The impact of targeted IRS, vector dynamics, and population movement on malaria in a high-transmission setting in northern Zambia

By

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DISSERTATION ABSTRACT

Background: The scale-up of malaria control efforts has led to a global decline in malaria burden, but progress has stalled or reversed in high-transmission regions. In Zambia, malaria cases increased annually since 2009 despite extensive malaria control activities, and malaria remains the most common cause of child mortality. To achieve the goal of malaria elimination in Zambia by 2021, drivers of transmission in high-burden areas must be identified and new intervention strategies must be developed and evaluated.

Methods: The study was conducted in Nchelenge District, a high-transmission area in northern Zambia. The region has two main malaria vectors, *Anopheles funestus s.s.* and *An. gambiae s.s.*, whose distribution varies spatially and temporally. Household surveys were conducted bimonthly from April 2012 to July 2017. Parasite prevalence was measured using rapid diagnostic tests (RDTs), and malaria vectors were collected with indoor light traps. Correlates of parasite prevalence and household vector abundance were identified, and the relationships between vector abundance and prevalence were defined. An evaluation was conducted to determine the impact of three years of targeted indoor residual spraying (IRS) with pirimiphos-methyl on vector abundance and parasite prevalence. Individual movement patterns were characterized using global positioning systems (GPS) data loggers and linked to malaria risk.

Results: Parasite prevalence was approximately 50% across all participants, and an average of 7.0 *An. funestus* and 0.8 *An. gambiae* were collected per household. *An. funestus* counts were positively correlated with both rainy- and dry-season malaria transmission, and *An. gambiae* counts were positively associated with rainy-season transmission only. Within the area targeted for IRS, there was a 28% decline in parasite prevalence in the rainy season, and a 51% and 36% decline in *An. funestus* and *An. gambiae* counts. Three-quarters of participants spent time in both sprayed and unsprayed areas, and half spent at least an hour away from home per night during times of peak vector biting activity.

Conclusions: Malaria transmission in Nchelenge District remains high with many barriers to control. Novel intervention strategies are needed to successfully reduce and interrupt transmission in high-burden areas, including year-round comprehensive vector control. Population movement patterns have the potential to increase malaria risk and must be considered in malaria control activities.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1: Introduction

OVERVIEW

Global malaria burden and control

Malaria is a vector-borne disease caused by infection with the *Plasmodium* parasite and transmitted to humans by female *Anopheles* mosquitoes. Worldwide, there were an estimated 216 million malaria cases and 445,000 deaths in 2016, with the majority of morbidity and mortality attributed to the species *P. falciparum* [1]. These values represent a 40% decline in malaria cases and a 61% decline in malaria mortality since 2000 due to the global scale-up of funding and malaria control interventions following the launch of the Roll Back Malaria Initiative in 1998, the Global Fund to Fight AIDS, Tuberculosis, and Malaria (GFTAM) in 2002, and the President's Malaria Initiative (PMI) in 2005 [2-6].

The World Health Organization (WHO) has goals to reduce malaria cases and deaths 90% by 2030 and to eliminate transmission in 35 countries [7]. Current strategies to achieve these targets include expanded surveillance, enhanced vector control, improved case management, and chemoprevention [1, 7]. Vector control in particular has been identified as a key priority for malaria control, including increased coverage of long-lasting insecticide-treated nets (LLINs), expanded indoor residual spraying (IRS) and management of insecticide resistance [1, 7]. Larval source management or other environmental interventions can also be implemented where appropriate [8]. Current recommendations emphasize the integration of vector control methods into local health systems and the use of evidence-based methods to guide intervention strategies [9, 10].

Priorities for case management include community and health provider education, prompt identification of infection through rapid diagnostic tests (RDTs), and the use of standardized artemisinin-combination therapy (ACT) to ensure quality and slow the development of drug resistance [1, 7].

Chemoprevention recommendations include intermittent preventive treatment (IPT) of pregnant women and children [1].

Malaria in high-burden areas

Despite the widespread expansion of malaria control, gains have been uneven both within and between countries. Approximately 90% of malaria cases and deaths continue to occur in sub-Saharan Africa, with 14 countries in this region accounting for 74% of all global cases (Figure 1.1) [1]. Progress in malaria control has occurred disproportionately in regions with low- to medium-transmission intensity, while areas with high baseline transmission have maintained large malaria burdens [11]. As of 2010, there continued to be 327 million people in sub-Saharan Africa living in high-risk areas for malaria, defined as having parasite prevalence above 40% among children aged 2-10 years [12]. Furthermore, the rate of progress has slowed or reversed in many areas. Global malaria incidence has increased yearly since 2015, largely in high-burden areas, and cases rose by approximately 5 million in 2016 for the first time since the 1990's [1, 13]. To maintain and restore progress in global malaria control and achieve elimination targets, the epidemiology and control of malaria in high-transmission areas must be investigated further.

Although there is a large degree of variation between sites, high-burden areas are characterized by stable parasite prevalence above 50% (hyperendemic) or 75% (holoendemic) among children aged 2-10 years [14, 15]. Regions that support high baseline transmission tend to be tropical, with supportive climates for malaria vectors including warm or hot temperatures throughout the year, and have substantial precipitation during at least one rainy season [16]. High transmission can persist due to a multiplicity of factors including insufficient vector control, limited health and transportation infrastructure, lack of health services and trained staff, rural location, afebrile infections, multiple vector species, insecticide or drug resistance, and lack of funding or political will for comprehensive malaria

control [1, 17]. Socioeconomic hardship is a consistent characteristic of high-transmission regions, and high malaria transmission has been implicated as both a cause and effect of poverty [18].

Challenges to malaria control in high-burden areas

High indices of transmission

There are many challenges to successful malaria control in areas with high parasite prevalence. In some regions, indices of malaria transmission are exceptionally high. The basic reproductive number R_0, or expected number of secondary cases from an infectious individual in a susceptible population, has been estimated to be up to 3,000 in some regions of sub-Saharan Africa [19]. The number of infectious bites per person per time, or entomologic inoculation rate (EIR), has similarly been recorded in excess of 1,000 per year [20], an average of almost three infectious bites per day. To interrupt transmission, it has been estimated that EIR must be less than 1 per year, and parasite prevalence rises rapidly above this value [20]. Prevalence over 50% has been recorded in sites where EIR is more than 15 per year, and prevalence between 15-40% has occasionally been recorded in areas with EIRs less than 1 per year [20, 21]. Therefore, in settings with high EIRs, the infectious contact between people and vectors (vectorial capacity) must be reduced substantially to see a noticeable reduction in malaria burden (Figure 1.2) [20-23]. Furthermore, multiplicity of infection is common in areas of high transmission [24]. If a considerable proportion of the population is exposed and superinfected with a new malaria parasite genotype before their previous infection has resolved, an extensive decline in transmission must occur before a decrease in cases is observed. Aggressive vector control measures will therefore be needed to reduce the burden of disease in these regions.

Multispecies vector ecology poses another challenge to malaria control in high-transmission settings. The dominant malaria vectors in sub-Saharan Africa are *An. funestus s.s.*, *An. gambiae s.s.*, and *An. arabiensis*, all efficient disease vectors. *An. funestus* and *An. gambiae* particularly tend to be

anthropophilic (feed on humans), endophagic (bite indoors), and endophilic (rest indoors) [25, 26]. If several of these species contribute to malaria transmission in one location, parasite prevalence tends to be high and vector control may be complicated due to differences in ecology as well as breeding, host-seeking, and biting behaviors between species [17]. For example, *An. gambiae* and *An. funestus* populations may peak at different times in relation to seasonal rains, making interventions harder to target temporally, and *An. arabiensis* often exhibits zoophilic behavior, which might confer some population-level resilience to indoor vector control measures [23, 27, 28]. In multispecies systems, the intersection of vector ecology and malaria epidemiology must be thoroughly investigated to inform appropriate control measures, otherwise interventions may inadvertently fail to address all sources of transmission.

Due to this differing ecology, multispecies systems may have extended transmission into the dry season [17, 27, 28]. A longer period of transmission may result in more infections and ongoing transmission past the peak effect of interventions, since malaria control activities like IRS are generally timed to occur before the start of the rainy season to most effectively reduce rainy-season malaria transmission. As a result, residual transmission into the following dry season undermines malaria control activities and prevents progress towards elimination. Dry season transmission has therefore been identified as a contributing cause of malaria control failure in both modeling and field studies, including in low-transmission areas and those with a single transmission season [29-31]. In high-transmission regions, where dry-season malaria transmission may continue at high levels, it is unlikely that addressing only rainy-season transmission will be sufficient to significantly reduce the malaria burden.

Intervention effectiveness

Another barrier to malaria control in high-transmission areas stems from the limitations of current interventions. The majority of malaria control activities are focused on case management and

the reduction of adult vector populations [1, 7]. However, high-burden areas are characterized by a large proportion of asymptomatic or afebrile infections among adults and older children, which can reduce healthcare-seeking and thus lead to chronic infection and a large human reservoir for ongoing transmission [32]. Furthermore, vector control interventions such as IRS and LLINs are generally limited to indoor biting and resting behavior while participants are asleep. These strategies do not account for improper bed net use, outdoor biting, outdoor resting, or indoor biting when a person is active, which may increase exposure to infectious mosquitoes. Only 54% of people at risk are estimated to sleep under a bed net, and studies have shown substantial outdoor biting behavior among *An. funestus* and *An. gambiae* if people are outside during active biting hours [1, 23, 31, 33]. The presence of indoor vector control interventions has also been shown to influence outdoor and daytime biting behavior of these vectors, perhaps due to behavioral selection pressures or insecticide avoidance [34-37].

In low- to moderate-burden areas, the efficacy of current methods may be adequate to interrupt transmission; however, in regions with high vector density and a large parasite reservoir in humans, the proportion of mosquitoes that avoid vector control measures may be sufficient to maintain high levels of transmission [23, 38]. Modeling studies have estimated that current vector control methods are insufficient to interrupt transmission in holoendemic areas, even at very high coverage levels [23, 29, 31, 39, 40]. Similarly, high vector pressure in these regions has led to rapid development of insecticide resistance, further reducing the effectiveness of vector control methods. The insecticidal action of DDT, pyrethroids, and carbamates has declined rapidly in sub-Saharan Africa in recent decades, leading to the need for novel chemicals to maintain the same level of vector control [1, 41]. The increased cost of these new formulations of insecticides may be prohibitive to achieving sufficient intervention coverage in many regions.

Human movement

Human movement patterns are another potential barrier to malaria control. Movement between high- and low-transmission areas contributes to continuing malaria transmission due to introductions of infectious individuals, and failure to account for these behaviors has been implicated in the failure of malaria elimination efforts [42-45]. Cross-border movement can facilitate heterogeneous exposures across transmission gradients due to differing control activities in different countries, and the porousness of borders in much of sub-Saharan Africa make these behaviors hard to measure or address [45-47]. Most research on this topic has focused on the impact of movement in areas with a low burden. However, heterogeneities in transmission exist at both large and small spatial scales within high-transmission regions; thus, local and cross-border movement patterns are expected to contribute to elevated malaria risk among individuals and populations [48-50]. Further investigation is warranted to determine the impact of population movement on malaria transmission in high-burden areas.

Accessibility and cost

Another challenge to malaria control in high-transmission regions is accessibility. Many regions with the highest malaria burdens are difficult to target for interventions due to political instability, poor transportation infrastructure, lack of sufficient health systems, or socioeconomic hardship [16, 51]. Provision of health care is challenging in remote settings, particularly during conflict, and local governments might not have the capacity to prioritize malaria interventions [52, 53]. The lack of maintained transportation networks makes delivery of medication and supplies difficult, especially for environmentally controlled insecticides, and this issue can be exacerbated due to flooding during seasonal rains. Indicators of poverty, such as housing construction and sanitation practices, may attenuate the impact of malaria control interventions, and financial constraints can reduce health-seeking behaviors [18]. Scarcity of trained staff to administer clinical care or implement intervention

programs can be an obstacle to progress, and surveillance systems in these regions are often inadequate or absent [52-55]. Given these issues, sustained control programs are not presently feasible in many settings given current funding, manpower and infrastructure challenges.

As a result of these overlapping factors, successful malaria control in high-transmission areas is expected to require an extremely high financial investment, which provides another significant barrier to control. The intensity of transmission necessitates high levels of intervention coverage, and the cost of implementation increases substantially as remaining hard-to-reach populations are sought out for inclusion. The need for novel insecticides will further increase required spending. A primary challenge for malaria control in these regions is the capacity for rapid resurgence of transmission if control measures lapse, and pre-intervention levels of parasite prevalence may return in a few years without sustained programs [56]. However, funding long-term interventions at sufficiently high coverage in high-transmission areas is challenging and requires secure funding streams to ensure consistent provision of programs and services.

Strategies for malaria control in high-burden areas

Given these challenges and barriers, the question remains of how best to implement malaria control activities in high-transmission regions. As mentioned, modeling studies have demonstrated that current methods are insufficient to interrupt transmission in holoendemic areas, even with high coverage [16, 23, 29, 31, 39, 40]. However, some strategies can reduce disease burden substantially, particularly if multiple methods are used together for a synergistic effect. Vector control continues to be a key priority, and LLINs are estimated to be the most cost-effective method to reduce malaria burden [40]. Current vector control recommendations include rapid scale-up of LLIN coverage to at least 80% and twice-yearly IRS with at least 85% coverage, which would target all vector species and both rainy- and dry-season transmission [29, 30, 39]. Achieving this level of IRS coverage is difficult in remote areas,

and requires intensive planning and investment to train staff, develop logistics, secure equipment, and sensitize communities to the intervention.

Vector control methods alone are expected to provide only a moderate reduction in prevalence in holoendemic settings, so steps must be taken to reduce the infectious human reservoir. Prompt diagnosis and treatment with ACTs should continue to be prioritized, but due to the high proportion of afebrile cases in high-transmission areas, treatment of only care-seeking individuals will be insufficient to appreciably reduce malaria prevalence [32]. Options to target asymptomatic individuals include IPT of pregnant women and children, mass screen and treat (MSAT), focal screen and treat (FSAT), and mass drug administration (MDA) [32, 40, 57]. MSAT aims to test all members of a population and treat parasitemic individuals with ACTs, FSAT does this for sub-populations that are thought to be at high risk, such as school children or migrant workers, and MDA aims to give a curative dose of ACTs to all members of a population without testing. MDA is anticipated to be more successful and cost-effective than MSAT in high-burden areas, and the inclusion of gametocidal drugs such as primaquine would further reduce transmission, although there are toxicity risks in some populations [40, 57-59].

In past trials of MDA in high-burden areas, with or without combinations of other interventions, results have generally shown substantial but transitory decreases in malaria indices and failure to interrupt transmission [60-62]. For instance, in the Garki Project in Nigeria in the 1970's, the addition of several rounds of MDA with sulfalene-pyrimethamine to a carbamate IRS campaign significantly reduced parasite prevalence and incidence more than IRS alone, but transmission returned to pre-intervention values within two years of stopping the intervention [60]. Due to the consistent risk of resurgence, MDA and all chemoprevention interventions must include multiple rounds with high coverage over a sustained period of time, and still may not fully interrupt transmission.

The combinations of these interventions over time are predicted to reduce parasite prevalence to approximately 10% in high-burden areas [29, 40]. Although not enough to interrupt transmission, this decline would represent a large number of cases averted and a massive reduction in malaria burden. However, it is predicted that this lower prevalence level would be unstable, necessitating indefinite continuation of control interventions to prevent resurgence and requiring a substantial financial investment over many years [29]. At this time, the WHO is not recommending MDA in high-transmission areas due to the risk of developing multi-drug resistance [63]; however, these strategies may be employed in the future with combination therapies if appropriate methods are developed. To further reduce transmission, vector control methods that target outdoor-biting or resting mosquitoes must be established to augment existing strategies [38]. These could include spatial or personal repellents, outdoor applications of insecticides, toxic sugar baits, odor-baited traps, and improved larval control [23, 38].

Surveillance and evaluation

Another noteworthy issue in high-transmission areas is the paucity of systematic malaria surveillance [54, 55], due in part to the aforementioned challenges in these regions. Due to the high risk of resurgence and the substantial investment required for malaria control in these regions, careful monitoring of parasite incidence and prevalence is essential to track progress or lapses in progress. In addition, high transmission may persist for a variety of reasons in different settings, and epidemiologic and entomologic investigations are needed in areas of differing ecology and economic development to discern the individual causes of residual transmission.

In part due to the lack of long-term surveillance data, many studies investigating optimal intervention methods for high-transmission areas have been conducted using models of simulated data rather than direct observation. These models are essential to help prioritize which combinations of interventions should be implemented, but few rigorous epidemiologic field evaluations of intervention

strategies have been conducted in high-burden areas. To properly inform malaria control strategies, an increase in field-based intervention evaluations is needed to supplement and inform mathematical models. However, malaria and vector dynamics vary naturally over time due to seasonality, climactic variation, and temporal trends, so long time series of surveillance data are necessary to establish baseline patterns in advance of interventions. Evaluations should also occur at varying points throughout the year to control for these fluctuations, rather than immediately following an intervention, which may confound results.

Furthermore, due to issues such as multiplicity of infection, high vector densities, and lack of symptoms, there are several epidemiologic challenges to accurately discern the impact of interventions in these regions [55]. Hospital and clinic records are an important benchmark of malaria surveillance and can provide information on changes in incident infections, but they are limited by health-seeking behavior, availability of accurate testing methods, and the proportion of afebrile infections. Conversely, serial community surveys using RDTs or microscopy can identify asymptomatic infections and determine changes in population disease burden over time, but they are prone to sampling bias and low diagnostic sensitivity for low-level parasitemia. Prevalence surveys also cannot identify the time of infection, which can attenuate the apparent impact of interventions. Emerging methods show promise for both surveillance and intervention evaluation. Genomic methods can provide information on transmission intensity and importations, and serologic surveys can provide markers of age at first infection and intensity of exposure [64-66].

Changes in vector abundance are typically measured through a series of household surveys before and after the intervention using baited or un-baited light traps, pyrethrum spray catches (PSCs), or vacuum aspiration. However, due to the difficulty of collecting live mosquitoes and the high degree of manpower required to process samples, these methods may suffer from low sensitivity and low sample sizes, and therefore may have low statistical power. Furthermore, despite the importance of

surveillance for both epidemiologic and entomologic outcomes, these data sources are rarely linked. As molecular methods for both parasite prevalence and vector identification improve, the interaction between vector dynamics and parasite prevalence must be further explored, particularly in the context of successful and unsuccessful malaria control. Given the limitations of these options, a variety of surveillance and evaluation methods are needed to accurately determine the impact of interventions in high-burden settings.

DESCRIPTION OF CURRENT RESEARCH

The aims of this research were to: 1) describe the dynamics of vector abundance and malaria epidemiology in a high-transmission setting in southern Africa and determine the relationship between vector abundance and malaria epidemiology; 2) evaluate a novel targeted IRS strategy in this region; and 3) describe the impact of human movement on malaria risk in a high-transmission setting. These analyses were conducted within the context of the Southern and Central Africa International Centers of Excellence for Malaria Research (ICEMR) in Zambia.

Malaria remains a leading cause of child morbidity and mortality in Zambia [1, 67]. Under Zambia's National Malaria Control Programme, scale-up of malaria control measures has reduced transmission considerably since 2000, with key strategies including free malaria rapid testing and treatment, LLIN distribution in antenatal and vaccination clinics, and yearly IRS in selected areas [68, 69]. However, despite these activities, high-burden regions persist in the north of the country, and there has been a resurgence of cases in recent years (Figure 1.3) [70, 71]. The heterogeneity of malaria control under the same intervention policy, with reversal of progress in high-burden regions, indicates the need to further investigate risk factors for malaria transmission and barriers to control in different epidemiologic settings.

To investigate trends in malaria transmission and inform intervention policy, the Southern and Central Africa ICEMR conducts active and passive surveillance in Nchelenge District, Luapula Province, northern Zambia. Nchelenge District is a holoendemic area with year-round malaria transmission and a resurgence in cases in recent years. The prevalence of malaria increased from 38% in 2006 to 53% in 2012 despite LLIN distributions and yearly IRS campaigns from 2008-2012 [72]. Since 2012, the prevalence of malaria by RDT averaged approximately 70% in school-age children, and the EIR is estimated to be 140 infective bites per year [73, 74]. The predominant malaria vectors in Nchelenge District are *An. gambiae s.s.* and *An. funestus s.s.*, which peak at different times of year in relation to the single rainy season [75, 76]. Malaria control activities in this region are consistent with the national policy described above. In 2014, a targeted IRS campaign was initiated in Nchelenge District using a novel formulation of the organophosphate insecticide pirimiphos-methyl following identification of vector resistance to pyrethroids, DDT, and carbamates [72, 77-79].

This setting provides an opportunity to conduct research in an area of ongoing high transmission despite active malaria control. Concurrent epidemiologic and entomologic active surveillance began in 2012, providing a long time series of both vector abundance and malaria prevalence. To determine risk factors for transmission, demographic, geographic, and climatological predictors of vector abundance and parasite prevalence were identified, and the relationship between vector abundance by species and malaria epidemiology was described. An evaluation was conducted of a three-year targeted IRS intervention using two years of baseline surveillance data to determine the focal and district-level impact of this vector control strategy in a high-transmission setting. Global positioning systems (GPS) data loggers were used to describe patterns of population movement in Nchelenge District and investigate the impact of movement on malaria risk. The overall goals of this research were to contribute to the body of knowledge on malaria epidemiology in high-transmission areas and inform the timing and strategy of interventions in this setting.

Figure 1.1: Annual malaria incidence per thousand people per year among children aged 2-10 in sub-Saharan Africa, data courtesy of Malaria Atlas Project

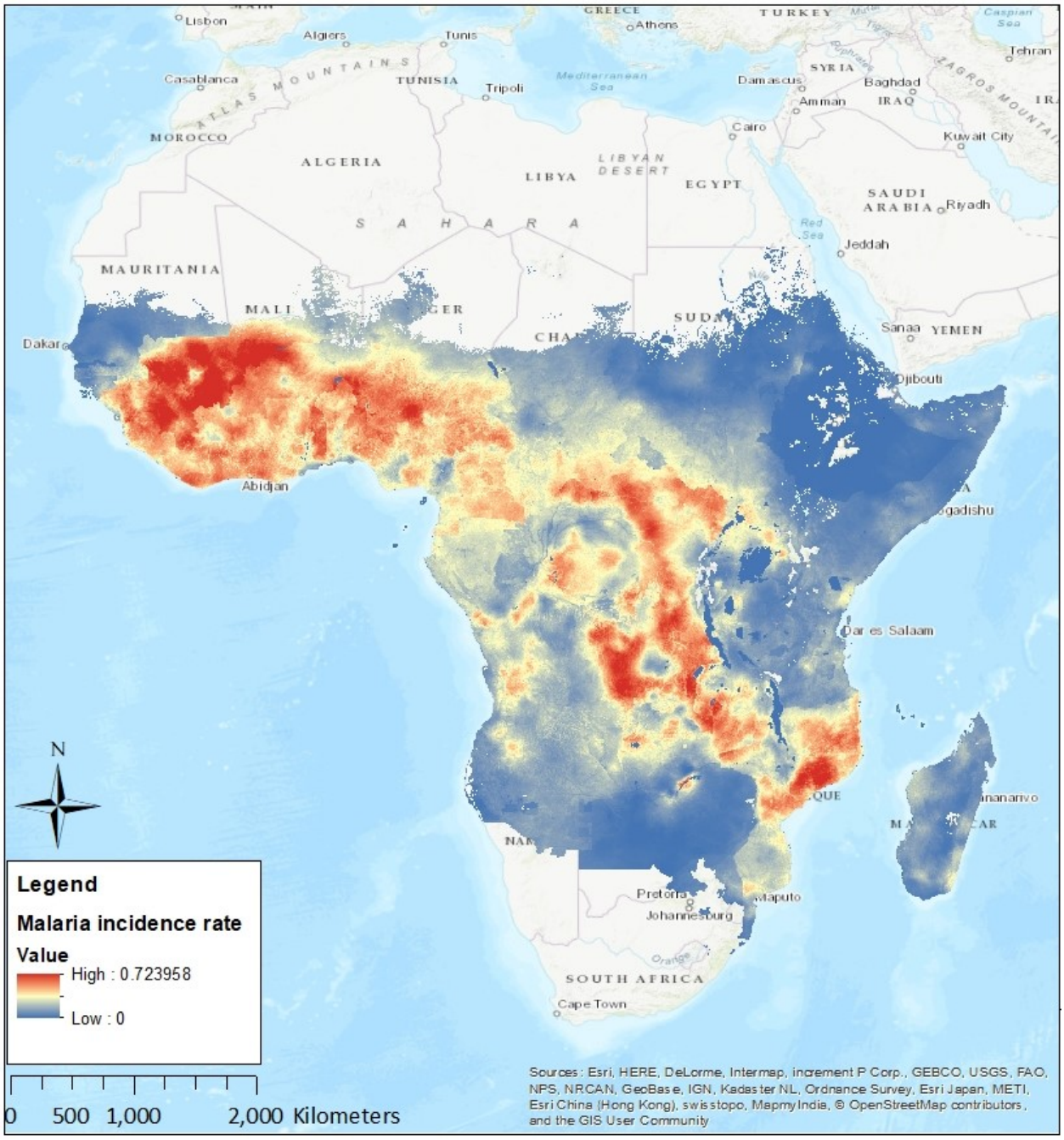


Figure 1.2: Relationship between annual EIR and prevalence of *P. falciparum* from 31 sites throughout Africa on A) linear and B) logarithmic scales, courtesy of: Beier, J.C., G.F. Killeen, and J.I. Githure, *Short report: entomologic inoculation rates and Plasmodium falciparum malaria prevalence in Africa.* Am J Trop Med Hyg, 1999. **61**(1): p. 109-13.

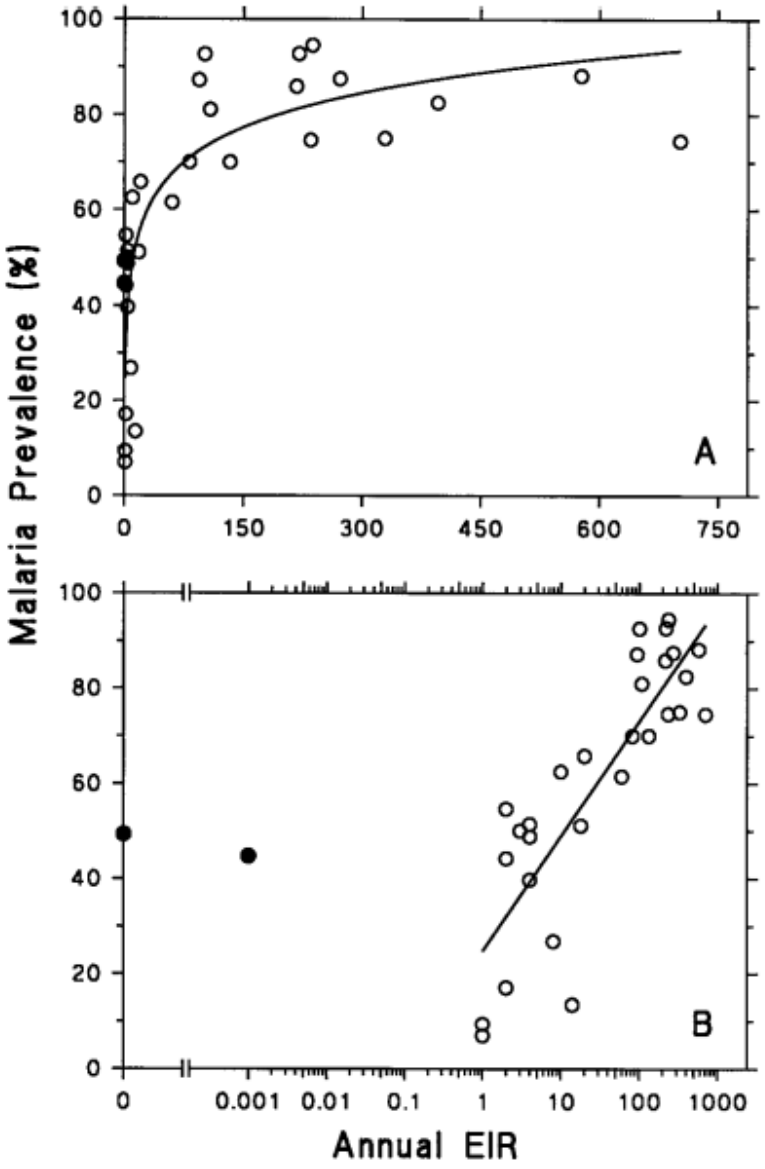
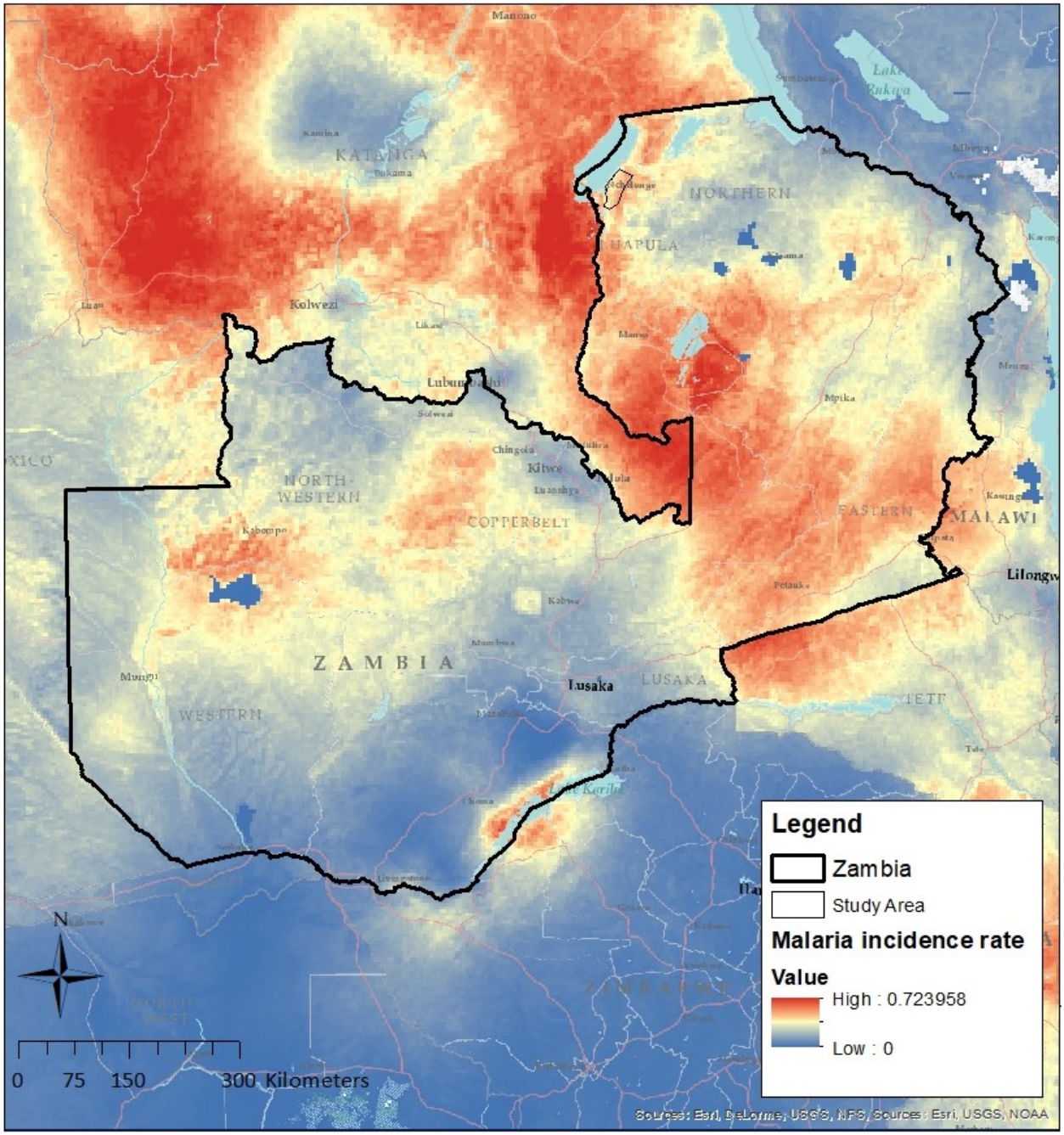


Figure 1.3: Annual malaria incidence rate per thousand people per year among children aged 2-10 in Zambia, data courtesy of Malaria Atlas Project



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Chapter 2: Characterizing the impact of vector abundance on malaria prevalence in a high-transmission area of northern Zambia

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ABSTRACT

Background: Malaria transmission is dependent on the density and distribution of mosquito vectors, but the relationship between vector abundance and malaria risk has not been adequately studied across a range of transmission settings. To inform intervention strategies for high-burden areas, further investigation is needed to identify predictors of vector abundance and to determine the impact of vector population dynamics on malaria epidemiology.

Methods: Active household surveillance was conducted in Nchelenge District, northern Zambia, a high-transmission setting with two dominant malaria vectors, *Anopheles gambiae s.s.* and *An. funestus s.s*. Between April 2012 and July 2017, mosquitoes were collected during household visits using Centers for Disease Control and Prevention (CDC) light traps, and malaria parasitemia was measured using rapid diagnostic tests (RDTs). Demographic, geographic, and climatological correlates of vector abundance and parasite prevalence were identified using regression models with robust standard errors. The relationship between household vector counts and parasite prevalence was estimated using regression models with appropriate time lags for vector counts, stratified by season. Variance in all models was adjusted for clustering of participants within households and repeat household visits.

Results: Among 3,520 individual participants, parasite prevalence averaged 50% across all ages and 60% in children under 5 years. Nearly 14,000 female anopheline mosquitoes were collected from 1,724 household visits, with a mean of 7 *An. funestus* and 0.8 *An. gambiae* per visit. Household vector counts were associated with parasite prevalence. In the rainy season, there was a 30% increase in the risk of parasitemia for each additional 10 *An. gambiae* mosquitoes, lagged by 1 month, and a 5% increase in risk with each additional 10 *An. funestus* mosquitoes, lagged by 4 months. In the dry season, there was a 2% increase in risk with each additional 10 *An. funestus* mosquitoes but no association with *An. gambiae*. Parasitemia and vector counts were associated with residence in rural areas, rudimentary

household construction, and lack of vector control interventions, including long-lasting insecticide-treated bed nets (LLINs) and indoor residual spraying (IRS).

Conclusion: Malaria control activities must be informed by vector population dynamics. In high-burden areas with multiple vector species, the contribution of each vector to transmission may be complex, necessitating appropriate vector control measures to interrupt transmission. In Nchelenge District, dry season vector abundance was high, and both rainy and dry season vector abundance impacted rainy season parasite prevalence, indicating that vector control is required year-round to achieve successful malaria control.

INTRODUCTION

Malaria transmission is dependent on the density and distribution of malaria vectors, and vector control has been identified as the key priority in reducing global malaria burden [1, 2]. Following the launch of the Roll Back Malaria Initiative in 1998, which pledged to halve the malaria burden by 2010 and again by 2015, there was a worldwide scale-up of malaria control activities including significant expansion of vector control and improved case management [3, 4]. With the addition of the Global Fund to Fight AIDS, Tuberculosis, and Malaria (GFTAM) in 2002 and the President's Malaria Initiative (PMI) in 2005, global malaria funding increased substantially between 2000 and 2015 and malaria cases and deaths fell by 41% and 62% worldwide [4-6].

While this reduction was substantial, the rate of progress fell short of stated goals, and gains in malaria control have been uneven both between and within countries. Regions with the highest burden have maintained high or resurgent transmission levels, and global cases have increased yearly since 2015 [1, 7]. In 2016, malaria cases rose by an estimated 5 million from the previous year, the first yearly increase of this magnitude since the 1990's [1, 8]. This reversal of progress indicates that current intervention strategies and coverage are not sufficient to reach global malaria reduction goals. In order to achieve successful malaria control, further research is needed to investigate appropriate and sustainable vector control methods across a variety of transmission settings.

Current strategies and benchmarks for integrated vector control include improved coverage of long-lasting insecticide-treated bed nets (LLINs), expanded indoor residual spraying (IRS), and management of insecticide resistance using sustainable and evidence-based policies [2, 9-11]. Where appropriate, larval source management and personal protection are also integrated into malaria control policies [2, 12]. In combination with rapid diagnostic testing (RDTs), artemisinin-combination therapies (ACTs), and intermittent preventive treatment (IPT) of pregnant women and children, these strategies have led to substantial reductions in the malaria burden throughout sub-Saharan Africa [1, 10].

However, improvements have occurred disproportionately in regions with low or moderate malaria transmission, with less success in high-transmission areas despite aggressive control activities [1, 7]. Moreover, increasing vector resistance to pyrethroid and carbamate insecticides and parasite resistance to artemisinins threatens to further reverse progress across epidemiologic settings [11, 13, 14].

In response to continuing transmission in the presence of active vector control, further investigation is needed on the failures and limitations of vector control strategies in areas of high transmission. One knowledge gap that has yet to be fully explored is the relationship between vector population dynamics and malaria epidemiology. The distribution and abundance of different malaria vectors vary by time and space at both large and small scales, and these patterns have been shown in modeling analyses to impact individual malaria risk [15-17]. However, few studies have incorporated this level of spatial and temporal heterogeneity concurrently for both entomology and epidemiology. Indices of vector-to-human transmission, such as the entomologic inoculation rate (EIR, average infectious bites per person per year) or the sporozoite rate (proportion of mosquitoes testing positive for sporozoites), are generally defined as a single number over the entire population without respect to local ecology, seasonality, or other variations in risk. Conversely, studies that examine risk factors for household vector counts or vector population dynamics rarely link these data to human malaria indices, such as parasite prevalence or incidence. To better design vector control interventions for unique epidemiologic settings, the relationships between malaria transmission and complex vector dynamics must be further elucidated.

The body of literature directly linking vector dynamics and malaria transmission is sparse. Modeling studies have included vector abundance or EIR as predictors of malaria transmission and have identified positive correlations between vector numbers and malaria risk [18-22]. However, these relationships have rarely been directly observed. Several papers show either observational or statistical correlations between vector abundance and parasite prevalence or incidence in Africa [23-27] and the

Amazon [28-32], but these associations are generally at the community level and are variable with regard to the duration of time investigated, vector collection, statistical methods, and incorporation of temporal delays between vector catches and malaria outcomes. Other studies using field-collected data failed to show a correlation or showed a negative correlation between vector abundance and malaria risk [33, 34].

There are several explanations for the scarcity of these types of studies. Most importantly, sufficiently long-term time series of epidemiologic and entomologic surveillance data are rare and infrequently collected concurrently due to the differing methodologies between entomologic and epidemiologic investigations [35]. The lack of long time series is a barrier to accurate model parameterization and limits the ability to account for short-term anomalies and longer temporal trends. Time series are also needed to adequately account for lags between vector collections and epidemiologic outcomes due to the length of mosquito and parasite life cycles and resulting delays between vector exposure and parasite detection. In addition, collection and processing of mosquitoes is labor-intensive, particularly for prolonged surveillance systems, and vector data are often difficult to statistically model due to the high number of zero counts and overdispersion. However, these basic relationships must be explored to properly time and target control measures for maximum impact.

Zambia is a country of particular interest to identify optimal vector control strategies for malaria control and elimination. Despite country-wide scale up of malaria control activities, including widespread LLIN distributions, expanded IRS, and free rapid testing and treatment with ACTs, there was a resurgence of cases in 2009 after nearly a decade of steady decline [36]. Cases increased nearly every subsequent year, and the country reported 3.1 million cases and 7,000 deaths in 2016, an increase of nearly a million cases from 2010 [1]. This resurgence was largely driven by provinces northeast of the border with the Democratic Republic of the Congo (DRC), while prevalence in Lusaka and the southern provinces continued to decline [37-39]. The heterogeneity of malaria control under this comprehensive

vector control strategy and the growing burden of disease in the north indicates a need to further investigate vector dynamics across malaria transmission settings in Zambia.

The primary malaria vectors in Zambia are members of the *Anopheles funestus* and *An. gambiae* complexes, which are predominantly anthropophilic (feed on humans), endophagic (bite indoors), and endophilic (rest indoors) [40, 41]. Ecologically, *An. gambiae* mosquitoes are dependent on rainfall, with populations typically peaking during the rainy season and breeding in temporary and man-made pools such as puddles, hoof prints, or tire tracks [40-43]. *An. funestus* mosquitoes prefer breeding in permanent bodies of fresh water, especially those with emergent or floating vegetation such as swamps, river banks, ponds, and marshlands [40, 41]. They are therefore more tolerant of dry weather conditions and can breed year-round in permissive environments, with populations often peaking directly after the rainy season ends [41-44]. Due to their preference for indoor biting and resting behavior, both mosquito species are vulnerable to indoor vector control interventions, including LLINs and IRS. However, increasing resistance to pyrethroids, DDT, and carbamate insecticides has reduced the efficacy of these interventions [39, 45].

The Southern and Central Africa International Centers of Excellence for Malaria Research (ICEMR) conducts active malaria surveillance in Zambia [38]. This study was based at a high-transmission site in Nchelenge District, Luapula Province in northern Zambia. In Nchelenge District, concurrent epidemiologic and entomologic surveillance have been conducted since 2012, providing a rare opportunity to link these data streams in a high-burden, multi-vector area over time. This study aimed to determine correlates of household vector abundance and malaria prevalence in this setting, and to define the relationship between multispecies vector abundance and patterns in malaria prevalence to inform malaria control interventions.

METHODS

Study Site

This study was conducted in Nchelenge District, Zambia by the Southern and Central Africa ICEMR. The project is led by the Johns Hopkins Malaria Research Institute (JHMRI) in collaboration with the Zambian Ministry of Health's Tropical Disease Research Center (TDRC), the Macha Research Trust (MRT), and other partners to investigate heterogeneity in malaria burden and transmission dynamics across distinct epidemiological settings in sub-Saharan Africa [46]. The study site in Nchelenge District represents high malaria transmission and ineffective control. As per Zambia's National Malaria Control Programme, malaria control strategies in this region include free malaria rapid testing and treatment, LLIN distribution in antenatal and vaccination clinics, and yearly IRS in selected areas using pyrethroid (2008-2010), carbamate (2011-2012), and organophosphate insecticides (2014-present) [47, 48]. Despite these activities, this region continues to experience holoendemic transmission, with a resurgence of cases since 2009 and an average malaria prevalence of approximately 70% in children under 17 years [39, 49].

Nchelenge District is located in the marshlands along Lake Mweru and the Luapula River, one of the early tributaries of the Congo River. These waterways form the border with the DRC to the west, and there is a land border to the north across which formal and informal movement is common. The inland area has an extensive stream network leading to the lake, and the lakeside and riverbank regions include swamplands that remain inundated with water throughout the year. This environment supports year-round malaria transmission due to the presence of both *An. gambiae s.s.* and *An. funestus s.s.*, two highly efficient malaria vector species with differing ecologic patterns and distributions across the study area [50, 51]. Both vectors exhibit a strong preference for feeding on people and have relatively long adult life spans (mean of 23 days for *An. gambiae*, 28 days for *An. funestus*), which can facilitate rapid and widespread malaria transmission [40, 52, 53]. *An. funestus* is the principle vector in Nchelenge

District, with high household vector counts collected throughout the year and a peak immediately following the end of the rainy season, which lasts from October to April. *An. gambiae* peaks at smaller numbers during the rainy season, particularly in the areas near the lake. Across both species, the cumulative EIR is estimated to be 140 infective bites per year [54]. Household construction in this region is generally rudimentary, with most people living in huts with natural flooring and walls, thatch roofs, and open eaves, and a smaller number living in finished housing with metal roofs and concrete walls.

Data Collection

Households were selected for active surveillance through a modified cluster sampling design. Quickbird™ satellite images of the study areas were purchased (DigitalGlobal Services, Denver, CO), and a 1x1 kilometer grid was overlaid on the study area in ArcGIS Version 10.2 (ESRI, Redlands, CA). Households were enumerated (Figure 2.1), and grid quadrants were selected using spatially-balanced random sampling to ensure inclusion of the full range of population density and ecology in the region. Households were randomly selected into either cross-sectional or longitudinal cohorts using population proportional to size sampling. Each month, between 1 and 6 households were selected per grid quadrant. If a grid quadrant ran out of houses to sample, an adjacent grid quadrant was selected. For longitudinal cohorts, 25-30 households were visited every other month for one year and then replaced with a new longitudinal cohort. In each alternating month, approximately 25 new households were recruited into cross-sectional cohorts.

At household visits, a questionnaire was administered to each consenting household member aged 16 and older and to guardians of children under 16 years. The questionnaire collected household coordinates, demographic information, history of recent malaria and treatment, reported LLIN use, history of household IRS, and malaria knowledge and practices. Participant temperature was taken using a digital ear thermometer, and a blood sample was collected by finger prick for hemoglobin testing,

RDTs to detect *Plasmodium falciparum* HRP-2 antigen, and dried blood spot collection for detection of *P. falciparum* DNA using polymerase chain reaction (PCR) to detect *Pfcytb* [55, 56]. Several RDTs were used throughout the study period in accordance with changing national policy; these included ICT (ICT Diagnostics, Cape Town, South Africa) from April 2012 to May 2013, First Response (Premier Medical Corporation LTD., Mumbai, India) from June to September 2013, and SD Bioline (Standard Diagnostics, Kyonggi, Republic of Korea) from October 2013 to the present. All participants with a positive RDT were treated with Coartem® (Novartis, Basel, Switzerland), the first-line standard of care in Zambia.

Mosquitoes were collected at each household visit. The evening before, study staff visited the selected household and placed Centers for Disease Control (CDC) light traps (John W. Hock, Ltd, Gainesville, FL) in sleeping spaces of participating households to collect mosquito vectors overnight. Traps were hung by a bed covered with an LLIN, and household participants were instructed to turn traps on at 18:00 and turn them off at 6:00 the following morning. Traps were collected at the time of the study visit the following day and transported to the TDRC field station in Kashikishi township for preliminary processing. Mosquitoes were killed by freezing, identified morphologically to genus and sex, enumerated, and stored dry on silica. Samples were transported to TDRC headquarters in Ndola once per month for final laboratory identification using standard morphological keys [57, 58] and molecular identification methods [59, 60]. More detailed methods of vector identification are described elsewhere [51].

Climatological data were collected from a HOBO Micro Station (Onset Computer Corporation, Bourne, MA) located on the grounds of the TDRC field station and from the African Flood and Drought Monitor (AFDM) online tool [61, 62]. Climate data were aggregated by day from January 2012 to July 2017. Both weather variables (rainfall in mm/day, minimum and maximum daily temperature in C°, windspeed in m/s) and hydrological variables (evaporation in mm/day, streamflow in m^3/s, percent soil moisture) were collected.

Data management

Survey and entomologic data collected at participating households were uploaded into REDCap secure file-sharing software and downloaded as .csv files [63]. Individual malaria status was determined by RDT results, and fever was defined as having a temperature above 38° C. Anemia was determined by WHO criteria for hemoglobin levels by age and sex [64].

Household locations were plotted in ArcGIS (Figure 2.1), and geographic covariates were created. Population density was calculated as the number of households within a 500-meter buffer around each participating household. Household elevation, slope, and normalized difference vegetation index (NDVI) were extracted from raster files downloaded from the Shuttle Radar Topography Mission (SRTM) version 3 and from LandSat 5 data [49]. NDVI values range from -1 to +1, with negative values corresponding to bodies of water and positive values increasing with increasing photosynthetic vegetation, or "greenness." Stream networks were developed using the SRTM elevation data in the ArcHydro Tools module of ArcGIS, as described previously [65]. Streams were classified as category 1-4 using the Strahler classification system, in which the smallest streams are defined as category 1, which join to form a category 2 stream, and so on [66]. The distances to roads, health clinics, Lake Mweru, and category 1-4 streams were calculated for each household. To investigate spatial distributions, vector counts were merged to sampling grid quadrants and plotted over the study area as a function of absolute and relative abundance by species and season.

Based on observed patterns in household density, households were classified as being in the lakeside area if they were within 3 km of Lake Mweru and as being in the inland area if they were further than 3 km from the lake. Shapefiles for the area targeted for IRS with pirimiphos-methyl were provided by the NGO Akros, based in Lusaka, Zambia [67].

Statistical analysis

The primary aims of this analysis were to determine correlates of malaria prevalence and household vector counts by species in Nchelenge District, and to determine the relationships between vector abundance by species and malaria prevalence. Data were analyzed using STATA 13.1 (Stata-Corporation, College Station, TX) and R version 3.4.2 (R Core Team, Vienna, Austria).

All epidemiological, entomological, geographic, and climatological data were merged by household and day. Epidemiological analyses were restricted to cross-sectional households and the first visit to longitudinal households to identify prevalent infection with *P. falciparum*. Epidemiologic models were also stratified by season to account for the different transmission dynamics in the rainy and dry seasons. The start and end of the rainy season each year was defined as the first and last weeks in which the average rainfall exceed 1 mm. Sensitivity analyses using different cutoffs and time intervals were conducted to ensure that this definition best represented the epidemiologic and entomologic relationships in this region. Vector count analyses were not restricted to first household visit because repeat visits were not shown to impact vector counts in adjusted analyses, so vector data from all longitudinal and cross-sectional visits were included. To preserve analytical power, vector models were not stratified by season due to the high degree of variation and overdispersion in the vector counts.

To explore correlates of household vector abundance, bivariate comparisons were conducted by species using negative binomial models with robust standard errors [68, 69]. The level of analysis was the household, with vector counts by species as the outcome. Generalized estimating equations (GEE) were used to account for repeat visits to longitudinal households [70, 71]. Potential household-level covariates included self-reported history of household IRS with pirimiphos-methyl, natural vs. finished household flooring, open vs. protected household water source, head of household (HOH) attending only primary school, HOH in permanent employment, roof type (thatch vs. metal) and presence of open

vs. closed household eaves. Geographic covariates as described above included households within 500 m, NDVI, elevation, slope, and distances to roads, health clinics, Lake Mweru, and category 1-4 streams. Household demographics were also considered, including the number of household occupants, proportion of occupants who slept under a bed net, and the proportion of occupants who were male or under age 5 years. Multivariate vector models were also developed using GEE negative binomial regression with robust variances.

To explore correlates of malaria prevalence, bivariate models were developed with individual malaria status by RDT as the outcome. Due to the high prevalence of malaria in the study population, the odds ratio estimated in logistic regression would overestimate the magnitude of relative risk, so models were run using the Poisson estimation of the binomial distribution with robust standard errors, which can directly estimate the prevalence rate ratio (PRR) [72-75]. Epidemiologic models also used GEE to account for clustering of individuals within households. All household-level and geographic variables described above were considered, as well as participant sex and age category (<5, 5-16, >16 years). Multivariate epidemiologic models were also developed using GEE Poisson models with robust variances.

For both vector and epidemiologic multivariate models, weather and hydrology covariates were included to account for drivers of interannual variation and temporal trends. The impact of climatological variables was expected to be delayed due to the duration of vector and parasite life cycles, but the optimal time lags to predict vector density and malaria prevalence have not been explored in this setting. A cross-correlation framework was developed in previous studies to explore the impact of weather variables on vector-borne disease at various time intervals and lags, and this approach was adapted for this analysis [76, 77]. The average value of each weather and hydrology variable was calculated at time intervals of 1-12 weeks and lags of 1-12 weeks from each day of data collection, returning a total of 144 potential variables for each climatological factor. For each outcome

(vector counts by species or parasite prevalence by RDT), the most predictive combination of covariates was identified using random forest algorithms in in R version 3.4.2 (R Core Team, Vienna, Austria), which are designed to handle a large number of collinear variables [78, 79]. These variables were confirmed in fully adjusted models using stepwise regression and AIC optimization methods [80, 81]. The variables for roof type and household eaves were measured in only a subsample of visited households and were excluded from multivariate analyses.

In preparation to fit models incorporating both vector counts and malaria prevalence, several challenges related to the vector data were encountered. Due to the lag between an infectious bite and malaria onset (approximately 6-23 days [82]) and the potentially long duration of prevalent infection [83], mosquitoes collected at the study visit were unlikely to have infected sampled participants. Therefore, simultaneous household vector counts may not be the most relevant measurement of vector abundance to predict malaria risk. However, due to the design of the surveillance system, vector collections were not conducted in the same household in consecutive months, so vector abundance from a more epidemiologically relevant time window was unknown. Furthermore, household vector counts may be underestimated due to the stochastic chance that any individual mosquito fails to be captured by the trap. To account for these discrepancies, several methods of spatial and temporal smoothing were performed with the intent to estimate household vector counts at the time of infection (Figure 2.2).

The variance within and between sampling grid quadrants was compared by month and year of collection to determine if household vector counts were spatially correlated, such that households near the house of interest should be more similar than households farther away. To account for stochasticity in nightly mosquito catches, estimated vector counts per household were adjusted using a random intercept at the grid quadrant-level for the month of collection (Figure 2.2). The expected vector count

by species $E(\widehat{M}_{jkt})$ for each household j, in grid quadrant k, at time t was calculated as an exponential function of the grid quadrant-level average for that month μ_{kt} plus a household-level offset β_{jkt} (Equation 1), which was normally distributed around zero with standard error σ (Equation 2). The observed vector count by species M_{jkt} was assumed to follow a Poisson distribution around $E(\widehat{M}_{jkt})$ (Equation 3).

Equation 1: $E\left(\widehat{M}_{jkt}\right) = \exp(\mu_{kt} + \beta_{jkt})$

Equation 2: $\beta_{jkt} \sim N(0, \sigma)$

Equation 3: $M_{jkt} \sim Poisson\left(\widehat{M}_{jkt}\right)$

Similarly, household vector abundance at the unknown time of infection $M_{jk(t-n)}$ at time t minus lag n was assumed to be correlated with both the adjusted household vector count $\widehat{M}_{jkt}$ and the grid quadrant-level mean at the time of infection $\mu_{k(t-n)}$. However, the optimal time lag n to model vector abundance on malaria prevalence has not been defined for this setting, and the degree to which each factor should be weighted is unknown. Time lags were thus explored between 0-5 months, with each factor weighted at 10%, 25%, 50% 75%, or 90%. The weight for the adjusted value $\widehat{M}_{jkt}$ was defined as α_1 and the weight for the grid-level average $\mu_{k(t-n)}$ was defined as $\alpha_2 = (1 - \alpha_1)$. These 30 pairs of values were compared using a modified cross-correlation analysis by season with the probability of a positive RDT as the outcome. This process was repeated with the unadjusted observed household count M_{jkt} as a sensitivity analysis. For each vector species, the estimated risk of malaria infection $E(Y_{ijkt})$ for individual i, in household j, in grid quadrant k, at time t would be best predicted by $(\alpha_1\widehat{M}_{jkt} + \alpha_2\mu_{k(t-n)})$ in addition to any other relevant variables X (Figure 2.2), modeled as:

Equation 4: $logE\left(Y_{ijkt}\right) \sim [\beta_0 + \beta_1\left(\alpha_1\widehat{M}_{jkt} + \alpha_2\mu_{k(t-n)}\right) + \beta_2X_2 + \beta_3X_3 \ldots]$

A simple mediation analysis was conducted to further guide the development of models. Several covariates were expected to be associated with parasite prevalence due in part to their direct effect on vector abundance, so these should be excluded from models investigating the impact of vector counts on malaria risk. To explore these relationships, each covariate that predicted either vector abundance or parasite prevalence at the P=0.1 level was individually added to robust Poisson GEE models with parasitemia by RDT as the outcome and containing only vector predictors. Any covariate that attenuated the relationship between vector abundance and parasite prevalence by more than 10% was excluded from further models to isolate the effect of the primary relationship of interest. Models were fit by season with the remaining covariates using stepwise regression and AIC optimization. As a sensitivity analysis, all relevant covariates were added back into the models to determine the overall best predictors of malaria prevalence in this population from all available data.

RESULTS

Climate and geography

Over the course of the study period, there was a mean of 6.8 mm/day of precipitation in the rainy season (range 0-64.6) and 0.06 mm/day in the dry season (range 0-6.1). The daily minimum temperature averaged 19.7 ^{o}C in the rainy season (range 11.4-25.3) and 15.7 ^{o}C in the dry season (range 10.2-23.7), and the daily maximum temperature averaged 29.2 ^{o}C in the rainy season (range 19.4-34.1) and 28.4 ^{o}C in the dry season (range 23.1-33.5). In the rainy season, there was an average of 56% soil moisture, 2.7 mm/day of evaporation, 1.5 m/s windspeed, and 2,983 m^3/s of streamflow. In the dry season, there was an average of 26% soil moisture, 0.3 mm/day of evaporation, 2.5 m/s windspeed, and 143 m^3/s of streamflow. Household elevation ranged from 920 to 1,055 m, and household slope ranged from 0 to 10.4 degrees. NDVI ranged from 0.29 to 0.78. For each household, there were between 10 and 1,021 households within 500 m.

Vector Abundance

Vector species composition

From April 2012 through July 2017, a total of 13,780 female anopheline mosquitoes were collected during 1,724 visits to 1,084 unique households. The species composition included 12,365 *An. funestus*, 1,371 *An. gambiae*, 43 *An. coustani*, and 1 *An. maculipalpis*. The distribution of household vector counts was highly skewed, with more than half of household visits yielding zero mosquitoes and 5% yielding between 50 and 230. Overall, there were a mean of 7.8 female anophelines per household visit (range 0-230, median 0, IQR 0-3), including 7.0 *An. funestus* (range 0-226, median 0, IQR 0-2) and 0.8 *An. gambiae* (range 0-35, median 0, IQR 0-0).

Across the study area, a higher number of vectors were collected in inland areas throughout the year, with several large collections in the dry season along a large inland lagoon (Figure 2.3). As anticipated, *An. funestus* was the predominant vector, with higher household counts than *An. gambiae* in both rainy and dry seasons. *An. funestus* counts peaked shortly after rains ceased and remained high throughout the dry season (Figure 2.4.A). *An. gambiae* was nearly absent in the dry season and was found in low numbers throughout the study area in the rainy season. In the lakeside area, *An. gambiae* was the predominant vector in some grid quadrants during the rainy season, although overall numbers remained low. *An. gambiae* counts rose at the start of the rains and then generally increased throughout the rainy season.

Correlates of household vector counts

In bivariate analyses, household construction, household composition, and geography were associated with vector abundance (Table 2.1). For *An. funestus,* higher counts were found in households with natural floors, natural roofing, open eaves, or an unprotected water source. Higher counts were observed in households with more occupants and a higher percentage of occupants under 5 years.

Counts were higher in households on a steeper slope, at a lower elevation, and with higher NDVI. Counts were higher in households that were closer to category 2 streams and farther from category 4 streams. Residence in more rural areas was associated with increased *An. funestus* abundance. Counts were higher in households farther from Lake Mweru and health clinics, and counts were lower with increasing population density. History of IRS with pirimiphos-methyl was associated with lower *An. funestus* abundance.

For *An gambiae*, higher counts were found in households with an unprotected water source and a higher percentage of occupants under 5 years, and counts were higher in households with higher NDVI and closer proximity to category 2 streams (Table 2.1). Residence in rural areas was also associated with *An. gambiae* abundance. Higher counts were observed with increasing distance from Lake Mweru, and lower counts were observed with increasing population density. There was no association between *An. gambiae* counts and history of IRS. Interestingly, higher numbers of both species were collected in households where more people slept under bed nets, potentially indicating that larger indoor vector populations encouraged bed net use.

In multivariate models, *An. funestus* counts were positively correlated with NDVI and proximity to roads and category 1 streams (Table 2.2). Protective factors included a history of household IRS, residence in lakeside areas, high population density, high elevation, and steeper slope. Correlations with weather were complex. Counts of *An. funestus* were 71% lower for each additional 10 mm of rain in the 2-week period lagged 2 weeks (IRR = 0.29, 95% CI = 0.17-0.47) and were 44% lower for each additional 10 mm of rain in the 2-week period lagged 4 weeks (IRR = 0.56, 95% CI = 0.36-0.86). Counts were 8% higher for each 1^{o} C increase in maximum temperature for the 2-week period lagged 2 weeks (IRR = 1.08, 95% CI = 1.00-1.2) but were 24% lower for each 1^{o} C increase in maximum temperature of the 8-week period lagged 4 weeks (IRR = 0.76, 95% CI = 0.69-0.85), indicating a potentially different relationship with temperature at different life stages.

For *An. gambiae*, household counts were positively correlated with proximity to roads and category 1 streams and were negatively correlated with high population density (Table 2.2). *An. gambiae* counts were lower in lakeside areas; however, when this variable was included in models, increasing distance from Lake Mweru appeared protective. In models stratified by lakeside vs. inland areas, this association was consistent, indicating that within both the lakeside and inland zones, *An. gambiae* counts were lower in eastern areas of each region, but that counts were lower in the lakeside area overall (Figure 2.3). Counts of *An. gambiae* were 34% lower for each additional 10 mm of rain in the 1-week period lagged 2 weeks (IRR = 0.66, 95% CI = 0.46-0.95) but were 230% higher for each additional 10 mm of rain in the 7-week period lagged 3 weeks (IRR = 2.3, 95% CI = 1.4-3.8). Counts were 25% lower with each 1^{o} C increase in maximum temperature for the 4-week period lagged 3 weeks (IRR = 0.75, 95% CI = 0.68-0.82) but were 30% higher for each 1^{o} C increase in minimum temperature over this period (IRR = 1.3, 95% CI = 1.2-1.4), again indicating a complex relationship with temperature.

Parasite Prevalence

Study population

A total of 3,520 individuals residing in 1,052 households participated in the study between April 2014 and July 2017 (Table 2.3). Between 1 and 13 people participated per household, with a mean of 3.3. Approximately 20% of participants were under 5 years, 34% were school age (between 5 and 16 years), and 45% were male. Nearly 60% of participants reported sleeping under a bed net, and 22% reported household IRS with pirimiphos-methyl, which began in October 2014. Two thirds lived within 3 km of Lake Mweru. This population reported evidence of substantial economic hardship. Nearly 90% had unfinished floors in their home, and half used an unprotected water source. Only 10% had a finished roof, and 8% had closed household eaves. Nearly 70% of participants lived in a house where the HOH had only a primary school education, and 7% had a HOH that was permanently employed.

The *P. falciparum* parasite prevalence by RDT among all participants was 50% at the time of the study visit, although only 2% had a fever, and 60% were anemic (Table 2.3). Twenty percent reported treatment for malaria in the past two weeks. Among children under 5 years, 60% were parasitemic, 6% had a fever, and 71% were anemic. Among school aged children, 71% were parasitemic, 3% had a fever, and 67% were anemic. Throughout the study period, parasite prevalence by month ranged from 21% to 78% in the overall population and from 25% to 91% among children under 17 years (Figure 2.4.B). Among both adults and children, there were two yearly peaks in parasite prevalence despite there being only one rainy season, with this pattern most visible before IRS with pirimiphos-methyl was initiated. One peak occurred at the start of the rainy season and the other occurred shortly after the rains ceased.

Correlates of parasite prevalence

In bivariate analyses, individual-level risk factors for parasite prevalence in both rainy and dry seasons included age under 5 years and school age. Household-level risk factor included having natural floors in the home, an HOH with only a primary education, higher household occupancy, and proximity to roads and category 1 streams (Table 2.4). Additional risk factors in the rainy season included male sex, using an unprotected water source, higher NDVI, increasing distance from Lake Mweru and health centers, and increasing proximity to category 2 streams. Additional dry season risk factors included steeper slope and increasing distance from category 3 streams. Across seasons, the primary protective factors against parasite prevalence were sleeping under a bed net, finished roofing, closed eaves, and higher household density within 500 m. In the rainy season, a history of IRS with pirimiphos-methyl was also protective, and elevation was protective in the dry season.

In multivariate models for the rainy season, parasite prevalence was positively correlated with age under 5 years, school age, male sex, and the HOH having only primary education (Table 2.5). Parasite prevalence was higher with increasing distance from health clinics and with increasing

proximity to roads and category 1 streams. Protective factors included sleeping under a bed net, history of IRS with pirimiphos-methyl, increasing population density within 500 m, and higher elevation. In the rainy season, parasite prevalence was positively correlated with minimum temperature, with a 10% increase in risk for each 1^{o} C increase in the 2-week period lagged 2 weeks (PRR = 1.1, 95% CI = 1.05-1.2), but was negatively correlated with maximum temperature, with a 4% decrease in risk for each 1^{o} C increase over the 2-week period lagged 1 week (PRR = 0.96, 95% CI = 0.93-0.99). This again indicates that rainy season vectors may transmit best within specific temperature ranges, as found in the results for *An. gambiae*.

In multivariate models for the dry season, parasite prevalence was positively correlated with age under 5 years, school age, having a dirt floor in their home, and HOH having primary education only (Table 2.5). Parasite prevalence was higher with increasing distance from health clinics and from category 3 streams. Protective factors included sleeping under a bed net, increased population density within 500 m, and higher elevation. In the dry season, parasite prevalence was positively correlated with maximum temperature, with a 20% increase in risk for each 1^{o} C increase for the 5-week period lagged 3 weeks (PRR = 1.2, 95% CI = 1.1-1.3), and was negatively correlated with increased rainfall, with a 23% decrease in risk for each 10 mm increase in rain over the 6-week period lagged 5 weeks (PRR = 0.77, 95% CI = 0.64-0.94). This largely corresponded to the results presented for *An. funestus*.

Relationships between vector abundance and parasite prevalence

For both vector species across seasons, spatially adjusted estimates were stronger and more stable predictors of parasite prevalence than directly observed household counts. In the rainy season, the strongest predictors of parasite prevalence were spatially adjusted *An. gambiae* counts weighted 90% by the grid quadrant mean at a 1-month lag, and spatially adjusted *An. funestus* counts weighted 90% by the grid quadrant mean at a 4-month lag. In the dry season, the strongest predictor of parasite

prevalence was spatially adjusted *An. funestus* counts weighted 10% by the grid quadrant mean at a 1-month lag. Based on these results, the relationship with rainy season parasite prevalence was largely predicted by average vector counts in previous months, and the relationship with dry season parasite prevalence was largely driven by observed values in the same month.

In mediation analyses, a large proportion of potential covariates were found to attenuate the relationship between vector counts and parasite prevalence, indicating that at least some of the association between these covariates and malaria risk is mediated through their relationship with vector abundance. Covariates found to be on the same causal path included all weather variables (rainfall, minimum and maximum temperature), population density within 500 m, having natural floors, use of an open water source, history of household IRS, residence in the lakeside area, and proximity to Lake Mweru, clinics, roads, and streams. Having natural floors was likely identified due to the correlation with other household features, such as natural roofing and open eaves, since these covariates could not be included in final models. Covariates that did not attenuate the relationship between vector abundance and parasite prevalence included age, HOH education, bed net use, NDVI, elevation, and slope. These covariates were carried on into final model selection.

In multivariate models for the rainy season (Table 2.5), there was a 30% increase in risk of parasitemia for each additional 10 *An. gambiae* (90% 1-month lag) (PRR = 1.3, 95% CI = 1.1-1.5) and a 5% increase in risk for each 10 *An. funestus* (90% 4-month lag) (PRR = 1.05, 95% CI = 1.03-1.07). In multivariate models for the dry season, there was a 2% increase in risk for each 10 *An. funestus* (10% 1-month lag) (PRR = 1.02, 95% CI = 1.01-1.03). These patterns were consistent with the observed time series data (Figures 2.5, 2.6), where a peak in prevalence in the rainy season (approximately October – April) coincided with an increase in household *An. gambiae* counts, and a peak in prevalence in the dry season (May-September) approximately coincided with a peak in household *An. funestus* counts.

In the rainy season, parasite prevalence remained positively correlated with age under 5 years, school age, male sex, and having an HOH with only primary education. Bed net use remained protective. In the dry season parasite prevalence remained positively correlated with age under 5 years, school age, and having an HOH with only primary education. Bed net use and higher elevation were protective. When all significant covariates were added back into models, vector covariates were no longer statistically correlated with parasite prevalence but contributed to model fit by AIC and likelihood methods, indicating that parasite prevalence was positively correlated with vector abundance but that the statistical relationship was not as strong as other measured covariates.

DISCUSSION

Vector abundance was a significant predictor of parasite prevalence in Nchelenge District. In the rainy season, the risk of parasitemia by RDT was positively correlated with both spatially adjusted *An. gambiae* and *An. funestus* at differing time lags. For each additional 10 *An. gambiae* mosquitoes predicted in the household the previous month, there was a 30% increase in risk of parasitemia with a high level of statistical significance, indicating a strong relationship between abundance of this vector and parasite prevalence. *An. funestus* was most strongly correlated with parasitemia at a 4-month time lag, with the risk increasing 5% for each additional 10 *An. funestus* mosquitoes. This result suggests that dry season vector abundance influences malaria prevalence in the rainy season but that concurrent *An. funestus* abundance does not, which is a surprising result biologically. Conversely, the risk of parasitemia in the dry season was most strongly correlated with spatially adjusted *An. funestus* counts at the time of collection and was not correlated with *An. gambiae* counts. With each additional 10 *An. funestus* mosquitoes captured in the household, weighted 10% by the grid quadrant mean the previous month, there was a 2% increase in the risk of parasitemia.

These results further strengthen previous conclusions that rainy-season malaria transmission in Nchelenge District is driven by *An. gambiae* and dry-season transmission is driven by *An. funestus* [51].

However, these results also suggest that dry-season *An. funestus* abundance is an unexpected driver of rainy-season malaria transmission, which has implications for the timing of interventions. Furthermore, given the uneven distribution of vector species, it is surprising that the impact of household *An. gambiae* abundance was much stronger per additional mosquito than *An. funestus*. Although *An. funestus* outnumber *An. gambiae* by approximately 10:1, the strength of the correlation for *An. gambiae* per additional collected mosquito was 6-15 times higher than for *An. funestus*. Explanations for this could include that a higher proportion of *An. funestus* transmission occurs outside or that the effect of *An. funestus* was saturated due to the extremely high number of mosquitoes found in some households. In a sensitivity analysis among the 43 dry-season households with 100-226 *An. funestus* collected in one visit, there was no additional risk of parasitemia associated with higher vector counts.

These findings have important implications for malaria control in this setting. Since *An. gambiae* abundance has a disproportionate influence on malaria transmission, reducing this species through targeted vector control should have a large impact on rainy-season parasite prevalence. However, *An. funestus* was the sole vector correlated with the risk of parasitemia in the dry season and significantly contributed to risk in the rainy season, indicating that failure to reduce the abundance of this vector will undermine malaria control efforts in both rainy and dry seasons. In this setting, successful malaria control will therefore depend on significant reductions of vector abundance during both rainy and dry seasons. Furthermore, the high counts of *An. funestus* observed in this study suggest that aggressive vector control measures will be necessary to decrease vector abundance in the dry season.

In addition to these relationships, other correlates of parasite prevalence and vector abundance have implications for malaria control in Nchelenge District. Among individual characteristics, younger age (children younger than 5 years and school-age children) and male sex were correlated with increased risk of parasitemia, as previously found in this setting [49, 84]. Among household-level and geographic characteristics, several consistent relationships were observed across outcomes. Markers of

socioeconomic status were strongly associated with both vector abundance and risk of parasitemia, including household construction, household water source, HOH education, and number of household occupants. Household construction particularly has been a consistent predictor of malaria and vector abundance across studies [85-90], and interventions to reduce mosquito entry could be impactful in future malaria control efforts. For example, interventions to close eaves have been shown to successfully reduce household entry by *An. gambiae* [90, 91].

Residence in more rural areas was strongly associated with higher vector counts and parasite prevalence, with predictors including low population density within 500 m and increasing distance from health clinics and Lake Mweru. This indicates a clear disparity and healthcare need among residents in inland regions. In support of this finding, only 38% of inland resident reported sleeping under a bed net compared to 62% of lakeside residents, and IRS was primarily conducted along the lakeside (Figure 2.3). Furthermore, vector counts and parasite prevalence were associated with geographic features, such that interventions can be targeted to households in areas of greatest risk. Higher vector counts and parasite prevalence were measured in areas close to roads and small streams, which may serve as mosquito breeding sites, and higher counts of *An. funestus* were found in areas of high NDVI, where vegetation cover may provide protection and moisture for mosquitoes. Both *An. funestus* counts and parasite prevalence declined with increasing elevation. Many of these findings are consistent with the literature, including the proximity to breeding sites, roads, NDVI, elevation, and number of household residents, among others [42, 43, 87, 88, 92-94].

Despite evidence of widespread pyrethroid resistance in this area [45], bed net use was consistently correlated with reduced parasite prevalence, indicating that this intervention continues to be successful and remains an important method of reducing vector-human contact. History of IRS with pirimiphos-methyl was also protective, with a 17% lower risk among sprayed households in the rainy season (the six months after application) and a 55% reduction in *An. funestus* counts. Despite being the

primary vector in the rainy season, there was no significant reduction in *An. gambiae*, potentially due to consistently low trap counts of this vector coupled with several outliers among sprayed households with very high *An. gambiae* counts.

The relationships between climate and both epidemiologic and entomologic outcomes were complex, but correlations were generally consistent between each season and its primary vector. Across analyses, temperature was the strongest predictor of vector abundance and parasite prevalence. For both *An. gambiae* abundance and rainy-season parasite prevalence, risk increased with increasing minimum temperature and decreased with increasing maximum temperature, suggesting transmission occurred most efficiently within a defined temperature range. Increased rainfall was generally associated with large increases in *An. gambiae* abundance. However, increased rainfall was associated with lower counts at very short time lags, and no association was found between rainy-season parasite prevalence and rainfall. This could be due to saturation effects, or the differing impact of rainfall in different ecologic areas of the district. Similarly, both *An. funestus* abundance and dry-season parasite prevalence declined with increasing rainfall and were higher with increasing maximum temperature within the past month, but higher temperatures at longer time lags were associated with lower vector counts. These results for rainfall and temperature by species generally agree with the published literature [18, 42, 43, 89, 93, 95-100].

A major strength of this analyses is the availability of long time series of both epidemiologic and entomologic surveillance data collected concurrently. Vector abundance has rarely been directly linked to malaria outcomes, particularly at the individual level. Furthermore, the interaction between vector dynamics and malaria prevalence has not been adequately described in high-transmission areas or in complex multi-species systems. Many high-burden regions are difficult to access for long-term surveillance due to logistic difficulties or instability, and this dataset is therefore unusual in both duration and scope. The length and frequency of data collection allowed a comprehensive investigation

into appropriate time lags and weights for each vector across seasons, accounting for other covariates, to determine the best time window to predict parasite prevalence in individuals. This has direct relevance for evaluating future vector control interventions.

In addition, this study clarified the vector-related drivers of the unique epidemiologic pattern in Nchelenge District. Despite having one rainy season, the presence of both *An. funestus* and *An. gambiae* generated two annual peaks in prevalence, and the synergy between these vectors leads to high transmission year-round. Although similar multispecies patterns have been described in Kenya and Cameroon [42, 43], most settings with two peaks in parasite prevalence are linked to bimodal annual rains [101], and the dynamics of this transmission pattern with a single rainy season have remained largely unexplored. The disproportionate contribution of *An. gambiae* to malaria transmission despite low numbers is another noteworthy result that may have been obscured without an individual-level study design, and the importance of both rainy- and dry-season vector abundance to rainy season parasite prevalence has direct implications for the timing of vector control interventions in this setting.

This analysis also had several limitations. Due to the study design, vectors were not collected in individual households in consecutive months, and therefore household vector collections were not etiologically linked to concurrent parasite prevalence. To address this issue, spatial and temporal smoothing was performed to approximate the vector counts at the time of infection. However, these methods assumed that repeated vector counts from the same household would be correlated in consecutive months and that vector counts would be correlated among neighboring households. If not correct, these assumptions may attenuate the relationship between vector abundance and parasite prevalence. For *An. funestus* particularly, dry-season parasitemia was driven largely by observed *An. funestus* counts at the time of collection, which could indicate that lagged grid quadrant means are a poor predictor for true lagged household counts due to the high variability in *An. funestus* abundance

between households. The relationship between *An. funestus* abundance and parasitemia might therefore have been harder to determine, which could have attenuated the observed PRR.

In addition, these analyses used vector abundance as the primary marker of vector-mediated transmission rather than EIR, a more commonly used metric of transmission intensity. The EIR is a function of both vector abundance and the sporozoite rate [102], so if the sporozoite rate varies independently from vector abundance throughout the year, vector counts would not be an accurate indicator of transmission intensity. Sporozoite data were not available, so the assumption was made that there was limited variability in sporozoite rates and that vector abundance was the primary driver of EIR, as reported in prior studies in high-transmission areas [103, 104]. If this assumption is incorrect, the relationship between vector abundance and parasite prevalence might not accurately reflect the underlying transmission processes. However, vector abundance was collected at the relatively fine temporal scale of one month, while EIR is generally measured over one year or a single transmission season, so the benefits of increasing the resolution of data collection may outweigh the drawbacks of these assumptions.

Furthermore, although vector counts were correlated with parasite prevalence, vector variables were no longer statistically significant when covariates on the same causal pathway were added back into models. This could indicate that the measured and weighted household counts are not as strong predictors of parasitemia as higher-level common causes such as geography and weather, which could be caused by measurement error in mosquito collections, incorrect specification of time lags or weights, or other factors. Vector collection is prone to measurement error due to the inherent difficulty of measuring stochastic animal behavior, and it is possible that upstream factors such as climate could be stronger predictors of malaria risk since they are easier to measure. Indoor vector collections also do not account for outdoor transmission, which was found to be a significant driver of total malaria

transmission in other settings and may increase in the presence of vector control interventions [44, 105-110].

Another potential limitation of this study is the use of cross-sectional parasite prevalence rather than incidence as the main outcome. Due to the potentially long duration of parasitemia without treatment [83], the relationship between vector abundance and prevalence may be less direct, and therefore the strength of associations may be attenuated. Because new longitudinal cohorts were re-enrolled yearly, reliable incidence data were not available in this population over sufficiently long time periods to conduct this analysis. However, time lags of up to 5 months were explored, which should somewhat account for this challenge.

CONCLUSION

Vector abundance was significantly correlated with parasite prevalence in Nchelenge District, Zambia. In the rainy season, parasitemia was associated with household counts of *An. gambiae* mosquitoes in the previous month and *An. funestus* mosquitoes four months prior, indicating that both rainy and dry season vector abundance influenced rainy season parasite prevalence. Dry season parasitemia was associated with household counts of *An. funestus* mosquitoes only. Although vector abundance was a significant predictor of parasite prevalence, geographic and climatological factors were more strongly associated with parasitemia, which highlights the difficulty of accurately collecting and modeling vector counts. These results have implications for vector control. Due to substantial dry season transmission and the contribution of dry season vectors to rainy season transmission, malaria control interventions in Nchelenge District should be conducted throughout the year with increased attention to rural areas.

Figure 2.1: Nchelenge District sampled and enumerated households from April 2012 – July 2017

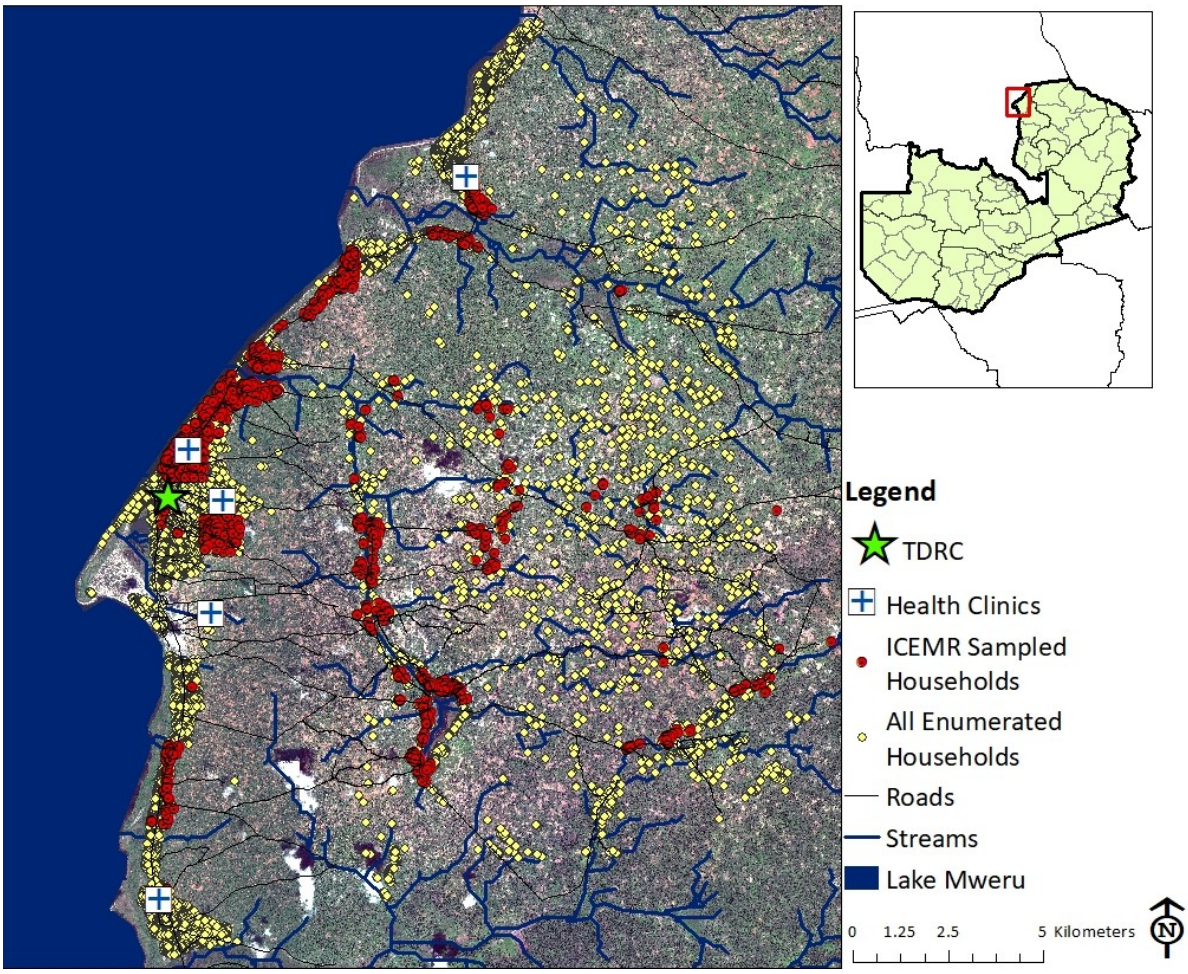
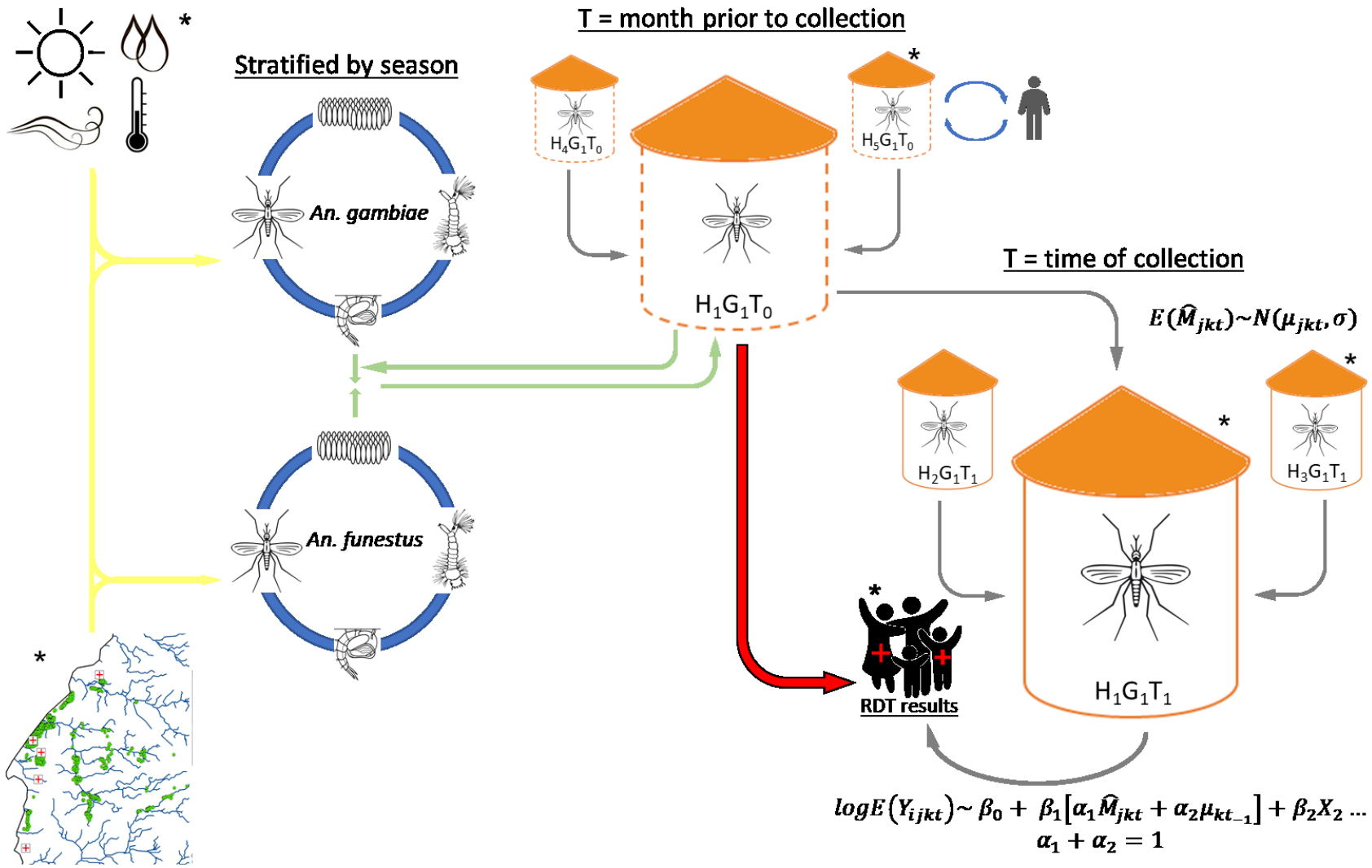


Figure 2.2: Schematic for relationship between household vector counts and individual malaria prevalence



If a one-month time lag is the most epidemiologically relevant, human malaria infection in household H_1 in grid quadrant G_1 at time T_1 is caused by exposure to vectors in the same household at time T_0. Household counts at T_0 are affect by weather, geography, vector and parasite life cycles, and ongoing transmission. However, vector counts in H_1 at T_0 are not measured, so vector counts at this time are inferred through the measured average vector counts in G_1 at time T_0.

Figure 2.3 Distribution of *An. funestus* and *An. gambiae* in sampled grid quadrants throughout the study area in Nchelenge District, Zambia in A) rainy and B) dry seasons

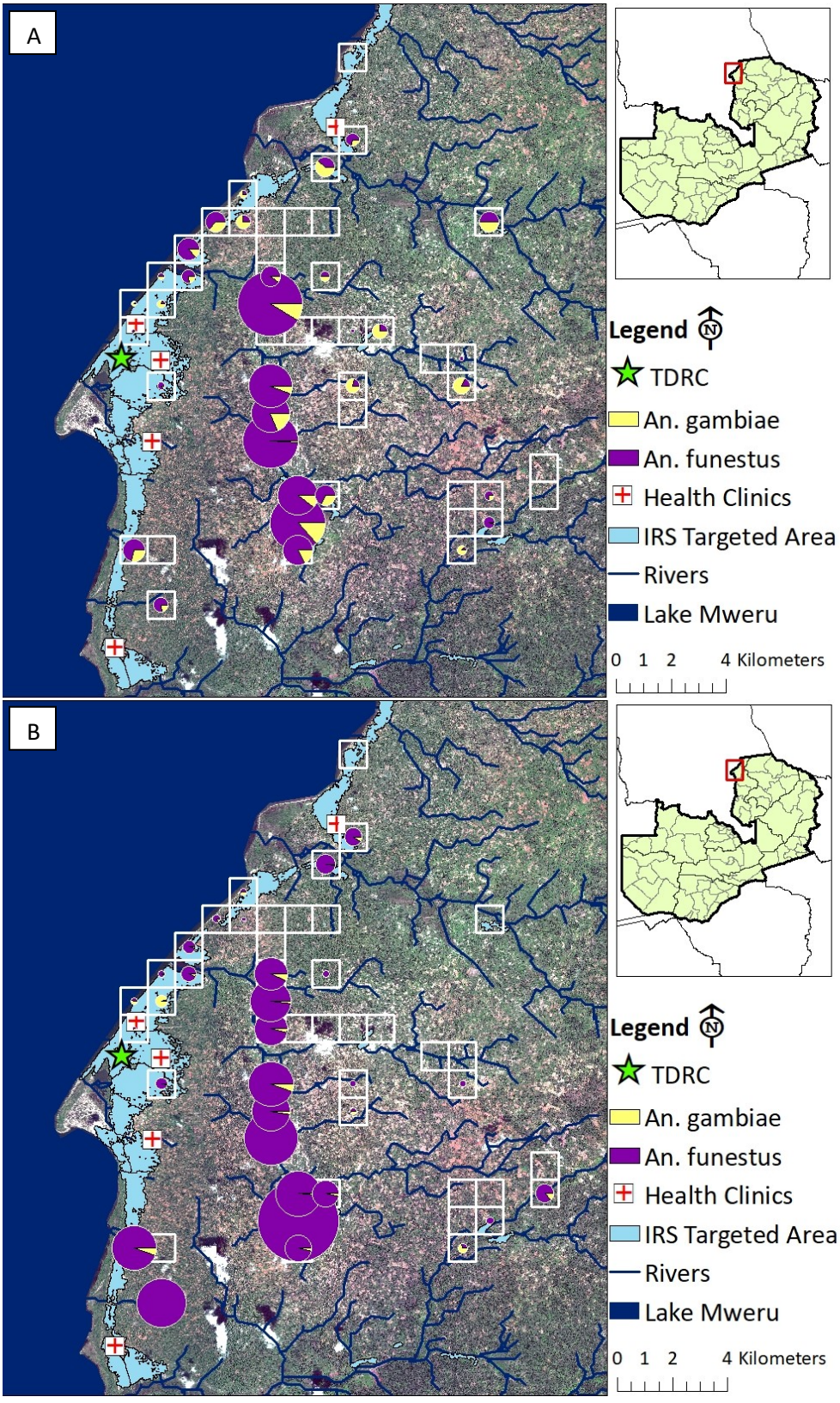


Figure 2.4: Time series of A) *An. funestus* and *An. gambiae* (x10) counts by months, and B) parasite prevalence among the study population and among children under age 17 years

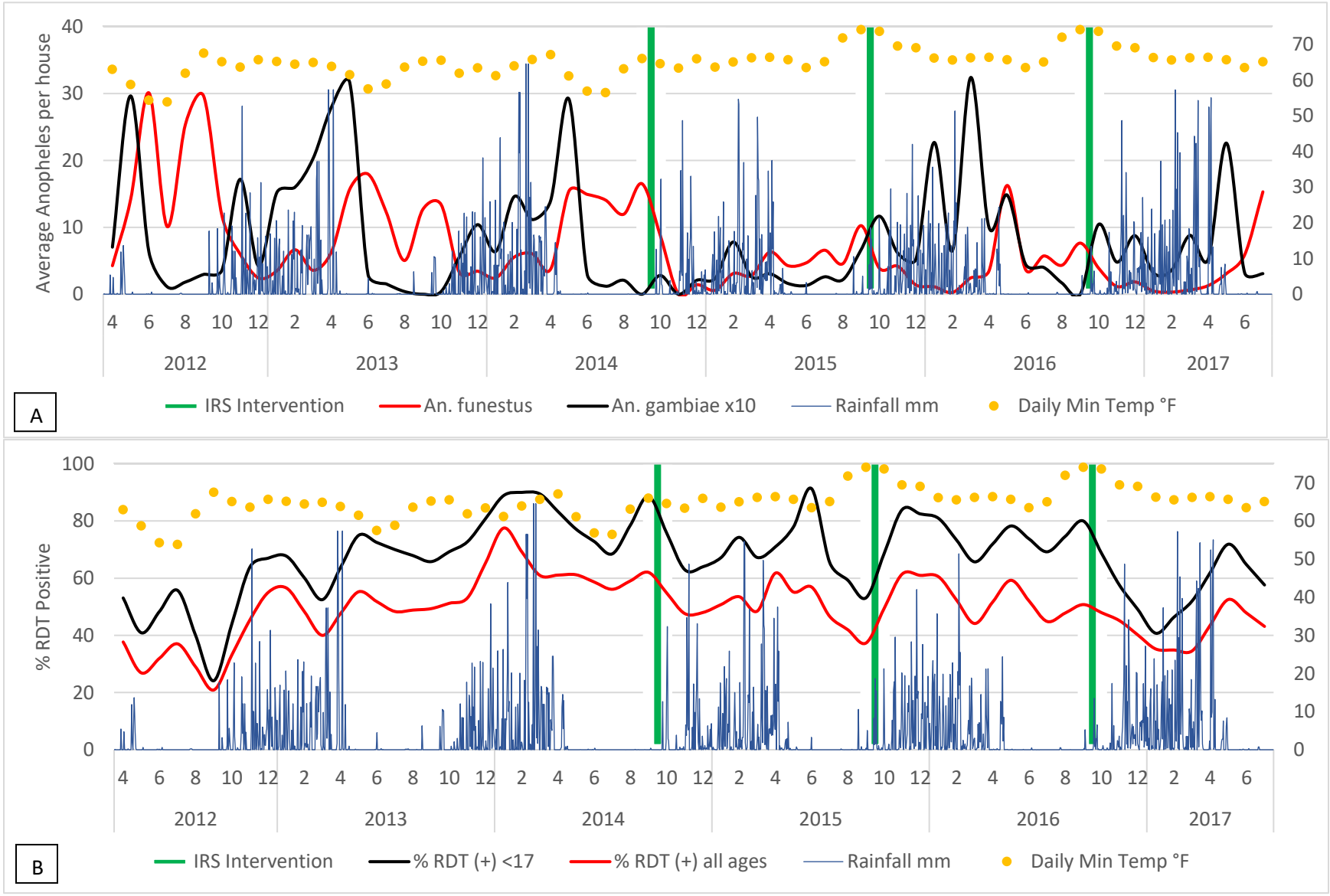


Table 2.1: Factors associated with household counts of *An. funestus* and *An. gambiae* using bivariate negative binomial models with robust standard errors and GEE clustered by household, N=1,732

	An. funestus			*An. gambiae*		
	IRR	95% CI	P value	IRR	95% CI	P value
HOUSEHOLD CHARACTERISTICS						
History of IRS by self-report[#]	0.35	(0.25, 0.49)	*<0.001*	0.92	(0.63, 1.3)	0.7
Dirt floor in home	4.3	(2.2, 8.2)	*<0.001*	1.3	(0.78, 2.2)	0.3
Use unprotected water source	2.0	(1.4, 2.9)	*<0.001*	1.9	(1.4, 2.7)	*<0.001*
Longitudinal HH type	1.01	(0.62, 1.6)	0.9	0.73	(0.52, 1.03)	0.08
Metal roof*	0.20	(0.10, 0.39)	*<0.001*	0.68	(0.40, 1.2)	0.2
Closed eaves*	0.23	(0.12, 0.46)	*<0.001*	0.70	(0.40, 1.3)	0.2
HOUSEHOLD DEMOGRAPHICS						
Number of household participants	1.1	(1.04, 1.2)	*0.001*	1.03	(0.96, 1.1)	0.4
Proportion who use bed net (by 10%)	1.09	(1.05, 1.1)	*<0.001*	1.06	(1.02, 1.1)	*0.002*
Proportion under 5 years (by 10%)	1.2	(1.1, 1.3)	*<0.001*	1.1	(1.02, 1.2)	*0.02*
Proportion male (by 10%)	1.00	(0.97, 1.03)	0.9	0.99	(0.95, 1.03)	0.6
GEOGRAPHIC VARIABLES						
HHs within 500 m (by 100 HH)	0.61	(0.50, 0.73)	*<0.001*	0.80	(0.74, 0.87)	*<0.001*
Elevation (by 10 m)	0.86	(0.82, 0.90)	*<0.001*	1.00	(0.93, 1.07)	0.9
Slope (by 1°)	1.1	(1.07, 1.2)	*<0.001*	0.96	0.89, 1.05)	0.4
NDVI (by 10%)	2.1	(1.8, 2.5)	*<0.001*	1.5	(1.2, 1.8)	*<0.001*
Distance from Lake Mweru (in km)	1.2	(1.1, 1.2)	*<0.001*	1.06	(1.02, 1.1)	*0.002*
Distance from health clinics (in km)	1.3	(1.2, 1.3)	*<0.001*	1.09	(1.05, 1.1)	*<0.001*
Distance from roads (in 100 m)	0.93	(0.82, 1.05)	0.2	0.89	(0.75, 1.05)	0.2
Distance from cat. 1 streams (in km)	0.63	(0.38, 1.04)	0.07	0.68	(0.40, 1.17)	0.2
Distance from cat. 2 streams (in km)	0.59	(0.47, 0.74)	*<0.001*	0.78	(0.67, 0.91)	*0.002*
Distance from cat. 3 streams (in km)	0.82	(0.72, 0.95)	*0.007*	0.95	(0.85, 1.06)	0.4
Distance from cat. 4 streams (in km)	1.2	(1.06, 1.3)	*0.001*	1.08	(0.99, 1.2)	0.09

IRR = incidence rate ratio, CI = confidence interval, HH = household

[#] IRS with pirimiphos-methyl, *subsample of all households, N=1,383

Table 2.2: Negative binomial multivariate models of predicting *An. funestus* (N=1,665) and *An. gambiae* (N=1,732) counts per household, using robust standard errors and GEE clustered by household

	An. funestus			*An. gambiae*		
	IRR	95% CI	P value	IRR	95% CI	P value
History of IRS by self-report[#]	0.45	(0.32, 0.62)	*<0.001*	-		
HH within 500 m (by 100 HH)	0.65	(0.54, 0.77)	*<0.001*	0.82	(0.75, 0.89)	*<0.001*
Elevation (by 10 m)	0.53	(0.47, 0.61)	*<0.001*	-		
Slope	0.88	(0.80, 0.97)	*0.01*	-		
NDVI (by 10%)	1.3	(1.08, 1.5)	0.004	-		
Lakeside	0.28	(0.16, 0.47)	*<0.001*	0.25	(0.14, 0.43)	*<0.001*
Lake Distance (in km)	-			0.82	(0.75, 0.89)	*<0.001*
Distance from roads (in 100 m)	0.80	(0.74, 0.86)	*<0.001*	0.82	(0.74, 0.90)	*<0.001*
Distance from cat. 1 streams (in km)	0.52	(0.32, 0.84)	*0.007*	0.64	(0.46, 0.89)	*0.007*
Lagged rainfall (by 10 mm) [1]	0.29	(0.17, 0.47)	*<0.001*	-		
Lagged rainfall (by 10 mm) [2]	0.56	(0.36, 0.86)	*0.008*	-		
Lagged rainfall (by 10 mm) [3]	-			0.66	(0.46, 0.95)	*0.02*
Lagged rainfall (by 10 mm) [4]	-			2.3	(1.4, 3.8)	*0.002*
Lagged maximum temperature (in C°) [1]	1.08	(1.00, 1.2)	*0.05*	-		
Lagged maximum temperature (in C°) [5]	0.76	(0.69, 0.85)	*<0.001*	-		
Lagged maximum temperature (in C°) [6]	-			0.75	(0.68, 0.82)	*<0.001*
Lagged minimum temperature (in C°) [6]	-			1.3	(1.2, 1.4)	*<0.001*

IRR = incidence rate ratio, CI = confidence interval, HH = household

[1] Interval=2 weeks, lag=2 weeks; [2] Interval=2 weeks, lag=4 weeks; [3] Interval=1 weeks, lag=2 weeks; [4] Interval=7 weeks, lag=3 weeks; [5] Interval=8 weeks, lag=4 weeks; [6] Interval=4 weeks, lag=3 weeks

Table 2.3: Characteristics of study population (N=3,520)

	n	%
INDIVIDUAL CHARACHTERISTICS		
Age <5	690	19.6%
Age 5-16	1,190	33.8%
Male	1,576	44.8%
Sleep under bed net	2,004	57.1%
CLINICAL RESULTS		
RDT positive	1,767	49.9%
Fever at visit	79	2.2%
Anemic at visit	2,125	60.4%
Taken Coartem in past 2 weeks	702	20.0%
HOUSEHOLD CHARACTERISTICS		
Longitudinal HH type	554	15.7%
History of IRS by self-report[#]	764	22.0%
Within 3 km of Lake Mweru	2,249	64.1%
Dirt floor in home	3,079	87.9%
Unprotected water source	1,711	48.7%
HOH primary school only	2,407	68.6%
HOH permanently employed	229	6.5%
Metal roof^	261	10.1%
Closed eaves^	202	7.8%

^subsample of population (N=2,577)

Table 2.4: Factors associated with parasite prevalence by RDT using bivariate Poisson regression models with robust standard errors and GEE clustered by household, N=3,533

	Rainy Season			Dry Season		
	PRR	95% CI	P value	PRR	95% CI	P value
DEMOGRAPHIC VARIABLES						
Age <5*	1.9	(1.7, 2.2)	*<0.001*	2.02	(1.8, 2.3)	*<0.001*
Age 5-16*	2.2	(2.0, 2.5)	*<0.001*	2.5	(2.3, 2.9)	*<0.001*
Male	1.3	(1.1, 1.4)	*<0.001*	1.06	(0.96, 1.2)	0.2
Longitudinal HH type	0.96	(0.82, 1.1)	0.6	1.3	(1.1, 1.5)	*<0.001*
Sleep under bed net	0.69	(0.62, 0.77)	*<0.001*	0.70	(0.62, 0.78)	*<0.001*
History of IRS by self-report#	0.80	(0.69, 0.92)	*0.003*	0.95	(0.80, 1.1)	0.5
Dirt floor in home	1.2	(1.02, 1.5)	*0.03*	1.4	(1.1, 1.8)	*0.004*
Unprotected water source	1.2	(1.08, 1.4)	*0.001*	0.99	(0.88, 1.1)	0.9
HOH primary school only	1.3	(1.1, 1.5)	*<0.001*	1.4	(1.2, 1.6)	*<0.001*
HOH permanently employed	0.87	(0.68, 1.1)	0.3	0.93	(0.72, 1.2)	0.6
Number of household participants	1.06	(1.04, 1.09)	*<0.001*	1.07	(1.04, 1.1)	*<0.001*
Metal roof^	0.66	(0.50, 0.88)	*0.004*	0.63	(0.46, 0.85)	*0.003*
Closed eaves^	0.54	(0.37, 0.80)	*0.002*	0.68	(0.50, 0.93)	*0.02*
GEOGRAPHIC VARIABLES						
HHs within 500 m (by 100 HH)	0.92	(0.90, 0.94)	*<0.001*	0.95	(0.93, 0.98)	*<0.001*
Elevation (by 10 m)	0.99	(0.96, 1.03)	0.7	0.95	(0.92, 0.99)	*0.008*
Slope (by 1°)	1.00	(0.97, 1.03)	0.9	1.03	(1.01, 1.06)	*0.02*
NDVI (by 10%)	1.08	(1.01, 1.2)	*0.02*	1.06	(0.98, 1.2)	0.1
Distance from Lake Mweru (in km)	1.03	(1.02, 1.05)	*<0.001*	1.0	(0.98, 1.01)	0.7
Distance from health clinics (in km)	1.04	(1.03, 1.06)	*<0.001*	1.01	(0.99, 1.03)	0.4
Distance from roads (in 100 m)	0.94	(0.91, 0.97)	*<0.001*	0.93	(0.88, 1.00)	*0.04*
Distance from cat. 1 streams (in km)	0.82	(0.72, 0.93)	*0.002*	0.78	(0.67, 0.90)	*0.001*
Distance from cat. 2 streams (in km)	0.92	(0.87, 0.97)	*0.002*	1.02	(0.96, 1.08)	0.5
Distance from cat. 3 streams (in km)	0.98	(0.95, 1.00)	0.1	1.04	(1.01, 1.07)	*0.02*
Distance from cat. 4 streams (in km)	1.02	(0.99, 1.05)	0.2	1.00	(0.97, 1.03)	0.8

PRR = prevalence rate ratio, CI = confidence interval, HOH = head of household, HH = household

*Compared to adults aged >16, # IRS with pirimiphos-methyl, ^subset of study population N=2,589

Table 2.5: Poisson multivariate GEE models, clustered by household, investigating correlates of parasite prevalence by season using both non-vector and vector-based predictors (non-vector model N=3,477, vector model N=3,493)

	Rainy Season			Dry Season		
	PRR	95% CI	P value	PRR	95% CI	P value
NON-VECTOR MODEL						
Age <5*	1.8	(1.6, 2.0)	*<0.001*	2.0	(1.7, 2.3)	*<0.001*
Age 5-16*	2.1	(1.9, 2.4)	*<0.001*	2.5	(2.2, 2.8)	*<0.001*
Male	1.2	(1.1, 1.3)	*<0.001*	-		
Sleep under bed net	0.82	(0.75, 0.91)	*<0.001*	0.88	(0.80, 0.96)	*0.006*
History of IRS by self-report[#]	0.83	(0.73, 0.94)	*0.005*	-		
Dirt floor in home	-			1.2	(0.98, 1.5)	0.07
HOH primary school only	1.2	(1.03, 1.3)	*0.01*	1.2	(1.04, 1.3)	*0.01*
HH within 500 m (by 100 HH)	0.93	(0.90, 0.96)	*<0.001*	0.96	(0.94, 0.99)	*0.01*
Elevation (by 10 m)	0.90	(0.86, 0.94)	*<0.001*	0.91	(0.87, 0.95)	*<0.001*
Distance from health clinics (in km)	1.05	(1.01, 1.08)	*0.005*	1.05	(1.02, 1.07)	*0.003*
Distance from roads (in 100 m)	0.95	(0.93, 0.98)	<0.001	-		
Distance from cat. 1 streams (in km)	0.91	(0.81, 1.02)	0.1	-		
Distance from cat. 3 streams (in km)	-			1.04	(1.02, 1.07)	*<0.001*
Lagged rainfall (by 10 mm) [1]	-			0.77	(0.64, 0.94)	*0.01*
Lagged minimum temperature (in C°) [2]	1.1	(1.05, 1.2)	*<0.001*	-		
Lagged maximum temperature (in C°) [3]	0.96	(0.93, 0.99)	*0.03*	-		
Lagged maximum temperature (in C°) [4]	-			1.2	(1.1, 1.3)	*<0.001*
VECTOR MODEL						
An. gambiae (per 10) [5]	1.3	(1.1, 1.5)	*<0.001*	-		
An. funestus (per 10) [6,7]	1.05	(1.03, 1.07)	*<0.001*	1.02	(1.01, 1.03)	*<0.001*
Age <5*	1.8	(1.6, 2.1)	*<0.001*	2.00	(1.7, 2.3)	*<0.001*
Age 5-16*	2.1	(1.9, 2.4)	*<0.001*	2.4	(2.2, 2.8)	*<0.001*
Male	1.2	(1.08, 1.3)	*<0.001*	-		
Sleep under bed net	0.83	(0.75, 0.92)	*0.001*	0.89	(0.81, 0.98)	*0.02*
HOH primary school only	1.2	(1.1, 1.4)	*0.001*	1.3	(1.1, 1.5)	*<0.001*
Elevation (by 10 m)	-			0.96	(0.93, 0.99)	*0.04*

PRR = prevalence rate ratio, CI = confidence interval, HOH = head of household, HH = household

*Compared to adults aged >16, [#] IRS with pirimiphos-methyl

[1] Interval=6 weeks, lag=5 weeks; [2] interval=2 weeks, lag=2 weeks; [3] interval=2 weeks, lag=1 weeks; [4] interval=5 weeks, lag=3 weeks; [5] adjusted by neighbors, weighted 90% by grid average lagged 1 month; [6] rainy season: adjusted by neighbors, weighted 90% by grid average lagged 4 months; [6] dry season: adjusted by neighbors, weighted 10% by grid average lagged 1 month

Figure 2.5: Time series of both parasite prevalence and household counts of A) *An. funestus* and B) *An. gambiae*

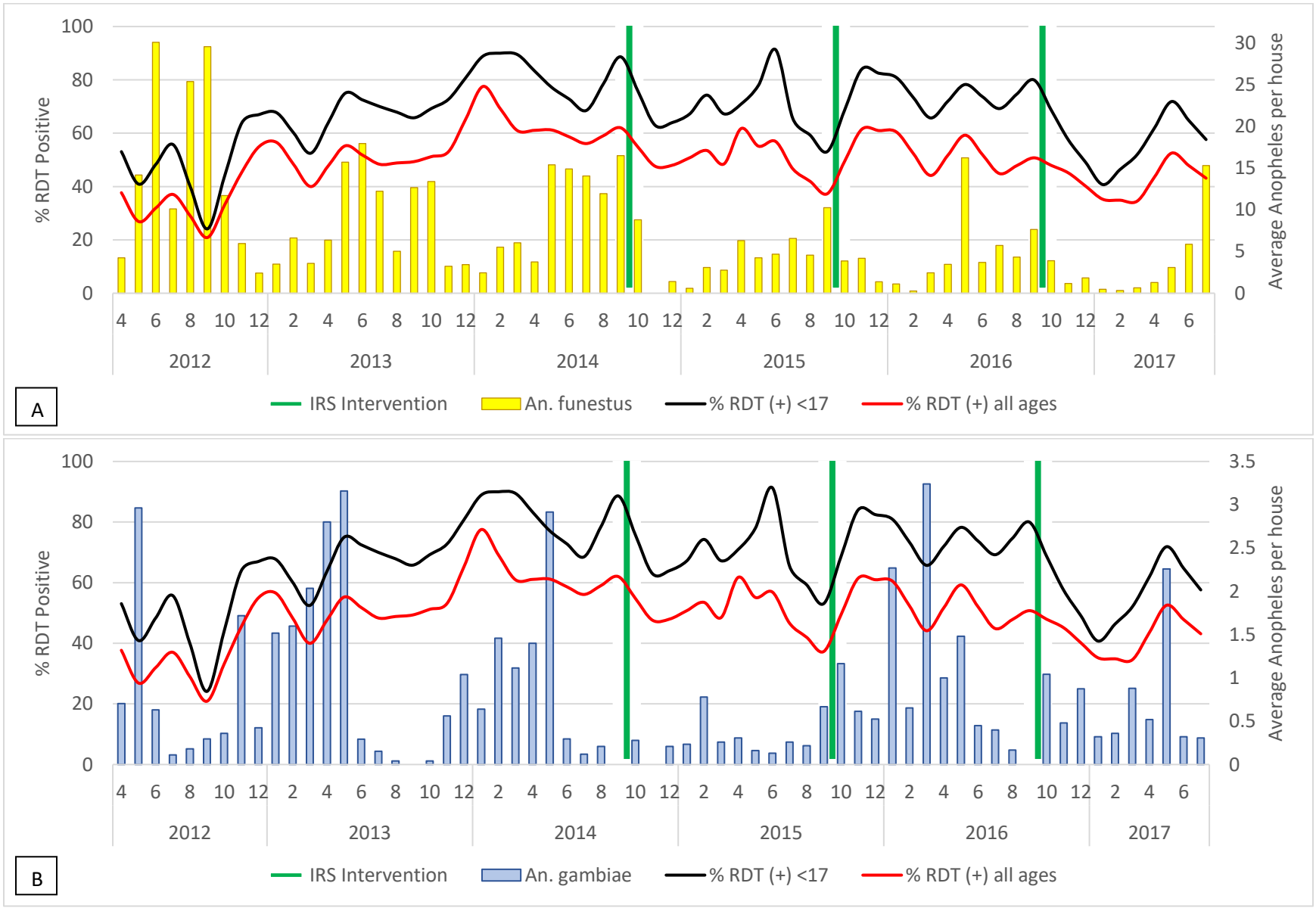
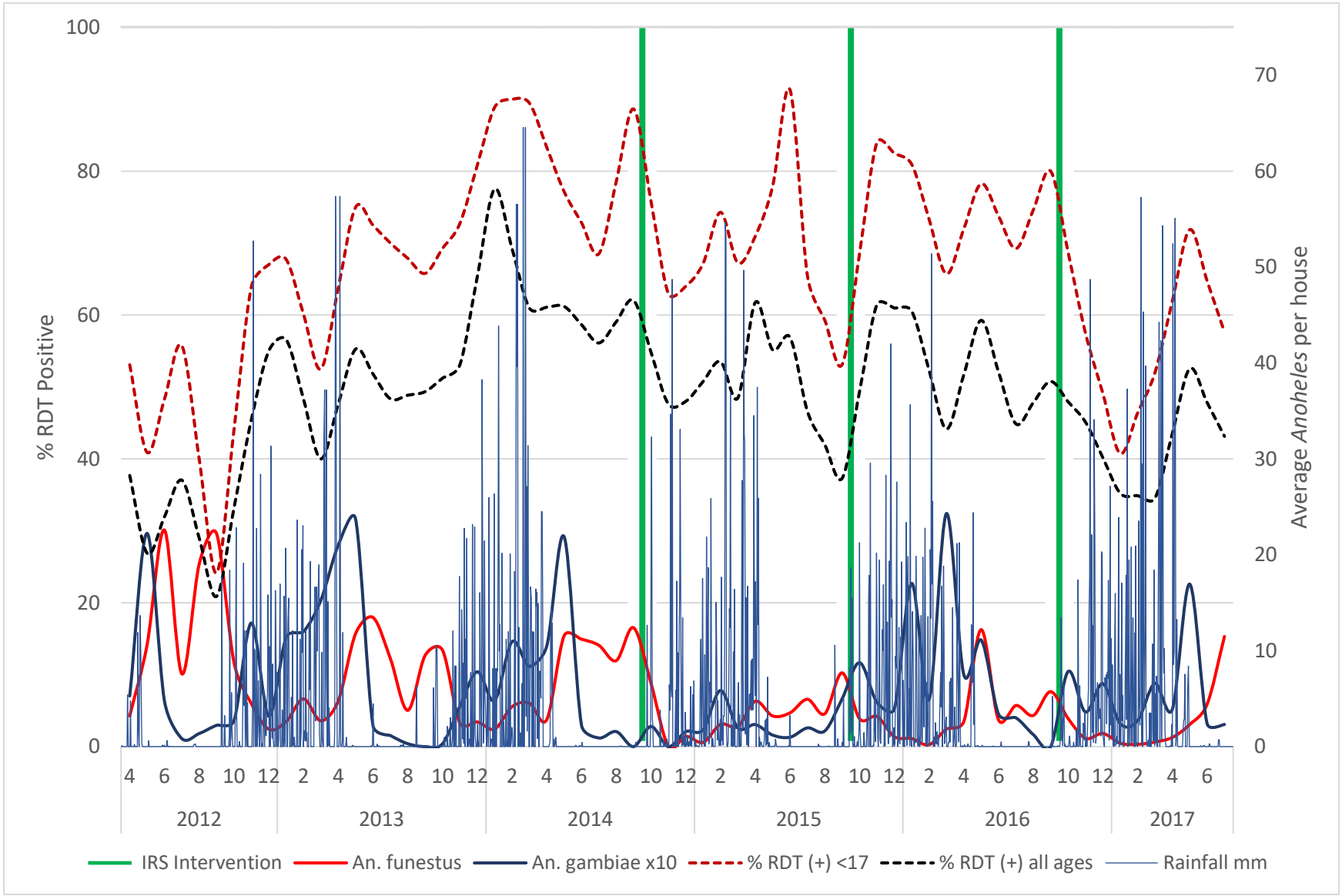


Figure 2.6: Time series of both parasite prevalence and household counts of *An. funestus* and *An. gambiae*



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Chapter 3: The impact of three years of targeted IRS with pirimiphos-methyl on malaria parasite prevalence and vector abundance in a high-transmission area of northern Zambia

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ABSTRACT

Background: The scale-up of malaria control efforts has led to a decline in the global malaria burden, but progress in some regions has stalled or reversed. Malaria transmission in northern Zambia increased in the past decade despite malaria control activities. Evidence-based intervention strategies are needed in this setting to effectively control and interrupt malaria transmission.

Methods: Targeted indoor residual spraying (IRS) was conducted in Nchelenge District, Luapula Province, northern Zambia using the organophosphate insecticide pirimiphos-methyl. An evaluation of three years of the IRS campaign was conducted using actively detected malaria cases and indoor vector counts collected in bimonthly household surveys from April 2012 to July 2017. Parasite prevalence was measured using rapid diagnostic tests (RDTs) and vectors were collected with indoor Centers for Disease Control and Prevention (CDC) light traps. Changes in prevalence and vector abundance by species before vs. after IRS were assessed using regression models with robust standard errors, controlling for demographic, geographic, and climatological covariates. Variances in all models were adjusted for clustering of participants within households and repeat household visits.

Results: Vector abundance and parasite prevalence decreased following IRS with pirimiphos-methyl. *Anopheles funestus* counts declined by 51% in both the areas targeted for IRS and the entire study area, and *An. gambiae* counts declined 36% in targeted areas and by 40% in the entire study area. Within the targeted area, *An. funestus* counts declined significantly in sprayed households but not unsprayed households, but the reverse was true for *An. gambiae.* Parasite prevalence declined by approximately 25% in areas targeted for IRS during the rainy season but did not decline during the dry season or in areas not targeted for IRS. Within the targeted areas, parasite prevalence declined in both sprayed and unsprayed households, indicating that the intervention had both direct and indirect effects on malaria transmission.

Conclusion: As more countries move towards malaria elimination, high-transmission regions remain a challenge for malaria control. Novel intervention strategies are needed to successfully reduce and interrupt transmission in these settings. In Nchelenge District, the moderate decrease in vector density and parasite prevalence following three years of IRS indicates that IRS with pirimiphos-methyl is an effective malaria control measure, but a more comprehensive package of interventions is needed to reduce malaria burden in this setting.

INTRODUCTION

Due to widespread scale-up of malaria control interventions and improved case management, there has been a substantial decrease in the global malaria burden following the launch of the Roll Back Malaria Initiative in 1998 and the President's Malaria Initiative (PMI) in 2005. From 2000 to 2015, global malaria incidence fell 41% and malaria mortality rates declined by 62% [1]. However, these gains have been uneven both within and between countries, and the rate of progress has slowed or reversed in some regions [2].

In Zambia, malaria remains the leading non-neonatal cause of child mortality, and the World Health Organization (WHO) estimated there were 3.1 million cases and 7,000 deaths in 2016, an increase of almost a million cases since 2010 [2, 3]. Both in-patient malaria cases and deaths in Zambia declined by approximately two thirds between 2000 and 2008 following a highly successful malaria control campaign that supported universal access to rapid diagnostic testing (RDTs), artemisinin-combination therapy (ACT), long-lasting insecticide-treated nets (LLINs), and expanded indoor residual spraying (IRS) [4]. However, these achievements were not equal across the country, with large gains made in Lusaka and southern Zambia but a high burden of malaria continuing in the northern and eastern regions of the country [5]. In subsequent years, low transmission was maintained in the south, but declining funds and intervention effectiveness led to an increase in malaria cases in northeast Zambia in 2009, and cases continued to increase nearly every subsequent year [2, 5-7]. By 2014, Luapula Province in the north reported over 50% malaria prevalence and increasing severe malaria in children under 5 years old, while Lusaka and the southern provinces reported parasite prevalence of <1% to 14% in children this age [4, 7]. This striking heterogeneity of malaria control under the same intervention policy, with reversal of progress in northern Zambia, indicates a need to continue to develop new intervention strategies for different epidemiologic settings.

Malaria epidemiology is highly dependent on the distribution and abundance of mosquito vectors, and integrated vector management (IVM) is a key priority for Zambia's national malaria control strategy [2, 8, 9]. The main malaria vectors in Zambia are *Anopheles gambiae sensu stricto (s.s.), An. arabiensis,* and *An. funestus s.s.*, all highly efficient disease vectors that may be anthropophilic (feed on humans), endophagic (bite indoors), and endophilic (rest indoors) [4, 10, 11]. Due to these behaviors, IRS in combination with other interventions has historically been a successful and cost-effective malaria control strategy in Zambia [4, 10, 12]. However, increasing resistance to DDT, pyrethroid, and carbamate insecticides has reduced the efficacy of this intervention and may be partly responsible for the rebound in cases in northern Zambia over the past decade [7, 10].

In response to this trend, a novel formulation of the organophosphate insecticide pirimiphos-methyl (Actellic 300CS) underwent sensitivity testing in 2013 in preparation for a national IRS campaign and was found to be 100% effective against the malaria vectors in Luapula Province [13, 14]. The standard for IRS is blanket coverage within a district, in which all household structures are sprayed; however, the increased cost of pirimiphos-methyl introduced resource constraints which limited the number of districts that could receive IRS. In an effort to maximize the impact of the intervention given limited resources, the Government of Zambia in collaboration with PMI and the African Indoor Residual Spraying (AIRS) project implemented targeted IRS in selected districts within Zambia in 2014 [15].

Targeted IRS is an emerging strategy in low-resource settings that has gained interest in recent years due in part to the high cost of new insecticide formulations [16]. The approach focuses spraying activities on transmission hotspots with high population density and high numbers of reported cases so that resources can be concentrated on clusters of households that have the most impact in sustaining local transmission [16]. The proposed advantages of this strategy include potential cost savings and logistical ease.

Although the need for targeted malaria control interventions has been discussed broadly [17-19], the body of published literature on targeted IRS is sparse. The current WHO recommendation is to use targeted IRS in areas of low endemicity for residual foci of high transmission, and several studies have evaluated the impact of this strategy [20-26]. Targeted IRS in meso-endemic valleys in Burundi in 2002-2005 resulted in substantial reductions in parasite prevalence and vector densities in targeted areas [21, 22], and targeted IRS in western Kenya in 2005, 2007, and 2016 produced similar beneficial results [23-25]. In northwest Tanzania, IRS targeted to epidemic-prone villages in 2007 was associated with reduced malaria prevalence in both targeted and neighboring villages [26]. However, studies have not previously been conducted in high-transmission settings and have not conclusively demonstrated interruption of transmission outside targeted areas. In Burundi, vector density but not parasite prevalence declined slightly in nearby untargeted highlands, and the most recent Kenya study found no impact of the targeted intervention outside the sprayed area.

As global interest in targeted interventions increases, research is needed to determine the effectiveness of this strategy in different settings. The continued rise in malaria transmission in northern Zambia despite active malaria control highlights the need for evidence-based intervention strategies. The objective of this study was to evaluate the impact of three consecutive years of targeted IRS on vector counts and malaria prevalence in Nchelenge District, Luapula Province, a high-transmission area in northern Zambia. Nchelenge District is one of the surveillance sites for the Southern and Central Africa International Centers of Excellence for Malaria Research (ICEMR) and was among the districts in Zambia selected for targeted IRS with pirimiphos-methyl from 2014-2016.

METHODS

Study Site

This study was conducted by the Southern and Central Africa ICEMR led by the Johns Hopkins Malaria Research Institute (JHMRI), in collaboration with the Zambian Ministry of Health's Tropical Disease Research Center (TDRC) and other partners [6]. The project uses active and passive surveillance to investigate heterogeneity in malaria burden across four distinct epidemiological settings and inform intervention policies [27]. Nchelenge District is one of these study sites, representing a setting of high transmission. Nchelenge District is located in the marshlands of the Luapula River along the banks of Lake Mweru, which forms the border with Haut-Katanga Province of the Democratic Republic of the Congo (DRC). There are approximately 150,000 residents with an average of 4.7 people per household [28]. There is a single rainy season from October to April, followed by a dry season with little to no rain. Nchelenge District experiences holoendemic malaria with year-round transmission and a resurgence in cases in recent years. The prevalence of malaria increased from 38% in 2006 to 53% in 2012 despite LLIN distributions and annual IRS campaigns from 2008-2012 [7]. Since 2012, the prevalence of malaria by RDT averaged approximately 70% in school-age children, and the entomological inoculation rate (EIR) is estimated to be 140 infective bites per year [29, 30].

The predominant malaria vectors in Nchelenge District are *An. gambiae s.s.* and *An. funestus s.s.*, and the distribution of these vectors varies across small spatial scales [31, 32]. *An. gambiae* breeding sites are typically found in shallow temporary pools such as wheel ruts and hoof prints, making this species dependent on rainfall, while *An. funestus* breeding sites are more frequently found in permanent bodies of water with emergent vegetation, such as marshlands and river banks, and are thus more robust to dry weather conditions [11, 33]. *An. funestus* is the predominant vector throughout the year in in both lakeside and inland areas, with a large peak in abundance in the dry season once rains have ceased, and *An. gambiae* abundance peaks in lakeside areas shortly after the start of the rainy season [31, 32]. The differing ecology of these vectors and the suitability of local breeding sites in Nchelenge District supports year-round malaria transmission.

Targeted IRS Campaign

In Zambia, PMI-supported IRS campaigns were conducted yearly in Nchelenge District from 2008-2012. Different formulations of pyrethroid insecticides were used from 2008-2010, and carbamate insecticides were used in 2011 and 2012 following identification of pyrethroid resistance [9]. During this time, the strategy for vector control was to use IRS in highly populated urban and peri-urban areas and to distribute LLINs in rural areas. No spraying occurred in Nchelenge District in 2013.

In 2014, Zambian IRS activities were transitioned to the AIRS program with Abt Associates as an implementing partner [34, 35]. Forty high-burden districts were identified in five provinces in Zambia, and sub-district areas were selected for targeted IRS with pirimiphos-methyl. Detailed methods for selecting targeted areas were described elsewhere [15, 16, 36]. In brief, structures were enumerated using publicly available satellite images and classified into clusters of >25 households. These clusters were linked to health center catchment areas and assigned a level of malaria burden based on the health center's malaria incidence reports and population size. The clusters were then ranked by malaria burden, and high-ranking clusters of sufficient population density were targeted for IRS. Spraying was initiated in October 2014 ahead of the rainy season with the goal of at least 85% coverage of targeted structures [15]. This strategy was repeated in 2015 and 2016 in fewer districts, with expanded criteria for selecting targeted areas [37, 38]. Specifically, low-population areas with high transmission adjacent to targeted clusters were included where logistically feasible, with the objective of linking isolated targeted areas. During this time, LLINs continued to be distributed in antenatal and vaccination clinics [9].

Data Collection

The study area is located along the banks of Lake Mweru in the center of Nchelenge District and constitutes about 20% of the land area of the district (Figure 3.1). Households in the study area were

enumerated using Quickbird™ satellite images (DigitalGlobal Services, Denver, CO). A 1x1 kilometer grid was overlaid on the study area and grid quadrants were randomly selected to represent the full range of ecology in the study area. Household sampling and data collection began in April 2012 and is ongoing. Households were randomly selected within each quadrant using population proportional to size sampling and were recruited into longitudinal or cross-sectional cohorts, with sampling alternating between cohorts every other month. In cross-sectional cohorts, approximately 25 new households were recruited each bimonthly period. Longitudinal cohorts were comprised of 25-30 households visited bimonthly six times over a year and then were replaced with a new longitudinal cohort.

At each study visit, household coordinates were recorded, and a questionnaire was administered to each consenting household member aged 16 and older and to guardians of residents under 16 years. The questionnaire gathered demographic data, history of recent malaria and treatment, reported LLIN use, history of household IRS, and malaria knowledge and practices. Participant temperature was taken using a digital ear thermometer, and a blood sample was collected by finger prick for hemoglobin testing, *Plasmodium falciparum* HRP-2 RDT, and dried blood spots for downstream cytochrome b (*cytb*) polymerase chain reaction (PCR) to detect *P. falciparum* DNA [39, 40]. In accordance with Zambian national policy, the RDT brands used throughout the study period were ICT (ICT Diagnostics, Cape Town, South Africa) from April 2012 to May 2013, First Response (Premier Medical Corporation LTD., Mumbai, India) from June to September 2013, and SD Bioline (Standard Diagnostics, Kyonggi, Republic of Korea) from October 2013 to the present. All participants positive for *P. falciparum* by RDT were offered treatment with Coartem® (Novartis, Basel, Switzerland), the first-line standard of care in Zambia. The day before each study visit, Centers for Disease Control (CDC) light traps (John W. Hock, Ltd, Gainesville, FL) were placed in participating households to collect mosquito vectors overnight. Traps were placed near a sleeping space with an LLIN, and participants were instructed to turn them on at 18:00 and off at 6:00 the following morning. Traps were collected at the time of the

study visit the following day. The number of *Anopheles* mosquitoes were counted and speciated morphologically and by PCR [32, 41, 42]. Additional details of study methods are described elsewhere [31, 32, 43].

Data Management

Data collected electronically at participating households were uploaded into REDCap secure file-sharing software and downloaded as .csv files [44]. Malaria status was determined by individual RDT results. Participants were defined to have a fever if their temperature exceeded 38 C°, and anemia was determined by WHO criteria for hemoglobin levels by age and sex [45]. Participating households were plotted in ArcGIS Version 10.2 (ESRI, Redlands, CA), and population density for each household was calculated as the total number of enumerated households within a 500-meter buffer. Geographic variables were created from previously developed geolocated raster and shapefiles for roads, stream networks, elevation, slope, and normalized difference vegetation index (NDVI) for the study area [43]. Streams were categorized using the Strahler classification system, in which two small category 1 streams join to form a category 2 stream, which joins another to form a category 3 stream, and so on [46]. Distances to Lake Mweru, health centers, roads, and category 1-4 streams were calculated for each household, and households were categorized as lakeside (rather than inland) if they were within 3 km of the lake. Residence in the area targeted for spraying in each year was determined using shapefiles provided by the NGO partner Akros based in Lusaka [16].

Meteorological and hydrological data were collected from a HOBO Micro Station (Onset Computer Corporation, Bourne, MA) located near TDRC offices in Kashikishi township and from the African Flood and Drought Monitor (AFDM) online tool [47, 48]. These variables included rainfall in mm/day, evaporation in mm/day, minimum and maximum daily temperature in C°, windspeed in m/s, streamflow in m^3/s, and percent soil moisture.

Statistical Analysis

The primary outcomes for this evaluation were the differences in malaria parasite prevalence and household vector abundance by species before vs. after IRS with pirimiphos-methyl. Due to the differing malaria transmission dynamics in lakeside and inland regions of Nchelenge District and the disproportionate targeting of IRS to lakeside areas, a direct comparison between sprayed and unsprayed areas would be biased and was not conducted. Epidemiological analyses were restricted to cross-sectional households and the first visit to longitudinal households to identify prevalent malaria infections. Analyses on vector density used data collected from all cross-sectional and longitudinal households, as household type and repeat visits were not shown to impact vector counts in adjusted analyses. Analyses were conducted for the overall study area as well as restricted to the area targeted for spraying. All epidemiological models were stratified by season. The start and end of the rainy season each year was defined as the first and last weeks in which the average rainfall exceed 1 mm. Sensitivity analyses using different cutoffs and time intervals were performed to ensure that this definition best represented the epidemiologic and entomologic relationships in this region. Vector abundance models were not stratified by season due to the high degree of variation in the data and a lack of power to run stratified analyses.

Data were analyzed using STATA 13.1 (Stata-Corporation, College Station, TX) and R version 3.4.2 (R Core Team, Vienna, Austria). Bivariate comparisons using demographic and geographic variables were conducted using chi-square tests and bivariate regression models. Demographic variables collected from the questionnaire included sex, age category (<5, 5-16, >16 years), reported LLIN use, natural vs. finished household flooring, open vs. protected household water source, head of household (HOH) attending only primary school, HOH in permanent employment, roof type (thatch vs. metal) and presence of open vs. closed household eaves. Geographic variables developed using ArcGIS were defined above.

For the epidemiologic analyses, multivariable regression models were fit comparing the probability of a positive RDT before vs. after IRS with pirimiphos-methyl, with the study participant as the unit of analysis. Standard logistic regression models are not ideal in this setting because malaria prevalence in Nchelenge District averages 50%, so models were fit using the Poisson estimation of the binomial distribution with robust standard errors, which can directly estimate the prevalence rate ratio (PRR) [49-52]. Multivariate regression models for vector analyses compared household vector counts before vs. after IRS with pirimiphos-methyl, stratified by species. Negative binomial regression models with robust standard errors were used to account for overdispersion [53, 54]. For all bivariate and multivariate models, generalized estimating equations (GEE) were used to account for clustering of participants within households in the epidemiological analyses and for repeat visits to households in vector analyses [55, 56].

For both the epidemiologic and vector analyses, preliminary multivariate models were fit with demographic and geographic variables as appropriate using stepwise regression and AIC optimization methods [57, 58]. The variables for roof type and household eaves could not be included in multivariate analyses since they were measured in only a subsample of the study population. Final models were fit with meteorological and hydrological covariates to explain inter-annual variation in malaria transmission. Due to the length of vector and parasite life cycles, the impact of climatological factors is expected to be lagged; however, optimum time lags have not been investigated for this setting. Previous studies introduced a matrix framework for investigating the impact of weather variables on malaria and vector densities at various time intervals and lags [59, 60], and this approach was adapted for this analysis. The mean values of each weather and hydrology factor were calculated at intervals of 1-12 weeks and lags of 1-12 weeks prior to each day of data collection. Final models were fit using random forest algorithms to control for the large number of collinear weather and hydrology variables [61, 62], and were confirmed using stepwise regression and AIC optimization methods. Indirect effects of the IRS

intervention within the sprayed area were investigated using fully parameterized models stratified by self-reported history of household IRS with pirimiphos-methyl.

A difference-in-differences analysis was conducted to further account for secular trends in malaria transmission. This analysis assumes that, although parasite prevalence and vector abundance were different in the sprayed and unsprayed areas, the proportionate change in these parameters over time would be equal if the IRS campaign had not been performed. The change in each outcome before vs. after the IRS intervention was compared between the sprayed and unsprayed areas using an interaction term. The value of this interaction term is interpreted as the ratio of risk ratios, or the ratio of the change in prevalence or vector density in the sprayed area and the change in the unsprayed area.

RESULTS

Characteristics of targeted IRS

Targeted IRS with pirimiphos-methyl started in Nchelenge District on October 20th in 2014, September 28th in 2015, and September 26th in 2016 and ran for 7-10 weeks [15, 37, 38]. Most areas targeted for IRS were in the peri-urban lakeside area where population density was highest (Figure 3.2). The number of targeted households in Nchelenge District increased from 18,315 in 2014 to approximately 26,000 in 2015 and 2016 [37, 38, 63]. Quality assurance activities conducted in five sentinel sites showed 100% mortality of *An. funestus* 24 hours after spraying in cone bioassays, declining to less than 80% mortality after five months in most sites [9].

Vector Abundance

Vector species composition

From April 2012 to July 2017, 13,780 female anopheline mosquitoes were collected from 1,724 visits to 1,084 cross-sectional and longitudinal households. These included 12,365 *An. funestus*, 1,371

An. gambiae, and 44 anophelines of other species. As expected, *An. funestus* abundance peaked in the dry season and *An. gambiae* peaked in the rainy season (Figure 3.3). Across all visits, households had an average of 7.2 and a median of 0 *An. funestus* (range = 0-226, IQR 0-2), and an average of 0.8 and a median of 0 *An. gambiae* (range = 0-35, IQR 0-0). There were a high number of households with counts of zero mosquitoes. No *An. funestus* were collected in 60.7% of household visits, and no *An. gambiae* were collected in 77.5% of household visits. Fifty three percent of household visits yielded no anopheline mosquitoes.

Impact of targeted IRS on vector densities

Over the entire study area, an average of 10.6 *An. funestus* and 0.96 *An. gambiae* were collected per household visit before the IRS campaign, which declined to 4.2 and 0.65 per visit, respectively, after IRS. Since more than half of households had no *Anopheles* mosquitos collected, the median values did not change; however, in Wilcoxon rank-sum tests, *An. funestus* counts were significantly lower after the intervention (P=0.01) but *An. gambiae* counts were not (P=0.6). In bivariate negative binomial models, which control for clustering within household but do not account for other covariates, there was a 58% decrease in household *An. funestus* counts (IRR = 0.42, 95% CI = 0.31-0.59) and a 31% decrease in household *An. gambiae* counts across the study area after targeted IRS (IRR = 0.69, 95% CI = 0.50-0.96). In bivariate models stratified by residence within the area targeted for IRS, *An. funestus* counts declined after the IRS intervention by 68% in the sprayed area (IRR = 0.32, 95% CI = 0.22-0.48) and by 50% in the unsprayed area (IRR = 0.50, 95% CI = 0.30-0.85), and this difference was statistically significant (P<0.001) (Table 3.1). In contrast, *An. gambiae* counts did not decline significantly in the sprayed area after the IRS intervention (IRR = 0.73, 95% CI = 0.49-1.08), but unexpectedly declined by 49% in the unsprayed area (IRR = 0.51, 95% CI = 0.28-0.94).

In multivariate models for the entire study area controlling for geographic and climate variables, there was a 51.0% decrease in *An. funestus* counts per household (IRR = 0.49, 95% CI = 0.29-0.82) and a 40.0% decrease in *An. gambiae* counts per household (IRR = 0.60, 95% CI = 0.44-0.80) over three years of IRS (Table 3.2). These results indicate that there was an overall decline in household vector counts throughout the study area following the IRS campaign.

In final multivariate models restricted to the sprayed area and controlling for all covariates, there was a 51% decrease in *An. funestus* counts (IRR = 0.49, 95% CI = 0.29-0.82) and a 36% decrease in *An. gambiae* counts (IRR = 0.64, 95% CI = 0.42-0.96) over three years of IRS with pirimiphos-methyl. (Table 3.3). For *An. funestus*, there were no significant differences from year to year (Figure 3.4), but there was a trend towards a larger change in year three. The impact on *An. gambiae* density was significantly higher in the first year of the IRS campaign, which had a 72% reduction, compared to the second and third years, which had 6% and 31% reductions, respectively. The addition of covariates and climatological factors did not substantially change the point estimates, however, there was a slight attenuation of the effect for *An. funestus* and a slightly increased effect for *An. gambiae* in the adjusted models (Figure 3.4).

Indirect effects of IRS

To investigate direct and indirect effects of the IRS intervention on household vector densities, models restricted to the sprayed area were further stratified by self-reported history of IRS. In unadjusted bivariate negative binomial models, there was 68% reduction in *An. funestus* counts in households that reported having been sprayed with pirimiphos-methyl (IRR = 0.32, 95% CI = 0.21-0.50), and a 65% reduction in households that reported not having been sprayed (IRR = 0.35, 95% CI = 0.22-0.54) (Table 3.1). These values were similar to the overall reduction in *An. funestus* counts in the sprayed area. In fully adjusted multivariate models, *An. funestus* counts decreased 67% in households that

reported IRS with pirimiphos-methyl (IRR = 0.33, 95% CI = 0.20-0.52), but did not decrease significantly in households that reported no history of IRS (IRR = 0.64, 95% CI = 0.32-1.27).

Within the area targeted for spraying, there was no significant reduction in *An. gambiae* counts among households that reported a history of IRS with pirimiphos-methyl in either adjusted or unadjusted models (unadjusted IRR = 0.90, 95% CI = 0.57-1.41; adjusted IRR = 0.74, 95% CI = 0.46-1.18). However, in households within the sprayed area that reported no history of IRS, *An. gambiae* counts decreased 41% and 48% in unadjusted and adjusted models, respectively, compared to household counts prior to the IRS campaign (unadjusted IRR = 0.59, 95% CI = 0.35-0.98; adjusted IRR = 0.48, 95% CI = 0.29-0.78).

Difference-in-differences analysis

Although household vector counts within the sprayed area were shown to decline after IRS with pirimiphos-methyl, there was no evidence of a significant impact of the intervention itself in the difference-in-differences analysis. As described above, these models include an interaction term which represents the ratio of the risk ratios for vector density before vs. after the IRS intervention in sprayed vs. unsprayed areas. For both species, vector counts were significantly lower post-IRS, but this decrease was only larger in the sprayed area for *An. funestus*, and the interaction term was not significant for either species (*An. funestus* = 0.67, 95% CI = 0.31-1.42; *An. gambiae* = 1.2, 95% CI = 0.62-2.40). These results indicate that there was a study area-wide reduction in vector counts, that this reduction was larger for *An. funestus* within sprayed areas, but that there was no significant difference in the change in vector counts between sprayed and unsprayed areas for either species. This suggests that the decrease in vector counts may not have been attributable solely to the IRS intervention, or that these models may not have the power to conclusively estimate these effects.

Impact of Covariates on Vector Abundance

In addition to the IRS campaign, several other factors were associated with household vector counts (Tables 2.1, 2.3). In both adjusted and unadjusted analyses, higher household density and increasing distance from category 1 streams was associated with lower vector abundance for both species, and increasing NDVI was associated with increased vector abundance. For *An. funestus*, higher elevation and steeper slope were associated with lower vector counts, and increasing distance from Lake Mweru and category 4 streams were associated with increased vector counts. For *An. gambiae*, household use of an open water source was associated with higher vector abundance. In unadjusted models, closed household eaves and metal roof type were additionally associated with lower *An. funestus* counts; however, these could not be included in final models since they were measured in only a subsample of the study population. Additional factors were associated with malaria risk in unadjusted models that no longer contributed to model fit in final multivariate models (Table 3.1).

The climatological variables that best predicted vector abundance were lagged rainfall and temperature (Table 3.3). For *An. funestus*, there was a 76% decrease in vector counts for each 10 mm increase in average daily rainfall over a two-week interval, lagged two weeks (IRR = 0.27, 95% CI = 0.16, 0.48). There was a 14% increase in vector counts for each 1° C increase in maximum temperature in the one-week period lagged 2 weeks (IRR = 1.14, 95% CI = 1.03-1.26), and a 21% decrease in vector counts for each 1° C increase in maximum temperature in the eight-week period immediately prior to this (IRR = 0.79, 95% CI = 0.66-0.94), indicating a more complex relationship with temperature depending on stage of life cycle and other factors. For *An. gambiae*, there was a 32% decrease in vector counts for each 10 mm increase in average daily rainfall over the two-week interval, lagged two weeks (IRR = 0.68, 95% CI = 0.47-0.99), but a nearly 400% increase in vector counts for each 10 mm increase in average daily rainfall for the prior ten-week period (IRR = 3.96, 95% CI = 1.88-8.37). There was a 21% decrease in vector counts for each 1° C increase in maximum temperature in the seven-week interval lagged two

weeks (IRR = 0.79, 95% CI = 0.67-0.92), and a 32% increase in vector counts for each one-degree C° increase in minimum temperature for this same time period (IRR = 1.32, 95% CI = 1.18-1.48), indicating that *An. gambiae* may survive and reproduce best within a specific temperature window.

Parasite Prevalence

Study population

A total of 3,309 individuals residing in 1,025 households participated in the study between April 2012 and July 2017, including 2,446 participants from within the area targeted for IRS and 863 participants outside the targeted area. Approximately 45% of participants were male, 19% were younger than 5 years, and 34% were school age (between 6 and 16 years). Sixty percent of participants were anemic and 2% had a fever at the time of the study visit. The overall parasite prevalence by RDT in the population over the duration of the study was 49.8%, with 59% prevalence in children under 5 years and 72% prevalence among school age children. Overall parasite prevalence ranged from 20% to 80% in the sprayed area and 22% to 78% in the unsprayed area (Figure 3.3). In most years there were two annual peaks in parasite prevalence, the first of which occurred soon after the start of the rainy season and the second occurring during the dry season, consistent with the changes in vector populations. Due to sampling issues, no households from the unsprayed area participated from November 2016 to March 2017. Over the three years of IRS, 54% of study households in the targeted areas reported that they were sprayed, corresponding to 55% of participants. By year, the proportion of eligible households in targeted areas that reported being sprayed was 56% in year 1, 48% in year 2, and 57% in year 3.

There were some demographic differences in the study population before and after the start of targeted IRS with pirimiphos-methyl (Table 3.4). Across the study population, there was a decrease in the proportion of participants with natural flooring and whose HOH was in permanent employment, and an increase in participants whose HOH had only primary school education. In sprayed areas, there was a

decrease in children under five years. In unsprayed areas, there was a decrease in participants using an open water source and an increase in participants who reported sleeping under a bed net.

Impact of targeted IRS on parasite prevalence

In analyses over the entire study area, there was no change in parasite prevalence by RDT before vs. after IRS in either unadjusted or adjusted analyses. This result was consistent in chi-squared analyses (OR = 0.99, 95% CI = 0.87-1.14) and in multivariate Poisson models stratified by season and adjusting for all covariates (rainy season PRR = 0.90, 95% CI = 0.80-1.02; dry season PRR = 0.99, 95% CI = 0.89-1.10).

Further analyses were restricted to the area targeted for IRS. In unadjusted chi-squared analyses, there was a 16% decrease in parasite prevalence in the sprayed area compared to a 46% increase in parasite prevalence in the unsprayed area (Table 3.4). In the sprayed area, there was also a 66% decrease in fever, a 30% decrease in history of taking malaria medication, but a 73% increase in anemia. In unadjusted bivariate Poisson models, however, which controlled for clustering within household but did not account for other covariates, the change in parasite prevalence after IRS with pirimiphos-methyl was not statistically significant for either rainy (PRR = 0.90, 95% CI = 0.78-1.06) or dry seasons (PRR = 0.89, 95% CI = 0.76-1.03) (Table 3.3). Similarly, when only demographic and geographic variables were included in models, there was no statistically significant change in parasite prevalence after IRS in rainy (PRR = 0.88, 95% CI = 0.77-1.01) or dry seasons (PRR = 0.94, 95% CI = 0.82-1.08) (Figure 3.5).

In final multivariate Poisson models for the sprayed area, which included weather and hydrology covariates, IRS with pirimiphos-methyl was associated with a 28% decrease in parasite prevalence in the rainy season (PRR = 0.72, 95% CI = 0.62-0.84), but was not significantly associated with decreased malaria risk in the dry season (PRR = 0.91, 95% CI = 0.80-1.05) (Table 3.6). There was no significant

difference year-to-year; however, there was a non-significant trend toward a greater impact of IRS with each subsequent year when climatological factors were included in models (Figure 3.5).

In all analyses, there was a trend towards a decrease in malaria prevalence after the IRS intervention, although which covariates were included had a large effect on the magnitude of the outcome. The addition of weather and hydrological covariates had a substantial impact on model precision and effect size (Figure 3.4). The rationale for including these variables in the model was to account for inter-annual differences in malaria risk based on climatological variation, and these results suggest that these patterns must be taken into account to accurately model the impact of malaria control interventions in this setting.

Indirect effects of targeted IRS on parasite prevalence

To investigate direct and indirect effects of the IRS intervention on parasite prevalence, models restricted to the sprayed area were stratified by self-reported history of IRS. In unadjusted Poisson models, parasite prevalence in the rainy season decreased 18% among participants who reported IRS with pirimiphos-methyl (PRR = 0.82, 95% CI = 0.69-0.97), but there was no significant change among participants who reported no IRS (PRR = 1.02, 95% CI = 0.84-1.21). In the dry season, there was no significant change in parasite prevalence among participants who reported IRS with pirimiphos-methyl (PRR = 0.93, 95% CI = 0.77-1.11) or those who did not (PRR = 0.86, 95% CI = 0.71-1.05).

In fully adjusted Poisson models restricted to the sprayed area, parasite prevalence in the rainy season decreased 33% among participants who reported IRS with pirimiphos-methyl (PRR = 0.67, 95% CI = 0.63-0.87) and 26% among participants who reported no IRS (PRR = 0.74, 95% CI = 0.63-0.87), compared to parasite prevalence prior to the IRS campaign. This indicates both direct and indirect effects of IRS with pirimiphos-methyl within the sprayed areas. In the dry season, there was no

significant change in parasite prevalence in either sprayed (PR = 0.88, 95% CI = 0.74-1.05) or unsprayed households (PR = 0.98, 95% CI = 0.83-1.15) within the targeted areas.

Difference-in-differences analysis

The difference-in-differences analysis was conducted to further control for secular trends and to account for the apparent increase in malaria prevalence in unsprayed areas (Table 3.4). As described above, the interaction term in these models is the ratio of the risk ratios for malaria prevalence before and after IRS with pirimiphos-methyl in the sprayed vs. unsprayed areas. For the rainy season, the value of this interaction term was 0.77 (95% CI = 0.62-0.95) indicating that there was a 23% larger decrease in parasite prevalence in the sprayed area compared to the unsprayed area. For the dry season, the interaction term was not statistically significant (PRR = 0.83, 95% CI = 0.65-1.05).

Impact of Covariates on Malaria Prevalence

In addition to the IRS intervention, several other factors were associated with parasite prevalence (Tables 3.5, 3.6). In both adjusted and unadjusted analyses across seasons, the strongest risk factors for RDT positivity were male sex, school age, and age under five years. The strongest protective factors were sleeping under a bed net, residence in areas with high population density, and higher elevation. The large protective effect of sleeping under a bed net is noteworthy given the high levels of pyrethroid resistance among vectors in this setting. Having a HOH with only a primary school education was another risk factor and contributed to model fit across analyses. In the rainy season, increasing distance from Lake Mweru was associated with increased malaria risk. In the dry season, increasing distance from Lake Mweru and category 1 streams was associated with decreased malaria risk, and increasing distance from health clinics was associated with increased malaria risk. In unadjusted models, closed household eaves and metal roof type were associated with lower malaria prevalence in both rainy and dry seasons, but these variables could not be included in final models since they were only

measured in a subsample of the study population. Additional factors were associated with malaria risk in unadjusted models that no longer contributed to model fit in final multivariate models (Table 3.5).

The climatological variables that best predicted malaria risk in the rainy season were lagged rainfall and minimum temperature (Table 3.6). There was a 24% increase in malaria risk for each 10 mm increase in average daily rainfall over a two-week interval and lagged three weeks (PRR = 1.24, 95% CI = 1.06-1.45). There was a 15% increase in risk for each 1° C increase in minimum daily temperature averaged over a three-week interval, lagged two weeks (PRR = 1.15, 95% CI = 1.08-1.22). In the dry season, the climatological variables that predicted malaria risk were lagged maximum temperature and streamflow. Malaria risk increased 17% for each 1° C increase in daily maximum temperature averaged over a five-week interval and lagged three weeks (PRR = 1.17, 95% CI = 1.09-1.17). Malaria risk decreased 10% for each 1000 m^3/s increase in streamflow over a two-week interval and lagged four weeks (PR = 0.90, 95% CI = 0.83-0.97).

DISCUSSION

After three years of targeted IRS with pirimiphos-methyl in Nchelenge District, Zambia, there was a decrease in both vector abundance and parasite prevalence in the area targeted for spraying. Household vector counts but not parasite prevalence decreased over the entire study area, and parasite prevalence in the targeted area decreased only within the first six months after IRS.

In all analyses, *An. funestus* counts per household decreased after the IRS intervention. This result was consistent in unadjusted analyses and in analyses adjusting for geographic and climatological covariates. Vector counts declined by approximately 50% over the entire study area, including both the sprayed and unsprayed areas. While there was a slightly larger decrease in the sprayed area than the unsprayed area, the ratio of risk ratios was not statistically significant in the difference-in-differences analysis, indicating that the change in vector counts post-intervention was not meaningfully larger in the

area targeted for IRS. However, within the targeted area, the decline in *An. funestus* vector abundance was twice as large in sprayed households as in unsprayed households.

Similarly, *An. gambiae* counts per household decreased after the IRS intervention by 40% in the entire study area and by approximately 36% in the area targeted for spraying. However, this result was less consistent across analyses, likely because of small sample sizes and resulting wide confidence intervals. The decline in the unsprayed area was larger than in the sprayed area, and therefore the ratio of risk ratios was not statistically significant in the difference-in-differences analysis. Furthermore, when households within the targeted area were stratified by history of IRS, households that had not been sprayed unexpectedly showed a larger decline in vector abundance after the intervention than households that had been sprayed.

These results generally indicate that there was a significant overall reduction in indoor *An. funestus* and *An. gambiae* abundance in the study area following IRS with pirimiphos-methyl, but that the cause of this decline is likely multifactorial and may not be wholly due to the intervention itself. Although it is feasible that the IRS intervention reduced vector counts district-wide due to unexpectedly long flight distances among host-seeking *Anopheles* mosquitoes [64], sampled unsprayed households were often 5-10 km away from the sprayed area and would not be expected to experience a large impact of IRS at these distances. The absence of a larger effect in the sprayed area than the unsprayed area is surprising, particularly for *An. gambiae*, as is the absence of a significant decline in *An. gambiae* counts in sprayed households.

Both unexpected findings may be explained in part by the high variability in the vector data and the large number of zero counts, which may reduce model stability and may increase the impact of outliers. For example, in the small inland lagoon area within the sprayed zone (Figure 3.2), both *An. funestus* and *An. gambiae* counts after the intervention averaged three times higher than in lakeside

sprayed areas, which may have had a disproportionate effect on model outcomes. Due to lack of power, models could not be further stratified to isolate the effects in different geographical areas, and these outliers were not excluded from analyses in order to provide a comprehensive evaluation of the intervention throughout sprayed areas in the district. Given these challenges, it is possible that these models lack the power to conclusively demonstrate the isolated effect of the intervention on vector abundance in Nchelenge District as compared with other factors.

The impact of the intervention on parasite prevalence was more readily interpretable. Over three years of IRS with pirimiphos-methyl in Nchelenge District, there was an approximately 25% decrease in rainy season malaria parasite prevalence in areas targeted for spraying. This result was consistent across adjusted analyses. Households with a self-reported history of IRS had a 32% larger effect size than unsprayed households within the sprayed area, but both sets of households experienced a decline in prevalence. This demonstrates that IRS with pirimiphos-methyl had both direct and indirect effects on malaria transmission in the targeted area. However, there was no impact of the IRS intervention on parasite prevalence in the dry season or at the district level. These results indicate that there was a moderate impact of targeted IRS with pirimiphos-methyl on parasite prevalence in Nchelenge District in the six months following the intervention, but only within the targeted area.

Although there was a significant reduction in parasite prevalence and vector abundance following the IRS intervention, the size of the impact was not as large as anticipated given the scale of the intervention and the efficacy of the insecticide used. While a 25% decrease in prevalence corresponds to a large number of cases averted in this high-transmission region, this result was observed for only half of the year, and malaria burden continues to be substantial in Nchelenge District. Furthermore, although overall vector abundance declined after IRS, high vector counts continued to be collected from sprayed households, with up to 93 *An. funestus* and 35 *An. gambiae* collected from single

sprayed households in the six months after the intervention. Several potential explanations for the lower than expected impact are explored below.

Nchelenge District is a particularly challenging setting for malaria control with many barriers to intervention effectiveness. Year-round transmission and high EIRs lead to a high force of infection, and the predominance of asymptomatic infections may result in a large untreated parasite reservoir. The population in Nchelenge is highly mobile, and movement between the inland and lakeside areas or across the border to the neighboring DRC can increase exposure to infected mosquitoes for people residing in intervention areas. There is only one paved road in the district, and flooding is common throughout much of the year, which creates substantial barriers to provision of malaria interventions and health services to the remote rural population. Furthermore, studies in other areas have shown *An. funestus* and *An. gambiae* may have substantial outdoor biting behavior if people are outside during peak hours [65-67]. This can reduce the effectiveness of many vector control activities, including IRS and LLINs, if people are outside in early mornings or late evenings. In fact, outdoor and daytime biting behavior of *An. funestus* and *An. gambiae* has been shown to increase after the implementation of vector control activities, which may select for this behavior in mosquito populations [68-71].

In addition to these structural barriers, there may be limitations of the current IRS strategy for this setting. Although pirimiphos-methyl was highly effective against malaria vectors in the region, studies have shown that this formulation produces only 5-8 months of insecticidal activity, with a particularly short duration of efficacy on the natural or mud walls common in this region of northern Zambia [13, 38, 72-74]. Since Nchelenge District experiences year-round transmission and *An. funestus* populations peak in the dry season, a single annual application of insecticide at the start of the rainy season would impact only rainy season malaria transmission and would not affect transmission by *An. funestus* for the other half of the year. This evaluation supports this hypothesis, as there was no significant impact of pirimiphos-methyl on parasite prevalence in the dry season, which typically started

6 months after IRS application. In addition, high parasite prevalence and vector densities exist in the areas not targeted for IRS. Prior to the intervention, approximately half of participants in unsprayed areas were RDT positive, but these regions were not included in the IRS campaign due to low population density and logistical challenges. Since the entire district has a high malaria burden, targeted IRS would not be expected to interrupt transmission in this setting.

There were additional challenges of the IRS campaign that may have limited the effectiveness of the intervention. In the active surveillance data, only 54% of participating households in IRS-targeted areas reported that their house had been sprayed, which corresponds to 55% of targeted participants and 42% of all participants in the study area. The 2015 Malaria Indicator Survey similarly reported that only 32% of households in Luapula Province received IRS in the past 12 months [75]. This level of coverage is substantially lower than the goal of 85% and would not be expected to reduce the impact of the IRS campaign [15]. Furthermore, in several years, IRS began after the first rainfall and continued into mid-November in all three years. The extension of spray operations into the rainy season could attenuate the impact of the intervention, since many households in the targeted area would not have been sprayed until after vector populations started to increase. The long duration of the spray campaign could also reduce acceptability of the intervention in the community, since home preparation for IRS includes placing all household items outside, which would be less tolerated during rainy weather. This could have contributed to the low household coverage by either active refusals or passive non-participation, in which household members are intentionally absent for the day of spray operations. In official reports, PMI confirmed that the inconvenience of home preparation contributed to low acceptability of IRS in Nchelenge District, among other factors [38].

Given these substantial barriers and challenges, the question remains of how best to implement malaria control activities in this high-transmission region. Zambia has stated the goal of national malaria elimination by 2021 [9], however this objective will be difficult to achieve in Nchelenge District with the

current rate of progress. To this end, a substantial increase in resources and intervention coverage will be needed if interruption of transmission in this area is going to be achieved.

In high-transmission areas, vectorial capacity must be reduced substantially to interrupt transmission [66, 76, 77]. To achieve this, IRS must be performed consistently at high coverage and for multiple years to have a considerable impact on parasite prevalence [78, 79]. Furthermore, dry season malaria transmission has been implicated in failure of malaria control, even in low-transmission areas and those with a single primary transmission season [65, 79, 80]. Mathematical models have demonstrated that once-yearly IRS is insufficient to substantially reduce malaria prevalence in high-transmission areas, although twice-yearly IRS in combination with other interventions could reduce prevalence to 10% [79]. These results indicate that the high degree of dry season transmission in Nchelenge District will undermine intervention effectiveness if malaria control measures are concentrated on rainy season transmission only.

Due to these factors, IRS should continue to be used in Nchelenge District as one of a suite of malaria control activities, but spraying must be conducted twice yearly with at least 85% coverage across the district to have a substantial impact on malaria burden. The significant reduction in parasite prevalence given the current strategy indicates that IRS can be an important part of an integrated vector management plan in this high-transmission setting and that much larger gains could be made if interventions were scaled up and coverage was increased. The evidence of indirect effects of IRS further suggests that the impact might increase disproportionately at high levels of coverage. In keeping with Zambia's current policy, monitoring for insecticide resistance should continue to ensure that IRS remains effective [8, 9]. Although achieving these goals will require overcoming substantial economic and logistical barriers, this level of investment will be required to achieve successful malaria control and elimination in Nchelenge District by 2021.

This evaluation had several strengths. The study included a long time series of both epidemiologic and entomologic surveillance, the combination of which is rare in areas of high transmission. This time series allows for the investigation of seasonality, inter-annual variation, and long-term temporal trends of parasite prevalence and vector abundance. Although both vector and epidemiologic data sets include a large degree of noise and variation due to sampling probabilities, natural fluctuations in weather, and life cycles of parasites and vectors, the availability of six years of data helps control for these effects in statistical models. If the evaluation were conducted for only the year before and after the start of the intervention, the decrease in vector abundance and parasite prevalence would be overestimated. The long time series furthermore allows for the inclusion of weather and hydrological covariates, which are known to have a large impact on vector abundance and malaria risk, but are often omitted due to the lack of appropriate multi-year data to parameterize models. This analysis shows that inclusion of these climatological factors was important to properly evaluate the impact of the IRS intervention. When climate variables were not included, the effect of the intervention was attenuated.

Another strength of this evaluation was the use of active surveillance data. Although health center case reports are one of the cornerstones of malaria surveillance, there are limitations to these data that active surveillance circumvents. In a high-transmission area like Nchelenge District, malaria is frequently asymptomatic or sub-clinical, particularly in older age groups. In this study population, fever was recorded in only 8% of RDT positive children under five years and in only 3% of all RDT positive participants. This low proportion of febrile illness in infected individuals indicates that people are less likely to seek health care, and thus will not be recorded as a case. Therefore, relying on health center incidence reports alone may lead to underestimation of the malaria burden.

Furthermore, although malaria testing and treatment is free of charge in Zambia, health centers in remote areas often experience stockouts of RDTs and antimalarial drugs, which can impact malaria

diagnosis and reporting. Over the course of this study in Nchelenge District, among 11 health centers that reported weekly case counts, RDT stockouts occurred in at least one health center in 60% of reported weeks, and stockouts occurred in at least six health centers in 13% of reported weeks. During these times, the number of confirmed cases declined but the number of patients treated for malaria increased, presumably because febrile patients were treated presumptively without confirmation (Figure 3.7). For this reason, health center data from Nchelenge District on either confirmed or suspected cases are unreliable to assess the impact of IRS with pirimiphos-methyl. In exploratory regression analyses clustered by health center, there was no apparent impact of the IRS intervention using either the number or percent of RDT positives in sprayed or unsprayed areas. The availability of active surveillance data allows for a comprehensive evaluation of the IRS intervention in this area without these limitations.

This study also had several limitations. Because the overall ICEMR study was designed as a surveillance system without this specific intervention in mind, there was limited power to investigate the impact of this IRS campaign at finer spatial and temporal scales. This was a particular limitation for the vector data, which had a large number of zero counts and was overdispersed, limiting the ability to examine intervention effects by season and geography and leading to large confidence intervals. A larger sample size would aid in performing intervention evaluations with vector data, but this is extremely labor- and laboratory-intensive and was not possible for the present study nor programmatically feasible for ongoing surveillance. Furthermore, although it is generally a strength of this analysis, the use of active surveillance data limits the generalizability of the evaluation to other areas of Zambia that have only health center-based surveillance. Although RDT stockouts precluded a comprehensive evaluation of the IRS intervention in Nchelenge District using passive surveillance, health center reports must be an integral part of large-scale intervention evaluations in Zambia.

Another limitation of this analysis is the inability to conclusively link declines in vector populations to declines in parasite prevalence. Vector counts per household decreased across the study area and were difficult to link to the intervention explicitly. In contrast, malaria prevalence decreased moderately in the area targeted for spraying only. This can once more be partially explained by the high degree of variation and difficulty modeling vector count data, which limits the inferential power of the vector analyses. Furthermore, EIRs must be reduced to very low numbers to have a large impact on malaria prevalence, and therefore reductions in vector density in Nchelenge District would need to be large before a direct relationship with parasite prevalence is observed.

The use of cross-sectional parasite prevalence data rather than malaria incidence data is another potential limitation, since prevalence is a less direct indicator of recent malaria transmission due to the long potential duration of natural infection. Using prevalence rather than incidence may therefore attenuate the calculated impact of the IRS intervention because the time of initial infection is unknown. However, the long time series of data collected post-intervention should somewhat mitigate the difference in metrics since reduced malaria transmission will ultimately result in reduced prevalence. Prevalence is also a useful indicator of population disease burden and therefore provides a programmatically relevant metric of intervention effectiveness.

Various sources of measurement error could also have impacted study outcomes. For low-level infections, RDTs may return false negatives due to low sensitivity or HRP-2 deletions, and frequent infection in high-transmission areas may result in false positives due to persistent HRP-2 antigenemia [81-84]. Although not available for the full period of this evaluation, future analyses will include PCR results to help control for these issues. In addition, the use of light traps may underestimate true indoor vector counts. For each individual host-seeking mosquito, there is a chance that it will not be captured and observed in the trap due to a variety of natural stochastic processes. Mathematically, this would have a larger impact for households with low vector abundance and may have contributed to the high

number of zero counts and thereby model instability. However, underestimation of household counts can occur with any vector collection method, and the considerable benefits of using light traps for active surveillance, including logistical ease, low cost, and high acceptability, outweigh any drawbacks in comparison to other methods of vector collection that are more time- and laboratory-intensive and costly [85].

CONCLUSION

Indoor residual spraying continues to be an important element of integrated vector control. Three years of targeted IRS with pirimiphos-methyl was associated with significant reductions in malaria parasite prevalence and indoor vector abundance within intervention areas in Nchelenge District, Zambia. Vector abundance also decreased in nearby unsprayed areas, although parasite prevalence did not. Across analyses, declines were not as large as anticipated due a variety of structural and logistical challenges. It is expected that substantial financial and logistical investments must be made in this region to interrupt malaria transmission, including twice-yearly IRS with high coverage across the district.

Figure 3.1: Nchelenge District sampled and enumerated households from April 2012 – July 2017

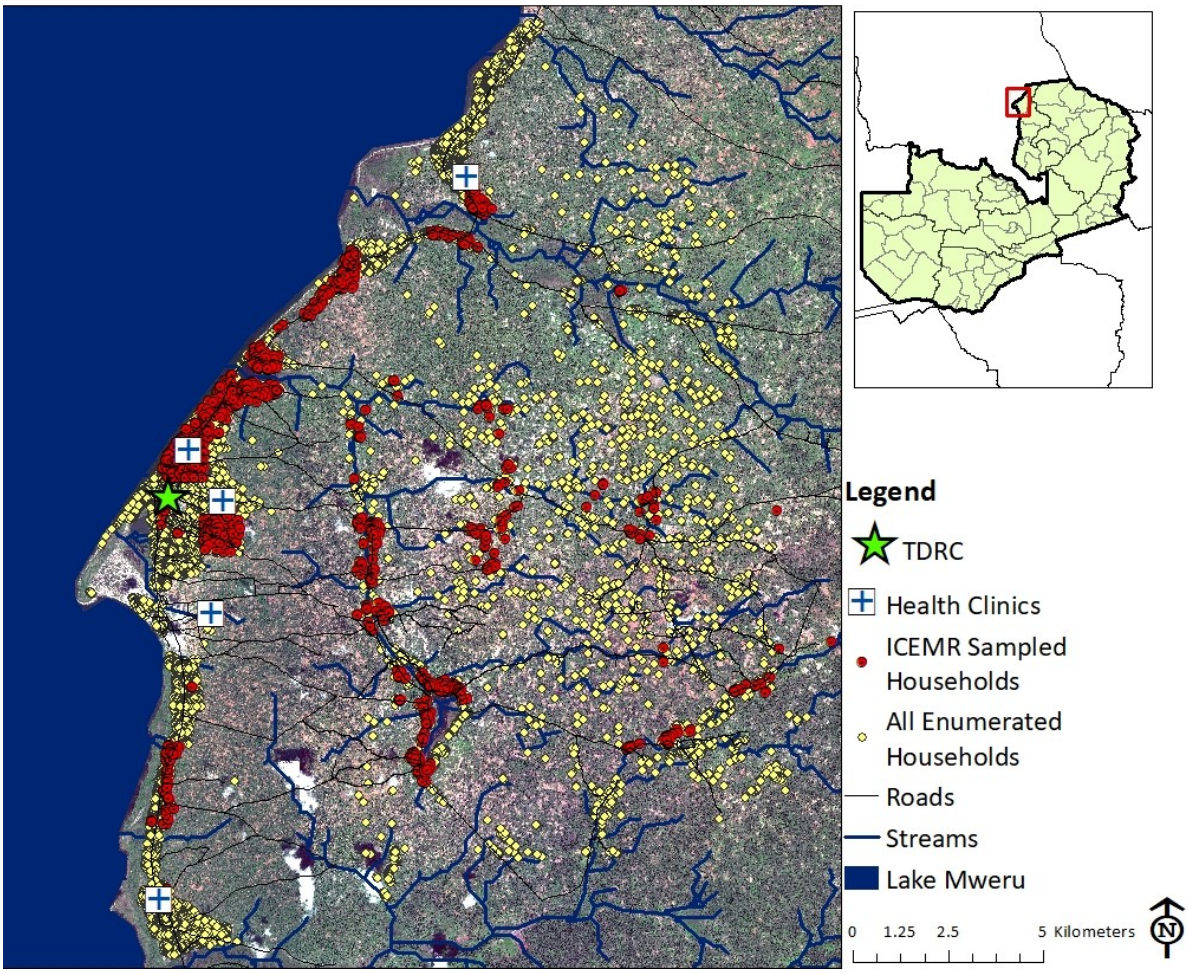


Figure 3.2: Areas in Nchelenge District targeted for IRS in year 1 (2014) and years 2 and 3 (2015, 2016)

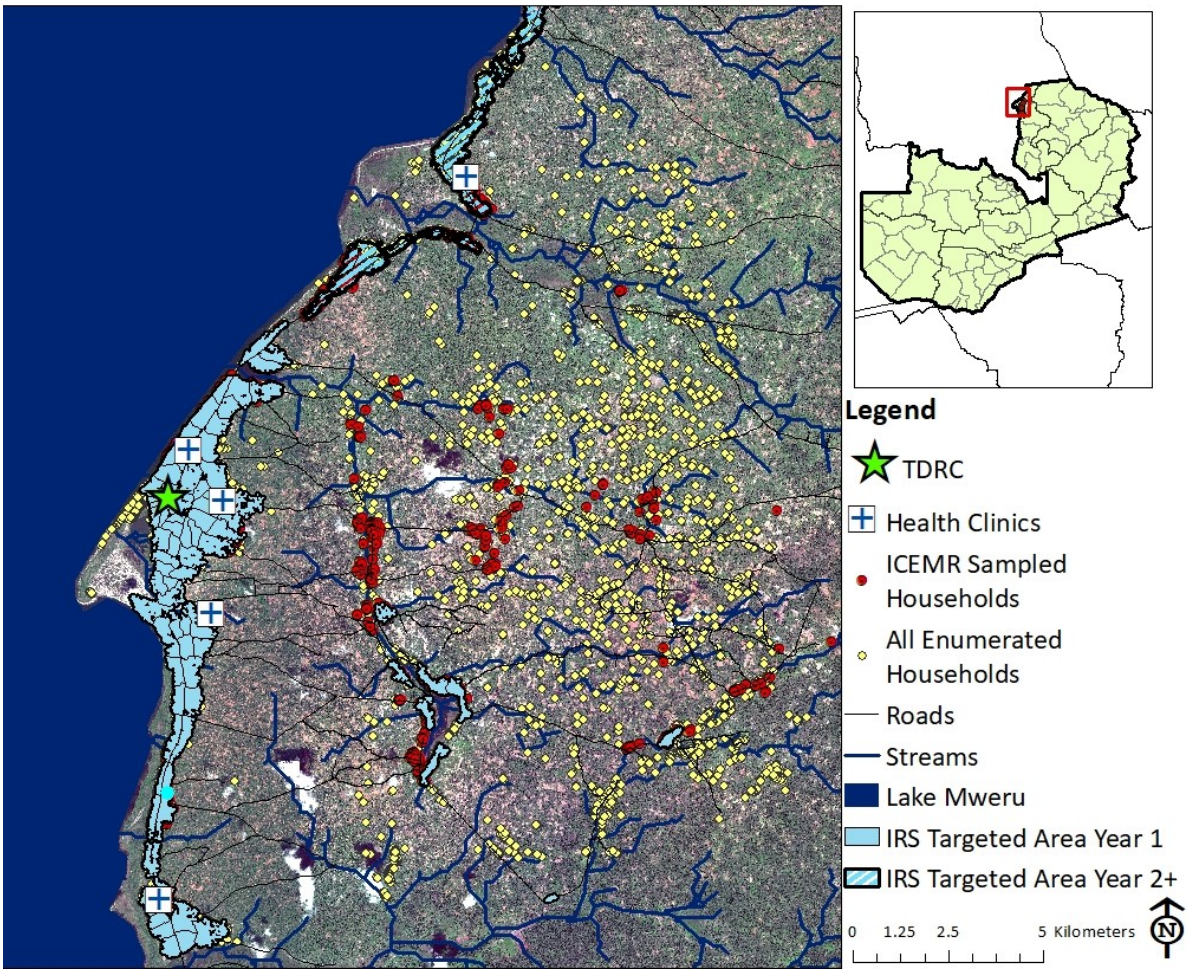


Figure 3.3 Time series of A) *An. funestus* and B) *An. gambiae* average counts per household in sprayed and unsprayed areas in Nchelenge District

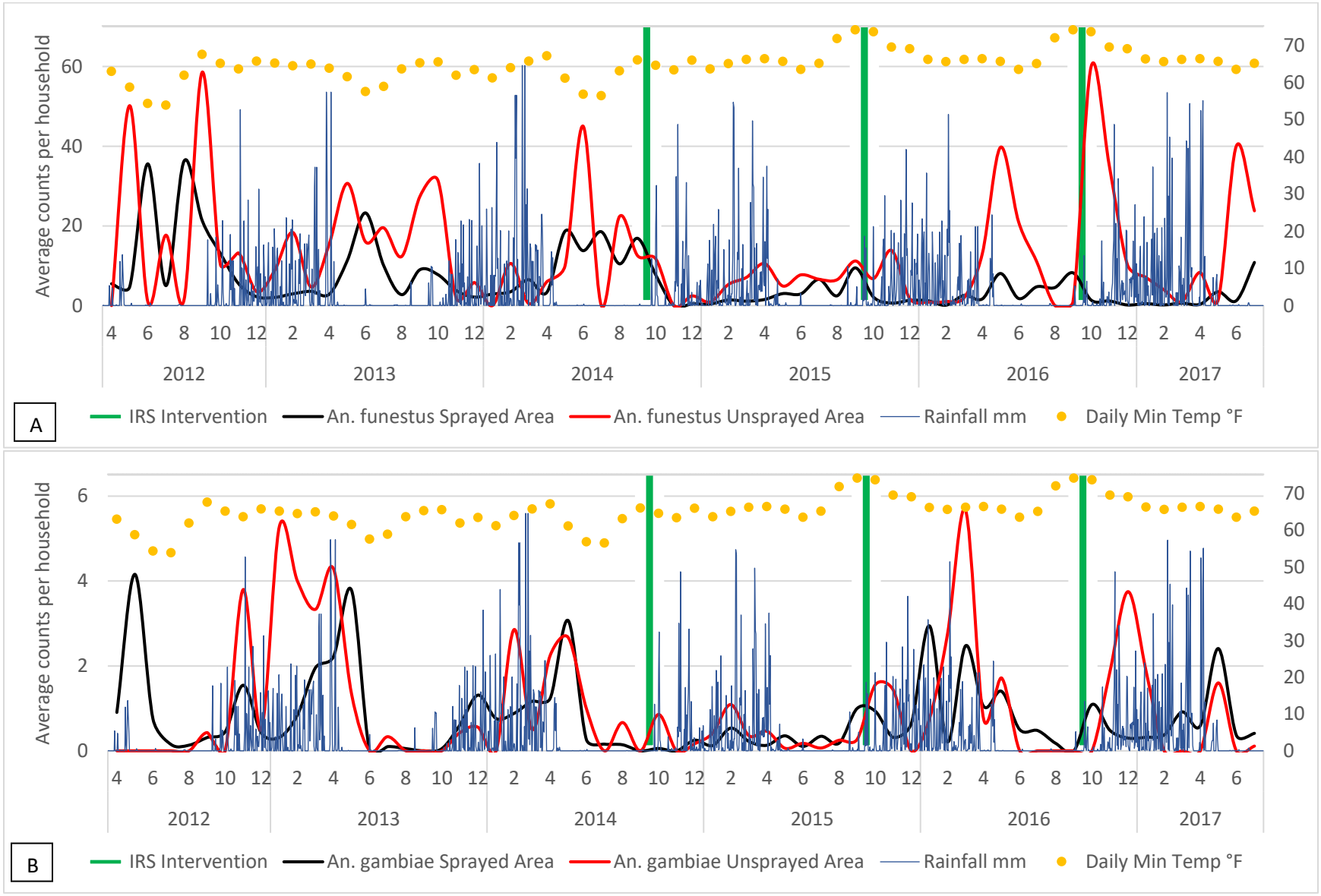


Table 3.1: Factors associated with household counts of *An. funestus* and *An. gambiae* within the areas targeted for spraying using bivariate negative binomial models with robust standard errors and GEE clustered by household, N=1,271

	An. funestus			*An. gambiae*		
	IRR	95% CI	P value	IRR	95% CI	P value
IRS VARIABLES						
Post-IRS	0.32	(0.22, 0.47)	*<0.001*	0.73	(0.49, 1.08)	0.1
Self-reported history of IRS[#]	0.32	(0.21, 0.50)	*<0.001*	0.90	(0.57, 1.41)	0.6
Self-reported history of no IRS[#]	0.35	(0.22, 0.54)	*<0.001*	0.59	(0.35, 0.98)	0.04
DEMOGRAPHIC VARIABLES						
Number of household participants	1.10	(1.03, 1.19)	*0.008*	1.00	(0.92, 1.08)	0.9
Longitudinal HH type	1.13	(0.63, 2.07)	0.7	0.62	(0.42, 0.92)	*0.02*
Dirt floor in home	4.70	(2.59, 8.53)	*<0.001*	1.49	(0.89, 2.52)	0.1
Unprotected water source	1.62	(1.02, 2.58)	*0.04*	2.11	(1.40, 3.16)	*<0.001*
Metal roof*	0.23	(0.11, 0.47)	*<0.001*	0.71	(0.40, 1.26)	0.2
Closed eaves*	0.27	(0.13, 0.55)	*<0.001*	0.74	(0.40, 1.36)	0.3
GEOGRAPHIC VARIABLES						
HHs within 500 m (by 100 HH)	0.53	(0.46, 0.62)	*<0.001*	0.78	(0.70, 0.86)	*<0.001*
Elevation (by 10 m)	1.21	(1.11, 1.32)	*<0.001*	1.05	(0.95, 1.16)	0.4
Slope (by 1°)	0.90	(0.82, 0.99)	*<0.001*	0.94	(0.84, 1.05)	0.3
NDVI (by 10%)	2.03	(1.63, 2.53)	*<0.001*	1.51	(1.17, 1.95)	*0.002*
Distance from Lake Mweru (in km)	1.22	(1.16, 1.30)	*<0.001*	1.08	(1.03, 1.14)	*0.004*
Distance from health clinics (in km)	1.47	(1.37, 1.57)	*<0.001*	1.16	(1.10, 1.22)	*<0.001*
Distance from roads (in 100 m)	0.58	(0.43, 0.80)	*0.001*	0.82	(0.53, 1.26)	0.4
Distance from cat. 1 streams (in km)	0.27	(0.13, 0.58)	*0.001*	0.62	(0.30, 1.27)	0.2
Distance from cat. 2 streams (in km)	0.63	(0.47, 0.82)	*0.001*	0.76	(0.62, 0.92)	*0.004*
Distance from cat. 3 streams (in km)	0.92	(0.76, 1.11)	0.4	1.02	(0.87, 1.19)	0.8
Distance from cat. 4 streams (in km)	1.39	(1.23, 1.56)	*<0.001*	1.12	(0.99, 1.26)	0.07

IRR = incidence rate ratio, CI = confidence interval, HH = household

[#] Comparison group is malaria prevalence pre-IRS, *subsample of all households, N=1,334

Table 3.2: Negative binomial multivariate models of the impact of targeted IRS with pirimiphos-methyl on *An. funestus* and *An. gambiae* counts per household over the entire study area, using robust standard errors and GEE clustered by household, N=1,724

	An. funestus			*An. gambiae*		
	IRR	95% CI	P value	IRR	95% CI	P value
Post-IRS	0.49	(0.30, 0.82)	*0.007*	0.60	(0.44, 0.80)	*0.01*
HH within 500 m (by 100 HH)	0.66	(0.54, 0.81)	*<0.001*	0.82	(0.75, 0.89)	*<0.001*
Elevation (by 10 m)	0.53	(0.46, 0.61)	*<0.001*	-		
Slope	0.88	(0.80, 0.97)	*0.007*	-		
NDVI (by 10%)	1.24	(1.03, 1.49)	0.02	-		
Lakeside	0.24	(0.14, 0.41)	*<0.001*	0.29	(0.16, 0.50)	*<0.001*
Distance from Lake Mweru (in km)	-			0.83	(0.77, 0.89)	*<0.001*
Distance from roads (in 100 m)	0.80	(0.74, 0.86)	*<0.001*	0.82	(0.75, 0.91)	*<0.001*
Distance from cat. 1 streams (in km)	0.55	(0.31, 0.98)	*0.04*	0.56	(0.41, 0.78)	*<0.001*
Lagged rainfall (by 10 mm) [1]	0.27	(0.16, 0.48)	*<0.001*	-		
Lagged rainfall (by 10 mm) [2]	0.62	(0.39, 0.96)	*0.03*	-		
Lagged rainfall (by 10 mm) [3]	-			0.67	(0.48, 0.94)	*0.02*
Lagged rainfall (by 10 mm) [4]	-			2.33	(1.40, 3.86)	*0.001*
Lagged maximum temperature (in C°) [1]	1.08	(1.01, 1.16)	*0.03*	-		
Lagged maximum temperature (in C°) [5]	0.80	(0.69, 0.92)	*0.003*	-		
Lagged maximum temperature (in C°) [6]	-			0.81	(0.73, 0.90)	*<0.001*
Lagged minimum temperature (in C°) [6]	-			1.30	(1.20, 1.41)	*<0.001*

IRR = incidence rate ratio, CI = confidence interval, HH = household

[1] Interval=2 weeks, lag=2 weeks; [2] Interval=2 weeks, lag=4 weeks; [3] Interval=1 weeks, lag=2 weeks; [4] Interval=7 weeks, lag=3 weeks; [5] interval=8 weeks, lag=4 weeks; [6] interval=4 weeks, lag=3 weeks

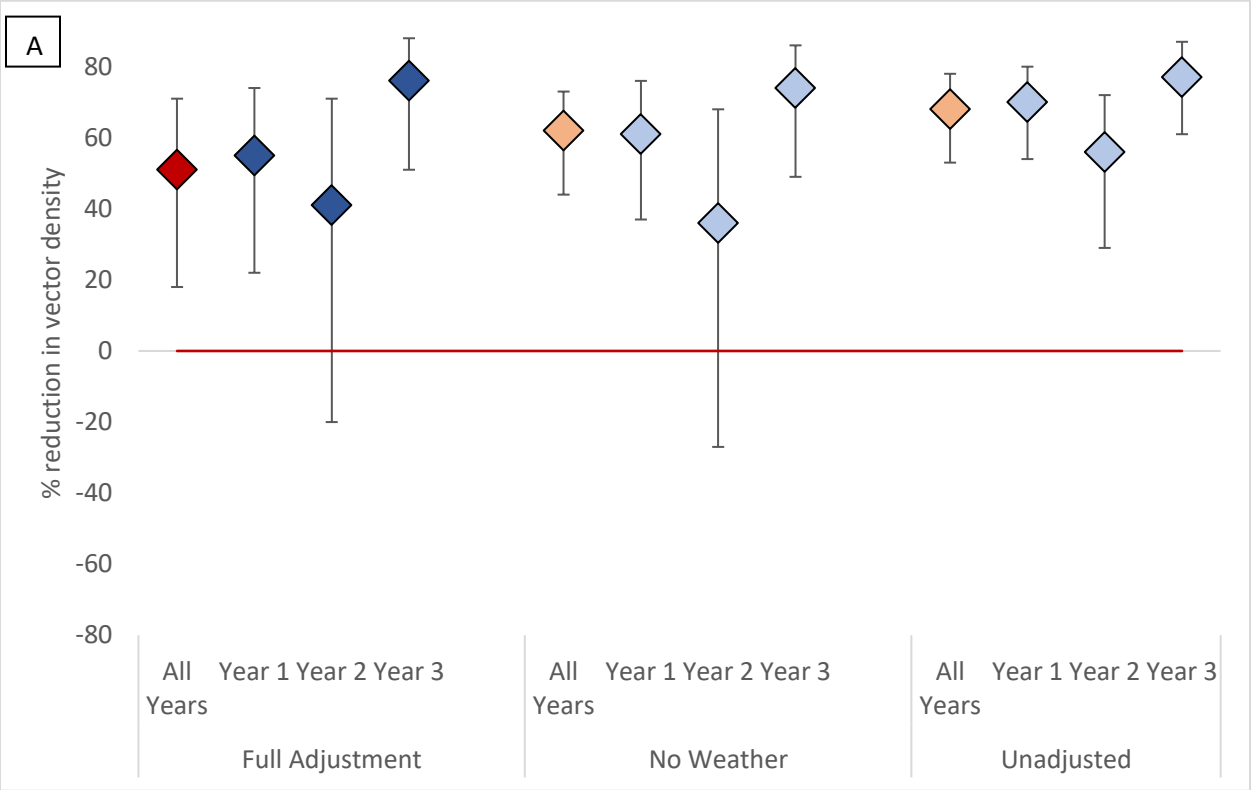
Table 3.3: Negative binomial multivariate models of the impact of targeted IRS with pirimiphos-methyl on *An. funestus* and *An. gambiae* counts per household within the areas targeted for spraying, using robust standard errors and GEE clustered by household, N=1,271

	An. funestus			*An. gambiae*		
	IRR	95% CI	P value	IRR	95% CI	P value
Post-IRS	0.49	(0.29, 0.81)	*0.005*	0.64	(0.42, 0.96)	*0.03*
Open water source	-			1.41	(1.02, 1.95)	*0.04*
HH within 500 m (by 100 HH)	0.60	(0.51, 0.70)	*<0.001*	0.81	(0.75, 0.88)	*<0.001*
Elevation (by 10 m)	0.43	(0.33, 0.55)	*<0.001*	-		
Slope	0.82	(0.73, 0.92)	*0.001*	-		
NDVI (by 10%)	1.29	(0.99, 1.66)	0.06	1.23	(1.00, 1.52)	*0.05*
Distance from Lake Mweru (in km)	1.12	(1.03, 1.21)	*0.005*	-		
Distance from cat. 1 streams (in km)	0.55	(0.31, 0.98)	*0.04*	0.49	(0.34, 0.73)	*<0.001*
Distance from cat. 4 streams (in km)	1.42	(1.18, 1.71)	*<0.001*	-		
Lagged rainfall (by 10 mm) [1]	0.24	(0.13, 0.47)	*<0.001*	0.68	(0.47, 0.99)	*0.05*
Lagged rainfall (by 10 mm) [2]	-			3.96	(1.88, 8.37)	*0.001*
Lagged maximum temperature (in C°) [3]	1.14	(1.03, 1.26)	*0.01*	-		
Lagged maximum temperature (in C°) [4]	0.79	(0.66, 0.94)	*0.007*	-		
Lagged maximum temperature (in C°) [5]	-			0.79	(0.67, 0.92)	*0.002*
Lagged minimum temperature (in C°) [5]	-			1.32	(1.18, 1.48)	*<0.001*

PRR = prevalence rate ratio, CI = confidence interval, HOH = head of household, HH = household

[1] Interval=2 weeks, lag=2 weeks; [2] Interval=10 weeks, lag=4 weeks; [3] interval=1 weeks, lag=2 weeks; [4] interval=8 weeks, lag=3 weeks; [5] interval=7 weeks, lag=2 weeks

Figure 3.4: Adjusted and unadjusted percent reduction in vector densities by year compared to pre-IRS time period in A) *An. funestus* and B) *An. gambiae*



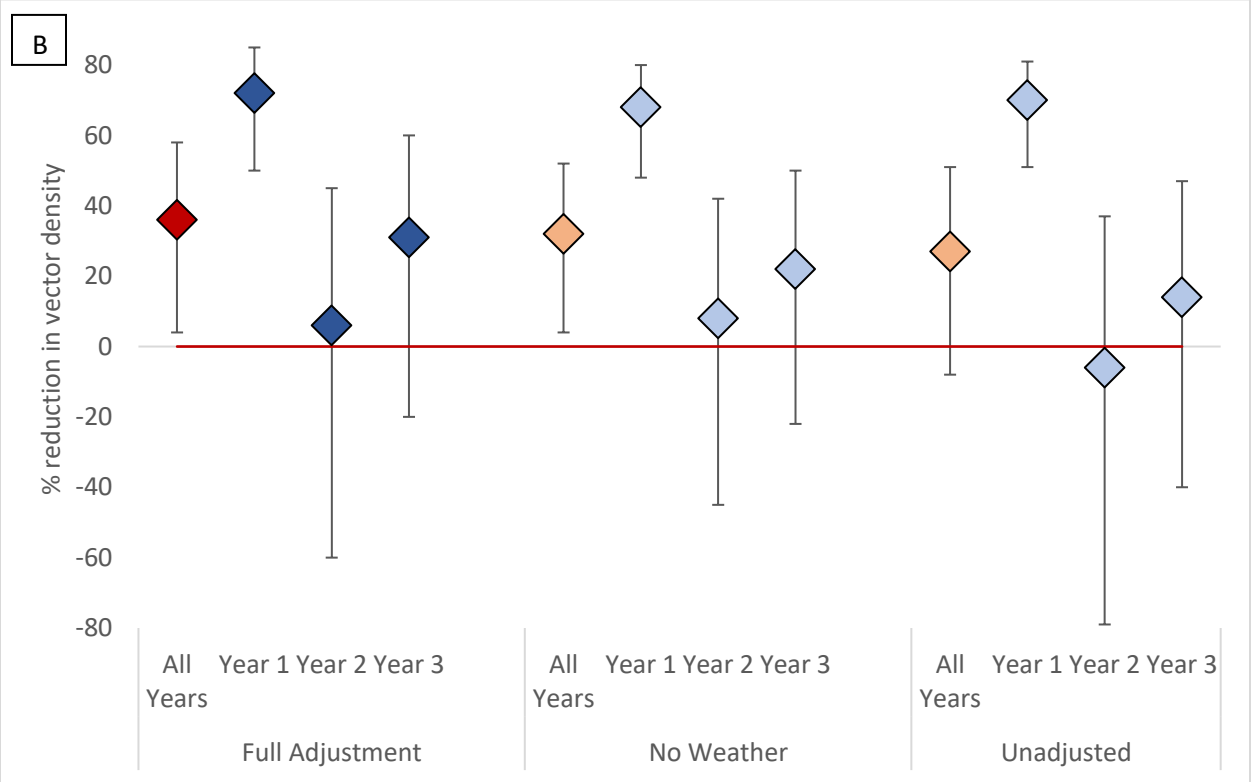


Figure 3.5: Time series of parasite prevalence among active surveillance participants in sprayed and unsprayed areas in Nchelenge District

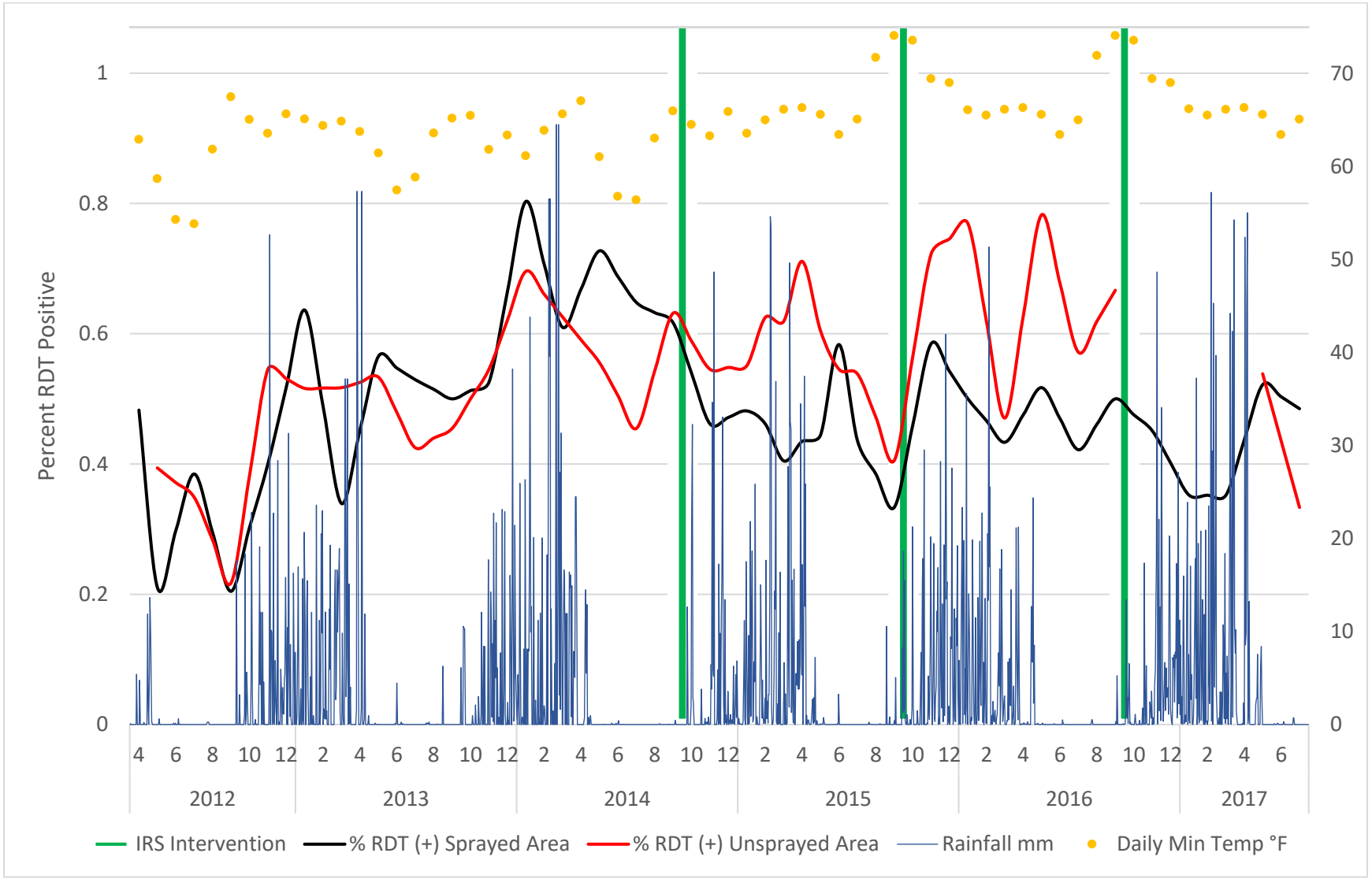


Table 3.4: Demographic and clinical characteristics of participants, before and after IRS with pirimiphos-methyl in sprayed and unsprayed areas, N=3,309

	Sprayed area			Unsprayed Area		
	Pre-IRS N=1,281	Post-IRS N=1,165	P value*	Pre-IRS N=353	Post-IRS N=510	P value*
DEMOGRAPHIC VARIABLES						
Male	592 (46.2%)	514 (44.1%)	0.3	160 (45.3%)	216 (41.4%)	0.2
Age <5	278 (21.7%)	181 (15.5%)	*<0.001*	78 (22.1%)	107 (20.5%)	0.6
Age 5-16	425 (33.2%)	422 (36.2%)	0.1	115 (32.6%)	160 (30.7%)	0.5
Sleep under bed net	671 (52.4%)	652 (56.0%)	0.08	210 (59.5%)	349 (70.0%)	*0.02*
Dirt floor in home	1,134 (89.1%)	918 (79.3%)	*<0.001*	347 (99.7%)	501 (96.0%)	*0.001*
Unprotected water source	474 (37.0%)	437 (37.7%)	0.7	322 (91.2%)	396 (75.9%)	*<0.001*
HOH primary school only	799 (62.6%)	811 (70.1%)	*<0.001*	252 (71.4%)	412 (79.2%)	*0.008*
HOH permanently employed	96 (7.5%)	63 (5.4%)	*0.04*	30 (8.5%)	12 (2.3%)	*<0.001*
CLINICAL RESULTS						
RDT positive	643 (50.2%)	534 (45.8%)	*0.03*	172 (48.7%)	303 (58.1%)	*0.007*
Taken Coartem in past month	294 (23.0%)	200 (17.2%)	*<0.001*	68 (19.3%)	98 (18.8%)	0.9
Fever	37 (2.9%)	15 (1.3%)	*0.006*	7 (2.0%)	11 (2.1%)	0.9
Anemic	674 (52.6%)	766 (65.8%)	*<0.001*	196 (55.5%)	368 (80.5%)	*<0.001*

*P value is from chi-squared analyses, HOH = head of household

Table 3.5: Factors associated with parasite prevalence by RDT within the areas targeted for IRS using bivariate Poisson regression models with robust standard errors and GEE clustered by household, N=2,446

	Rainy Season			Dry Season		
	PRR	95% CI	P value	PRR	95% CI	P value
IRS VARIABLES						
Post-IRS	0.90	(0.78, 1.06)	0.2	0.89	(0.76, 1.03)	0.1
Self-reported history of IRS[#]	0.82	(0.68, 0.99)	*0.04*	0.93	(0.77, 1.11)	0.4
Self-reported history of no IRS[#]	1.02	(0.85, 1.21)	0.9	0.86	(0.71, 1.05)	0.1
DEMOGRAPHIC VARIABLES						
Male	1.25	(1.12, 1.40)	*<0.001*	1.07	(0.95, 1.20)	0.3
Age <5*	1.83	(1.54, 2.17)	*<0.001*	1.91	(1.63, 2.25)	*<0.001*
Age 5-16*	2.33	(2.03, 2.67)	*<0.001*	2.43	(2.12, 2.80)	*<0.001*
Longitudinal HH type	0.97	(0.78, 1.22)	0.8	1.40	(1.19, 1.65)	*<0.001*
Sleep under bed net	0.64	(0.55, 0.74)	*<0.001*	0.67	(0.59, 0.77)	*<0.001*
Dirt floor in home	1.19	(0.95, 1.49)	0.1	1.39	(1.08, 1.77)	*0.01*
Unprotected water source	1.23	(1.07, 1.43)	*0.005*	0.93	(0.80, 1.09)	0.4
HOH primary school only	1.28	(1.08, 1.52)	*0.005*	1.32	(1.11, 1.56)	*0.001*
HOH permanently employed	0.90	(0.63, 1.29)	0.6	1.00	(0.74, 1.36)	0.9
Metal roof^	0.72	(0.53, 0.96)	*0.03*	0.67	(0.50, 0.91)	*0.01*
Closed eaves^	0.59	(0.39, 0.89)	*0.01*	0.73	(0.54, 1.00)	*0.05*
GEOGRAPHIC VARIABLES						
HHs within 500 m (by 100 HH)	0.92	(0.89, 0.94)	*<0.001*	0.94	(0.91, 0.97)	*<0.001*
Elevation (by 10 m)	0.97	(0.92, 1.02)	0.2	0.88	(0.84, 0.92)	*<0.001*
Slope (by 1°)	1.00	(0.96, 1.04)	0.8	1.04	(1.00, 1.07)	*0.04*
NDVI (by 10%)	1.07	(0.97, 1.12)	0.2	1.15	(1.04, 1.26)	*0.006*
Distance from Lake Mweru (in km)	1.03	(1.01, 1.05)	*0.001*	0.98	(0.96, 1.01)	0.3
Distance from health clinics (in km)	1.05	(1.03, 1.07)	*<0.001*	1.02	(0.99, 1.06)	0.2
Distance from roads (in km)	0.83	(0.29, 2.42)	0.7	0.56	(0.23, 1.36)	0.2
Distance from cat. 1 streams (in km)	0.77	(0.65, 0.91)	*0.002*	0.72	(0.60, 0.87)	*0.001*
Distance from cat. 2 streams (in km)	0.91	(0.85, 0.98)	*0.01*	1.06	(0.98, 1.14)	0.1
Distance from cat. 3 streams (in km)	0.99	(0.95, 1.03)	0.7	1.05	(1.01, 1.10)	*0.02*
Distance from cat. 4 streams (in km)	1.04	(1.00, 1.09)	*0.05*	0.99	(0.95, 1.03)	0.7

PRR = prevalence rate ratio, CI = confidence interval, HOH = head of household, HH = household

[#]Comparison group is malaria prevalence pre-IRS, *Compared to adults aged >16, ^subset of study population N=1,384

Table 3.6: Poisson multivariate models of the impact of targeted IRS with pirimiphos-methyl on malaria prevalence by season within the area targeted for spraying, using robust standard errors and GEE clustered by household, N=2,446

	Rainy Season			Dry Season		
	PRR	95% CI	P value	PRR	95% CI	P value
Post-IRS	0.72	(0.62, 0.84)	*<0.001*	0.91	(0.80, 1.05)	0.2
Male	1.16	(1.05, 1.29)	*0.004*	-		
Age <5	1.70	(1.44, 2.01)	*<0.001*	1.88	(1.61, 2.20)	*<0.001*
Age 5-16	2.12	(1.84, 2.45)	*<0.001*	2.32	(2.02, 2.67)	*<0.001*
Sleep under bed net	0.75	(0.66, 0.86)	*<0.001*	0.87	(0.78, 0.97)	*0.01*
HOH primary school only	1.16	(1.01, 1.34)	*0.04*	1.17	(0.97, 1.29)	0.1
HH within 500 m (by 100 HH)	0.92	(0.89, 0.95)	*<0.001*	0.95	(0.92, 0.98)	*0.003*
Elevation (by 10 m)	0.88	(0.83, 0.93)	*<0.001*	0.90	(0.85, 0.95)	*<0.001*
Distance from Lake Mweru (in km)	1.04	(1.02, 1.07)	*0.001*	0.95	(0.91, 0.99)	*0.02*
Distance from health clinics (in km)	-			1.07	(1.02, 1.12)	*0.006*
Distance from cat. 1 streams (in km)	-			0.85	(0.72, 1.01)	0.07
Lagged rainfall (by 10 mm) [1]	1.24	(1.06, 1.45)	*0.007*	-		
Lagged minimum temperature (in C°) [2]	1.15	(1.08, 1.22)	*<0.001*	-		
Lagged maximum temperature (in C°) [3]	-			1.17	(1.09, 1.17)	*<0.001*
Lagged streamflow (in m^3/s) [4]	-			0.90	(0.83, 0.97)	*0.005*

PRR = prevalence rate ratio, CI = confidence interval, HOH = head of household, HH = household

[1] Interval=2 weeks, lag=3 weeks; [2] interval=3 weeks, lag=2 weeks; [3] interval=5 weeks, lag=3 weeks; [4] interval=2 weeks, lag=4 weeks

Figure 3.6: Adjusted and unadjusted reduction in parasite prevalence compared to pre-IRS time period in A) rainy and B) dry seasons

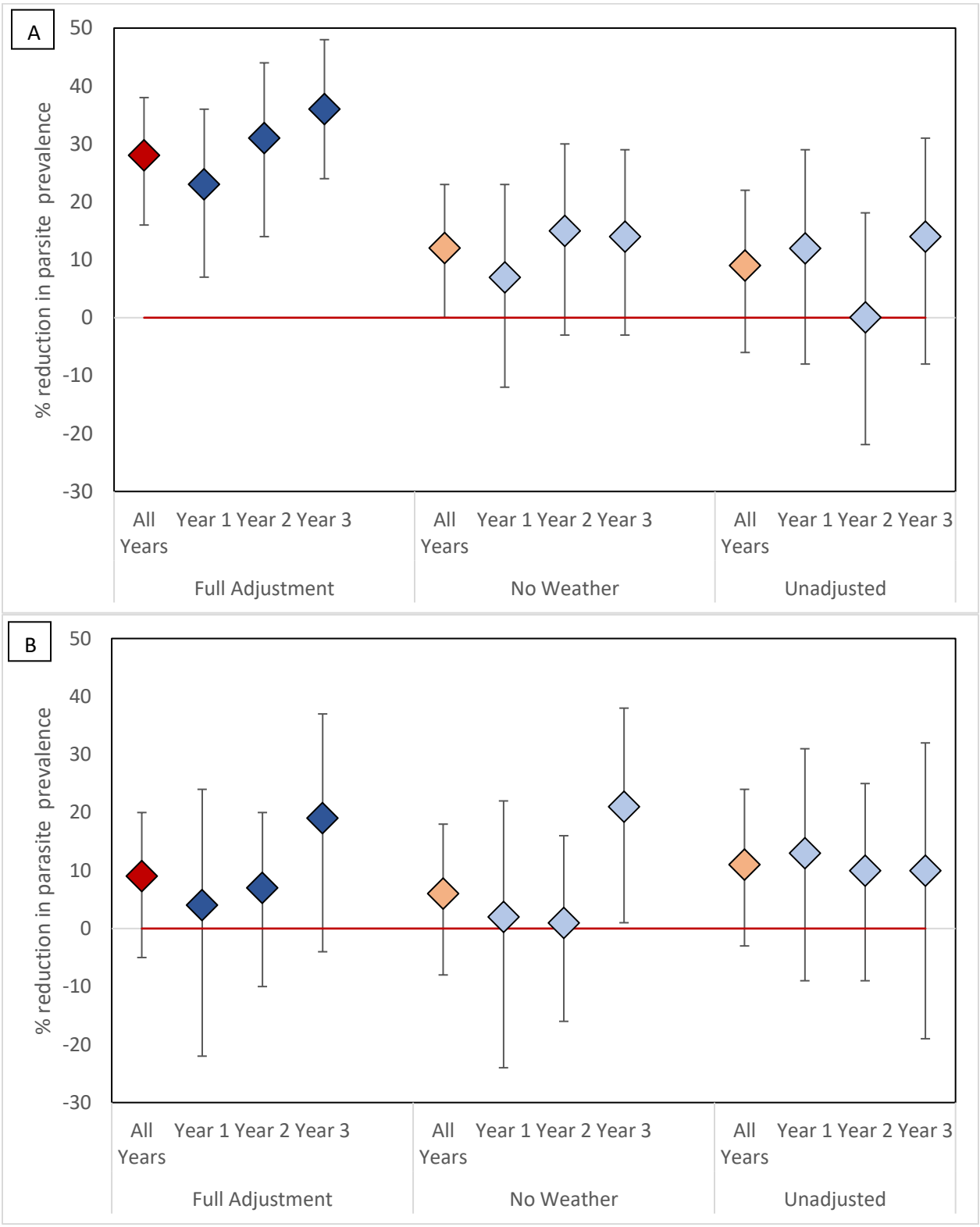
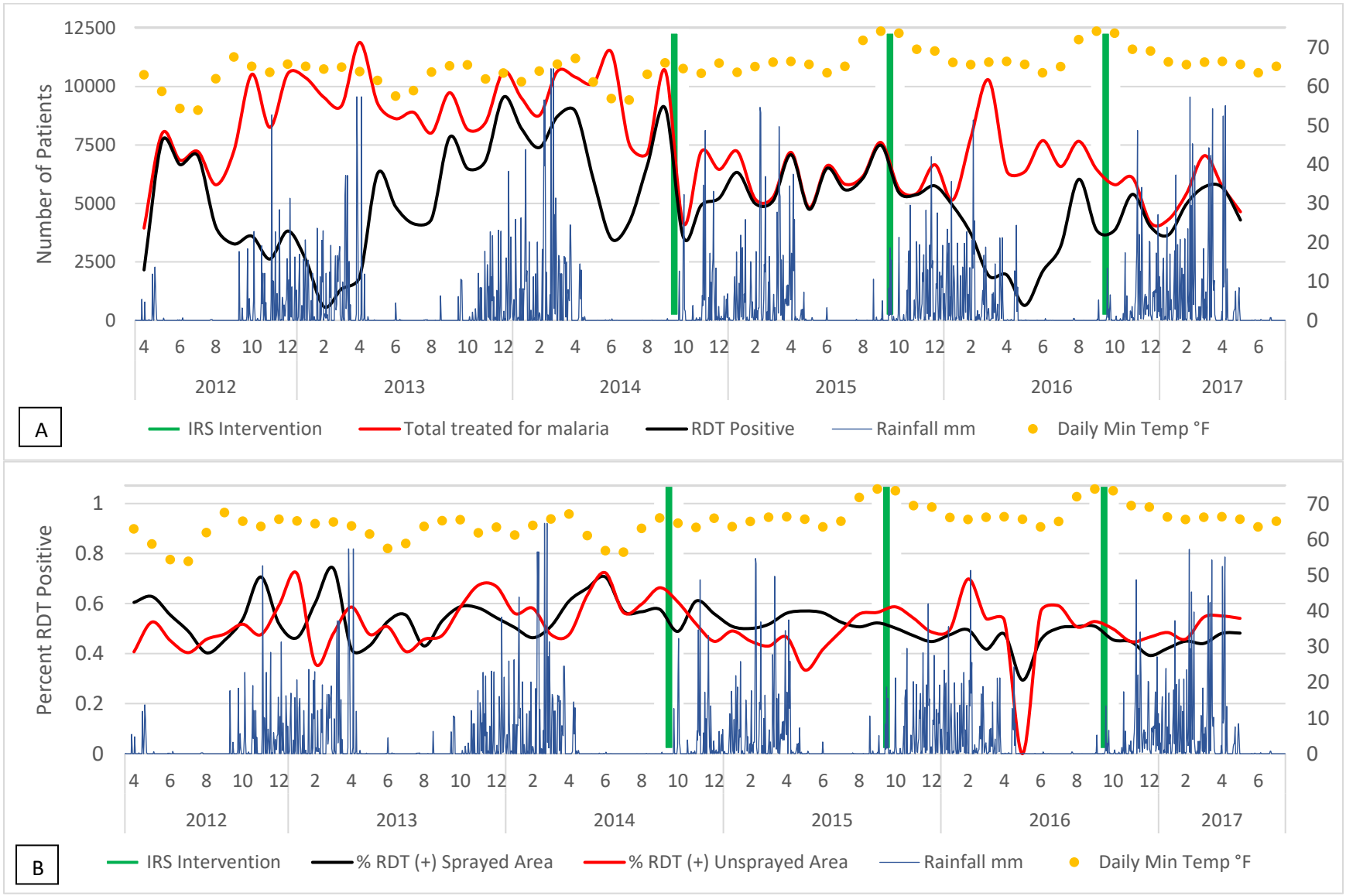


Figure 3.7: Time series from 11 health centers in Nchelenge District for A) total treated for malaria vs. total RDT positive, and B) percent RDT positive in sprayed and unsprayed areas



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Chapter 4: The use of GPS data loggers to describe spatio-temporal movement patterns and the impact on targeted malaria control in a high-transmission area of northern Zambia

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ABSTRACT

Background: Human movement is a driver of malaria transmission and has implications for sustainable malaria control. However, little research has been done on the impact of fine-scale movement, especially in high-transmission settings. In particular, the effect of individual movement patterns on targeted intervention strategies has not been investigated. As interest in targeted malaria control increases, evaluations are needed to determine the appropriateness of these strategies in the context of human mobility across a variety of settings.

Methods: A GPS data logger study was conducted in Nchelenge District, a high-transmission setting in northern Zambia. Over one year, 84 participants were recruited from ongoing active surveillance cohorts to wear a data logger for one month during all daily activity. Participants completed a survey and malaria testing and treatment at logger distribution and collection, and incident malaria infections were identified using polymerase chain reaction (PCR). Participant movement was characterized throughout the study area and across areas targeted for an indoor residual spraying (IRS) intervention. Participant movement patterns were compared by incident parasitemia and demographic characteristics using movement intensity maps, activity space plots, and statistical analyses. Malaria risk was characterized across participants using risk maps and time spent away from the home during peak vector biting hours.

Results: Movement data was collected from 82 participants, and 63 completed a second visit, for a loss to follow-up of 25%. Participants exhibited diverse mobility patterns across ecological zones and in and out of areas targeted for IRS, with implications for malaria control in this region. Movement patterns did not differ significantly by season of participation or age, but male participants traveled longer distances and spent more time away from home. By PCR, malaria incidence was 22%. Participants with incident parasitemia traveled a shorter distance and spent more time away from home during peak biting hours, but these relationships were not statistically significant. Malaria risk was characterized to be high across

participants and did not differ by parasitemia incidence. Time spent outside during peak biting hours was identified as a likely source of malaria transmission in this setting.

Conclusion: Individual movement patterns in Nchelenge District had significant implications for malaria control and the effectiveness of a targeted IRS campaign. Large and fine-scale population mobility must be considered when planning intervention strategies across transmission settings.

INTRODUCTION

Human population movement is increasingly known to be an important driver of malaria transmission. The movement of infected individuals between high and low-transmission settings has long been recognized as a source of ongoing disease introductions and continued transmission, and cross-border movement has been shown to facilitate the spread of drug resistance [1-6]. These types of movement patterns have been cited as contributing causes for the failure of the Global Malaria Eradication Program (GMEP) in the 1950s and 60s [7, 8].

Following the end of the GMEP in 1969, a number of studies were published on the impact of human mobility on malaria control [3, 8-10]. These described large-scale population movements across borders and between urban and rural areas, which resulted in reimportation and reemergence of malaria in areas with previously successful malaria control. Common socioeconomic factors contributing to these types of movement included economic migration, agricultural labor, familial networks, displacement, and tourism. These studies broadly demonstrated that accounting for large-scale human mobility is necessary for sustainable malaria control. If high-transmission regions continue to exist, there remains a risk of local reintroductions from incoming visitors or returning residents. In an area with competent vectors, this can lead to a rapid resurgence of malaria transmission in the absence of ongoing malaria control activities [10]. Therefore, successful malaria control in an area receptive to transmission can be unstable. Studies in recent years have further strengthened this conclusion using novel methods of characterizing human mobility patterns, including anonymized cellular phone records, census-based migration data, and records of ticketed travel [11-17].

Despite the recognized importance of large-scale movement to malaria control and elimination efforts, little is known about how small-scale movement patterns affect malaria risk [8]. There is evidence of heterogeneity in malaria transmission across relatively fine spatial and temporal scales, and mathematical models have shown that heterogeneity in individual disease risk for vector-borne

pathogens can increase population-level transmission [18-22]. Consequently, individual movement patterns are likely to contribute to both personal and population-level malaria risk. In support of this hypothesis, mathematical models have shown that daily movement increases heterogeneity of vector-borne disease risk for individuals, which in turn can lead to an overall increase in transmission for the population [20, 23, 24]. In one modelling study, individual movement patterns during times of active vector biting were a more significant predictor of disease risk than vector density in the individual's home [20]. However, these relationships have rarely been directly observed.

Small-scale movement may also impact the effectiveness of malaria control activities. While large-scale movement has been shown to undermine interventions at the national and sub-national levels, mobility between areas of heterogeneous malaria control at finer levels of spatial resolution could also be expected to adversely affect intervention effectiveness. This is particularly relevant amid increasing interest in targeted malaria control strategies, in which hotspots of malaria transmission are preferentially selected for malaria interventions with the intention of interrupting transmission in a wider area [25-27]. This approach has the potential to reduce cost, time, and manpower. However, if not all transmission hotspots are successfully targeted, and areas of residual high transmission remain, this strategy could unintentionally increase transmission heterogeneity at the local level. Small-scale population mobility between areas of differing malaria control could then attenuate the impact of the targeted intervention through inward movement of infected people from neighboring non-intervention areas and outward movement from intervention areas into areas with greater risk of exposure. Targeted intervention strategies must therefore be designed and assessed within the framework of human mobility patterns.

While the connection between human movement and malaria has been explored in the context of low-transmission and elimination settings, few studies have investigated the importance of human mobility to malaria risk in high-transmission settings. For areas with continued high burden despite

malaria control efforts, the individual drivers of transmission must be more clearly delineated to better inform intervention strategies. This is particularly urgent for countries with local elimination goals and areas at risk of spreading artemisinin resistance [28]. Further work is therefore needed to investigate the association between fine-scale, individual mobility patterns and malaria risk across a variety of transmission settings.

Several methods have been explored to investigate the impact of individual movement on infectious disease transmission, most notably travel histories, cellular phone data, and portable geographic positioning systems (GPS) devices [8, 14, 29-33]. Each method has strengths and limitations. Cellular phone data are limited to people who own a phone in an area with reliable cellular service, which excludes some groups by rural residence or socioeconomic status [34]. Cellular phone studies are not linked to individual users, can only present data at the spatial level of the cell tower, and are generally conducted within a single country due to network restrictions that prevent the capture of cross-border movement [14]. Travel histories, in contrast, collect data on individuals at potentially detailed spatial levels, but are prone to recall bias and are difficult to validate [14, 29, 35].

Commercially available GPS devices can provide data at a much finer level of spatial and temporal resolution. These devices allow for collection of demographic and household data for individuals, have been shown to be acceptable to study participants, and have high reliability in rural settings [30, 36, 37]. Several studies using GPS data loggers successfully demonstrated the impact of individual movement patterns on transmission of vector-borne and parasitic diseases, such as malaria, dengue, schistosomiasis, and hookworm [31, 38-40]. Most notably, recent studies in Iquitos, Peru and Choma District, Zambia explored the use of GPS data loggers to quantify human movement patterns at a fine scale in resource-poor environments with the aim of explaining epidemiologic patterns of disease transmission [31, 40]. In both settings, detailed data on movement behavior was found to be informative in explaining transmission patterns of vector-borne disease. Particularly in the low-burden

setting in southern Zambia, mobility patterns were correlated with seasonal increases in clinical cases and reinforced concerns about the risk of importation from neighboring areas [40].

Informed by the methodology and results of these studies, a population movement study was conducted in Nchelenge District, Luapula Province, Zambia using commercially available GPS data loggers. The aims of this study were to describe human movement patterns in a remote rural area of sub-Saharan Africa, to assess the relationships between movement and malaria risk in a high-transmission setting, and to investigate the impact of fine-scale mobility on a targeted indoor residual spraying (IRS) campaign.

METHODS

Study site

This study was conducted by the Southern and Central Africa International Centers of Excellence for Malaria Research (ICEMR) in Nchelenge District, Luapula Province, Zambia [41], in collaboration with the Zambian Ministry of Health's Tropical Disease Research Center (TDRC), the Macha Research Trust (MRT), and the Johns Hopkins Malaria Research Institute (JHMRI). The Southern and Central Africa ICEMR collects active and passive malaria surveillance data from four distinct epidemiological settings to monitor trends in malaria transmission and inform national and international policies [42].

One of these research sites is located in Nchelenge District, a setting with holoendemic transmission and ineffective malaria control. Despite national policies to provide universal access to long-lasting insecticide-treated bed nets (LLINs), rapid diagnostic tests (RDTs), and artemisinin-combination therapy (ACTs), malaria cases in Nchelenge District increased steadily from 2007-2012 [43]. Parasite prevalence with *Plasmodium falciparum* continues to average almost 70% in children under 17 years, and residents receive approximately 140 infective bites per year [44-46]. The district is located in the marshlands surrounding the Luapula River along the bank of Lake Mweru, and year-round

transmission is sustained by environmental suitability and the presence of two effective mosquito vectors with differing ecology. *Anopheles funestus* is the dominant vector and has a distinct peak in the dry season from May to September, and *An. gambiae s.s.* populations peak near the lake during the rainy season from October to April [47, 48]. Both vectors have shown high resistance to pyrethroid and carbamate insecticides, and their distributions vary across the study area at relatively small spatial scales [43, 48, 49]. The population of Nchelenge District is largely agrarian, with fishing in Lake Mweru as the main economic activity during the dry season and agricultural practices occurring further inland during the rainy season when an annual fishing ban is imposed [48]. Nchelenge District and Lake Mweru form the border with Haut-Katanga Province of the Democratic Republic of the Congo (DRC), and a large degree of formal and informal movement across this border is presumed to occur.

In October 2014, a targeted IRS campaign using the organophosphate insecticide pirimiphos-methyl was conducted in Nchelenge District and other high-burden districts in northern Zambia [50]. Targeted areas were selected using population density and case reports of nearby health centers [50, 51]. In Nchelenge District, spray activities occurred mostly in the highly-populated peri-urban areas along Lake Mweru and the main road. Targeted IRS with pirimiphos-methyl was repeated annually in subsequent years and continues to be the strategy in this region. Bed nets were distributed in antenatal and vaccination clinics throughout the study period.

GPS data loggers

The GPS data loggers used were IgotU® GT-600 devices (Mobile Action Technology, New Taipei City, Taiwan). These were selected based on their acceptability for field study, which included ease in programming, light weight (37 grams), large memory (>250,000 points), long battery life (30 hours of continuous use), water resistance, and relatively low cost [36]. Outside under open sky, they are accurate within 20 m more than 90% of the time, and they have an average error of 4.4 m while

stationary and of 10.3 m while in motion [30, 36]. Data loggers were programmed to record geographic position, date, time, and altitude every 2.5 minutes. They were password protected, and recorded data could only be accessed with a specific software program and unique connection cable, which were kept at the locked field station in Kashikishi. Therefore, if a logger was lost in the field, the data on the device could not be accessed by anyone other than the field manager. To extend battery life, data loggers were programmed to be motion activated, so no points were logged if the participant was asleep or stationary. To reduce errors, the power button was disabled so participants could not accidentally turn off the device.

Study population and recruitment

The population for the GPS data logger study was recruited from the Southern and Central Africa ICEMR active surveillance cohorts. Households were originally enumerated in 2012 using Quickbird™ satellite images (DigitalGlobal Services, Denver, CO) and 1x1 kilometer grid quadrants were randomly selected to represent the full range of ecology in the study area. Beginning in April 2012, households were randomly selected within quadrants using population proportional to size sampling and were recruited into longitudinal or cross-sectional cohorts with alternating frequency. Approximately 25 new households were recruited bimonthly into cross-sectional cohorts, and 25-30 households were recruited into longitudinal cohorts to be visited six times over a year and then replaced with a new longitudinal cohort.

Sensitization activities for the data logger study occurred in June 2014, coinciding with enrollment of a new longitudinal cohort. New households were visited by study teams, were recruited into the longitudinal cohort, and were informed of the additional option to participate in the data logger study in the following year. Study procedures were explained, including the purpose of the study and protection of confidentiality, and pamphlets describing the study were distributed in Bemba as

appropriate. Consent was obtained from participating household members or the parents of children under 16 years.

Recruitment for the data logger study occurred every other month from August 2014 to June 2015. During each bimonthly study visit, a convenience sample of 12-15 participants aged 13 years and older were recruited to wear a data logger for approximately 30 days, with no more than two household members participating concurrently. Participants were recruited from the longitudinal cohort only from August 2014 to January 2015; however, due to some refusals and absences among longitudinal participants, cross-sectional participants also were recruited starting in February 2015 to maintain the desired sample size. Participants were instructed to wear the data logger at all times during their normal daily activity, except when bathing or engaging in other activity where the logger could be submerged in water. Data loggers could be worn around the wrist like a watch, around the neck on a lanyard, or in a pocket or bag. Wrist straps and lanyards were provided to participants according to their preference. To ensure battery life, the first data logger distributed to each participant was collected after 15 days and replaced with a fully charged one to be worn for another 15 days. If participants were not available at the time of logger replacement or final collection, the study team returned to the household until the logger was located or confirmed to be lost. Data logger serial numbers were matched to unique participant ID numbers, and the date and time were recorded at each logger distribution and collection for every participant.

As part of the standard longitudinal study visit at data logger distribution, consenting household members completed a survey to collect information on demographic characteristics, history of recent malaria and treatment, reported LLIN use, history of household IRS, and malaria knowledge and practices. Participant temperature was measured using a digital ear thermometer, and household coordinates were recorded on a handheld tablet. Blood samples were collected by finger prick for hemoglobin testing, *Plasmodium falciparum* HRP-2 RDT (Standard Diagnostics, Kyonggi, Republic of

Korea) and spotted onto filter paper (Whatman 903™ Protein Saver card) as dried blood spots (DBS) for polymerase chain reaction (PCR) detection of *P. falciparum* DNA [52]. Participants with a positive RDT were offered treatment with Coartem® (Novartis, Basel, Switzerland), the first-line standard of care in Zambia.

To identify incident malaria parasitemia, these procedures were repeated at the final data logger collection with the aim of having two complete study visits approximately 30 days apart, referred to as visit 1 and visit 2. Positive test results at visit 2 were considered incident malaria infections, as preexisting infections confirmed by RDT were identified and treated at logger distribution. Thus, this study design permitted assessment of both movement patterns and incident parasitemia in the same individual.

Laboratory procedures

Filter paper with DBS were sealed in plastic bags containing a desiccant and stored at the field station at -20 ^{o}C. They were transported to the TDRC laboratory in Ndola, where they were stored at -20 ^{o}C prior to DNA extraction and PCR. Chelex® extraction was used to recover parasite DNA from DBS within one year of sample collection [53]. Within one month of DNA extraction, a nested PCR assay was conducted to detect asexual stage *P. falciparum* DNA targeting a segment of the mitochondrial cytochrome b gene (*cytb*) [54]. Reactions were run in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA). Amplified product was detected by electrophoresis on 1% agarose gel and viewed under UV light as an 815-base pair DNA band [52].

Data management and processing

Data from returned GPS data loggers were uploaded onto a password-protected computer in the Kashikishi field office using the @trip software developed for use with IgotU products (Mobile Action Technology, New Taipei City, Taiwan) and exported as .csv files. De-identified files were sent to the data

manager at Macha Research Trust, who checked the raw data for errors or inconsistencies and removed data points not within the appropriate time interval for each participant. Cleaned data logger files and survey data from visits 1 and 2 were uploaded into REDCap secure file-sharing software and downloaded as .csv files [55].

Participant data logger files and geolocated household locations were uploaded into ArcGIS Version 10.2 (ESRI, Redlands, CA). Logger data were cleaned using a software extension developed for GPS-based trajectory analysis in ArcGIS, which removes potentially erroneous data points based on improbable speeds or abrupt changes in direction [56]. For each participant, the distances from each logged point to their household and to Lake Mweru were calculated. Several participants had two households due to seasonal agricultural practices. For these participants, distance was calculated to the household closest to the logged point. Distance was also calculated from the participant's household at logger distribution to Lake Mweru and to the nearest health center.

Images of participant data points and movement intensity plots were developed using the ArcGIS extension software and plotted on high definition satellite maps of the study area. Intensity plots indicate the cumulative time spent in each location using color and height, and were created by month of data collection and season. Data points were projected over shapefiles of the targeted IRS intervention to visualize the degree of movement in and out of targeted areas. Data points were also projected onto previously created malaria risk maps of the study area, which were created from the first two years of active surveillance data in Nchelenge District from 2012-2014. To produce these maps, environmental correlates of household malaria prevalence by RDT were identified, and kriging methods were used to create a prediction surface of malaria risk across the study area as a high-resolution raster file, stratified by rainy or dry season [57]. For each geolocated data logger point, the value of the appropriate underlying raster pixel was extracted to obtain a measure of calculated malaria risk for that location, R_k.

Data analysis and activity space

Datasets developed in ArcGIS were exported as .csv files and loaded into STATA 13.1 (Stata-Corporation, College Station, TX). Movement data were merged by participant ID and date to household survey data for visits 1 and 2. Malaria status at each visit was determined by RDT and PCR results, with positive malaria results at visit 2 considered incident infections. Participants were defined to have a fever if their temperature exceeded 38 C°, and anemia was determined by WHO criteria for hemoglobin levels by age and sex [58]. Differences in characteristics between retained participants and participants lost to follow-up were calculated using chi squared tests to assess differential loss to follow-up. Individual and household-level correlates of PCR results at visits 1 and 2 were identified using chi squared tests.

For each individual i, the total time carrying each of their two data loggers λ_{i1} and λ_{i2} was calculated as the time difference between logger distribution and logger collection or battery death for each device, as noted in the field and verified in ArcGIS. Total participation time T_i was defined as the sum of $\lambda_{i1} + \lambda_{i2}$. This excludes missing time between the two logger distributions caused by early battery failure or other complications. The amount of time spent at each logged point location τ_{ij} was estimated to be half the time between the previous and subsequent points. The proportion of participant time spent at each logged point location was calculated as the ratio of the time spent at the point over the total participation time for that participant (τ_{ij}/T_i). Similarly, participation time during peak vector biting hours $\bar{T}_i$ was calculated as all participation time between the hours of 6 pm and 6 am [59-61], and the proportion of peak biting time at each location was calculated correspondingly as $\tau_{ij}/\bar{T}_i$.

For each participant, the total distance, maximum distance, and average daily distance traveled were calculated for the total time recorded T_i and for peak biting hours $\bar{T}_i$. To determine whether

participants were at home, sensitivity analyses were conducted using different radii from the household, and 50 m was identified as the distance that most accurately captured movement and epidemiological patterns. Participants were therefore classified to be at home if they were within 50 m of their household; however, due to limitations in the precision of GPS devices, it could not be confirmed whether participants were inside or directly outside the home. Values were calculated for the number of hours per day and proportion of time spent at home, within 3 km of the lakeside, and within areas targeted for IRS for both total time T_i and peak biting hours $\bar{T}_i$. These values were stratified by sex, age, and parasite positivity at visits 1 and 2, and were compared using Wilcoxon rank-sum tests to account for non-normality of the data.

To further describe participant activity space, plots were created of the cumulative proportion of time spent at increasing distances from the household and lakeside, stratified by sex, age, season, and incident parasitemia at visit 2. For total time contributed, activity space plots were examined at the full spatial extent of distance travelled to compare patterns of large-scale movement. For peak biting hours, activity space plots were examined within 1 km of the household to better compare patterns of fine-scale movement and time spent near the home when at risk of malaria transmission.

Using the risk maps describe above [62], the study area was divided into quartiles of malaria risk. After risk values R_k were extracted for each logger point, the proportion of time spent at each quartile of risk was calculated and compared by demographic factors and parasite positivity at visits 1 and 2 using Wilcoxon rank-sum tests. Each individual was then assigned a risk score R_i, calculated by multiplying the time spent at each logged point by the extracted risk value for that point, summing these risks over the time of individual's participation, and dividing by the number of days contributed:

$$R_i = \frac{\sum_{j=1}^{n}(\tau_{ij} * R_k)}{T}$$

Calculating this value as an average daily risk controls for different participation times per person. Nighttime risk $\bar{R}_i$ was calculated similarly, using total participation time during peak biting hours $\bar{T}_i$. To help control for the impact of malaria control interventions, including use of LLINs and IRS, outdoor nightly risk $\bar{\bar{R}}_i$ was also calculated for the total time spent farther than 50 m from the participant's household during peak biting hours $\bar{\bar{T}}_i$. For all calculations involving risk scores, time outside the study area was censored due to lack of information.

A risk analysis was also conducted using data from multiple studies on reported indoor vs. outdoor vector biting rates [61, 63-66]. For *An. funestus* and *An. gambiae*, the mean biting rate across sites was calculated for each hour between 6 pm and 6 am. From these studies, the proportion of outdoor vs. indoor biting rates was determined for each species every hour and overall. In Nchelenge District, approximately 10 times as many *An. funestus* as *An. gambiae* were collected in indoor light traps throughout the year, and this ratio was assumed to be similar outdoors [48]. Using these values, and assuming that each vector species is equally likely to be infectious, the potential indoor vs. outdoor risk across species was calculated for this setting. The proportion of participant time farther than 50 m from any household during peak biting hours was determined, during which participants were assumed to be outside, and the resulting proportion of malaria risk caused by time spent outdoors was calculated. This value was then adjusted for the proportion of participants that report sleeping under a bed net, assuming either 100% or 50% protection for peak biting time spent at home.

RESULTS

Participant characteristics

Over six recruitment periods from August 2014 to June 2015, 84 participants were enrolled from 44 unique households. Sixty-one participants were recruited from longitudinal households and 23 were recruited from cross-sectional households (Figure 4.1). At least one participant was enrolled from each

longitudinal household. Data loggers with usable movement tracks were collected from 82 participants. Full data from the second visit, including movement data and a blood sample, was collected from 63 participants, for a loss to follow-up of 25%. Data loggers were successfully collected from participants without a second visit because the device was left at the house or was returned from other locations by a family member. Despite the loss to follow-up, acceptability of the data logger study was high among participants, with approximately 10% refusals among approached participants and few questions or concerns reported by the field team.

At the first visit, 37% of participants were male and the median age was 33 years, with age ranging from 13-72 years (IQR 20-47 years) (Table 4.1). Fifty-seven percent of participants lived within 3 km of Lake Mweru, and 41% lived within a 30-minute walk from a health clinic. Nearly 40% of participants reported residing in another household for part of the year, with the most common reason being farming (80%), followed by the other household being the primary home (7%), and one participant reported sleeping away from home in the past month. Health-related behaviors were common, with 86% of participants reporting sleeping under a bed net, 19% reporting a history of household IRS with pirimiphos-methyl, and 56% reporting a visit to a health center in the past month. More than half (55%) of participants were anemic, 4% had a fever, and 28% reported having a fever in the past two weeks. Thirty-five percent of participants tested positive for malaria by RDT and 51% tested positive by PCR. At visit 1, there were no significant differences between participants who completed the second visit and those who were lost to follow-up.

Participant characteristics were compared by presence or absence of parasitemia by PCR (Table 4.2). Head of household employment, floor type, and history of sleeping away from home were excluded from bivariate analyses due to lack of variability. At visit 1, individuals with parasitemia by PCR were more likely than those without to live more than 3 km from Lake Mweru, to live more than 30 minutes from a health center, and to use an unprotected water source, but were less likely to have a

history of household IRS with pirimiphos-methyl. Although not statistically significant, individuals with a positive PCR were more likely to be younger than 18 years.

Malaria incidence

At visit 2, 38% of participants tested positive for parasitemia by RDT and 22% tested positive by PCR (Table 4.1). Due to the discrepancy between RDT and PCR results, only PCR results are reported for further analyses. Because participants with a positive RDT at visit 1 were treated with Coartem, participants with parasitemia by PCR at visit 2 were considered incident malaria infections, so the 1-month malaria incidence in the study population was 22%. There were no statistically significant differences in participant characteristics between individuals with a positive and negative PCR at visit 2 (Table 4.2), however confidence intervals were wide due to the small sample size.

Overall participant movement patterns

A total of 179,443 geolocated GPS data points were collected from 82 participants, comprising 2,407 days of data and covering a combined distance of 11,456 km. Individual participants contributed a median of 1,882 GPS points (IQR = 888-2,989; range = 101-6,388), and 31.1 days of data (IQR = 25.0-34.0; range = 9.9-48.4). The average distance traveled per day ranged from 0.1 – 42.7 km/day, with a median of 3.1 km/day (IQR=1.6-6.9). The maximum distance participants travelled from their home ranged from 0.1 – 212.4 km, with a median of 4.2 km (IQR=1.9-10.1). Participants spent a median of 4.7 hours away from home per day (IQR=1.9-8.4, range=0.1-23.9), and a median of 1.6 hours away per night during peak biting hours (IQR=0.6-4.3, range=0.01-12.0). Forty-six participants (56%) spent at least 10% of peak biting time away from home. Five participants averaged >20 hours away from home per day and >11 hours away from home per night, suggesting they had a second household that was unknown to the study team or they regularly slept at another location.

Maps of participant movement patterns were created using all logged points overlaid on satellite imagery of the study area and the areas targeted for IRS (Figure 4.2.A). Movement is visible across the study area, including between lakeside and inland areas and between sprayed and unsprayed areas. Using a boundary of 3 km from Lake Mweru as the dividing line, 40 participants (49%) spent time in both lakeside and inland areas and made between 1 and 19 round trips between them (median=3, IQR=2-4). Thirty participants (36.6%) spent time in both lakeside and inland areas during peak biting hours. Similarly, 59 participants (72%) spent time in both sprayed and unsprayed areas and made between 2 and 144 round trips between them (median=17, IQR=8-30). Thirty-eight participants (46%) spent time in both sprayed and unsprayed areas during peak biting hours. Participants were more likely to travel between sprayed and unsprayed areas if they lived within the sprayed areas, both for total time and peak biting hours (P=0.01, P=0.008).

Some participants also traveled longer distances (Figure 4.2.A inset). Eight participants (10%) made trips outside Nchelenge District, and at least one trip was made in each sampled month except February. Time outside the district borders ranged from 3 hours to 17 days, and five participants spent at least 24 cumulative hours outside the district. The number of trips outside the district during the study period ranged from 1-14 (median=4). Of these, the two persons with the highest number of trips were fisherman, who left the borders of the district 14 and 7 times, respectively, to go onto the lake in boats (Figure 4.2.A). No participants left Luapula Province. By distance, 21 participants made trips of at least 10 km away from their homes, with the number of trips of this distance ranging from 1-9. Nine participants traveled at least 20 km from their homes (1-3 trips), 5 participants traveled at least 30 km away from home (1-2 trips), and 2 participants travelled at least 100 km from their homes (1 trip).

Additional information on movement patterns was collected from participant surveys. Between visits 1 and 2, eight participants reported sleeping away from home in the past month. Three of these traveled for work, two visited friends or family, two traveled for funerals, and one travelled to buy or sell

goods. All eight reported sleeping in the homes of friends or family while they were away, and trips lasted a median of 7 nights (range=1-14 nights). Six participants reported staying in another village in the district and two reported staying in another district within Luapula Province. These reports are not entirely consistent with the GPS data because participants who left Nchelenge District were more likely to be lost to follow up.

Seasonal movement patterns

Maps of seasonal movement patterns were created using logged points overlaid on the study area and stratified by season (Figure 4.3). Contrary to expectations, participants frequently moved throughout the study area during both dry and rainy seasons. Due to agricultural practices, it was anticipated that movement would concentrate along Lake Mweru during the fishing period in the dry season but would concentrate in the inland area during the rainy season when fishing was banned. Fishing in Lake Mweru was observed in the dry season (Figure 4.3.A), but movement occurred across the district in both seasons, and participants with two known households went back and forth between them throughout the year.

In movement intensity plots by month and season (Figure 4.4, 4.5), most participant time was spent in the high-population density area near the lake in both seasons, but local maxima were observed throughout the study area across all months and seasons. In these plots, straight line segments indicate high speeds and thus longer distances between points, for example travel in a car, and this type of travel was also observed throughout the year. Fishing activity was observed in the dry season in August (Figure 4.4.A) and June (Figure 4.4.F).

Participants also engaged in long-distance movement throughout the year. In both dry and rainy seasons, participants travelled outside Nchelenge District, although longer distances were traveled in the dry season (Figure 4.3.A, 4.3.B insets). Activity space plots of total time contributed also show that

participants traveled a longer distance in the dry season, and they indicate that participants spent a larger proportion of time near their home in the rainy season (Figure 4.6). Activity space plots of peak biting hours, shown within 1 km of participant households, further confirm that participants spent a larger proportion of time near their home in the rainy season (Figure 4.7).

In statistical comparisons, no significant seasonal differences were observed between rainy and dry seasons for the number of points, total time contributed, total or maximum distance travelled, or average hours spent away from home, for either total time or peak biting hours.

Movement patterns by sex and age

Male participants contributed a higher mean number of points than female participants (P=0.008), but not a higher mean number of days. Male participants also traveled longer total distances, longer distances per day, farther from home, and spent more hours away from home during both total time and peak biting hours (Table 4.3). Male participants were more likely to travel between lakeside and inland areas (P=0.05) but were not more likely to travel in and out of areas targeted for IRS. Male participants were also more likely to travel more than 10 km away from home (P=0.005), but there was no difference at longer distances, and half of the participants who travelled outside Nchelenge District were women. There were no significant differences in these metrics by age.

In activity space plots for total time contributed, male and female participants travelled similar distances, but female participants spent a greater proportion of time near the home (Figure 4.6), as noted in statistical analyses. Female participants also spent a higher proportion of time near the home during peak biting hours, while male participants spent most of this time within 200 m of the home (Figure 4.7). In activity space plots for total time contributed, adult participants traveled further and spent less time near the home than adolescent participants between 13 and 17 years (Figure 4.6), but both age groups spent a similar proportion of time near the home during peak biting hours (Figure 4.7).

Movement patterns and malaria incidence

In statistical comparisons, movement patterns were not associated with incident infections in this population (Table 4.3). No significant differences were observed between participants with and without incident parasitemia by PCR at visit 2, including the number of points per participant, time contributed, total or maximum distance travelled, or average hours spent away from home, for both total time contributed and peak biting hours. Furthermore, no significant differences were observed in the probability of traveling between lakeside and inland areas or into and out of areas targeted for IRS, and there was no difference in the probability of having travelled various distances from home. However, all participants who travelled at least 20 km away from home were PCR negative at visit 2. These metrics also did not differ by PCR status at visit 1.

Although there were no significant differences in movement patterns by incident parasitemia in statistical analyses, several patterns were evident in plots of participant activity space (Figure 4.8). Participants with incident parasitemia traveled shorter distances than those without. However, at a finer spatial scale, participants with incident parasitemia spent a smaller proportion of peak biting hours at home, suggesting that these individuals spent more time outside the home during times of increased risk. Furthermore, although participants with both positive and negative PCRs travelled throughout the study area, participants with incident parasitemia spent less time within one km of the lakeside and spent slightly more time farther than 5 km from the lake. Patterns during peak biting hours were similar.

Movement patterns and malaria risk

Participant movement was largely concentrated in areas that were at highest risk for malaria (Figure 4.2.B). Approximately 70% of all participant time was spent in areas within the top two quartiles of malaria risk, and this value was consistent during peak biting hours and across demographic groups. Risk scores were calculated for individuals during peak biting hours and for time spent outside during

peak biting hours. There were no differences in risk score between individuals with parasitemia by PCR and those without at visits 1 or 2 (Figure 4.9), consistent with the statistical analyses. In general, malaria risk was determined to be high for all participant movement patterns in this high transmission setting.

Participants spent an average of 10.3% of time away from all households during peak biting hours. Based on previous estimates of vector biting rates across various sites, approximately 46% of *An. funestus* and 27% of *An. gambiae* bite outside. As described, there are on average 10 times as many *An. funestus* as *An. gambiae* throughout the year [48]. Assuming that each vector species has an equal chance of being infectious, and assuming that participants are inside when they are within 50 m of a household, the proportion of malaria risk from time spent outside during peak biting hours is approximately 8%. Reported bed net usage in this population is 86%, so if bed net usage perfectly protects people for all times they are inside, this would remove 86% of the household-related risk, and outdoor risk would proportionally increase to 37% of total malaria risk. However, if bed net use protects only 50% of household-related risk due to time spent outside the net or improper net usage, bed nets would remove only 43% of indoor risk, and outdoor risk would proportionally increase to 12% of total malaria risk.

DISCUSSION

In this high-transmission setting in northern Zambia, participants exhibited a high degree of mobility at both small- and large-scales throughout the year. A large proportion of time was spent near the home and in the densely populated area near the lake, including in areas targeted for IRS with pirimiphos-methyl, but half of participants traveled between lakeside and inland regions, and nearly three quarters spent time in both sprayed and unsprayed areas. During their month of participation, 10% of participants traveled outside the district. One third of participants resided in multiple homes, and movement between homes was common throughout the year, contrary to expectations based on local agricultural practices. In general, movement was less seasonal than anticipated, with both intra-

district travel and long-distance trips occurring with similar frequency throughout the year despite the known occurrence of flooding during the rainy season.

Movement patterns during peak biting hours were particularly informative for assessing the effectiveness of malaria control activities. Participants spent an average of 27% of time at risk away from the home. One third spent time in both lakeside and inland areas during peak biting hours, and nearly half spent time in both sprayed and unsprayed areas during these times. Male and adult participants spent more time away from home during peak biting hours, and males exhibited higher overall nighttime mobility. Long-distance movement patterns during peak biting hours were similar to overall patterns, likely due to overnight trips in other districts. Participants furthermore spent nearly three-quarters of peak biting time in areas predicted to be at high risk for malaria.

These movement patterns have clear implications for malaria control in this population. In particular, the high degree of movement observed into and out of areas targeted for IRS raises concerns about the effectiveness of a targeted intervention strategy in this setting. Due to the proximity of and continuing high transmission in unsprayed areas [57], this frequency of circulation between sprayed and unsprayed areas would be expected to attenuate the impact of the intervention because of the ongoing risk of reintroductions and exposures. The year-round transmission in Nchelenge District and the lack of clear seasonality of movement patterns may further exacerbate this relationship, since there is no prolonged period of low transmission or reduced movement during which interventions can be conducted. In addition, the presence of multiple households and lack of firm seasonal residence patterns may undermine malaria control efforts because people might not be at a particular home to consent to and receive interventions such as household IRS. Given these factors, the movement patterns observed in Nchelenge District likely decrease the effectiveness of targeted IRS and highlight the need to better understand such movement patterns when targeting malaria control interventions.

The observed 10% of time spent away from home during peak biting hours may also reduce the effect of indoor vector control interventions. Given the high vector density in this region [47, 48] and reports of outdoor biting from other research sites [61, 63-66], time spent outside during peak biting hours is likely to contribute to malaria transmission. Without accounting for the impact of bed nets, outdoor transmission was calculated to be 8% of total transmission among this population, representing a large number of infections in a high-burden area. If LLINs provide only 50% protection for time spent at home, outdoor transmission was calculated to comprise 12% of total malaria transmission. In addition, the time spent outside is probably underestimated due to the inability of GPS data to discern whether participants are inside or outside their home with sufficient precision. Time considered at the home would consequently include both indoor and outdoor fractions, so the true proportion of time spent outside is likely higher than 10% and would therefore contribute an even greater degree to malaria transmission in this region.

The frequency of long-distance travel among the study population is also of great interest due to the risk of both imported and exported malaria. In this high transmission area, it is likely that outbound travelers serve as a source of malaria introductions in regions with lower transmission, for example in the provincial capital Mansa. Although no trips outside of the province were observed, the high mobility of this population indicates that longer-distance travel may reasonably be expected at lower frequencies, with implications for malaria elimination in Zambia. The large proportion of participants that spent time in both lakeside and inland areas could furthermore be expected to increase heterogeneity of risk due to the differing patterns of ecology across study area.

At the individual level, there was a 22% monthly malaria incidence by PCR among participants with a second visit. For a short period of follow-up, this indicates a very high level of transmission. Different movement patterns were expected to emerge among those with or without incident malaria, however, few clear relationships were evident. In plots of the activity space, participants with

parasitemia by PCR at visit 2 spent more time away from home during peak biting hours and did not travel more than 20 km, but these relationships were not statistically significant.

There are several potential explanations for this result. Due to the small sample size and short follow-up time, only 14 participants were PCR positive at visit 2, which resulted in wide confidence intervals and limited the ability to draw inferences between groups. One possible explanation is that the study may have lacked sufficient power to compare movement patterns at the individual level, and that true relationships exist that would be observable with a larger study population. Alternatively, if these results are not due to chance, it would denote that there is no strong relationship between mobility patterns and malaria transmission in this high-burden setting, perhaps due to lack of variation between participants or saturation of malaria risk. If transmission is sufficiently high, movement patterns may not emerge as a significant predictor of individual risk. This hypothesis is supported by the comparison of malaria incidence by individual risk score (Figure 4.9), which showed a high level of risk across participants which did not vary significantly between those with and without parasitemia by PCR at either visit 1 or 2.

This study had several limitations. The sample size was not sufficiently large to conduct all statistical comparisons with precision, particularly between participants with and without incident parasitemia. This limitation was exacerbated by a 25% loss to follow-up for the second visit. Participants who were lost to follow-up did not differ from retained participants by demographic or clinical characteristics at visit 1, but their movement data indicated that they were more likely to travel longer distances, so they may not be fully comparable. Overall, a larger sample size and greater retention would have improved the inferential power.

A further limitation was the exclusion of young children and the use of non-probability sampling, so the study population may not fully represent the underlying population at risk. In Nchelenge District,

children aged 5-16 years have the highest malaria prevalence; however, children younger than 13 years were excluded from participation due to concerns about logger care, so a large proportion of the population at greatest risk was not accounted for in this analysis. Children aged 13-17 years and adult men were also sampled at lower proportions than the underlying population due to lack of availability in homes at recruitment, so these groups were somewhat underrepresented in this study.

Furthermore, although fishing activity and long-distance movement up to 200 km were observed, the study did not capture movement outside of Luapula Province or cross-border movement into the DRC, which is presumed to be common in this population due to porous borders. Potential explanations for this outcome include the short duration of participation time and low sample size. Since participants needed to be available at two-week intervals to swap out and return data loggers, this may have excluded people who were planning longer trips. If these movement patterns are relatively rare, a higher sample size may also have improved the chances of capturing these behaviors.

Another limitation was the potential for inaccuracies in the GPS logger data caused by imprecision and user error. Although data loggers performed well in rural areas in previous field tests, points recorded from within a household had some random scatter. After sensitivity analyses, logger data were not considered accurate to more than 50 m while the participant was at the home, limiting the precision with which they could be classified as inside or outside. As mentioned above, this had an impact on the indoor vs. outdoor risk calculation, and the proportion of outdoor risk is likely higher than reported. GPS data may also have been incorrect if the logger was forgotten at home, intentionally not used, or used by another participant. Since these scenarios were difficult to verify, logger data was analyzed as collected unless a specific issue was reported to the field team.

A further concern was the high level of discordance between RDT and PCR results for both visits 1 and 2. At visit 1, RDT results underestimated true parasite prevalence by PCR by 31%, and several PCR

positive individuals were not treated at their first visit as a result. Although 80% of these infections resolved by visit 2, two participants were RDT-/PCR+ at visit 1 and remained PCR+ at visit 2, so it is unknown if these were new or persistent infections. If these two people were excluded, the 1-month incidence in this population was 20%. These participants were classified as PCR positive in analyses, but sensitivity analyses were conducted to ensure that their inclusion in this group did not impact inferences. The main causes of false negatives among RDTs are infections below the level of detection (approximately 100 parasites/μL), or HRP-2 deletions [67]. HRP-2 deletions have not been found in Nchelenge District [Kobayashi, unpublished data], so false negatives are likely due to low-level parasitemia, which corroborates the spontaneous resolution of infection among most discordant participants as described above. Conversely, RDT results overestimated malaria incidence by PCR by 73% at visit 2. Several studies have reported false positives among RDTs after a prior malaria infection due to HRP-2 antigen persistence, and therefore a one-month interval may not be long enough to detect incident infections with RDTs in this high-transmission setting [67-70]. PCR results were therefore used for all analyses involving malaria incidence.

Despite these limitations, this study had considerable strengths. The project built off research conducted in Peru and Southern Zambia, but was the first to attempt to link movement patterns with disease incidence through a second study visit. Although loss to follow up occurred, incident malaria infection was identified in 1 of 5 participants, indicating that this study design is feasible in high-transmission settings. Also, despite the small sample size, a large amount of geolocated data was collected over a year in a challenging research setting. High-burden rural populations in sub-Saharan Africa are often difficult to access for logistical or political reasons, and so this dataset is unique among human movement studies. Moreover, a wide variety of movement patterns were captured with direct implications for targeted malaria control. High acceptability was observed among study participants, suggesting that a larger sample size or longer follow-up would be feasible.

CONCLUSION

Population movement has significant implications for malaria control at both large and small spatial scales. Over one month of participation in a GPS data logger study, residents of Nchelenge District, Zambia spent a large proportion of time in high-risk areas and exhibited a wide range of movement patterns. These behaviors can increase individual malaria exposures, amplify population-level transmission, and attenuate targeted interventions. In this high-transmission setting, movement patterns are thought to attenuate the impact of a targeted IRS strategy due to frequent movement in and out of targeted areas. This is the first time that fine-scale movement data has been directly linked to malaria incidence, and although there was insufficient power to conclusively draw inferences at the individual level, this study provides evidence of the importance of individual movement patterns for malaria transmission and the feasibility of similar investigations to inform malaria control policy. Overall, human mobility should be considered when selecting intervention strategies for similar high-transmission settings, particularly when designing targeted control strategies.

Figure 4.1: Flowchart of participation

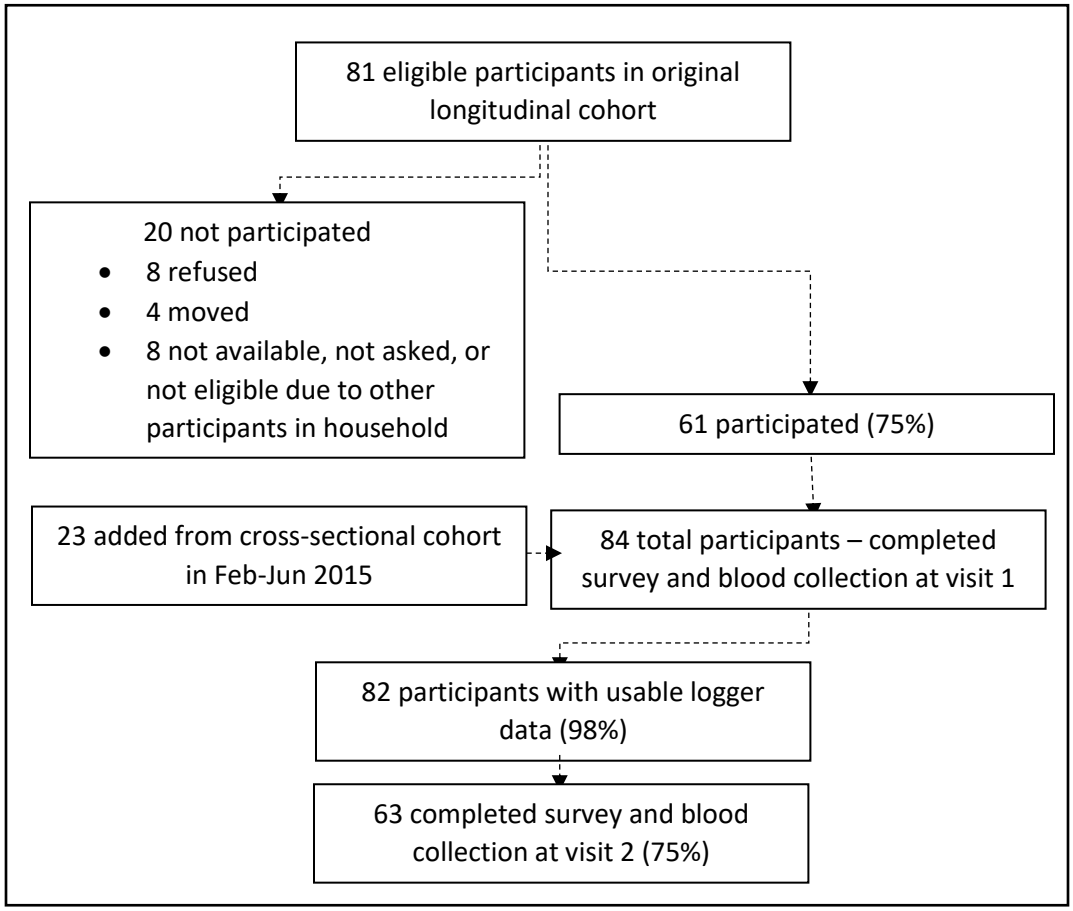


Table 4.1: Participant characteristics at visit 1 (N=84)

	n	%
Demographics		
Age < 18	12	14.3%
Male	31	36.9%
Household visit type		
First ICEMR visit	40	47.6%
Follow-up visit	44	52.4%
Household characteristics		
Within 3 km of lakeside	49	58.3%
Within 30-minute walk^ of health clinic	34	40.5%
Reside in different home part of year	31	36.9%
Head of household permanently employed	6	7.1%
Household uses open water source	37	44.1%
Household has dirt floor	81	96.4%
Health-related behaviors		
Sleeps under bed net	72	85.7%
House sprayed with pirimiphos-methyl	16	19.3%
Visited health center in past 6 months*	47	56.0%
Slept away from home in past month*	1	1.2%
Clinical results		
RDT positive	29	34.5%
PCR positive*	43	51.2%
Fever at visit	3	3.6%
Report fever in past 2 weeks	23	27.7%
Anemic at visit	46	55.4%

*Significant difference between visit 1 and visit 2 in chi squared analysis at P<0.05
^2.5 km

Table 4.2: Bivariate comparisons of PCR status by participant characteristics at visits 1 and 2

	Visit 1			Visit 2		
	PCR + n (%) N=43	PCR – n (%) N=40	**OR (95% CI)**	PCR + n (%) N=14	PCR – n (%) N=49	**OR (95% CI)**
Age						
Age < 18	9 (75.0%)	3 (25.0%)	3.2 (0.8, 13.1)	2 (25.0%)	6 (75.0%)	1.2 (0.2, 6.8)
Age >=18	34 (47.9%)	37 (52.1%)	ref	12 (21.4%)	44 (78.6%)	ref
Sex						
Male	16 (53.3%)	14 (46.7%)	1.1 (0.5, 2.7)	6 (25.0%)	18 (75.0%)	1.3 (0.4, 4.3)
Female	27 (50.9%)	26 (49.1%)	ref	8 (20.5%)	31 (79.5%)	ref
Household visit type						
First ICEMR visit	25 (62.5%)	15 (37.5%)	2.3 (0.96, 5.6)			
Follow-up visit	18 (41.9%)	25 (58.1%)	ref	14 (22.2%)	49 (77.8%)	-
Distance to lakeside						
Lakeside < 3 km	20 (40.8%)	29 (59.2%)	*0.3 (0.1, 0.8)**	9 (22.5%)	31 (77.5%)	1.0 (0.3, 3.6)
Lakeside >= 3 km	23 (67.7%)	11 (32.4%)	ref	5 (21.7%)	18 (78.3%)	ref
Distance to health center						
Health center < 30-minute walk	10 (29.4%)	24 (70.6%)	*0.2 (0.08, 0.5)****	7 (24.1%)	22 (75.9%)	1.2 (0.4, 4.0)
Health center >= 30-minute walk	33 (67.4%)	16 (32.6%)	ref	7 (20.6%)	27 (79.4%)	ref
Reside in different home part of year						
Yes	15 (50.0%)	15 (50.0%)	0.9 (0.3, 2.2)	2 (9.1%)	20 (90.9%)	0.2 (0.05, 1.2)
No	28 (52.8%)	25 (47.2%)	ref	12 (29.3%)	29 (70.7%)	ref
Household uses open water source						
Yes	25 (67.6%)	12 (32.4%)	*3.2 (1.3, 8.0)***	7 (30.4%)	16 (69.6%)	2.0 (0.6, 6.7)
No	18 (39.1%)	28 (60.9%)	ref	7 (18.0%)	32 (82.0%)	ref
Sleeps under bed net						
Yes	36 (50.7%)	35 (49.3%)	0.7 (0.2, 2.5)	11 (22.0%)	39 (78.0%)	0.9 (0.2, 4.0)
No	7 (58.3%)	5 (41.7%)	ref	3 (23.1%)	10 (76.9%)	ref
House sprayed with Actellic						
Yes	3 (18.8%)	13 (81.2%)	*0.2 (0.04, 0.6)***	2 (20.0%)	8 (80.0%)	0.9 (0.2, 5.0)
No	39 (59.1%)	27 (40.9%)	ref	11 (21.2%)	41 (78.8%)	ref

*Chi squared P<0.05, ** P<0.01 ***P<0.001

Figure 4.2: All GPS data logger points recorded from August 2014 to July 2015 in Nchelenge District overlaid on A) the study area with IRS targeted areas highlighted and B) a malaria risk in the study area

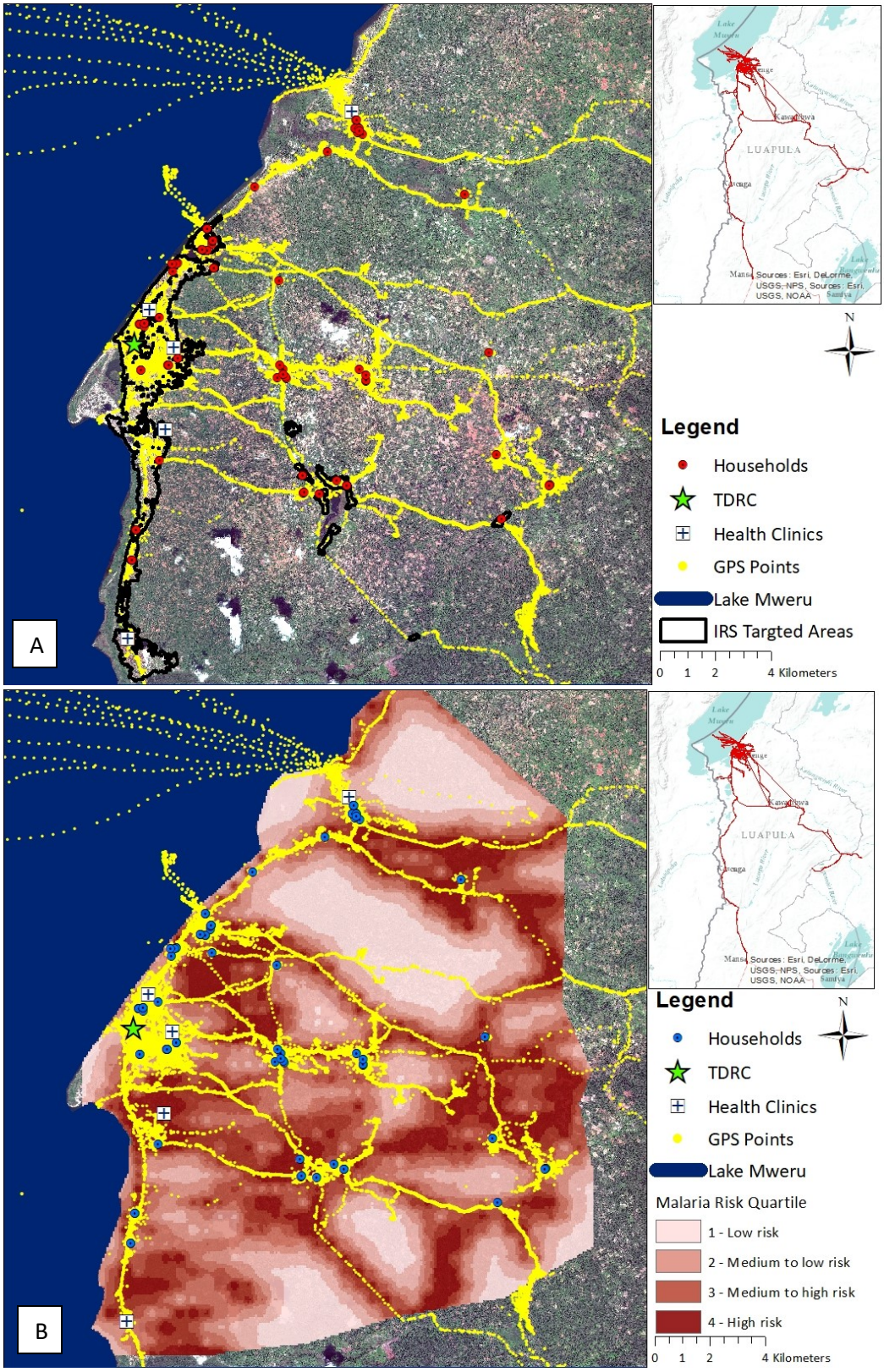
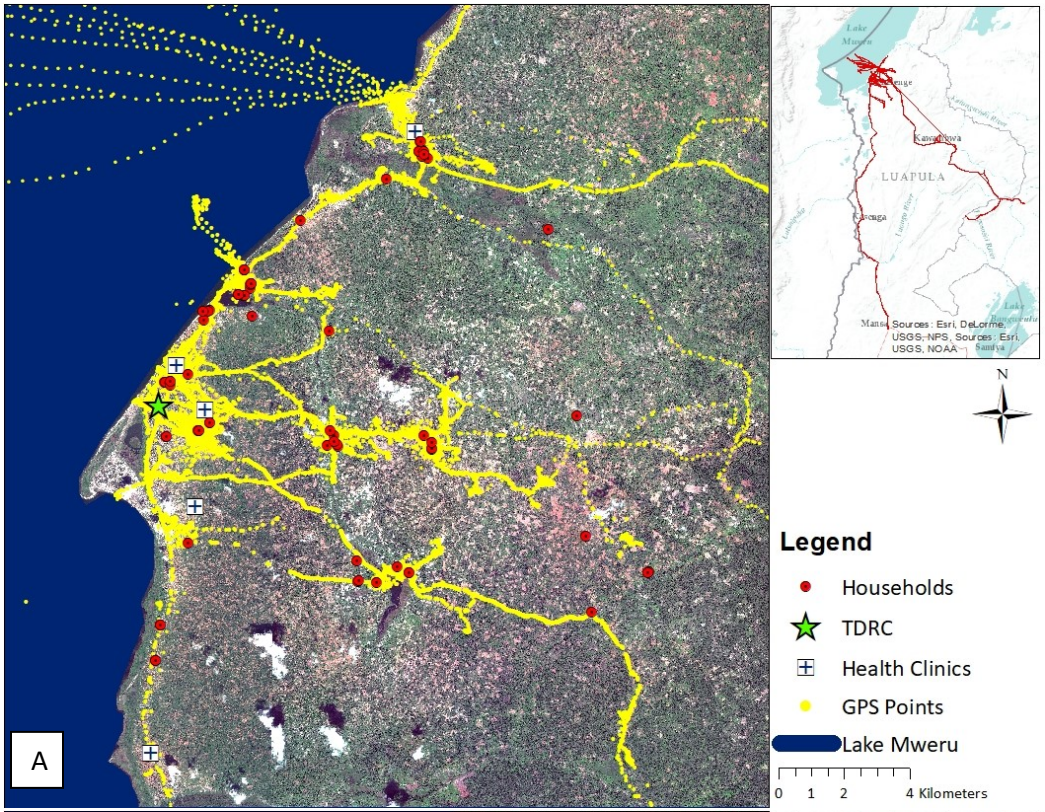


Figure 4.3: GPS data logger points stratified by A) dry season and B) rainy season. Boat travel on Lake Mweru is visible for two dry season participants



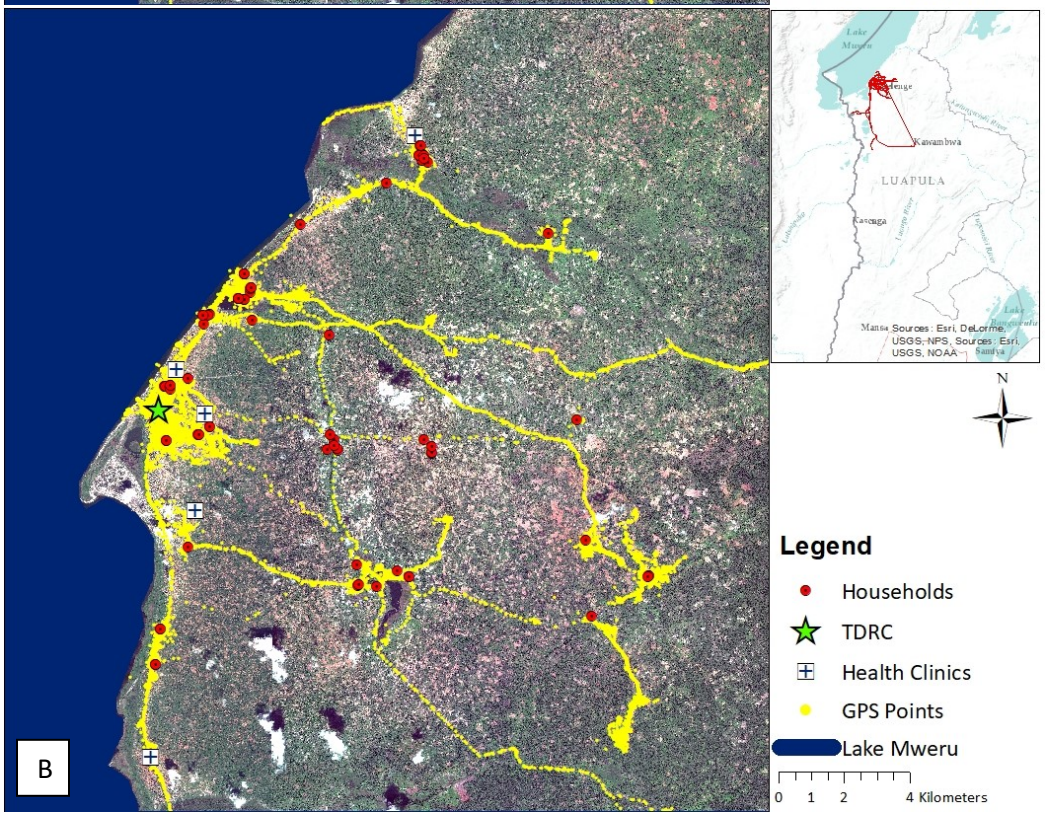


Figure 4.4: Intensity maps of population movement in Nchelenge District from August 2014 – June 2015 by month in A) August 2014, B) October 2014, C) December 2014, D) February 2015, E) April 2015, and F) June 2015. Boat travel on Lake Mweru is visible for panels A and F.

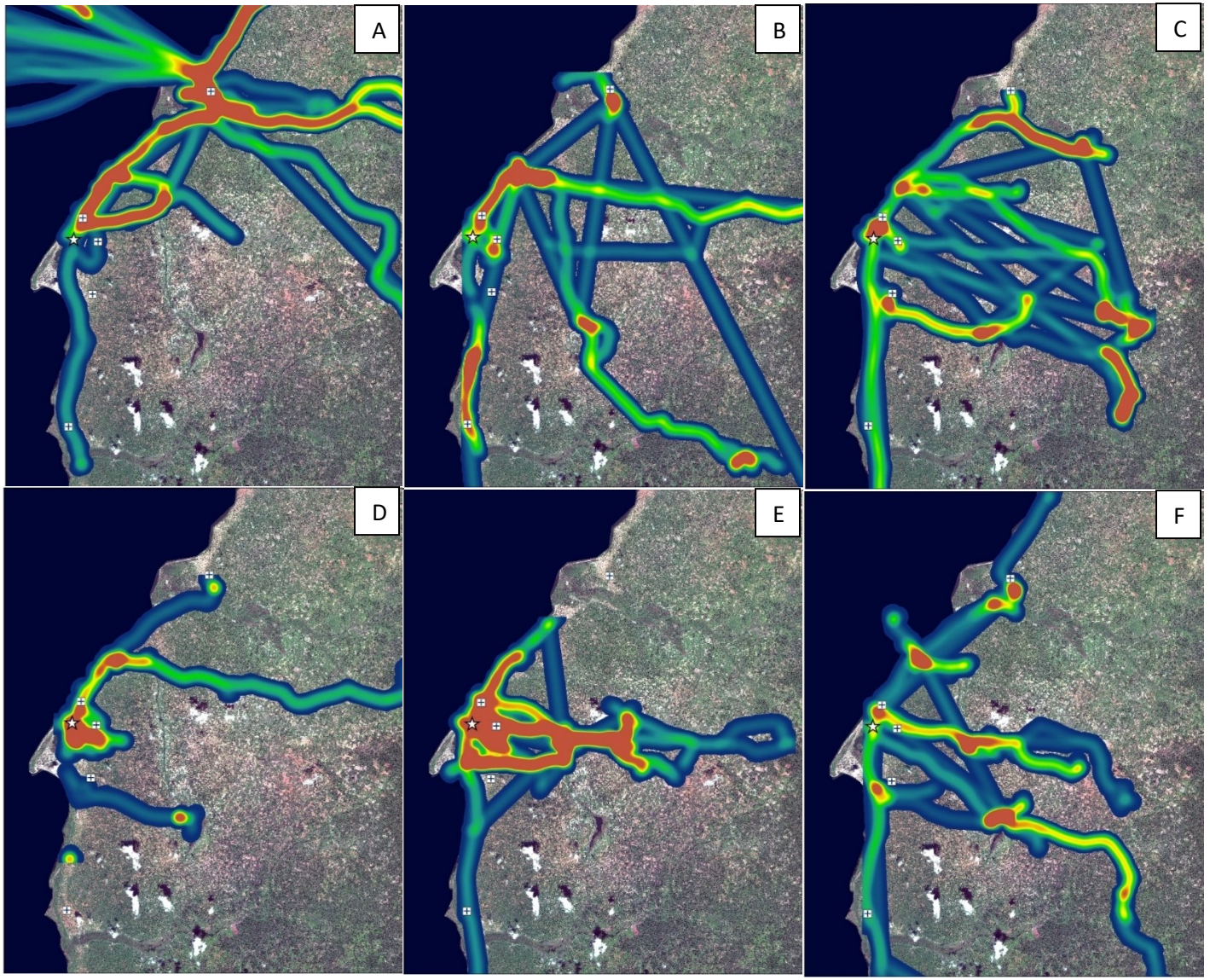


Figure 4.5: Three-dimensional intensity maps of population movement in Nchelenge District in A) dry season, and B) rainy season

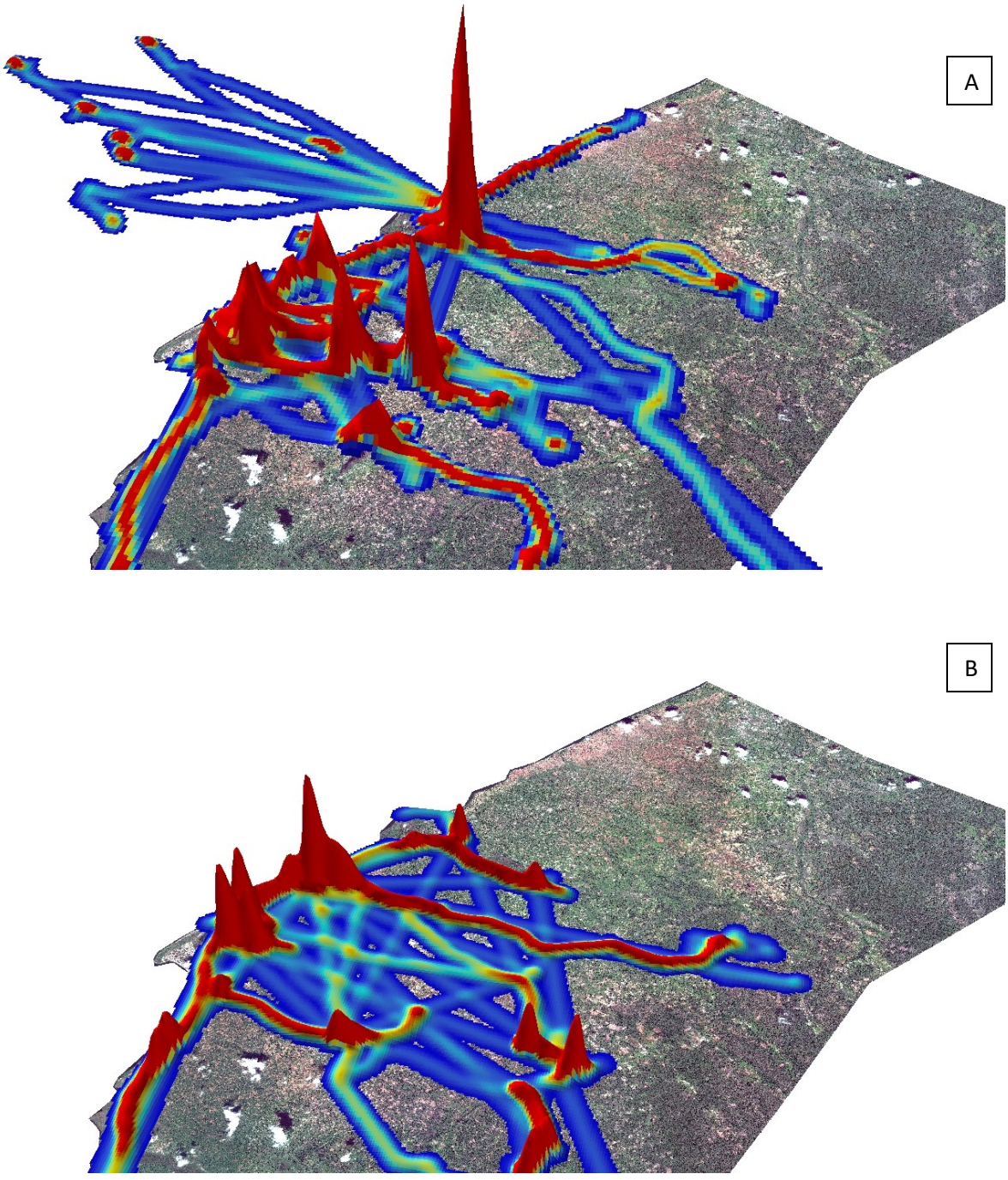


Table 4.3: Metrics of movement patterns among participants, stratified by sex, PCR positivity at visit 2, and vector peak biting hours

	Males		Females		
	Median (IQR)	Range	Median (IQR)	Range	P value*
OVERALL					
Total distance traveled (km)	145.0 (58.3 – 255.5)	14.0 – 1,169.0	79.7 (28.9 – 158.2)	2.1 – 511.8	*0.01*
Average distance per day (km)	5.9 (2.4 – 8.9)	0.7 – 42.7	2.4 (1.0 – 4.5)	0.1 – 15.5	*0.003*
Maximum distance from home (km)	8.7 (2.0 – 14.1)	0.2 – 165.9	3.2 (1.5 – 7.1)	0.1 – 212.4	*0.03*
Average hours away from home per day (>50m)	6.4 (2.6 – 14.3)	0.5 – 23.9	3.8 (1.4 – 6.5)	0.1 – 23.8	*0.02*
PEAK BITING HOURS^					
Total distance traveled	26.8 (14.2 – 42.7)	1.9 – 174.8	17.3 (7.9 – 34.1)	0.9 – 74.8	*0.04*
Average distance per day	2.4 (1.3 – 3.1)	0.2 – 11.7	1.4 (0.6 – 2.2)	0.1 – 9.2	*0.01*
Maximum distance from home	2.4 (0.6 – 9.2)	0.06 – 154.9	1.7 (0.4 – 3.4)	0.07 – 212.4	0.2
Average hours away from home per night (>50m)	2.9 (0.9 – 6.3)	0.01 – 12.0	1.3 (0.5 – 2.5)	0.1 – 12.0	0.06

	PCR +		PCR -		
	Median (IQR)	Range	Median (IQR)	Range	P value*
OVERALL					
Total distance traveled (km)	78.3 (28.2 – 130.0)	7.4 – 346.1	88.2 (52.8 – 181.7)	6.7 – 1,169.0	0.4
Average distance per day (km)	3.0 (1.2 – 3.9)	0.3 – 10.1	2.8 (1.8 - 6.5)	0.3 – 42.7	0.4
Maximum distance from home (km)	4.2 (2.3 – 8.0)	0.3 – 16.4	3.7 (1.9 – 8.8)	0.2 – 212.4	0.9
Average hours away from home per day (>50m)	2.6 (1.3 – 6.4)	0.5 – 10.0	4.5 (2.3 – 6.8)	0.8 – 23.9	0.3
PEAK BITING HOURS^					
Total distance traveled	15.9 (8.3 – 30.1)	2.2 – 58.4	21.6 (10.1 – 37.0)	1.9 – 174.8	0.7
Average distance per day	1.8 (0.6 – 2.2)	0.2 – 3.9	1.8 (0.8 – 2.6)	0.2 – 11.7	0.6
Maximum distance from home	2.2 (0.7 – 4.0)	0.1 – 9.2	1.8 (0.6 – 4.0)	0.1 – 212.4	0.7
Average hours away from home per night (>50m)	1.0 (0.5 – 2.7)	0.2 – 5.0	1.6 (0.8 – 2.8)	0.01 – 12.0	0.5

*Wilcoxon rank sum test

^Peak biting hours from 6 pm to 6 am

Figure 4.6: Activity space plots for participants showing proportion of time spent by distance from participant household, stratified by season of participation, sex, and age

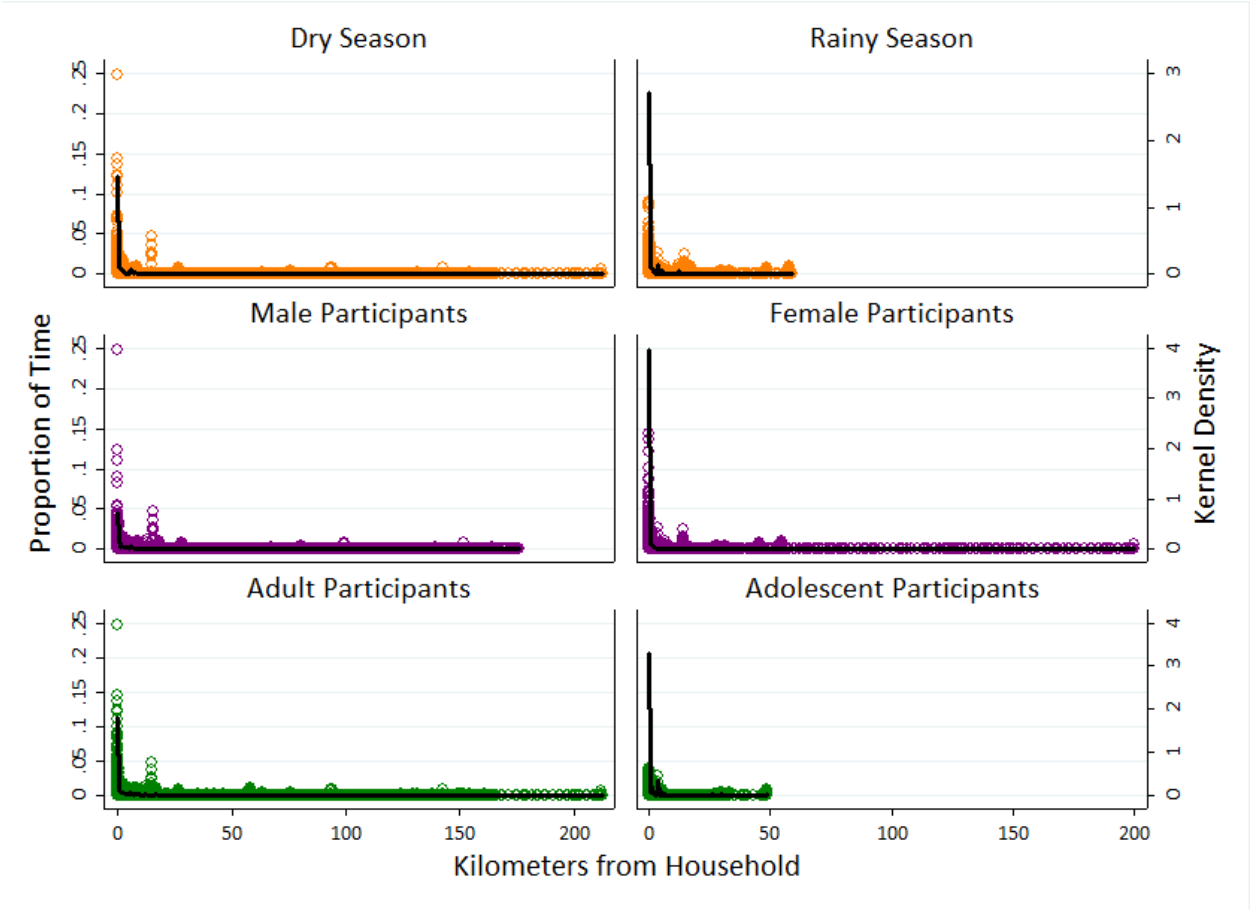


Figure 4.7: Activity space plots for participants showing proportion of peak vector biting time spent by distance from participant household, stratified by season of participation, sex, and age

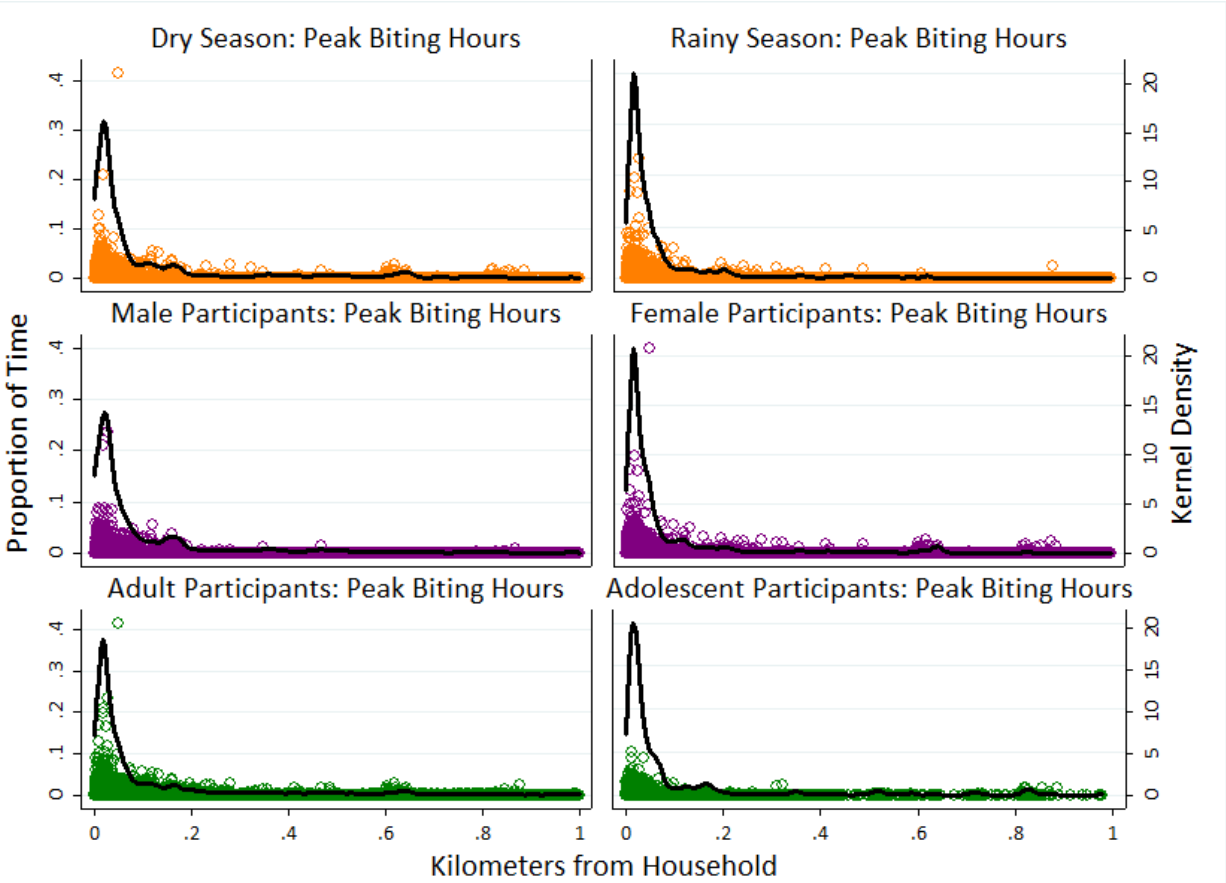


Figure 4.8: Activity space plots for participants showing proportion of time spent by distance from participant household or Lake Mweru, stratified by season of PCR positivity at visit 2

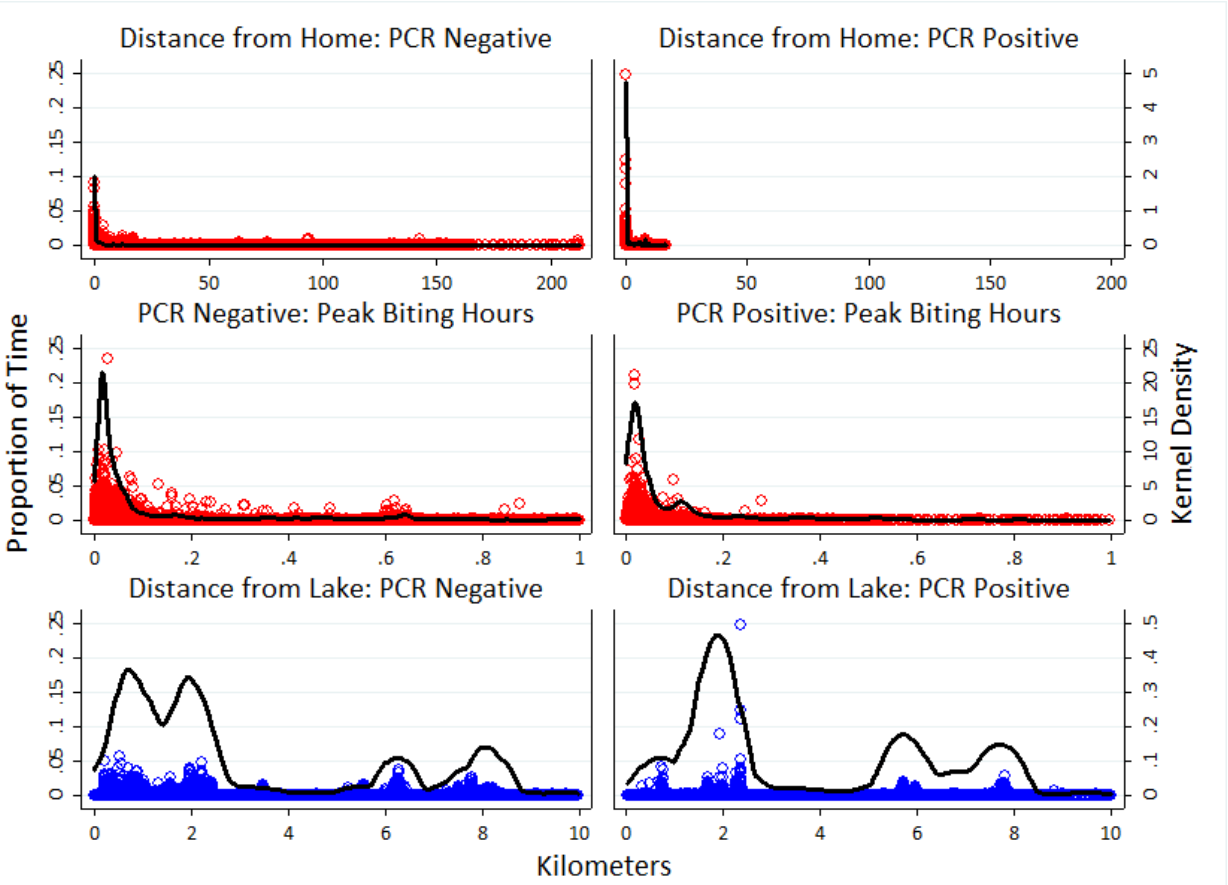
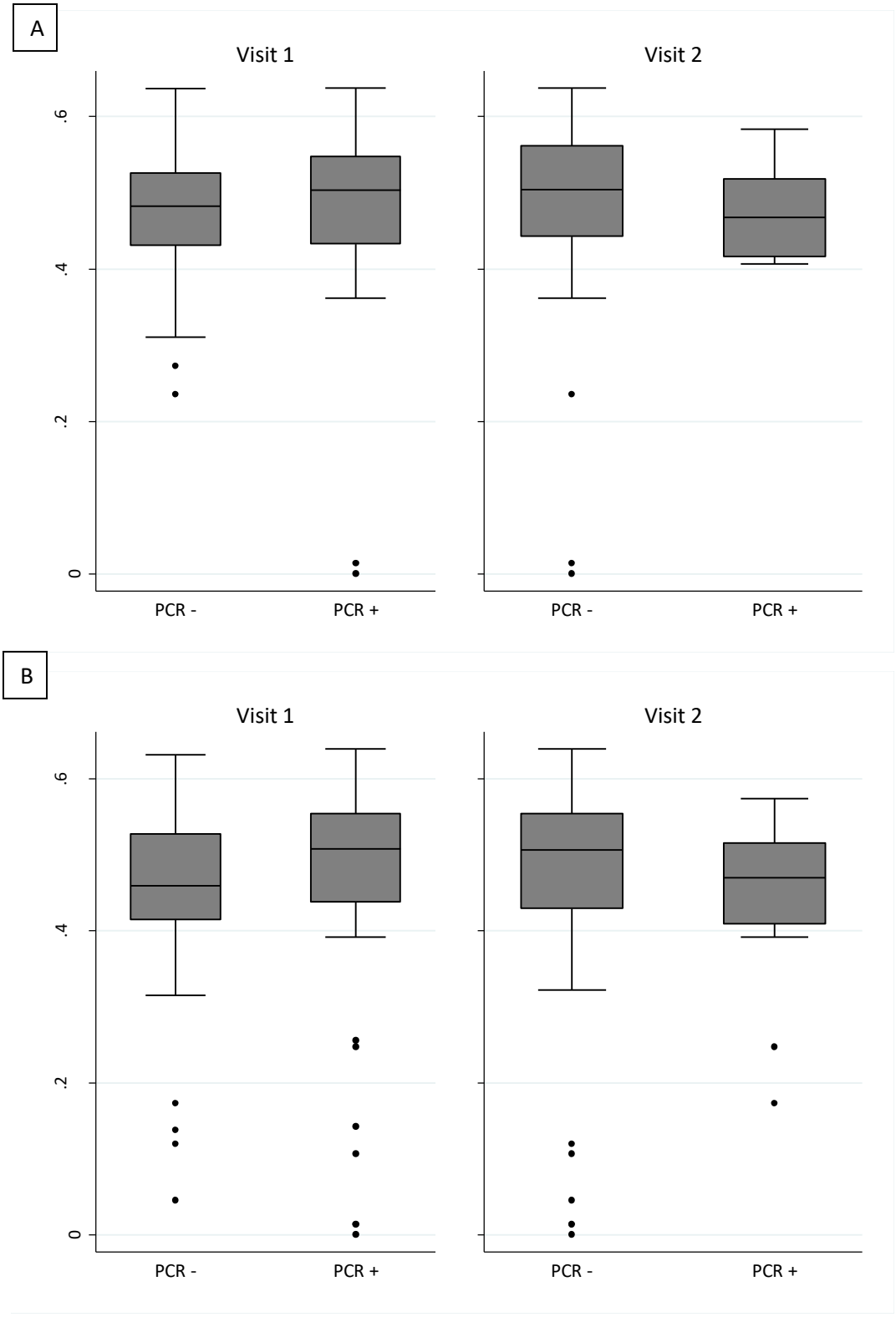


Figure 4.9: Boxplots and histograms of average nightly malaria risk assigned to participants for A) all peak vector biting times and B) time spent away from the household during peak vector biting times



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Chapter 5: Conclusions

SUMMARY OF FINDINGS

In Nchelenge District, northern Zambia, many factors contributed to persistent high malaria transmission despite standard control interventions (Figure 5.1). The presence of a permissive environment and two efficient vectors, *An. funestus s.s.* and *An. gambiae s.s.*, with differing ecology and breeding behavior resulted in year-round transmission. People had a high parasite prevalence, averaging approximately 50% by rapid diagnostic test (RDT), but only 4% were febrile, signifying a large asymptomatic or afebrile human reservoir. High levels of poverty were associated with malaria risk as approximately 90% of study participants lived in rudimentary housing with dirt floors, thatch roofs, and open eaves, facilitating the entry of mosquitoes, and half of residents used an unprotected water source such as streams, ponds, or surface water. Only 7% of residents lived in a home with a head of household in formal full-time employment, indicating economic insecurity, and only 30% of heads of household completed more than primary school education. Furthermore, people in this area were highly mobile and many had two homes due to seasonal agricultural cycles, which complicated the implementation of targeted malaria control interventions.

Malaria control activities in Nchelenge District were extensive. Under Zambia's National Malaria Elimination Programme, malaria testing and treatment were free of cost at health centers, and LLINs were distributed at antenatal and vaccination clinics. Most recently, a targeted IRS campaign with a novel formulation of pirimiphos-methyl was conducted in Nchelenge District. However, these interventions were not sufficient to interrupt transmission. Use of LLINs was associated with a 12-25% reduction in malaria risk across adjusted analyses, including a 24% decline in households with IRS in the prior 6 months, but less than 60% of the population reported sleeping under a net. Over three rounds of targeted IRS, there was a 51% decrease in indoor counts of *An. funestus*, a 36% decrease in indoor counts of *An. gambiae*, and a 28% reduction in parasite prevalence by RDT in sprayed areas. However,

the effect on parasite prevalence was only evident during the six months after IRS, and there was no impact in untargeted areas or during the dry season. Unexpectedly, household vector counts decreased throughout the study area, indicating that factors other than IRS may have caused the decline in mosquitoes, and high counts of malaria vectors continued to be collected in some sprayed households. Limitations to the IRS intervention may have reduced its impact. Despite high malaria burden throughout the study area, IRS was targeted only to areas with high population density, allowing continuing transmission in untargeted regions. Furthermore, coverage within targeted areas averaged only 54% of eligible households across the three years, far below the goal of 85%, resulting in protection of only 55% of residents of targeted areas and 42% of all residents of the district.

The high mobility of the population and participant movement patterns further served to attenuate the effectiveness of malaria control interventions. Nearly half of participants spent time in both the sprayed and unsprayed areas during peak biting hours, and 56% of participants spent at least 10% of peak biting time outside the home, both of which should reduce the impact of indoor vector management interventions. Movement was common between sprayed and unsprayed areas, lakeside and inland areas, and outside the study area, which increased the heterogeneity of malaria risk and potential exposure to infectious vectors.

PUBLIC HEALTH SIGNIFICANCE AND RECOMMENDATIONS FOR MALARIA CONTROL

In this high transmission setting in northern Zambia, ongoing obstacles to malaria control include the high baseline rate of transmission, low intervention coverage, high population mobility, and the low proportion of symptomatic infections. Given current strategies, interruption of transmission is unlikely to occur by the 2021 Zambian national malaria elimination goal. However, scale-up of malaria control interventions could reduce transmission substantially. Given the moderate impact of the targeted IRS intervention at low levels of coverage, a higher impact would be expected if coverage were increased. Moreover, evidence of indirect effects of IRS suggests that the population-level impact of the

intervention will increase non-linearly with increasing coverage, so higher coverage levels may have a larger impact than expected. Increasing coverage of LLINs will also reduce malaria transmission on both an individual and population level, particularly among school-age children, children under five years, and adult men, for whom parasite prevalence is highest. For future interventions, coverage of at least 85% for both LLINs and IRS will be necessary to see a sustained impact on parasite prevalence.

Furthermore, IRS in Zambia has historically been conducted in urban or peri-urban areas due to logistical ease and lack of surveillance in more rural regions. The recent IRS campaign with pirimiphos-methyl was the first to use systematic methods to maximize the impact of IRS; however, IRS activities in Nchelenge District continued to be concentrated in areas with the highest population density despite a high malaria burden in surrounding rural areas. These more rural, inland residents have lower LLIN coverage and lower socioeconomic indicators that increase malaria risk, including a higher proportion of natural housing construction and open water sources and lower education attainment. To reduce malaria transmission throughout Nchelenge District, a significant increase in resources must be allocated to rural, inland regions, including IRS throughout the district, LLIN distributions, and improved surveillance, such as through the malaria indicator surveys (MIS), community health workers, or additional health centers. Also, movement between sprayed and unsprayed regions was common, and residents of sprayed areas spent a significant amount of peak vector biting time in unsprayed rural areas, undermining the effectiveness of targeted IRS.

In addition, due to year-round transmission, vector control interventions in Nchelenge District should be conducted in both rainy and dry seasons. Current IRS policy includes once-yearly application of pirimiphos-methyl in targeted areas, which is expected to only affect rainy season transmission due to the short insecticidal action of residual pirimiphos-methyl. This strategy does not impact the substantial degree of dry season transmission. Dry season *An. funestus* populations were also found to impact parasite prevalence in the rainy season, so neither rainy nor dry season transmission can be interrupted

without vector control interventions targeted to dry season transmission. Therefore, IRS must be conducted twice per year or longer-lasting insecticides must be used in Nchelenge District to provide continuous protection.

Another significant barrier to malaria control in this region is the high proportion of asymptomatic or afebrile infections, which would reduce health-seeking and maintain a large parasite reservoir. Although these analyses did not specifically address this issue, successful malaria control in this region may necessitate enhanced chemoprevention methods to reduce the parasite population. Due to the high prevalence, mass drug administration (MDA) among the whole population or high-risk groups may be instrumental in reducing the malaria burden. For example, school age children were found to have the highest parasite prevalence, and school-based MDA twice per year may improve indicators and reduce sub-clinical incident infections in this population. However, these methods have not previously been shown to interrupt transmission in high-burden settings, and new strategies must be developed to maintain high coverage and sustainability over time to ensure a lasting impact.

A further issue in this region is the limitations of routine passive surveillance to monitor trends and evaluate interventions. Routine data collection in Nchelenge District occurs through 11 health centers, which provide free testing and treatment to patients and send monthly case counts to the Ministry of Health. Clinical surveillance data is an important benchmark of malaria surveillance and can provide information on incident infections over time. However, in high-transmission areas, these data are limited by health-seeking behavior, which may underrepresent true incidence due to the high proportion of afebrile infections. The availability of accurate testing methods has also been an issue in Nchelenge District, and frequent stockouts of RDTs have limited the usefulness of these data to evaluate interventions. Furthermore, the health centers in this district are along the main road near Lake Mweru, which could overrepresent people in this region and underrepresent people in more rural areas, who may have to travel 15-20 km to reach the nearest health facility. Additional health centers in the inland

region or an enhanced role of community healthcare workers would provide services for a neglected population. Ensuring that all clinics have consistent supplies of RDTs and medications would enable these data to be used more accurately to monitor long-term trends.

Overall, malaria control recommendations for this region include ensuring 85% coverage of LLINs and IRS with pirimiphos-methyl twice per year across the entire district, with efforts particularly focused in rural areas. Monitoring insecticide resistance must be a priority, due to the rapid development of resistance to pyrethroids and carbamates in this area in the past. Improved surveillance would further inform malaria control interventions. If sustainable and scientifically sound methods are developed to ensure high coverage, these strategies can be supplemented by MDA or other chemoprevention methods. The financial and logistical investment required to accomplish these goals will be substantial, and therefore additional sources of funding will be required to carry out these recommendations. However, without this investment, substantial reductions in malaria transmission are unlikely to occur in this high-burden area.

STRENGTHS AND LIMITATIONS

A major strength of these analyses was the use of five consecutive years of epidemiologic and entomologic active surveillance data, which are rarely collected concurrently. These long time series allowed for the investigation of seasonality, inter-annual variation, and long-term temporal trends in parasite prevalence and vector abundance, as well as the interaction between vector counts and malaria risk. The use of active surveillance data facilitated the identification of asymptomatic infections and helped ensure representativeness of results for the target population due to the use of random sampling techniques. Furthermore, the ongoing relationship between study staff and the local population increased study participation and compliance, including in an individual population movement study that required sustained participant involvement over a month. Finally, the findings of

these studies contribute to the body of knowledge of malaria in high-transmission areas and may help inform future malaria control policy in this region and other similar settings.

Limitations of these studies included a low sample size for some comparisons, particularly in rural, inland areas. Due to the logistical difficulties of conducting research in this region and the time- and laboratory-intensive nature of active surveillance, the number of households sampled per month was restricted. This issue was particularly limiting for the population movement study, which required a large additional time investment per participant. This was compounded by underrepresentation of males and older children due to work and school, which could lead to underestimates of risk due to the high parasite prevalence among these groups. Models were particularly challenging for entomologic analyses due to the high variability and overdispersion of vector count data, which limited statistical power. The use of cross-sectional prevalence data rather than incidence may also have attenuated some associations due to the unknown original time of infection and resulting dilution of causal relationships.

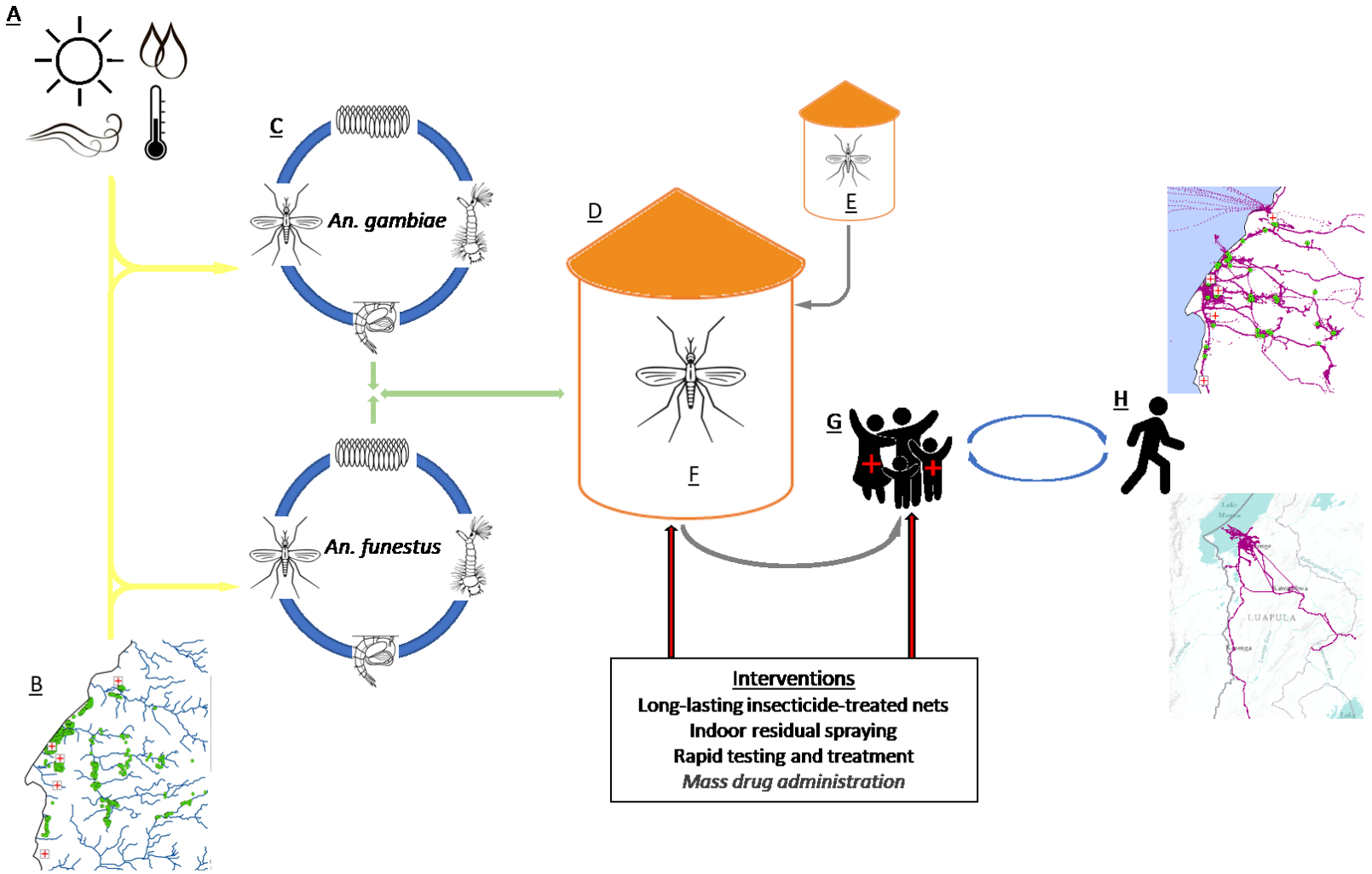
FURTHER RESEARCH QUESTIONS

The results of these analyses highlight ongoing gaps in knowledge for Nchelenge District and other high-transmission areas in rural sub-Saharan Africa. Given the challenges highlighted in these studies, the primary remaining question is how to identify optimal combinations of interventions to successfully reduce malaria transmission in similar settings. Ongoing active surveillance will allow future evaluations of new interventions as they are implemented, and modeling studies can make predictions of the anticipated impact of different combinations of interventions at various levels of coverage. The synergy between these methods can be leveraged to optimize intervention strategy in this region. For example, evidence of indirect effects of IRS in Nchelenge District in active surveillance data can inform modeling analyses to identify the level of coverage needed to reduce or interrupt transmission.

Outdoor transmission is expected to be a considerable source of malaria transmission in high-burden areas, but it has not been investigated in this setting. Also, although these studies used vector abundance as a proxy for EIR, measuring time-varying sporozoite rates would more accurately inform each species' proportionate contribution to malaria transmission. Future studies will conduct outdoor vector collections to determine the importance of outdoor transmission and will investigate sporozoite rates by species to further elucidate transmission patterns in this region. This information will help inform vector control interventions.

The impact of population movement on malaria risk in this region requires further investigation. The low sample size limited inference in this study, but future work using other methods could enroll a higher number of participants over a longer time period in order to capture long-distance and cross-border movement. As GPS and cellular technology improves, new GPS devices will be developed with longer battery life, better accuracy, and higher acceptability to populations, and cell phone-based methods will become more accessible to people across socioeconomic settings as cell coverage expands and costs decline. These emerging technologies can therefore be used to measure more extensive movement behaviors in this population. Parasite genotyping is another novel method to measure movements of people and parasites. As genetic signatures of local parasite populations are identified across settings, parasite introductions from other regions can be recognized and investigated to ultimately build a network of mobile transmission.

Figure 5.1: Schematic for factors influencing parasite prevalence in Nchelenge District, Zambia



A) Climactic factors, including rainfall and temperature, and B) geographic factors, such as distance to breeding sites, impact C) vector life cycles and regional vector abundance. Regional vector abundance, D) household construction, and E) neighboring vector abundance impact F) household vector abundance, which impacts G) individual parasite prevalence. Parasite prevalence is also impacted by H) short- and long-distance movement patterns and malaria control interventions.

CURRICULUM VITAE

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EDUCATION

Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD **Expected May 2018**
Doctor of Philosophy, Epidemiology, Infectious Disease

- Accepted as Mary B. Meyer Memorial Scholar (competitive full tuition scholarship and stipend)
- Dissertation title: *The effect of targeted indoor residual spraying, vector dynamics, and population movement on malaria risk in a high-transmission setting in northern Zambia*

Rollins School of Public Health, Emory University, Atlanta, GA **May 2011**
Master of Public Health, Global Epidemiology

- Accepted as Merit Scholar (competitive half tuition scholarship and Graduate Assistantship)
- Recipient of Certificate in Complex Humanitarian Emergencies
- Master's Thesis Title: *HIV Knowledge and Receipt of Targeted Training among UN Peacekeepers in a Post-Conflict Setting*

Brown University, Providence, RI **May 2006**
Bachelor of Science, Biology

- Graduated Magna Cum Laude
- Honors Thesis Title: *Snag Demographics and Dynamics in a Neotropical Rainforest*

RELEVANT PROFESSIONAL EXPERIENCE

Bloomberg School of Public Health, Baltimore, MD **September 2013-Present**
Research Assistant – Drs. William Moss, Justin Lessler, Susan Sherman

- Conducting multivariate and spatio-temporal analyses on malaria in rural Zambia as part of Southern and Central Africa International Centers of Excellence in Malaria Research surveillance studies
- Additional international projects: review on cholera in refugee camps, data entry and quality control for international cholera mapping project, analysis on influenza transmission in China
- Baltimore based projects: development of spatial sampling frame for street-based recruitment and analysis of risk and health outcomes among high risk women for HIV in Baltimore

Teaching Assistant – Drs. Kenrad Nelson, David Dowdy, Rosa Crum, Susan Sherman

- Assisted with Epidemiology of Infectious Disease (3 years), Epidemiology Methods 3, Principles of Epidemiology, and Survey Research Methods courses and labs

Centers for Disease Control and Prevention (CDC), Atlanta, GA **May 2011-April 2013**
Epidemiologist, Violence against Children Surveillance Team, Health Systems Reconstruction Office

- Co-investigator on nationally representative quantitative Violence Against Children Surveys in Haiti, Cambodia, Malawi to investigate physical, sexual, and emotional violence among girls and boys
  - Studies each enrolled over 2,000 children 13-24 years old in 188x35 cluster design survey
  - Duties included secondary data analysis, questionnaire and survey tool development, on-site supervision of implementing partners for pilot, mapping and listing, survey implementation
- Co-researcher to design and implement novel laboratory-enhanced sentinel surveillance system in 4 hospitals in Haiti to investigate pathogenesis of priority syndromes (acute watery diarrhea, AFI, ARI)
- Named official CDC Assistant to Incident Commander for 2011 Horn of Africa Famine Response

Centers for Disease Control and Prevention (CDC), Atlanta, GA **August 2010-May 2011**
Student Research Assistant, International Emergency and Refugee Health Branch

- Data entry and analysis for 150-person case-control study on Nodding Syndrome in Uganda
- Data interpretation, entry, and weekly reporting for acute watery diarrhea outbreak Pakistan 2010
- Quantitative analysis of HIV KAP survey among 667 UN military peacekeepers stationed in Liberia
- Analysis of data for novel infant formula-feeding centers established after earthquake in Haiti
- Assisted medical countermeasures team in the Emergency Operations Center for cholera in Haiti
- Translated technical documents into Spanish for public health course in Tunisia

Emory University, Rollins School of Public Health, Atlanta, GA **August 2009-May 2011**
Student Research Assistant - Dr. Patrick Sullivan and Dr. Rob Stephenson

- Organized venue time-space sampling dataframe for longitudinal cohort study investigating racial disparities in HIV incidence among MSM, recruited participants using venue-based sampling
- Coordinated research team and venue-based participant recruitment for 5 focus groups on interpersonal violence (IPV) among MSM in Atlanta metro area
- Developed, coordinated, and implemented qualitative study on IPV and acceptability of couples HIV testing among MSM in Namibia including leading 7 focus groups and 7 in-depth interviews in 5 cities

Teaching Assistant

- Coordinated two team-taught courses taught cooperatively between CDC and Emory: Health in complex humanitarian emergencies, Epidemiology methods in complex humanitarian emergencies

The BODY Project, NYU Medical Center*,* New York, NY **February-December 2008**
Research Assistant, Unit Supervisor

- Served as supervisor/team leader for one of two schools participating in research project testing 500 New York public high school students for metabolic syndrome in school-based health centers
- Coordinated medical screens with fasting blood draws and proctored surveys in English and Spanish
- Assisted in design and delivery of personalized health reports for students, developed databases, performed data entry and abstraction of data collection forms for analysis

PEER REVIEWED PUBLICATIONS AND REPORTS

Sherman, S., **Hast, M**., Flyn, C., Holtgrave, D., German, D. 2018. *Correlates of transactional sex among high-risk heterosexuals in Baltimore.* AIDS Care. 1-9.

Gilbert, L., Reza, A., Mercy, J., Lea, V., Lee, J., Xu, L., Marcelin, L.H., **Hast, M.**, Vertefeuille, J., Domercant, J.W. 2017. *The experience of violence against children in domestic servitude in Haiti: Results from the Violence Against Children Survey, Haiti 2012*. Child Abuse & Neglect. 76: 184-193.

Jiang, CQ, Lessler, J., Kim, L., Kwok, KO, Read, J., Wang, S., Tan, L., **Hast, M.**, Zhu, H., Guan, Y., Riley, S., Cummings, D. 2016. *Cohort Profile: A study of influenza immunity in urban and rural Guangzhou region of China, the Fluscape Study*. International Journal of Epidemiology. 46 (2): e16.

Stephenson, R., **Hast, MA**, Finneran, C., Sineath RC. 2014. *Intimate Partner, Familial and Community Violence among Men who have sex with Men in Namibia.* Culture, Health, and Sexuality. 16 (5): 473-487.

Steenland, MW, Joseph, AG, Lucien, MAB, Freeman, N, **Hast, MA**, Nygren, BL, Leshem, E, Juin, S, Parsons, MB, Talkington, DF, Mintz, ED, Vertefeuille, J, Balajee, SA, Boncy, J, Katz, MA, 2013. *Laboratory-Confirmed Cholera and Rotavirus among Patients with Acute Diarrhea in Four Hospitals in Haiti, 2012-2013*. American Journal of Tropical Medicine and Hygiene. 89 (4): 641-646.

Boncy, J., Rossignol, E., Dahourou, G., **Hast, MA**, Buteau, J., Stanislas, M., Moffett, D., Bopp, C., Balajee, A., 2013. *Performance and utility of a rapid diagnostic test for the diagnosis of cholera: notes from Haiti*. Diagnostic Microbiology and Infectious Disease. 76 (4): 521-523.

Boyle, WA, Ganong, CN, Clark, DB, and **Hast, MA**. 2008. *Density, Distribution, and Attributes of Tree Cavities in an Old-Growth Tropical Rain Forest*. Biotropica. 40: 241-245

Cambodia Ministry of Women's Affairs, UNICEF Cambodia, US Centers for Disease Control and Prevention. 2014. *Findings from Cambodia's Violence Against Children Survey 2013* (Listed as co-investigator) http://www.unicef.org/cambodia/UNICEF_VAC_Full_Report_English.pdf

Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, PEPFAR, Republic of Haiti, Together for Girls, and Interuniversity Institute for Research and Development. 2014. *Violence Against Children in Haiti: Findings from a National Survey, 2012.* (Listed as co-investigator) http://www.togetherforgirls.org/wp-content/uploads/Haiti_Final-Report_English.pdf

Centers for Disease Control and Prevention, Interuniversity Institute for Research and Development. 2011. *Report for the Comité de Coordination: Findings of Focus Groups to Inform the Violence Against Children Survey (VACS) in Haiti*. (Listed as co-investigator)

POSTERS PRESENTED AT SCIENTIFIC CONFERENCES

Hast, MA, M. Chaponda, J. Lupiya, M. Muleba, JB Kabuya, T. Kobayashi, T. Shields, F. Curriero, J. Lessler, M. Mulenga, JC Stevenson, DE Norris, WJ Moss. *Evaluating three years of a targeted IRS campaign in a high transmission area of northern Zambia.*

- American Society of Tropical Medicine and Hygiene Annual Meeting, Baltimore, MD 2017
- Johns Hopkins Malaria Research Institute Future of Malaria Research Symposium, Baltimore, MD 2017
- GIS Day Poster Session, Johns Hopkins Bloomberg School of Public Health, Baltimore MD 2017
- World Malaria Day Symposium, Johns Hopkins Bloomberg School of Public Health, Baltimore MD 2017

Hast, MA, JC Stevenson, M. Muleba, M. Chaponda, JB Kabuya, M. Mulenga, CM Jones, J Lessler, T. Shields, WJ Moss, DE Norris. *Characterizing the impact of dynamic vector abundance on individual malaria prevalence in a high transmission area of northern Zambia*

- Epidemics International Conference on Infectious Disease Dynamics, Sitges, Spain 2017
- American Society of Tropical Medicine and Hygiene Annual Meeting, Atlanta, GA 2016
- Johns Hopkins Malaria Research Institute Future of Malaria Research Symposium, Rockville, MD 2016
- GIS Day Poster Session, Johns Hopkins Bloomberg School of Public Health, Baltimore MD 2016

Hast, MA, M. Chaponda, K. Searle, J. Lupiya, T. Kobayashi, T. Shields, M. Mulenga, F. Curriero, W.J. Moss. *The effectiveness of a targeted indoor residual spray campaign with pirimiphos-methyl in Nchelenge District, northern Zambia.*

- American Society of Tropical Medicine and Hygiene Annual Meeting, Philadelphia, PA 2015
- Johns Hopkins Malaria Research Institute Future of Malaria Research Symposium, Rockville, MD 2015

Hast, MA, M. Chaponda, K. Searle, J. Lupiya, J. Lubinda, T. Kobayashi, T. Shields, M. Mulenga, F. Curriero, W.J. Moss. *The use of GPS data loggers to describe spatio-temporal movement patterns and correlations with malaria risk in an area of hyperendemic malaria in northern Zambia.*

- American Society of Tropical Medicine and Hygiene Annual Meeting, Philadelphia, PA 2015
- Epidemics International Conference on Infectious Disease Dynamics, Clearwater, FL 2015
- GIS Day Poster Session, Johns Hopkins Bloomberg School of Public Health, Baltimore MD 2015
- World Malaria Day Symposium, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 2016

Desormeaux, MA, M. Steenland, M. A. B. Lucien, S. Juin, N. Freeman, R. Emmanuel, **M. Hast**, A. Balajee, J. Boncy, G. Joseph, M.A. Katz. *Burden, epidemiology and seasonality of Cholera and Rotavirus among Patients with Acute Diarrhea in Four Hospitals in Haiti, 2012-2013.*

- American Society of Tropical Medicine and Hygiene Annual Meeting, Washington, D.C. 2013

Desormeaux, MA, M. Steenland, M. A. B. Lucien, S. Juin, N. Freeman, R. Emmanuel, **M. Hast**, A. Balajee, J. Boncy, G. Joseph, M.A. Katz. *Etiology of acute febrile illness in four hospitals in Haiti, April 2012-January 2013.*

- American Society of Tropical Medicine and Hygiene Annual Meeting, Washington, D.C. 2013

Steenland, MW, Joseph, AG, Lucien, MAB, Freeman, N, **Hast, MA**, Nygren, BL, Leshem, E, Juin, S, Parsons, MB, Talkington, DF, Mintz, ED, Vertefeuille, J, Balajee, SA, Boncy, J, Katz, MA *Laboratory-Confirmed Cholera among Patients with Acute Diarrhea in Four Hospitals in Haiti, 2012.*

- American Society of Tropical Medicine and Hygiene Annual Meeting, Washington, D.C. 2013

Little, K., Harrison, C., Kanago, M., **Hast, M.**, Thornton, A. *Extra Hands in Emergencies: Emory's Student Outbreak and Response Team and Mutually Beneficial Collaborations with Public Health Partners.*

- Public Health Preparedness Summit, Atlanta, GA 2011

Hast, M., *Interpersonal Violence among Men who Have Sex with Men in Namibia*

- Public Health Scholars event, Emory University, Atlanta, GA 2011

Hast, M., *Investigating the Disparities in HIV Incidence and Prevalence among White and Black MSM*.

- Public Health Scholars event, Emory University, Atlanta, GA 2010

ACADEMIC AND PROFESSIONAL HONORS

2018 Selected for 68th Lindau Noel Laureate Meeting for young scientists
2017 Accepted into 2018 Epidemics Intelligence Service Cohort
2015 R. Bradley Sack Family Scholarship Award, Johns Hopkins Bloomberg School of Public Health
2015 Miriam E. Brailey Fund award, Department of Epidemiology, Johns Hopkins University
2015 Health Resources and Services Administration Trainee Fellow, Office of Public Health Practice and Training, Johns Hopkins University
2015 Student Assembly Merit Award, Johns Hopkins Bloomberg School of Public Health
2014 Global Health Established Field Placement Grant recipient, Johns Hopkins University
2013 Mary B. Meyer Memorial Fund Award recipient, Johns Hopkins University
2013 CDC National Center for Injury Prevention and Control Honor Award for Excellence in Surveillance and Health Monitoring presented to Violence Against Children Survey Team
2012 CDC Center for Global Health Director's Medal of Excellence in Global Health
2012 CDC Center for Global Health Honor Award for Excellence in Partnering presented to DB Contractors working in Haiti
2012 DB Consulting Group Certificate of Achievement
2011 CDC Center for Global Health Certificate of Appreciation for Excellence in Emergency Response presented to Haiti Emergency Response Team
2011 CDC & ATSDR Honor Award for Public Health Impact presented to Haiti Cholera Response Team
2011 DB Consulting Group Certificate of Appreciation presented to Health Systems Reconstruction Office
2011 Graduate Certificate in Global Complex Humanitarian Emergencies, Emory University
2010 Global Field Experience grant recipient, Emory University
2009 Merit Scholar Awards Program, Emory Rollins School of Public Health
2006 Sc.B. Awarded Magna cum Laude, Brown University
2005 Research Experience for Undergraduates grant recipient, Organization for Tropical Studies

GRANTS

Impact of Targeted IRS and Population Movement on Malaria in Northern Zambia, National Institutes of Health F31AI124645, Ruth L. Kirschstein National Research Service Award (NRSA), Individual Predoctoral Fellowship to Promote Diversity in Health, 7/01/2016 – 6/30/2018

Malaria Transmission and the Impact of Control Efforts in Southern Zambia, National Institutes of Health 3U19AI089680-06S1, Research Supplements to Promote Diversity in Health-Related Research, 8/25/2015 – 6/30/2016

TEACHING EXPERIENCE

Instructor, Université Protestante au Congo, Kinshasa, Democratic Republic of Congo
- Principles of Epidemiology 1-week intensive course for medical fellows, 2017

Teaching assistant, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
- Infectious Disease Epidemiology, 2014 – 2016
- Principles of Epidemiology, 2015
- Epidemiologic Methods III, 2015
- Survey Research Methods, 2014

Health Teacher, Commodore Johns Rogers School, Baltimore Maryland
- Co-taught 1 semester health class to 8th grade students, 2016

Teaching assistant, Emory University Rollins School of Public Health, Atlanta, GA
- Health in complex humanitarian emergencies, 2011
- Epidemiology methods in complex humanitarian emergencies, 2011

Math and science tutor, various organizations
- Seven Tepees After-School Program, San Francisco CA, 2006-2007
- Students Teaching Students, Providence RI, 2003-2004
- National Honors Society tutoring program, Boulder High School, Boulder, CO, 2001-2002

VOLUNTEER EXPERIENCE

2016	Volunteer Health Teacher, Commodore John Rodgers Middle School, Baltimore MD
2014-current	Student Outbreak Response Team, Johns Hopkins University
2009-2011	Logistics officer, Student Outbreak Response Team, Emory University
2009-2010	Volunteer HIV Tester and Counselor, AID Atlanta, Atlanta GA
2009	Community and Advocacy Volunteer, Refugee Women's Network, Atlanta GA
2008	Clinical Assistant, Early Options Women's Health, New York, NY
2006-2007	Math and Science Tutor, Seven Tepees After-School Program, San Francisco CA
2004	International Health Care Volunteer, St. Joseph Hospital, Moshi Tanzania
2003-2004	Math Tutor, Students Teaching Students, Providence RI
2003	Health Care Volunteer, Peoples Clinic, Boulder CO

LANGUAGE SKILLS

Spanish: Fluent
French: Beginner

CERTIFICATIONS, TRAININGS AND MEMBERSHIPS

- Member of American Society for Tropical Medicine and Hygiene
- Member of Society for Epidemiologic Research

- Member of Johns Hopkins Biomedical Scholars for underrepresented minorities in the sciences
- Completed the Meaningful Modeling in Epidemiological Data Program through the University of Florida and African Institute for Math Sciences 2016
- Completed three courses of the University of Washington Summer Institute in Statistics and Modeling in Infectious Disease 2016
- Recipient of Graduate Certificate in Global Complex Humanitarian Emergencies 2011
- NIMS certified, completed ICS trainings 100, 200, 300, 700, 800
- Completed CITI Trainings and Johns Hopkins HIPAA trainings
- Completed Red Cross disaster training, Master the Disaster trainings 1-2 with DeKalb Board of Health
- Certified HIV Counselor/Tester, completed training in HIV rapid testing and AIDS 101

COMPUTER AND RESEARCH SKILLS

- Skilled in SAS and STATA statistical software, Microsoft Office Suite, ArcGIS
- Proficient with statistical packages R, SUDAAN, EpiInfo, JMP
- Extensive experience with research into primary scientific and medical literature
- International field research experience in Zambia, Zimbabwe, Democratic Republic of the Congo, Haiti, Cambodia, Namibia, Costa Rica, Tanzania
- Experience with diverse populations in medical and educational settings, including people of varied income, ethnicity, sexual preference and gender identity