demonstrates that habitat fragmentation reduces biodiversity by as much as 75% (*2*). The general effects of land-use change on ecological communities include predator loss, decline in species richness, and the simplification of ecological communities that favour generalist species exhibiting high adaptability to human-modified environments (*7, 33-35*). A previous study found that overall species richness was significantly more sensitive to human disturbance than species abundance (i.e. k-selected) (*36*). Studies from the Americas (*37*);

Asia (*38*), and Africa (*39*), have shown large declines in k-selected species followed by similarly large increases in the abundance of r-selected species in degraded forest habitat (*36*).

A recent meta-analysis supporting this relationship showed that in terrestrial biomes, land-use change and species invasions had the largest impact on species richness, again, with the general pattern of increasing population levels of generalists and decline of specialists (*40*). However, in tropical systems, habitat loss and land-use change were identified as the two most significant processes contributing to declines in species richness, potentially due to the extremely high taxonomic diversity in tropical biomes (*41*), which is particularly affected by reduction in suitable habitat (*40*). Despite the large and growing literature on the impacts of land-use change on ecosystems, communities and populations, little is known about how habitat fragmentation and loss influence pathogen diversity in wildlife hosts.

1.3 Effects of fragmentation on pathogens

Understanding the influence of fragmentation on disease dynamics is important but challenging given the relative scarcity of both empirical and theoretical studies. Nonetheless, theory suggests the existence of host population thresholds for the invasion or persistence of infectious diseases in host populations (*42*). Deterministic epidemiological models show the existence of such thresholds for pathogen invasion, while stochastic models show host population size and/or density thresholds for both pathogen invasion and persistence, emphasizing the increasing chance of non-persistence of a pathogen in small host populations (*43*).

For directly-transmitted pathogens, fragmentation is expected to increase pathogen prevalence due to edge effects and crowding. In fragmented landscapes, habitat edges

significantly increase for a given amount of core habitat (*44*). This results in the juxtaposition of distinct ecosystems, which can facilitate cross-species movement between habitats, increasing the potential for novel contact and disease exposure. Within small and isolated patches, an increase in pathogen burden in host species may occur given crowding of hosts with limited dispersal capabilities (*45, 46*).

This, in turn, could lead to increased vulnerability to infections due to heightened competition for resources and increases in host stress. Further, rodents, which can be important reservoir hosts for diseases such as Lyme disease (*47*) and Hantavirus (*48*), often increase in abundance in tropical and temperate forest fragments (*49*). For vector-borne diseases such as malaria, deforestation has been shown to increase malaria risk by significantly increasing the abundance of open-habitat preferring mosquitoes such as *Anopheles* (*50*). In some cases where fragmentation decreases host diversity, pathogen prevalence may increase, as vectors are concentrated on the remaining individuals, particularly if the remaining species are more competent hosts.

Recent studies have examined the relationship between fragmentation and pathogen dynamics by comparing pathogen prevalence and richness in different sized forest fragments most frequently using primates as study models (*46*) and, less frequently, other mammals (*51*) and avian species (*52*). Although several studies have demonstrated an increase in pathogen burden accompanying habitat loss and fragmentation (*46*), other studies found no effects at all (*51, 52*). In the tropical rainforests of Northern Australia, (*49*) found a significantly higher prevalence of the blood-borne parasite (*Haemoproteus*) in continuous forest than in habitat fragments. Similarly, in Brazil, levels of exposure to the pathogens *Brucella abortus* and *Leptospira*

spp were significantly higher in white-lipped peccary herds sampled in larger fragments compared to those in smaller fragments (*53*).

Another mechanism through which fragmentation may influence disease dynamics is better viewed from the perspective of the pathogen rather than that of the host species. In accordance with metapopulation theory (*54*), this mechanism predicts that as patches become smaller and more isolated, rates of pathogen extinction are likely to increase as rates of (re) colonization decrease lowering pathogen pressure in small populations. The expected outcome is a decrease in pathogen richness within smaller patches that are more isolated. However, Vögeli, Lemus, Serrano, Blanco and Tella (*55*) found that an increase in prevalence, richness and diversity of the pathogen community in samples collected from Dupont's larks (*Chersophilus duponti*) was best predicted by its population size, without effect from geographical isolation and habitat area. From this perspective, the 'size' of potential habitat may be better represented by the number of potential hosts rather than by patch area.

1.4 Land-use change and disease risk

Human impact on natural landscapes can influence the prevalence of infection in hosts by altering human-wildlife interactions (e.g. how and when contact with wildlife occurs), types of human-animal contact (e.g. hunting, butchering or consumption of wild meat), and the likelihood of infection given contact (*6*). In addition, land-use changes may also influence the prevalence of infection in host reservoirs by disrupting the community ecology of pathogens and/or their hosts and vectors, causing changes in species' abundance and distribution, community composition, and ecosystem function (*6, 56*). Thus, understanding the interactions between biodiversity and

human activities is crucial in unravelling the likely complex relationship among biodiversity, disease risk and land-use change.

In tropical regions, changes in land-use have been linked to the occurrence of Chagas disease, yellow fever, and leishmaniasis (*57*). Such changes are particularly intense in tropical regions where primary forest is opened for a range of land-use activities including mining, logging, gold mining, road construction and oil and gas extraction. In fact, there is compelling evidence that human activities, such as hunting and butchering wild meat, played a key role in the initial spillover of several well-known zoonoses including HIV and Ebola virus (*13*). Yet despite increasing attempts to detect a general relationship between land-use change, biodiversity and disease risk, recent research has emphasized the idiosyncratic nature of these relationships.

Perhaps the clearest process linking land-use change and disease risk is through the spread of invasive species and pathogens. Under land-use change, the introduction of new species can also facilitate the introduction of exotic pathogens to new areas, with sometimes devastating effects on native species. Examples include avian malaria and subsequent declines in endemic bird fauna in Hawaii (*58*) and the introduction of parapoxvirus from gray squirrels (*Sciurus carolinensis*) to endemic red squirrels (*S. vulgaris*) across the United Kingdom (*59*). Yellow fever and malaria have also crossed biogeographic barriers due to human-induced disturbances to the environment and emerged in new populations.

A growing body of research has shown that biodiversity can influence disease risk through 1) an 'amplification effect,' in which biodiversity positively correlates with infectious disease risk or 2) via a 'dilution effect' which describes a negative relationship between

biodiversity and infectious disease risk (*60, 61*). Yet, examination of the theoretical and empirical evidence has produced mixed support as to which of these hypotheses is generally more likely to occur under a land-use change scenario (*6, 62-64*).

The best-studied example supporting the dilution effect is Lyme disease caused by the spirochete *Borrelia burgdorferi.* This pathogen is vector-borne and transmitted between numerous host species by ticks. The dilution effect proposes that as biodiversity in a region is reduced through land use change, the assemblage of hosts that are non-competent or poorly-competent for pathogen transmission is reduced, and the density of transmission-competent hosts increases. Thus, the probability of ticks feeding on transmission-competent hosts is increased, leading to an increase in the risk of infection to people. Specifically, studies have shown that the presence of a diverse ecological community reduces the proportion of ticks that feed on the highly competent white-footed mouse (*Peromyscus maniculatus)* (*56, 61*).

However, other factors may be relevant. Recent work has shown that the loss of predators (*65*) in the Northeastern USA may have altered Lyme disease prevalence in ticks and risk of human infection. Similarly, recent research has shown that West Nile virus exposure in the United States increases as avian biodiversity decreases, with heightened risk in suburban regions compared to intact forests in the Northeastern USA. However, the main drivers seem to be the presence of a highly competent, abundant and mosquito-preferred species, the American robin (*Turdus migratorius*) in the former and its absence in the latter, coupled with increased abundance of the key vectors (*Culex* spp. mosquitoes) in the former (*66-69*). Patterns consistent with the dilution effect have also been reported for Chagas disease in Latin America (*70*), where risk of human exposure is positively correlated with reduced vertebrate host diversity and for

hantavirus, where in field experiments, disease exposure increases as mammalian diversity decreases (*48*). A similar pattern was also reported for haemoparasite infections in the fruit bat (*Artibeus jamaicensis*), where trypanosome prevalence increased with loss of species richness in forest fragments (*71*).

While the generality of the dilution effect across different diseases and ecosystems remains unclear, recent work has shown an increase disease risk from regions of high biodiversity (lower-latitude developing countries in the tropics), particularly for zoonotic diseases of wildlife origin (*8, 72*). The specific mechanism is not clearly demonstrated yet, but it is assumed that pathogen diversity is a function of host diversity, with higher levels of biodiversity inferring greater pathogen diversity. Thus human disturbances to highly biodiverse regions could result in novel exposure to a more diverse 'pool' of pathogens and elevated risk of spillover (*6*).

These disturbances can also result in exposure of both human and wildlife populations to novel human pathogens, as cross species transmission (spillover) can occur in both directions (*11, 73, 74*). A good example of this is infection by several human pathogens in gorillas exposed by the ecotourism industry in central Africa. In western Uganda, humans and chimpanzees shared genetically similar gastrointestinal communities in areas where their habitats overlapped, indicating an exchange of bacteria between humans and non-human primates even where there was no direct contact involved (*74*). Researchers also found that forest fragmentation increases bacterial transmission between primates and humans and their livestock, as the level of disturbance of forest fragments positively correlated with human-primate bacterial genetic similarity (*75*).

Another potential mechanism linking biodiversity, disease risk and land-use change proposed by McFarlane et al. (2013) involves contact rate, and changes in species richness and abundance. Synanthropic species, defined as those able to tolerate anthropogenic disturbances, are more likely to be sources of EIDs of wildlife origin than those that do not (*9*). The authors acknowledge that this relationship is likely an effect of exposure opportunity rather than a more pathogenic microbial community, which corresponds with the findings of previous studies (*76-78*). They reason that because specialist species are highly adapted to particular habitats, thus more vulnerable to habitat loss, they have less opportunity to interact with humans than generalist species and are more likely to become extirpated from an ecological community.

1.5 Specific mechanisms underlying disease emergence

Current research suggests that land-use change and biodiversity loss may play significant roles in increasing transmission of many emerging zoonotic infectious diseases, however the specific mechanisms underlying these linkages remain poorly understood. In a recent review, Murray and Daszak (2013) propose two conceptual models for how land-use change drives disease emergence: the perturbation and/or pathogen pool hypotheses (*6*). The first focuses on a more dynamic model for disease emergence, where land-use change forces perturbations in pathogen dynamics within the reservoirs, before emergence occurs in humans or livestock. For instance, work from the Peruvian Amazon has demonstrated that land-use change, human-made or natural, can increase the risk of malaria due to effects on the behaviour, abundance and/or distribution of open-habitat preferring mosquitoes such as *Anopheles* (*50, 79*). The latter assumes exposure to novel diseases from a diverse pool of pathogens to wildlife to which humans and

livestock, as naïve hosts have not had prior exposure. Consistent with the pathogen pool hypothesis, disease risk is also predicted to correlate with baseline microbial diversity in host species (*80*). In reality, it seems unlikely that these two hypotheses are mutually exclusive, and disentangling them will likely prove difficult, but necessary in understanding disease emergence and developing effective prevention and control strategies.

1.6 Conclusions

Anthropogenic activities are transforming many of Earth's natural landscapes in ways that are pervasive and increasing. Mounting evidence suggests that limiting human influence in natural systems and protecting biodiversity could help to reduce the risk of emerging zoonotic diseases (*6, 61*). Unfortunately, there is currently minimal uptake of this as a conservation or disease management strategy, given our lack of knowledge of the specific mechanisms involved, as well as a significant lack of knowledge of the diversity of pathogens present in potential reservoir hosts and host-pathogen ecology.

However, the interactions among land-use change, host diversity, cross-species transmission, human behaviours and disease risk are major focuses of disease ecology and substantial work has been published on the disease systems described here. Although recent studies are beginning to examine these linkages at the human-animal interface, there are few studies that elucidate the specific mechanisms through which they interact. Here, we adopt a more systematic and comprehensive approach focusing on robust study design that allows for replication and involve controls or reference sites. Studies that integrate an understanding of human ecology with host-pathogen ecology will likely be critical to determining if there are

predictable relationships between host and pathogen diversity, and how these change with the landscape. Lastly, robust knowledge of how habitat fragmentation affects not only macro-biodiversity, but also microbial diversity, is needed if we are to comprehend adequately the implications of forest fragmentation.

1.7 Brazil as a case study

This study was conducted in two regions of Brazil: 1) Amazonia, and 2) the interior Atlantic Forest in São Paulo state. Household surveys quantifying animal-human contact took place in both regions, while bat and viral surveys were only conducted in the Atlantic Forest region. These regions define the extremes of the fragmentation process and were chosen as model systems as they represent patterns of fragmentation across the globe.

During the past three centuries, much of the Brazilian Atlantic Forest has been converted to agriculture or pasture land and/or logged for timber. The remaining forest is predominantly comprised of small fragments less than 1000 ha in size, which are located within 1 km of a forest edge (*81*). In contrast, although Amazonia is a rapidly changing frontier, much of the forest in this region remains contiguous and less fragmented. In Amazonia, the proportion of forest farther than 1 km from the forest edge has decreased from 90% (historical) to 75% (today). While this decrease is significant, it is less severe compared to the Atlantic Forest where the proportion of forest farther than 1 km from the forest edge has declined from 90% (historical) to less than 9% at present (*81*).

1.7.1 Amazonia

Amazonia is the world's largest tropical rainforest spanning nine countries in South America and hosting approximately a quarter of the world's terrestrial species (*82*). The Brazilian Amazon contains over half of Amazonia's forests, over 1 million km^2 of freshwater ecosystems (*83*) and more fish species than the Congo and Mekong basins combined (*84*). It is also increasingly threatened by deforestation, stress from climate change, and the rising resource demands of growing urban populations. In fact, urbanization of forest wilderness is particularly relevant in Brazil, where little is known about the scale and drivers of urban bushmeat consumption. Here, we examine the extent of human-wildlife contact in communities situated along a rural-to-urban gradient ranging 50 km north from the Amazon's largest city, Manaus (population 2.1 million people). Our sites were located in a preserved primary forest (pristine), in forest within a highly fragmented, developing landscape (intermediate) and in a forest patch within a highly deforested area (urban). The urban site was located in urban neighbourhoods in the city of Manaus. The majority of the population in this area descends from early European settlers, chiefly the Portuguese, and assimilated indigenous peoples. The intermediate site was located in the municipality of Rio Preto da Eva, specifically in the Beija-Flor Indigenous Community, comprised of different ethnic groups including Sataré-Mawe, Tukano, Dessano, Twiuca, Apurinâ, Baniwa, Arara, Marubo, Mayuruna, which are distributed among three communities: Beija flor I, Beija Flor II and Beija Flor III (*85*). The pristine site is located in an area that has been protected by Universidad Federal do Amazonas since 1974, and is part of an extensive stretch of forest (30 km^2). The community of Nova Canaã is located directly beside the preserved primary forest that is managed by UFAM.

1.7.2 Atlantic Forest

Along a habitat fragmentation gradient that ranges from 36,000 ha of continuous forest to habitat fragments less than 2 ha, the interior Atlantic Forest in Brazil is one of the most threatened tropical regions in the world. In terms of understanding the effects of habitat fragmentation on bat and viral assemblages, the Pontal do Paranapanema region provides an ideal model of extreme habitat fragmentation as the landscape matrix has been largely maintained as homogenous, comprised of pasture, sugarcane and now soy, with low human density for the last 50 years (*86*). These homogenous landscape characteristics minimize factors that could influence species richness in the region apart from the spatial structure of forest patches and its dynamics.

In Pontal do Paranapanema, our field sites were located along a habitat fragmentation gradient in and around Morro do Diabo State Park (MDSP), which contains the largest preserved area of inland Atlantic Forest in São Paulo State (~33,000 ha). Scattered fragments on farms and rural agrarian settlements total an additional 15,000 ha. For this component of the study, we sampled bat communities from four different land-use categories including reference sites in continuous forest in Morro do Diabo State Park, large and small fragments in the region, and matrix sites surrounding the park. To date, approximately 300+ families of small producers have been settled in the matrix area surrounding MDSP, with each family given a plot of ~15 ha. In addition, more than 1,500 additional families are camped along highways and ranches awaiting land titles. As a consequence, new colonies of farming families are cultivating land along the borders of forest fragments (*87-89*). To ensure a range of fragment sizes were represented in the study, I selected four large (1000-2000 ha) and three small (<100 ha) fragments. I did not choose

patches smaller than 50 ha because generally these patches are highly degraded due to the edge effects, which is very intense in this type of tropical semi-deciduous forest.

1.8 Bats and emerging infectious diseases

I focus specifically on bats as the order *Chiroptera* comprises over one-fifth of all extant mammals, with more than 1,300 described species (*90*). In Brazil, there are currently 182 known species (*90*), with this number likely to increase as several new species have been described each year on average during the past two decades (*91*). Their diversity alone, along with their global distribution may contribute to the diversity of their pathogens.

Further, bats have been widely recognized as important host reservoirs for EIDs as they possess several unique features that may make them 'special' in terms of EID transmission and maintenance. First, bats are the only mammals that are capable of true flight, allowing them to disperse and transmit their pathogens during seasonal migrations and foraging flights. This mobility, coupled with their broad food range and their ability to inhabit a wide range of ecological niches has facilitated intra- and inter-species transmission in various locations (*92*). For example, several species of bats known to harbour lyssaviruses (*Eptesicus fuscus* and *Eptesicus serotinus*) commonly roost in men-made structures. Further, bats often inhabit and forage in agricultural areas, which brings them into closer contact with humans and domesticated animals. In the tropics, frugivorous bats can be found roosting in urban areas and feeding on fruit trees in plantations (*93*).

The ability to fly also has immunological implications. As flight requires a low body mass, similar to many birds, bats have evolved to have hollow bones which decreases body mass and allows them to fly. As a result, their bones do not contain bone marrow and B-cells are produced in different locations (*94*). Unlike other mammals, these unique anatomical and physiological characteristics may contribute to the diversity of pathogens associated with this taxon.

In addition to their ecological vagility, many bat species roost together in large and dense colonies, which may provide increased opportunities for viral exchange between and among bat species (*95*). Many bat species, such as *Myotis* spp, have high levels of interspecies contact and, have been found to harbour a diverse range of RABV (rabies virus), suggesting that increased inter-species contact increases viral transmission (*96*). Several infectious agents, including NiV, have been isolated from the urine of fruit bats and also from mutually-groomed fur contaminated by urine, which may allow for viral transmission between individuals (*95*).

In South America, frugivores and insectivores are the most common dietary guilds. In addition, hematophagous bats (i.e. vampire bats) are only found in Central and South America. These dietary requirements can affect pathogen transmission as well as bat-human contact. For example, habitation degradation and fragmentation and urban expansion into natural habitats can deplete natural food sources. When their natural food sources are scarce, frugivores will switch preferences to resources in human-occupied areas such as small orchards or fruit plantations. Likewise, vampire bats will switch preferences and feed on humans and domestic animals (*97*). The way in which they consume their food may also influence viral transmission. For example, Chua et al. and Dobson both hypothesized that the way frugivores feed can facilitate

the transmission of viruses because multiple bats will often eat the same fruit or leave partially eaten fruit behind which another bat eventually consumes (*94, 95*). It is widely accepted that the consumption of partially-eaten fruits played a key role in the spillover of NiV to pigs and ultimately humans (*95*). Environmental factors can also influence pathogen transmission and inter-specific transmission; during periods where resources are scare, inter-specific contact may increase (*98*).

During the past decades, several infectious diseases have emerged or re-emerged in South America either as part of larger pandemics or as local processes involving endemic pathogens. These have included arthropod-borne viral diseases, such as Dengue Fever, Chikungunya and Zika as well as several viral haemorrhagic fevers, such as Hantavirus Pulmonary Syndrome, Junin, Machupo and Guanarito viruses (*99*). Parasitic diseases such as Malaria, Leishmaniasis and Chagas Disease also seem to be expanding geographically(*100*). Several social and environmental processes have contributed to the emergence of these pathogens, including human migration, deforestation, road and dam building and climate shifts. Thus, we chose two regions of Brazil as our study sites, the Atlantic Forest and the Amazon regions, because of the high biodiversity they contain and the large-scale anthropogenic pressures they face. While, the Atlantic Forest does not possess high species richness of bats relative to other regions of Brazil such as Amazonia and Cerrado, which host around 100 species (*101*), there are high levels of endemism of bat species.

1.9 Thesis aims and structure

In this thesis, I integrate methods from ecology, biology, virology and public health to study the effects of land-use change on bat and viral communities, as well as human-wildlife

contact rates in the Atlantic Forest, Brazil. **In Chapter 2,** I designed and administered a household survey in two regions of Brazil, the Interior Atlantic Forest and Amazonia, to obtain basic demographic information and determine whether the relationship between land-use change and human-animal contact rates is predictable. Questionnaires were informed by formative research, which provided the basis for establishing categories of contact modes and the "who", "what" "when and "where" of human-animal contact. Potential transmission pathways were identified by nature and frequency of human-animal contact, as well as demographic and socioeconomic factors that may put individuals at increased risk of zoonotic infections.

In Chapter 3, I investigated the effects of deforestation on the prevalence of five viral families and host abundance and diversity in a multi-host, multi-pathogen community. Here, I address the following questions: (1) Does bat abundance and diversity differ in forested versus non-forested areas? (2) Does viral prevalence differ between bat communities in forested versus deforested areas? (3) Is viral prevalence influenced by bat abundance?

Chapter 4 demonstrates how habitat fragmentation structures both viral and bat host assemblages. I apply methods from community ecology and virology to show that viral diversity is a function of bat host diversity, and that risk of novel spillover is likely highest in matrix environments with intermediate levels of disturbance. I discuss how my findings will contribute to the development of more realistic mechanistic models of disease emergence, as well as a better understanding of host-pathogen ecology.

Finally, **Chapter 5** is a general discussion of the results, emphasizing the implications of my work and future research directions.

2 Wildlife consumption and human-animal contact along an anthropogenic disturbance gradient in Brazil

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2.1 Abstract

Tropical forests are incredibly biodiverse. Yet, in many regions of the world, they are being converted to other types of land-use, resulting in biodiversity loss and ecosystem services. Land-use change has also been linked to emerging infectious diseases, particularly those that originate in animals, which account for an estimated 60% of all human pathogens. The nature and frequency of human-animal contact determine the potential transmission pathways through which a zoonotic disease may emerge. Here, we examine how human-animal contact varies across an anthropogenic disturbance level gradient. A representative survey of two regions of Brazil, Amazonia and the Atlantic Forest, indicates that human-animal contact rates are predictable at the landscape level under land-use change, with higher levels of direct contact with wildlife found in lower disturbance areas and higher levels of indirect contact in more disturbed areas. In both regions, nearly all households in the least disturbed sites consumed wildlife (Amazonia 87%; Atlantic Forest 85%) compared to less than one-third of urban households in Amazonia (29%) and approximately half (52%) of urban households in the Atlantic Forest. Interestingly, urban households in both regions reported consuming the widest range of species compared to less disturbed areas, suggesting that demand from city-dwellers may be driving the harvesting of wildlife from low disturbance areas. We suggest that if the patterns of human-animal contact encountered in our study regions are typical under other land-use change scenarios, that different intensities of land-use change may create different risk profiles, providing important guidance for conservation and public health policy.

Keywords: human-animal contact, zoonoses, wildlife consumption, land-use change

2.2 Introduction

Tropical forests are essential for conserving biodiversity, however in many regions of the world, they are being converted to other land-uses, resulting in losses of biodiversity and the valuable ecosystem services they provide (*102*). Land-use change, through deforestation, mining, agriculture, and urbanization, is also thought to drive the emergence of infectious diseases (*103-106*). Such influences have rapidly increased over the last half century to meet rising global demand for food and natural resources, and have resulted in changes to species assemblages and contact rates that can promote zoonotic disease emergence (*107, 108*). It is estimated that between 60 and 80% of newly emerging diseases are zoonotic in origin and of these, at least 70% have a wildlife origin (*8, 109*). Although both wildlife and domesticated animals are considered important sources of EIDs (*110*), it is likely that changes in human ecology brought about by land-use change largely determine the level of disease risk at the human-animal interface (*6*).

Previous studies have shown that frequent contact with wildlife puts people at risk of infection with zoonotic pathogens. For example, people highly exposed to non-human primates occupationally, together with those hunting these species or keeping them as pets, are at higher risk of infection by their viruses (*72, 111-113*). Examples of pathogens that are transmissible to humans from wildlife include simian immunodeficiency virus, human T-cell lymphotropic virus, simian foamy viruses, monkeypox virus, hantavirus, Ebola and Marburg filoviruses, anthrax, herpes viruses, hepatitis viruses, paramyxoviruses and various other parasites among others (*114-116*). Previous studies have demonstrated that prolonged exposure to wildlife and arthropods in occupational settings results in higher incidence of zoonotic infections than

working primarily in administration or management (*117-119*). These studies imply that contact rate with disease reservoirs/vectors correlates positively with risk of zoonotic infection.

The reservoirs of emerging zoonotic pathogens are primarily mammals(*110*). However, among mammals, ungulates, primates, bats and rodents each harbour a greater total number of viruses than other taxa, and continue to be highlighted as important sources of zoonoses (*110*). Perhaps the most notable example of cross species transmission from wildlife into the human population is that of pandemic HIV. It is now widely accepted that a frequent mechanism of zoonotic disease transmission from wild animals to humans is through hunting and butchering (*112, 120*). Further, due to these practices, past research has revealed other simian retroviruses to be actively spilling over from wild primates into human populations (*112, 121*). Yet compared to primates, rodents and bats are far more abundant and geographically widespread (*122*); they also host a high number of viral species (*110*).

Rodents are well-known reservoirs for a number of zoonotic diseases (e.g. plague, monkeypox, Lassa virus, leptospirosis, leishmaniasis, hantavirus)(*123*), act as amplifier hosts for diseases transmitted by arthropod vectors (e.g. Lyme disease)(*124*), and many species are able to adapt to various habitats (*125*). Bats harbour the highest number of zoonotic viruses per host species, and in many regions of the world, human exposure to bats has increased due to habitat encroachment (*126, 127*) and increased use of bats as bushmeat (*128*). Bats have also received growing attention due to their role in the transmission of several recent outbreaks of global health concern (e.g. Ebola, Nipah, Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome) (*116, 126, 129*).

The nature and frequency of human contact with these and other wildlife taxa determine the potential transmission pathways through which a zoonotic disease may emerge. In basic disease models, R_0, defined as the basic reproductive number of a pathogen (e.g. transmission potential of a pathogen), describes the initial growth of a pathogen in a previously unexposed host population (*130, 131*). Human-animal contact is of fundamental importance in zoonotic disease emergence dynamics as R_0 is closely linked to the rate of contact between susceptible and infectious individuals (*125*). While numerous studies have examined levels of bushmeat hunting and consumption in West and Central Africa (*72, 112*) and Asia (*112, 132*), few studies have examined human-wildlife contact beyond hunting and bushmeat consumption (*133*). Paige et al. (2014) examined human-animal interactions in Western Uganda identifying patterns of injuries from animals and contact with non-human primates. However, to our knowledge, no research exists on patterns of human-wildlife contact along gradients of land-use change.

The mitigation of EIDs requires an improved understanding of potential transmission pathways and how these vary across gradients of land-use change. In Brazil, bushmeat is an important source of food and consumption spans socio-economic levels from the poorest and most vulnerable (*134*) to wealthier urban residents (*135*). Recent evidence shows that urban consumption of bushmeat in Amazonia is an important driver of declines in tropical wildlife populations (*136*). Although illegal, the bushmeat trade in Brazil functions under an organized supply chain which provides an important source of income for many rural people, and forms the basis of a highly lucrative trade (*137*). While bushmeat can be obtained directly from a hunter, it is more commonly sold in open markets despite regulations, which are often poorly enforced (*136*). In Amazonia, consumption of bushmeat is also common among indigenous people, who

do not obtain bushmeat through the market but rather directly from the forest or through close rural-urban social networks (*138*).

Despite the diversity and abundance of bats across Brazil, the majority of studies to date suggest the consumption of bats is much less common in South America compared to regions in Africa, Asia and Oceania (*139*). However, it does occur in highly localized indigenous areas with regular consumption of several bat species from the *Phyllostomidae* family (*139*). Further, although all native species are legally protected in Brazil, bats have traditionally been regarded as dangerous and have been hunted with the goal of exterminating populations.

In this study, I designed and administered a questionnaire in two regions of Brazil, the Interior Atlantic Forest and Amazonia, to obtain basic demographic information and determine how human-wildlife contact varies between regions and across an anthropogenic disturbance gradient. Specifically, I hypothesize that patterns of human-animal contact will vary predictably across an anthropogenic land-use gradient. Questionnaires were informed by formative research, which provided the basis for establishing categories of contact modes. Formative research is an iterative, multistage participatory process that draws on multiple methods (e.g. focus groups, pile sorts) and actors to generate community-congruent interventions (*140*). I specifically identify potential transmission pathways by nature and frequency of human-animal contact, as well as demographic and socioeconomic factors that may put individuals at increased risk of zoonotic infections.

2.3 Materials and methods

Here, following methods by (*141*), we use a mixed-methods approach implemented in two-phases: qualitative research and a quantitative survey. This approach accommodates the need for quantification while also recognizing the importance of local context in the information gathering process.

2.3.1 Formative research

The first phase used qualitative methods, which provides an in-depth understanding of human-animal interactions, including the "how," "when", "where" and "why" of contact. Data collected during this phase was then used to develop the quantitative survey accounting for local knowledge such as local words for animals and relevant places. In this phase, we used several qualitative approaches including participatory rural appraisal (PRA) methods (*142*) and structured anthropologic methods (*143*). PRA methods, such as participatory mapping, are employed to elicit critical local knowledge. For instance, we used participatory mapping in both regions. Working in focal groups of up to four people, local people were able to map out the physical space in their communities and areas where they encountered animals, including inside the forest, at markets, in their orchards and gardens, and in residential areas, among others. We also conducted transect walks in both regions where several key informants conducted walking tours through areas of interest. For example, during a transect walk through a forest fragment located inside the city of Manaus, we learned that people, specifically women, often encountered wildlife while they were inside the forest washing their clothes in the stream. Structured anthropologic methods allowed for the collection of data exploring how local people categorized

animals into groups. For instance, pile sorts were used during which participants were given a set of cards containing pictures of local animals. They were then asked to sort them by groups. This method allowed us to collect data on local animal names, animal categories, animal roles (food, medicinal, spiritual, etc) and different types of human-animal contact. For example, using pile sorts, we were able to determine that people were more likely to groups bats with birds rather than other mammals, and cockroaches with rats as "pests".

2.3.2 Household survey

The second phase of the human-animal contact study was a structured questionnaire survey administered at the household level to quantify the types and frequencies of human-animal interactions (Appendix 2). The questionnaire was structured into sections according to the most frequent ways (activities and locations) people interact with animals. If formative research identified activities or locations associated with human-animal contact that were not previously included, the questionnaire was amended accordingly.

While the use of household surveys is a well-established method of assessing behaviour, recall error and biases represent a major concern to the validity of studies that rely on self-reported data (*144*). For instance, not all human-animal interactions are direct or memorable thus may be overlooked or forgotten. To improve recall error, survey questions were supported with systematic probes focusing on the taxa of interest – bats, primates and rodents. We also used cognitive interviewing (*145*) to ensure the survey questions were understandable and clear. This approach involved pretesting sections of the survey to assess how people would respond. This method identifies questions that are difficult to answer and determines if response categories are

clear. Ten households in each region were administered the survey and were excluded from final analyses. Information from this pre-test in each region was used to modify the questionnaire before it was administered.

Questionnaires were administered by enumerators in two regions of the country, Amazonia, and the Interior Atlantic Forest, and were deployed within the framework of community-based health-outreach campaigns organized by the Department of Health Surveillance of Teodoro Sampaio (Departamento da Vigilância Sanitária de Teodoro Sampaio) in the Atlantic Forest and the Health Department in Amazonia. We engaged 724 participants between October 2014 and October 2015, using systematic random sampling whereby every third house was selected in a village and across neighbourhoods in urban areas. In each sampled household, the household head, who fulfilled the inclusion criteria and who gave his/her consent to participate in the survey, completed the questionnaire.

In each region, surveys were administered in three land-use type categories with different characteristics: (a) low disturbance sites defined as natural forest environments; (b) intermediate disturbance sites defined as forest fragments surrounded by pasture/agroecosystems; and (c) high disturbance areas located in urban areas. In Amazonia, study sites were distributed along a gradient spanning approximately 50 km from the city of Manaus. Here, 512 households in Amazonia were included in the study, comprising 306 urban households, 100 households in the intermediate area and 99 households in the low disturbance area. In the Atlantic Forest, sites spanned Morro do Diabo State Park, which contains the largest preserved area of inland Atlantic Forest in São Paulo State (~33,000 ha) to the city of Teodoro Sampaio, with a population of 22,675

people. In this region, questionnaires were administered to 132 households in low disturbance areas, 42 households in the intermediate areas, and 50 households in the high disturbance area, totalling 224 surveys (Table 2.1). Participants included in the study were individuals of both sexes aged 18 years and older. Persons below 18 years of age, and those who did not give their consent were excluded from the study (Manaus n=2).

Table 2.1. Number of participants interviewed in each land-use type by region.

Region	Low	Intermediate	High
Amazonia	99	100	306
Atlantic Forest	132	42	50
Total	231	142	356

Data were collected using a closed ended questionnaire delivered in Portuguese by trained enumerators, and designed to collect demographic variables (age, sex, length of residency) and to measure the extent of human-animal contact in households. Data collectors were trained on the administration of the questionnaires by the lead author. The questionnaire was piloted in twenty households in one community, which was not included for the survey. After field testing, certain questions were modified for better comprehension. The survey personnel then orally administered the questionnaire with study participants.

The questionnaire included ten questions to determine the extent of human-animal contact: five to assess direct contact with animals, and five to assess indirect contact (Table 2.2).

Table 2.2. List of study outcome variables

	Variable number	Variable (Question)
Direct contact	Q1	Have you consumed wildlife in the last 12 months?
	Q2	What types of wildlife have you consumed in the last 12 months?
	Q3	Where did you get the animals consumed?
	Q4	Did you butcher any of these animals yourself in the last 12 months?
	Q5	How often have you experiences blood-to-skin contact in the last 12 months?
Indirect contact	Q6	Have you seen wildlife inside your house in the last 12 months?
	Q7	How often does wildlife come into your house?
	Q8	What types of wildlife come into your house?
	Q9	Do bats roost in your house?
	Q10	Have you found animal faeces in your food during the past 12 months?

2.3.3 Ethics Statement

The University of California Davis Institutional Review Board (protocol #609404-1) and the Human Research Ethics Committee at the University of Sao Paulo (protocol CEPSH.157.12) approved all research activities. All participants provided informed oral consent. We did not obtain written consent due to concerns about confidentiality. We documented oral consent with the signature of the individual responsible for obtaining consent (Appendix 3).

2.3.4 Analyses

Data were entered into Microsoft Excel spread sheets by enumerators, independently checked for consistency by the lead author, and exported to R for statistical analyses. To determine whether there were significant differences in reported human-animal contact between respondents in different land-use types, I used chi-square tests with the significance level set at $p<0.05$. I used generalized linear models with binomial error terms to fit response variables, wildlife consumption, and butchering, to identify significant predictor variables of these

behaviours, as well as indirect animal contact. Definitions of the variables used are given in Table 2.3. After testing for collinearity among the response variables, no variables were excluded based on their variance inflation factor (VIF) scores. These variables were then subjected to a logistic regression analysis. In a "stepwise backwards-selection", factors were eliminated from the full model in an iterative process based on the Akaike information criterion (AIC) (Akaike 1874) with the stepAIC function of the MASS package (*146*) in the statistical software R 3.5.1.

Table 2.3. Description of predictor variables used in the generalized linear models**.**

Predictor variables	Definition
Residency	The number in years a respondent has resided in the area
Age	Age of respondent in years
Region	Amazonia versus Atlantic Forest
Land-use type	Low disturbance, intermediate disturbance, high disturbance
Butchers wildlife	Whether a respondent reports butchering wildlife (yes or no)

2.4 Results

2.4.1 Demographic information

Among the 724 households interviewed, nearly half (49%) were in urban areas, 25% were in areas categorized as intermediate disturbance, and 27% were in low disturbance areas. The average household age was 41; it was slightly lower in intermediate disturbance areas (Mean = 38.4, SD = 13) than in high (Mean = 42, SD = 15.1) and low (Mean = 43.4, SD = 16.3) disturbance areas. The average age of participants was lower in Amazonia (Mean = 39.6, SD = 15.4) compared to the Atlantic Forest (Mean = 45.9, SD = 13.3). More than half of participants interviewed were female (58%). In the Atlantic Forest, 39% of respondents were male and 61% were female, compared to 43% male and 57% female in Amazonia. Overall, the average length of household

residency was higher in urban areas (Mean = 30.7, SD = 19.5) than in intermediate (M = 23.7, SD = 14.8) and low disturbance areas (Mean = 24.4, SD = 16.8). The average household residency in Amazonia was more than double of that in the Atlantic Forest.

2.4.2 Direct Contact

2.4.2.1 Wildlife Consumption: In Amazonia**,** nearly half of all households (44%) reported eating wildlife during the previous 12 months compared to over three-quarters (78%) in the Atlantic Forest. In Amazonia, the proportion of respondents consuming wildlife was varied significantly across the land-use gradient χ^2 (2, N = 467) = 97.3, $p < 0.001$). The vast majority of households (87%) in the least disturbed sites reported eating wildlife, compared to over half (58%) in areas of intermediate disturbance and less than one-third (29%) in urban areas. Similarly, in the Atlantic Forest, the proportion of respondents consuming wildlife across the land-use gradient was significantly higher in the least disturbed areas, χ^2 (2, N = 219) = 29.2, $p < 0.001$). In this region, 85% of households in the least disturbed areas reported consuming wildlife, compared to 81% in intermediate areas and approximately half (52%) in the urban areas (Figure 3.1a). Urban households in both Amazonia and the Atlantic Forest reported consuming the widest range of species, (n=18; n=16, respectively) compared to intermediate (n=11; n=8) and low disturbance (n=12, n=9) areas, a trend that did not differ between regions, $\chi^2 = 0.157$, $p > 0.05$). The nine-banded armadillo, *Dasypus novemcinctus* was the wildlife species most commonly consumed in the Atlantic Forest compared to the lowland paca, *Cuniculus paca* in Amazonia. In Amazonia, 37 households in the urban area and one household in the least disturbed area chose not to respond

to the question asking whether they had consumed wildlife during the previous 12 months compared to only one household in the least disturbed areas in the Atlantic Forest.

2.4.2.2 Butchering Wildlife: In Amazonia, nearly half of all households (42%) claimed to have butchered wildlife in the previous 12 months. Respondents in low disturbance areas were more likely to have contact with wildlife through butchering χ^2 (2, N = 514) = 79.5, $p < 0.001$); the vast majority (84%) of households in the least disturbed areas had butchered wildlife, compared to just under one-third in intermediate areas (29%) and urban areas (32%). In the Atlantic Forest, nearly three-quarters (73%) reported butchering wildlife in the previous 12 months. Across the land-use gradient, there is a significant difference in the proportion of respondents who butcher wildlife χ^2 (2, N = 219) = 106.2, $p < 0.001$); nearly all respondents (91%) in the least disturbed areas reported butchering wildlife, compared to 87% in the intermediate areas and 16% in the urban areas (Fig 2.1b).

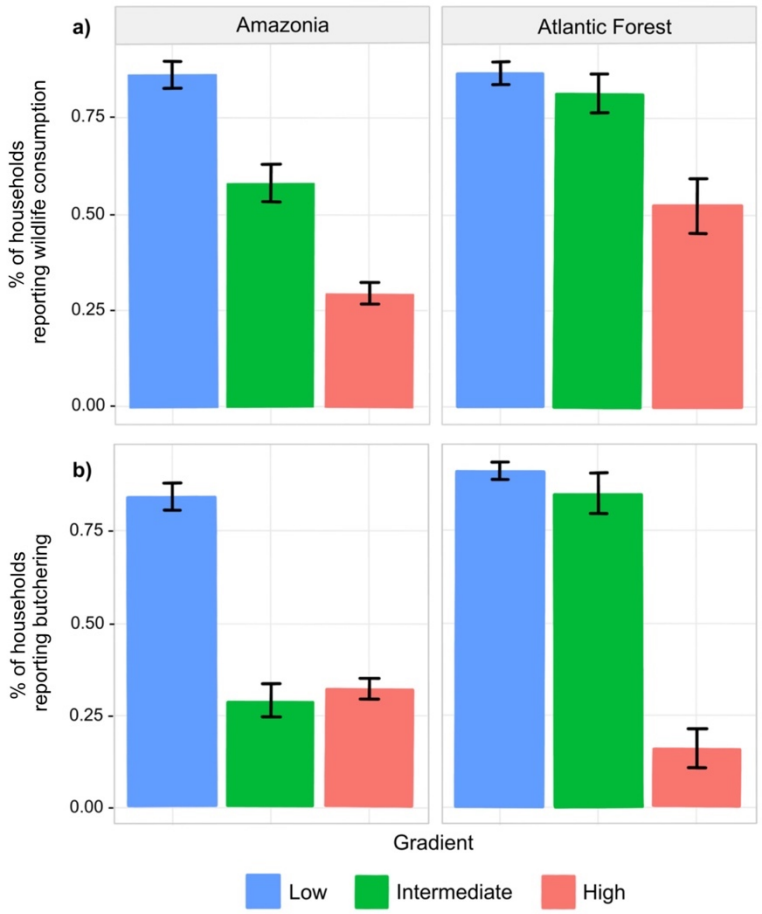


Figure 2.1: Illustrates patterns of direct wildlife contact across the land-use gradient. Panel a) shows the proportion of people that reported eating wildlife within the last twelve months along an anthropogenic disturbance gradient. Panel b) shows the proportion of people that reported butchering wildlife along the disturbance gradient.

According to Brazilian law, eating wildlife is illegal unless for rural subsistence so many of the households interviewed were breaking the law. Further, in Amazonia, many of the species consumed are vulnerable to overharvesting, reflected by their threat status assessed by the IUCN (for mammals, birds, and reptiles), including Endangered (n = 1 species), Vulnerable (n = 8) and Near Threatened (n = 2) species inclusion in the CITES appendices. In addition, there was

prevalent consumption (~20% of all households) of threatened species, spanning mammals (e.g., *Priodontes maximus, Tayassu pecari, Taipirus terrestris)* reptiles (e.g., *Podocnemis unifilis, Cheloniidae spp*); and birds (e.g.*Tinamus spp, Penelope spp, Amazona spp*) (Figure 2.2). In the Atlantic Forest, only two of the species consumed were threatened (e.g. *Tapirus terrestris and Penelope spp.).* Respondents in both regions reported eating primates, however no species were confirmed to species-level (Figure 2.2).

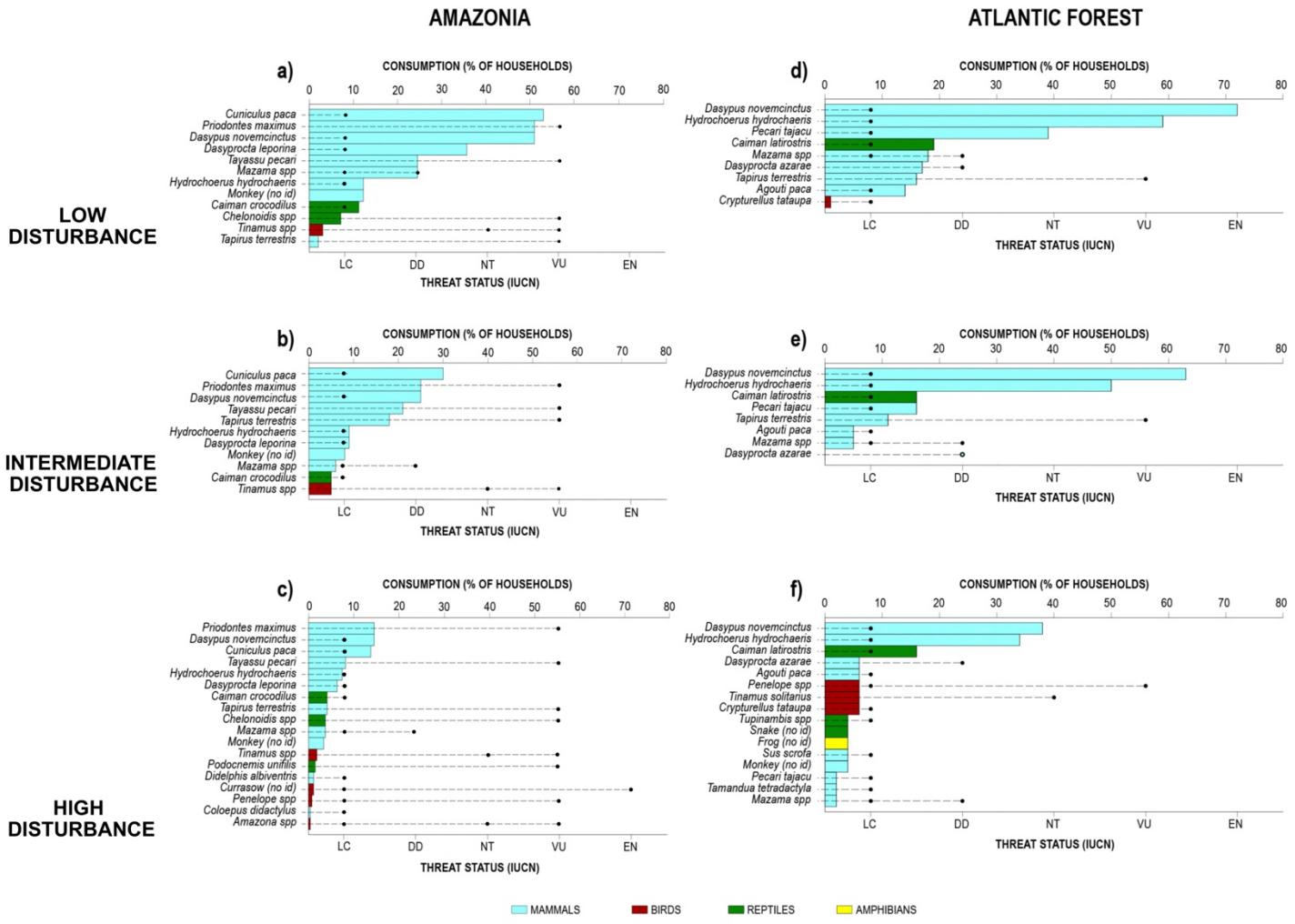

AMAZONIA
ATLANTIC FOREST
LOW DISTURBANCE
INTERMEDIATE DISTURBANCE
HIGH DISTURBANCE
a)
b)
c)
d)
e)
f)
CONSUMPTION (% OF HOUSEHOLDS)
0 10 20 30 40 50 60 70 80
Cuniculus paca
Priodontes maximus
Dasypus novemcinctus
Dasyprocta leporina
Tayassu pecari
Mazama spp
Hydrochoerus hydrochaeris
Monkey (no id)
Caiman crocodilus
Chelonoidis spp
Tinamus spp
Tapirus terrestris
Podocnemis unifilis
Didelphis albiventris
Currasow (no id)
Penelope spp
Coloepus didactylus
Amazona spp
Caiman latirostris
Agouti paca
Pecari tajacu
Tamandua tetradactyla
Snake (no id)
Frog (no id)
Sus scrofa
Tupinambis spp
Crypturellus tataupa
LC DD NT VU EN
THREAT STATUS (IUCN)
MAMMALS
BIRDS
REPTILES
AMPHIBIANS

Figure 2.2: Wildlife consumption along anthropogenic disturbance gradients in two regions of Brazil, ranging from low (natural forest environments with >90% of original forest cover), medium (agroecosystems with adjacent forest patches) to high (urban areas) disturbance. Horizontal bars show the percentage of households that had consumed each species during the previous 12 months and bars are coloured according to broad taxonomic groups (mammals, birds, reptiles, amphibians). Black dots indicate the conservation threat status of species and groups defined by the IUCN (*136*). All common names, Latin names and IUCN status are also included as Supplementary Table 1.

2.4.3 Indirect Contact

2.4.3.1 Wildlife in and around the home: In both regions, the proportion of respondents that reported seeing wildlife in and around their home was significantly different across the land-use gradient (Amazonia: χ^2 (df=2, N = 496) = 69.5, $p < 0.001$; Atlantic Forest: χ^2 (2, N = 219) = 29.2, $p < 0.001$)). In Amazonia, a much lower proportion of respondents in the least disturbed areas (54%) reported seeing wildlife in and around their homes compared to nearly all (91%) households in disturbed and 79% in intermediate areas (Figure 3.3a). However, in the Atlantic Forest, we found the opposite trend whereby more respondents in the intermediate areas (95%) and low disturbance areas (93%) reported seeing wildlife in their homes compared to those in areas that were highly disturbed (68%). When broken down by the type of species seen in and around the home, the majority of households in Amazonia specifically reported seeing rodents. When focusing on rodents exclusively, a similar trend was evident for both regions with a higher proportion of respondents in the disturbed areas reporting seeing wildlife in their homes compared to the intermediate and low disturbance areas (Figure 2.3b). Although rodents are considered by some as peri-domestic or synanthropic animals, several zoonoses of notable importance including hantavirus, plague, leptospirosis, lyssavirus, have rodent reservoirs (*123*). We therefore opted to include them in analyses.

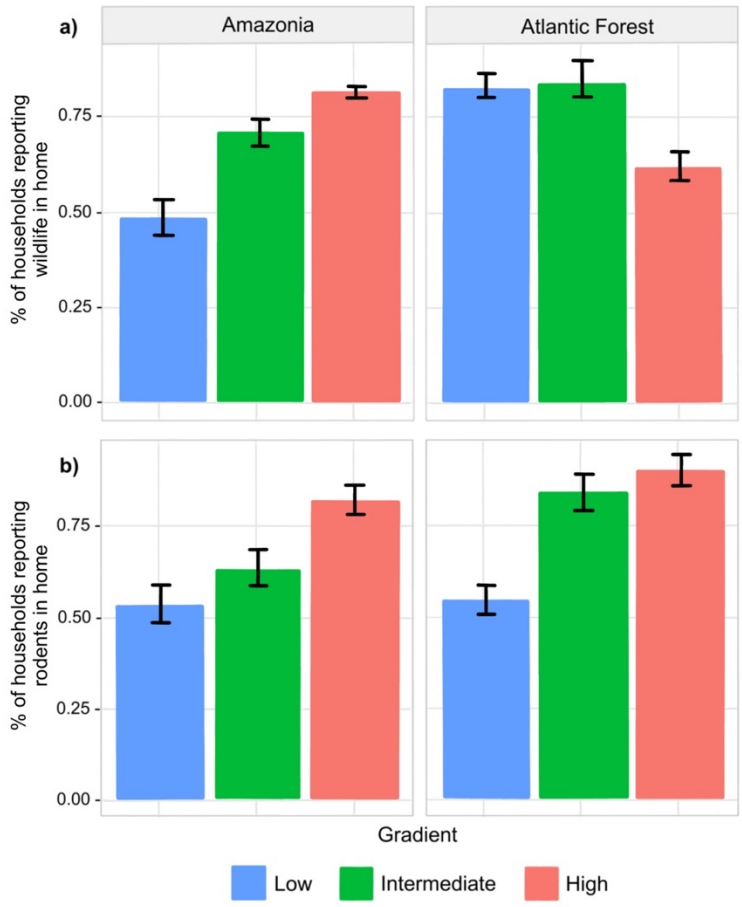


Figure 2.3: The proportion of people reporting seeing wildlife in their homes (a) and specifically rodents (b) within the last twelve months in the Amazonia and Atlantic Forest regions of Brazil. Results are reported in areas of high, medium and low disturbance.

2.4.3.2 Faces found in stored food: Across both regions, a higher proportion of respondents in more disturbed areas reported finding animal faeces in their stored food at higher frequency than those in the least disturbed areas. In Amazonia, nearly a quarter of respondents in areas of high disturbance reported finding faeces, compared to <5% in intermediate and low disturbance areas. In the Atlantic Forest, over a quarter of respondents in areas of high and intermediate disturbance reported finding faeces in their

stored food compared to <10% in the least disturbed areas (Figure 2.4).

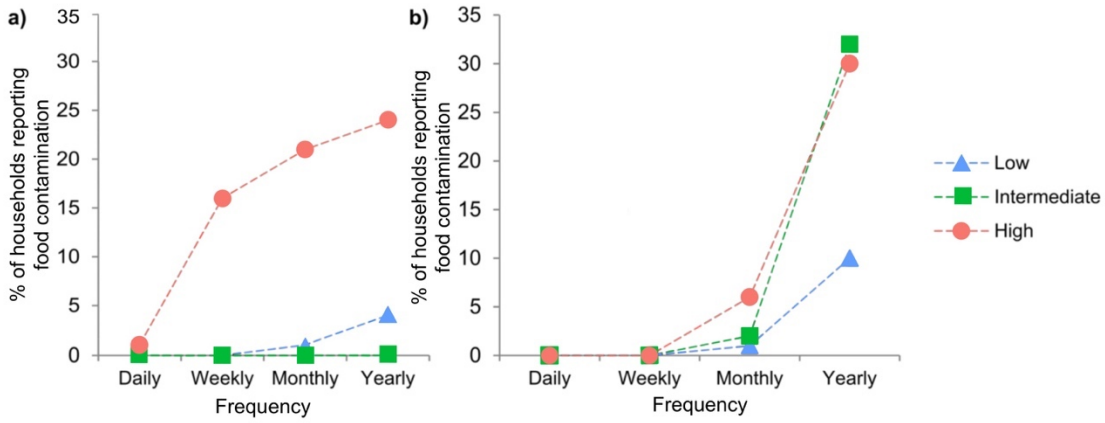


Figure 2.4: Minimum frequency of finding faeces in stored food along the disturbance gradient in a) Amazonia and b) Atlantic Forest. Categories were Almost every day/ At least once per week/ At least once per month/ At least once during the year/ Never.

We used generalized linear models with binomial error terms to fit our response variables, wildlife consumption, and butchering, to identify significant predictor variables of these behaviours, as well as indirect animal contact. Five variables were selected for the final analysis. To identify significant predictor variables of wildlife consumption, butchering, and indirect animal contact (defined by wildlife reported inside the home, we used generalized linear models with binomial error terms. Each logistic regression model explained a significant portion of outcome variability for wildlife consumption ($R^2 = 0.58$, Table 2.4) and butchering wildlife ($R^2 = 0.60$), though less so for indirect wildlife contact ($R^2 = 0.14$). Land-use type demonstrated a significant impact on each of the model outcomes. The odds of butchering wildlife (OR = 2.02) were highest for the low disturbance areas as compared to high disturbance areas, whereas the odds of having

indirect contact with wildlife was lowest for low disturbance areas (OR = -1.32) when demographic variables are held constant. The odds of wildlife consumption were highest for intermediate areas when compared to urban and low disturbance areas. Wildlife consumption and butchering meat seem to be confounders for each other, as the odds of butchering meat were approximately three times higher for households that consumed wildlife and vice versa. Demographic variables were inconsistently significant across different models: the odds of wildlife consumption were higher for Atlantic Forest households (OR = 0.86). Age and residency may also have a moderate effect on odds of butchering wildlife and indirect wildlife contact, respectively (Table 2.5).

Table 2.4. Fitted generalized linear models examining predictor variables for wildlife consumption.

Model description	AIC	ΔAIC	Null d.f	Residual deviance	Residual d.f.
Wildlife consumption ~					
~ region + land-use + butcher	561.0		*684*	552.0	680
~ region + land-use + butcher + age	563.1	2.1	684	*551.1*	679
~ region + land-use + butcher + age + residency + sex	566.1	5.1	683	550.1	676
Butchering ~					
~ land-use + wildlife consump + age + residency	553.1		683	541.1	678
~ land-use + wildlife consump + age + residency + region	554.9	1.8	683	540.9	677
~ land-use + wildlife consump + age + residency + region + sex	556.8	3.7	683	540.8	676
Wildlife in home ~					
~age + residency + land-use	612.7	0.5	721	602.65	717
~ age + residency + land-use + sex	612.2		721	600.2	716
~ age + residency + land-use + sex + region	614.15	1.5	721	600.2	715

Table 2.5: Logistic regression analysis comparing wildlife consumption with different categories of land-use change, region and reported butchering

	odds ratio	95% CI	P-value
Wildlife consumption			
Intercept	-2.924	-2.299, -1.576	<2e-16
Region (Amazonia vs Atlantic Forest)	0.864	0.343, 1.393	0.0012
Land-use (High vs. Intermediate)	1.605	1.092, 2.133	1.36e-09
Land-use (High vs. Low)	0.905	0.336, 1.479	0.0019
Butcher (yes or no)	3.022	2.563, 3.508	<2e-16
Butchering			
Intercept	-3.447	-4.294, -2.565	<2e-16
Age	0.037	0.020, 0.054	1.34e-05
Residency	-0.012	-0.026, 0.002	0.084
Land-use (High versus Intermediate)	0.266	-0.253, 0.780	0.313
Land-use (High versus Low)	2.023	1.452, 2.620	9.96e-12
Wildlife consumption (no or yes)	3.009	2.547, 3.498	<2e-16
Wildlife in home			
Intercept	2.595	1.914, 3.311	2.98e-13
Age	0.020	0.004, 0.037	0.019
Residency	-0.043	-0.058, -0.029	8.27e-09
Land-use (High versus Intermediate)	-0.411	-0.958, 0.149	0.144
Land-use (High versus low)	-1.318	-1.802, -0.845	6.18e-08

2.5 Discussion and Conclusions

Land-use change is a major driver of emerging infectious diseases (*105*). The nature and frequency of human contact with wildlife may determine the potential transmission pathways through which a zoonotic disease may emerge. Pathogens can be spread by direct contact (e.g. hunting and butchering), indirect contact (ingesting food or water contaminated with faecal material can cause infection), via aerosol transmission (inhaling tiny particles contaminated by a pathogen) or by vectors (ticks and mosquitoes can spread viruses and bacteria between hosts) (*106*). Here, we show that the types and frequencies of contact with wildlife vary according to the disturbance level of the underlying landscape, with direct contact with wildlife more common in areas characterized as low disturbance and indirect contact more common in high disturbance areas.

People involved in wildlife consumption, hunting and butchering are at risk of transmission of infection from direct contact with live and dead animals. Zoonotic infections resulting from these practices are well documented, and include past Ebola outbreaks that were traced back to bats in the Democratic Republic of the Congo (*147*), the handling of infected primates and duiker carcasses in Gabon and Republic of Congo (*148*), the detection of human T-lymphotropic virus Type 4 in hunters (*149*), and the regular spillover of retroviruses from wild primates into human populations across Central Africa (*112*). Further, food-borne pathogens from wildlife span the taxonomic spectrum from helminths to viruses and have been reported globally. Hepatitis E from raw or undercooked venison in Japan, and brucellosis and trichinellosis from wild boar are examples of hunter-acquired food-borne infections (*150-152*). There is also evidence that SARS, monkeypox, and Nipah virus may have

been transmitted through food-related incidents (*153, 154*).

We found that region, disturbance level, and whether a person butchered wildlife were all significantly associated with wildlife consumption. Respondents from low and intermediate disturbance areas were more likely to consume wildlife compared to those from urban areas, and respondents from the Atlantic Forest were more likely to consume wildlife than those from Amazonia, although this result can most likely be attributed to an issue of scale. Specifically, in Amazonia, our sites along the disturbance gradient were located more the 50 km apart, and included the urban centre of Manaus with a population of nearly two million, where a significantly lower proportion of respondents reported eating wildlife compared to the intermediate and pristine areas. In contrast, our sites in the Atlantic Forest were in much closer proximity to one another and located in a more compact and highly fragmented landscape. Thus, the proportion of respondents consuming wildlife in the region overall would have likely decreased had we included respondents from a larger urban centre that was comparable to Manaus in Amazonia.

In a study of two pre-frontier cities in the Brazilian Amazon, defined as municipalities with over 90% of their original forest cover intact, Parry et al. 2014 found that nearly all (79%) respondents reported consuming wildlife during the previous 12 months (*136*). We also found that wildlife consumption was most common in low disturbance areas, where the majority of households in both Amazonia (87%) and the Atlantic Forest (85%) reported eating wildlife during the previous 12 months, compared to under one-third of households (29%) and just over one-half (52%) of households in high disturbance/urban areas in Amazonia and the Atlantic Forest, respectively.

Across both regions, our results indicate that respondents were more likely to butcher

wildlife in low disturbance areas. Nearly all households in the low disturbance areas (84% Amazonia; 91% Atlantic Forest) reported butchering wildlife compared to under one-third of respondents in urban areas (32% Amazonia; 16% Atlantic Forest). This suggests that respondents in areas of high disturbance (large urban areas) are less likely to be at risk for disease transmission through direct contact (blood-to-skin transmission pathway).

Interestingly, while wildlife consumption was more common in low disturbance areas, urban households in both Amazonia and the Atlantic Forest reported consuming a wider range of species, (n=18; n=16, respectively) compared to intermediate (n=11; n=8) and low disturbance (n=12, n=9) areas, indicating that demand from city-dwellers, who purchase wildlife for consumption, may be driving the harvesting of wildlife from low disturbance areas. Indeed, recent work has demonstrated that urban markets can facilitate species declines deep into rainforest wilderness by providing rural fishers with reliable access to fish buyers, as well as the necessary equipment including city-based boats and ice (*135*). Our results suggest that this is likely the case for terrestrial wildlife species as well.

For indirect contact, we found that respondents in more disturbed areas were more likely to report seeing wildlife, particularly rodents, in and around their homes. A higher proportion also reported seeing faecal matter in stored food and at higher frequency than those in intermediate and low disturbance areas in Amazonia, and low disturbance areas in the Atlantic Forest. This discrepancy across regions could potentially be explained by the difference in scale between our two regions. In Amazonia, our sites along the disturbance gradient are located more than 50km apart, whereas in the Atlantic Forest region, our study area is much more compact and more highly fragmented.

Pathogens that are transmitted via indirect contact are generally transmitted through

the ingestion of food or water contaminated with faecal material or through aerosolized particles (*106*). Studies have shown that urban adapted (referred to here as synanthropic) wildlife is most abundant in cities, and is comprised of species that respond to behavioural and resource-based selection pressures imposed by urban environments (*35, 125, 155*). For example, *Mus musculus, Rattus rattus* and *R. norvegicus* are all highly adapted to urban environments due to several biological characteristics including their generalist diets, enormous reproductive potential, and feeding behaviours (*156*). Synanthropic species have also been shown to carry zoonotic pathogens and in some cases act as reservoir hosts for these pathogens (*125, 157*). Rodents, for example, host important zoonoses such as plague, leptospirosis, and hantavirus infection (*158, 159*). In West Africa, Lassa fever, an acute viral haemorrhagic illness caused by Lassa virus, is most commonly transmitted to people from contact with the urine or faeces of the Natal multimammate mouse (*Mastomys natalensis*), its natural host. The disease yields up to 300,000 human cases annually, with a 15% fatality rate of those who are hospitalized and a 1% overall fatality rate (*160*). Because *Mastomys* rodents often live in and around homes and scavenge on poorly-stored human food, transmission via indirect contact is the most common (*161*). Similar to Lassa virus, there are several neotropical arenaviruses including Junin, Machupo, Guanarito, and Sabia virus that are rodent-borne, and can be transmitted via the inhalation of aerosolized excretions or via direct contact with rodents (*162*).

In terms of potential risk from ingesting food or water contaminated with faecal material or inhaling particles contaminated with rodent excretions, our findings suggest that respondents in more disturbed areas are more likely to have contact with wildlife via these potential pathways as anthropogenic disturbances associated with urbanization can bring

rodents into closer and more frequent contact with humans and their domestic animals. As such, human activities that increase the abundance of synanthropic wildlife, thus increasing contact, will certainly increase the risk of cross-species spillover of diseases to people.

Our findings highlight the value of understanding how land-use change influences human-animal contact. From a landscape perspective, anthropogenic pressures that alter natural habitats have been associated with several wildlife zoonoses including yellow fever, West Nile virus, Nipah virus, influenza, malaria, lyme disease and hantavirus (*69, 124, 163-165*). Here, we consider the role that land-use change plays in the emergence of zoonoses, by providing critical information on how likely an individual is to have contact with different types of wildlife directly, indirectly, or inadvertently, and how patterns of contact may vary at the landscape level. In doing so, we suggest that if the patterns of human-animal contact encountered in our study regions are typical under other land-use change scenarios, that different intensities of land-use change may create different risk profiles. From a public health perspective, this information is crucial in the design of relevant intervention and control strategies, as well as in determining at which point along the anthropogenic disturbance gradient, spillover of zoonotic diseases is most likely to occur and through which pathway. This research, coupled with further research into the microbial diversity in wildlife species and how this varies across gradients of land-use change, will contribute to the development of more realistic mechanistic frameworks for cross-species spillover.

3 Prevalence of bat viruses associated with land-use change in the Atlantic Forest, Brazil

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* The laboratory analyses in this chapter were conducted by Angelica Campos at the Institute of Biological Sciences at the University of Sao Paulo.

3.1 Abstract

Viruses that originate in wildlife are key threats to global health, and their emergence is often associated with anthropogenic factors, including land-use change. However, few studies have assessed how host communities and their viruses change with human disturbance, particularly in highly biodiverse sites. We investigated the effects of deforestation on bat abundance and diversity, and viral prevalence across five forested sites and three nearby deforested sites in the interior Atlantic Forest of southern Brazil. Nested-PCR was used to amplify and detect viral genetic sequence from five viral families (corona-, adeno-, herpes-, paramyxo-, and astro-viridae) in 944 blood, saliva and rectal samples collected from 335 bats. We found that deforested sites had a less diverse bat community than forested sites, but higher viral prevalence and richness after controlling for confounding factors. Our findings suggest that deforestation can result in reduced bat diversity and increased abundance of generalist synanthropic species, which host higher viral prevalence.

Keywords: deforestation; land-use change; bat hosts; viruses; disease risk

3.2 Introduction

Emerging viruses with wildlife origins are a significant threat to global health (e.g. Ebola virus, SARS and MERS coronaviruses) (*166*). Analyses of recent emerging infectious disease (EID) events show that anthropogenic changes including land-use change (e.g. habitat degradation, deforestation, forest fragmentation), intensification of food production, and global trade and travel are key factors in disease emergence (*106, 167, 168*). Further, nearly one-third of all EIDs, and a higher proportion of zoonoses, are associated with land-use change specifically (*106*). This suggests that increasing and/or novel interactions among hosts, vectors and pathogens following land-use change are significant contributors to disease emergence.

In tropical and subtropical environments, the pace of land-use change is unprecedented and continues to increase globally as demand for natural resources grows (*102*). Yet, the relationship between land-use change and disease emergence is poorly understood. Recent studies have hypothesized that land-use change may increase the risk of zoonotic disease emergence through increased human-animal contact, or by influencing pathogen diversity, either directly by changing pathogen prevalence and/or diversity, or indirectly via impacts on host community diversity (*6, 164, 168*). Correlational studies have tended to focus on how abundance and prevalence of specific pathogens, or their vectors and hosts, vary over the landscape (*47, 68, 124, 164, 169, 170*). For example, decades of research on Lyme disease has shown that infection risk varies inversely with vertebrate host diversity (*124, 171*). Negative correlations between diversity and disease have also been reported for West Nile virus in birds, and hantavirus in rodents. These phenomena termed the 'dilution

effect' suggests that the net effects of biodiversity (including host and non-host species) reduce the effects of certain disease in ecological communities. Others have used meta-analyses to try to identify generality and mechanisms involved (*172-174*). Yet, few empirical studies have taken a community approach to examine how viral prevalence in host communities varies with land-use change, particularly in the neotropics.

Here, we investigate the effects of deforestation on the host abundance and diversity and pathogen prevalence and richness, of a multi-host, multi-virus community. We worked with bats because they are generally the most rich and abundant mammal in Neotropical landscapes (*175*), comprising species from nearly every trophic level, with wide differences in their dispersal abilities (*176*). Further, in the last two decades, several zoonotic diseases of significant public health concern have been linked to bat-borne viruses including SARS coronavirus, Hendra virus and Nipah virus (*177*). In addition, previous studies have linked ebolaviruses and MERS coronavirus to bats (*178, 179*). Research findings accumulated over the last few decades have identified several viral families that are: (1) more prevalent in bats and (2) with proven potential for inter-species transmission including *Coronaviridae, Paramyxoviridae, Reoviridae, and Filvoridae* (*180*). Further, numerous known and novel viruses have been detected in bats from the families *Astroviridae, Circoviruidae, Parvoviridae, Partitviridae, Coronaviridae, Picobirnavirdae, Adenoviridae, Herpesviridae, Papillomaviridae, Phenuiviridae, and Picornaviridae* among others (*181, 182*). These families also possess viruses with spillover potential to humans (*180*).

Finally, bats are the reservoir hosts of pathogens that have caused a series of critical EIDs (e.g. Ebola, SARS, MERS, rabies), and harbour the highest proportion of zoonotic viruses

of any mammal order (*166, 183*). Here we build upon previous work to further expand the diversity of the bat virome by detecting known and novel viruses from the following viral families: *Coronaviridae, Paramyxoviridae, Herpesviridae and Astroviridae*. We focus on these viral families because this group of pathogens is responsible for several of the recent human pandemics, as well as significant emerging diseases of people, livestock and wildlife (*183*). Finally, we chose the Atlantic Forest, Brazil as our study site because of the high biodiversity it contains and the large-scale deforestation it has undergone (*2*).

Our study focuses on three questions: (1) Does bat abundance and diversity differ in forested versus non-forested areas? (2) Do viral prevalence and species richness differ between bat communities in forested versus deforested areas? (3) Is viral prevalence influenced by bat abundance? I hypothesize that deforested areas will be under-resourced, supporting lower bat abundance and diversity and higher viral prevalence.

3.3 Materials and methods

3.3.1 Ethical Statement

This study was carried out with animal handling permits issued from the Brazilian Ministry of the Environment (#33078-4). Animal handling ethics approval was provided by the University of California, Davis (#16048). Bat handling followed strict personal protection and biosafety requirements and short capture times to minimize stress on individual animals. All captured individuals were released at the point of capture.

3.3.2 Study site

Morro do Diabo State Park (municipality of Teodoro Sampaio, São Paulo state, Brazil, (Figure 1) is located in the Pontal do Paranapanema region and contains the largest preserved area of interior Atlantic Forest in Sao Paulo State. The park covers an area of 33,845 ha (*184*) and is comprised of mesophytic semideciduous forest and a small area of Cerrado (savanna-like vegetation). The climate is characterized as subtropical, with dry winters and wet summers. Mean annual temperature is 22°C, and annual rainfall ranges between 1100 and 1300 mm (*185*). The matrix around the park is comprised of 63 small properties of agrarian reform settlements, as well as pasture (~60%) and agriculture (~15%), and forest fragments ranging from 2 to 2000 ha in area, most of which are privately owned (*186*). The forested study sites were chosen to control for similar characteristics including elevation, vegetation structure and rainfall. We sampled bats and viruses at five intact forested sites (>200ha) and three nearby deforested sites, located 3-5km away, converted from the original forest to agrarian reform settlements (Figure 4.1).

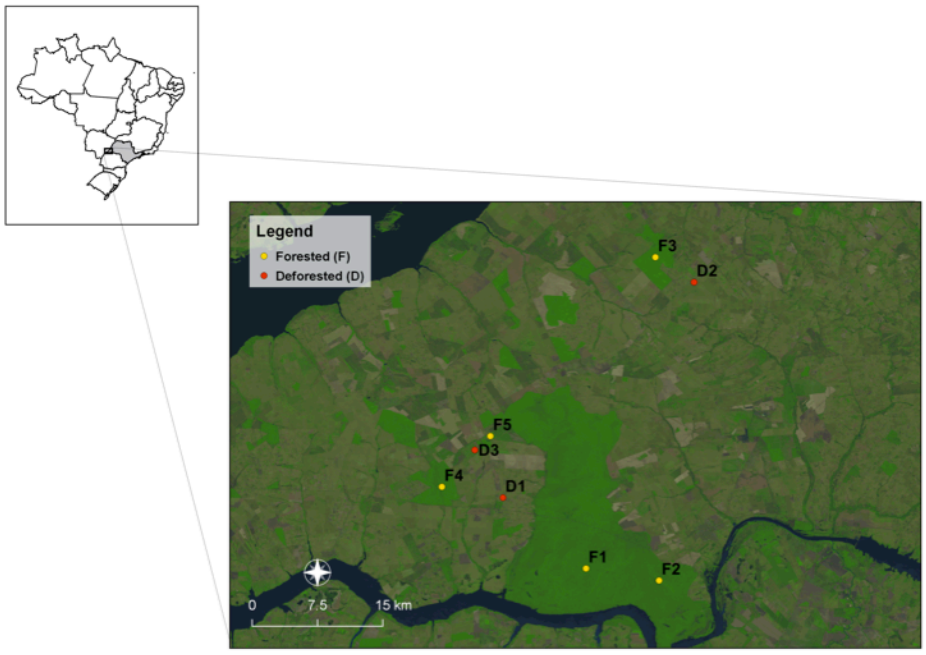


Figure 4.1: Map of the study site in the Pontal do Paranapanema region in the interior Atlantic Forest, Brazil.

3.3.3 Bat capture and sample collection

Bats were sampled during the dry season from April to September of 2014. At each site we sampled a 100m x 100m grid using eight horizontal mist nets (9m x 3m), one canopy mist net (6m x 3m) and one harp trap (1.5m x 1.5m). At least 150 individuals were collected from deforested sites and from forested sites each. Additional sampling effort was required in the forested areas due to lower catch rates. Bats were captured for a period of five consecutive nights at each site, totalling 2040 m^2/270 hours capture effort across all sites. Mist nets were opened at sunset and remained open for six hours. Nets were checked at 30-minute intervals and bats processed immediately.

Samples were collected from bats deemed healthy by trained veterinarians, and all animals were released immediately following processing. Blood, saliva, and rectal swabs were

collected from each captured animal, with faeces and urine opportunistically collected. All samples were placed in cryovials containing 200 ml of Viral Transportation Media (VTM) and stored in liquid nitrogen in a dry shipper while in the field, then transferred to -80C freezers at the Institute of Biomedical Sciences at the University of Sao Paulo. External morphological measurements (including forearm/radius length, body length, head length) were collected to assist in taxonomic identification, and sex and age class (e.g. juvenile, sub-adult, adult) were determined for all individuals. Before release, each individual was marked with a non-toxic pen to determine the rate of within-trip recapture. This was used to ensure that the same bat was not re-sampled within sampling trips. Detailed protocols for all bat capture methods used are provided as Appendix 3.

3.3.4 Viral detection

Total nucleic acid was extracted from all samples using the EasyMag (bioMérieux, Inc.) platform, and cDNA synthesis performed using SuperScript III first-strand synthesis supermix (Invitrogen), all according to the manufacturer's instructions. Viral discovery was performed using nested-PCR assays targeting coronaviruses (*187*), astroviruses (*188*), paramyxoviruses (*189*), and herpesviruses (*190*) PCR results were visualized in Agarose gel 2%, then sequenced. Sequences were analysed and edited using Geneious (version 6.0.3). Sequences were aligned with ClustalW and MUSCLE, and phylogenetic trees (see Text S1) constructed with neighbor-joining (p-distance, pairwise deletion, 1,000 bootstraps), maximum-likelihood (1,000 bootstraps), and Bayesian (GTR+I - Mr Bayes) algorithms. In Mr. Bayes, we discarded the first 25% of trees as burn-in, and used the remaining trees to estimate the posterior probability value (PP) of 0.7. The chains ran for 2,000,000 cycles

(mcmc ngen = 2,000,000). Trees were reconstructed with unconstrained branch lengths and unrooted. Tree convergence was checked by PP value with numbers equivalent to the bootstrap values. In MEGA 7 (form macOS available in: https://www.megasoftware.net/) we used Maximum Likelihood with heuristic search and GTR+gamma+I algorithm. For the ML tree, we conducted 1,000 fast bootstrap ML replicates to assess the support values of internal nodes and visualized the trees in FigTree software version 1.4.4 with Midpoint Root (available in: http://tree.bio.ed.ac.uk/software/figtree/). Sequences were segregated into discrete viruses, defined as a viral species, based on distinct monophyletic clustering following (*191*). Detailed protocols for all cPCR assays used are provided in the Supplementary Methods and Supplementary Table 2.

3.3.5 Data analysis

Statistical analysis was performed using R 3.5.1, with ggplot2 for graphing. To compare estimated bat species diversity between forested and deforested sites, we calculated abundance-based diversity profiles with Hill numbers (effective number of species) using the iNEXT package based on the parameter q (*192*). This parameter controls the relative emphasis placed on rare or common species. In addition to providing information on species richness, this diversity profile estimator also accounts for species abundances to differing degrees. With increasing order q, the weight of dominant species increases in the calculation of species diversity. We used three widely used species diversity measures: Species richness (number of observed species; q=0), Shannon diversity (number of typical species; q=1) and Simpson diversity (number of most common species; q=2). We then applied a bootstrap method (1,000 bootstraps) using observed detections to obtain approximate variances of the proposed

profiles and to construct the associated confidence intervals. These estimations account for the effect of undetected species in samples. We compared viral species richness and overall viral prevalence across treatments using a Fisher's Exact Test. To account for the uneven number of captures per bat species, we used Bartels rank test of randomness to determine whether viruses were randomly distributed among species.

We used a generalized linear model (GLM, with a logistic regression link) to fit our response variable, the presence or absence of a viral detection, to identify significant predictor variables of a positive viral detection. Previous studies have shown that species life history and ecological traits (e.g. dietary guild, pregnancy status, age) can influence pathogen prevalence (*49, 193*). Previous studies have shown that the dietary requirements and feeding habits of bats may also be an important factor that may contribute to a bat's exposure to and spread of viral pathogens (*94, 95*). Further, bats are also strongly impacted by land-use changes which may also affect the viruses they host (*194*). After testing for collinearity among the response variables, no variables were excluded based on their variance inflation factor (VIF) scores. Nine variables were selected for the final analysis. Definitions of the variables used are given in Table 3.1. These variables were then subjected to a logistic regression analysis. In a "stepwise backwards-selection", factors were eliminated from the full model in an iterative process based on the Akaike information criterion (AIC) (Akaike 1874) with the stepAIC function of the MASS package (*146*) in the statistical software R 3.5.1.

Table 3.1. Description of predictor variables used in the generalized linear models.

Predictor variables	Definition
Guild	Five dietary functional groups including: insectivore, frugivore, nectarivore, omnivore, sanguivore
Family	Three families including: *Phyllostomidae*, *Molossidae*, and *Vespertillionidae*
Treatment	Forested versus Deforested
Pregnancy status	Yes or No
Age class	Three categories including: juveniles, subadults and adults
Genus	12 unique genera
Species	18 unique species
Abundance	Total number of individuals captured per species

3.4 Results

3.4.1 Bat diversity

We recorded 18 bat species from three families (*Phyllostomidae, Molossidae, and Vespertilionidae*) and five dietary guilds (frugivorous, insectivorous, nectarivorous, sanguivorous, omnivourous) from 335 mist-net captures. No bats were captured using the vertical canopy net or harp trap. After accounting for sampling effort, capture rates were similar between forested (n = 163 captures) and deforested (n = 172 captures) sites (Paired t-test, t = 1.883, p = 0.081). Bat species richness in the deforested sites (n=11 species) was slightly higher than the forested sites (n = 9 species); however, this difference was not significant as indicated by the empirical diversity profiles that show overlap between the 95% confidence intervals at q = 0 (Figure 3.2). In contrast, at q>1 (i.e. measures of diversity that incorporate abundance information) the forested sites were found to be more diverse than deforested sites. When correcting for the bias introduced by the non-detection of species in the samples, bat diversity was reduced in deforested sites; species richness was

slightly higher in forested areas (n=15 species) compared to deforested areas (n = 11 species). However, for q > 1.25, this difference in community diversity was statistically significant, as reflected by the two non-overlapped confidence intervals.

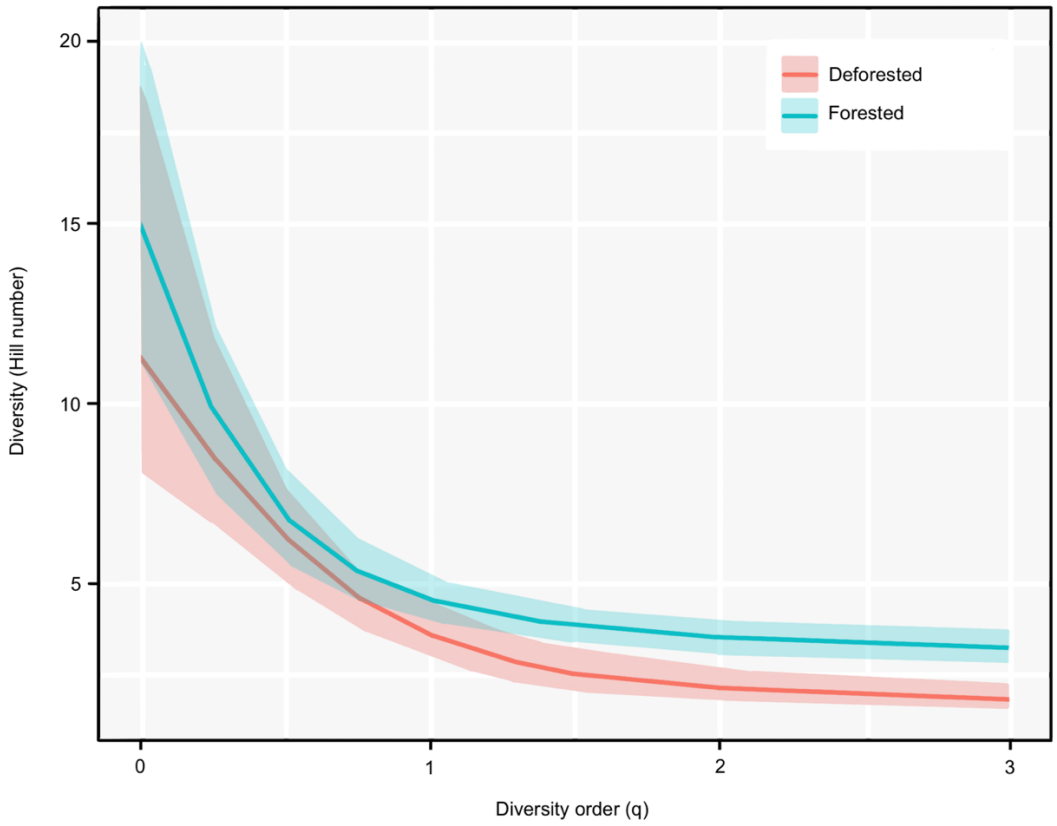


Figure 3.2: Estimated diversity profiles for bat species data in forested (green line) and deforested (red line) sites for q between 0 and 3 with 95% confidence interval (shaded areas based on a bootstrap method of 1000 replications). The numbers show the estimated diversities for q = 0, 1, 2 and 3.

3.4.2 Viral prevalence and species richness

Overall, a total of 21 individual bats from three families (*Phyllostomidae, Molossidae, Vespertilionidae*) were positive for 12 viral species in the following viral families: *Astroviridae, Coronaviridae*, *Herpesviridae*, and *Paramyxoviridae*, with a combined viral prevalence of 6.2%

(21/335) (Table 3.2). These families have been selected to account for variation in i) biological properties of viruses (genome organization, replication strategies, prevalence) and ii) families that contain existing and significant pathogens to humans. None of the samples were positive for adenoviruses, despite previous studies documenting their presence in other bat species (*195, 196*). Only one individual bat yielded more than one viral species - a coinfection by a coronavirus and herpesvirus was found in *Artibeus planirostris*. Viral species were not evenly distributed among bat species, with all detected viruses coming from just five of 18 sampled bat species. However, after accounting for the number of captures per bat species, we found that viral taxa were randomly distributed among bat species ($P > 0.05$, Bartel's Rank Test). Viral prevalence differed significantly among viral families; *Coronaviridae* had the highest prevalence of 3.6%, followed by *Astroviridae* (1.2%), *Paramyxoviridae* (0.6%), and *Herpesviridae* (0.9%).

Table 3.2. Total captures of bat species and total viral detections in forested and deforested habitat in the Interior Atlantic Forest.

FAMILY/Species	Captures	*Corona-*	*Herpes-*	*Astro-*	*Paramyxo-*
PHYLLOSTOMIDAE					
Artibeus lituratus	66	1	0	0	0
Artibeus fimbriatus	34	0	0	0	0
Artibeus planirostris	130	6	3	4	1
Carollia perspicillata	55	4	0	0	0
Desmodus rotundus	1	0	0	0	0
Diaemus youngi	2	0	0	0	0
Glossophaga soricina	1	0	0	0	0
Phyllostomus hastatus	3	0	0	0	1
Sturnira lilium	17	1	0	0	0
Vampyrodes caracciol	9	0	0	0	0
MOLOSSIDAE					
Molossus molossus	3	0	0	0	0
Eumops glaucinus	2	0	0	0	0
VESPERTILIONIDAE					
Lasiurus blossevillii	1	0	0	0	0
Myotis nigricans	1	0	0	0	0
Myotis albescens	3	0	0	0	0
Myotis riparius	3	0	0	0	0
Myotis unidentified A	1	0	0	0	0
Myotis unidentified B	3	0	0	0	0

Treatment (forested vs. deforested) was the only significant predictor of overall viral prevalence ($P < 0.05$, $df = 1$) (Table 3.3). This result was supported by the logistic regression model with the lowest AIC value (Table 3.4), which demonstrates that the odds of a positive viral detection decreases in forested habitat.

Table 3.3. Fitted generalized linear models examining predictor variables for a positive viral detection.

Model description	AIC	ΔAIC	Null d.f	Residual deviance	Residual d.f.
~ *sex + treatment*	159.1	0	334	153.2	332
~ *sex + abundance + treatment*	**159.5**	**.4**	**334**	**151.5**	**331**
~ *sex + pregnancy + abundance + treatment*	**161.5**	**2.4**	**334**	**151.5**	**330**
~ *sex + pregnancy + age + abundance + treatment*	**164.3**	**5.2**	**334**	**150.3**	**328**
~ *sex + pregnancy + age + species + abundance + treatment*	179.8	20.7	334	133.8	312
~ *sex + pregnancy + age + genus + species + abundance + treatment*	179.8	20.7	334	133.8	312
~ *sex + pregnancy + age + genus + species + guild + abundance + treatment*	179.8	20.7	334	133.8	312
~ *sex + pregnancy + age + genus + species + guild + family + abundance + treatment*	179.8	20.7	334	133.8	312

Table 3.4: Logistic regression analysis comparing viral detection with different categories of land-use change and host sex.

	odds ratio	95% CI	P-value
Intercept	-2.588	-3.413, -1.909	8.47e-12
Sex (male vs female)	0.712	-0.176, 1.665	0.1250
Treatment (forested vs deforested)	-1.310	-2.444, -0.345	0.0127

With all viral families combined, viral prevalence in deforested sites (9.3%) was significantly higher than in forested sites (3.68%) ($P<0.05$, Fisher's Exact Test). Deforested sites also had higher observed species richness (n = 12 unique viral taxa) compared to forested sites (n = 2).

3.5 Discussion

In the Atlantic Forest of Brazil, viral prevalence and observed viral species richness were significantly higher in bat communities in deforested sites compared to those in nearby forested areas. However, higher bat host diversity was not associated with higher viral

prevalence or diversity. This result does not appear to be associated with the abundance of bat hosts, which was not significantly different (based on mist-net capture frequency) in deforested versus forested areas. We also found no direct relationship between foraging strategy and resources use (i.e. guild) and viral prevalence.

Previous studies examining the link between forest loss/fragmentation and disease prevalence in wildlife have been equivocal (*168, 173, 174, 197*). Some studies of single-pathogens (e.g. West Nile virus, Hantavirus, the Lyme disease pathogen *Borellia burgdorferi*) in multi-host systems have found that higher pathogen prevalence is associated with decreased continuous forest area (*66, 124, 163*). However, studies of *Plasmodium* infections in Australia (*49*), Cameroon (*198*), and Brazil (*199*) found a positive correlation between continuous forest area and pathogen prevalence. Our study provides evidence from a multi-pathogen, multi-host species system that deforestation can result in increased viral prevalence in bat hosts. In Sabah, Seltmann et al. 2017 found that reduced body mass in bats in logged forests was associated with chronic stress and impaired health status for some species of bats. Interestingly, this did not translate into a change in coronavirus and astrovirus detection rates among disturbance gradients (*200*), perhaps due to the extent of disturbance. Unlike our system, which is more than 30 years post-fragmentation and fully converted, Seltmann's study sites in Sabah are still ongoing active deforestation and fragmentation, which may result in delays in species' responses.

Deforestation generally results in increased abundance of r-selected, generalist species that are able to thrive in a variety of environmental conditions (*201*). The strategies they employ such as greater dispersal ability, and the ability to exploit a variety of resources, allow

these species to tolerate a wide range of habitats, leading to higher colonization rates throughout human-modified landscapes.

Previous studies of bats have demonstrated that even moderate forest disturbance can result in an increase of certain generalist species that can successfully adapt to human-modified landscapes (*202, 203*). In our study area, *A. planirostris and A. fimbriatus* were the two species most commonly captured in deforested sites. Both species are large-bodied frugivores, which feed heavily on figs in the canopy (*204*). In many tropical landscapes, figs are not regularly available throughout the year, thus *Artibeus* species are more likely to occupy human-influenced landscapes which provide a variety of food resources (*205*). Although *Artibeus* spp. bats accounted for 73% (n=16/22) of all viruses detected, after accounting for the number of captures per bat species, our results show that viral taxa are randomly distributed among bat species.

While we did not measure disease risk directly, the presence of a rich assemblage of viruses from families that contain known zoonoses suggests that if contact rates are held constant across a landscape, disease risk would be higher in deforested sites. Bats are known to harbour a wide variety of coronaviruses, and have received growing attention due to their role in the transmission of several recent outbreaks of coronaviruses (e.g. Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome) (*116, 126, 129*). To date, over 200 novel coronaviruses have been identified in bats and approximately 35% of the bat virome sequenced to date is composed of coronaviruses (*206*). Similar to our findings, previous studies have detected CoV in bats from urban areas of São Paulo State (*207*) and the Atlantic Forest (*129, 208*). In contrast, while bat astroviruses have been detected in Hong Kong

, China, North America, Germany, Hungary and the Czech Republic (*209*) from numerous bat species, our results, to our knowledge, provide evidence of the first detection of astroviruses from bats in Brazil, as most studies in Brazil have focused on avian and human astroviruses (*210, 211*). Lastly, both paramyxoviruses and herpesviruses have been previously described in Brazil (*212-214*). While data on wildlife-human contact are not presented here, it is possible that deforestation increases contact along increased forest edges, through changes in host and vector abundance, or through changed environmental conditions that expands host extent. Urbanized and agricultural areas that have undergone deforestation have been associated with higher rates of disease transmission of West Nile Virus in the United States, increased risk of malaria in Peru (*164*), Leishmaniasis in Costa Rica (*215*) and hantavirus in Panama (*163*), in part because human-modified areas have higher host and vector abundance.

Our study examines some of the complexities in the relationship among deforestation, pathogen prevalence and host and viral community assemblages by addressing how viral diversity in bat hosts varies with land-use change. Our findings suggest that deforestation can increase the abundance of generalist species that, in our case, host the majority of viruses detected. While this doesn't test the 'dilution effect' as laid out for Lyme disease and other single pathogen systems (*69, 124*), it does provide further evidence that anthropogenic land-use change can in some cases, lead to increased abundance of reservoirs that harbour a higher diversity and prevalence of potential pathogens.

3.6 Supplementary information

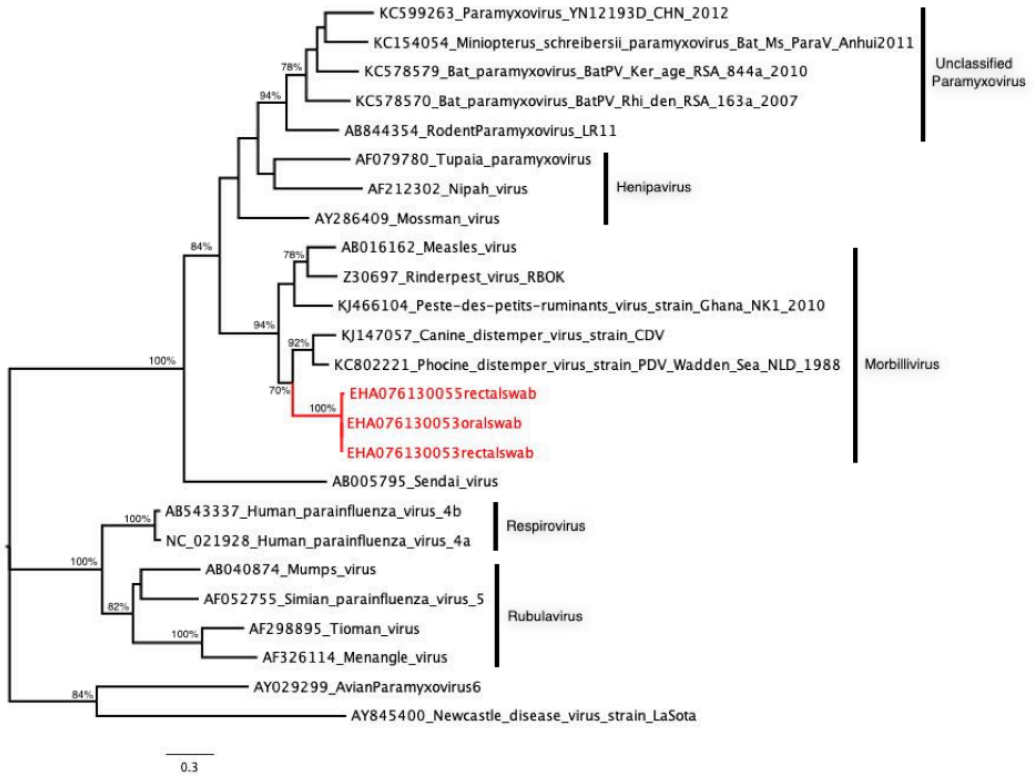


Figure S3.6.1 Paramyxovirus Maximum Likelihood Phylogenetic Tree.
Genetic analysis of 558 nucleotide partial L gene. Tree reconstructed by MEGA7 with heuristic search and Neighbor-Joining "NJ" algorithm. The principal node values superior to 70% represent 1,000 bootstrap replicates

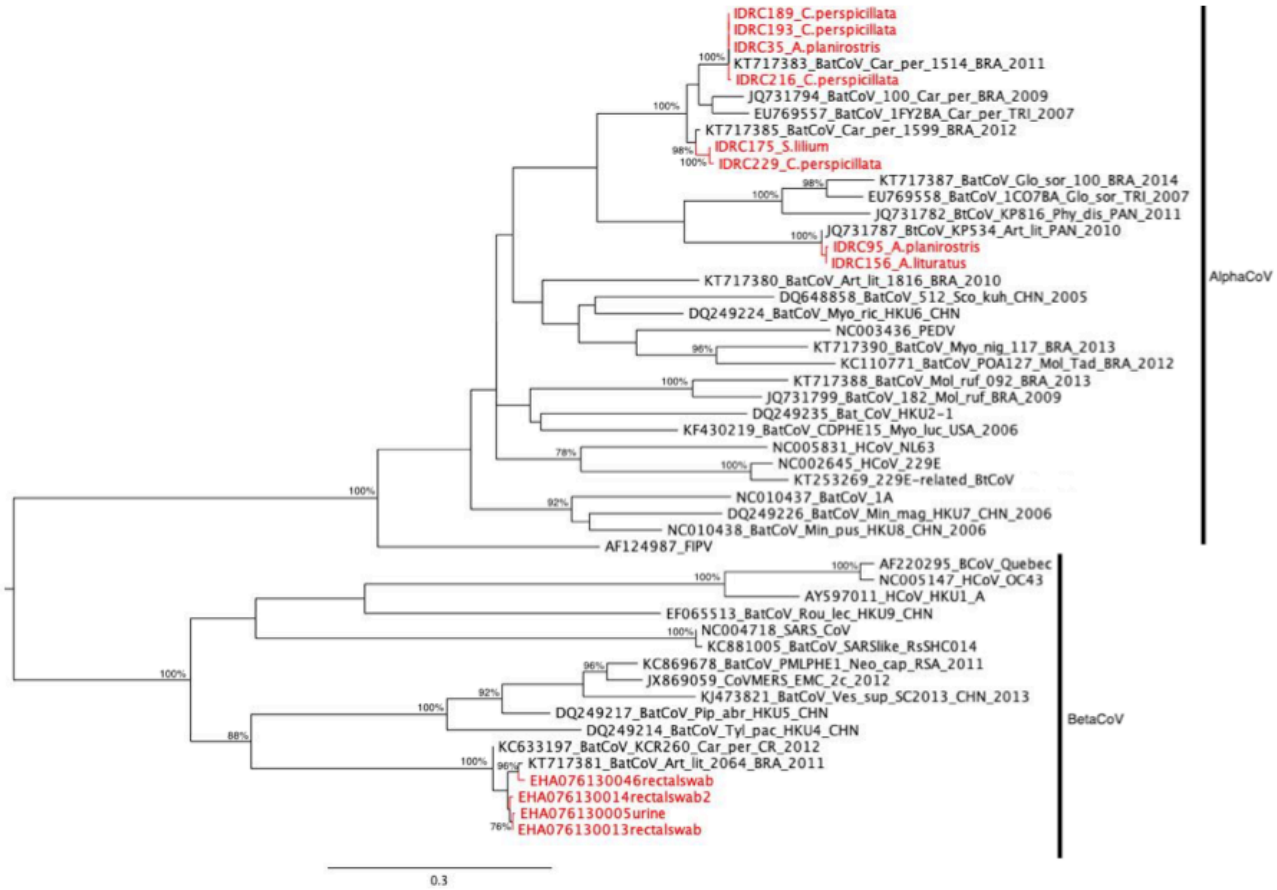


Figure S3.6.2 Coronavirus Maximum Likelihood Phylogenetic Tree.

Genetic analysis of 394 nucleotide partial RdRp gene. Tree reconstructed by MEGA7 with heuristic search and Neighbor-Joining "NJ" algorithm. The principal node values superior to 70% represent 1,000 bootstrap replicates.

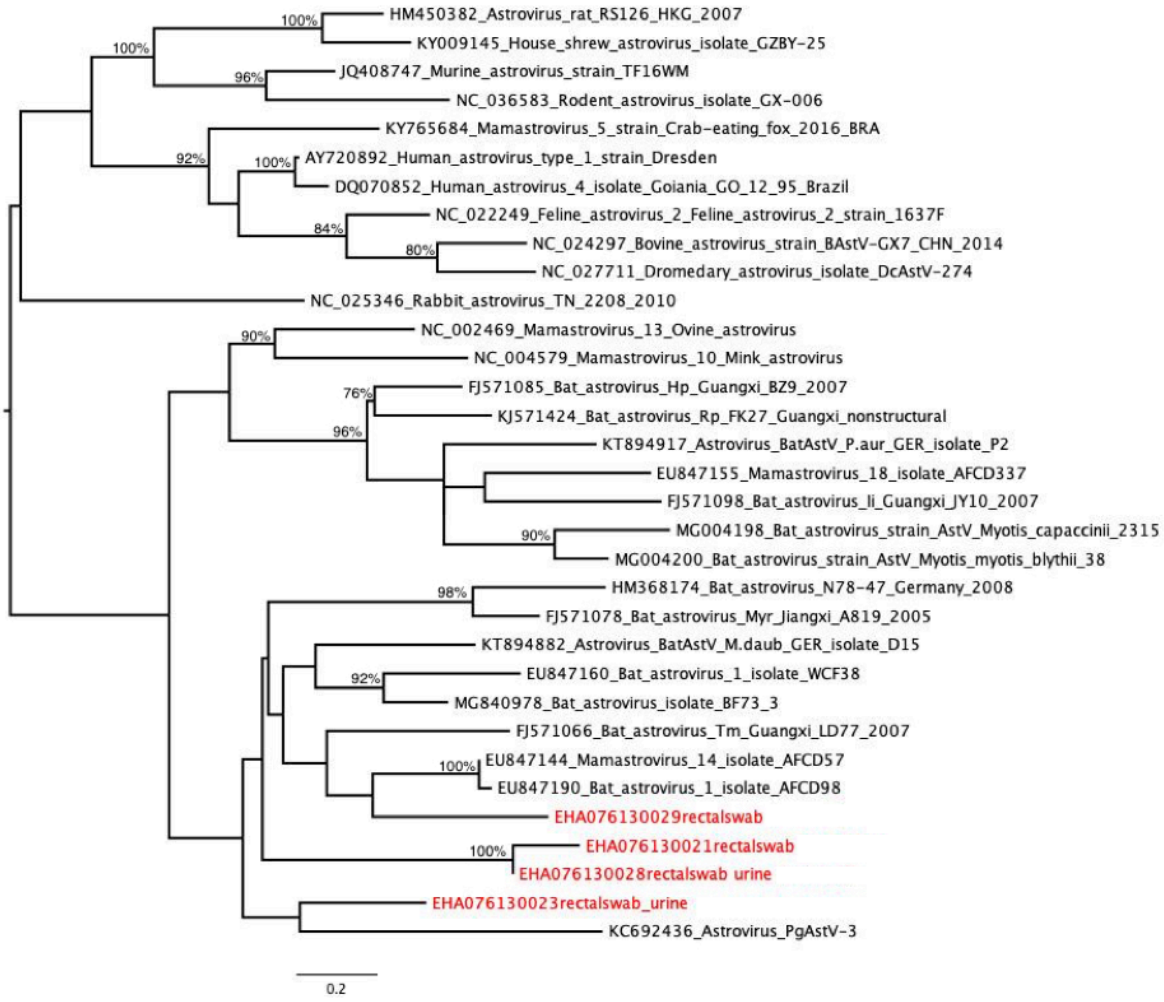


Figure S3.6.3 Astrovirus Maximum Likelihood Phylogenetic Tree.

Genetic analysis of 369 nucleotide partial RdRp gene. Tree reconstructed by MEGA7 with heuristic search and Neighbor-Joining "NJ" algorithm. The principal node values superior to 70% represent 1,000 bootstrap replicates.

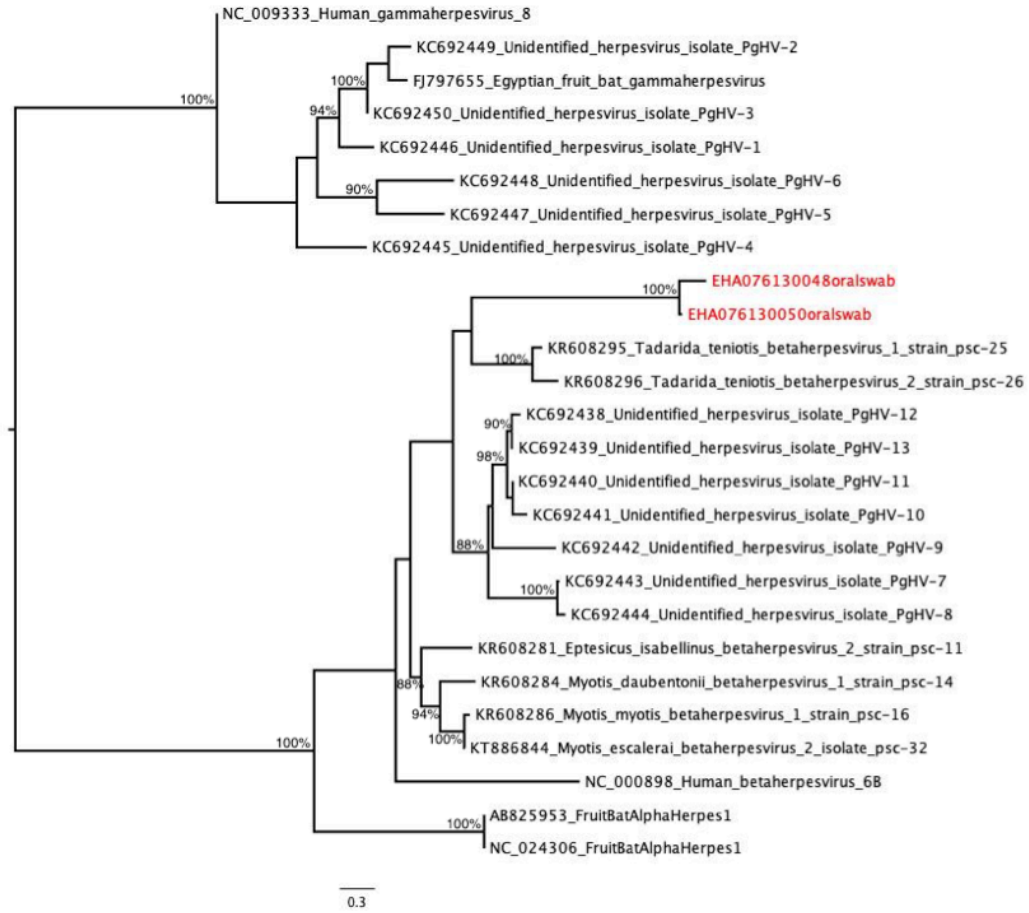


Figure S3.6.4 Herpesvirus Maximum Likelihood Phylogenetic Tree.

Genetic analysis of 189 nucleotide partial Polymerase (pol) gene. Tree reconstructed by MEGA7 with heuristic search and Neighbor-Joining "NJ" algorithm. The principal node 475 values superior to 70% represent 1,000 bootstrap replicates.

4 Habitat fragmentation structures host-virus assemblages in the Atlantic Forests of Brazil

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* The following laboratory analyses were supervised by Dr. Simon Anthony at the Center for Infection and Immunity at Columbia University.

4.1 Abstract

Microorganisms comprise much of the biodiversity on Earth. Estimates vary widely, but a recent analysis suggests there could be as many as 1.6 million distinct microbes on Earth, of which each is undoubtedly associated with at least one virus. Thus, viruses likely comprise the most abundant source of genetic diversity on Earth, yet the majority of research on viral ecology has been conducted in marine environments. This chapter uses multidisciplinary insights to identify factors that drive viral diversity within changing terrestrial landscapes. Importantly, this research addresses the relative importance of stochastic vs. deterministic processes in structuring host-virus assemblages across varying types of land-use. We sampled bats along a fragmentation gradient across 15 sites in Brazil and use consensus PCR and sequencing to discover 75 viruses from four viral families. We use ecological analyses to show that patterns of viral alpha- and beta-diversity correlate with those of bat diversity. We then use network analyses and ordination methods to show the presence of non-random deterministic patterns across the fragmentation gradient. We show that landscape factors including area and connectivity are important in structuring bat assemblages, while the composition of viral communities is dependent upon host community composition. In showing that determinism is an important process in viral community assembly across different land-use types we conclude that it should be possible to forecast how viral diversity responds to land-use change, which could be useful in allowing us to potentially mitigate the impact of those changes and potentially reduce zoonotic disease emergence.

Keywords: habitat fragmentation; viral communities; bat communities; zoonoses; PCR; microbial diversity

4.2 Introduction

Tropical forests are incredibly biodiverse, supporting at least two-thirds of the world's biodiversity (*216*). Moreover, they play key roles in ecosystem functioning, performing functions such as carbon storage, nutrient retention and water supply and purification (*20*). In recent decades, anthropogenic land-use change has led to unprecedented loss of tropical forests across the globe (*217*), with hotspots concentrated mainly in Asia and Brazil (*20*). Habitat fragmentation, defined as the transformation of contiguous natural habitats into smaller and more isolated patches embedded in a matrix of human-modified landscapes (*218, 219*), can reduce biodiversity by influencing resource availability, population carrying capacities, species persistence, and the community composition of plants and animals (*32, 220, 221*). In addition to these ecological implications, mounting evidence suggests that land-use change can influence the cross-species transmission of pathogens among wildlife, humans and domestic animals. Yet, despite this evidence and nearly a century of empirical and theoretical research devoted to the study of macroscopic organisms including vertebrate and plant communities, considerably less is known about microbial diversity and the factors underlying its variation.

Viruses likely comprise the most abundant source of genetic diversity on Earth. They drive major ecological processes like carbon cycling, modulate microbial populations (*222*) and shape the evolutionary trajectories of the host taxa they infect (*223, 224*). While the diversity of some microbial communities has been shown to vary predictably along gradients or in response to disturbances (*225, 226*), viral ecology in terrestrial systems remains largely unexplored (*227*). This knowledge gap is even greater for parasites of wildlife and has recently

taken on added urgency in light of the global rise in zoonotic infectious disease outbreaks (*166, 228*). Anthropogenic land-use change has been associated with infectious disease emergence (*106, 166, 172*), and nearly all recent pandemics have a viral aetiology with animal origins including HIV/AIDS, Middle East Respiratory Syndrome coronavirus (MERS-CoV)(*229*), the continual appearance of novel subtypes and strains of influenza A virus (*230, 231*) and the recent outbreak of Ebola in West Africa (*232*). Further, in the last two decades, several zoonotic diseases of significant public health concern have been linked to bat-borne viruses including SARS coronavirus, Hendra virus and Nipah virus (*177*). In addition, previous studies have linked ebolaviruses and MERS coronavirus to bats (*178, 179*). It is increasingly suspected that bats may be reservoirs of numerous known and novel viruses with potential for inter-species transmission. Thus, understanding the potential effects of land-use change on host and viral diversity is key to attempts to predict and mitigate emerging threats to global health.

Bats harbour the highest proportion of zoonotic viruses of any mammal order (*166, 183*) and their suitability as hosts of pathogenic viruses has garnered much attention in recent years. The wide differences in their dispersal abilities (*176*) and their diverse feeding strategies spanning multiple guilds likely facilitate the acquisition and dispersal of viruses across regions, as well as interspecies transmission. Within fragmented landscapes, multiple interrelated factors contribute to species responses. While some species may thrive, particularly those associated with edge environments (*19, 233*), habitat fragmentation generally results in a loss of species diversity (*2*).

Ecological theory predicts that the abundance and diversity of parasitic organisms likely correlates with the hosts in which they reside (i.e. the "host diversity begets parasite diversity hypothesis) (*234-236*). Given the intimate association between hosts and parasites as well as the expectation that many parasites are specialized to infect a small number of host species (*236*) an increase in host species richness would likely correlate with an increase in parasite diversity, including a greater number of pathogenic species (*237*). One would therefore also expect a close linkage between host and virus community composition (*238*). Assuming this to be the case, we predict that declines in host diversity driven by habitat fragmentation will likely include corresponding loss of potentially pathogenic microbial agents, the majority of which remain uncultured or described.

Here, we investigate the effects of habitat fragmentation on bat and viral assemblages in a multi-host, multi-parasite community. We sample bat communities across 15 sites in the interior Atlantic Forest, Brazil and use consensus PCR with degenerate viral family-level primers to screen for both known and novel members of four viral families. Building from community ecology theory, we hypothesize that land-use change, specifically habitat fragmentation will structure bat and viral richness (number of species) and diversity (functional α- diversity) and that viral assemblages among land-use types (β diversity) will be non-randomly structured, possibly indicating competitive exclusion or inherent structuring of viral assemblages.

4.3 Methods

4.3.1 Study area and sites

Along a habitat fragmentation gradient that ranges from 36,000 ha of continuous forest to habitat fragments less than 2 ha, the interior Atlantic Forest in Brazil is one of the most threatened tropical regions in the world(*86*). The Pontal do Paranapanema constitutes a unique model system in which to understand the effects of habitat fragmentation on bat and viral assemblages, since the landscape matrix was maintained as homogenous , composed of pasture and sugarcane with low human density for the last 50 years (*86*), thus minimizing factors that could influence species richness in the region apart from the spatial structure of forest patches and its dynamics. This area is characterized by a tropical semi-deciduous forest, one of the most threatened sub-types within the Atlantic Forest. An important feature of this area is a contiguous forest tract, Morro do Diabo State Park (36,000 ha), which is one of the only four patches within this physiognomy larger than 10,000 ha. Adjacent to the park are forest fragments ranging from 2 to 2,000 ha, most of which are located within private properties. The remnant forest, including the State Park, now occupies about 18% of the whole region. The matrix is composed mainly of pasture (59.9%) and agriculture (14.6%), both of which are strong impediments to dispersal (*186*).

We sampled bat communities from four different land-use categories including reference sites in continuous forest, large and small fragments, and matrix sites. Study sites were located at least 5 km apart with the aim of achieving sampling independence. During year one, each land-use category was sampled at three sites (replicates), each for five nights. During year one, a full set of three replicates for each land-use category was sampled twice

per year, once in the wet season and once in the dry season. During year two, each site was revisited apart from two matrix sites from year one due to issues with access to private property; two new matrix sites were chosen (Table 4.1). Additional sampling effort was required in the large fragments due to lower catch rates. To ensure a range of fragment sizes, we selected four large (1000-2000 ha) and three small (<100 ha) fragments (Figure 4.1). We did not choose patches smaller than 50 ha because generally these patches are highly degraded due to the edge effects, which is very intense in this type of tropical semi-deciduous forest.

Table 4.1. Study design and sampling effort sampling bats in the Pontal do Paranapanema region of Atlantic forest, Brazil.

Bats			Continuous		Large		Small		Matrix		
			Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Total
Year 1	Replicate	Site 1	5	5	5	5	5	5	5	5	
		Site 2	5	5	5	5	5	5	5	5	
		Site 3	5	5	5	5	5	5	5	5	
Year 2	Replicate	Site 1	5	5	5	5	5	5	5	5	
		Site 2	5	5	5	5	5	5	-	-	
		Site 3	5	5	5	5	5	5	-	-	
		Site 4	-	-	5	5	-	-	5	5	
		Site 5	-	-	-	-	-	-	5	5	
Total nights			30	30	35	35	30	30	30	30	**250**

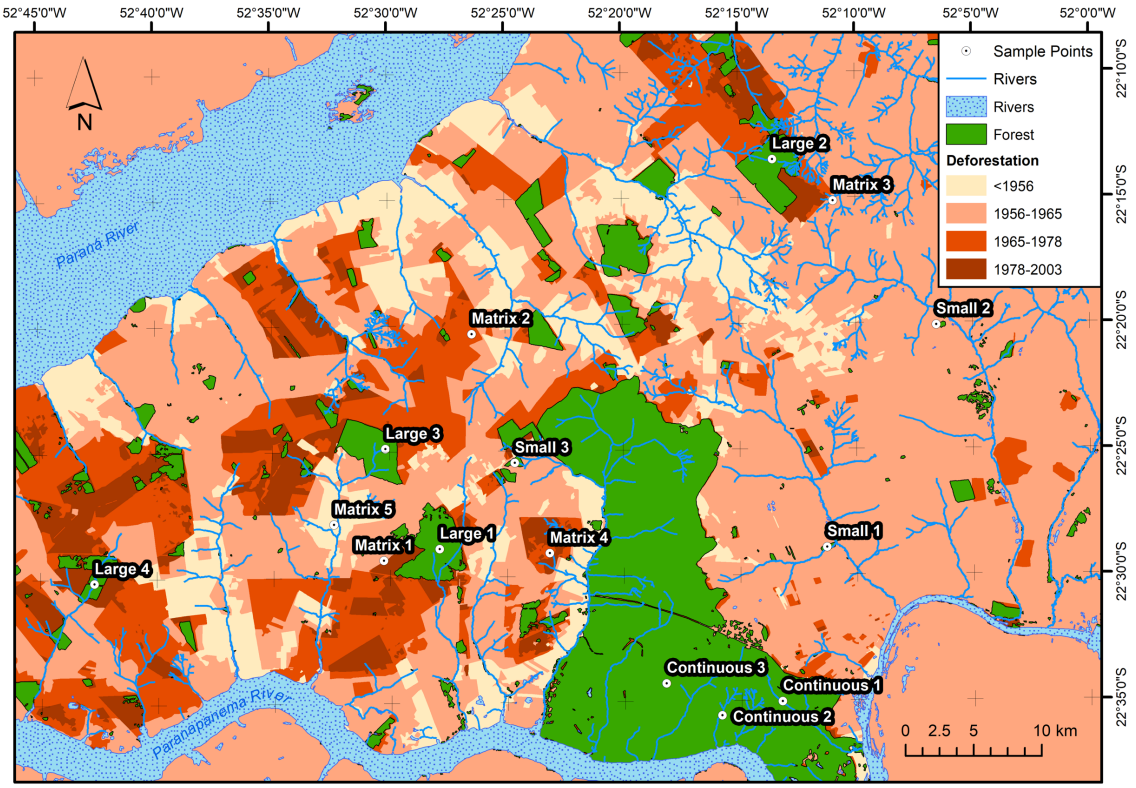


Figure 4.1: Map of the study site in the Pontal do Paranapanema region in the interior Atlantic Forest, Brazil. The study area was mapped for six different years: 1965, 1978, 1984, 1993 and 2003. Aerial photographs were used to map the study areas in 1965 using ArcGIS (scale 1:35,000). Data from 1965 and 1978 were collected by digitalization of topographic maps (scales 1:50,000 and 1:10,000 respectively), and from 1993 to 2003, Landsat Imageries were classified. For each year, we mapped two categories of occupation: forest and non-forest. Maps were geo-referenced using the same projection and datum and, because the sources were of different resolution, we converted these to raster type and resampled them for a pixel size of 30 m prior to the analysis.

4.1.1. Ethical Statement

This study was carried out with animal handling permits issued from the Brazilian Ministry of the Environment (#33078-4). Animal handling ethics approval was provided by the University of California, Davis (#16048). Bat handling followed strict personal protection and biosafety requirements and short capture times to minimize stress on individual animals. All captured individuals were released at the point of capture.

4.1.2 Bat capture and sample collection

Bats were sampled during the dry and wet seasons from December 2014 to December 2016. At each site we sampled a 100m x 100m grid using eight horizontal mist nets (9m x 3m), one canopy mist net (6m x 3m) and one harp trap (1.5m x 1.5m). Bats were captured for a period of five consecutive nights at each site. Mist nets were opened at sunset and remained open for six hours. Nets were checked at 30-minute intervals and bats processed immediately.

Samples were collected from bats deemed healthy by trained veterinarians, and all animals were released immediately following processing. Blood, saliva, and rectal swabs were collected from each captured animal, with faeces and urine opportunistically collected. All samples were placed in cryovials containing 200 ml of Viral Transportation Media (VTM) and stored in liquid nitrogen in a dry shipper while in the field, then transferred to -80C freezers at the Institute of Biomedical Sciences at the University of Sao Paulo. Once all samples were collected, they were imported to the United States and transferred to the Center for Infection and Immunity at Columbia University. External morphological measurements (including forearm/radius length, body length, head length) were collected to assist in taxonomic identification, and sex and age class (e.g. juvenile, sub-adult, adult) were

determined for all individuals. Before release, each individual was marked with a non-toxic pen to determine the rate of within-trip recapture. This was used to ensure that the same bat was not re-sampled within sampling trips. Detailed protocols for all bat capture methods used are provided as Appendix 3.

4.1.3 Genetic Barcoding

To identify species in the field, we used a morphology-based approach. However, this approach has several limitations including significant variation in taxonomically important traits which can lead to incorrect identification. To overcome these limitations, genetic barcodes were generated to confirm species identification. This was achieved using PCR amplification of *cytochrome b* (Cyt b) *or cytochrome oxidase subunit I* (COI). To generate a barcode, PCR product from at least one sample per animal was sequenced (either by direct sequencing of sense and antisense strands, or by cloning and sequencing). This method has been particularly successful in the identification and description of both known and novel species of various taxonomic groups (*239-242*).

4.1.4 Landscape structural analysis

To visualize the historical process of deforestation in the region, the study area was mapped for six different years: 1965, 1978, 1984, 1993 and 2003. We interpreted aerial photographs to map the study areas in 1965 using ArcGIS (scale 1:35,000). Data from 1965 and 1978 were collected by digitalization of topographic maps (scales 1:50,000 and 1:10,000 respectively), and from 1993 to 2003, Landsat Imageries were classified. For each year, we mapped two categories of occupation: forest and non-forest. Maps were geo-referenced using

the same projection and datum and, because the sources were of different resolution, we converted these to raster type and resampled them for a pixel size of 30 m prior to the analysis.

We used the Integral Index of Connectivity (IIC) as a measurement of habitat fragmentation (Pascual-Hortal and Saura 2006), which is based on a binary connections model in which two patches are linked (directly connected) if the distance between them is below a certain threshold dispersal distance. The IIC metric was calculated as follows:

$$\mathrm{IIC} = \frac{\sum_{i=1}^{n}\sum_{j=1}^{n} a_i a_j / (1 + \mathrm{nl}_{ij})}{A_L^2} \qquad (1)$$

where a_i is the area of each habitat patch, nl_{ij} is the number of links in the shortest path (topological distance) between patches i and j, and A_L is the total landscape area (area of the study region, comprising both habitat and non-habitat patches). For patches that cannot be reached from each other (no path exists between them) the numerator in the sum of Eq. (1) equals zero ($\mathrm{nl}_{ij} = \infty$). When $i = j$ then $\mathrm{nl}_{ij} = 0$ (no links needed to reach a certain patch from itself). IIC ranges from 0 to 1 and increases with improved connectivity. IIC = 1 in the hypothetical case that all the landscape is occupied by habitat.

To calculate the landscape connectivity for each year, initially the region was divided in hexagons of 21.6 ha (distance between parallel edges of 500 m). For each one of these hexagons we determined its relative importance for landscape connectivity. As hexagon attribute we considered the area of the largest forest patch that intersect them, and the link among hexagons was defined based on the distance among their centroids. The analysis was restricted to a radius of 5000 m around the survey points. Indexes were calculated using the software Conefor 2.6.

4.1.5 Viral Discovery

During the last few decades, findings from previous studies have drawn attention to several viral families as being both more prevalent in bats compared to other taxon, and with proven potential for interspecies transmission including: *Coronaviridae, Paramyxoviridae, Reoviridae,* and *Filvoridae (180).* Further, a growing number of known and novel viruses have been detected in bats from the following families: *Astroviridae, Circoviruidae, Parvoviridae, Partitviridae, Coronaviridae, Picobirnavirdae, Adenoviridae, Herpesviridae, Papillomaviridae, Phenuiviridae, and Picornaviridae* among others (*181, 182*), illustrating the breadth of viral richness in bat populations. Here, we build upon previous work to further expand the diversity of the bat virome by detecting known and novel viruses from the following viral families: *Coronaviridae, Paramyxoviridae, Herpesviridae and Flu. Herpesviridae* was chosen as this family is generally regarded as highly successful with most vertebrates infected with at least one herpesvirus. Because they are so ubiquitous and diverse, *Herpesviridae* is an ideal model system to examine how viral assemblages change across a fragmentation gradient. *Flu* was included because influenza virus is known for zoonotic transmission and two novel subtypes have been discovered previously in bats; and lastly, *Coronaviridae* and *Paramyxoviridae* both contain bat viruses of recent public health concern, as well as novel viruses which may possess spillover potential (*180*).

Following methods from Anthony et al. (2014), samples were viral particle enriched through (i) filtration to remove cellular debris and bacteria and (ii) nuclease treatment to remove unencapsulated RNA/DNA. For this process, samples were thawed on ice and 500 µl of viral transport medium (Viral Transport Medium (VTM); BD Universal Viral Transport

System) added, vortexed to homogenise, and centrifuged for 5 min at 8,000*g*. Supernatant was transferred to an Ultrafree-MC HV Centrifugal Filter 0.45 μM (Milipore Cat. No. UFC30HVNB) and centrifuged for 3 min at 12,000g. The flow (~130–150 μl) was collected and 1 μl RNase A (Ribonuclease protection assay Grade, 1 mg ml^{-1}, Life Technologies Cat. No. AM2272) added and incubated at room temperature for 15 min. If the flow volume was close to 200 μl then 2 μl of RNase A was used. Following RNase treatment, 1.5 μl of $MgCl_2$ (1M), 4 μl of Turbo DNase (2 U ml^{-1}, Ambion Cat. No. AM2238) and 1 μl of Benzonase. (Novagen, Cat. No. 70664-3), were added, mixed gently and incubated at room temperature for 45 min. Roche MagNa Pure lysis solution was added immediately to inactivate nucleases and lyse viral particles, and total nucleic acids extracted using the Roche MagNA Pure 96 platform according to the manufacturer's instructions. Samples were processed for viral detection and discovery using consensus PCR, which allows the 'universal' amplification of sequences from viruses within a given family or genus, and the subsequent discernment of viral strains within. Total nucleic acids were reverse transcribed into cDNA using SuperScript III (Invitrogen) according to the manufacturer's instructions, and a total of 7 assays representing 4 viral families or genera used for the detection of viral sequences. Two synthetic plasmids were constructed for use as 'universal controls' to confirm successful execution of each assay and check for contamination. Detailed protocols for all cPCR assays used are provided in the Supplementary Methods and Supplementary Table 2. Bands of the expected size were excised from 1% agarose, cloned into Strataclone PCR cloning vector, and 24 white colonies sequenced to confirm detection and look for co-occurring viruses.

4.1.6 Statistical analysis

Asymptotic viral richness was estimated from observed bat captures and viral detections using two estimates of richness, Chao 1 and ACE, as well as the Shannon index (*208*), using the 'vegan' package in open source R (3.4.0). The Shannon index, was chosen over the Gini–Simpson index because it does not place disproportionate weight on dominant species. Correlation between the effective number of viruses and bats at each site was then tested by applying Pearson *r* correlation.

To evaluate the relative importance of landscape structure and dynamics upon bat and viral diversity we related bat and viral richness indices with landscape variables (i.e. area and IIC metrics, log transformed). Bat and viral richness (number of species, i.e. alpha diversity) were obtained from the survey data and backward stepwise multiple linear regressions were conducted. We then constructed Rank Abundance Distributions (RAD), which are simply vectors of species abundances sorted in decreasing order (*243*). For viruses, we used two approaches to visualize viral species richness and species evenness. The first measured the abundance of each virus in a single infected host (i.e. individual bat) across each land-use type, while the second measured the abundance of viruses regardless of host across each type of land-use category.

All multivariate analyses were conducted using open source R version 3.4.0. We used the Bray-Curtis distance to create a matrix of dissimilarity. To compare differences in community composition of viruses and bats, we ordinated samples using non-metric multidimensional scaling (NMDS), which is regarded as the most robust unconstrained ordination method in community ecology (*244*). The main objective of NMDS is to plot

dissimilar objects far apart in the ordination space and similar objects close to one another ('metaMDS function, package 'vegan'). In addition, we partitioned the Bray-Curtis dissimilarities (BC_{sum}) into two components following Baselga (2013): (1) balanced variation in abundance (BC_{bal}), that is dissimilarity caused by changes in species abundances among site categories with different signs for different species so the changes balance (i.e. analogous to turnover with incidence-based metrics), (2) abundance gradients (BC_{gra}), that represents changes in species abundances among site categories in the same direction (increase or decrease; analogous to nestedness). The R package 'betapart' ver 1.3 was used to calculate BC_{bal} and $BC_{gra(245)}$. The adonis function was used to conduct Permutational multivariate analysis of variance (PERMANOVA) to test for statistical differences in within-group variance among site categories. We also used the base R statistical package to run two-way ANOVAs and the 'agricolae' package to perform Tukey post-hoc analyses for differences in bat and viral abundances and estimated species richness between site categories. We used a Mantel test to assess whether there was a correlation between bat and viral beta-diversity. Distance matrices were constructed using the Bray-Curtis distance. We performed 100,000 permutations and calculated the Pearson correlation coefficient for each permutation. For all statistical analyses, we applied $\alpha = 0.05$. All R scripts can be found in the Supplementary Information.

4.2 Results

5.4.1 Alpha diversity

We captured 1230 individuals from 27 bat species, six guilds and five bat families. Observed species richness was highest at matrix sites (n=19), followed by continuous forest (n=17), large (n=9) and small fragments (n=6). Estimated species richness was highest at

matrix sites (n=23, Chao estimator), which was four species greater than the observed value. The flat-faced fruit-eating bat, *Artibeus planirostris* was the most frequently captured species representing 27% of all captures, followed by the great fruit-eating bat, *Artibeus lituratus* (25%), and Sebas's short-tailed bat, *Carollia perspicillata* (16%). Conversely, eight species were captured only once, four in the matrix sites and four in continuous forest sites. Across the fragmentation gradient, estimated species richness (Chao 1 and ACE) declined significantly in small and large fragments, while the Shannon index increased in large fragments as compared to small suggesting fragmentation induced changes in alpha diversity in small fragment sites (Figure 4.2).

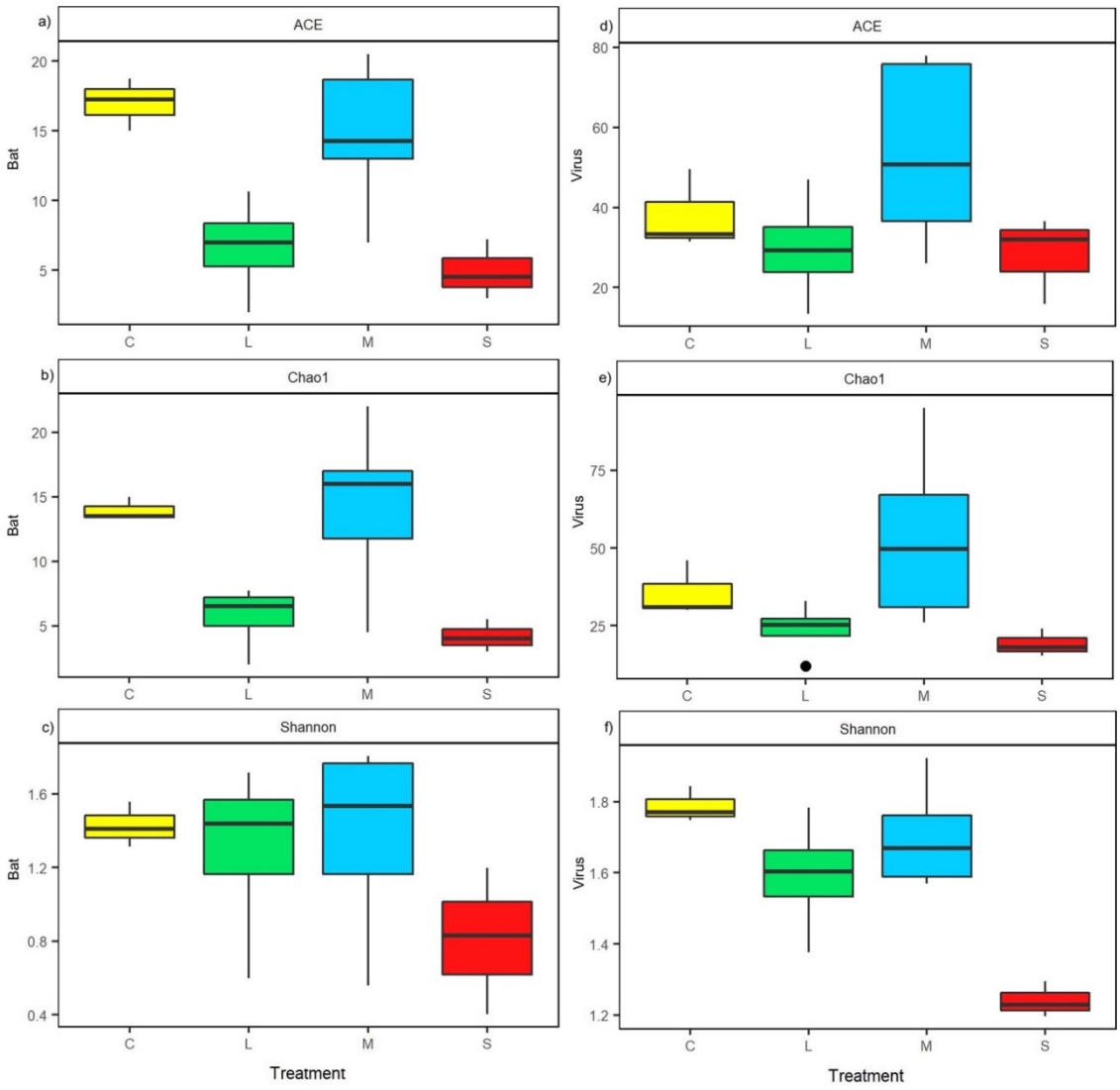


Figure 4.2: Panels a-c; Similar richness of bat species in continuous forest and matrix sites. **(a)** Richness indices ACE **(a)** and Chao 1 **(b)** were significantly reduced in fragment sites, but no significant difference was found between continuous forest and matrix sites, whereas Shannon index was similar in all groups, excluding small forest sites (**c). Panels d-f**; Increased richness of viral species in matrix sites (**d-e**). Richness indices ACE **(d)** and Chao 1 **(e)** were significantly increased in matrix sites, whereas Shannon index was similar in all groups, excluding small forest sites. Box plots represent median (black horizontal line), 25th and 75th quartiles (edge of boxes), upper and lower extremes (whiskers). Outliers are represented by a single data point. C= continuous, L = large fragments, S = small fragments, and M = matrix sites.

Bat functional diversity was higher in matrix sites due to the occurrence of higher proportions of sanguivores, insectivores, carnivores, and omnivores. The 27 bat species recorded fell into six guilds, of which two (i.e. frugivores and insectivores) were recorded across all site categories. The frugivore guild was the most abundant (95%, 98%, 97%, and 88% of the total number of individuals collected in continuous, large and small fragments, and matrix sites, respectively). Insectivores nearly doubled in proportion among matrix-species compared to continuous forest species, tripled when compared to small fragment species, and quadrupled when compared to large fragment species. Sanguivores and omnivores were only detected in continuous forest and matrix sites, with a higher proportion of both guilds recorded in matrix sites.

A total of 3608 consensus PCR assays were performed for the detection of viruses from four different families/genera including corona-, herpes-, influenza, and paramyxo- viruses. A total of 1032 viral sequences were detected in 22 bat species. These sequences were segregated into 75 discrete viruses based on distinct monophyletic clustering, and a virus was considered novel if the sequence identity to its closest relative was less than or equal to the identity between the two closest species for a given viral family (*246*). The numbers of viral species (observed viral species richness) in continuous, matrix, and large and small fragments were 38, 58, 23 and 18 respectively; the richest land-use type was the matrix, and the poorest land-use type were the small fragments. Estimated viral species richness was higher in matrix sites as compared to continuous forest and fragment sites. Similar to bat communities, the Shannon index was significantly lower for the small fragment sites (Figure 4.2). Viral distribution was more even within bat hosts in continuous forest and settlement sites (i.e. all

the bats are similarly infected) as compared to small and large fragments sites where only a few host individuals appear to host a higher abundance of viruses (Fig 4.3 panel c). Whereas, there was no clear difference in the abundance of each virus, regardless of bat hosts, across land-use types. Viral α-diversity was significantly correlated with bat α-diversity and this association was maintained across several diversity metrics (Fig. 4.4). In nearly all cases, the highest diversity was found in matrix and continuous forest sites, as compared to fragments.

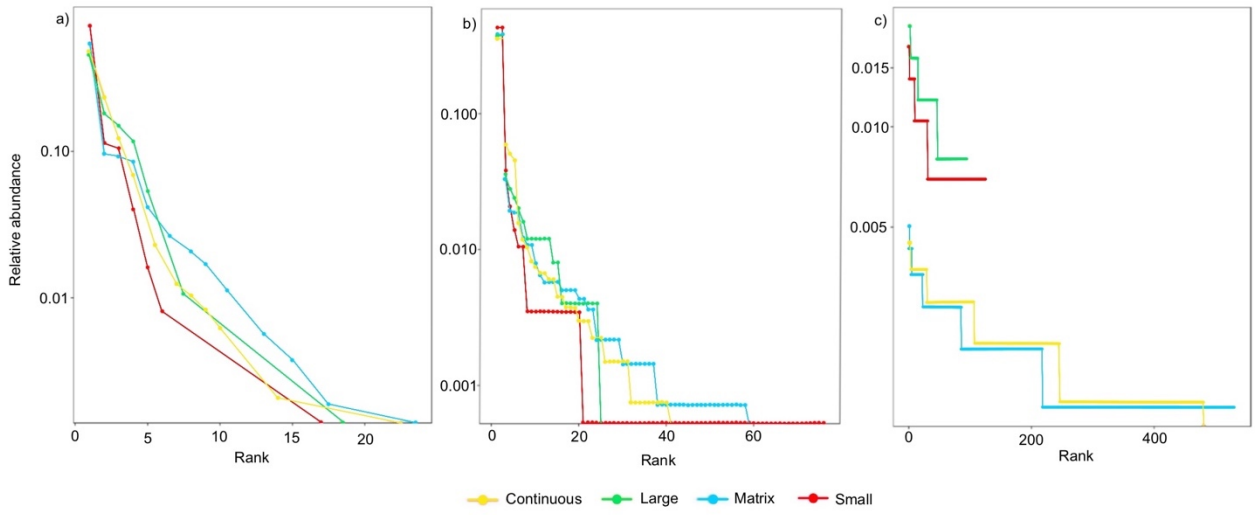


Figure 4.3: Rank abundance distributions among different land-use categories for a) bat host abundance; b) viral abundance across land-use categories, and c) viral abundance in individual host across land-use categories.

Both area and connectivity were significant in explaining bat species richness, with a negative effect from area and a positive effect from connectivity (Table 4.2). Interestingly, this result shows that for bats, the context of the landscape matters more than the local area, which is likely related to their dispersal ability. Viral species richness was not significantly associated with any landscape metric.

Table 4.2. Multiple linear regression analysis predicting bat and viral species richness from landscape factors.

	β value	95% CI	P-value	B value	95% CI	P-value
		Bat model			**Virus model**	
Intercept	8.546	4.670, 12.393	0.001 **	42.912	15.33, 70.50	0.009**
Area	-0.005	-0.009, -0.001	0.020 *	-0.020	-0.047, 0.008	0.127
diic_2003	0.624	0.189, 1.060	0.012 *	2.341	-0.784, 5.467	0.120

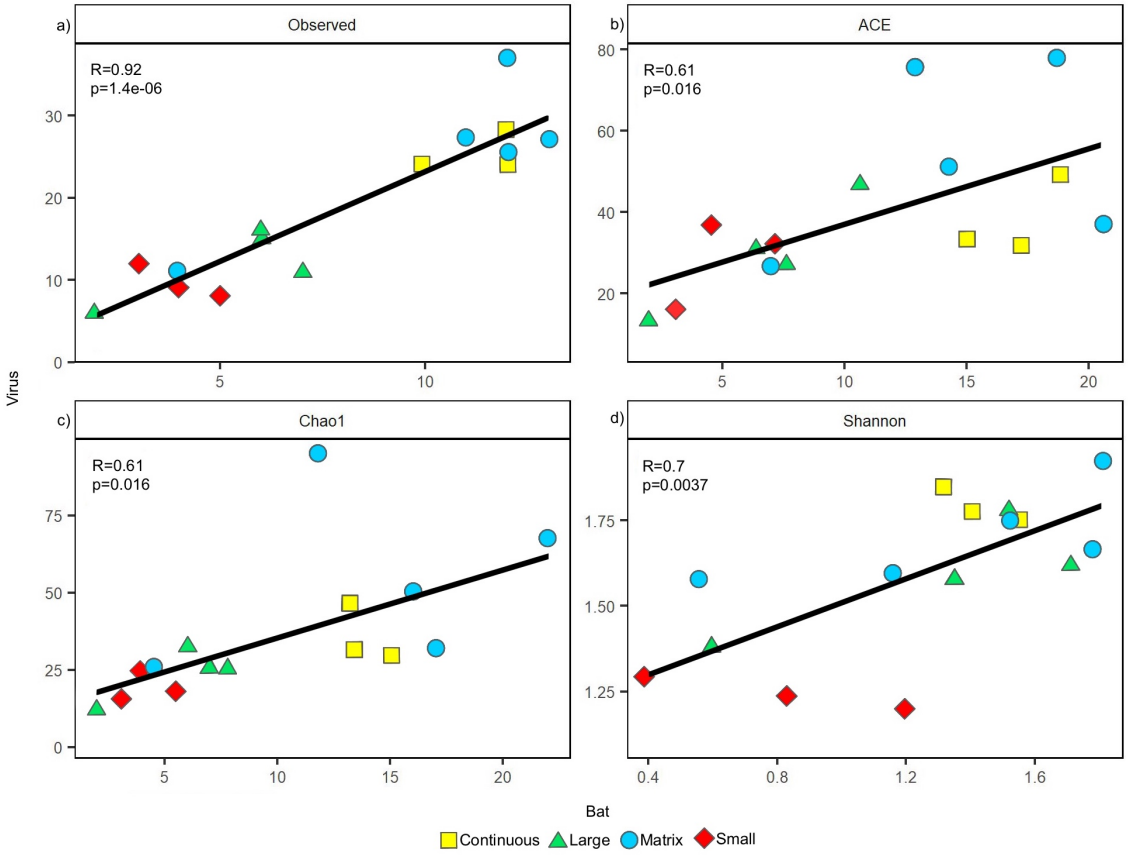


Figure 4.4: Viral alpha-diversity is significantly correlated with bat alpha diversity, across a range of metrics including a) observed species richness; b) abundance-based coverage estimator (ACE); c) Chao 1 estimator and d) Shannon diversity index.

4.4.2 Beta-diversity

Alongside changes in species richness and diversity, biotic communities may change in their species composition with increasing habitat fragmentation. Non-metric multidimensional scaling, based on the presence and absence data of 27 bat species, and abundance of 75 unique viral taxa, revealed a strong directional change (e.g. decline in abundance) of both bat and viral communities from continuous forest to habitat fragments in the Atlantic Forest, Brazil (bats, BC_{sum}=69%, BC_{bal}=5%, BC_{gra}=64%; viruses, BC_{sum}=69%, BC_{bal}=2%, BC_{gra}=67%). In contrast, bat and viral communities exhibited less change between large and small fragments, 33% and 25% dissimilarity respectively, and a significant proportion of the changes were attributed to balanced variation, that is, the abundance of some species declined over time in the same magnitude as the abundance of other species increased (viruses, BC_{bal} = 20%, BC_{gra}= 5%; bats, BC_{bal} = 22%, BC_{gra}= 11%). From small fragments to matrix sites, the total dissimilarity was 86% for bat communities, with changes largely explained by balanced variation (64%) and a smaller proportion by a directional change (22%). While viral communities also exhibited a significant change from small fragments to the matrix (66% overall dissimilarity), they showed a different pattern with changes in viral species composition almost exclusively driven by a directional change (62%), suggesting that the viruses detected in the small fragments were a subset of the matrix community. Interestingly, while the total dissimilarity of bats communities between continuous forest to matrix sites was high (70%) and driven primarily by balanced variation (68%), the viral communities were much more similar (21% dissimilar), suggesting that the abundance of host species plays an important role in structuring viral communities.

The bat communities exhibited differentiation among land-use categories (PERMANOVA: $n = 4$, $R^2 = 0.31$, $F = 23.83$ $P = 0.001$;), indicating that bat assemblages were significantly structured by habitat fragmentation (Fig 4.5a). A post-hoc Tukey analysis indicated that bat species richness was highest in continuous forest sites, and lowest in the forest fragments, which is consistent with previous studies on habitat fragmentation and bat diversity (*247*). Land-use category did not affect the average bat abundance across site categories (that is, the average number of bats caught per sample; Fig. 4.5c). Viral communities, like the bat communities, were also significantly structured by land-use (PERMANOVA: $n = 4$, $R_2 = 0.31$, $F = 23.83$ $P = 0.001$;). However, unlike bat communities, a post-hoc Tukey analysis showed that average viral species richness was not significantly different between continuous forest and matrix sites, but decreased significantly in large and small fragments. Viral species richness was highest in the matrix, then continuous forest, and significantly decreased in large and small fragments, respectively. In terms of co-infection, matrix sites hosted the highest number of individual bats that were co-infected, and a larger proportion of individuals that hosted more than three viruses. Viral abundance did not change between the four site categories (Fig. 5.5f).

Results from the Mantel test revealed a moderate effect size, with a standardized Mantel statistic of r = 0.31. This correlation was significant, with p = 0.004, showing that dissimilarity in virus species is positively, but weakly, correlated to dissimilarity in bat species, suggesting that the composition of virus communities is dependent upon host community composition, not landscape factors. To visualize this relationship, we constructed a two-mode affiliation network to illustrate the connectivity between viruses and their hosts.

This revealed a dominant (though not exclusive) pattern of genera-specific diversity consistent with determinism (Appendix 1).

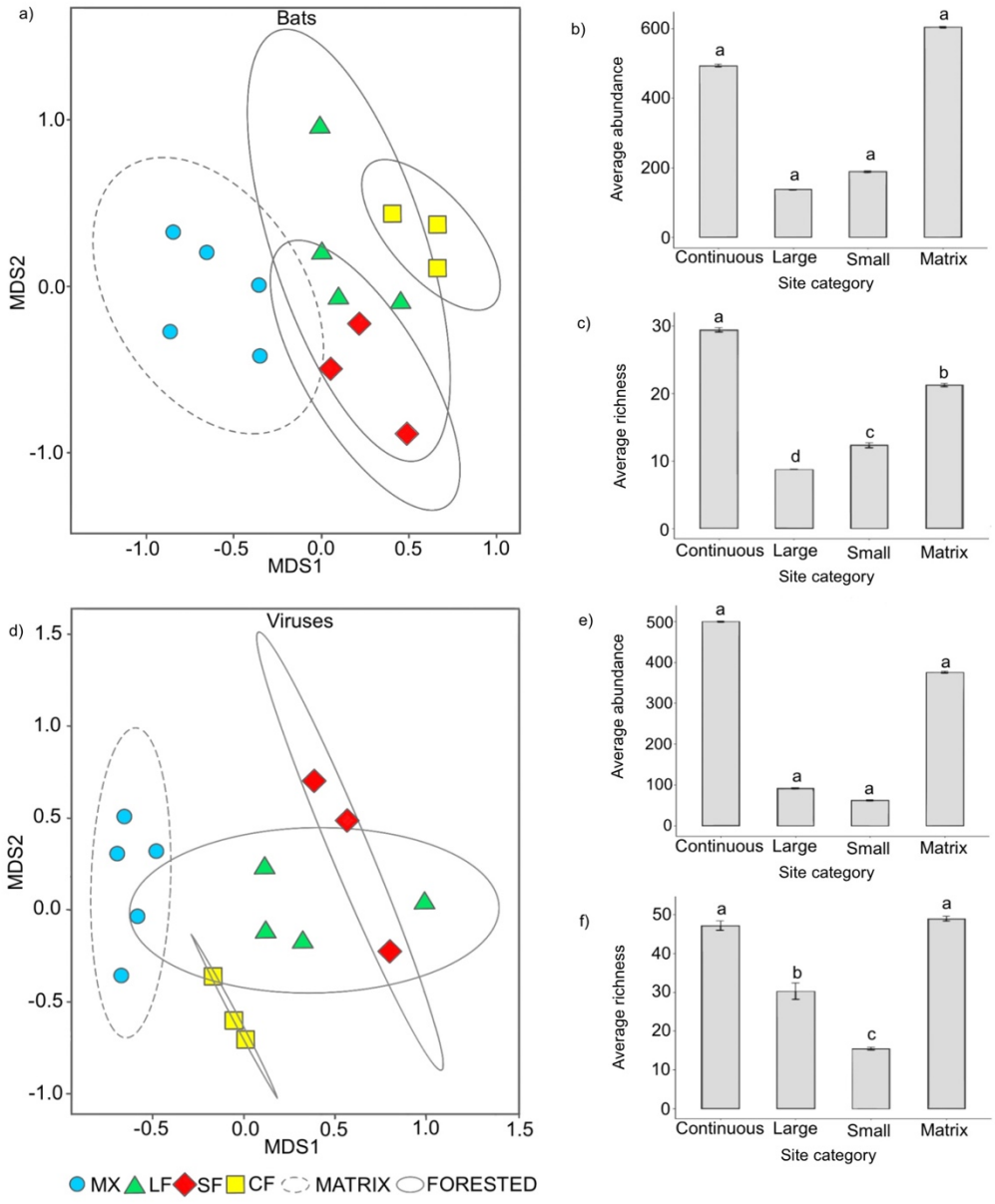


Figure 4.5: Effect of habitat fragmentation on beta diversity, abundance and alpha diversity of bat and viral assemblages**. a-c,** NMS ordination **(a;** final stress value 0.16), **(b)** average bat abundance (number of individuals caught) under each land-use **(c)** and average estimated species richness for bats using Chao 1. d–f, NMS ordination (d; final stress value 0.15), (e) average viral abundance (number of viruses

detected) under each land-use and (f) average estimated species richness for viruses using Chao 1. In all cases, error bars represent standard error and letters indicate post-hoc Tukey's HSD significance at $\alpha = 0.05$.

4.3 Discussion

Global declines of biodiversity have driven efforts to understand how anthropogenic land-use change can affect ecological processes, including potential effects on disease dynamics. Recent work on the biodiversity-disease relationship has focused on two seemingly competing hypotheses: host biodiversity can correlate positively with overall parasite diversity (i.e. the 'diversity begets diversity' hypothesis (*235, 237, 248*)); whereas the maintenance of biodiversity can reduce pathogen prevalence and consequently human disease ('dilution effect' (*104, 249*)). As predicted by the 'diversity begets diversity' hypothesis, we show that viral alpha-diversity correlates with bat host diversity suggesting that areas higher in biodiversity will support higher parasite diversity, including a greater number of pathogenic species(*237*). Further, both bat and viral community assemblages exhibited site-specific, non-random patterns, suggesting that the effects of deterministic factors on viral diversity should be predominantly predictable.

Indeed, recent work has demonstrated the role of deterministic processes in structuring viral diversity. Network models of viral families have shown that viral communities are likely to be inherently structured and heavily influenced by deterministic factors including geographic region, host family (*129*) and across spatial sites (*250*). We were able to further unravel such patterns by determining how viral diversity responds to habitat fragmentation. Perhaps, the most striking results are the changes in both bat and viral

communities in the human settlements located in the matrix: higher taxonomic and functional diversity of bat host species (e.g. higher proportions of sanguivores, insectivores, omnivores and nectarivores), and the increase of both viral richness and coinfection in matrix sites. Indeed, a growing number of studies are applying matrix-inclusive approaches to fragmentation studies (*251*) , highlighting the importance of the type and quality of matrix landscapes in which fragments are embedded. In our system, the matrix habitats likely mitigate some of the negative effects of fragmentation on bat communities, particularly given their ability to disperse.

The decline in bat species richness and abundance, along with the concomitant decrease in viral richness and prevalence from continuous forest sites to habitat fragments is not surprising and aligns with the conclusion of global assessments that habitat fragmentation significantly reduces species diversity (*2*). A recent synthesis revealed strong and consistent responses of organisms and ecosystem processes to fragmentation from decreased fragment area, increased isolation, and creation of edge habitats (Haddad). At the population level, consistent community and ecosystem responses emerged. Reduced area within fragments decreased animal residency, and increased isolation reduced movement among fragments, thus reducing fragment recolonization after local extinction.

Reduced fragment area and increased fragment isolation generally reduced the abundance of birds, mammals, insects and plants. This overall pattern emerged despite complex patterns of increases or declines in abundances of individual species with various proximate causes such as release from competition or predation, shifts in disturbance regimes, or alteration of abiotic factors. As predicted by theory, fragmentation strongly reduced species

richness of plants and animals across experiments, often changing the composition of entire communities.

While previous meta-analyses suggest that habitat fragmentation impacts mammal diversity to a lesser extent than other taxonomic groups (*36*), such analyses tend to overlook important responses in community structure. In our study sites, the observed changes in bat community composition are consistent with other neotropical studies that demonstrate habitat fragmentation, through altered habitat structure and changes in resource availability, result in changes to bat assemblages by decreasing bat species richness and abundance (*247*) while concurrently increasing the abundance of generalist species that are highly tolerant to environmental changes (*202, 252*). However, at the population level, previous research has shown that responses in bat abundance are highly species and guild specific (*202*). For example, in the Neotropics, the abundance of gleaning carnivorous and piscivorous bats, as well as certain aerial insectivores often decline in response to fragmentation (*202, 251, 253*) in contrast to frugivourous and nectarivorous bats which tend to increase (*203, 252*). Similarly, Struebig et al. 2009 showed that in the Paleotropics, cave-roosting species are less vulnerable to fragmentation than insectivorous bat species that roost in tree cavities or foliage (*254*). At the assemblage level, the results of studies that have compared fragmented and continuous forest in terms of species richness, diversity, and composition have been idiosyncratic (*255, 256*) likely due to differences in fragmentation history and the surrounding matrix.

We found that the effects of fragmentation were greatest in the smallest and most isolated fragments which harboured the lowest bat and viral diversity. However, we did find

a significant increase in viral burden in host species within the habitat fragments, which corresponds with previous work showing that pathogen burden in host species may occur given crowding of hosts and competition for resources (*45, 46*). Unexpectedly, we found that bat functional diversity and viral diversity were highest in the matrix sites, with matrix viral species richness comparable to that found in continuous forest sites.

Recent work modelling disease dynamics under various scenarios of land-use change has shown that at low levels of disturbance, disease outbreaks tend to die out due to a lack of susceptible individuals in the matrix that can sustain local chains of transmission. This is despite a large infectious pool of core species, a high force of infection and intermediate edge effects. However, as more land is converted, spillover risk increases due to growing matrix populations and high edge effects, with the highest probability of an outbreak occurring at intermediate levels of conversion(*257*). In our system, abundant bat populations, as well as high taxonomic and functional diversity can likely be attributed to the aggregation of food resources found in the matrix including fruit orchards (e.g. figs, acerola, mango, guava), and domestic animal species (e.g. cows, chickens, pigs). For many wildlife species, human-driven changes such as habitat fragmentation can alter the abundance, distribution and timing of food resources (*258, 259*). In some cases, human activities such as agroforestry can provide wildlife with abundant and reliable food sources (*260, 261*). As a result, human-modified environments can function as important breeding and foraging habitat as wildlife species adapt their behaviour to capitalize on these resources.

In terms of disease risk, changes in resource availability specifically can result in the formation of novel assemblages of species around human food sources facilitating inter- and

intra-species transmission via an increase in contact rates (*258, 262, 263*). For example, in songbirds, the emergence of *Mycoplasma gallisepticum* and trichomoniasis *(Trichomonas gallinae)* has been linked to shared resources at bird feeders (*258*). Further, the emergence of Nipah virus in Malaysia (*126*), Hendra virus in Australia (*264*) and the 2014 Ebola outbreak in West Arica (*168*) also highlight the importance of understanding how land-use change can influence disease dynamics by altering host community structure, changing contact patterns and altering resource availability. At our study site in the Atlantic forest region, the matrix predominantly comprises agriculture and pasture with adjoining agroforestry systems, which have been shown to support a high diversity of birds (*260*), bats (*261*), other small mammals and amphibians (*265*). As such, our results show that the pattern of resource provisioning in matrix sites influences host dispersal patterns, foraging behaviour and possibly disease dynamics by increasing contact rates. Thus, while habitat fragmentation can result in species decline along a fragmentation gradient, it can simultaneously create diverse, species-rich wildlife-human interfaces in the matrix with adequate populations to sustain disease transmission.

At the macro-scale, regions of high biodiversity are associated with the emergence of novel zoonotic infectious diseases (*166*). Moreover, the number of zoonotic mammalian hosts increases with species richness among mammals (*168, 266*). Yet, at the local scale increased host diversity leading to higher parasite diversity has, in some cases, been shown to decrease infection prevalence and pathology (*267, 268*). While we acknowledge that parasite diversity alone does not equate disease risk, we suggest that high host and viral diversity, paired with increasing human contact may increase the exposure of people to novel infectious agents from

wildlife, thereby influencing transmission rates (i.e. pathogen pool hypothesis (*6*)).

In this study, many of the captured bat species depend on fruits and nectars that are unevenly distributed and irregular throughout native forests (*269*). In the Atlantic Forest, the percentage of forest located more than 1 km from the forest edge has decreased in magnitude from 90% historically to less than 9% today (*2*). Given this decrease in native habitat, frugivorous bats are likely seeking alternative food sources in nearby human settlements, which provide abundant and reliable floral resources in areas with reduced canopy. Opportunities such as these may facilitate novel wildlife-human-domestic animal interactions and are likely to increase given the impacts of expanding human populations.

As such, we surmise that human behaviours that change the interplay between host species and the environment by altering foraging behaviours, the extent of host ranges and host community composition, will also likely affect the potential 'pool' of viruses. Given the growing threat of emerging viral diseases in the face of anthropogenic changes (e.g. climate change, habitat loss and fragmentation, urbanization), describing and cataloguing the viruses of wildlife is crucial, as well as understanding how viral assemblages vary in response to these changes. Ultimately, we must continue to decipher patterns in the virosphere to be able to predict the emergence of the next pandemic.

5 Discussion and Conclusions

In this thesis, we consider the role that habitat change and fragmentation play in structuring bat and viral communities, and influencing patterns of human-animal contact within changing tropical landscapes. In doing so, we argue that a better understanding of both the ecological and social factors that may drive zoonotic disease emergence is needed. Despite a large body of theoretical and empirical studies on established pathogens, relatively little is understood about the processes underlying disease emergence. As signaled by the burgeoning field of disease ecology, new approaches are required to investigate disease emergence, that shift focus from the pathogen to understanding the underlying mechanisms.

This thesis uses approaches from community ecology, virology and public health to identify the factors that structure host-virus assemblages. Here, we do not attempt to quantify zoonotic disease risk directly, rather we focus on whether viral and bat responses to habitat fragmentation are predictable at the assemblage level and provide evidence of the different types and rates of human contact with wildlife. Research outlined in Chapter 2 highlights how human-animal contact rates are predictable at the landscape level under land-use change, with higher levels of direct contact found in lower disturbance areas and higher levels of indirect contact, particularly with rodents, in more disturbed areas. While bat hunting for consumption and medicine is much less common in South America, compared to Africa, Asia and across Oceania (*139*), it does occur in highly localized areas and affects primarily species in the family *Phyllostomidae*. While we found no evidence for direct contact with bats via consumption or butchering, contact with bats via indirect contact (e.g. faeces in stored food and bite marks on fruit) occurred across the gradient with the highest rates in least disturbed

areas. Bats are hosts to some of the most significant emerging zoonoses, and transmission from bats to humans often occurs when bats abandon natural habitats to take advantage of resources associated with human settlements. We argue that although we found no evidence of direct bat-human contact, that the dual use of land to cultivate fruit, raise livestock, and establish human settlements may lead to increased bat-human encounters and increase disease transmission.

Further, we argue that land-use change will continue to influence the likelihood of disease spread through effects on human behaviour and human dispersal. In tropical regions, these changes are particularly intense where primary forest is being opened up logging, large-scale agriculture, and extractive industries (*57*). As discussed throughout this thesis, habitat fragmentation might increase the risk of zoonotic diseases by changing habitat and vector community composition, modifying the distribution of wildlife populations and domestic animals, and increasing exposure to known and novel pathogens through increased human contact with animals (*7*). Yet, habitat fragmentation is often also accompanied by processes such as road building that has been shown to increase human mobility (*112*), bringing new human populations into contact with wildlife through increased levels of hunting, and bushmeat consumption. Further, if new sites are poorly managed, increased populations can strain existing infrastructure, leading to overcrowding, poor sanitary conditions, and improper disposal of waste. Additionally, new human inhabitants might not have immunity to zoonotic diseases endemic to an area, making them particularly susceptible.

Chapter 3 showed that deforested sites had a less diverse bat community than forested sites, but higher viral prevalence and richness, suggesting that deforestation can result in reduced bat diversity and increased abundance of generalist synanthropic species, which host higher viral prevalence. Building on Chapter 3, Chapter 4 highlights how bat host community ecology is predictive of viral community ecology under land-use change, such that human activities in areas of high host diversity may result in novel exposure to a more diverse pool of viruses and an elevated risk of novel spillover. This final section focuses on how habitat fragmentation structures both bat and viral assemblages and identifies future research priorities.

Understanding the complex relationships among multi-host/multi-pathogen systems, environmental changes, and human populations presents a daunting challenge. Looking forward, research priorities will most certainly include developing integrative models that combine a better understanding of human ecology with host-pathogen ecology. These models will require empirical investigation that explicitly quantifies how cross-species tranmission rates between varying land-use types differ as a function of contact and relevant enviromental parameters (e.g. edge effects, area, etc). The key challenge will be the development of approaches that isolate each hypothesized variable. Even for single-pathogen systems (e.g. Lyme disease in the Northeastern USA), many years of detailed fieldwork were needed to develop and test hypotheses (*56, 61, 171*).

Further, to better characterize human-ecology in the landscape, additional methods are needed to validate the results outlined in Chapter 2. In Brazil, wildlife consumption is illegal except for rural subsistence; because of this, many of the households included in our

study are likely underreporting contact with certain species, particularly primates that are more highly protected. While the use of household surveys is a well-established method of assessing behaviour, recall error and biases represent a major concern to the validity of studies that rely on self-reported data (*144*). To account for this, we recommend future studies that utilize direct observation of human behaviour through the use of focal subject follows to characterize the true nature of human-animal contact. Focal subject follows involve observing a particular individual for up to a day at a time and recording the specific activity in which an individual is engaged (*270*). This method is the gold standard for assessing behavioural data and has previously been used by human ecologists interested in assessing foraging patterns (*271*) and human-animal interactions(*272, 273*). This method has also been used to assess potential pathogen transmission from humans to non-human primates (*274*).

Across the habitat fragmentation gradient, we suggest that the matrix sites located in areas of intermediate disturbance represent a critical point for potential cross-species transmission and emergence of pathogens into new host populations, due to increased novel interactions, paired with high host and viral diversity (i.e. higher zoonotic pool). Considering this, further characterization of the viruses detected and a better understanding of the nature of human-animal interactions is necessary to identify suitable interventions to mitigate the risk of disease emergence. To achieve this, these sites must be studied as complex, multi-host communities that are structured by both ecological and social factors.

Here, we show how viral diversity in bat host species (i.e. pathogen pool) varies across a habitat fragmentation gradient. Given the current rate of habitat change in tropical regions globally, and the potential for associated changes in wildlife communities and the pathogen communities they host, we recommend an interdisciplinary approach that collaborates among

disciplines (e.g. wildlife biology, microbiology, theoretical and applied ecology, veterinary medicine and the social sciences), but maintains a strong grounding in ecological approaches to field studies. These build on strong theoretical underpinnings in disease ecology, use the latest microbial diagnostic techniques, but infuse an understanding of human behaviour, complexity, and practical ecological approaches.

Further study of these coupled natural-human systems will provide clear implications for land-use and development policies, as well as wildlife conservation by developing ways to alter land management plans to minimize the risk of spillover, or developing prevention strategies to limit exposure for populations at risk. For example, as a result of our study the Municipal Health Secretary of Teodoro Sampaio (Secretaria Municipal de Saude) implemented a series of community workshops to discuss zoonotic disease awareness and potential mitigation strategies including (i) the potential establishment of a buffer zone around Morro do Diabo State Park, as well as a transition zone where future human settlements could be established; (ii) planting fruit orchards a minimum distance away from the household to minimize indirect bat-human contact; and (iii) housing livestock in ways to decrease wildlife-livestock contact. While a detailed discussion of control measures and intervention strategies is beyond the scope of this thesis, certain interventions could be implemented at the site level (i.e preventative action such as agricultural and husbandry practices, as detailed above; behavioral changes) or at the policy level (i.e. zoning, land-management).

Lastly, we argue that although bats are the known reservoirs of several recent notable zoonoses, they also comprise a critical element of all terrestrial ecosystems. Bats are worth billions of dollars annually to human economies (*275*) and play key roles in the health of

ecosystems upon which we depend (*276*). They help to control many agricultural pests, pollinate numerous plants and disperse seeds, reseed cut forests, and even their guano is a valuable commodity as a fertiliser (*276*). Instead of blaming bats as 'disease-carrying' reservoirs of EIDs, simple public health measures could be implemented to protect against potential health threats including altering land management plans to minimize human-bat contact and education initiatives to educate the public about bats, zoonotic diseases and ecosystem services that they provide. The intention of this research is not to stoke fears about the possibility that bats might cause future pandemics, but rather provide evidence to show how human disturbances to the environment, such as habitat fragmentation, can lead to predictable changes in viral communities and patterns of human-animal contact.

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Supplementary materials

Supplementary Table 1:

Region	Latin name	Common name	IUCN status
Amazonia	*Agouti paca*	Lowland Paca	LC
	Priodontes maximus	Giant Armadillo	VU
	Dasypus novemcinctus	Nine-Banded Armadillo	LC
	Dasyprocta leporina	Red-rumped agouti	LC
	Tayassu pecari	White-lipped peccary	VU
	Mazama spp	Brocket deer	DD
	Hydrochoerus hydrochaeris	Capybara	LC
	Monkey (no id)	Monkey (no id)	
	Caiman crocodilus	Spectacled caiman	LC
	Chelonoidis spp	Tortoise spp	VU
	Tinamus spp	Tinamou spp	NT, VU
	Tapirus terrestris	Lowland tapir	VU
	Podocnemis unifilis	Yellow-spotted river turtle	VU
	Didelphis albiventris	White-eared oppossum	LC
	Currasow (no id)	Currasow spp.	LC, EN
	Penelope spp	Penelope spp	LC, VU
	Coloepus didactylus	Two-toed sloth	LC
	Amazona spp	Parrot spp.	LC, NT, VU
Atlantic Forest	*Dasypus novemcinctus*	Nine-Banded Armadillo	LC
	Hydrochoerus hydrochaeris	Capybara	LC
	Pecari tajacu	Collared peccary	LC
	Caiman latirostris	Broad-snouted caiman	LC
	Mazama spp	Brocket deer	DD
	Dasyprocta azarae	Azara's agouti	DD
	Tapirus terrestris	Lowland tapir	VU
	Agouti paca	Lowland Paca	LC

	Crypturellus tataupa	*Tataupa* tinamou	LC
	Penelope spp	Penelope spp	LC, VU
	Tinamus solitarius	Solitary tinamou	NT
	Tupinambis spp.	Lizard spp	
	Snakes (no id)	Snakes	
	Frogs (no id)	Frogs	
	Sus scrofa	Wild boar	LC
	Monkey (no id)	Monkey spp	
	Tamandua tetradactyla	Collared anteater	LC

Supplementary Table 2: A summary of the consensus PCR assays used in this study (n=9). Table intended to be used for quick ('at-a-glance') review. Detailed description of all assays is given in the Supplementary Methods.

Assay	Family/Genus	Target	Reference	Amplicon(bp)	PCR Enzyme	Control
P-001	Astrovirus	RdRp	Atkins	~342	QIAGEN Fast Cycle Chemistry	UC2
P-002	Astrovirus	RdRp	Chu	421	QIAGEN Fast Cycle Chemistry	UC2
P-005	Coronavirus	RdRp	Quan	~328	QIAGEN Fast Cycle Chemistry	UC1
P-006	Coronavirus	RdRp	Watanabe	434	QIAGEN Fast Cycle Chemistry	UC1
P-031	Herpesvirus	Polymerase	Van de Vante	215-315	QIAGEN Fast Cycle Chemistry	UC2
P-032	Herpesvirus	Terminase	Chmielewicz	419	QIAGEN Fast Cycle Chemistry	UC2
P-025	Influenza A	M	Anthony	~240	AmpliTaq Gold 360	UC2
P-026	Influenza A	PB1	Liang	422	QIAGEN Fast Cycle Chemistry	Influenza cDNA
P-014	Paramyxovirus	POL	Tong	~561	QIAGEN Fast Cycle Chemistry	UC1

Supplementary methods

Consensus PCR (cPCR) Assays

Below is a detailed description of all cPCR assays used in this study, accompanied by anecdotal notes that may be useful to investigators wishing to use these methods for their own purposes.

RNA Virus Protocols:

P-001 Astroviruses

REFERENCE: Atkins, A. et al (2009)
TARGET: RNA-Dependent RNA Polymerase (RdRp)
CONTROL: Universal Control 2 (UC2)
ENZYME: QIAGEN Fast Cycling PCR Kit (Cat. No. 203745)

	Primers	Protocol	Amplicon
Round 1	Astr4380F-GAYTGGRCNCGNTWYGATGGNACIAT	95°C 5 min, then 45 cycles of 96°C for 8 sec, 45°C for 8 sec and 68°C for 15 sec. Finish with 72°C for 3 min	~431 bp
	Astr4811R-GGYTTNACCCACATNCCAAA		
Round 2	Astr4380F Same as for first round	95°C 5 min, then 45 cycles of 96°C for 8 sec, 45°C for 8 sec and 68°C for 12 sec. Finish with 72°C for 3 min	~342 bp (variable)
	Astr4722R-ARNCKRTCATCNCCATA		

NOTES: This assay works well to detect novel astroviruses, however investigators should be aware that it does cross-react fairly readily and often produces false-positive bands of the expected size. Sequencing will be required to rule out false positives.

P-005 Astroviruses

REFERENCE: Chu, D.K.W. et al (2008)
TARGET: RdRp
CONTROL: UC2
ENZYME: QIAGEN Fast Cycling PCR Kit (Cat. No. 203745)

	Primers	Protocol	Amplicon
Round 1	AstroFWD1;GARTTYGATTGGRCKCGKTAYGA	95°C 5 min, then 40 cycles of 96°C for 5 sec, 50°C for 8 sec and 68°C for 15 sec. Finish with 72°C for 2 min	~436 bp
	AstroFWD2-GARTTYGATTGGRCKAGGTAYGA		
	AstroRVS1-GGYTTKACCCACATNCCRAA		
Round 2	AstroFWD3-CGKTAYGATGGKACKATHCC	Same protocol for both rounds	~421 bp
	AstroFWD4-AGGTAYGATGGKACKATH CC		
	AstroRVS1 Same as for first round		

NOTES: None

P-005 Coronaviruses

REFERENCE: Quan, P.L. et al (2010)
TARGET: RdRp
CONTROL: UC1
ENZYME: QIAGEN Fast Cycling PCR Kit (Cat. No. 203745)

	Primers	Protocol	Amplicon
Round 1	CoV-FWD1; CGTTGGIACWAAYBTVCCWYTICARBTRGG CoVRVS1;GGTCATKATAGCRTCAVMASWWGCNACATG	95°C 5 min, then 14 cycles of 96°C for 5 sec, 65°C (-1°C per cycle) for 8 sec and 68°C for 18 sec, the 35 cycles of 96°C for 5 sec, 50°C for 8 sec and 68°C for 18 sec. Finish with 72°C for 3 min	~520 bp
Round 2	CoV-FWD2;GGCWCCWCCHGGNGARCAATT CoV-RVS2;GGWAWCCCCAYTGYTGWAYRTC	95°C 5 min, then 14 cycles of 96°C for 5 sec, 65°C (-1°C per cycle) for 8 sec and 68°C for 12 sec, the 35 cycles of 96°C for 5 sec, 50°C for 8 sec and 68°C for 12 sec. Finish with 72°C for 3 min	~328 bp

NOTES: This assay has been used to identify a large diversity of novel coronaviruses in different species.

P-006 Coronaviruses

REFERENCE: Modified from Watanabe, S. et al (2010)
TARGET: RdRp
CONTROL: UC1
ENZYME: QIAGEN Fast Cycling PCR Kit (Cat. No. 203745)

	Primers	Protocol	Amplicon
Round 1	CoV-FWD3;GGTTGGGAYTAYCCHAARTGTGA CoV-RVS3;CCATCATCASWYRAATCATCATA	95°C 5 min, then 15 cycles 96°C for 5 sec, 65°C (-1°C per cycle) for 8 sec and 68°C for 15 sec, then 35 cycles of 96°C for 5 sec, 50°C for 8 sec and 68°C for 15 sec. Finish with 72°C for 3 min	440 bp
Round 2	CoV-FWD4/Bat;GAYTAYCCHAARTGTGAYAGAGC CoV-FWD4/Other; GAYTAYCCHAARTGTGAUMGWGC CoV-RVS3 Same reverse primer as round 1	Same protocol for both rounds	434 bp

P-014 Paramyxoviruses

REFERENCE: Tong, S. et al, (2008)
TARGET: RdRp
CONTROL: UC1
ENZYME: QIAGEN Fast Cycling PCR Kit (Cat. No. 203745)

	Primers	Protocol	Amplicon
Round 1	PAR-F1;GAAGGITATTGTCAIAARNTNTGGAC	95°C 5 min, then 40 cycles of 96°C for 5 sec, 48°C for 8 sec and 68°C for 20 sec. Finish with 72°C for 4 min	~639 bp
	PAR-R;GCTGAAGTTACIGGITCICCDATRTTNC		
Round 2	PAR-F2;GTTGCTTCAATGGTTCARGGNGAYAA	Same for both rounds	~561 bp
	PAR-R Same Reverse primer as round 1		

NOTES: The paper provides good validation of the primers against various paramyxoviruses, but when applied to clinical samples this assay can cross-react with host sequences quite readily. It has been observed that this is a particular problem with bat samples. When a paramyxovirus is present in a sample, the assay does seem to work well, and (mostly) amplifies a single and specific product, with very little non-specific amplification. Investigators should therefore proceed cautiously with any sample that looks to have products that are close to the right size, but which also have a high background of non-specific amplification. It is probable that such samples are in fact negative.

P-025 Influenza A Viruses

REFERENCE: Anthony, S.J. et al (2012)
TARGET: Matrix
CONTROLS: UC2
ENZYME: AmpliTaq Gold 360 Master Mix (Applied Biosystems Cat. No. 439881)

	Primers	Protocol	Amplicon
Round 1	FLUAV-MU44;GTCTTCTAACCGAGGTCGAAACG	95°C for 10 mins, then 14 cycles of 95°C for 30 sec, 65°C (-1°C/cycle) for 30 sec, 72°C for 1 min. Then 35 cycles of 95°C for 30 sec, 50°C for 30 sec, 72°C for 1 min. Final extension of 72°C for 7 min	~240 bp
	FLUAV-M-L287;GCATTTTGGACAAAGCGTCTACG		

NOTES: None

P-026 Influenza A Viruses

REFERENCE: Liang, E. Unpublished. Developed at CII.
TARGET: PB1
CONTROL: Influenza A cDNA. Not included in either UC1 or 2
ENZYME: QIAGEN Fast Cycling PCR Kit (Cat. No. 203745)

	Primers	Protocol	Amplicon
Round 1	FLUAPB1-F;ATGATGATGGGNATGTTYAAYATG	95°C for 5 min, then 14 cycles of 96°C for 5 sec, 65°C for 8 sec (-1°C /cycle) and 68°C for 15 sec. Then perform 35 cycles of 96°C for 5 sec, 50°C for 8 sec and 68°C for 15 sec. Finish with 72°C for 4 min	~407 bp
	FLUAPB1-R;GCNGGNCCNAKDTCRYTRTTDATCAT		

Round 2	FLUAPB1-NF;GATGGGNATGTTYAAYATGYTDAGYAC	Same protocol for both rounds	402 bp
	FLUAPB1-R Same reverse primer as Round 1		

NOTES: This assay was designed to be more degenerate than P-025 above. It was developed to capture the highly diverse bat influenza viruses (recently discovered) in addition to all 'classic' influenza A viruses. While a nested assay is presented here, we have to date not observed any increased sensitivity from the nested round.

DNA Virus Protocols:

P-031 Herpesviruses

REFERENCE: Van DeVanter, D.R. et al (1996)
TARGET: Polymerase
CONTROL: UC2
ENZYME: QIAGEN Fast Cycling PCR Kit (Cat. No. 203745)

	Primers	Protocol	Amplicon
Round 1	DFA;GAYTTYGCNAGYYTNTAYCC	95°C 5 min, then 45 cycles of 96°C for 5 sec, 46°C for 8 sec and 68°C for 12 sec. Finish with 72°C for 2 min	-
	KG1;GTCTTGCTCACCAGNTCNACNCCYTT		
	ILK;TCCTGGACAAGCAGCARNYSGCNMTNAA		
Round 2	TGV;TGTAACTCGGTGTAYGGNTTYACNGGNGT	Same protocol for both rounds	215-315 bp
	IYG;CACAGAGTCCGTRTCNCCRTADAT		

P-032 Herpesviruses

REFERENCE: Chmielewicz, B. et al (2001)
TARGET: Terminase
CONTROL: UC2
ENZYME: QIAGEN Fast Cycling PCR Kit (Cat. No. 203745)

	Primers	Protocol	Amplicon
Round 1	TS-TERM_707s;TTGTGGACGAGRSIMAYTTYAT	95°C 5 min, then 45 cycles of 96°C for 5 sec, 46°C for 8 sec and 68°C for 12 sec. Finish with 72°C for 2 min	519 bp
	TS-TERM_707as;ACAGCCACGCCNGTICCIGAIGC		
Round 2	TS-TERM_708s;GCAAGATCATNTTYRTITCITC	Same protocol for both rounds	419 bp
	TS-TERM_708as;TGTTGGTCGTRWAIGCIGGRT		

Appendix I Network analyses of bat-virus assemblages in the Atlantic Forests of Brazil

Methods: A presence/absence matrix was constructed to show the distribution of viral sequence clusters across all bat host species. Using the R package igraph, a network model was constructed, connecting bat species to all viral clusters that were identified within that species in our data. The network is made up of 19 connected components, 31 viral clusters (75 viral nodes), 22 bat species (# bat nodes), and 862 edges. Networks were plotted using Gephi using the force-directed algorithm ForceAtlas2. Specifically, we plotted a two-mode affiliation network demonstrating the link between viruses and their hosts at the genus level.

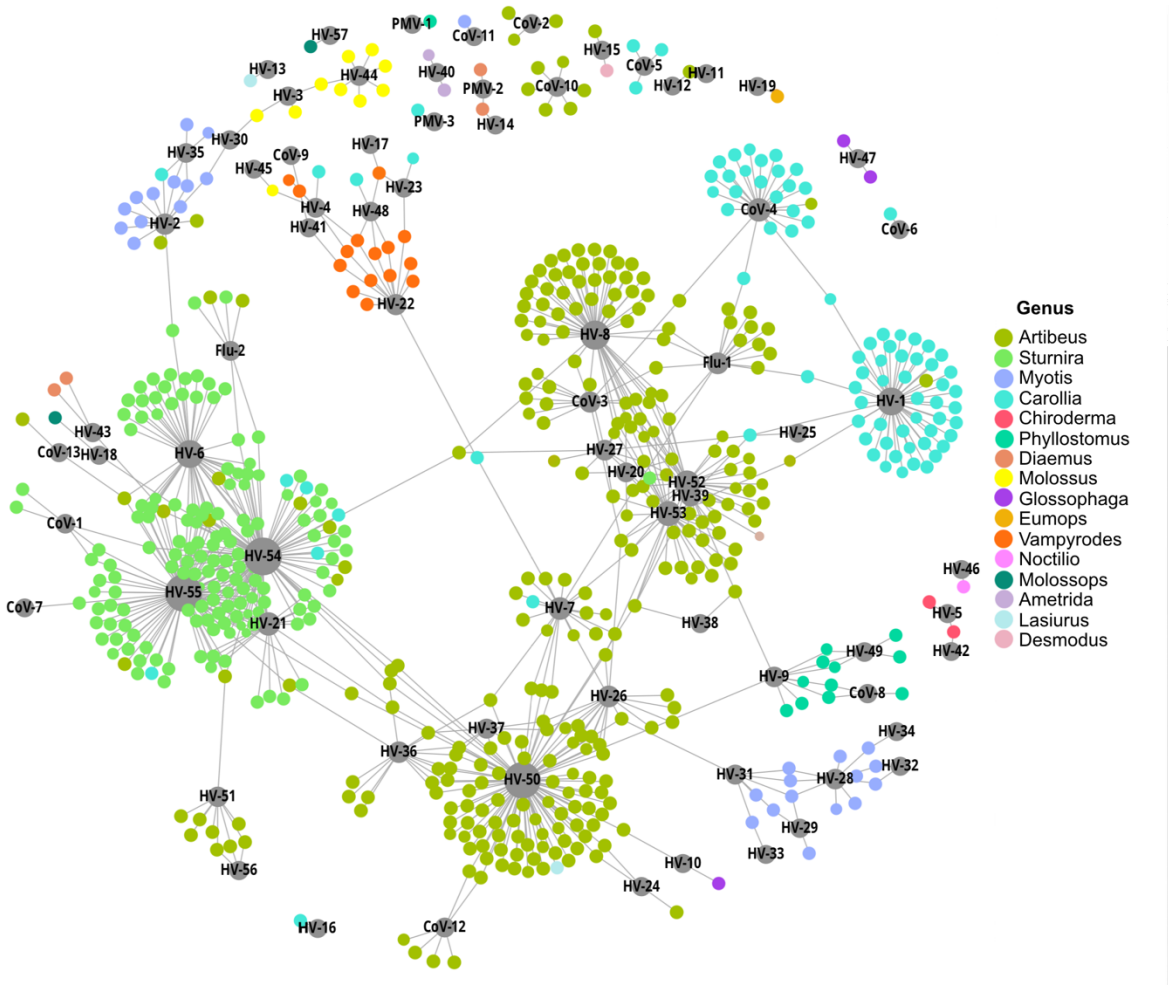


Figure 1: Two-mode affiliation network demonstrating the link between viruses and their hosts. Viruses are shaded grey. Size of node indicates abundance. Coloured nodes indicate individual bats, coloured by genera.

Results: A two-mode affiliation network was used to illustrate the connectivity between viruses and their hosts. This revealed a dominant (though not exclusive) pattern of genera-specific diversity consistent with determinism. We then performed a Mantel test comparing the bat distance matrix to a geographical distance matrix and then the virus matrix to the geographical distance matrix. We found that the bat matrix was not related to spatial distance ($r = -0.089$, $p = 0.788$), but the virus matrix was ($r = 0.190$, $p = 0.046$), suggesting that these non-random patterns in viral communities may be emerging due to dispersal limitation.

PESQUISA DE EXPOSIÇÃO DE DOMICÍLIO IDRC
PARA USO COM HOMENS E MULHERES

SEÇÃO 1 – Formulário de identificação, triagem e consentimento de questionário

Q 001 Número de identificação de questionário ☐☐☐☐

Q 002 Bairro

Q 003 Nome e código da cidade ☐☐☐

Q 004 Data da entrevista ___ / ___ / ___

Q 005 Nome e código do entrevistador ☐☐☐☐

Q 006 Nome e código do supervisor ☐☐☐☐

Q 007 Grupo de estudo 1 3
2 4

Q 008 Tipo de amostra 1 Homem 2 Mulher

Q 009 Número do domicílio ☐☐☐

Q 010 Alguma pessoa (do grupo de estudo) mora neste domicílio?
Sim 1 – *Continue*
Não 2 – *Agradeça a pessoa; comece a contar o próximo intervalo*

Q 011 Quantos (grupo de estudo) que vivem neste domicílio têm entre 18 e 50 anos de idade? Vamos trocar os objetos amostrais e deixar só grupo de estudo.

☐☐

Se 0, agradeça a pessoa, comece a contar o próximo intervalo

Entrevistador(a): coloque o numero

Entrevistado(a) # ☐☐

Q 012 {Nome do(a) entrevistado(a)} está disponível agora? Se não estiver disponível, marque um horário para voltar para conversar com ele(a).

Data marcada: __ / __

Horário marcado: __ : __ AM PM circule

Questionário número ☐☐☐☐ Horário de início: __ : __

Por favor, **entrevistador(a),** descreva os animais que estão no entorno da casa e sua interação (se estão juntos ou separados por cercas ou jaulas) *Essa questão deve ser respondida pelo entrevistador .

SEÇÃO 2: Características de domicílio e respondente

Q 201 Sexo do respondente (observe)
Masculino 1
Feminino 2

Q 202 Qual seu nome?

Q 203 Qual sua idade? ☐☐

Q 204a Há quanto tempo você mora continuamente em _____ (bairro e interior)? Utilizar bairro e interior (qual?)

Sempre 1 - *Vá para Q206*

Número de anos 2 ☐☐ - *Continue*

Ou

Número de meses ☐☐ - *Continue*

Visitante 3 - ***PARE***

Q 204b Imediatamente antes de se mudar para cá, você morava (leia as opções)
No campo 2
Em um vilarejo 3
Na cidade 4
Em outro país

Outro _____ ☐☐

Q 204c Quanto tempo você morou naquele lugar?

Número de anos 2 ☐☐

Ou

Número de meses ☐☐

Q 205a Qual é a principal língua falada na sua casa? Boa pergunta. Quem sabe aparece alguma língua indígena.

Que outras línguas você fala? (*Múltiplas respostas possíveis*)

Língua materna (resposta única)	Outra (múltiplas respostas)		
1	1		
2	2		
3	3		
Outra			

Q 205b Qual é a renda da família?

SEÇÃO 3: Animais de criação

Agora eu gostaria de conversar sobre os animais que vocês criam atualmente. Procurei marcar o " você " para que utilizemos sempre no plural, assim damos dimensão que as perguntas mesmo sendo respondidas por um representante da casa pode expressar a visão de todos. Acredito que não correremos o risco de perder informações relevantes, dado que a pessoa não confundirá a pergunta dirigida a ela como sendo restritiva.

Q 301a Primeiramente, que animais você ou outras pessoas que moram aqui criam atualmente – como fonte de renda, para comida, para trabalho ou como animais de companhia?
Explore: **Que outros animais?** (*Escreva o nome do animal na primeira coluna e circule 1 na coluna "espontânea"; para animais chave, apenas circule 1*)

Q 301b Pergunte para cada animal chave que não tenha sido mencionado: **Você cria (nome do animal chave) atualmente?** (*Circule 2 para Sim, 3 para Não na coluna "Direcionada"*)

Para galinhas, patos e cada tipo de animal criado, pergunte Q 302 a – e, animal por animal:

Q 302a Por volta de quantos (nome do animal) você possui atualmente? *(Escreva o número na coluna 5*)

Q302b Nos últimos 12 meses; ou seja, desde (mês) do ano passado, por quantos meses você manteve ao menos um (nome do animal)? (*Escreva o número na coluna 6*)

Q 302c Você é a pessoa primariamente responsável por cuidar destes (nome do animal)? *(Circule 1 para **Sim**, 2 para **Não** na coluna 7)*

Q 302d Com que frequência um dos (nome do animal) entra na casa? É todo dia, algumas vezes por semana, algumas vezes por mês, apenas uma vez nos últimos 12 meses*? (Escreva o código da resposta na coluna 8)*

Q 302e Com que frequência alguém da casa foi mordido(a) ou arranhado(a) a ponto de sangrar por um dos (nome do animal) nos últimos 12 meses? *(Leia as frequências e*

escreva a resposta na coluna 9) Essas perguntas podem incluir não só a pessoa entrevistada, mas alguém da família ou morador da casa, ok?!

Q 301				Q302a	Q302b	Q302c	Q302d	Q302e
Animais que possui atualmente	Espontânea	Direcionada		Quantos (nome do animal) você possui atualmente?	Nos últimos 12 meses, por quantos meses você possuiu ao menos um (nome do animal)?	É você mesmo o responsável por cuidar destes (nome do animal)?	Com que frequência o(a) (animal) entra na sua casa?	Com que frequência você foi mordido/ arranhado por (animal)?
							Todo dia 1 Algumas vezes /semana 2 Algumas vezes / mês 3 Algumas vezes / ano 4 Uma vez nos últimos 12 meses 5 Não nos 12 meses passados 6	
Nome do animal		S	N			Sim Não		
Pergunte apenas para galinhas, patos e mamíferos								
[galinhas]	1	2	3			1 2		
[ovinos]	1	2	3			1 2		
[suínos]	1	2	3			1 2		
Bovinos	1	2	3			1 2		
Felinos	1	2	3			1 2		
Caninos	1	2	3			1 2		
Equinos	1	2	3			1 2		
Patos	1	2	3			1 2		
	1	2	3			1 2		

Agora eu gostaria de falar sobre os animais que você não cria atualmente mas já criou no último ano.

Q303a Quais animais vocês criaram nos últimos 12 meses; ou seja desde (mês) do ano passado? As perguntas direcionadas ao entrevistado pode abranger outros moradores da residência. Por vezes a pessoa entende que ela não tem animal, mas alguém da casa pode ter.
(Escreva os nomes dos animais na primeira coluna e circule 1 na coluna "espontânea"; para animais chave, apenas circule 1)

Q 303b *Se galinhas, patos ou porcos não forem criados atualmente e não forem mencionados, pergunte para cada animal:* **Você criou [galinhas/patos/porcos] por qualquer período nos últimos 12 meses?**
(*Circule 2 para **Sim**, 3 para **Não** na coluna "Direcionada")*

Se a coluna 1 estiver vazia e apenas 3 estiver circulado sob "Direcionada" – Pule para Seção 4

Para galinhas, patos e cada mamífero mencionado, pergunte Q304a – e, animal por animal:

Q 304a Durante o período que vocês criavam (nome do animal), cerca de quantos (nome do animal) vocês possuiam? *(Escreva o número na coluna 5)* Abranger a pergunta para demais moradores.

Q 304b Nos últimos 12 meses; ou seja, desde o último mês de (nome do mês), por quantos meses vocês possuíram ao menos um(a) (nome do animal)? (*Escreva o número na coluna 6)*

Q 304c Era você mesmo a pessoa responsável por cuidar destes(as) (nome do animal)? *(Circule 1 para **Sim**, 2 para **Não** na coluna 7)*

Q 304d Durante o período que vocês criaram (nome do animal), com que frequência um dos(as) (nome do animal) entrou na casa*? (Leia as freqüências e escreva o código de resposta na coluna 8)*

Q 304e Durante o período que vocês criaram (nome do animal), com qual frequência alguém foi mordido(a) ou arranhado(a) a ponto de sangrar por qualquer um dos(as) (nome do animal)? *(Leia as freqüências e escreva o código de resposta na coluna 8)*

Q 303 a-b				Q304a	Q304b	Q304c	Q304d	Q302e
Animais que possui não agora; mas nos 12 últimos meses	Espontânea	Direcionada		Quantos (nome do animal) você possuía?	Nos últimos 12 meses, por quantos meses você possuiu ao menos um (nome do animal)?	Era você mesmo o responsável por cuidar destes (nome do animal)?	Com que frequência o(a) (animal) entrou na sua casa?	Com que frequência você foi mordido/ arranhado por (animal)?
							Todo dia 1 Algumas vezes /semana 2 Algumas vezes / mês 3 Algumas vezes / ano 4 Uma vez 5 Não nos 12 meses passados 6	
Nome do animal		S	N			Sim Não		
Pergunte apenas para galinhas, patos e mamíferos								
[galinhas]	1	2	3			1 2		
[bovinos]	1	2	3			1 2		
[suínos]	1	2	3			1 2		
Equinos	1	2	3			1 2		
Caprinos	1	2	3			1 2		
Ovinos	1	2	3			1 2		
	1	2	3			1 2		
	1	2	3			1 2		
	1	2	3			1 2		

SEÇÃO 4: Outros animais (não criados) que entram na casa

Agora eu gostaria de falar sobre outros animais, aqueles que vocês não criam e entram na sua casa. No caso dessas perguntas com você, podemos considerar vocês como demais moradores da residência.

Há muitas maneiras pelas quais vocês podem saber que outros animais entraram na sua casa: vocês podem vê-los, sentir seu cheiro ou ouvi-los. Vocês também podem ver animais mortos.

Os animais também deixam evidências de que estiveram por perto – podem ser encontradas fezes, roupas roídas ou outros objetos ou sinais de que os animais levaram ou comeram alimentos que vocês comem.

Q401a. Pensando em todas essas formas você afirmaria que outros animais entraram na sua casa durante os últimos 12 meses; ou seja, desde (mês) do ano passado?
Explore: **Que outros animais?** (*Escreva o(s) nome(s) do(s) animal(is) na primeira coluna e circule 1 na coluna "espontânea"; para animais chave, apenas circule 1).*

Q401b. Pergunte para cada (animal chave) que não tenha sido mencionado:
Nos últimos 12 meses vocês viram, ouviram ou sentiram o cheiro de (animal chave) ou notaram sinais de que qualquer tipo de [roedores] na casa*? (Circule 1 para **Sim**, 3 para **Não** na coluna "Direcionada")*

Se nenhum animal for mencionado nas questões Q 401a – b, pule para Q403

Obrigado(a). Agora peço que me conte como vocês souberam que estes animais estiveram na sua casa e com que frequência vocês viram cada um destes sinais

Q 402a Para cada mamífero listado, pergunte:

i. Com que frequência vocês notaram (nome do animal) morto(a) na sua casa nos últimos 12 meses; ou seja, desde (mês) do ano passado*? (Leia as frequências e escreva o código de resposta na coluna 5)*

ii. (*Se uma vez ou mais*) Com que frequência vocês notaram (nome do animal) morto(a)(s) nos alimentos e bebidas da sua família? *(Leia as frequências e escreva o código de resposta na coluna 6)*
Após passar pela lista, pergunte:

iii. Nos últimos 12 meses; ou seja, desde (mês) do ano passado, que outros animais mortos vocês viram na sua casa, pelo seu sítio ou quintal? (*Adicione os nomes dos animais à lista; para cada animal, faça Q 402a i-ii)*

Q 402b *Para cada mamífero citado, pergunte:*
i.Com que frequência vocês notaram fezes ou urina de (nome do animal) em sua casa nos últimos 12 meses?
(Leia as frequências e escreva o código de resposta na coluna 7)
ii. (S*e uma ou mais vezes*) Com que frequência vocês notaram fezes ou urina de (nome do animal) em alimentos ou bebidas da sua família? (*Leia as frequências e escreva o código de resposta na coluna 8)*
Após passar pela lista, pergunte:
iii. Nos últimos 12 meses; ou seja, desde (mês) do ano passado, fezes ou urina de que outros animais vocês notaram na sua casa, quintal ou sítio? (*Coloque os nomes dos animais na lista; para cada mamífero pergunte Q402i-ii)*

Q 402c *Para cada mamífero citado, pergunte*: Uma dúvida minha, como vamos fazer essa diferenciação entre os animais? O que é um mamífero que deva ser considerado? Como eu e as demais estudantes saberemos distinguir?

i. Com que frequência nos últimos 12 meses o(a) (nome do animal) roeu ou danificou objetos, tais como roupas, materiais ou roupas de cama? (*Leia freqüências e escreva o código de resposta na coluna 9*)

Após passar pela lista, pergunte:
ii. Nos últimos 12 meses; ou seja, desde (mês) do ano passado, que outros animais roeram ou danificaram objetos, tais como roupas, materiais ou roupas de cama? (*Adicione os nomes dos animais à lista; para cada mamífero pergunte Q 402e i)*

Q 402d *Para cada mamífero citado, pergunte:*
i. Com que frequência nos últimos 12 meses vocês perceberam que (nome do animal) roubaram ou comeram alimentos ou grãos ou outros alimentos consumidos por sua família? (*Leia as frequências e anote o código de resposta na coluna 10)*

Após passar pela lista, pergunte:
ii. Nos últimos 12 meses; ou seja, desde (mês) do ano passado, que outros animais roubaram ou comeram alimentos ou grãos ou outros itens de alimentação da sua família? (Adicione os nomes dos animais à lista; para cada mamífero, pergunte Q 402d i)

Q 402e *Para cada mamífero citado, pergunte:*
i. Com que frequência vocês vêem, ouvem ou sentem cheiro de (nome do animal) nos últimos 12 meses? (*Leia freqüências e anote o código de resposta na última coluna)*

Após terminar a lista, pergunte:

ii. Nos últimos 12 meses, que outros animais vocês sabiam estar em casa porque viam, ouviam ou sentiam o cheiro deles? *(Adicione os nomes dos animais à lista; para cada mamífero pergunte Q 402e – i)*

Q 401a-b				Q402a		Q402b		Q402c	Q402d	Q402e
Que outros animais entraram na sua casa?	Espontânea	Direcionada		Percebeu animal morto		Percebeu fezes/urina		Percebeu objetos roídos	Percebeu alimentos ou grãos comidos	Viu, ouviu ou sentiu cheiro
				Dentro de casa	Na comida, água	Dentro de casa	Na comida, água			
Nome do animal		S	N	Todo dia 1 Algumas vezes /semana 2 Algumas vezes / mês 3 Algumas vezes / ano 4 Uma vez nos últimos 12 meses 5 Nunca 6						
Pergunte apenas para mamíferos										
[roedores]	1	2	3							
[morcegos]	1	2	3							
[primatas]	1	2	3							
baratas	1	2	3							
	1	2	3							
	1	2	3							
	1	2	3							
	1	2	3							
	1	2	3							

Q 403 Pensando nos últimos 12 meses; ou seja, desde (mês) do ano passado, com que frequência vocês notaram fezes/excrementos deixados por animais em alimentos ou cereais que sua família consome? *(Leia as respostas*)

Todo dia	1
Algumas vezes por semana	2
Algumas vezes por mês	3
Algumas vezes por ano	4
Apenas uma vez nos últimos 12 meses	5
Nunca	6 – *Pule para Q 405*
Não sei	9 – *Pule para Q 405*

Q 404 Se for o caso, o que você faz quando isso acontece?

Recolho as fezes	1	
Jogo fora os cereais/alimentos	2	
Outro	☐	☐

Q 405 Nos últimos 12 meses, com que frequência vocês varreram ou limparam fezes/excrementos deixados por animais em sua casa ou outros lugares?

Todo dia	1
Algumas vezes por semana	2
Algumas vezes por mês	3
Algumas vezes por ano	4
Apenas uma vez nos últimos 12 meses	5
Nunca	6 – *Pule para Q 407*
Não sei	9 – *Pule para Q 407*

Q 406 Quando vocês fazem isso com que frequência há poeira sobre vocês ou que vocês possam inalar?

Sempre	1
Algumas vezes	2
Nunca	3

Agora eu gostaria de fazer algumas perguntas sobre o que vocês podem fazer para evitar problemas com animais.

Q 407 Se é este o caso, o que vocês fazem para impedir que (roedores) entrem em sua casa?
Explore: **Algo mais?** *(NÃO leia as respostas*)

	Sim	Não
Uso armadilhas/ratoeiras	1	2
Uso veneno	1	2
Crio gatos	1	2
Mantenho a casa limpa	1	2
Vedo a casa	1	2
Nada	1	2
Outro		

Q 408 Se é este o caso, o que vocês fazem para impedir que [roedores] comam alimentos da sua casa ou áreas de estocagem? (*NÃO leia as respostas*)
Explore: **Algo mais?**

	Sim	Não
Uso armadilhas/ratoeiras	1	2
Uso veneno	1	2
Crio gatos	1	2
Mantenho janelas e portas fechadas	1	2
Mantenho comida em lugares fechados	1	2
Nada	1	2
Outro		

Q 409 Se é este o caso, o que vocês faz para impedir que [morcegos] entrem em sua casa?
Explore: **Algo mais?** (*NÃO leia as respostas*)

	Sim	Não
Uso armadilhas para morcegos	1	2
Fecho as portas	1	2
Espanto os animais	1	2
Mantenho janelas e portas fechadas	1	2
Mantenho comida em lugares fechados	1	2
Nada	1	2
Outro		

Q 410 Morcegos abrigam-se nas árvores próximas a sua casa?

Sim	1
Não	2

Q 411 Se é este o caso, o que vocês fazem para impedir que [morcegos] abriguem-se nas árvores próximas a sua casa?
Explore: Algo mais? *(NÃO leia as respostas)*

	Sim	Não
Espanto os animais	1	2
Atiro neles	1	2
Nada	1	2
Outro		

SEÇÃO 5: Animais perto de jardins/plantações

Agora eu gostaria de falar sobre os alimentos que você planta, seja uma plantação comercial, um pomar, um jardim ou mesmo uma pequena horta. Podemos especificar que a horta é um item importante, dado que por estar em área urbana teremos dificuldade de encontrar plantação. Caso a pessoa não plante nada, pularemos essa sequencia de perguntas?

Q 501 Você mesmo(a) cultiva a(o) [plantação principal]: *Leia as opções:* Você prepara a terra, cuida das plantas em crescimento, faz a colheita, algo mais? *(Circule o código da resposta para cada um que se aplique)*

	Sim	Não	
Preparo da terra	1	2	
Plantio	1	2	
Cuidado com as plantas	1	2	
Colheita			
Envolvimento com plantações	1	2	*- Pule para Q 506*

Há diferentes maneiras pelas quais você pode notar animais enquanto cuida de plantações; por exemplo, você poderia ver o animal mesmo, notar fezes do animal ou você poderia notar que um animal destruiu ou danificou as plantas.

Q 502a Pensando nestas maneiras, você pode me dizer que animais você mesmo(a) notou durante os últimos 12 meses; ou seja, desde (mês) do ano passado enquanto cuidava da plantação?

Explore: **Que outros animais?** (*Anote o nome do animal na primeira coluna da tabela abaixo e circule 1 em "Espontânea")*

Q 502b Pergunte para cada animal chave que não tenha sido mencionado: Alguma vez você mesmo(a) encontrou nos últimos 12 meses algum(a) (nome do animal) enquanto cuidava da plantação?
*(Circule 2 para **Sim**, 3 para **Não** na coluna "Direcionada")*

Se as respostas direcionadas são todas "3" e nenhum outro animal for citado, pule para Q 506

Q 503 i *Para cada mamífero chave não mencionado, pergunte:* Nos últimos 12 meses, com que frequência você notou fezes de (nome do animal) enquanto cuidava da plantação*? (Leia as frequências e anote o código da resposta na coluna 5)*

Depois que você tiver terminado toda a lista de animais, pergunte:
ii. Nos últimos 12 meses você notou as fezes de quaisquer outros animais enquanto cuidava da plantação?

*(Se **Sim**, adicione o nome do(s) animal(is) à lista e pergunte Q 503 i (com que frequência))*

Q 504 i *Para cada mamífero mencionado, pergunte:* Nos últimos 12 meses com que frequência você notou ninhos de (nome do animal) enquanto trabalhava na plantação? *(Leia as frequências e anote o código da resposta na coluna 6)*

Depois que você tiver terminado toda a lista de animais, pergunte:
ii. Nos últimos 12 meses você notou ninhos de quaisquer outros animais?
*(Se **Sim**, adicione o nome do(s) animal(is) à lista e pergunte Q 504 i (com que frequência))*

Q 505 i Nos últimos 12 meses, com que frequência você notou danos ou destruição das plantações devido a (nome do animal)?
(*Leia as frequências e anote o código da reposta na coluna 7)*

Q 505 ii Que plantações (nome do animal) danificou ou destruiu? (*Anote a resposta na última coluna*)
Após passar por toda a lista de animais, pergunte:
Nos últimos 12 meses, algum outro animal destruiu ou danificou sua plantação:
Se Sim: adicione os nomes dos animais à lista e pergunte Q 505 i-ii (com que frequência; quais plantações))

Q 502				Q 503		Q 504		Q505i	Q 505 ii
Nos últimos 12 meses que animais você encontrou ao trabalhar nas plantações?	Espontânea	Direcionada		Com que frequência notou fezes de (animal)?		Com que frequência notou fezes de (animal) em ninhos?		Com que frequência notou que (animal) destruiu ou danificou as plantações?	Em qual(i)s plantação(ões)
				Dentro de casa	Na comida, água	Dentro de casa	Na comida, água		
Nome do animal		S	N	Todo dia 1 Algumas vezes /semana 2 Algumas vezes / mês 3 Algumas vezes / ano 4 Uma vez nos últimos 12 meses 5 Não nos últimos 12 meses 6					
Pergunte apenas para mamíferos									
[roedores]	1	2	3						
[primatas]	1	2	3						
[morcegos]	1	2	3						
Porco do mato	1	2	3						
Tatu	1	2	3						
Onça	1	2	3						
Capivara	1	2	3						
Cervo	1	2	3						
Coelho	1	2	3						

Q 506 Nos últimos 12 meses; ou seja, desde (mês) do ano passado, vocês usaram fezes de animais como fertilizantes em qualquer plantação sua?

Sim 1
Não 2 – *Pule para Q 510*

Q 507 De que animal elas vem? (*Explore*: **Algum outro animal**?)

Q 508 Em quais plantações vocês usam? (*Explore:* **Algum outro vegetal?**)

Q 509 Durante que estações do ano vocês o utilizam? *Explore: Alguma outra estação?* (*Múltiplas respostas possíveis*) As estações no Amazonas são os períodos de sol e chuva, conhecido como verão e inverno.

[Seca] 1
[Chuvosa] 2
[Outra] 3

Q 510i Vocês cultivam ou coletam frutas de arbustos ou árvores? Podemos pergunta quais são os frutos? Assim poderemos ter uma noção se há deslocamento ou não para essa atividade. Exemplo: o tucumã certamente terá que ser colhido na mata, já um abacate pode ser do quintal da pessoa.

Sim 1
Não 2 – *Pule para Seção 6*

Q 510ii Que frutos vocês e onde vocês os coletam?

510i Fruto coletado	510ii Local de coleta

Q 511 Nos últimos 12 meses; ou seja, desde (mês) do ano passado, ao coletar frutas de árvores onde os animais se abrigam, com que frequência vocês entraram em contato com fezes de animais, [morcegos] ou aves nas árvores ou frutas?

Todo dia 1
Algumas vezes por semana 2
Algumas vezes por mês 3
Algumas vezes por ano 4
Apenas uma vez nos últimos 12 meses 5
Nunca 6
Não coletei frutas nos últimos 12 meses 88

SEÇÃO 6: Animais que servem de alimento

Agora eu gostaria de falar sobre os animais para alimentação.

Primeiramente gostaria de falar sobre os animais que foram comidos durante o último mês; ou seja, nas últimas quatro semanas.

Q 601 Que animais – carne ou outras partes ou sangue – vocês comeram no mês passado?
Explore: **Que outros animais?** (*Explore duas vezes; escreva o(s) nome(s) do(s) animal(is) na primeira coluna)*

Para cada animal citado, pergunte Q 602 a-b, animal por animal:

Q 602a No mês passado, quantos dias vocês comeram (nome do animal)? (*Escreva o número na segunda coluna*)

Q 602b Destas vezes, quantos dias vocês comeram carne mal passada ou crua, inclusive comendo sangue não cozido? (*Anote o número na terceira coluna)*

Inclua TODOS os animais nesta tabela (inclusive peixe e insetos)

Q601	Q602a	Q602b
Que animais você comeu no mês passado?	No último mês quantos dias você comeu (nome do animal)	Dentre estas oportunidades, quantos dias você comeu carne crua ou mal cozida, inclusive sangue
Nome do animal	Todo dia 1 Algumas vezes por semana 2 Algumas vezes por mês 3 Nenhuma vez 4	
Bovino		
Suíno		
Peixe		
Frango		

Agora irei perguntar com que frequência você comeu algum dos animais específicos nos últimos 12 meses; ou seja, do (mês) do ano passado para cá. Quando pensar na sua resposta, considere todas as vezes que você os comeu durante o último ano.

Para cada animal listado na tabela abaixo, pergunte:

Q 603 Nos últimos 12 meses, com que frequência vocês comeram (nome do animal)? (*Leia as frequências, registre o código de resposta na coluna 2)*

Para cada animal que o(a) respondente não tenha consumido, pergunte Q 604 a-b, animal por animal:

Q 604a Você evita comer (nome do animal)? *(Circule 1 para **Sim** e 2 para **Não** na coluna 3)*

Q 604b Qual é a principal razão pela qual você não consome (nome do animal)?

Nome do animal	Durante os últimos 12 meses com que frequência você comeu (nome do animal) Todo dia 1 Algumas vezes por semana 2 Algumas vezes por mês 3 Algumas vezes por ano 4 Uma vez nos últimos 12 meses 5 Regularmente nas estações de pico 6 Algumas vezes na estação de pico 7 Não nos últimos 12 meses 8	Você evita ingerir este animal? Y N	Qual a principal razão para evitar comer (nome do animal)
[capivara]		1 2	
[tatu]		1 2	
[porco do mato]		1 2	
macaco		1 2	

Agora eu gostaria de fazer uma pergunta sobre frutas e vegetais

Q 605 Nos últimos 12 meses, com que frequência vocês comeram frutas ou vegetais que apresentavam mordidas de animais?

Todo dia	1
Algumas vezes por semana	2
Algumas vezes por mês	3
Algumas vezes por ano	4

Apenas uma vez nos últimos 12 meses	5
Nunca	6
Não sei	9

SEÇÃO 7: Caça ou captura de animais

Agora vamos falar de caça ou captura de animais. Teremos que ter muit atençãoo nessa parte. As pessoas, mesmo que consumam carne de carça, dificilmente falarão de maneira aberta e clara sobre o assunto.

Q 701a Vocês já comeram carne de caça?

Sim	1
Não	2 – *Pule para Seção* 8

Q701b Que tipo de carne vocês comeram? Listar as espécies.

Q701c Onde você adquiriu? De quanto em quanto tempo? Essa pergunta não será respondida. No máximo a pessoa pode responder a frequência que faz ou fazia uso, mas dizer como adiquiriu...

Q701d Estava vivo ou morto?

Q 702 Nos últimos 12 meses, a quais tipos de lugares as pessoas vão para caçar ou capturar animais? *(leia as respostas)* Acho que essa eles também não irão responder.

	Sim	Não	
Mata	1	2	
Caverna	1	2	
Pasto	1	2	
Algum outro lugar?	1	2	
Algum outro lugar?	1	2	

Q 703 Que tipo de animais você pegou neste último mês?
(Anote os nomes dos animais na primeira coluna da tabela)
(Se nenhum, escreva "nenhum" na primeira coluna e Pule para Q 705)

<u>Pergunte Q 704 a-b sobre cada animal, um a um</u>

Q 704a Quantos (nome do animal) você pegou no último mês? (*Anote o número na coluna 2)*

Q 704b Alguém da casa já foi mordido(a) ou arranhado(a) a ponto de sangrar por um(a) (nome do animal) no último mês? (*Circule 1 para **Sim** e 2 para **Não** na coluna 3)*

Q 703	Q704a	Q704b	
Último mês Que tipo de animal?	Quantos (nome do animal)?	No último mês alguém foi mordido(a) ou arranhado(a) por (nome do animal)?	
		Sim	Não
		1	2
		1	2
		1	2
		1	2
		1	2

Agora vamos falar sobre o(a) (estação 1)

Q705a Tem alguns animais que se comem em uma estação e outros em outra? No(a) (estação 1)?

Sim	1
Não	2 – *Pule para Seção 8*

Q705b Listar as espécies e as estações

[dependendo dos resultados da pesquisa formativa, adicione outra estação]

SEÇÃO 8 – Animais nos espaços comuns: Mercados

Agora eu gostaria de falar sobre animais que você pode ter encontrado em mercados. Vamos deixar claro que estamos querendo tratar de animais vivos? No caso não vamos abordar a respeito de carne comprada em supermercado ou açougue, correto?

Q 801 Durante os últimos 12 meses; ou seja, desde (mês) do ano passado, com que frequência vocês foram a um mercado no qual as pessoas vendem animais vivos ou carne de qualquer tipo de animal?

Todo dia	1
Algumas vezes por semana	2
Algumas vezes por mês	3
Algumas vezes por ano	4
Apenas uma vez nos últimos 12 meses	5

Nenhuma vez nos últimos 12 meses	6
Não sei / NA	9

Q 802 Vou ler uma lista de animais para você. Para cada animal, por favor, me diga se você o viu para venda nos últimos 12 meses. *(Circule 1 para **Sim,** 2 para **Não** na coluna 1)*

Para cada animal visto, pergunte Q 803 a-b, animal por animal:

Q 803a Pensando nas vezes que você viu (animal), com que frequência você tocou nele(a)? Você o(a) tocou todas as vezes, algumas vezes ou nunca? *(Leia as frequências e anote as respostas na última coluna)*

Q803b Pensando nas vezes que você tocou (nome do animal), em que estado você o(a) tocou? O(a) (nome do animal) estava vivo, era uma carcaça inteira, em pedaços ou em alguma outra forma? Se você tocou diferentes formas de (nome do animal), por favor conte-me todas elas. *(Circule 1 na coluna abaixo de cada forma mencionada; anote a resposta na coluna "Outra")*

802			803a	803b			
Nome do animal	Você viu (nome do animal) a venda?		Com que frequência você o tocou? Toda vez 1 Algumas vezes 2 Nunca 3	Que forma(s) de (nome do animal) você tocou?			
	Y	N		Vivo	Carcaça	Pedaços	Outra
Peixe	1	2		1	1	1	
Galinha	1	2		1	1	1	
Suínos	1	2		1	1	1	
Vaca	1	2		1	1	1	
Capivara	1	2		1	1	1	
Tatu	1	2		1	1	1	
Veado	1	2		1	1	1	

Q 804 Quando vocês vão ao mercado, com que frequência há sangue, vísceras ou fezes no chão?

Toda vez	1
Algumas vezes	2
Nunca	3

SEÇÃO 9: Abate, limpeza de carcaça e corte de animais

Agora eu gostaria de falar sobre animais que vocês abatam, limpem ou cortem.

Q 901 Primeiramente, vamos falar sobre abater; ou seja, matar um animal, seja aquele que vocês criaram, aquele capturado vivo ou comprado vivo em um mercado.

Nos últimos 12 meses; ou seja, desde (mês) do ano passado, vocês abateram algum animal, inclusive aqueles que vocês não comeram?

Sim	1
Não	2 – *Pule para 905*

Q 902a Que animais vocês abateram nos últimos 12 meses?
Explore: Que outros animais? *(Anote o nome do animal na primeira coluna da tabela abaixo e circule 1 abaixo de "Espontânea").*

Q 902b Pergunte para cada animal chave mencionado: Nos últimos 12 meses, vocês alguma vez abateram um(a) (nome do animal)? *(Circule 2 para **Sim** e 3 para **Não** na coluna "Direcionada")*

<u>Para galinhas, patos e cada mamífero citado, pergunte Q 902 a-c, animal por animal</u>:

Q 903a Nos últimos 12 meses, com que frequência vocês abateram um(a) (nome do animal)? (*Leia as frequências e anote o código da resposta na coluna 5)*

Q 903b Na maioria das vezes que vocês abateram um(a) (nome do animal), qual método vocês usaram? (*Anote a resposta na coluna 6)*

Q 903c Ao abater um(a) (nome do animal), com que frequência o sangue do animal entrou em contato com a pele desprotegida? *(Leia as frequências e anote o código da resposta na última coluna)*

Q902				Q903a	Q903b		Q903c
Durante os últimos 12 meses, que animal você limpou?	Espontânea	Induzida		Com que frequência você abateu (nome do animal)	Qual método você usou?		Com que frequência sangue entrou em contato com pele desprotegida?
Nome do animal		S	N	Todo dia 1 Algumas vezes por semana 2 Algumas vezes por mês 3 Algumas vezes por ano 4 Uma vez no último ano 5 Nunca 6			Todas as vezes 1 Algumas vezes 2 Nunca 3
Pergunte apenas para galinhas, patos e mamíferos							
[galinhas]	1	2	3				
[suínos]	1	2	3				
[bovinos]	1	2	3				
[primatas]	1	2	3				
[peixe]	1	2	3				
morcegos	1	2	3				
roedor	1	2	3				
	1	2	3				

Q 904a Ao abater animais nos últimos 12 meses, com que frequência vocês se cortaram? *(Leia as frequências)*

Todo dia	1
Algumas vezes por semana	2
Algumas vezes por mês	3
Algumas vezes por ano	4
Apenas uma vez nos últimos 12 meses	5
Nenhuma vez nos últimos 12 meses	6
Não sei	9

Q 904b Ao abater animais nos últimos 12 meses, com que frequência vocês foram mordido(a)s? *(Leia as freqüências)*

Todo dia	1
Algumas vezes por semana	2
Algumas vezes por mês	3
Algumas vezes por ano	4
Apenas uma vez nos últimos 12 meses	5
Nenhuma vez nos últimos 12 meses	6
Não sei	9

A seguir vamos falar sobre limpeza de carcaças. Por limpeza de carcaça, entenda cortar um animal inteiro, seja ele grande ou pequeno, em pedaços ou remover as vísceras ou pele do animal.

Q 905 Nos últimos 12 meses; ou seja, desde (mês) do ano passado, vocês limparam animais, inclusive aqueles que vocês não comeram?

Sim	1
Não	2 – *Pule para 908*

Q 906a Que animais vocês limparam nos últimos 12 meses?
Explore: Que outros animais? (*Anote o nome do animal na primeira coluna da tabela abaixo e circule 1 sob "Espontânea").*

Q906b *Pergunte para cada animal chave que não tenha sido mencionado*: Nos últimos 12 meses vocêsalguma vez limparam um(a) (nome do animal)? (*Circule 2 para **Sim** e 3 para **Não** na coluna "Direcionada")*

<u>Para galinhas, patos e cada mamífero citado, pergunte Q 907 a-c, animal por animal</u>:

Q 907a Nos últimos 12 meses, com que frequência vocês limparam um(a) (nome do animal)? (*Leia as frequências e anote o código da resposta na coluna 5)*

Q 907b Na maioria das vezes que vocês limparam um(a) (nome do animal), há quanto tempo ele estava morto? A carcaça estava ainda morna, foi depois de um dia do abate do animal ou mais tempo que isso? *(Anote o código da resposta na coluna 6*)

Q 907c Ao limpar um(a) (nome do animal), com que frequência vocês se cortaram? *(Leia as frequências e anote o código da resposta na última coluna)*

Q906				Q907a	Q907b		Q907c
Durante os últimos 12 meses, que animal vocês limparam?	Espontânea	Induzida		Com que frequência vocês limparam (nome do animal)	Há quanto tempo o animal estava morto?		Com que frequência vocês se cortaram? Todo dia 1 Algumas vezes por semana 2 Alguma vezes por mês 3 Algumas vezes por ano 4 Uma vez no último ano 5 Nenhuma no último ano 6
Nome do animal		S	N	Todo dia 1 Algumas vezes por semana 2 Algumas vezes por mês 3 Algumas vezes por ano 4 Uma vez no último ano 5 Nenhuma no último ano 6	Carcaça ainda morna 1 Um dia depois do abate 2 Mais de um dia 3		
Pergunte apenas para galinhas, patos e mamíferos							
[galinhas]	1	2	3				
[suínos]	1	2	3				
[roedores]	1	2	3				
[primatas]	1	2	3				
[morcegos]	1	2	3				
Bovinos	1	2	3				
Peixe	1	2	3				

Agora vamos falar sobre cortar carne; ou seja, cortar carne ou partes de animais que já estão em pedaços grandes em pedaços menores.

Q 908 Nos últimos 12 meses; ou seja, desde (mês) do ano passado, vocês cortaram a carne de algum animal, inclusive aqueles que vocês não comeram?

Sim 1
Não 2 – *Pule para Seção 10*

Q 909a Quais animais vocês cortaram nos últimos 12 meses?
Explore: **Que outros animais**? (*Anote os nomes dos animais na primeira coluna da tabela abaixo e circule 1 sob "espontânea").*

Q 909b *Pergunte para cada anima chave não mencionado*: **Nos últimos 12 meses vocês alguma vez cortaram carne de (nome do animal)**? *(Circule 2 para **Sim** e 3 para **Não** na coluna "Direcionada")*

Para galinhas, patos e cada mamífero citado, pergunte Q 910 a-c, animal por animal:

Q 910a Nos últimos 12 meses, com que frequência vocês cortaram carne de um(a) (nome do animal)? (*Leia as frequências e anote o código da resposta na coluna 5*)

Q 910b Na maioria das vezes que vocês cortaram carne de um(a) (nome do animal), há quanto tempo ele estava morto? A carcaça ainda estava morna,foi depois de um dia do abate do animal ou mais tempo que isso? (*Anote o código da resposta na coluna 6*)

Q 910c Ao cortar a carne de um(a) (nome do animal), com que frequência vocês se cortaram? (*Leia as frequências e anote o código da resposta na última coluna*)

Q909				Q910a	Q910b		Q910c
Durante os últimos 12 meses, que animal vocês cortaram?	Espontânea	Induzida		Com que frequência vocês cortaram (nome do animal)	Há quanto tempo o animal estava morto?		Com que frequência vocês se cortaram?
Nome do animal		S	N	Todo dia 1 Algumas vezes por semana 2 Algumas vezes por mês 3 Algumas vezes por ano 4 Uma vez no último ano 5 Nenhuma vez no último ano 6	Carcaça ainda morna 1 Um dia depois do abate 2 Mais de um dia 3		Todo dia 1 Algumas vezes por semana 2 Alguma vezes por mês 3 Algumas vezes por ano 4 Uma vez no último ano 5 Nenhuma no último ano 6
Pergunte apenas para galinhas, patos e mamíferos							
[galinhas]	1	2	3				
[suínos]	1	2	3				
[roedores]	1	2	3				
[primatas]	1	2	3				
[morcegos]	1	2	3				
Peixes	1	2	3				
Bovinos	1	2	3				
	1	2	3				

SEÇÃO 10: Animais encontrados mortos

Agora eu gostaria de falar sobre animais que vocês encontraram mortos. Qualquer tipo de animal? Cachorro, gato, rato?

Q1001 Pensando nos últimos 12 meses, vocês alguma vez viram algum animal morto no quintal ou longe da sua casa?

Sim	1
Não	2 – *Pule para Seção 11*

Q 1002 Que tipo de animal(is) vocês encontraram morto(s)?
Explore: **Que outros animais**? (*Anote o nome do animal na primeira coluna da tabela abaixo e circule 1 sob "espontânea")*

Q 1003 Pergunte para cada animal chave não mencionado: Nos últimos 12 meses vocês alguma vez viram um(a) (nome do animal) morto(a)? *(Circule 2 para **Sim** e 3 para **Não** na coluna "Direcionada")*

Para cada tipo de mamífero ou ave mortos, pergunte Q 1004 a-d, animal por animal

Q 1004a Quantas vezes vocês encontraram um(a) (nome do animal) morto(a)? *(Escreva o número de encontros na coluna 5)*

Q 1004b *Pergunte sobre cada vez que o respondente ou alguém da casa encontrou um animal morto*:

Quantos (nome do animal) vocês encontraram na (primeira/segunda/terceira...) vez?

Anote o número na coluna adequada sob "encontro". Quando o respondente tiver contado tudo sobre todos os encontros, some os números. Coloque o total sob "total"

Q 1004c Vocês encontraram um total de (total) (nome do animal) mortos(as). Quantos destes (nome do animal) mortos vocês tocaram? *(Anote o número na penúltima coluna)*

Q 1004d Quantos destes(as) (nome do animal) vocês comeram? *(Anote o número na última coluna)*

				Q1004a	Q1004b						Q1004c	Q1004d
Durante os últimos 12 meses, que animais vocês encontraram mortos?	Espontânea	Induzida		Quantas vezes vocês encontram (nome do animal) mortos?	Quantos (nome do animal) vocês encontraram mortos?						Quantos destes (nome do animal) vocês tocaram com mãos desprotegidas?	Quantos destes (nome do animal) vocês comeram?
Nome do animal		S	N		encontro					Total		
					1	2	3	4	5			
Pergunte apenas para mamíferos e aves												
[roedores]	1	2	3									
[primatas]	1	2	3									
[morcegos]	1	2	3									
galinha	1	2	3									
Cão	1	2	3									
Bovino	1	2	3									
Cervo	1	2	3									
Capivara	1	2	3									
	1	2	3									
	1	2	3									

SEÇÃO 11: Crenças e atitudes

Q 1101 Agora eu gostaria de ler algumas frases. Para cada uma, por favor, me diga se você concorda totalmente, concorda parcialmente, discorda parcialmente ou discorda totalmente.

a A carne de animais que são caçados/capturados tem gosto melhor do que a carne de animais que são criados.
Discordo totalmente 1 Discordo 2 Concordo 3 Concordo totalmente 4

b Pacas são boas para comer
Discordo totalmente 1 Discordo 2 Concordo 3 Concordo totalmente 4
Pergunta não cabe.

c Apenas pessoas velhas comem macacos
Discordo totalmente 1 Discordo 2 Concordo 3 Concordo totalmente 4
Pergunta não cabe.

d Pessoas comem mais carne de caça do que comiam cinco anos atrás
Podemos dizer animais selvagens ou de caça?
Discordo totalmente 1 Discordo 2 Concordo 3 Concordo totalmente 4

e As pessoas deveria evitar comer [macacos]
Discordo totalmente 1 Discordo 2 Concordo 3 Concordo totalmente 4

f A carne de caça ajuda na alimentação da minha família
Discordo totalmente 1 Discordo 2 Concordo 3 Concordo totalmente 4

g Os animais podem estar doentes sem que as pessoas saibam
Discordo totalmente 1 Discordo 2 Concordo 3 Concordo totalmente 4

h É impossível manter ratos longe da farinha; você tem apenas que viver com eles Poderíamos deixar só farinha? Ou podemos alongar a lista de alimentos tais como feijão, arroz. Definir o que seria grão ou cereal?
Discordo totalmente 1 Discordo 2 Concordo 3 Concordo totalmente 4

i A carne de caça é melhor do que a carne de animais que são criados
Discordo totalmente 1 Discordo 2 Concordo 3 Concordo totalmente 4

Q 1102 Agora mencionarei diferentes formas através das quais as pessoas podem ter contato com animais. Para cada uma, por favor, me diga **quão arriscado** você acredita ser contrair doenças de animais: muito arriscado, um pouco arriscado, possivelmente arriscado e nada arriscado.

a Ser arranhado por um(a) [animal selvagem comumente mantido como de estimação]
Muito 1 Um pouco 2 Possivelmente 3 Nada 4

b Permitir que galinhas entrem livremente em casa
Muito 1 Um pouco 2 Possivelmente 3 Nada 4

c Ser mordido por um cachorro
Muito 1 Um pouco 2 Possivelmente 3 Nada 4

d Tocar animais encontrados mortos na mata ou no campo.
Muito 1 Um pouco 2 Possivelmente 3 Nada 4

e Consumir sangue animal não cozido
Muito 1 Um pouco 2 Possivelmente 3 Nada 4

f Limpar um animal doméstico ou de caça para comer
Muito 1 Um pouco 2 Possivelmente 3 Nada 4

g Ter animais domésticos perto da casa e não ter fossa?
Muito 1 Um pouco 2 Possivelmente 3 Nada 4

h Ser lambido por um cão
Muito 1 Um pouco 2 Possivelmente 3 Nada 4

i Beber água dos mesmos locais que animais bebem
Muito 1 Um pouco 2 Possivelmente 3 Nada 4

j Ficar empoeirado ao varrer fezes secas de animais
Muito 1 Um pouco 2 Possivelmente 3 Nada 4

k Limpar a carne de um animal doente
Muito 1 Um pouco 2 Possivelmente 3 Nada 4

l Comer carne de um animal que se encontrava morto há mais de três dias
Muito 1 Um pouco 2 Possivelmente 3 Nada 4

m Comer alimento que continha fezes de ratos tiradas antes de se cozinhar
Muito 1 Um pouco 2 Possivelmente 3 Nada 4

n Cuidar de uma vaca doente
Muito 1 Um pouco 2 Possivelmente 3 Nada 4 Essa pergunta fica mesmo em área urbana?

o Deixar cair sangue em um ferimento enquanto limpa a carcaça de um [animal grande]
Muito 1 Um pouco 2 Possivelmente 3 Nada 4

p Entrar em uma caverna onde ficam muitos morcegos (AMAZONIA)
Muito 1 Um pouco 2 Possivelmente 3 Nada 4 Se aplica para área urbana?

Q 1103 Você já ouviu falar de alguma doença que as pessoas podem contrair dos animais?

Sim	1
Não	2 – *Pule para Q 1106*
Não sei	3 – *Pule para Q 1106*

Q 1104a De quais doenças você já ouviu falar? Quais outras doenças? (*explore duas vezes). (Marque tantas quantas o respondente citar.)*

Q 1104b *Para cada doença que o respondente não mencionar, pergunte: Você já ouviu falar de (nome da doença*)?

	Espontânea	Direcionada	
	Sim	Sim	Não
Raiva	1	2	3
Carbúnculo	1	2	3
Brucelose	1	2	3
Leptospirose	1	2	3
Gripe, influenza	1	2	3
Influenza aviária	1	2	3
Tuberculose	1	2	3
	1		

Outra (Especifique)		1
Outra (Especifique)		1

Q 1105a De que tipos de animais as pessoas podem contrair doenças?
Explore duas vezes: ***Que outros animais****? (Múltiplas respostas possíveis)*

Q 1105b *Para cada animal chave que o respondente NÃO mencionar, pergunte:* **As pessoas podem contrair doenças de (nome do animal)?**
(Circule a resposta sob "Direcionada")

		Espontânea	Direcionada	
		Sim	Sim	Não
Animais selvagens		1	2	3
Macacos		1	2	3
[Cachorro]		1	2	3
Tatu		1	2	3
[Morcego]		1	2	3
Ratos		1	2	3
Capivara		1	2	3
Rato selvagem		1	2	3
Catita/Camundongo doméstico		1	2	3
Porco		1	2	3
Vaca		1	2	3
Galinha		1	2	3
Pato		1	2	3
Gato		1	2	3
Cachorro		1	2	3
Onça		1	2	3
Mucura		1	2	3
Outro (Especifique)		1		
Outro (Especifique)		1		
Outro (Especifique)		1		

Q 1106 Em quem você confia mais para lhe dar informações sobre doenças que os animais podem transmitir para as pessoas? Vamos acrencentar agente de saúde? Acho que seria interessante.
Explore duas vezes: **Em quem mais você confia?**
(Circule a primeira resposta na primeira coluna; circule as outras respostas na segunda coluna)

			1ª	Outra (múltipla)
Médico/Enfermeira			1	1
Equipe do governo (prefeitura)			2	2
Veterinário			3	3
Trabalhador extensionista			4	4
Vizinho			5	5
Guardas florestais			6	6
Rádio			7	7
TV			8	8
Ator da TV			9	9
Jornais			10	10
Outdoors			11	11
Centros de saúde			12	12
Hospitais			13	13
Feira			14	14
Escola			15	15
Revista			16	16
Liderança do bairro/ assentamento			17	17
Profissional de saúde do bairro/cidade			18	18
Outro (Especifique)			19	19
Não sei / Sem resposta			99	99

SEÇÃO 12: Informação sobre o domicílio

Agora eu gostaria de fazer algumas perguntas sobre você

Q 1201 Qual sua principal ocupação? Poderíamos acrescentar trabalhador da zona franca de Manaus ou do Distrito (como eles se referem). Mesmo que depois tenhamos que agrupar no item operário.

Nenhuma		1
Agricultor/ Pecuarista (ou outro)		2
Funcionário da usina		3
Açougueiro		4
Dona de casa		5
Lojista / Comerciante (no mercado)		6
Servidor público		7
Trabalhador de escritório		8
Operário		9
Estudante		10
Trabalhador em restaurante		11
Autônomo		12
Trabalhador da Zona Franca de Manaus		13
Outro (Especifique)		
Não sei / Sem resposta		99

Q 1202 Qual seu estado civil atual?

Solteiro(a)	1
União consensual	2
Casado(a)	3
Divorciado(a)/Separado(a)/Viúvo(a)	4
Não sei / Sem resposta	9

Q 1203 Qual seu nível de escolaridade?

Nunca fui à escola			0
Ensino fundamental			1
Ensino médio			2
Ensino técnico			3
Faculdade/Universidade			4
Outro			
Não sei / Sem resposta			9

Q 1204 Qual é o principal combustível que sua família geralmente usa para cozinhar? (*uma resposta*)

Energia elétrica		1
Gasolina líquida		2
Gás metano		3
Petróleo		4
Carvão de turfa, carvão vegetal, carvão mineral		5
Lenha, palha, feno		6
Outro (Especifique)		

Q 1205 Que tipo de banheiro seu domicílio utiliza? (Peça para usar no final e escreva o que encontrou)

Sem banheiro/mato/campo		1
Embrulhar e jogar		2
Armazenamento de metano		3
Fossa de chão ventilada e aperfeiçoada		4
Fossa de chão tradicional		5
Fossa com descarga		6
Banheiro com descarga		7
Mato		8
Outro (Especifique)		9

Q 1206 A sua família não tem acesso a energia elétrica, tem acesso a energia elétrica de gerador próprio ou tem acesso a rede de energia elétrica?

Não tem acesso	1
A partir de gerador	2
Rede elétrica	3

Q1207 Qual dos seguintes bens você ou alguém da sua família que more neste domicílio possui? (*leia a lista de bens)*

	Sim	Não
Rádio	1	2
TV	1	2
Telefone fixo	1	2
Celular	1	2
Refrigerador	1	2
Fogão a gás	1	2
Fogão elétrico	1	2
Bicicleta	1	2
Motocicleta	1	2
Barco	1	2
Carro	1	2

Q 1208 Com que frequência você ouve rádio?

Diariamente	1
2 ou 3 dias / semana	2
Ao menos uma vez / semana	3
2 ou 3 vezes durante último mês	4
Uma vez no último mês	5
Nunca	6
Não sei	9

Q 1209 Com que frequência você assiste TV?

Diariamente	1
2 ou 3 dias / semana	2
Ao menos uma vez / semana	3
2 ou 3 vezes durante último mês	4
Uma vez no último mês	5
Nunca	6
Não sei	9

Q 1210 Com que frequência você lê jornal ou revista

Diariamente	1
2 ou 3 dias / semana	2
Ao menos uma vez / semana	3
2 ou 3 vezes durante último mês	4
Uma vez no último mês	5
Nunca	6
Não sei	9

Q 1211 Qual é a principal fonte de água potável para os membros de seu lar na estação quente/seca?

ÁGUA ENCANADA	
Encanamento dentro de casa	1
Encanamento dentro de terreno ou campo	2
Torneira pública	3
ÁGUA DE POÇO ABERTO	
Poço aberto no terreno / campo	4
Poço aberto público	5
ÁGUA DE POÇO FECHADO	
Poço fechado no terreno / campo	6
Poço fechado público	7
ÁGUA DE POÇO ARTESIANO	
Poço artesiano no terreno / campo	8
Poço artesiano público	9
ÁGUA DE SUPERFÍCIE	
Nascente	10
Rio ou córrego	11

Lago ou reservatório		12
Represa		13
Água de chuva		14
Caminhão pipa		15
Água engarrafada		16
Outro; especifique		
Não sei		99

Q 1212 Há animais, inclusive [roedores] ou aves, que têm acesso à sua fonte de água potável?

Sim	1
Não	2
Não sei	9

Q 1213 O que é feito com o lixo orgânico em seu domicílio? (*Marque todas as que se aplicarem)*

Coletado		1
Levado a um ponto de coleta		2
Enterrado		3
Queimado		4
Jogado no terreno próprio		5
Jogado em rio / reservatório / mato		6
Jogado em outro lugar		7
Outro (Especifique)		

Q 1214 Com que frequência você faz isso? (*Leia as respostas*)

Diariamente		1
2 a 3 dias / semana		2
Ao menos uma vez / semana		3
1 a 3 vezes / mês		4
Jogado no próprio terreno		5
Uma vez / mês		6
Outro (Especifique)		
Não sei		9

Q1215 Qual o principal material usado para o chão da casa?
(*Observe o chão)*

Tábua de madeira		1
Terra / areia		2
Parquet, madeira envernizada		3
Pedra envernizada, asfalto, cimento, tijolos		4
Outro (Especifique)		

Q 1216 Qual o principal material usado na parede da casa?

Tábua de madeira		1
Terra / areia		2
Parquet, madeira envernizada		3
Pedra envernizada, asfalto, cimento, tijolos		4
Outro (Especifique)		

Q 1217 Qual o principal material do telhado da casa? Só acrescentei um L que faltou.

Tábua de madeira		1
Coberto de colmo		2
Junco / bambu		3
Chapa ondulada		4
Outro (Especifique)		

Este é o fim de nosso questionário. Muito obrigado(a) por doar seu tempo para responder estas perguntas. Agradecemos sua ajuda. Por favor, diga-me se você tiver qualquer dúvida sobre o estúdio, e eu ficarei muito feliz por respondê-las.

Entrevistador: Horário de finalização: ____ / ___

Língua na qual a entrevista foi realizada	
Alguém ajudou com a entrevista?	
Qualidade da entrevista:	

Baixo 1 2 3 4 5 Alto

EcoHealth Alliance

UCDAVIS VETERINARY MEDICINE
One Health Institute





deep forest

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Field methods to capture bats

The nocturnal and complex behavior of bats makes monitoring bat diversity a challenging task. There are many capture biases inherent in the survey techniques commonly used for bats. Thus, it is best to employ a variety of methods to provide the most robust inventory results possible. For the Deep Forest (DF) Project we will combine three techniques to capture bats:

1. horizontal ground-based mist nets,
2. vertical canopy mist nets,
3. harp traps.

For a more detailed methodological review for capturing bats we recommend the chapter "Methods of Capturing and Handling Bats" by Kunz et al. (2009).

Horizontal ground-based mist nets

Ground-based mist nets are the most common and efficient devices for capturing flying bats (Figure 11). Standard net sizes are 6, 12 or 18 m long and 2.1 - 2.4 m high when set. The net usually has several horizontal shelf strings that form 4 or 5 loose pockets that catch and tangle the bats. Mist nets also come in various mesh sizes, with finer mesh being used for smaller bats. Mist nets are usually deployed using aluminum tent poles or bamboo culms. All debris such as leaves or twigs should be removed from the net prior to bat capture or before pushing the shelves together to close the net.



Figure 11: Examples of deployed horizontal ground-base mist nets (Photos: K Murray, Amazonas - Brazil, 2012).

Vertical canopy mist nets

Bat assemblage composition differs between the ground and canopy level (e.g., Francis, 1994, Kalko and Handley, 2001) and some species may never be captured at ground level because of their exclusive canopy lifestyle (e.g. molossid bats, pteropodids or some phyllostomids). It is thus important to consider the vertical stratification of a bat assemblage when assessing local bat species diversity. In general, two methods are available for capturing bats at canopy height:

1. suspended horizontal mist nets, and
2. suspended vertical canopy mist nets.

Either technique requires training and sufficient time for preparation. Both techniques require the trapper to locate a site that provides 1) sufficient open space so that the net does not get entangled with twigs and branches, and 2) some large sturdy branch from which a rope can suspend the net.

Vertical canopy mist nets have the great advantage that they can be hoisted and handled by a single person. They are made out of the same material as the ground-based mist nets, but have a vertical instead of a horizontal rectangular orientation. These mist nets have loops at the bottom and the top of the net to attach the supporting poles and they are usually 6 m (6 shelves) or 12 m (12 shelves) high (Figure 12). Vertical mist nets can be deployed by attaching a rope to the top of net and position this rope over a horizontal branch by throwing a rock attached to the rope or by using a slingshot. To prevent leaves and debris on the ground from getting tangled in the net a plastic tarp should be laid on the ground underneath the canopy net for when it is lowered. We recommend practicing the deployment and the review of mist nets (lowering the mist net to check for bats) prior to the sampling period in order to avoid any difficulties when in use. Detailed information on this technique is described in Rinehart and Kunz (2001).

For DF we will use one (**6 m high x 3 m width**) vertical canopy mist net. These mist nets can be purchased at Avinet at `http://www.avinet.com/avi_order.taf?_function=view&ct_id=20` .

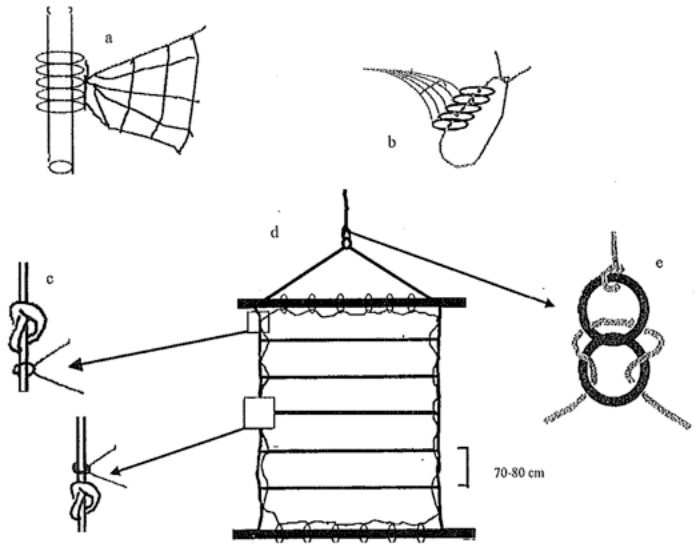


Figure 12: Vertical canopy mist net deployed using *horizontal* poles (from Rinehart and Kunz (2001)

Harp traps

The harp trap is another standard device used for trapping bats. Harp traps may trap bats that are not captured with mist nets (Kingston et al., 2003) because they use fishing lines (strings) that are more difficult for bats to detect acoustically (i.e., with echolocation) and/or visually. After the bat hits the strings, it will slide down into the canvas catch bag suspended underneath the trap. Once

in the bag, the bats will typically climb the material and be safely hidden away under a flap attached to the bag. Harp traps get their name because they look similar to the musical instrument (i.e., a large frame with numerous strings strung from top to bottom). Harp traps operate as if you linked a number of harps strapped together (one in front of the other) to catch bats - if the bats manage to evade the first bank of strings, the next bank behind them may catch them instead. Harp traps usually consist of 2 – 4 banks of strings supported between parallel rectangular metal frames (usually 2 m x 2 – 4 m) that are arranged about 4 – 6 cm apart. The distance between the strings in any one bank of vertically oriented monofilament fishing lines is usually about 2 – 3 cm (Figure 13). Fishing line strings may break from time to time; these broken strings should be replaced immediately using fishing line with a weight of 3 – 4 kg. Thicker strings will be more noticeable and reduce the trap efficiency.

- **For DF we will use two harp traps (2 m x 2 m with 3 banks)** separated by at least 20 m, but preferably placed further apart when sampling the 1 ha site.

Harp traps should be positioned along trails, over small streams or in forest gaps if available. Harp traps can be custom made in-country to minimize costs or purchased from a reputable manufacturer. Some possible suppliers include Faunatech (`http://www.faunatech.com.au/products/harptrap.html`) and Tropzea Resources (tropzea@yahoo.com).



Figure 13: Harp trap deployed in a natural flyway in the pristine site in Manaus. Bats flying the pathway hits the harp trap strings and slide down into the catch bag underneath the trap. Photos R Mendonça, Brazil 2013

Trapping procedure

For all trap types, capture success will be enhanced when traps are placed in natural flyways. You can and should move the traps around within the 100 x 100 m (1 ha) sampling site during the trapping period. Many people choose to rotate the direction of the nets on different nights by swiveling the net around one of the poles. You can also choose to change the net location completely. The only restriction is that the traps remain inside the 1 ha sampling site (the corners of which should be flagged upon site setup for your reference). A good rule of thumb to ensure that you are not sampling more than the 1 ha area is that the maximum distance between the two farthest mist nets should not exceed 140 m (Figure 14a and 14b). For DF we will deploy:

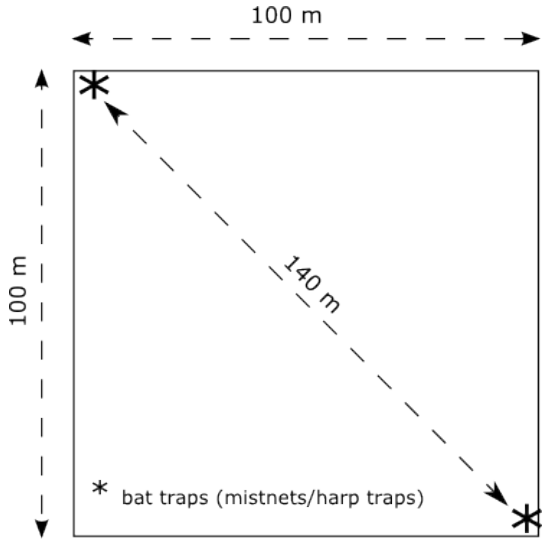
1. Eight ground-based mist nets, each 6 m wide x 2.6 m high. These should be fairly evenly distributed over the 1 ha site.

2. One vertical canopy mist net, 6 m high x 3 m width. This can be deployed anywhere within the 1 ha site, but should be separated by at least 20 m.

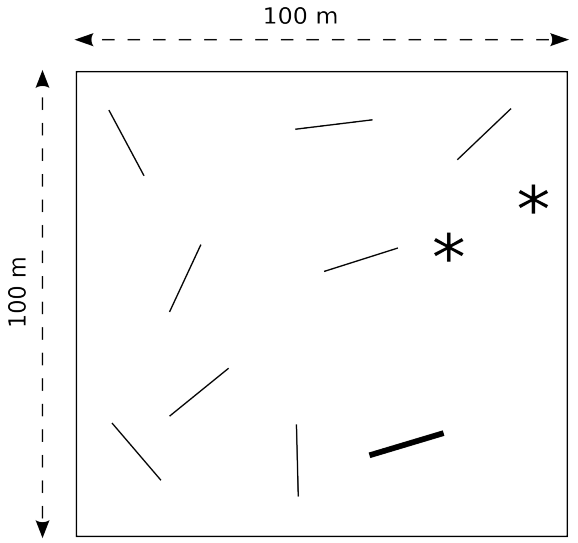
3. Two harp traps. These can be deployed along trails, small streams or gaps if available anywhere within the 1ha site.

Mist nets and harp traps should be reviewed regularly, but this frequency will depend on the number of captures. In general, mist nets should be reviewed every 15 minutes when bats are being frequently captured, or every 30 minutes if less frequent.

Note Unlike a biological inventory survey, we are not trying to survey all species in the "region" around our sampling sites. We are only interested in the bats that occur in the 1 ha sampling site. Since the 1 ha sampling site is chosen within a particular patch of vegetation (typically available forest), we do not need to include as many habitat types as possible that may occur nearby. Our protocol is designed to standardize the sampling method at the chosen sites in order to limit the unmeasured variation in our study as much as possible. Hence, once the 1 ha site is chosen, it is important that the corners are marked and that all traps are set within the boundaries of the site.



(a) Mist nets and harp traps can be located anywhere within the 1 ha (100 x 100 m) sampling site. As a simple rule of thumb, the maximum distance between two bat traps should not exceed 140 m.



(b) Example of deployment of bat traps (* 2) and both horizontal (8) and vertical (**in bold**, 1) mist nets within a 1 ha (100 x 100 m) site.

Figure 14: Deployment of bat traps within the 1 ha sampling site.

Time of capture and capture effort

To minimize the number of birds caught, mist nets should be deployed but not opened before sunset. Certain species of bats are at the peak of their activities at dusk and during the first few hours after sunset. For DF mist nets will be open at sunset and will remain open for a total of 6 hours. Bat capture effort is expressed in terms of the total time of mist netting and total area of mist nets used. Following this protocol, each day of bat sampling will have a total of 150.8 m^2 of net over 6 hours. For DF we will capture bats for a period of 5 consecutive days per site, having a total of 754 m^2/30 hours of capture effort per sampling site (Table 3).

Table 3: A combination of mist nets (vertical and horizontal) and harp traps will be used to maximize the sampling effort per day.

Type of bat trap	**Individual size (in meters)**	**Area (m^2)**	**Number of bat traps**	**Total area/day (m^2)**
Horizontal	6 x 2.6	15.6	8	124.8
Vertical	3 x 6	18	1	18
Harp trap	2 x 2	4	2	8
TOTAL	–	–	11	150.8

NOTE: Occasionally too many bats that can safely be processed will be captured in the misnets at the same time, which will also increases the bats' stress levels. In this rare event, **the first twenty (20) individuals of the same species will be sampled**. If more individuals of the same species are captured during the night these will not be sampled and can be released, unless the quantity of bats being captured per hour is decreased sufficiently enough for these animals to be safely sampled. Any animals that are released without sampling should be recorded in the data sheet and corresponding GAINS file. This rule tries to maximize both the number of species and the number of individuals per species sampled.

Bat sampling and processing

Captured bats will be sampled for blood (no serum), saliva, urine and rectal swabs. All samples should be placed in cryovials containing 200 µl of Viral Transportation Media (VTM) and stored in liquid nitrogen or a dry shipper while in the field. Sampling collection should follow PREDICT protocols (available on Basecamp). Blood should be collected only by a veterinarian or trained field biologist. To ensure animal welfare, each trained person will only attempt to draw blood two times, then if no blood is collected, the bat should be released. To ensure proper taxonomic identification and to generate information about the variation in body size and body weight between and within species, additional standard biometric information should be taken, including photographs of all animals (See below **Pictures**) – record the image number on the data sheet (Appendices A and B).

Acoustic surveys

Ultrasonic bat acoustic surveys will be employed to estimate the number of bat species unaccounted for by using our current methodology (Skalak et al., 2012). These findings will be used to correct estimations of the number of bat species present at a site, and subsequently the number of viruses estimated in the bat assemblage at each sampling site. Acoustic surveys can be performed using ultrasonic bat detectors (e.g., Anabat, Petterson). For DF we will use the *SM2BAT+ Passive Ultrasonic Bat Recorder* available at `http://www.wildlifeacoustics.com/products/song-meter-sm2-bat-plus`. Using an ultrasonic omnidirectional microphone, this unattended automatic bat detector records calls in full spectrum. Detailed specifications and configuration details are available in the section **Bat acoustic survey details and configuration** section). For DF we will install one *SM2BAT+* detector in or around the center of the 1 ha sampling site that is being sampled. The bat detector will be automatically recording bat calls for 12 hours between 6:00 pm to 6:00 am, for 5 consecutive days. There is an increasing availability of repositories with recorded bat calls identified at species level for different parts of the world (e.g., `http://users.lmi.net/corben/BatsOfBorneo.htm`). In addition to these libraries we will create local libraries that will be used to identify the recorded species and to estimate the total number of bat species that were not detected in the traps.

Recaptured individuals

Originally we suggested that captured bats should be marked using microchips: This idea was based on the following considerations:

1. to make sure we do not sample the same animal twice within a trip
2. to follow individual infection histories through time
3. to estimate the population size

Considering the low recapture rates we are having in Brazil, the time investment and cost of microchipping bats, we have decided to remove the permanent marking of bats from DF. The most compelling reason to microchip bats is long-term individual identification, which can be used to build up a history of infection through time. However, this is not a central aim of DF and so cannot be used to justify the costs. The other considerations above can be equally well achieved using more temporary, less invasive, less time consuming and less costly marking techniques. For short term marking we will paint toenail's bat using a non – toxic nail polish, please make sure the toes/claws do not get stuck together by the polish. It is not necessary to mark animals the last night of sampling. Animals that are recaptured during the same sampling trip should be reviewed for previous marks and then released without resampling, the capture should be recorded on the field sheet as a recapture. All individuals recaptured during separate sampling trips should be re-sampled.

Biometric data

For each bat captured, the sex and age (juvenile, subadult and adult) will be determined for all individuals (Notes 3), and standard biometric measures will be taken (body mass, head-body length, tail length, hind foot length, ear length and forearm length, see Notes 4). A standardized field datasheet is supplied below on page 37.

Pictures

When possible pictures of the face (front and side view) of each animal will be taken. If biometric information is difficult to take (e.g., very small animals) then a picture of the complete body next to a scale as reference will be taken.

Notes 3

Category

Juveniles are generally defined as non-volant young individuals that are smaller and weigh less than adult individuals. They are often captured together with their mother. The epiphyses of their bones are not fused yet, i.e. there is a light area of a few mm close to the joints of the finger bones (examine the finger bones on the posterior/dorsal side of the wing by shining a light through the wing from the anterior/ventral side of the bat), the pelage is often grayer than in adults and they have deciduous teeth.

Subadults are volant and fully-grown, but still show the unfused epiphyses (Anthony, 1988).

Adults show mature size, fused epiphyses, pelage and are often reproductively active, i.e. males may have large (scrotal or abdominal) testes and females may be either pregnant or lactating.