



**COMMUNITY-BASED MONITORING OF VECTOR CONTROL INTERVENTIONS IMPACT UPON
MOSQUITO POPULATION DYNAMICS IN RURAL ZAMBIA**

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ABSTRACT

Over the last decade, the malaria burden has reduced drastically across many parts of sub-Saharan Africa. This is mainly due to effective implementation of integrated malaria control programmes that include large scale application of vector control in the form of long-lasting insecticidal net (LLINs) and indoor residual spraying (IRS), both of which target the most efficient human-seeking malaria vector species. However, in spite of these efforts, malaria has yet to be eliminated from most of Africa. However, recent increases in the physiological resistance of vector populations, especially to the pyrethroids that remain the only active ingredients currently used on nets threaten these achievements. Furthermore, various forms of behavioural resilience and resistance exhibited by some vector species to LLIN and IRS delivery formats for insecticides respectively limit and undermine these valuable impacts upon malaria transmission. To monitor the impact that LLINs and IRS have on vector population dynamics and malaria transmission, more effective, practical and affordable entomological surveillance systems are required. Currently, surveillance of mosquito populations are conducted by the centralized specialist teams with limited personnel, resources and geographic outreach. None of these existing systems can adequately monitor vector population dynamics longitudinally across the vastness of entire countries.

The overall goal of the study was to demonstrate how a community-based surveillance system can be applied to longitudinally monitor vector population dynamics and assess the impact that LLINs and IRS have on malaria transmission in rural Zambia. To achieve this overall goal, the following specific objectives were addressed: (1) To evaluate the efficacy of exposure-free mosquito trapping methods for measuring malaria vector density, as alternatives to human

landing catch; (2) To assess the cost-effectiveness using a community-based (CB) mosquito trapping scheme for monitoring vector population dynamics; (3) To determine the extent to which a community-based mosquito trapping scheme captures trends in epidemiological indicators of malaria infection risk; (4) To determine the impact of indoor residual spraying with different classes of insecticides on malaria infection burden and vector abundances in an area of high coverage with insecticide treated nets using a community-based platform.

To address objective 1, a 3 x 3 Latin square method was used to evaluate the sensitivity of the Center for Disease and Control and Prevention miniature light traps (LT), the Ifakara tent trap (ITT), window exit traps (WET) and the resting boxes (RB) using the golden standard human land catch (HLC) as the reference method. The mean catches of HLC indoor, HLC outdoor, CDC-LT, ITT, WET, RB indoor and RB outdoor, were 1.687, 1.004, 3.267, 0.088, 0.004, 0.000 and 0.008 for *Anopheles quadriannulatus* Theobald respectively, and 7.287, 6.784, 10.958, 5.875, 0.296, 0.158 and 0.458, for *An. funestus* Giles, respectively. The LT (Relative rate (RR) [95% Confidence Interval] = 1.532 [1.441, 1.628] $P < 0.001$) and ITT (RR = 0.821 [0.765, 0.881], $P < 0.001$), were the only exposure-free alternatives which had comparable sensitivities relative to HLC indoor for sampling *An. funestus*.

To address objectives 2 and 3, the two most sensitive of these exposure-free trapping methods, the LT and ITT, were applied through a CB longitudinal entomological surveillance system implemented by local community health workers (CHW) trained in basic entomology. This surveillance platform was conducted using a monthly sampling cycle for over 2 years in 14 population clusters distributed across two rural districts covering over 4,000km² of south-east Zambia. Parallel active surveillance of malaria parasite infection rates amongst humans was also

conducted by CHWs in the same population clusters to determine the epidemiological relevance of these CB entomological surveys. Prior to the end of the study, a controlled quality assurance (QA) survey was conducted by a centrally supervised expert team using HLC, LT and ITT to evaluate accuracy of the CB trapping data. While the relative sampling efficiencies of both CB surveys were less than their QA counterparts, the costs of implementing per sampling night were far less expensive than any QA survey. The cost per specimen of *Anopheles funestus* captured was lowest for CB-LT (\$5.3), followed by potentially hazardous QA-HLC (\$10.5) and then CB-ITT (\$28.0). Time-trends of malaria diagnostic positivity (DP) followed those of *An. funestus* density with a one-month lag and the wide range of mean DP across clusters was closely associated with mean densities of *An. funestus* caught by CB-LT ($P < 0.001$).

To address objective 4, the same 14 cluster populations, with pre-existing high coverage of pyrethroid-impregnated long-lasting insecticidal nets (LLINs), were quasi-randomly assigned to receive IRS with either of two pyrethroid formulations, namely Deltamethrin (Wettable granules (WG)) (DM-WG) and Lambda-cyhalothrin (Capsule suspension (CS)) (LC-CS), or with an emulsifiable concentrate (EC) or CS formulation of the organophosphate pirimiphosmethyl (PM), or with no supplementary vector control measure. DP conducted is described in objective 2. Over the first 3 months, the PM-CS IRS supplement offered the greatest level of protection against malaria followed by LC-CS and then by DM-WG. Neither pyrethroid formulation provided protection beyond 3 months after spraying, but both PM CS and EC formulations persisted for 6 months and 12 months respectively. The CS formulation of PM provided greater protection than the combined pyrethroid IRS formulations throughout its effective life (Incremental protective efficacy (IPE) [95%CI] = 0.79 [0.75, 0.83]) over 6 months. The EC

formulation of PM provided incremental protection for the first three months (IPE [95%CI] = 0.23 [0.15, 0.31]) that was approximately equivalent to the two pyrethroid formulations (LC-CS, IPE [95%CI] = 0.31 [0.10, 0.47] and DM-WG, IPE [95%CI] = 0.19 [-0.01, 0.35]) but the additional protection provided by the former, apparently lasted an entire year. There were no obvious differences in the densities of *An. funestus* during the first three months post-spraying for both pyrethroid formulations (DM-WG (IPE[95%CI]=0.01[-0.56,0.37],P=0.103) and LC-CS (IPE[95%CI]=-0.03[-0.88,0.44],P=0.195) and PM-EC (IPE[95%CI]=-0.04[-0.30,0.17], P=0.103). However, where PM-CS was applied, mosquito densities were dramatically reduced during the same period (IPE [95%CI] =0.93[0.87, 0.97], P<0.001). Between the fourth and the sixth month after spraying with DM-WG, there was an apparent, but presumably spurious, three-fold increase in *An. funestus* densities while LC-CS, PM-EC and PM-CS achieved 5, 3 and 71-fold reductions, respectively. However, from the seventh to twelfth months after spraying, DM-WG and PM-EC had no obvious effect on the *An. funestus* densities while insufficient data was available to examine the incremental impact of LC-CS or PM-CS. When applied at this pilot scale, this CB mosquito-trapping scheme provided entomological evidence that complements epidemiological monitoring data to demonstrate how supplementing LLINs with IRS can reduce malaria transmission beyond levels achieved with LLINs alone in this setting where physiological resistance to pyrethroids occurs, especially when a non-pyrethroid organophosphate insecticide is used.

Overall, it appears that CB trapping schemes are affordable, cost-effective, and epidemiologically relevant. It also appears, based on the evidence from this pilot scale

evaluation, that they may be applicable to routine programmatic monitoring of vector population dynamics on unprecedented national scales.

DECLARATION

None of the work presented in this thesis has been published or submitted here or any other university. Any other works done by others has been done by others has been duly acknowledged in text. Chapters 2, 3 after the introduction have been published in peer-reviewed journals and are therefore presented here with slight modifications were appropriate.

Chapter 1: Introduction and literature review

Chadwick Haadezu Sikaala (CHS) wrote the entire chapter while DR. Gerry F. Killeen (GFK) edited the text.

Chapter 2: Evaluation of alternative mosquito sampling methods for malaria vectors in Lowland South-East Zambia.

CHS, Aklilu Seyoum (AS) and GFK designed the study collected data analysed and drafted the manuscript. Tanya L Russell (TLR)): Assisted at the inception of the study through data entry and field design operations. Javan Chanda (JC) and Dingani Chinula (DC): Collected field data and morphologically identified all the mosquitoes in the field. John Miller (JM): Provided the study map and assisted in the data analysis. AS: Together with CHS and GFK designed the study and oversaw the field implementation of the study and contributed to the formulation of the hypothesis and drafting the manuscript.

All the authors read and approved the final chapter.

Chapter 3: A cost-effective, community-based mosquito trapping scheme that captures spatial and temporal heterogeneities of malaria transmission in rural Zambia.

GFK, AS, and CHS conceived and planned the study. CHS, AS, DC, JC, Busiku Hamainza (BH), and Mulakwa Kamuliwo (MK) supervised execution of the study. Neil F. Lobo (NFL), Mulenga Mwenda (MM) and Isabel Mukali (IM) conducted the laboratory analyses. CHS analyzed the data and drafted the manuscript in consultation with GFK. All authors contributed to editing of the manuscript. All authors read and approved the final manuscript.

Chapter 4: Incremental impact upon malaria transmission of supplementing pyrethroid-impregnated long-lasting insecticidal nets with indoor residual spraying using pyrethroids or the organophosphate Pirimiphosmethyl.

BH, AS, CHS, HM, MK and GFK: Conceived, designed and supervised all field activities of the study. BH, CHS and GFK: Developed the data analysis plan. BH, CHS and GFK: Drafted the manuscript in consultation with the other authors, all of whom reviewed it and provided comments. All authors read and approved the final version of the manuscript.

Chapter 5: General discussions and conclusions.

CHS drafted the section and GFK gave an overview comment



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LIST OF ABBREVIATIONS

AL	Artemether Lumefantrine
BMGF	Bill & Melinda Gates Foundation
<i>Bti</i>	<i>Bacillus var. thuringiensis israelensis</i>
CB	Community-Based
CDC-LT	Centre for Disease and Control miniature Light Trap
CHW	Community Health Worker
CS	Capsule Suspension
CSP	Circum-sporozoite Protein
DDT	Dichloro-Diphenyl-Trichloroethane
DM-WG	Deltamethrin Wettable Granule
EBA	Erythrocyte Binding Antigen
EC	Emulsifiable Concentrate
EIR	Entomologic Inoculation Rate
EM	Environmental Management
ELISA	Enzyme Linked Immuno-Sorbent Assay
GFATM	Global Fund to fight AIDS Tuberculosis and Malaria
GMEP	Global Malaria Eradication Programme
GLMM	Generalized Linear Mixed Model
HBR	Human Biting Rate
HF	Health Facility
HLC	Human Landing Catch
HRP-2	Histidine Rich Protein-2 antigen
ITN	Insecticide Treated Net
IPE	Incremental Protective Effect
IRB	Institutional Review Board
IRS	Indoor Residual Spraying
ITT	Ifakara Tent Trap
ITT-B	Ifakara Tent Trap design B
ITT-C	Ifakara Tent Trap design C
<i>Kdr</i>	Knock down resistance
LC-CS	Lambdacyhalothrin CS
LLIN	Long Lasting Insecticidal Net
LSM	Larval Source Management
LT	Light Trap
MCP	Merozoite Cap Protein
MRL	Malaria Research Laboratory
MSP	Merozoite Surface Protein
MOH	Ministry of Health
NA	Not Applicable
NE	Not Estimable
NMCC	National Malaria Control Centre
NMCP	National Malaria Control Programme

NMSP	National Malaria Strategic Plan
PCR	Polymerase Chain Reaction
PM-CS	Pirimiphosmethyl CS
PM-EC	Pirimiphosmethyl EC
PMI	Presidential Malaria Initiative
PSC	Pyrethrum Spray Catch
QA	Quality Assurance
RDT	Rapid Diagnostic Test
RB	Resting Box
RR	Relative Rate
SPSS	Statistical Package for Social Science (Software programme for statistical analysis)
TRAP	Thrombospondin-Related Adhesive Protein
US \$	United States Dollar
WG	Wettable Granule
WET	Window Exit Trap
WHA	World Health Assembly
WB	World Bank
WHO	World Health Organization
ZMW	Zambia Kwacha

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.0 A brief description of the history of malaria disease and etiology

Malaria is an ancient disease with its probable origins in ancient Africa, from where it spread across the world during the human migration out of Africa (Bruce-Chwatt 1965; Carter and Mendis 2002; Cox 2010). The spread of *Plasmodium falciparum* malaria parasites through growing human populations has been associated with the expansion of agriculture, which transformed landscapes and created more conducive environs for breeding of *Anopheles* mosquitoes, which are now known to be the vectors of transmission (Senior 2001). Historical evidence of malaria-related fevers and symptoms were recorded in ancient scripts from Egypt, China, India and other parts of the Asia (Bruce-Chwatt 1965; Bruce-Chwatt 1985; Carter and Mendis 2002). However, due to inadequate medical understanding at the time, the cause of periodic fevers remained subject to speculation, often immersed in cultural and religious beliefs. These assertions were first challenged by Hippocrates, a Greek physician in the fifth century BC, who observed that patients residing near marshy areas often acquired fevers and enlarged spleens at particular times of the year (Bruce-Chwatt 1965; Bruce-Chwatt 1985; Carter and Mendis 2002; Cox 2010) and in England this phenomenon was referred to as *Ague* (Hutchinson and Lindsay 2006). For many centuries, texts referring to malaria fevers as consequences of bad air or *miasmas* - as it was referred to - arising from swamps were recorded in Greece, Italy, England and other parts of Europe (Hackett 1937). In Italy, the bad smell emanating from the swampy places was strongly linked to fevers and therefore referred

to as *mal'aria* meaning bad air, from which the name malaria finds its origin. Based on this association, Italians and Greeks pioneered vector control activities as early as the sixth century B.C, through the practice of improved land irrigation and draining water bodies to improve both agricultural food production and public health. Later on, other parts of Europe such as in France, Holland and England adopted these methods for public health purposes (Warrell and Gilles 2002). Furthermore, discovery of the therapeutic effects of the 'Peruvian bark' for treating malaria fevers in the seventeenth century marked the advent of antimalarial drug treatment, often called the Jesuit's powder by Europeans, because of the Roman Catholic missionaries in South America who first brought this indigenous knowledge to their attention (Sant 2014). Subsequently, in the eighteenth and nineteenth centuries, the tree was called *Cinchona ledgerianna* and quinine was isolated and identified as the main active ingredient respectively (Bruce-Chwatt 1985; Carter and Mendis 2002; Warrell and Gilles 2002).

However, the parasite that actually causes malaria fevers was not discovered until in the nineteenth century. This was precipitated by the work of microbiologist Pasteur and Koch when they demonstrated the bacterial causes for many diseases (Cox 2010). Following this discovery, malaria parasites were discovered in the red blood cells of individuals by the French surgeon Laveran whilst working in Algeria (Bruce-Chwatt 1985; Cox 2010). Even with this discovery, the mode of transmission of these parasites from one person to another remained unknown. However, Patrick Manson's 1878 discovery in China that mosquitoes were the invertebrate hosts of the filarial worms which also infect humans, and David Bruce's later demonstration

that African Tsetse flies transmit trypanosome parasites between animals, stimulated speculations that malaria was also transmissible by insects. Later in 1897, Ronald Ross showed that mosquitoes do transmit avian malaria (Cox 2010). This was a ground-breaking discovery in the quest to fully understanding the cycle of malaria infection. Further works by Battista Grassi and other scientists in Italy provided evidence which suggested malaria parasites were present in both human (vertebrate) and mosquitoes (invertebrate) hosts. Field experiments conducted by Patrick Manson and colleagues in Italy and England then provided evidence that suggested that it is mosquitoes from the specific genus *Anopheles* that transmit human malaria parasites and advocated for avoiding mosquito bites as a means to prevent malaria infection (Bruce-Chwatt 1985).

Over the years, there has been increasing interest and investigation into whether malaria parasites originated from the invertebrate or vertebrate hosts, and whether human malarias originated from humans or from other primates (Krief, Escalante et al. 2010). Bruce-Chwatt (1965) argued that fossil evidence suggests that mosquitoes are unlikely to be the hosts in which the human malaria parasites assumed their origins. Since malaria parasites have similar developmental stages to the intestinal Coccidiida, it was thought that the Haemosporidia evolved from the Coccidia and later adapted to living in the blood stream. This consequently led to the acquisition of a blood-seeking second host (Bruce-Chwatt 1965). The human malaria parasites, all of which are classified under the genus *Plasmodium*, are among hundreds of other species that transmit malaria to other mammals, birds and reptiles classified under the Phylum

Protozoa, Subphylum Sporozoa, Class Hemosporidiid (Cook and Zumla 2009). *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* are the commonly-known malaria parasites of humans across the globe but recent reports have implicated *Plasmodium knowlesi*, a known parasite of long-tailed and pig-tailed macaque monkeys in parts of Asia, causes significant morbidity and mortality among humans but this is a quite local phenomenon which has not been documented in Africa (Cox-Singh, Davis et al. 2008; Baird 2009; Cook and Zumla 2009). Of all these species, *P. falciparum* is the most virulent and is associated with high mortalities and morbidity across the tropics and subtropics, wherever conditions for its sporogonic development within mosquito vectors are conducive.

1.1.1 The parasite life cycle

The life cycles of the four common human malaria parasites exhibit many of the same basic biological processes and interactions between the vertebrate host and the female mosquito vector. *Plasmodium falciparum*, like all the other parasites causing human malaria, undergoes its development in phases that involve asexual and sexual stages, referred to as schizogony and sporogony, respectively. The asexual schizogony phase occurs in the vertebrate hosts' erythrocytes (red blood cells) producing schizonts whilst sporogony is a process of sexual reproduction of sporozoites in the female *Anopheles* mosquito. Recent literature (Bogitsh, Carter et al. 2005) suggests further separation than previously suggested (Warrell and Gilles 2002), to delineate the sporogony process further so that the sexual process of male and female gamete fusion is treated as a distinct intermediate phase known as gamogony. The

whole process is initiated by a blood-thirsty female *Anopheles* mosquito requiring a blood meal for its egg development requirements as a pre-requisite for oviposition (Beier 1998). The male mosquitos' mouth parts are incapable of penetrating through the skin of humans or animals to get a blood meal and they depend solely on nectar and juices extracted from plant material.

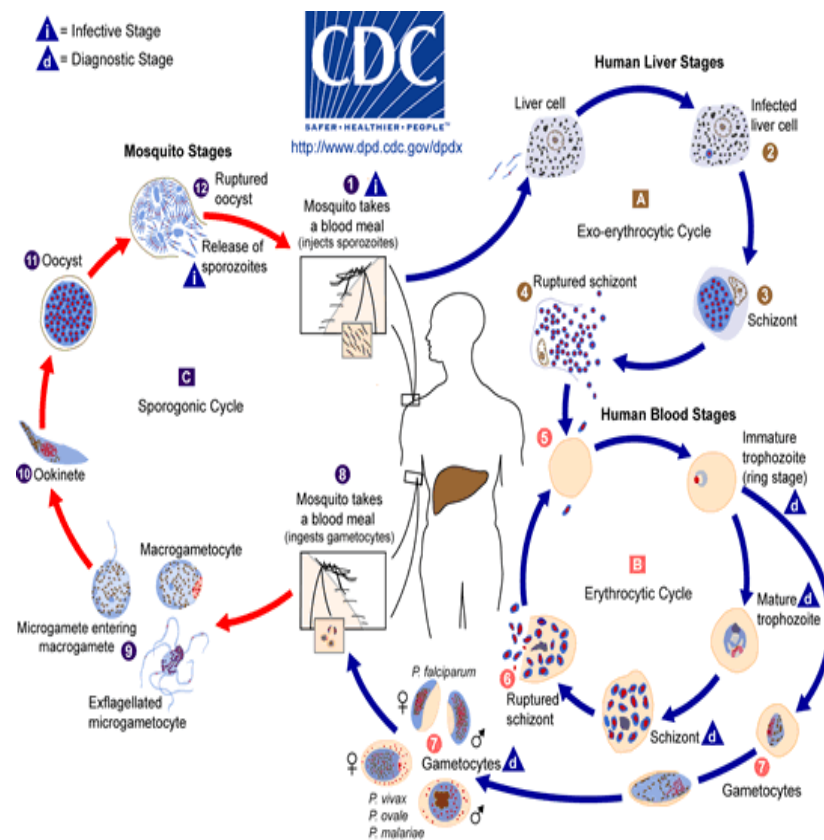


Figure 1.1: Diagrammatic presentation of life cycle of human malaria as described by Centre for Diseases Control (www.dpd.cdc.gov/dpdx).

1.1.2 Schizogony in the vertebrate host and the associated pathology

Infection of the vertebrate host occurs when an infected female *Anopheles* mosquito bites and uses its proboscis to pierce the epidermal cells of the skin and reach the blood vessels where it injects sporozoite-bearing saliva (Beier 1998; Warrell and Gilles 2002) (Figure 1.1). The release of both the anti-coagulant and the elongate infectious sporozoite-stage parasites from its salivary glands facilitates transmission to humans. These sporozoites are then circulated within the blood stream towards the liver within an hour. The attraction of these parasite stages to hepatocytes cells within the liver is enabled by the presence of receptors that are recognized by the sporozoite surface coating of the parasite. The proteins that facilitate this process on the apical surface of the sporozoite are the circumsporozoite protein (CSP) and the thrombospondin-related adhesive proteins (TRAP) (Silvie, Franetich et al. 2004), also sometimes referred to as sporozoite surface protein 2 (Florens, Washburn et al. 2002). These proteins are secreted through the rhoptry and microneme organelles of the sporozoites to facilitate formation, within 24 to 48 hours, of the parasitophorous vacuole inside the hepatocytes which envelops the sporozoites during the invasion process. Within the hepatocyte cells, the sporozoites undergo pre-erythrocytic schizogony, otherwise known as exo-erythrocytic schizogony. These hepatic schizonts develop from immature into mature stages by feeding on the cytoplasmic nutrients of the host cells and through pinocytosis of food extracted outside its confinement before becoming trophozoites. After 6 to 15 days they divide repeatedly through fission, so that numerous (10,000 to 30,000) merozoites are formed which are then released into the blood stream. Note, however, that in the *P. vivax* and *P. ovale* species, some of the schizonts develop into hypnozoites (hepatic schizonts) that stay dormant in the liver for weeks,

months or years until they are re-activated to undergo normal exo-erythrocytic schizogony and release merozoites in the blood stream (Carter and Mendis 2002).

Through their apical ends, merozoites release a complex of proteins that aid in penetrating the red blood cells (Bruce-Chwatt 1985; Blackman, Fujioka et al. 1998; Florens, Washburn et al. 2002; Murhandarwati, Wang et al. 2010). The commonly identified proteins aiding this process are the merozoite cap proteins (MCP), merozoite surface proteins (MSP), both of which are strongly associated with host immune evasion, and the erythrocyte binding antigens (EBA) (Florens, Washburn et al. 2002). The roles of these proteins are believed to be independent of each other (Woehlbier, Epp et al. 2010). Interestingly, individuals lacking Duffy antigens (Fy^{a+} or Fy^{b+}) are resistant to *P. vivax* because merozoite penetration is restricted by the absence of these membrane receptors (Bogitsh, Carter et al. 2005). Within the red blood cells remarkable changes occur after penetration; the merozoites lose two of their outer membrane coatings and progressively develop into ring form trophozoites which digest the haemoglobin, leaving haemozoin as a by-product of the process. These trophozoites have distinguishable species-specific features that allow them to be identified microscopically. *P. falciparum* trophozoites are smaller than the other species because they are characterized with an outer large ring and an inner vacuole containing two chromatin materials. The trophozoites then undergo fission, developing asexually through a process of erythrocytic shizogony to form schizonts which fill the inside of the red blood cells they have grown within. The matured schizonts form several merozoites following which the red blood cells rupture, releasing them into the blood stream to

begin the process of invading fresh new red cells. This process of erythrocytic shizogony occurs repeatedly in a cyclical manner, leading to an exponential growth of the parasitaemia load in the blood circulation. While other species invade only mature or older erythrocytes, *P. falciparum* is non-selective, thereby potentially invading more erythrocytes of all age groups so that it can cause the greater, more dangerous parasitaemia loads that underlay its higher virulence (Bruce-Chwatt 1985).

When the parasitized erythrocytes ruptures, they release cellular debris and by-products of haemoglobin digestion (hemozoin) into the host blood stream, thereby bringing about the symptoms of clinical illness associated with the asexual phase of the parasite in the vertebrate hosts, which include fever, shivering, nausea, vomiting, mild diarrhoea, headaches, joint pains and abnormal temperature variations. Severity of these conditions is variable, depending on the species of malaria. However *P. falciparum* is associated with higher severity of these conditions than any other malaria species because of its ability to invade both young and older erythrocytes. When *P. falciparum* infections are not early treated enough they may result in complications such as jaundice, convulsions and splenomegaly. For *P. falciparum*, the duration of the erythrocytic cycle of schizogony tends to range between 36 to 48 hours following invasion of the red blood cells and this schizogony cycle typically takes 48, 72 and 50 hours for *P. vivax*, *P. malariae* and *P. ovale*, respectively (Bogitsh, Carter et al. 2005). Because the timing of this cycle occurs at varying instances during early infection, febrile symptoms do not occur periodically in the first few days after blood-stage infection. Only after the timing schizogony by

all asexual stages in the blood stream becomes systematically synchronized do the symptoms of infection become more defined and pronounced, with peaks of clinical illness occurring with an obvious periodicity. Some of the merozoites differentiate into granulated sexual gametocyte forms: either a female form referred to as macrogametocyte containing heavily stained cytoplasm with a compact nucleus or a diffusely nucleated male form called a microgametocyte with pale-staining cytoplasm. The density of gametocyte carriage within the peripheral blood circulation is dependent on their production from the asexual parasites (Bousema and Drakeley 2011). When these sexual forms are ingested by a blood-feeding female *Anopheles* mosquito, the gamogony and sporogony processes commence (Figure 1.1).

1.1.3 Sporogony in the invertebrate mosquito host

The sexual phase commences with the ingestion by a mosquito of a blood meal that contains both the microgametocytes and the macro-gametocytes. After the mature gametocytes are swallowed and enter the mosquito stomach, the cell membranes of the erythrocytes are digested and release the male and female gametocytes into the stomach lumen. The nucleus of the male gametocyte undergoes mitotic multiplications, producing 4 to 8 nuclei which then further differentiate into individual flagellated thread-like forms, called microgametes, through exflagellation (Warrell and Gilles 2002; Bogitsh, Carter et al. 2005). Simultaneously, the female gametocytes undergo maturation to form macrogametes which assumes a form suitable for fertilization by developing an outer membrane coat by which the microgamete adheres to. Synogamy then occurs once the microgamete migrates, fuses with, and fertilizes the macrogamete to form a diploid zygote. Within 24 hours, the zygote develops into a motile

elongate vermicule known as ookinete, which is surrounded by a double pellicle wall and is conically shaped at its interior side (Warrell and Gilles 2002).

The ookinete moves to the interior stomach wall and penetrates the epithelium by secreting a proteolytic substance, and then establishes itself between the basal and epithelial cells of the mid-gut as a round structure called an oocyst. The initiation of oocyst development takes approximately 40hrs from the time a mosquito ingests the gametocytes. The cytoplasm within the oocyst then develops into multiple interconnecting lobes from the proliferation of haploid cells called sporoblast. The sporoblast enclose elongate, motile sporozoites inside a membrane which ruptures to release them into the cavity of oocyst. The motile sporozoites then burst the elastic cytoplasmic wall of the oocyst, enter the body cavity of the mosquito, and then migrate towards and invade the salivary glands, at which point the mosquito becomes infectious to subsequently-bitten vertebrate hosts.

1.2.0 Malaria distribution and epidemiology

The most recent World Malaria Report estimates 3.4 billion people to be at risk of exposure to malaria infection globally, with approximately 219 million cases annually, of whom 660,000 die (WHO 2013). About 86% of this estimated burden is in sub-Saharan Africa with most of the remainder (9%) occurring in south East Asia while the eastern Mediterranean and Americas contribute only 3% and 2%, respectively. An estimated 8.8 million children under the age of five

die from infectious diseases annually worldwide with 4.2 million of these cases reportedly coming from Africa (You *et al.*, 2010), of which close to 16% of these are attributable to malaria originating from sub-Saharan Africa (Black *et al.*, 2010).

Children under the age of five are the most affected in settings where malaria transmission is stable and endemic because they have not yet acquired immunity (Rowe, Rowe *et al.* 2006). Pregnant women, the immune-compromised, refugees and individuals visiting malarious areas from non-endemic countries are also at higher risk because they lack or have diminished acquired immunity (Warrell and Gilles 2002; Doolan, Dobano *et al.* 2009). Immunity to malaria infections is acquired as individuals are repeatedly exposed to infectious bites, so immunity increases with age (Marsh and Snow 1997; Snow, Omumbo *et al.* 1997). The older members of populations with regular exposure to malaria suffer less severe clinical symptoms due to acquired immunity and are characterized by reduced infection densities and incidence of clinical malaria but high rates of infection, many of which may be chronic or even cryptic (Snow, Omumbo *et al.* 1997; Mwangi, Ross *et al.* 2005; Smith, Ross *et al.* 2006; Bejon, Williams *et al.* 2010). Conversely, areas of low to moderate endemicity, as well as those which are epidemic-prone, where most members of the population will have had little if any prior exposure to infection, have relatively higher parasite densities circulating in the blood of those who do become infected and therefore higher detectability of infection. These populations have little or no acquired immunity and are therefore exposed to high risk of severe malaria symptoms associated with these higher parasitemias (Mouchet 1998; Bejon, Williams *et al.* 2010).

P. falciparum is the most commonly transmitted malaria parasite species in Africa south of the Sahara, south-east Asia and the western Pacific regions affecting about 2.4 billion people and therefore contributing the bulk of the global malaria burden (WHO 2013). *P. ovale* is also common in Africa and west Pacific; *P. malariae* is widely distributed at relatively low prevalence across Africa but relatively rare outside it. *P. vivax* is common all across the tropics and occurs alongside similar levels of *P. falciparum* across parts of Latin America, south-east Asia and Oceania but is rare in sub-Saharan Africa (Carter and Mendis 2002; Guerra, Howes et al. 2010). The Duffy-negative allele that occurs among most individuals of African descent renders them refractory to *P. vivax* infection by inhibiting this parasite from infecting erythrocytes. Its frequency is high in much of sub-Saharan Africa so the contribution of *P. vivax* to the malaria burden in the region is negligible compared to that of *P. falciparum*. However, elsewhere in the tropics *P. vivax* contributes in an important way to malaria morbidity and mortality (Nosten, McGready et al. 1999; Price, Tjitra et al. 2007; Guerra, Howes et al. 2010; Gething, Elyazar et al. 2012; Baird 2013) but the effect is much more pronounced where it occurs in combination with *P. falciparum* (Feachem, Phillips et al. 2010; Gething, Elyazar et al. 2012) (Figure 1.2).

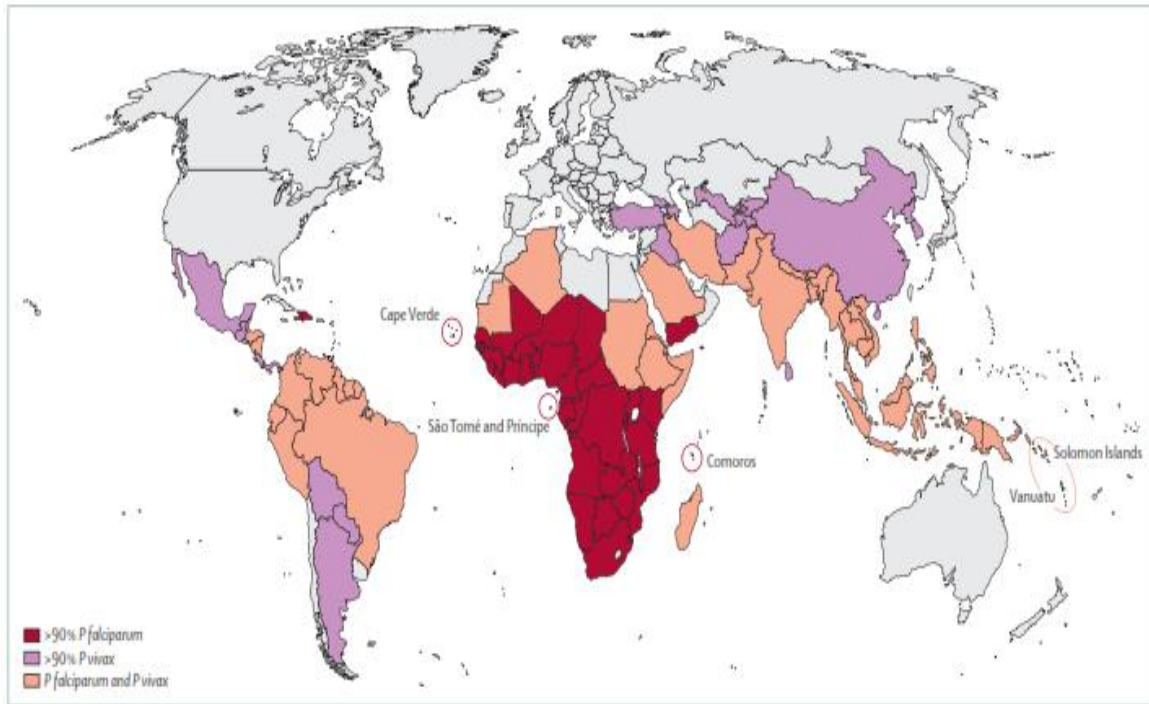


Figure 1.2: Distribution of *Plasmodium falciparum*, *plasmodium vivax* or both species (Feachem, Phillips et al. 2010).

1.2.1 Afro tropical vector distribution and their bionomics

In addition to the frequency of Duffy-negative alleles, malaria distribution is also strongly influenced by the climatic conditions, with warmth and humidity favouring survival and propagation of both vector mosquitoes and sporogonic-stage parasites. Climate is therefore most conducive to malaria transmission in the tropics and sub-tropics where transmission intensity is further compounded by the presence of very effective vectors that prefer to feed on human blood (Small, Goetz et al. 2003; Hay, Guerra et al. 2004; Kiszewski, Mellinger et al. 2004; Sinka, Bangs et al. 2012; Killeen, Seyoum et al. 2014). Across the globe, a total of 460 species of *Anopheles* are known, of which 70 are considered to regularly transmit malaria parasites (Sinka,

Bangs et al. 2012). In Africa, over 140 *Anopheles* species exists, of which 8 are considered to be effective primary vectors (Gillies and Demeillon 1968; Gillies and Coetzee 1987) (Figure 1.3). A small subset of species from the *Anopheles gambiae* complex and *Anopheles funestus* group are by far the most important primary vectors of malaria transmission all across sub-Saharan Africa (Gillies and Coetzee 1987). *An. gambiae* is a complex of seven sibling species which are morphologically indistinguishable but have quite different ecologic niches and behavioural traits, which also directly influence their vectorial capacities (Gillies and Demeillon 1968; Gillies and Coetzee 1987; Hunt, Coetzee et al. 1998). *Anopheles gambiae* sensu stricto Giles is highly anthropophilic (prefers to feed on humans), rests indoors and breeds profusely in a wide variety of fresh water habitats so it is a widespread and efficient vector. Although its endophilic (indoor-feeding) and endophilic (indoor-resting) characteristics contribute to its efficiency as a vector, this also makes it vulnerable to control because targeting these behaviours is the fundamental principle on which indoor application of insecticide-based interventions like indoor residual spraying (IRS) and long lasting insecticidal nets (LLINs) are based. Both *Anopheles arabiensis* Patton and *Anopheles quadriannulatus* Theobald Dönitz are indistinguishable from *An. gambiae* s.s. in their breeding habitat characteristics but these are both more inclined to bite humans both outdoors and are also highly zoophilic (prefer feeding on other animal hosts). Unlike *An. gambiae* s.s., *An. arabiensis* has adapted to survive in drier semi-arid, arid and desert (Gillies and Coetzee 1987; Coetzee, Craig et al. 2000; Sinka, Bangs et al. 2010). While both *An. quadriannulatus* species A and B commonly live in sympatric with *An. gambiae* and *An. arabiensis*, they have not been incriminated as vectors of any disease of medical interest (Coluzzi 1984). The other three members of the *An. gambiae* complex breed in

salt water and distribution is much more restricted. *Anopheles bwambae* is found in Bwamba area of Uganda alone where it breeds in warm natural spring water and, though it is not the main vector, it is known to contribute to malaria transmission in that locality. *Anopheles merus* breeds in the coastal regions of east Africa and *Anopheles melas* is mainly found on the west coast of Africa. Both these species contribute to local malaria transmission although usually less than *An. gambiae* or *An. arabiensis* (Temu, Minjas et al. 1998; Pock Tsy, Duchemin et al. 2003; Overgaard, Reddy et al. 2012; Kipyab, Khaemba et al. 2013).

An. funestus is divided into two subgroups; the funestus subgroup comprising *Anopheles aruni*, *Anopheles confusus*, *Anopheles parensis*, *Anopheles vaneedeni* and *Anopheles funestus* sensu stricto, whilst the *rivulorum* subgroup consists of *Anopheles brucei*, *Anopheles fuscivenosis* and *Anopheles rivulorum*. *An. funestus* breeds in permanent or semi-permanent fresh water bodies with emergent vegetation. *Anopheles confusus*, *An. rivulorum*, *An. brucei*, *An. fuscivenosis* and *An. lesoni* are morphologically distinguishable and can be identified at the egg and larval stages whilst *An. aruni*, *An. funestus* s.s, *An. parensis* and *An. vaneedeni* are morphologically indistinguishable from each other at any stage from egg to adult. *An. funestus* Giles is by far the most efficient malaria vector among the nine member group because it is highly anthropophilic and endophilic (Gillies and Coetzee 1987), which fortunately also makes it highly susceptible to adult vector control measures such as IRS and LLINs (Durnez and Coosemans 2013; Govella, Chaki et al. 2013; Killeen, Seyoum et al. 2013).

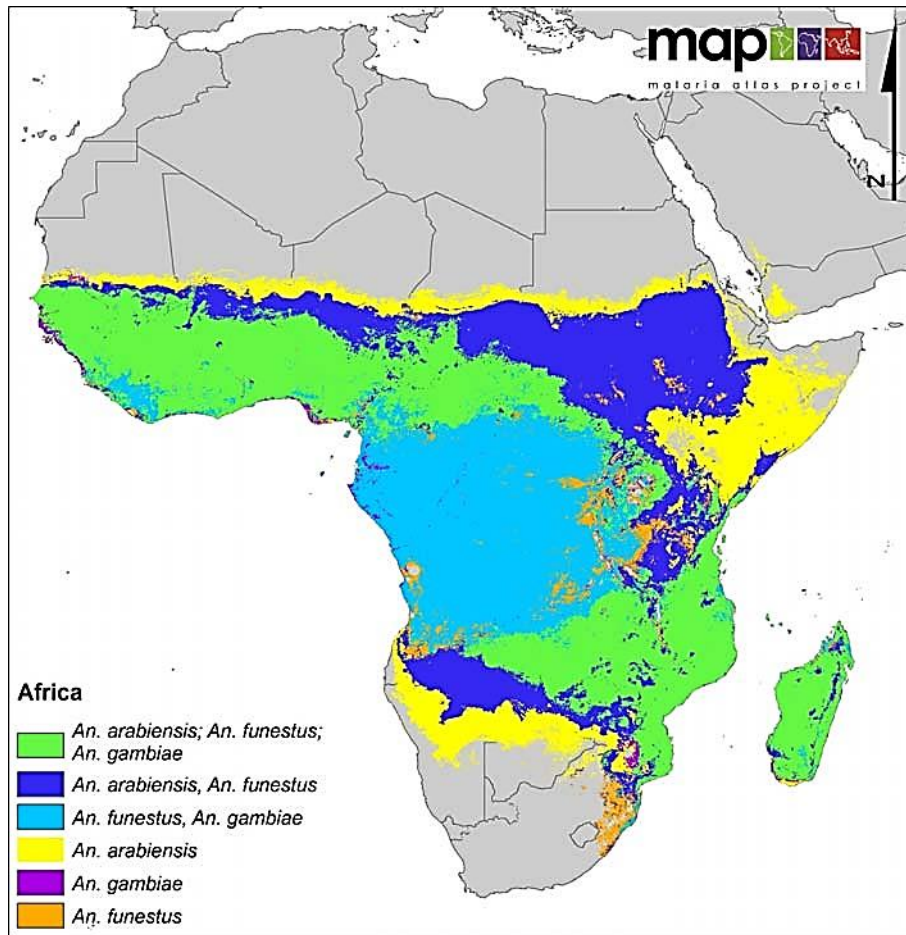


Figure 1.3: Map of major malaria vectors in sub-Sahara Africa (Sinka, Bangs et al. 2012)

1.2.2 Parasitological measures of malaria transmission

Different methods have been used to measure or classify malaria endemicity, with varying degree of success. In the nineteenth century, measurement of splenomegaly as the proportion of children between the ages of 2 and 9 with enlarged spleens, was mostly applied in India to detect malaria (De, Chandra et al. 1990). This crude method was improved by grading it on a scale of 0 to 5, referred to as Hackett's method. This method is rarely used nowadays, mainly

because a left rib or a faecal bolus in the large intestines can be mistaken for an enlarged spleens by a non-specialist, and splenomegaly may also be caused by leishmaniasis or Manson's schistosomiasis (Bruce-Chwatt 1985). Aside from these factors, this method is also unable to determine the malaria species involved in transmission. In spite of these limitations, it is still advocated for use in remote areas where infrastructure and resources are inadequate to support microscopy or any of the more recently developed detection tools (Shukla, Singh et al. 2011). Even though spleen rates correlated poorly with infant parasite rates by microscopy in some settings (De, Chandra et al. 1990), the former crudely reflects long-term levels of endemicity, whilst the latter acts as a proxy for recent transmission. Microscopy is considered the gold standard for diagnosing malaria infection rates (WHO 1988), particularly in endemic areas where parasite prevalence is stable, and has been used extensively to identify both the blood stages and the *Plasmodium* parasite species present in infected human blood. Microscopy conducted by preparing a thick smear for quantitative analysis and a thin smear for qualitative evaluation, by staining blood prepared on a slide is more specific but less sensitivity when compared to other diagnostic tools developed recently (Anonymous 2011). In health facilities (HF), it is the main diagnostic method that World Health Organization (WHO) advocates for (WHO 1988). However, because adequately trained personnel, quality assurance systems and infrastructure are required (Payne 1988; Speybroeck, Praet et al. 2011), the effectiveness of its practical application in African countries with weak health systems has been a subject of debate for decades. While other alternative diagnostic methods which use serological or nucleic acid markers have been evaluated, usually in comparison with the microscopy gold standard (Craig and Sharp 1997; Corran, Coleman et al. 2007; Baliraine, Afrane

et al. 2009; Bousema, Youssef et al. 2010; Hsiang, Hwang et al. 2012; Kobayashi, Chishimba et al. 2012), their routine applications in most rural African settings may be impractical due to inadequately infrastructure, trained specialized personnel and quality assurance systems (Hailegiorgis, Girma et al. 2010).

Since the launch of the Roll back Malaria (RBM) in 1998, new diagnostic methods with which to estimate malaria infection burden and measure the impacts of interventions have been advocated for (Attaran 2005) and subsequently developed successfully (WHO 2010). The rapid diagnostic test-kits (RDTs) are now widely used to survey malaria infection rates all across sub-Saharan Africa (Hamer, Ndhlovu et al. 2007; Abeku, Kristan et al. 2008; Chanda, Castillo-Riquelme et al. 2009; WHO 2010; Chanda, Hamainza et al. 2011; Bruxvoort, Kalolella et al. 2013). RDTs are widely used in settings with no microscopy, particularly because they yield results in a much shorter time (Moody 2002; Abeku, Kristan et al. 2008; Bruxvoort, Kalolella et al. 2013), are easy to use and require much less rigorous training than microscopy, so they can be applied at grass-roots level by non-specialist, community-based staff (Wongsrichanalai, Barcus et al. 2007; Counihan, Harvey et al. 2012; Zhao, Lama et al. 2012). Even though RDTs have proven highly sensitivity and specific in some settings, their reported performance has been somewhat inconsistent, partly because of variations in the study designs (Wongsrichanalai, Barcus et al. 2007) and also because their stability may be compromised under poor transport and storage conditions in many hot parts of tropical Africa (2011). Equally, RDTs specific for detecting HRP-2 antigen found in *P. falciparum* can give false positives caused

by the persistence of the antigenemia even weeks after effectively treatment with an anti-malaria drug, hence over-estimating the true prevalence (Bell, Wilson et al. 2005; Wongsrichanalai, Barcus et al. 2007; Brooker, Kolaczinski et al. 2009; Keating, Miller et al. 2009). However, despite these limitations, they are widely accepted as invaluable tools with which to conduct parasitological surveys and can estimate parasitological point prevalence with >90% precision (Batwala, Magnussen et al. 2010). Parasitological primary indicators of malaria transmission test for the presence of blood stages of the parasite within the human population while the entomological secondary indicators of transmission measure rates of human exposure to bites of mosquitoes infected with salivary gland sporozoites.

1.3.0 Entomological measurement of transmission

The longevity of the vector directly affects malaria transmission because the sporogonic cycle or development of the parasite within the mosquito needs to be complete whilst it is still surviving. Conducive temperature accelerates development of the parasites within the vector so the highest levels of transmission occur in the tropics (Bruce-Chwatt 1985). Across the sub-Saharan Africa there is great variability in transmission intensity (Beier, Killeen et al. 1999; Kelly-Hope and McKenzie 2009), best measured entomologically as the entomologic inoculation rate (EIR), which is the product of the mosquito biting-rates times the proportion of mosquitoes with sporozoites in their salivary glands (Macdonald 1957). The relationship between malaria prevalence and annual EIRs across varying epidemiological settings in Africa has clearly been demonstrated (Beier, Killeen et al. 1999; Smith, Dushoff et al. 2005) and how it impacts on

vector control and elimination of malaria parasites (Shaukat, Breman et al. 2010). Reviewed data from 31 sites across Africa shows that even with very low or non-detectable EIRs below one infectious bite per person per year, high prevalence rates of malaria in the human population could still be observed (Beier, Killeen et al. 1999).

1.3.1 Epidemiological significance of adult mosquito sampling methods

To assess the impact on vector population dynamics and malaria transmission by vector control interventions, a robust entomological sampling scheme is of paramount importance (WHO 1992). The selection of a sampling framework is heavily reliant on the question being addressed, which will typically determine the choice of a mosquito collecting tool (WHO 1992; Silver and Service 2008) and the cost associated with the sampling design. Since the discovery that malaria transmission is mediated by mosquitoes bites, at the beginning of the nineteenth century numerous methods of capturing mosquitoes have been developed with varying degrees of efficiencies (Service 1977; Service 1993). These collecting tools for measuring biting rate and EIR are broadly characterized into two categories; baited host-seeking traps which capture mosquitoes that are seeking a blood meal, or un-baited resting traps which typically capture mosquitoes when they rest before or after obtaining a blood meal.

1.3.2 Host seeking sampling methods

The human landing catch (HLC) has traditionally been considered the gold standard sampling method for estimating the human-vector interactions (Service 1977) and measuring malaria transmission intensity (Service 1993; Beier, Killeen et al. 1999; Mboera 2005; Kelly-Hope and McKenzie 2009) for evaluating of the impact of vector control programmes and, more importantly, the behaviours that underpin it (Huho, Briet et al. 2013). HLC directly measures EIR by estimating the frequency by which humans are exposed to the vectors or the human biting rate and the proportion of mosquitoes with sporozoites in their salivary glands, often called the sporozoite rate or sporozoite prevalence (Beier, Killeen et al. 1999). Even more importantly, because HLC is capable of monitoring the interactions between mosquito behaviours with human behaviours by sampling both indoors and outdoors, it reliably quantifies the distribution of human exposure to mosquito bites while they are indoors and outdoors (Seyoum, Sikaala et al. 2012; Huho, Briet et al. 2013) and thus aids in gathering vital information to programmatically select appropriate control measures (Pates and Curtis 2005; Geissbuhler, Chaki et al. 2007; Killeen, Seyoum et al. 2014).

HLC involves exposure of the lower parts of the limbs by the catcher so that mosquitoes get attracted and are captured using an aspirator as they attempt to get a blood meal. Because HLC involves exposure of individuals during capture of mosquitoes, there are ethical concerns that inevitably arise due to increased exposure to the risk of parasitic and arbovirus infections carried by these vectors (Service 1993). HLC is extremely labour-intensive because catchers are

required to remain alert to capture mosquitoes for a long time, usually for at least 12 hours in the African settings where the most virulent vectors are active throughout the hours of the night when most humans prefer to sleep. While the catchers acting as baits for the HLC vary in terms of their attractiveness to mosquitoes (Lindsay, Adiamah et al. 1993; Knols, de-Jong et al. 1995; Mukabana, Takken et al. 2002; Mukabana, Takken et al. 2004) and also possess different skills of mosquito collections (Kelly-Hope and McKenzie 2009), these sources of variance can be addressed with appropriate experimental design and statistical analysis. Nevertheless, several attempts to standardizing different alternative sampling methods by comparison with the HLC has yet to yield a consistent picture across different ecological zones in Africa (Kelly-Hope and McKenzie 2009).

However, in spite of these limitations, efforts have been made over the years to evaluate alternatively safer, cost effective and sensitive sampling methods. The most utilized or standardized sampling method for capture host seeking mosquitoes is the Centre for Disease miniature light trap (CDC-LT). This trap was typically used by hanging it close to a human bait (Odetoyinbo 1969) and later improved to an exposure-free trap by placing it near a bed net occupied by a sleeping human (Garret-Jones and Magayuka 1975). The CDC-LT has shown to be relatively successful in capturing different mosquito species across varying settings (Mbogo, Glass et al. 1993; Githeko, Service et al. 1994; Davies, Hall et al. 1995; Shiff, Minjas et al. 1995; Sithiprasasna, Jaichapor et al. 2004; Dia, Diallo et al. 2005; Okumu, Kotas et al. 2008; Dusfour, Carinci et al. 2010; Govella, Chaki et al. 2011; Duo-quan, Lin-hua et al. 2012) as a tool to

measure rates of human exposure to mosquito bites while indoors. For instance, while in Tanzania CDC-LTs were reliably able to estimate the HBRs (Lines, Wilkes et al. 1991; Davies, Hall et al. 1995), on the Bioko island this trap was not recommended for programmatic use as a surveillance tool to estimate biting rates of *An. gambiae* s.l. in this setting because; 1) different statistical analyses methods gave inconsistent results and, 2) the variations in collection methods could not reliably provide conversion of indoor and outdoor data mosquito collections (Overgaard, Saebo et al. 2012). These contrasting results suggest that light traps are imprecise in some settings and outcomes cannot always be reliably related to absolute human biting rates, as was also observed from recent trap evaluations in Tanzania and Kenya (Govella, Chaki et al. 2009; Wong, Bayoh et al. 2013). However, they may be reliably useful in estimating, spatial and temporal trends in vector densities to evaluate the impact of the intervention. To my knowledge, no comparable study has been reported with other vector trapping methods that suggests any of these alternatives are any more precise than light traps placed beside occupied bed nets.

In spite of the trap Because the trap depends on the battery to light the bulb and also propel insects in the holding chamber, its reliability for routine programmatic surveillance remains unproven in rural Africa, where electricity for recharging is often unavailable and access to procure essential spare parts, such as light bulbs poses challenges.

Modified bed nets to capture host-seeking mosquitoes have been developed and evaluated as alternatives to HLC in the past. The most common design combines two bed nets, with one intact inner net which keeps the human host exposure-free while the second outer net covering the entire inner one that either has many holes or is raised lightly to leave a small gap between its fringes and the floor as mosquito entry points. This design is not considered sensitive enough to warrant its application as a tool for the surveillance of host-seeking mosquito across different epidemiological set-up (Service 1977). Nonetheless, efforts to standardize exposure-free bed net trapping methods for mosquitoes have continued. For instance, the Mbita trap was developed in Kenya to capture host-seeking mosquitoes while protecting the bait (Mathenge, Killeen et al. 2002). It is conically shaped net with an upper funnel designed such that an inner aperture allows entry of mosquitoes in one direction so that they are unable to escape once captured (Mathenge, Killeen et al. 2002). When it was tried in the semi-field conditions in Kenya for sampling laboratory reared *An.gambiae* s.s., its sensitivity was close to half the capture rate of HLC (Mathenge, Killeen et al. 2002). Under controlled field trials in western Kenya, the trap was comparable to HLC in sampling *An. gambiae* s.l. and *An funestus* (Mathenge, Omweri et al. 2004; Mathenge, Misiyani et al. 2005), but performed poorly under different conditions elsewhere in the highlands of Madagascar and in rural and urban Tanzania (Laganier, Randimby et al. 2003) in sampling the local vectors that mediate transmission. Under natural environment in western Kenya, the numbers of mosquitoes captured for both *An. gambiae* s.l. and *An. funestus* were proportional to HLC for all methods regardless of the mosquito densities (Mathenge, Omweri et al. 2004) while again elsewhere in western Kenya

the trap performed less sensitive when evaluated against HLC (Mathenge et al. 2005, Filingir et al. 2008, Okumu et al. 2008).

Recently, an Ifakara tent trap (ITT) was developed in Tanzania which consisted of a rectangular canvas box with six funnels tilted at an angle so that mosquitoes can enter through and get trapped inside a chamber. It consists of a netting partition between the mosquito collecting chambers situated above and another chamber below where the person acting bait sleeps (Govella, Chaki et al. 2009). When standardized against HLC in urban Tanzania, the trap performed relatively well in capturing both the endophagic and exophagic vectors but, because it also captured blood fed mosquitoes, the authors questioned whether the occupant was really fully protected against mosquito bites under conditions of normal practical application so they recommended some modifications of the trap. The redesigned versions that ensured no contact between the host and the vector was also evaluated and were shown to be apparently exposure-free and also more sensitive than the previous version (Govella, Moore et al. 2010). Even though, capture rates with these bed net traps may not equal HLC in absolute terms in many settings, they are advantageous because they do not require electricity, they are exposure free, and they require less labour to operate simply because the bait just lies down and can sleep all through the time of collection.

1.3.3 Methods for capturing mosquitoes while resting and exiting

Understanding preferential resting behaviour of mosquitoes is paramount in the selection of control measures. Fundamentally, endophilic vectors are vulnerable to the intra-domiciliary interventions which can thus suppress their populations by reducing their longevity and densities. On the other hand, mosquitoes that are endophagic but exophilic exit houses after upon getting a blood meal indoors so they are far less vulnerable to intradomiciliary interventions like LLINs and IRS. Mosquitoes resting indoors are commonly sampled using a mouth aspirator with a torch to search and collect mosquitoes resting in the inside human dwellings (WHO 1992; Service 1993). This method has several limitations: 1) the mosquito collector can only cover a portion of the interior surfaces, especially in dwellings with unplastered walls such as those commonly found in rural Africa, 2) such a method relies on the skills and motivation of the collectors, therefore there is great individual variability in the catch of mosquitoes (Dias 1993; Harbison, Mathenge et al. 2006) and 3) generally it appears to be an inconvenience to household owners, particularly when they are requested to vacate their homes in the early morning hours when collections are done. This method has, over the years, been overtaken by the much more sensitive pyrethrum spray catch (PSC) which applies insecticidal aerosols to knock down mosquitoes resting indoors and collect them, upon falling, on an under-laid calico white sheet. This method however requires about half a month before another sampling can be done due to the persistence of the insecticides sprayed (Dias 1993; Chareonviriyaphap, Roberts et al. 1997). Furthermore, this method underestimates vector densities in houses with IRS or LLINs because these control measures may possess insecticides that can repel mosquitoes so that those which feed indoors usually exit and rest elsewhere

because of the excito-repellent effects of the insecticides (Chareonviriyaphap, Roberts et al. 1997; Kulkarni, Kweka et al. 2006; Killeen, Chitnis et al. 2011) while other mosquitoes naturally prefer to leave soon after taking a blood meal (Fornadel and Norris 2008). More recently, a CDC backpacker aspirator has been developed that operates similarly to the aspirator hand collection but with a battery operated suction tube, and one evaluation suggests that it is a much simple and quicker tool for sampling resting mosquitoes (Maia, Robinson et al. 2011) with similar sensitivity to the PSC.

A variety of methods have been used in the past to capture outdoor resting mosquitoes (Service 1977; Service 1993). The pit trap consists of a deeply dug hole in the grounds with pockets of smaller holes on its sides present conducive environments for mosquito resting (Service 1993), but this method is rarely used because it is immobile and may be hazardous to community members who may fall inside (Odiere, Bayoh et al. 2007). Resting boxes have been employed as alternatives to capture outdoor resting mosquitoes in a rice growing area in Tanzania (Kweka, Mwang'onde et al. 2009) but elsewhere in urban Tanzania their sensitivity was poor in comparison to other sampling methods (Sikulu, Govella et al. 2009; Govella, Chaki et al. 2011). Similarly, clay pots that work on the same principles as the resting boxes by providing a suitable micro-environment for the mosquitoes to rest in have shown to be effective in certain settings in Kenya for collecting outdoor resting mosquitoes (Odiere, Bayoh et al. 2007; Wong, Bayoh et al. 2013) but was less sensitive in collecting indoor resting mosquitoes in an arid area in the north of Tanzania (Van den Bijllaardt, ter Braak et al. 2009).

However, all these sampling methods have limited capture efficiency because resting mosquitoes are disturbed by the collection process, and because the surveyed surfaces represent a fraction of the resting potential places for mosquitoes such that a larger proportion rests elsewhere therefore representing a small and unknown fraction of the vector density populations (Killeen, Seyoum et al. 2014).

Window exit traps (WET) have been used in southern and west Africa to measure the impact of interventions by estimating the vector densities (Mouatcho, Hargreaves et al. 2007; Sharp, Ridl et al. 2007; Ridl, Bass et al. 2008; Chanda, Hemingway et al. 2011). For instance, on the island of Bioko in west Africa, *An. gambiae* s.l., and *An. funestus* densities and sporozoite infection rates were routinely monitored using WETs to evaluate the impact of a 2 year IRS programme using pyrethroids and carbamate-based IRS in the first and second year of spraying, respectively. Similarly, using WETS Chanda et al., (2011) monitored and evaluated the impact that IRS and LLINs programmes had on the densities, sporozoite rates and insecticide resistance profiles of *An. gambiae* s.l., and *An. funestus* in selected sentinel sites in Zambia, while Mouatcho and colleagues in South Africa equally used the same trapping methods to register the presence of pyrethroid resistance in *An. funestus* populations in Kwazulu Natal (Mouatcho, Hargreaves et al. 2007). The WET catches the fraction of mosquitoes that enter, bite and leave through the window to rest elsewhere (Mboera 2005; Pates and Curtis 2005; Fornadel and Norris 2008) or to go and oviposit in an aquatic larval habitat. However, this approach only works by leaving other spaces, such as the eaves or other windows in the same house, open so that mosquitoes

can enter in the first place. These open entry points can also be used by mosquitoes to exit without leaving through the window trap, so sensitivity is highly affected by the design of the houses (Govella, Chaki et al. 2011) and exiting mosquitoes are systematically under-sampled, consequently resulting in under-estimation of the vector density.

1.4.0 Historical perspective of malaria control

Traditional remedies for malaria illness date back over two million years. The Chinese used the plant *Artemisia annua* to treat fever for over 2000 years and use of extracts from the bark of the tree *Cinchona ledgerianna* has been documented since the 17th century. Quinine was not only isolated from *C. ledgerianna* and then synthesized as a drug in its own right, it was also used as a lead molecule to formulate chloroquine for routine malaria treatment. Similarly, *A. annua* was used to isolate and identify the synthetic artemisinins that have replaced chloroquine today (Bruce-Chwatt 1985). It is notable that these ancient therapies remain relevant today (Meshnick 1997).

1.4.1 Malaria control before Dichloro-Diphenyl-Trichloroethane (DDT)

Larval source management (LSM) that targets immature mosquitoes (larvae and pupae) before they emerged as adults were the main efforts implemented to control malaria-transmitting vectors in the early 20th century across many parts of the world including Africa (Utzinger, Tozan et al. 2001). LSM is classified as environmental or habitat manipulation, environmental or

habitat modification, application of chemical or biological larvicides and the application of biological control using natural predators of mosquito larvae. Historically, LSM was the primary vector control activity that contributed to the elimination of malaria transmission across large tracts of the Americas, the middle east, Europe and Africa (Watson 1953; Kitron 1987; Kitron and Spielman 1989; Utzinger, Tozan et al. 2001; Utzinger, Tozan et al. 2002). For instance in the south-eastern United States, a combination of environmental management (EM) and larviciding with oil was used largely to control *An. quadrimaculatus* which was the local vector mediating malaria transmission. Similarly during the construction of the Panama Canal, in Latin America, malaria and yellow fever were controlled by EM. In Brazil, *An. gambiae*, which was accidentally introduced from Africa in the mid-20th century was eliminated mainly by larviciding using Paris Green (Killeen, Fillinger et al. 2002) and the same larvicide was used to eliminate *An. gambiae* in Egypt a few years later in the middle of the Second World War. Similarly, LSM was crucial to the massive decline of malaria in the copper belt of Zambia from the 1930s onwards (Watson 1953). In recent years, reports have suggested that larval control may be effectively implemented in selected settings in Tanzania and Kenya (Fillinger and Lindsay 2006; Majambere, Lindsay et al. 2007; Geissbuhler, Kannady et al. 2009; Mwangangi, Kahindi et al. 2011) with more environmentally friendly biological larvicides, such as *Bacillus var. thuringiensis israelensis* (*Bti*) but not in other, more challenging settings such as the floodplain of the river Gambia (Majambere, Pinder et al. 2010). These historical and present reports of applying LSM tailored to local ecology suppress the vector population drastically even to the point of elimination because the cost fitness of the vectors is reduced when their habitats are limited and scarce; and located far from human habitations which provides their source of

blood (Killeen, Seyoum et al. 2004; Gu, Regens et al. 2006). Larviciding did not reduce malaria transmission in most rural tropical areas mainly due to the extensiveness and cryptic nature of the breeding sites preferred by the vectors (WHO 1982; Fillinger and Lindsay 2011; Tusting, Thwing et al. 2013).

1.4.2 House screening and urbanization

While LSM was the main activity conducted, construction and modification of houses to screen windows was also conducted on a large scale in America and this intervention was considered to be perhaps the most important contributor to elimination of malaria (Lindsay, Emerson et al. 2002; Lindsay, Jawara et al. 2003). Urbanization also reduces vector biting densities per person, thus limiting human-vector contact transmission of disease (Hay, Guerra et al. 2005; Tatem, Gething et al. 2013).

The majority of houses in rural Africa are constructed with mud and thatched roofing, usually with open eaves, through which human-seeking vectors can enter so that the occupants are more at risk of getting infected (Kirby, Green et al. 2008). Recent studies have reported that screening of mosquito entry points reduces exposure to infectious pathogens and subsequently disease prevalence in rural Africa (Lindsay, Emerson et al. 2002; Lindsay, Jawara et al. 2003; Gimnig and Slutsker 2009; Kirby, Ameh et al. 2009; Ogoma, Lweitoijera et al. 2010). Generally, construction of houses in the peri-urban and urban settings are characterized with improved

roofing made of either corrugated asbestos or iron sheets with minimal entry points for mosquitoes. However, in addition to screened houses, the improved economic status, reduction of potential breeding sites for the malaria transmitting mosquitoes, higher population density as well as access to health care, sanitation and other municipal services, are thought to be the major factors that contribute to the lower transmission of malaria in urban areas (Hay, Guerra et al. 2005; Tatem, Gething et al. 2013).

1.4.3 DDT and the Global Malaria Eradication Programme

The discovery of dichloro-diphenyl-trichloroethane (DDT) in 1939 and, later its application as a persistent residual insecticide for controlling adult mosquitoes resting indoors was perceived as a milestone for malaria vector control. Previously, pyrethroid extracts were used for small scale and domestic application purposes to control insect bites, but these were immediately superseded by DDT because of its superior residual potency, requiring far less frequent applications so that full scale up became economically viable (Bruce-Chwatt 1985; Najera, Gonzalez-Silva et al. 2011). Based on the understanding that spraying of interior walls of human dwellings would effectively shorten the longevity of indoor-resting vectors, and therefore disrupt the development of the parasite within the vectors, DDT-based IRS was accepted as the main vector control tool to be widely applied (Macdonald 1957; Garrett-Jones 1964; Smith, Battle et al. 2012). Indeed, DDT successfully combined with chloroquine therapy was successful in reducing malaria burden across many parts of Europe and Asia (Najera, Gonzalez-Silva et al. 2011). As a result of these successful demonstrations, the 8th World Health Assembly (WHA)

launched a Global Malaria Eradication Programme (GMEP) for all endemic countries, with low to moderate transmission, using DDT-based IRS with case management by administering the new cost-effective drug, chloroquine (WHO 2008). However, the sub-Saharan Africa was not included, apart from a few countries with marginal transmission levels where the application was restricted to focal areas of major economic importance (Zimbabwe, Ethiopia and South Africa) (Feachem and Sabot 2008; Mills, Lubell et al. 2008). Poor infrastructure and other resources in most impoverished Africa, and lack of trained malariologist, were cited as the major justifications used to exclude sub-Saharan Africa from participating in the GMEP (Atkinson, Vallely et al. 2011; Najera, Gonzalez-Silva et al. 2011). Eradication was defined as “the ending of the transmission of malaria and the elimination of the reservoir of infective cases in a campaign limited in time and carried out to such a degree of perfection that when it comes to an end, there is no resumption of transmission” (WHO 1957). This ambitious campaign partially achieved its objective such that by 1978, malaria was successfully eliminated from 37 out of the 143 countries that were previously endemic in Europe, the Americas and Asia (Mendis, Rietveld et al. 2009). Encouragingly, even countries that did not achieve elimination outside sub-Sahara Africa did experience drastically reduced the burden of the disease. For instance India was recording well above 100 million malaria cases by the mid-1950s but this figure drastically reduced to less than a million and remarkably no mortality was recorded after the ten years of the GMEP campaign (Najera, Gonzalez-Silva et al. 2011).

However, in spite of these efforts, the GMEP could not achieve its main objective, largely because these efforts underestimated the complexity and efficiency of the vectors mediating transmission in subtropics and the tropics, the collapse of political will exacerbate the situation coupled with inadequate funding for the campaign (Najera 2001; Najera, Gonzalez-Silva et al. 2011). Other factors were the inadequate clearing of the parasite reservoirs especially *P. vivax* and *P. ovale* species that have latent liver infections that causes relapses even after years of infection, the inability to provide site specific research to provide specific tailored control programmes (Alonso, Brown et al. 2011), the resistance of parasites to chloroquine (Payne 1987) and the inability to completely eliminate malaria in the tropics with varying transmission intensities. For instance, during the Garki project in northern Nigeria, where malaria transmission intensity was recorded as reaching 120 infectious bites per person per year in the 1970s, could not be reduced to the threshold ($<<1$ infectious bite per person per year) in order to eliminate transmission (Beier, Killeen et al. 1999) after several rounds of IRS with propoxur achieving over 95% coverage. One of the reasons attributed to this failure was the ability of the local vectors to feed and rest outdoors (Molineaux, Shidrawi et al. 1976). The GMEP later realized the need for local feasibility studies so that eradication should be implemented within the overall framework of the health sector development agenda of individual countries, rather than as a stand-alone vertical agenda (WHO 1968; 1974).

1.4.4 Post Global Malaria Eradication Programme

However, when the GMEP revised its strategic direction from malaria eradication to control, resurgences began to occur in some parts of the globe where malaria control efforts were not sustained. Malaria resurgence was described as “the return to a stable equilibrium after disturbances by malaria control efforts” by Najera et al., (1998) and later refined by Cohen and colleagues as “an increasing trend in malaria incidence or prevalence following suppression achieved through implementation of control efforts ” (Cohen, Smith et al. 2012). There were a number of reasons to which the malaria resurgence observed in some countries that had previously reported malaria free-zones could be attributed. Apart from dwindling resources, growing mosquito resistance to DDT, the increasing parasite resistance to chloroquine and lack of community-participation (Mendis, Rietveld et al. 2009; Najera, Gonzalez-Silva et al. 2011; Cohen, Smith et al. 2012), in some instances political and economic challenges (Feachem, Phillips et al. 2010) also contributed to the downward fall of the programmes. In Africa where this period coincided with transition to independence from one governance system to the next, there was inadequate knowledge transfer of health care service delivery skills and experience, so malaria resurged particularly aggressively in many parts of the continent (Cohen, Smith et al. 2012).

1.4.5 Roll Back Malaria and Long Lasting Insecticidal Nets

The global economic challenges that most African countries faced in the 1970s and 80s meant that insufficient support was provided for malaria control operations. Therefore, concerned

about the devastating effect of malaria on the economies of the poor nations of sub-Saharan Africa in particular, the WHO convened a ministerial conference in Amsterdam in 1992 where access to prompt diagnosis and treatment where the main focus for malaria control but renewed support for preventive measures were also emphasized (WHO 1993). Political will to reduce the burden of malaria, especially amongst the young children and pregnant women who are most susceptible to *P. falciparum* infections, was further strengthened by the endorsement of the African heads of states in Abuja Nigeria in 2000 (WHO 2000). Additionally, the Abuja declaration set an ambitious target that by 2015, malaria should not be a major cause of mortality or a deterrent for socio-economic growth around the world. Furthermore, the launch of the Roll Back Malaria (RBM) partnership which brought together multilateral, bilateral, non-governmental organizations and private organizations to support its objective of halving malaria deaths by 2010 was a milestone in renewing efforts to control malaria (Nabarro and Tayler 1998; Nabarro 1999; Nabarro and Mendis 2000). The RBM has prioritized case management, intermittent presumptive treatment for pregnant women and vector control with insecticide treated nets (ITNs) and indoor residual spraying (IRS) where appropriate (Nabarro 1999).

However, insecticide treated nets were a relatively new priority that had not been adopted as a major vector control tool until Alonso and colleagues demonstrated that these could reduce mortality in Gambian children (Alonso, Lindsay et al. 1991). Consequently, several studies were conducted across endemic countries in Africa, notably in Ghana (Binka, Indome et al. 1998),

Burkina Faso (Habluetzel, Diallo et al. 1997) and Kenya (Nevill, Some et al. 1996; Phillips-Howard, Nahlen et al. 2003; ter Kuile, Terlouw et al. 2003; ter Kuile, Terlouw et al. 2003) which further restored confidence in vector control as a means to prevent malaria by demonstrating the efficaciousness and effectiveness of ITNs in reducing child mortality and related anaemia. These studies provided the evidence that encouraged the heads of African states to give their political support and this further prompted the RBM to endorse efforts aimed at supporting endemic countries to apply scientifically proven vector control interventions.

1.4.6 Modern Control to Elimination Era

Upon realizing the efficacy of treated mosquito nets across varying epidemiological settings in Africa, RBM increased its effort to advocate for countries to implement malaria control programmes that are anchored on distribution of ITNs or regular IRS application as the main vector control tools, coupled with presumptive treatment for pregnant women and effective case management (WHO 2008). Therefore multi-national organization such as the Global Fund to Fight AIDS Tuberculosis and Malaria (GFATM), the World Bank (WB) and the United States President's Malaria Initiative (PMI) provided financial support at unprecedented levels since the end of the GMEP. Between 2006 and 2010 an estimated \$ 8.9 billion was used for malaria control and research activities with the larger amount disbursed to Africa (Pigott, Atun et al. 2012) where malaria infections are the most. The annual disbursement of funds has been increasing from less than US\$ 100 million in 2000 to an estimated 1.84 billion in 2012, although there was a slight fall of about 4% per year between 2005 and 2009 partly attributed to the

slow in disbursements of funds (WHO 2013). However, these figures are estimated to be far much less than the USD 4 – 5 billion that is required annually (Pigott, Atun et al. 2012) to achieve satisfactory coverage with all the proven core interventions recommended by WHO.

Despite this funding shortfall, there has been tremendous progress in the last two decades with many countries reporting reductions in malaria morbidity and mortality due to the scale-up of both combination artemisinin drugs and LLINs and IRS (Fegan, Noor et al. 2007; Sharp, Kleinschmidt et al. 2007; Chizema-Kawesha, Miller et al. 2010; D'Acremont, Lengeler et al. 2010; O'Meara, Mangeni et al. 2010; Steketee and Campbell 2010; van Eijk, Hill et al. 2011; Eisele, Larsen et al. 2012; Murray, Rosenfeld et al. 2012; van Eijk, Hill et al. 2013; WHO 2013; Gething, Battle et al. 2014) in Africa where *Plasmodium falciparum* malaria is commonly transmitted by potent vectors from the *Anopheles gambiae* complex and the *Anopheles funestus* group (Gillies and Demeillon 1968; Gillies and Coetzee 1987).

High coverage with ITNs, now available as long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) can dramatically reduce densities, survival rates and malaria transmission mediated by highly endophagic (indoor biting) and endophilic (indoor resting) vectors such as *An. gambiae* sensu stricto Giles and *An. funestus* Giles (Pluess, Tanser et al. 2010). While ITNs and IRS have proved to be effective (Curtis, Mnzava et al. 1999; Misra, Webber et al. 1999; Mnzava, Dlamini et al. 1999; Lengeler 2000; Mabaso, Sharp et al. 2004; Pluess, Tanser et al. 2010), there are several reports suggesting that a combination of these

strategies can have an even greater impact than either strategy alone (Pardo, Descalzo et al. 2006; Kleinschmidt, Schwabe et al. 2009; Bekele, Belyhun et al. 2012; Fullman, Burstein et al. 2013; West, Protopopoff et al. 2014). Nonetheless, in settings where insecticide resistance has been associated with disappointing impact of LLINs and/or IRS using a different class of insecticides with IRS has an additive advantage (N'Guessan, Boko et al. 2010; Akogbeto, Padonou et al. 2011; Ngufor, N'Guessan et al. 2011; Osse, Aikpon et al. 2012; Rowland, Boko et al. 2013) while other reports also suggest that LLINs have an impact even when physiological resistance is present (Strode, Donegan et al. 2014; Tokponnon, Ogouyemi et al. 2014).

1.4.7 Persisting obstacles to the elimination of malaria

Historical evidence from the Global Malaria Eradication Programme (GMEP) era suggests populations of the most potent vectors can be eliminated where they are physiologically and behaviourally susceptible to the insecticides delivered in the form of LLINs or IRS because they are highly dependent on human blood (Durnez and Coosemans 2013; Govella, Chaki et al. 2013; Killeen, Seyoum et al. 2013). In the Pare-Taveta region along the border of Tanzania and Kenya, *An. funestus* populations were eliminated over the course of three years of IRS with dieldrin, and this vector only re-appeared five years after stoppage of IRS (Smith and Draper 1959; Smith 1962; Smith 1966). In the coastal district of Malindi in Kenya, and in KwaZulu Natal region of South Africa, *An. funestus* were also reportedly eliminated with IRS using DDT (Gillies and Furlong 1964; Sharp and le Sueur 1996). On the Bioko island west Africa, *An. funestus* were eliminated and drastic reduction in the population density of the molecular S form of *An.*

gambiae s.s. were noticeable following IRS, first with pyrethroids until 2004 and then with bendiocarb in subsequent years (Sharp, Ridl et al. 2007; Overgaard, Reddy et al. 2012; Hemingway, Vontas et al. 2013). Also, during the GMEP era, populations of the *An. gambiae* complex were dramatically reduced in the northern savannah of Nigeria where propoxur was used for IRS (Molineaux, Shidrawi et al. 1976).

More recently, spectacular reductions in the densities of *An. gambiae* populations have been documented as insecticidal nets have been scaled up (Curtis, Maxwell et al. 1998; Bayoh, Mathias et al. 2010; Russell, Lwetoijera et al. 2010; Mutuku, King et al. 2011; Russell, Govella et al. 2011; Derua, Alifrangis et al. 2012; Mwangangi, Mbogo et al. 2013). These observational studies bolster historical evidence that effective IRS and LLINs implementation may even approach elimination of the vectors which are both behaviourally vulnerable to these approaches and physiologically susceptible to their active ingredients (Govella, Chaki et al. 2013; Killeen, Seyoum et al. 2013).

However, the increased application of these insecticide-based intra-domiciliary vector control interventions has inevitably seen the widespread emergence of physiological resistance to their active ingredients among mosquitoes (Ranson, N'Guessan et al. 2011; WHO 2012), sometimes resulting in rebounding vector populations and resurgent malaria transmission (Sharp and le Sueur 1996; Trape, Tall et al. 2011; Cohen, Smith et al. 2012; Moss, Norris et al. 2012; WHO 2013; Trape, Tall et al. 2014). For instance, in Senegal, the density of *An. gambiae* and the

malaria transmission they mediate were reduced in the first two years following introduction of LLINs, but then rapidly returned to their initial levels as the knock down resistance (*kdr*) allele increased from 8 to 48% within the population (Trape, Tall et al. 2011; Trape, Tall et al. 2014) suggesting that partial coverage of expired nets creates an opportunity for resistance to emerge with subsequent rebounds in the vector populations so that malaria transmission resurgences occurs. In South Africa, where *An. funestus* had been eliminated with DDT in the 1950s but this species later re-entered from Mozambique, a shift to the use of pyrethroids in the 1990s was rapidly followed by the emergence of physiological resistance to these new active ingredients, and then an increase of malaria incidence of more than six fold (Sharp and le Sueur 1996; Hargreaves, Koekemoer et al. 2000).

However, the emergence of physiological resistance to insecticides does not necessarily or inevitably result in control failure and resurgence of transmission. For instance, the phenotypic expression of physiological resistance traits may gradually reduce as mosquitoes grow older (Lines and Nassor 1991; Hunt, Brooke et al. 2005; Glunt, Thomas et al. 2011; Rajatileka, Burhani et al. 2011; Jones, Sanou et al. 2012) and tolerance levels to insecticides also depend on the age at which mosquitoes obtain their first blood meal (Lines and Nassor 1991; Hunt, Brooke et al. 2005; Rajatileka, Burhani et al. 2011). These much older mosquito populations are more epidemiologically relevant to the control of malaria (Jones, Sanou et al. 2012) because they have survived long enough to become infectious and potentially transmit the parasite to humans. Also, recent data from a physiologically-resistant *An. gambiae* s.s. field populations on

Bioko Island, illustrate how individual mosquitoes that are homozygous for the *kdr* allele have lower sporozoite prevalence rates than the combined heterozygous and non-*kdr* homozygous members of the same population, so *kdr* status alone does not reduce the effectiveness of pyrethroid-based IRS (Hemingway, Vontas et al. 2013). Several other examples have been documented in which vector control impact has been sustained despite clear emergence of physiological resistance traits that are clearly manifested in the laboratory but do not obviously undermine the impact of interventions relying upon their active ingredients. Effective control has thus far been sustained in several well characterized settings like South Africa, west Kenya and Zanzibar where physiological resistance has clearly emerged (Mathias, Ochomo et al. 2011; Maharaj, Morris et al. 2012; Jones, Haji et al. 2013). These examples may be explained by the hypothesis that mosquitoes with shorter lifespans may be selected for when intervention pressure is applied because they invest more reproductive resources into early gonotrophic cycles at the expense of the later ones that mediate transmission of mature sporozoite-stage malaria parasites (Ferguson, Maire et al. 2012).

Beyond physiological traits which render mosquitoes invulnerable to insecticides, normally endophagic vector species (Huho, Briet et al. 2013) have been observed to exhibit the atypical, in some cases clearly altered, behaviours following scale up of LLINs or IRS, specifically biting during the early evening and early morning when communities are unprotected because they are active outdoors (Bugoro, Cooper et al. 2011; Reddy, Overgaard et al. 2011; Russell, Govella et al. 2011; Durnez and Coosemans 2013; Gatton, Chitnis et al. 2013; Govella, Chaki et al. 2013;

Russell, Lwetoijera et al. 2013; Moiroux, Damien et al. 2014; Sougoufara, Diedhiou et al. 2014). This increasingly common phenomenon has been attributed to mosquitoes altering the way in which they express innately flexible behaviours to simply continue searching so that feeding is delayed until they find exposed, unprotected hosts which may be more abundant around dawn and dusk (Durnez and Coosemans 2013; Gatton, Chitnis et al. 2013; Govella, Chaki et al. 2013; Huho, Briet et al. 2013) or to evolutionary selection of heritably altered behavioural preferences that becomes progressively more frequent within the mosquito population over several generations (Russell, Lwetoijera et al. 2013; Killeen and Chitnis 2014). Reductions of the proportion of blood meals obtained from humans, commonly referred to as the human blood index (HBI) (Garrett-Jones 1964; Garrett-Jones 1980), are also commonly observed following scale up of LLINs or IRS, with vector populations exhibiting flexible behaviour by feeding on animals rather than humans where the latter are protected by LLINs or IRS. This effect can be further increased by the physical or chemical deterrent actions of those protective measures (Garrett-Jones 1964; Garrett-Jones 1980; Pates and Curtis 2005; Killeen, Chitnis et al. 2011; Durnez and Coosemans 2013) or even by heritably selected changes in host preference (Gillies 1964). Therefore, such apparently altered behavioural patterns do not necessarily represent emerging behavioural resistance but rather pre-existing behavioural resilience and are therefore by no means inevitably associated with vector population rebound or malaria transmission resurgence (Durnez and Coosemans 2013; Govella, Chaki et al. 2013; Killeen, Seyoum et al. 2013).

However, such apparent changes in biting times might not even necessarily reflect a behavioural shift of a mixed mosquito population that consists of a complex of sibling species or other distinct subsets within structured populations but may simply suggest that effective control of a dominant species or other cryptic sub-taxon in favour of another that was originally less important but then predominates the residual population that persists after IRS or LLIN scale up (Durnez and Coosemans 2013; Gatton, Chitnis et al. 2013; Govella, Chaki et al. 2013). For instance historical evidence provides several examples in which taxonomical alterations of proportional composition of member species within a complex after successful application of intra-domiciliary interventions (Bugoro, Iro'ofa et al. 2011; Govella, Chaki et al. 2013; Killeen, Seyoum et al. 2013; Russell, Beebe et al. 2013). For example, in Kenya and Tanzania, the extremely potent populations of anthropophagic, endophilic and endophagic *An. funestus* were replaced by the exophagic and zoophagic sibling species *An. rivulorum* and *An. parensis* (Smith and Draper 1959; Smith 1962; Gillies and Furlong 1964). In the Solomon Islands in the Pacific, the potent indoor-biting vectors *An. koliensis* and *An. punctulatus* have both been eliminated or dramatically reduced in their geographic distribution by LLINs and IRS, leaving only the outdoor biter *An. farauti* as the main vector of residual transmission (Bugoro, Iro'ofa et al. 2011; Russell, Beebe et al. 2013). In Guiana in Latin America, *An. darlingi* was eliminated after three years with IRS using DDT, while populations of zoophagic and exophagic species (*An. aquasalis*, *An. albitarsis* and *An. triannulatus*) appeared to remain at unaltered densities (Giglioli 1951). The introduction of insecticidal nets in nearby Suriname has also resulted in the collapse of *An. darlingi* and *An. nuneztovari* (Hiwat, Mitro et al. 2012), the two most important vectors of malaria in the country. This phenomenon of mosquito population composition

alteration is also strongly influenced by the levels of phenotypic plasticity exhibited by the various taxa present. While those that are suppressed, eliminated or replaced tend to be those that exhibit rigid phenotypic behaviours that makes them responsive to LLINs and IRS, the more resilient taxa ones portray plasticity in their choice of hosts, time of biting and resting places (Pates and Curtis 2005; Durnez and Coosemans 2013; Govella, Chaki et al. 2013; Huho, Briet et al. 2013; Killeen, Seyoum et al. 2013). So while the emergence of genuine behavioural resistance and associated malaria resurgence is indeed a worrying possibility that merits vigilance, it remains to be clearly documented in the field.

1.4.8 Implications for surveillance needs

The inability of programmatic vector control implementation to eliminate malaria transmission can be explained by either a vector population that exhibits inherent, stable behavioural traits making it resilient (Figure 1.4A) or by one exhibiting emerging behavioural or physiological resistance (Figure 1.4B) that allows it to recover from initial suppression. The former is characterized by some sustained but incomplete levels of impact in the context of a sustained programme while the latter is characterized by outright failure of a programme despite sustained implementation practice, resulting in rebounding vector populations and malaria transmission (Cohen, Smith et al. 2012; Durnez and Coosemans 2013; Govella, Chaki et al. 2013). There is therefore a clear distinction between the two phenomena as described in figure 1.4 but these remain unclearly understood across sub-Saharan Africa. There is therefore a need to develop robust, cost effective and reliable longitudinal surveillance systems to monitor such

vector population dynamics process. Monitoring of vector population dynamics will play a critical role in rational vector control management if behavioural and physiological traits that limit or undermine intervention impact are to be mitigated sustainably (Cohen, Smith et al. 2012; Durnez and Coosemans 2013; Govella, Chaki et al. 2013; Killeen, Seyoum et al. 2013).

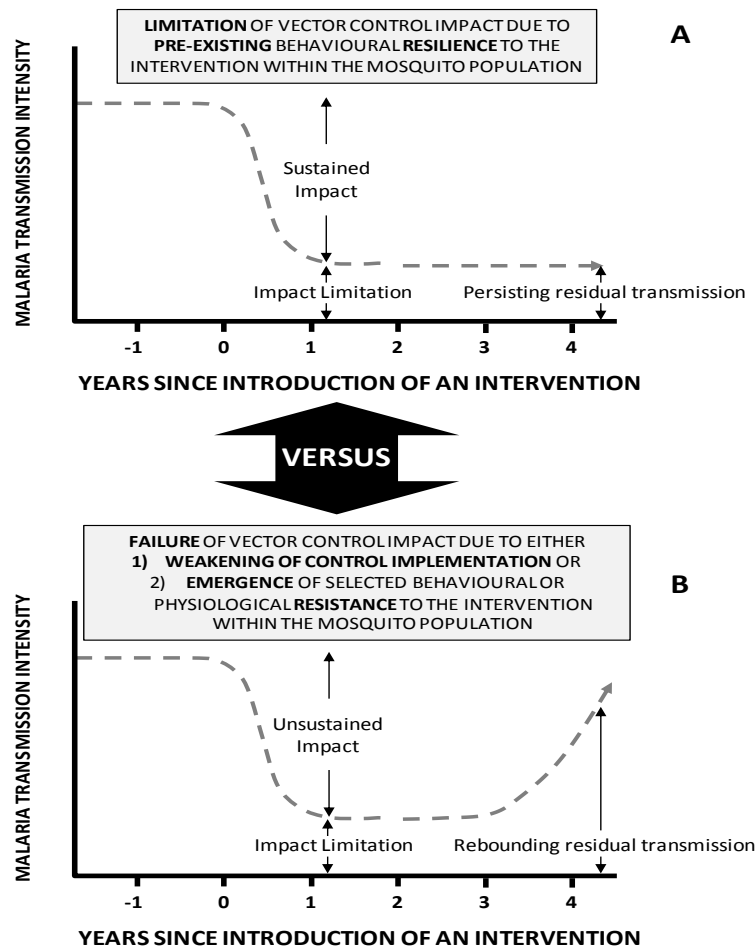


Figure 1.4: A schematic illustration of the differing trajectories of impact of an intervention upon malaria transmission by a vector population under the distinctive scenarios of either (A) Stable limitation of sustained impact arising from expression of pre-existing behavioural traits within a resilient vector population, or (B) Failure of impact and resurgence of malaria transmission when, either intervention programme implementation quality and coverage weakens, or selected behavioural or physiological traits emerge within an increasingly resistant, rebounding vector population.

1.5 Historical perspective of malaria control in Zambia

The history of malaria control in Zambia dates back to the early twentieth century when the copper was being explored for economic reasons on the Copper belt (Watson 1953). As early as 1929, LSM was introduced to control *An. funestus* and *An. gambiae* and the malaria transmission they mediate, to enhance staff retention and financial viability of the large Copper mines (Utzinger, Tozan et al. 2002). At the inception of the LSM programmes, concerted efforts were applied to the identification of breeding sites along the main river (Luanshya) its tributaries, swamps and any other water bodies perceived to potentially sustain breeding of mosquitoes. Adult collections showed that 80% of the catches were *An. funestus* while larval collections contained higher proportions of *An. gambiae*, presumably due to the difference in the ecology of the breeding sites of the two species. *An. gambiae* breeds in a wide variety of accessible, partially shaded natural and manmade habitats so it was much easier to control, and indeed to sample as larvae, than *An. funestus* that breeds in inaccessible heavily-shaded swamps and fringes of rivers and lakes even during the dry season. A systematic approach to LSM was planned and executed; Drains and river banks were cleared of vegetation to allow water flow, and static water bodies with flooded or stagnant water such as swamps were drained so that the immature aquatic stages of mosquitoes are disrupted. Coupled with LSM, house screening of doors and windows were systematically applied to prevent entry of insects, bed nets were introduced to complement the efforts and quinine was used for both treatment and prophylaxis.

Plasmodium falciparum contributed 86.8% of malaria infections while the rest was accounted for by *P. malariae*. Between 1929 and 1930, the annual mortality rate was 23.41 per 1000 and the first reported annual incidence rate was 514 per 1000. Malaria incidence was reduced to 135 per 1000 per year by 1939 – 1940 (Utzinger, Tozan et al. 2002) and these figures later dropped to 16 per 1000 per year by 1950, after indoor residual spraying (IRS) using DDT was introduced as a further measure efforts to target endophagic and endophilic vectors (Utzinger, Tozan et al. 2002). These efforts also reduced annual malaria-related mortality from 10.3 to 0.5 per 1000 per year, averting approximately 4173 deaths and 161,205 malaria attacks by the mid late 1960s. By the period from 1969 to 1972, this part of the Copper belt had reduced splenomegaly for children less than 15 years from the initial level of 36% down to 6% (Utzinger, Tozan et al. 2002).

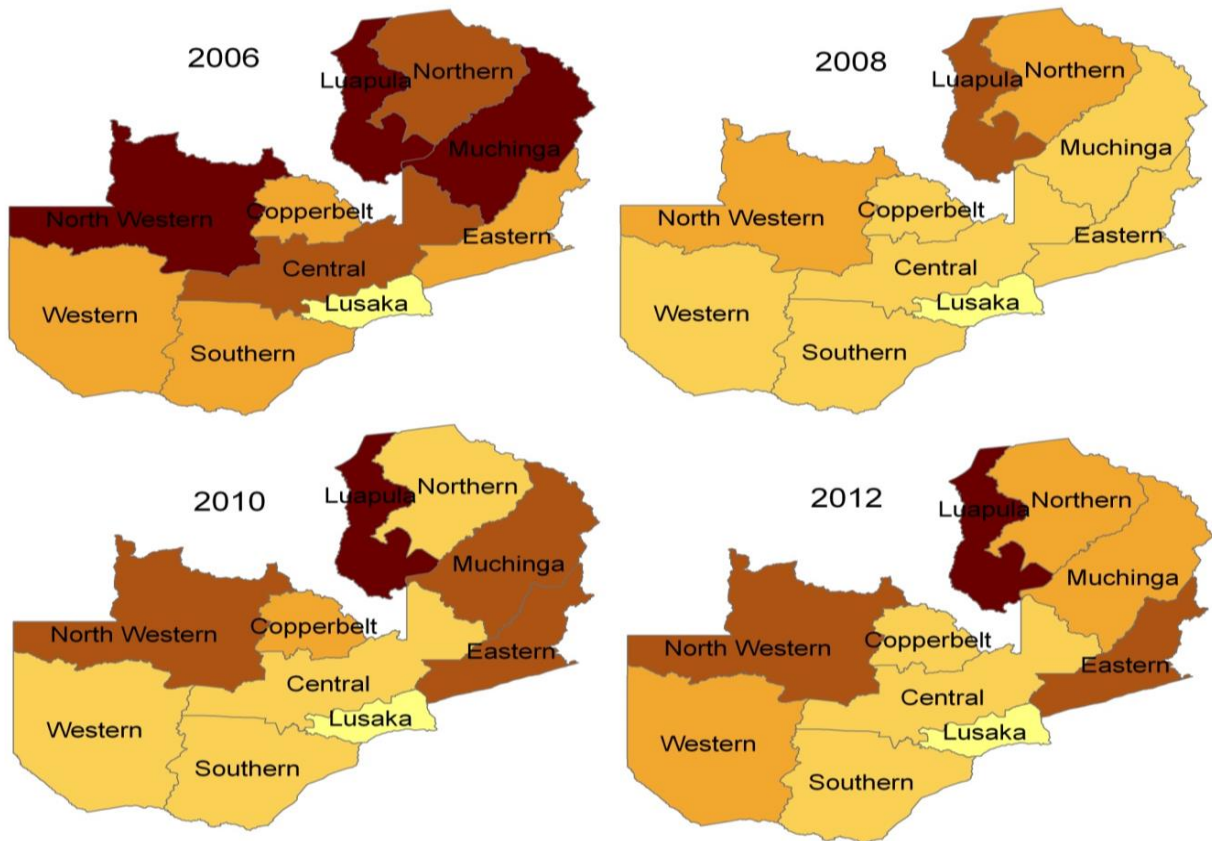
However, when GMEP was halted in the late 1960s, and this collapse of political will to support malaria control was compounded by the global economic recession that occurred in the 1970s and 80s, malaria vector control efforts were neglected (Najera, Gonzalez-Silva et al. 2011). Therefore as the GMEP failed to eradicate malaria globally and in Zambia, reliance was placed on drug therapies as sole control efforts with no deliberate preventive strategies. In spite of wide spread usage of chloroquine, the country experienced dramatic increases in malaria burden such that from an incidence rate of 121.5 per 1000 per year in 1976, this figure tripled by 1999 and case fatality rates due to malaria and impatient cases almost quadrupled (NMCC

2000), which was attributed to the cessation of active vector control activities combined with the emergence of chloroquine resistance (NMCC 2000; Masaninga, Chanda et al. 2013).

However in 1992 Zambia went through a series of health reforms that paralleled global policy changes, which prioritized malaria control with emphasis on using ITNs, especially for the children under-five and the pregnant women. IRS was restarted by the mines in 2000 using both pyrethroids and DDT (Sharp, van Wyk et al. 2002) and the first national malaria strategic plan (NMSP) was developed in the following year with the view of reducing malaria morbidity and mortality by 50%. This plan was further developed in subsequent years, with greater ambition so that the 2006 to 2010 NMSP laid out a vision of “a malaria free Zambia through scaling up for impact”, with the goal of reducing malaria incidences by 75% and under-five mortality due to malaria by 20% (Steketee, Sipilanyambe et al. 2008). This was anchored on the principal that both LLINs and IRS will be the front-line vector control interventions with LSM supplementing where feasible. The 2011 to 2015 NMSP maintains these targets with some minor modifications, aiming to reduce malaria incidence by 75%, reduce mortality due to malaria to zero, and reduce all-cause child mortality by 20% by 2015 (NMCC 2012). Over the period of development of the NMSPs spanning over 10 years, Zambia has recorded dramatic reductions of malaria cases, mainly due to the scaling up of vector control (LLINs and IRS) and case management interventions through the support of resources from the GFATM the WB and the PMI through USAID, with the annual budget increasing from USD\$ 10 million in 2003 to USD\$ 41 million in 2008 (Masaninga, Chanda et al. 2013).

1.5.1 Brief epidemiological and geographical profile of malaria burden in Zambia

Malaria remains among the leading causes of mortality and morbidity in Zambia accounting for over 40% of all child mortality and 20% of maternal mortality with about 8000 reported deaths annually, although the latter figure is undoubtedly a gross underestimate (NMCC 2012). Over 98% of malaria cases are attributed to *P. falciparum*, with the remainder accounted for by *P. malariae* and *P. ovale*. Malaria is still predominately transmitted by *An. funestus*, *An. gambiae* s.s. and *An. arabienisis* (NMCC 2000; Masaninga, Chanda et al. 2013). In 2010, the country conducted a review, with the assistance of both local and regional stakeholders, of the malaria programme and the epidemiologic profile, whereby the country was stratified into three epidemiologic zones. A low transmission region in south-east Zambia with a parasite prevalence among children under the age of 5 of < 1%, a low stable transmission zone occupying the north-western, south and central parts of the country with a parasite prevalence of 1 to 10%, and a high transmission zone in the north-east with a prevalence rate of over 20% (NMCC 2012; NMCC 2013). The programme has been conducting bi-annual malaria indicator surveys since 2006, revealing that malaria transmission profile has been consistent (Figure 1.5).



Parasitaemia prevalence

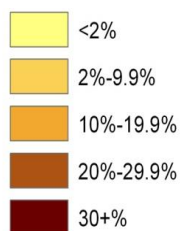


Figure 1.5: Malaria prevalence per province in Zambia 2006 to 2012.

Source: National Malaria Control Programme Strategic Plan for FY 2011-2015: "Consolidating Malaria Gains for Impact"

1.5.2 Historical vector distribution and mosquito infection rates in Zambia

Over the years, surveys of malaria transmission intensity have typically been based exclusively on clinical or point prevalence indicators, without any supporting secondary entomological surveys of vector densities or infectivity rates. The malaria vector distribution in Zambia conforms to the sub-Saharan Afro-tropical zoogeographical region (Gillies and Demeillon 1968; Gillies and Coetzee 1987) where *An. gambiae* s.s., *An. arabiensis* and *An. funestus* are the main vectors. The earliest records examining vector distribution in Zambia were conducted in the Copper belt district of Luanshya during implementation of the historical integrated vector control programme described above. Chimumbwa (2003) has provided a clear historical account of the events that unfolded that have contributed to understanding the vector distribution in Zambia. When the Roan Antelope Mine Corporation introduced EM as the major vector control activity to control the proliferation of *An. gambiae* s.l. and *An. funestus* in 1929, this clearly marked the beginning of vector identifying vectors in Zambia (Watson 1953). Even though this was implemented at a relatively small scale considering the vast geographic landscape of Zambia, important lessons were learnt that understanding of mosquito behaviour is fundamental to the control of malaria. Importantly, the flight ranges of both species were also observed during this period providing vital information by which optimal application of control efforts can be designed by providing buffer zones around the designated areas for application of interventions (Watson 1953). However, because this was only a very local study, it could only be assumed that *An. gambiae* s.l. and *An. funestus* were the main mediators of transmission across the country because Zambia belongs to the Afro-tropical zoogeographical region (Gillies and Demeillon 1968; Gillies and Coetzee 1987).

In the early 1960s, what were then known as *An. gambiae* species A, B and C (now known as *An. gambiae* s.s., *An. arabiensis* and *An. quadriannulatus*, respectively), were found living sympatrically in Chirundu in the south of Zambia by Paterson (Paterson 1962; Paterson 1963; Paterson 1964) and were observed to be endophilic and anthrophagic. Apparently, the first evaluation of Hexachlorocyclohexane (commonly known as Lindane), an insecticide for IRS was conducted in some huts in Chirundu village targeting the local *An. gambiae* s.l., vectors (Hadjinicoulou 1963). From 1969 to 1970, the Malaria Research Laboratory (MRL) was established in Lusaka by the government of Zambia to provide evidence-based scientific research findings to provide guidance in implementation of malaria control activities. The MRL conducted entomological collections in Ndola, the provincial city of the Copper belt and, again, in Chirundu district in the southern part of the country, finding that both *An. gambiae* s.l. and *An. funestus* were present in both the areas (Zahar 1985). Even though the climatic conditions were conducive for *An. funestus* in Chirundu, this was the first time it was documented in this part of the country. However, in the central parts of Zambia in Lusaka City and to the east in Chipata district, reports by the MRL suggests that indoor human biting collections conducted in these areas only yielded *An. arabiensis* (Bransby-Williams 1979). It must be emphasized that, at this time, little insecticidal vector control was undertaken, especially in rural areas.

Chimumbwa (2003) also conducted entomological surveys in two different epidemiologic settings: one in a village in the northern, wetter part of Zambia situated in Mwense districts and

another in Kafue district south of Lusaka which is characterized by mild rainfall patterns. After a series of mosquito collections, two distinctive outcomes were observed; in both villages all the three vectors *An. gambiae* s.s., *An. arabiensis* and *An. funestus* were observed but in varying proportions. In the village characterized with high rainfall patterns, the species predominately was *An. funestus*, followed by *An. gambiae* and then *An. arabiensis*, while in the southern area *An. arabiensis* was the predominant species, followed by *An. funestus* and then *An. gambiae* s.s.

The current survey platforms for mosquito collection that are active in Zambia today are implemented primarily to determine physiological resistance status but also capture species composition and relative abundance to some degree. The surveys are mainly conducted by the central entomological team at the NMCP and by other affiliated research institutions. These collections do not capture vector population dynamics in any substantive way and are characterized by sporadic implementation and are restricted in scope by 1) the cost associated with conducting longitudinal surveillance systems to cover sentinel sites across the vast area of the country, and 2) a proven system that will captures both physiological and behavioural resistance and can capture malaria infection risk on nationally representative scales that are relevant to NMCC operations. In order to address these limitations in current vector surveys, a community-based entomological surveillance is required that allows survey procedures to be decentralized to local level but be supervised and quality assured by the central NMCC team so

that reliable data on vector population dynamics is gathered and synthesized to appropriately guide and apply vector control intervention management.

1.6 Goal and Objectives

The overall goal of the study was to demonstrate how a community-based surveillance system can be applied to longitudinally monitor vector population dynamics and assess the impact that LLINs and IRS have on malaria transmission in rural Zambia. To achieve this overall goal, the following specific objectives were addressed.

1.6.1 Specific Objectives

1. To evaluate the efficacy of exposure-free mosquito trapping methods for measuring malaria vector density, in direct comparison with human landing catch.
2. To assess the cost-effectiveness of using a community-based mosquito trapping scheme for monitoring population dynamics.
3. To determine the extent to which a community-based mosquito trapping scheme captures temporal and spatial trends in epidemiological indicators of malaria infection risk.
4. To determine the impact of indoor residual spraying with different classes of insecticides on malaria infection burden and malaria vector abundance in an area of high coverage with insecticide treated nets using a community-based platform.

CHAPTER TWO

EVALUATION OF ALTERNATIVE MOSQUITO SAMPLING METHODS FOR MALARIA VECTORS IN LOWLAND SOUTH-EAST ZAMBIA

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2.0 Abstract

Background

Sampling malaria vectors and measuring their biting density is of paramount importance for entomological surveys of malaria transmission. Human landing catch (HLC) has been traditionally regarded as a gold standard method for surveying human exposure to mosquito bites. However, due to the risk of human participant exposure to mosquito-borne parasites and viruses, a variety of alternative, exposure-free trapping methods were compared in lowland, south-east Zambia.

Methods

Centres for Disease Control and Prevention miniature light trap (CDC-LT), Ifakara Tent Trap model C (ITT-C), resting boxes (RB) and window exit traps (WET) were all compared with HLC using a 3×3 Latin Squares design replicated in 4 blocks of 3 houses with long lasting insecticidal nets, half of which were also sprayed with a residual deltamethrin formulation, which was repeated for 10 rounds of 3 nights of rotation each during both the dry and wet seasons.

Results

The mean catches of HLC indoor, HLC outdoor, CDC-LT, ITT-C, WET, RB indoor and RB outdoor, were 1.687, 1.004, 3.267, 0.088, 0.004, 0.000 and 0.008 for *Anopheles quadriannulatus* Theobald respectively, and 7.287, 6.784, 10.958, 5.875, 0.296, 0.158 and 0.458, for *An. funestus* Giles, respectively. Indoor CDC-LT was more efficient in sampling *An. quadriannulatus* and *An. funestus* than HLC indoor (Relative rate [95% Confidence Interval] = 1.873 [1.653, 2.122] and

1.532 [1.441, 1.628], respectively, $P < 0.001$ for both). ITT-C was the only other alternative which had comparable sensitivity (RR = 0.821 [0.765, 0.881], $P < 0.001$), relative to HLC indoor other than CDC-LT for sampling *An. funestus*.

Discussion and Conclusion

While the two most sensitive exposure-free techniques primarily capture host-seeking mosquitoes, both have substantial disadvantages for routine community-based surveillance applications: the CDC-LT requires regular recharging of batteries while the bulkiness of ITT-C makes it difficult to move between sampling locations. RB placed indoors or outdoors and WET had consistently poor sensitivity so it may be useful to evaluate additional alternative methods, such as pyrethrum spray catches and back packer aspirators, for catching resting mosquitoes.

2.1 Background

In measuring malaria transmission intensity under varying epidemiological settings, entomological sampling methods that catch mosquitoes with high sensitivity are very useful, particularly as vector densities drop in response to increasingly effective vector control and elimination of transmission is prioritised by an increasing number of countries (Service 1993; Kelly-Hope and McKenzie 2009; Bugoro, Iro'ofa et al. 2011; Meyrowitsch, Pedersen et al. 2011; Russell, Govella et al. 2011). Generally, these sampling methods involve collection of adult mosquitoes either indoors or outdoors, with the host-seeking females that mediate transmission as the primary target for trapping (Service 1993; Service and Townson 2002). Human landing catch (HLC) is the gold standard method for collection of host-seeking mosquitoes (Service 1977) to determine their biting rate, infection prevalence, and consequently the intensity of malaria transmission they mediate. However, HLC raises ethical concerns because catchers are exposed to vectors that could be potentially infective. It is also labour intensive and unreliable due to variation in attractiveness and skill of the catchers who act as bait hosts (Lindsay, Adiamah et al. 1993; Knols, de-Jong et al. 1995; Mukabana, Takken et al. 2002; Kelly-Hope and McKenzie 2009). The continued application of this tool in the surveillance of malaria transmission in sub-Saharan Africa requires careful re-examination and re-justification, with a view to developing and characterizing safer alternative tools that are comparably sensitive.

Over the years, a number of alternative sampling tools that avoid human contact with mosquitoes have been evaluated. These have exhibited wide variations in efficacy and cost, and may not be practical for adoption on programmatic scales in poor malaria-endemic countries (Service and Townson 2002; Kelly-Hope and McKenzie 2009). One of the most commonly employed tools for catching host-seeking malaria vectors in particular is the Centres for Disease Control and Prevention miniature light trap (CDC-LT), which is typically positioned indoors near an occupied net (Sudia 1962; Garret-Jones and Magayuka 1975). Numerous studies have shown the effectiveness of CDC-LTs over a wide range of transmission systems in Africa (Sudia 1962; Odetoyinbo 1969; Mbogo, Glass et al. 1993; Davis 1995; Shiff, Minjas et al. 1995; Mboera, Kihonda et al. 1998). The positioning of the CDC-LT during sampling influences the sensitivity with which it samples adult female mosquitoes (Mboera, Kihonda et al. 1998) and this trap is almost equally effective when occupants are sleeping under a treated or untreated bed net (Magbity and Lines 2002; Killeen, Tami et al. 2007).

However, where indoor-targeted insecticidal based interventions such as long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS) have drastically reduced endophilic and endophagic vectors (Griffin, Hollingsworth et al. 2010; Kiware, Chitnis et al. 2012), traps for capturing host-seeking mosquitoes outside of houses are considered more suitable to sample the exophagic vectors that become increasingly important contributors to the residual vector population as intervention coverage is scaled up (Molineaux, Shidrawi et al. 1976; Bugoro, Iro'ofa et al. 2011; Mutuku, King et al. 2011; Reddy, Overgaard et al. 2011;

Russell, Govella et al. 2011). While capture methods primarily targeting host-seeking mosquitoes are ideal for quantifying human exposure to bites and studying host attack behaviours, resting and exit traps are more appropriate for studying resting behaviours and sampling fed mosquitoes to determine the source of blood obtained (Service 1993; Service and Townson 2002).

The characteristic indoor resting (endophilic) behaviour of *Anopheles gambiae* Giles, *An. arabiensis* Patton and *An. funestus* Giles underpins the common use of indoor knockdown pyrethrum spray catches (PSC) and hand collections using a mouth aspirator when surveying (Service and Townson 2002). The major drawbacks associated with the hand collection method for resting mosquitoes is poor sensitivity, the laborious nature of rigorous searches through all the irregular surfaces of rural houses, and the great variability in skills and motivation among collectors (Service 1993; Harbison, Mathenge et al. 2006). PSC may be expensive to sustain for routine monitoring (Harbison, Mathenge et al. 2006) while the repellence and persistence of the pyrethrum used precludes sampling in the same dwelling more than twice a week (Service 1993; Chareonviriyaphap, Roberts et al. 1997). Other sampling methods such as, resting boxes (RB), clay pots and bed net traps have been evaluated under different epidemiological settings in Africa with varying degrees of success (Edman, Kittayapong et al. 1997; Kittayapong, Linthicum et al. 1997; Yasuno, Rajagopalan et al. 1997; Laganier, Randimby et al. 2003; Mathenge, Omweri et al. 2004; Mathenge, Misiani et al. 2005; Odiere, Bayoh et al. 2007; Okumu, Kotas et al. 2008; Kweka, Mwang'onde et al. 2009; Sikulu, Govella et al. 2009). While

window exit traps (WET) have been used for monitoring vector density trends in parts of southern Africa and Bioko island in central Africa (Mouatcho, Hargreaves et al. 2007; Sharp, Ridl et al. 2007), their efficacy is undoubtedly affected by variations in house design and behavioural patterns of both mosquitoes and humans (Govella, Chaki et al. 2011).

A recent review (Kelly-Hope and McKenzie 2009) has highlighted the lack of consistency, comparability and characterisation of the numerous, diverse entomological survey tools used to measure malaria transmission. Recent evaluations of a newly developed Ifakara Tent Trap Design C (ITT-C) (Govella, Moore et al. 2010) show that, unlike the B design that preceded it (Govella, Chaki et al. 2009; Sikulu, Govella et al. 2009) the ITT-C is a genuinely exposure-free tool that probably represents a relatively sensitive and practical mode of sampling malaria vectors for routine surveillance purposes (Govella, Chaki et al. 2011), notably through community-based trapping schemes with epidemiological predictive power (Chaki, Mlacha et al. 2012). Here we report a comparative evaluation of the ITT-C, CDC-LT, RB and WET methods that do not necessitate increased human exposure to mosquito bites, compared to the gold standard HLC which does, in a rural part of Zambia with stable endemic transmission mediated primarily by *Anopheles funestus* Giles (Keating, Miller et al. 2009; MoH 2010) Insecticidal interventions, such as LLINs and IRS can alter survival rates, as well as entry, feeding, resting and exiting behaviours within houses (Pates and Curtis 2005), and these two interventions are sometimes combined in parts of Zambia and elsewhere in Africa, with the intention of achieving greater impact than with either alone (Kleinschmidt, Schwabe et al. 2009; Okumu and Moore

2011; Chanda, Mukonka et al. 2013). The influence of supplementing LLINs with IRS upon the efficacy of these trapping methods was, therefore, also assessed by comparing capture rates and sample composition in and immediately outside of houses with both interventions versus those with LLINs alone.

2.2 Methods

2.2.1 Study area

The study was conducted in Chisobe and Nyamumba villages situated between Kasinsa and Chitope rural health centres in Luangwa district (Figure 2.1) which is about 255km east of Lusaka. Chisobe and Nyamumba are about 2 – 3 kilometres apart. Luangwa is at latitude - 15°41' E, and longitude 30°08' S. It is approximately 370m above sea level. The wet season runs from November to April and the dry season from June to September with October and May being transitional months. Annual rainfall varies from 600 to 1,400mm with mean daytime temperatures ranging from 10°C to 44°C. There are about 26,000 inhabitants in the district who predominantly practice fishing. They also practice animal husbandry and grow seasonal crops.

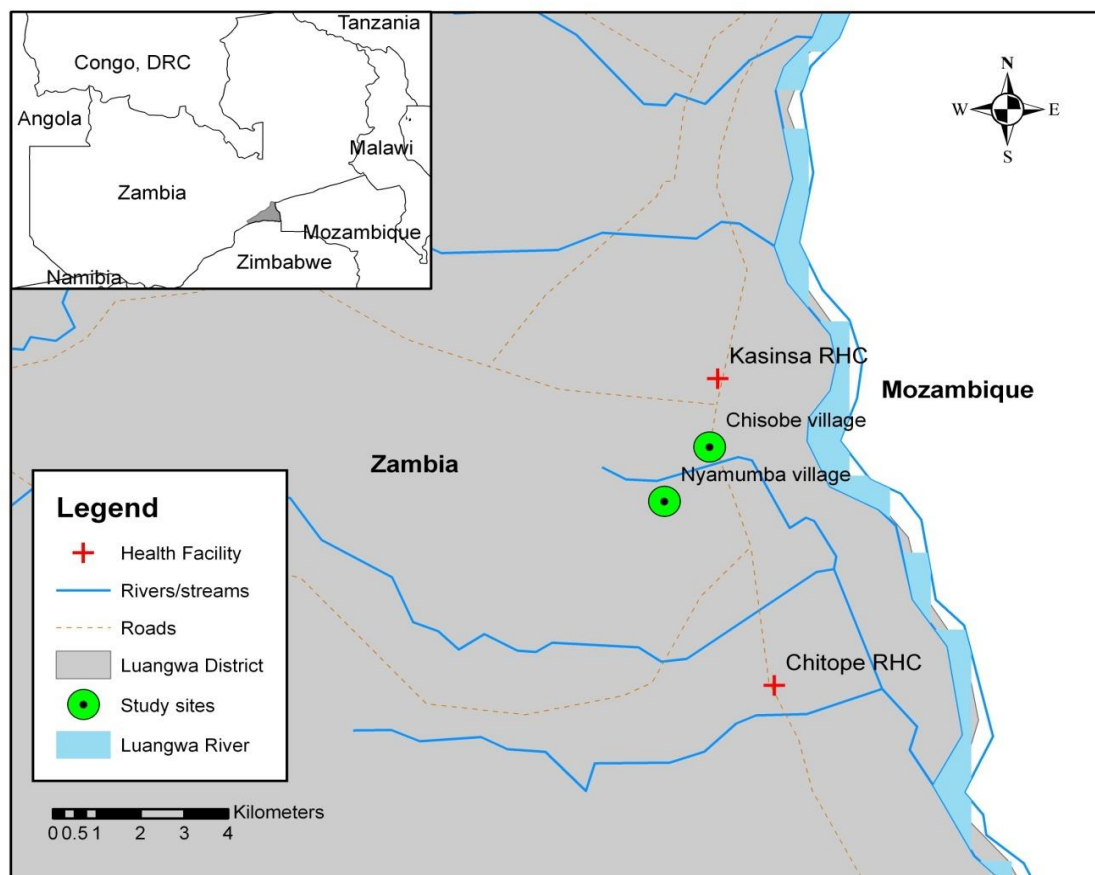


Figure 2.1: Location of study site (Chisobe and Nyamumba) in Luangwa district

2.2.2 Study design

The study was conducted during two intervals chosen within the dry and wet seasons, specifically from September to October 2009 and from February to March 2010, respectively. A 3×3 Latin Square design was used for the rotated assignment of mosquito sampling methods to experimental units (houses). In each village (Chisobe and Nyamumba), two groups of three houses which were clustered together and identified as distinct experimental blocks with one group comprising those containing LLINs whilst the other also had LLINs but were also treated

with IRS. IRS was applied using a deltamethrin formulation (K-Othrine® WG 250, Bayer Environmental Sciences) at a rate of 20mg of active ingredient per m² by an experienced spray operator trained at the National Malaria Control Centre (NMCC).

At the time of the experiment, the only major intervention in the district was the use of PermaNet 2.0® LLINs (Vestergaard Frandsen SA) distributed through mass distribution campaigns and ante-natal clinics by the Ministry of Health and its partners. As IRS was not an intervention implemented in the district at that time, we therefore purposely sprayed only the selected houses in the LLINs plus IRS blocks to conform to the study design.

Each block was treated as a self-contained trio of numbered (1, 2 and 3) houses in which a Latin Square rotation sequence was followed throughout the study period. In each of the blocks, the first treatment comprised the HLC conducted both indoors and outdoors and was randomly assigned to one of the numbered houses. The second treatment consisted of a CDC light trap beside an occupied LLIN inside the house, with an ITT-C (Elastic Products Manufacturing Co. Ltd, 67 Bibi Titi Mohamed Road, P.O. Box: 20872, Dar es Salaam, United Republic of Tanzania) placed approximately 5 metres outside of the house, and was assigned to the next highest number. The ITT-C is a canvas tent trap which is about 2000mm long, 1000mm wide and 1250mm high with six funnel-shaped mosquito entrances which enables entry while restricting mosquito exit (Govella, Moore et al. 2010). Two netting compartments are 700mm apart and have sealable cotton sleeves to enable the collection of mosquitoes while avoiding bites. The

collecting chambers are further supported with two strings to avoid collapse and further human-mosquito contact. The third treatment consisted of two resting boxes (one placed indoors and the other outdoor) and a window exit trap and was assigned to the next highest number. These collection methods have been described in detail in a similar study conducted in urban Tanzania (Govella, Chaki et al. 2011). Each of the three sets of indoor and outdoor collection methods was rotated through the three different houses in increasing order according to their assigned house number, for three consecutive nights in each of 10 rounds, to achieve a balanced data set reflecting an equal number of samples from each treatment-house combination, and time period (rounds). This series of 10 rounds of Latin Squares rotations in 4 blocks over a period of 30 consecutive nights was conducted in both the dry and wet seasons. To compensate for the relative attractiveness of individuals to mosquitoes (Lindsay, Adiamah et al. 1993; Knols, de-Jong et al. 1995) as a confounding factor, the same individual volunteers, who were retained in each house for the duration of the study, were exchanged between indoor (HLC or CDC-LT) and outdoor (HLC or ITT-C) stations each night in a crossover design. For the third treatment, where no human-baited outdoor catches were conducted, both volunteers slept within the house if they were from the same household, otherwise only the volunteer who owned the house and who subsequently conducted HLC indoors and slept under an LLIN when applying CDC-LT occupied the house. In order to ensure comparability, all methods for trapping host-seeking mosquitoes were conducted from 19:00hrs to 07:00hrs and all the RBs and WET were emptied at 07:00hrs after operating for 12hrs using hand held aspirators as described by Sikulu *et al.* (2009). Collections from the hourly catches from each catcher conducting HLC were placed in separate cups. Individuals collecting mosquitoes by HLC

were allowed to rest for 15 minutes in each hour of collection. Approximately 20 minutes were required to aspirate mosquitoes from each of the ITT-C, CDC-LT, RBs and the WET methods. A team of supervisors conducted random and regular on spot checks to ensure that acceptable standards of execution were maintained by the volunteers.

2.2.3 Mosquito processing

Mosquitoes were collected from each trap and identified in the field. Female *Anopheles* mosquitoes were identified to species morphologically (Gillies and Demeillon 1968) and preserved individually in silica gel. Male anophelines were only identified, recorded and discarded so they did not form any part of the analysis. *An. gambiae* sensu lato and *An. funestus* sensu lato samples were preserved for circumsporozoite ELISA for infectivity rates (Burkot, Williams et al. 1984) and Polymerase Chain Reaction (PCR) for species identification (Scott, Brogdon et al. 1993; Koekemoer, Lochouarn et al. 1999). Approximately 83% (1387) and 11% (932) of all the specimens which were morphologically identified as *An. gambiae* s.l. and *An. funestus* s.l., respectively, were analysed to determine species identity by PCR (Scott, Brogdon et al. 1993; Koekemoer, Lochouarn et al. 1999) and those which successfully amplified were used for further circumsporozoite ELISA analysis (Burkot, Williams et al. 1984). All identified culicine mosquitoes were recorded as either male or female and discarded.

2.2.4 Data Analysis

All the data were entered using the 2007 Microsoft Excel version. Analysis was performed following the Generalized Linear Mixed Models (GLMM) using R software version 2.15.1. augmented with the Matrix, lattice and lme4 packages. Mixed effects models were used so that fixed effects variables could be used to estimate the effect of factors of interest while accounting for repeated measurements and the influence of other variables such as date and household with many levels as random effects.

2.2.5 Relative abundance, mean catches and sensitivity per sampling method

The relative catches of the female *An. quadriannulatus*, *An. funestus*, and other anopheline and culicine mosquitoes by the different mosquito sampling methods, as compared to the reference method (HLC-indoor), were analysed by fitting GLMMs as follows. The number of catches of the specific mosquito taxon was treated as the dependent variable, to which a Poisson distribution with a logarithm link function was applied. The sampling method, village, treatment (LLINs alone versus LLINs plus IRS) and season were fitted as fixed effects while date (d.f. = 60) and household (d.f. = 12) were treated as random effects. The exponential of the parameter estimates (and 95% confidence intervals) for each method was calculated to represent the relative rate of catching mosquitoes compared to the standard reference method (HLC indoor). We calculated the mean by fitting GLMM with the sampling method treated as a categorical factor and both date and house as random effects using a Poisson distribution with logarithm link function and determined as described above. Similarly, we used the outputs from GLMM

model to test for and quantify the effect of treatment, season and village on the abundance of mosquitoes of different taxa.

2.2.6 Influence of indoor residual spraying upon the numbers of human-feeding *An. funestus* caught by all sampling methods

In order to analyse the effect of treating a house with IRS upon house entry and feeding on humans by mosquitoes, we fitted GLMMs with Poisson distribution, treating the number of mosquitoes caught with each trapping method in each house and station (in versus out) as the dependent variable and IRS treatment status, village and season as fixed effects. Household (d.f. =12) and date (d.f. =60) were treated as random effects.

2.2.7 Influence of sampling method on the proportion of all *An. quadriannulatus* and *An. funestus* caught which were fed

In order to analyse the effect of trapping method upon the proportion of mosquitoes which had fed, we applied binomial logistic regression by fitting a GLMM with a logit link function for the proportion of fed female mosquitoes caught by each method, defined by the total number of fed mosquitoes as the numerator and the total catch of all female mosquitoes of all physiological status as the denominator. Abdominal status was classified as either fed (partly fed and fed) or unfed (unfed, partly gravid and fully gravid) and the fixed effects included

village, season, and IRS treatment status while date and household were included as random effects.

2.3 Results

2.3.1 Mean catches and relative sensitivities of alternative sampling methods in relation to indoor HLC

Summary of total catches, mean catches per trap night and relative sensitivities of alternative sampling methods in relation to HLC indoor are indicated in Table 2.3.1. A total of 19664 female mosquitoes were caught in 60 sampling nights, with 7.4% comprising *An. gambiae* s.l., 38.9% *An. funestus* s.l., 22.6% other anophelines and 31.1% culicine mosquitoes. The other anophelines constituted mainly *An. coustani*, *An. pretoriensis*, *An. squamosus* and *An. rufipes*. Out of the 932 (11%) specimens of *An. funestus* s.l. that were tested by PCR, only 47% (n = 440) successfully amplified. Most of the successfully tested mosquitoes were identified as *An. funestus sensu stricto* (72.2%, n = 317) with the remainder being *An. rivulorum* (16.2%, n = 71), *An. parensis* (9.8%, n = 43), *An. vaneedeni* (1.4%, n = 7) and *An. lessoni* (0.5%, n = 2). The low amplification rate for the *An. funestus* group may be as a result of the limitation of the PCR assay used, which only works for a subset of 4 out of 9 species within the group (Koekomer et al., 1999). So while at least some modest proportion of the remaining 53% that failed to amplify was a consequence of technical reasons of DNA quality and quantity or technician error, it is also probable that many of these specimens were never possible to amplify in the first place because they are other species, specifically *An. aruni*, *An. confusus*, *An. brucei* or *An.*

fuscivenosis. From the total of 1387 (83%) *An. gambiae* s.l. specimens tested by PCR, 1169 (85%) successfully-amplified. The vast majority were *An. quadriannulatus* (95.2%, n = 1112) with only a very small number of *An. arabiensis* (3.9%, n = 46) and *An. gambiae sensu stricto* (0.9%, n = 11). In subsequent analysis, we therefore report results for the *Anopheles funestus* group and the *An. gambiae* complex as approximately representing *Anopheles funestus* s.s. and *An. quadriannulatus*, respectively. *Anopheles rivulorum* (18.3%, n = 13) and *An. funestus* s.s. (2.2%, n = 7) were the only species from the *An. funestus* group, or any other *Anopheles* taxon, found to be infected with *P. falciparum* sporozoites. However, none of these specimens were re-tested following heating of the homogenates, so the possibility of exaggerated sporozoite prevalence due to false positives, for *An. rivulorum* in particular, cannot be excluded (Durnez, Van Bortel et al. 2011).

Statistical estimates of the magnitude and significance of differences in relative rates at which each trapping method captured mosquitoes are presented in Table 2.3.1. Of all the alternative methods, only CDC-LT performed better than HLC indoor for sampling both *An. quadriannulatus* and *An. funestus*, being over one and a half times more sensitive for both species. For *An. funestus*, ITT-C placed outdoors exhibited over three fourths the sensitivity of HLC and may therefore be useful for trapping this malaria vector species. However, for *An. quadriannulatus*, other anophelines and culicines, indoor CDC-LT proved the only reasonably sensitive alternative to HLC. For culicines, indoor CDC-LT exhibited more than three fourths the sensitivity of HLC which yielded approximately equal catches indoors and outdoors. While the ITT-C was the only

alternative method other than CDC-LT that caught any useful numbers of culicines, it exhibited quite low sensitivity and might have limited utility for this important taxon that transmits a wide range of parasites and viruses of public health importance. ITT-C also exhibited extremely poor sensitivity for *An. quadriannulatus* and other anophelines. However, the RBs and the WET sampled much lower catches for all the mosquito taxa. Mosquitoes were observed on several occasions escaping from the RBs placed outdoors at sun rise prior to collection time.

Table 2.3.1: Number of mosquitoes caught by different sampling methods for 240 trap nights each and their relative rates in reference to the human landing indoor as determined by fitting generalized linear mixed models^a.

Sampling method	Catch ^b		Relative Sensitivity ^c	
	Total	Mean [95% CI]	RR [95% CI]	P value
<i>Anopheles quadriannulatus</i>				
HLC indoor	405	1.687 [1.531, 1.860]	1.00 ^d	NA ^e
HLC outdoor	242	1.004 [0.885, 1.139]	0.597 [0.509, 0.700]	< 0.001
CDC light trap	784	3.267 [3.046, 3.504]	1.873 [1.653, 2.122]	0.997
Ifakara tent trap – C	21	0.088 [0.057, 0.134]	0.050 [0.032, 0.078]	< 0.001
Window exit trap	1	0.004 [0.001, 0.030]	0.002 [0.000, 0.015]	< 0.001
Resting boxes indoor	0	NE ^f	NE ^f	NE ^f
Resting boxes outdoor	2	0.008 [0.002, 0.033]	0.004 [0.001, 0.016]	< 0.001
<i>Anopheles funestus</i>				
HLC indoor	1749	7.287 [6.954, 7.637]	1.00 ^d	NA ^e
HLC outdoor	1635	6.784 [6.463, 7.121]	0.928 [0.868, 0.993]	0.030
CDC light trap	2630	10.958 [10.547, 11.385]	1.532 [1.441, 1.628]	< 0.001
Ifakara tent trap – C	1410	5.875 [5.576, 6.190]	0.821 [0.765, 0.881]	< 0.001
Window exit trap	71	0.296 [0.234, 0.373]	0.040 [0.032, 0.051]	< 0.001
Resting boxes indoor	38	0.158 [0.115, 0.218]	0.022 [0.016, 0.030]	< 0.001
Resting boxes outdoor	110	0.458 [0.380, 0.553]	0.063 [0.052, 0.076]	< 0.001
<i>Other anophelines</i>				
HLC indoor	1661	8.046 [7.695, 8.413]	1.00 ^d	NA ^e
HLC outdoor	2064	9.685 [9.300, 10.086]	1.207 [1.137, 1.287]	< 0.001
CDC light trap	661	2.754 [2.552, 2.972]	0.337 [0.308, 0.369]	< 0.001
Ifakara tent trap – C	28	0.117 [0.081, 0.169]	0.014 [0.010, 0.021]	< 0.001

Window exit trap	7	0.029 [0.014, 0.061]	0.003 [0.002, 0.007]	< 0.001
Resting boxes indoor	4	0.017 [0.006, 0.044]	0.002 [0.001, 0.005]	< 0.001
Resting boxes outdoor	20	0.083 [0.054, 0.129]	0.010 [0.006, 0.015]	< 0.001
<u>Culicine species</u>				
HLC indoor	1971	8.296 [7.939, 8.668]	1.00 ^d	NA ^e
HLC outdoor	1921	8.033 [7.683, 8.399]	0.971 [0.912, 1.0349]	0.349
CDC light trap	1782	7.425 [7.088, 7.778]	0.871 [0.817, 0.930]	< 0.001
Ifakara tent trap – C	369	1.538 [1.388, 1.703]	0.180 [0.161, 0.202]	< 0.001
Window exit trap	54	0.225 [0.172, 0.294]	0.025 [0.019, 0.033]	< 0.001
Resting boxes indoor	6	0.025 [0.011, 0.056]	0.003 [0.001, 0.006]	< 0.001
Resting boxes outdoor	18	0.075 [0.047, 0.119]	0.008 [0.005, 0.013]	< 0.001

^a As described in the method section, village, season and treatment were all included as fixed effects while household and date as random effects. In sampling *An. quadriannulatus*, both village and treatment did not significantly affect ($P = 0.894$ and 0.0845 respectively) the catches of mosquitoes by all methods. The catches of *An. funestus* were also significantly affected by village ($P = 0.004$) and treatment ($p = 0.011$). The catches of other anophelines and culicines were not significantly affected by village ($P = 0.268$ and 0.265) and treatment ($P = 0.717$ and 0.721) respectively. The catches of all the mosquito taxa were significantly affected by season ($P < 0.001$).

^b Mean and 95% confidence interval (CI) estimated by fitting generalised linear mixed models as described above except that only method, date and house were included in a model without intercept..

^c Sensitivity of the sampling method catch with reference to HLC placed indoors (RR indicate Relative Rate).

^d Reference method.

^e Not applicable.

^f Not estimable due to no mosquito catch.

2.3.2 Influence of indoor residual spraying on the catches of *An. funestus* by all sampling methods

While no community-level effect of IRS upon vector densities was expected with only 3 houses in each of the two villages having been treated with IRS using deltamethrin, some degree of personal and household protection arising from deterrent effect of such pyrethroids upon house entry might be expected based on previous studies (Okumu and Moore 2011). However, supplementation of LLINs with IRS had no influence on the catches of *An. funestus* by indoor HLC ($P = 0.270$), outdoor HLC ($P = 0.242$) and CDC-LT ($P = 0.229$) placed indoors. While IRS appeared to increase catches in ITT-C placed outdoors (RR [95%CI] = 1.399 [1.016, 1.929], $P = 0.040$), this apparent effect is most likely spurious, arising from the relatively small number of houses assigned to each treatment.

2.3.3 Influence of sampling method on the proportion of all fed *An. quadriannulatus* and *An. funestus* captured

All specimens of *An. quadriannulatus* caught with RBs placed outdoors had previously fed. HLCs, CDC-LT and ITT-C each collected less than a third of the fed *An. quadriannulatus* while RBs placed indoors and WETs caught none (Table 2.3.2). Out of the total number of fed *An. quadriannulatus* caught, the proportion fully fed only exceeded 80% for the ITT and the RB placed outdoors, while both HLCs and the LTs collected higher proportions of partly fed mosquitoes (Table 2.3.3). However, RBs placed both indoors and outdoors collected high proportions of fed *An. funestus*. Approximately over a third of *An. funestus* mosquitoes caught

by HLC and WET had fed. The former is a remarkably high proportion for a sample of host-seeking vectors and it is reassuring that this proportion is reduced in samples from both ITT-C and CDC-LT that are assumed to protect the human participant from exposure to the collected mosquitoes (Table 2.3.2). Furthermore, of the total proportion fed *An. funestus* collected by ITT, about two thirds were partly fed, while other sampling methods captured proportionally high numbers of fully fed mosquitoes (Table 2.3.3).

Table 2.3.2: Influence of sampling method on the proportion of all *An. quadriannulatus* and *An. funestus* captured that were fed.^a

Sampling method	Percentage (Proportion fed)	OR ^b [95% C.I.]	P value
<u><i>Anopheles quadriannulatus</i></u>			
HLC indoor	24.4 (99/405)	1.00 ^c	NA ^d
HLC outdoor	29.3 (71/242)	1.900 [1.253, 2.881]	0.003
CDC light trap	12.9 (101/784)	0.417 [0.292, 0.596]	< 0.001
Ifakara tent trap – C	28.6 (6/21)	1.251 [0.430, 3.642]	0.682
Window exit traps	(0/1)	NE ^e	NE ^e
Resting boxes indoor	(0/0)	NE ^e	NE ^e
Resting boxes outdoor	100 (2/2)	NE ^e	NE ^e
<u><i>Anopheles funestus</i></u>			
HLC indoor	34.8 (608/1749)	1.00 ^c	NA ^d
HLC outdoor	37.2 (608/1635)	1.188 [1.017, 1.387]	0.030
CDC light trap	20.6 (541/2630)	0.543 [0.467, 0.633]	< 0.001
Ifakara tent trap – C	14.1 (199/1410)	0.261 [0.215, 0.317]	< 0.001
Window exit trap	38.0 (27/71)	1.086 [0.643, 1.835]	0.758
Resting boxes indoor	73.7 (28/38)	4.486[2.059, 9.776]	< 0.001
Resting boxes outdoor	72.7 (80/110)	5.899[3.688, 9.434]	< 0.001

^a As described in the method section, village, season and treatment were all included as fixed effects while household and date as random effects. The proportions of fed *An. quadriannulatus* and *An. funestus* were not affected by village, season and treatment ($P > 0.05$).

^b Odds Ratio represents the relative probability of sampled mosquitoes being fed compared to the reference indoor HLC method.

^c Reference method

^d Not applicable

^e Not estimable due to small or no numbers observed or no mosquito catches.

Table 2.3.3: Crude estimates of the numbers and proportions of *An. quadriannulatus* and *An. funestus* captured which were fed, partly fed or unfed, broken down by sampling method.

Sampling method	Numbers caught					Proportion of fed (%)	
	unfed	Partly fed	Fully fed	Total fed	gravid	Partly fed	Fully fed
<u><i>Anopheles quadriannulatus</i></u>							
HLC indoor	306	79	20	99	0	79.8	20.2
HLC outdoor	171	50	21	71	0	70.4	29.6
CDC LT	683	90	11	101	0	89.1	10.9
ITT	15	1	5	6	0	16.7	83.3
WET	1	0	0	0	0	0.0	0.0
RB indoors	0	0	0	0	0	0.0	0.0
RB outdoors	0	0	2	2	0	0.0	100.0
<u><i>Anopheles funestus</i></u>							
HLC indoor	1137	262	346	608	4	43.1	56.9
HLC outdoor	1024	295	313	608	3	48.5	51.5
CDC LT	2078	224	317	541	11	41.4	58.6
ITT	1204	132	67	199	7	66.3	33.7
WET	40	9	18	27	4	33.3	66.7
RB indoors	9	4	24	28	1	14.3	85.7

2.4 Discussion

Amongst the methods that capture host-seeking mosquitoes, the CDC-LT placed near an occupied net compares well with HLC. This observation is consistent with many reports from elsewhere in the tropics in sampling various pathogen-carrying mosquito species (Odetoyinbo 1969; Mbogo, Glass et al. 1993; Githeko, Service et al. 1994; Shiff, Minjas et al. 1995; Laganier, Randimby et al. 2003; Sithiprasasna, Jaichapor et al. 2004; Dia, Diallo et al. 2005; Okumu, Kotas et al. 2008; Govella, Chaki et al. 2009; Dusfour, Carinci et al. 2010; Stoops, Gionar et al. 2010; Govella, Chaki et al. 2011; Duo-quan, Lin-hua et al. 2012) except for an evaluation in Dar es Salaam, Tanzania which showed very poor sensitivity of CDC-LT in this urban environment. While previous studies were limited to *An. gambiae* s.s. and *An. arabiensis* in Tanzania (Govella, Chaki et al. 2009; Govella, Moore et al. 2010; Govella, Chaki et al. 2011), this is the first report showing that ITT-C appears to be a useful option for sampling host-seeking *An. funestus* in an external trial site in Zambia. This species is among the most important malaria vectors in Africa generally and Zambia specifically, and it is notable that the ITT-C sampled considerably more *An. funestus* than any other mosquito taxon in this study. This is particularly noteworthy because ITT-C is the only sampling tool that has yet been successfully applied through quality assured community-based trapping schemes with epidemiological predictive power as a malaria risk indicator (Chaki, Mlacha et al. 2012). ITT-C might, therefore, be applicable as an option for programmatic use across much of Africa where *An. funestus* is an important vector of malaria (Sinka, Bangs et al. 2010; Sinka, Bangs et al. 2012). Nevertheless, the poor sensitivity ITT-C exhibited for culicines, *An. quadriannulatus* and other anophelines suggests caution, and that it requires evaluation across a broader diversity of contexts before it can be recommended

for wide spread use. Indeed it has recently been emphasised that there is a great need to consistently compare sampling methods across diverse transmission patterns in Africa and that such comparative evaluations are conspicuous by their absence from the literature (Kelly-Hope and McKenzie 2009). Critically, this study used a very similar design to that previously implemented in Dar es Salaam, so that the two evaluations from two very different contexts can be directly compared.

The observation by Govella *et al.* (Govella, Chaki et al. 2011) that houses have many, highly variable entry and exit points, was also noted in our study area and might well explain the very low sensitivity of WET. The poor sensitivity of RBs is most likely explained by the fact that they represent too small a proportion of the total suitable resting surface area available to mosquitoes indoors and especially outdoors. Outdoor resting tools are also prone to natural mosquito predators which may contribute to the low catches (Seyoum, Sikaala et al. 2012) and mosquitoes also tend to leave when illumination increases as sunrise approaches. While other reports describe useful sensitivity levels of boxes (Harbison, Mathenge et al. 2006; Kweka, Mwang'onde et al. 2009) and pots (Odiere, Bayoh et al. 2007) as resting traps, our observation that both the RB and WET methods exhibited poor sensitivities for sampling all mosquito taxa are consistent with some previous reports from neighbouring Tanzania (Sikulu, Govella et al. 2009; Govella, Chaki et al. 2011). Much of the dramatic drop in capture efficacy reported by these recent studies in Zambia (Table 2.3.1) and Tanzania (Govella, Chaki et al. 2011), relative to previous reports from Kenya (Harbison, Mathenge et al. 2006; Odiere, Bayoh et al. 2007) and

Tanzania (Kweka, Mwang'onde et al. 2009) may well be explained by the presence and coverage levels of insecticidal nets. Given that insecticide-treated nets are estimated to prevent an average of 93% of exposure for people sleeping under them (Okumu and Moore 2011), it is inevitable that this study and a similar recent one in Tanzania in which all occupants used nets (Govella, Chaki et al. 2011) both report far lower catches in resting traps than host-seeking traps because only a small minority of host-seeking mosquitoes will successfully survive, acquire a blood meal and consequently rest in the same house they entered.

However, this cannot entirely explain the comparatively low numbers of mosquitoes caught with resting boxes ($\leq 1\%$ sensitivity relative to HLC for all taxa except *An. funestus*). In the case of the WET, any deterred mosquitoes are readily available for capture upon exit, as demonstrated by recent trials of completely intact nets in experimental huts combining baffled entry points with comprehensive exit trapping of all remaining eaves and windows (Okumu, Moore et al. 2012; Okumu, Mbeyela et al. 2013). Fundamental limitations of sampling sensitivity of these RBs and WET formats are therefore probably important so more sensitive approaches such as PSC (Service 1993) and backpack aspirators (Maia, Robinson et al. 2011) should be evaluated in a similarly standardised way. While these resting traps may be useful for some applications in some settings, the inferring quantitative levels of human exposure based on absolute numbers of mosquitoes caught may not be reliably recommended or readily interpreted in a standardized way. However, it is crucial to consider whether the focus of a given entomological survey is to quantify human exposure, understand vector resting

behaviour, or identify blood meal sources of fed mosquitoes when selecting appropriate sampling tools (Service 1993). Therefore sensitivity may not be the most important criterion in many cases.

In our study site, *An. quadriannulatus* appears to be the predominant species amongst the *An. gambiae* complex and was caught more indoors than outdoors by the CDC-LT and HLC methods. While these results seem unexpected because *An. quadriannulatus* is usually associated with outdoor biting and a preference for non-human hosts (Gillies and Demeillon 1968; Laganier, Randimby et al. 2003), it does occasionally bite people (Pates, Takken et al. 2001; Torr, Della Torre et al. 2008) but is thought to contribute negligibly to malaria transmission (Pates, Takken et al. 2001; Torr, Della Torre et al. 2008). Torr and colleagues (2008) showed that, when humans are indoors, their odour attracts more zoophilic species than those stationed outdoors and this may partially explain the results obtained in this study (Torr, Della Torre et al. 2008). The high numbers of *An. quadriannulatus* caught indoors here may also result from the fact that, apart from the catcher, other household inhabitants were present but covered with nets inside these homes, whereas the human single baits collected outdoors were alone. While the preference of *An. funestus* for feeding indoors was statistically significant (Table 2.3.1), it was quantitatively very small and of little biological significance as appears to be the case for most malaria vector populations in Africa (Huho, Briet et al. 2013). The vast majority of human exposure occurs indoors in this setting, and elsewhere in Africa (Huho, Briet

et al. 2013) simply because the peak hours of *An. funestus* biting activity coincide with almost all humans going into their houses to sleep (Seyoum, Sikaala et al. 2012).

Although IRS treatment of houses which already had LLINs appeared to have no impact on the catches of *An. funestus* across all trap types, it appeared to increase catches by ITT-C placed outdoors. This presumably spurious result probably arises from the small number of houses assigned to each treatment because it is inconsistent with results reported here for the gold-standard HLC method and reported previously using logistic models of the proportion of mosquitoes caught indoors rather than outdoors at a given house (Seyoum, Sikaala et al. 2012). So overall, it is notable that IRS with deltamethrin had so little apparent impact on house entry and subsequent host attack rates. This observation is consistent with a number of recent experimental hut evaluations (Ngufor, N'Guessan et al. 2011; Briet, Smith et al. 2012; Okumu, Moore et al. 2012) of modern pyrethroid formulations, confirming that this intervention format provides little direct protection to individual households and acts exclusively through community-level suppression of vector populations and malaria transmission. High coverage of houses within a community is therefore needed to reduce density and survival of *An. funestus* populations; so that even people living in unsprayed houses experienced reduced vector densities and malaria transmission exposure.

It is worth noting that whilst large numbers of both *An. quadriannulatus* and *An. funestus* s.l. were caught indoors by the CDC-LT and HLC, the majority that had fed were sampled by the

sampling methods placed outdoors. It is disconcerting that 24 to 37% of mosquitoes caught by the HLC methods, especially *An. funestus*, were blood fed. Presumably most of these either partially fed elsewhere before landing on the human bait to complete the blood meal, or obtained the blood meal from the human bait conducting the HLC. This supports the efforts to search for safer alternatives because these findings suggest that the catchers may have lacked concentration due to exhaustion and were therefore bitten extensively. High proportions of fed mosquitoes were also sampled in the RBs indoors and outdoors because these represent artificial resting places for mosquitoes, which rest most during the gestation phase of their life cycle. The lower proportions of fed *An. funestus* that were sampled by the ITT-C and fed *An. quadriannulatus* that were sampled by CDC-LT (Table 2.3.2) suggest that these methods do protect the human participants acting as bait and confirm the findings of Govella et al. (2011) in an urban Tanzanian setting (Govella, Chaki et al. 2011). It is possible that the substantial proportions of fed *An. funestus* and *An. quadriannulatus* in the ITT-C could have used the tent trap as an alternative resting place after feeding elsewhere or were simply attracted to the host for further feeding after being partially fed elsewhere. This appeared to be the case for *An. funestus*, of which less than 70% of those that had fed were partly fed, while the case is less clear for *An. quadriannulatus* because over 80% of the fed specimens were fully fed. However, we could not substantiate this because our study did not include host blood meal analysis.

Despite these ambiguities and study limitations, these experiments do demonstrate the importance of evaluating the efficacy of alternative exposure-free sampling tools for routine

monitoring of malaria transmission, in comparison with each other and with gold standard HLC in different settings. It further highlights the need to specifically evaluate sampling methods based on their ability to selectively trap either host-seeking, exiting, or resting mosquitoes, and to capture them with sufficient sensitivity relative to absolute house entry and host attack rates within houses.

Only a subset of *An. gambiae s.l.* and *An. funestus s.l.* specimens that were successfully identified to species by PCR were further analysed for the presence of circumsporozoite protein by ELISA in this study. In retrospect, this can be considered a substantial limitation of the study. Future work to investigate the roles of all vectors in transmission should probably take the opposite approach, by testing for sporozoite infection among all *Anopheles* specimens and then identifying all confirmed positives by PCR, or even by DNA sequencing where necessary.

2.5 Conclusions

Although CDC-LT seems to be the most sensitive option for trapping host-seeking mosquitoes in this setting, the continuous need to recharge batteries might be challenging for surveillance systems in rural communities, particularly where electricity is not readily available. This may pose particular challenges for routine programmatic monitoring applications outside of research studies, notably community-based trapping schemes with little supervision and only occasional quality assurance (Chaki, Mlacha et al. 2012). The ITT-C appears to offer a

reasonable alternative that does not depend on electrical power. However, its bulkiness could be a significant disadvantage that may limit its application in routine malaria surveillance systems, especially community-based schemes with little or no motorized transport. While RBs collect high proportions of fed mosquitoes, they have very low relative sensitivity in comparison with host-seeking methods, so similarly standardized evaluation of more promising methods for capturing resting mosquitoes, such as mechanized aspirators (Maia, Robinson et al. 2011) and pyrethrum spray catch (Service 1993) should be considered. The efficacy of neither CDC-LT nor ITT-C appears to be affected by the application of pyrethroid-based IRS to houses already containing LLINs.

CHAPTER THREE

AN AFFORDABLE, COMMUNITY-BASED MOSQUITO TRAPPING SCHEME THAT CAPTURES SPATIAL AND TEMPORAL HETEROGENEITIES OF MALARIA TRANSMISSION IN RURAL ZAMBIA

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3.1 Abstract

Background

Monitoring mosquito population dynamics is essential to guide selection and evaluation of malaria vector control interventions but is typically implemented by mobile, centrally managed teams who can only visit a limited number of locations frequently enough to capture longitudinal trends. Community-based (CB) mosquito trapping schemes for parallel, continuous monitoring of multiple locations are therefore required that are practical, affordable, effective, and reliable.

Methods

A CB surveillance scheme with a monthly sampling and reporting cycle for capturing malaria vectors, using Centers for Disease Control and Prevention light traps (LT) and Ifakara Tent Traps (ITT), were conducted by trained community health workers (CHW) in 14 clusters of households immediately surrounding health facilities in rural south-east Zambia. At the end of the study, a controlled quality assurance (QA) survey was conducted by a centrally supervised expert team using human landing catch (HLC), LT and ITT to evaluate accuracy of the CB trapping data. Active surveillance of malaria parasite infection rates amongst humans was conducted by CHWs in the same clusters to determine the epidemiological relevance of these CB entomological surveys.

Results

CB-LT and CB-ITT exhibited relative sampling efficiencies of 39 and 9%, respectively, compared with QA surveys using the same traps. However, cost per sampling night was lowest for CB-LT (\$13.6), followed closely by CB-ITT (\$18.0), both of which were far less expensive than any QA

survey (HLC: \$138, LT: \$289, ITT: \$269). Cost per specimen of *Anopheles funestus* captured was lowest for CB-LT (\$5.3), followed by potentially hazardous QA-HLC (\$10.5) and then CB-ITT (\$28.0), all of which were far less expensive per specimen caught than QA-LT (\$141) and QA-ITT (\$168). Time-trends of malaria diagnostic positivity (DP) followed those of *An. funestus* density with a one-month lag and the wide range of mean DP across clusters was closely associated with mean densities of *An. funestus* caught by CB-LT ($P < 0.001$).

Conclusions

CB trapping schemes appear to be far more affordable, epidemiologically relevant and affordable than centrally supervised trapping schemes, and may well be applicable to enhance intervention trials or even enable routine programmatic monitoring of vector population dynamics on unprecedented national scales.

3.2 Background

Despite the impressive successes of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) which selectively target malaria vectors when they feed or rest inside human habitations, these front line vector control tools have rarely achieved complete elimination of malaria outside of areas that had marginal transmission levels to begin with (Moonen, Cohen et al. 2010; Killeen, Seyoum et al. 2013; Smith, Cohen et al. 2013). These fundamental limits of what can be achieved with IRS or LLINs are primarily defined by the behavioural traits of mosquitoes (Ferguson, Dornhaus et al. 2010; Griffin, Hollingsworth et al. 2010; Durnez and Coosemans 2013; Eckhoff 2013; Govella, Chaki et al. 2013; Killeen 2013; Killeen, Seyoum et al. 2013; Russell, Beebe et al. 2013), most of which appear to have always been present in these populations (Ferguson, Dornhaus et al. 2010; Durnez and Coosemans 2013; Govella, Chaki et al. 2013; Killeen 2013; Killeen, Seyoum et al. 2013) so they are better described as pre-existing behavioural *resilience* (Figure 1.4A) (Govella, Chaki et al. 2013; Killeen 2013). On the other hand, recent modelling analyses (Killeen and Chitnis 2014) have illustrated how apparently altered distributions of feeding times and locations following scale-up of LLIN or IRS cannot be simply explained in terms of deferred feeding by hungry mosquitoes and may represent emergence of selected, heritable behavioural *resistance* in the strict sense (Figure 1.4B) (Govella, Chaki et al. 2013; Killeen 2013). Furthermore, resurgent malaria has been repeatedly associated with, not only failures of implementation and funding for vector control programmes, but also with emergence of physiological resistance to insecticides (Cohen, Smith et al. 2012). It is therefore crucial to distinguish between such fundamental *limitations* of a given vector control strategy, reflecting incomplete but nevertheless valuable levels of

sustainable impact (Figure 1.4A), and a genuine *failure* of an intervention programme that results in *rebounding* vector populations and malaria transmission (Figure 1.4B).

The only way in which suppression or resurgence of malaria transmission can be unambiguously attributed to the success or failure of interventions to control responsible vectors will be to monitor their population dynamics longitudinally. Currently, across sub-Saharan Africa, almost all monitoring of vector populations is limited to detecting physiological resistance to prioritize optimal selection of active ingredients for intra-domiciliary insecticidal-based interventions. It has therefore been suggested that robust longitudinal sentinel surveillance systems need to be established so that national malaria control programmes (NMCPs) can continually monitor physiological and behavioural traits, and assess their relevance to intervention selection, by evaluating their impact upon the population dynamics of target vector species (Gatton, Chitnis et al. 2013; Govella, Chaki et al. 2013).

However, the cost of implementing adult mosquito surveillance through conventional teams of specialist entomologists may be prohibitive in impoverished African countries (Mukabana, Kannady et al. 2006; Chaki, Mlacha et al. 2012). Conventional longitudinal entomological monitoring strategies rely operationally upon trained specialist technical staff managed centrally usually by academic and research institutions, so they are usually limited in both their geographic scope and the frequency of sampling at any survey location. The availability and

cost of the expert human resources required to sustain such specialist teams is also limiting (Killeen, Tanner et al. 2006; Mukabana, Kannady et al. 2006; Chaki, Mlacha et al. 2012). Mosquito species composition, abundance and transmission potential is not only altered by successful implementation of vector control measures (Gatton, Chitnis et al. 2013; Govella, Chaki et al. 2013; Killeen, Seyoum et al. 2013; Russell, Beebe et al. 2013), it also varies dramatically geographically and seasonally. It is therefore difficult to envision how conventional, centralized entomological surveillance teams could capture such spatial and temporal patterns in a representative manner on national scales because they simply cannot reach all sentinel survey locations often enough to provide a robust representation of longitudinal trends at each one.

Decentralized systems that adapt affordable, effective trapping methods to local, longitudinal application by resident community-based (CB) staff therefore represent an attractive alternative (Mukabana, Kannady et al. 2006; Chaki, Mlacha et al. 2012). Implementation of CB trapping schemes presents two important challenges: 1) selection of traps, and protocols for their use, that are safe practical and convenient enough for CB staff to apply them reliably in the absence of daily supervision, and 2) independent quality assurance (QA) of this unsupervised surveillance process so that the accuracy and limitations of the derived data can be quantified as a prerequisite to critical interpretation. To date, however, only one CB mosquito-trapping scheme, designed to support a municipal-scale, larval, source management programme in Dar es Salaam in Tanzania, has been critically evaluated through both QA of the

derived entomological data and appraisal of its epidemiological relevance in terms of its ability to predict malaria infection risk among humans (Chaki, Mlacha et al. 2012). This first validated CB trapping scheme was also more sensitive, in terms of total numbers of mosquito caught, than the centrally supervised scheme used to conduct QA, because it was much more intensive and at the same time spatially extensive (Chaki, Mlacha et al. 2012). Furthermore, CB trapping results in Dar es Salaam were predictive of malaria risk infection amongst humans despite the fact that vector populations were remarkably sparse in this low transmission urban area (Chaki, Mlacha et al. 2012). However, the generalizability of this study to a wider variety of settings is not only limited by its local geographic scope, but also by the fact that it relied on entirely upon a locally designed Ifakara Tent Trap (ITT) (Govella, Chaki et al. 2009; Govella, Moore et al. 2010) because this was shown to be the only safe, sufficiently sensitive capture method in this context where *Anopheles gambiae* is the predominate species maintaining transmission (Govella, Chaki et al. 2011).

Over the last decade, Zambia has made substantial progress toward implementing an ambitious strategic plan aiming to protect every at-risk individual in the country against malaria with either LLINs or IRS (NMCC 2012). As insecticide resistance has now been clearly identified within the country (Chanda, Hemingway et al. 2011; Thomsen, Strode et al. 2014), it is essential to develop a sustainable platform to monitor vector species composition, behaviour and transmission capacity on a national scale for the first time. Recent comparative evaluations of various mosquito-trapping methods, in rural south-east Zambia (Sikaala, Killeen et al. 2013)

where malaria transmission is primarily maintained by *Anopheles funestus* demonstrated that the Centers for Disease Control and Prevention miniature Light Trap (LT) and ITT (Govella, Moore et al. 2010) both performed reasonably well as methods for capturing host-seeking mosquitoes and also suggested that they could be applied across a much larger geographic area through a more practical and scalable CB system. This manuscript describes an evaluation of the applicability of CB trapping schemes, using these two candidate capture methods, to assess their effectiveness for sampling malaria vectors across different times and locations, as well as their overall cost effectiveness and ability to predict human malaria infection risk in the same rural Zambian transmission system (Sikaala, Killeen et al. 2013).

3.3.0 Methods

3.3.1 Study area

The study was conducted in Luangwa and Nyimba districts, located approximately 255 km and 325 km, respectively, east of Lusaka, the capital city of Zambia (Figure 3.1). There are about 25,000 and 85,000 inhabitants in Luangwa and Nyimba, respectively, who predominantly practice seasonal farming, fishing and animal husbandry as their primary livelihood (Hamainza, Moonga et al. 2014). Malaria prevalence in this part of Zambia ranges from 9 to 22%, with by far the lowest prevalence in the flat sandy southern half of Luangwa (NMCC 2013). Within this study area, intensive monitoring of malaria infection among the humans, and of human-biting mosquito densities in and around their houses, was carried out on a monthly survey cycle by

resident community health workers (CHWs) in 14 population clusters centred around health facilities between January 2011 and March 2013 (Hamainza, Moonga et al. 2014).

Between 2005 and 2012, Luangwa and Nyimba districts received repeated mass distributions of LLINs, complemented by routine distribution through antenatal clinics. As a result, 66 and 43%, respectively of children under five years of age in Luangwa and Nyimba reported using a net the previous night by 2010 (MoH 2010). IRS was implemented between October and November 2010, using deltamethrin (K-Othrine WG® 250, Bayer Environmental Science, South Africa) in the south of Luangwa district. At the same time of year in 2011, some of these villages in southern Luangwa district were sprayed with lambdacyhalothrin (Icon® 10 Capsule Suspension (CS) formulation, Syngenta Crop Protection AG, Switzerland) while others, as well as several in Nyimba district, were sprayed with an emulsifiable concentrate (EC) formulation of the organophosphate pirimiphos methyl (Actellic® EC, Syngenta Crop Protection AG, Switzerland) to mitigate against the resistance to pyrethroids among *An. funestus* populations in the area (Chanda, Hemingway et al. 2011). A year later 2012, the same regime was applied except that the selection of villages sprayed with pirimiphos methyl in Nymiba was changed and, in some of them, the EC formulation was replaced with a micro-encapsulated formulation of the same active ingredient (Actellic® 300SC, Syngenta Crop Protection AG, South Africa).

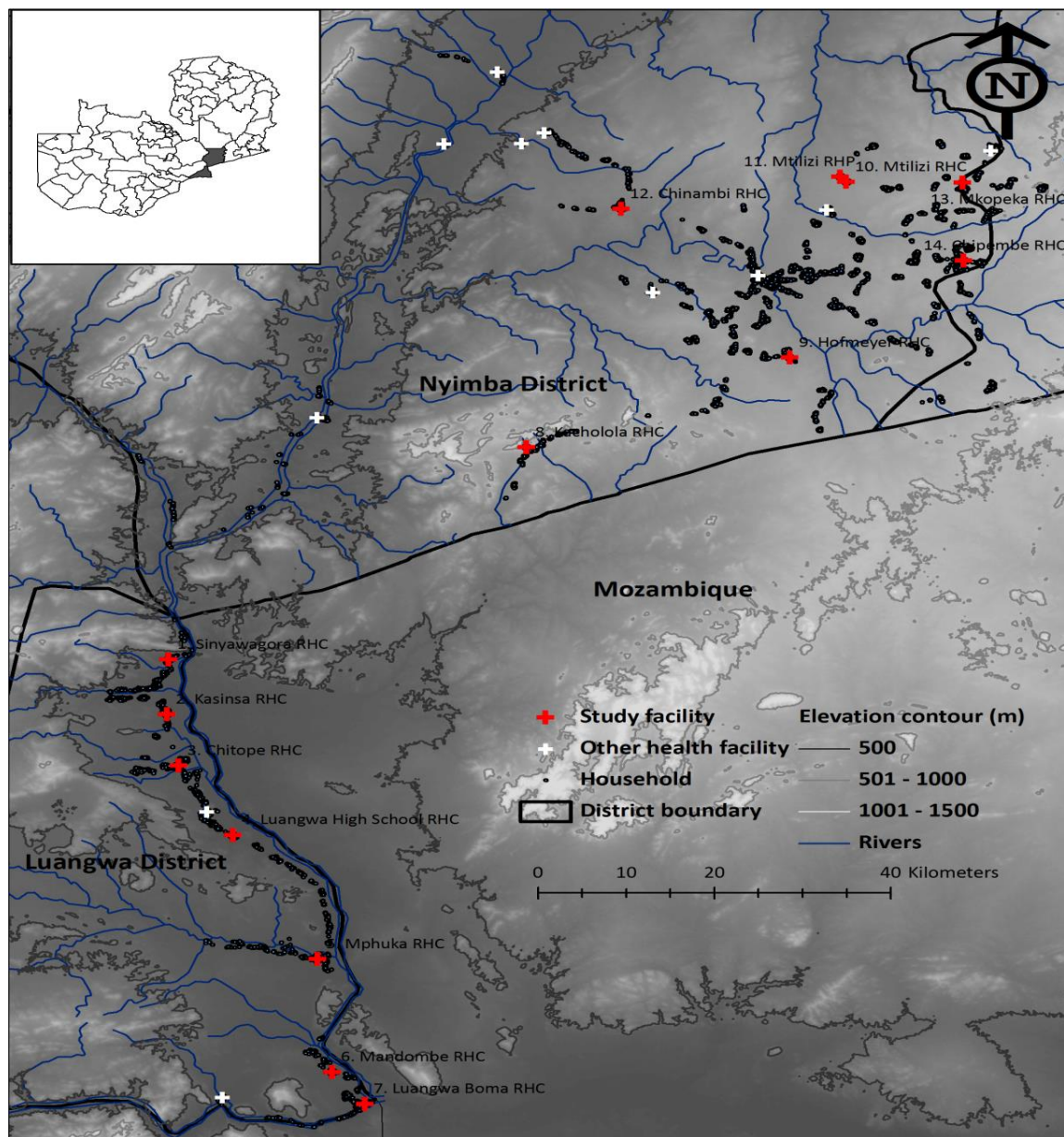


Figure 3.1: Location of study site, and numbered survey clusters around health facilities, in Zambia.

3.3.2 Longitudinal malaria parasites surveillance in human population

Fourteen population clusters of approximately 1,000 residents were selected in both districts (seven per district), each centred around a public sector health facility, in which each household was visited monthly by a CHW offering testing and treatment for malaria (Hamainza, Moonga et al. 2014). Three CHWs were recruited for this task, of which two enrolled approximately 60 households while the third, who was also responsible for CB mosquito trapping as described below, enrolled 45 households for parasitological surveillance. CHWs enlisted households in order of their proximity to the health facility and collected small finger-stick blood samples from all consenting and assenting household members who were present on a designated date each month for each household and tested on the spot using the MAL Pf® Rapid Diagnostic Test (RDT) kit (ICT Diagnostic, Cape Town, South Africa) that detects histidine rich protein-2 (HRP-2) antigen. All individuals that tested positive in the field for the presence of malaria parasite antigen were provided with artemether-lumefantrine (Coartem®, Novartis Pharma AG, Basel, Switzerland) free of charge in accordance with the national guidelines. Between these active visits, individuals who felt sick or had symptoms were encouraged to seek medical care from their assigned CHW or the nearest health facility so cases were also detected passively. All the recruited CHWs were remunerated as casual labourers at a rate of ZMW 350 (\$66.9) per month. Mean diagnostic positivity rates of residents in individual clusters tested during monthly activity visits to their households ranged from 6 to 47% with an overall mean of 20.3% (11,851/58,500) across all age groups (Hamainza, Moonga et al. 2014), approximately consistent with the recent National Malaria Indicator Survey (NMCC 2013) that describes a

mean infection prevalence of 19.5% in cross sectional household surveys in the rural districts of Zambia.

3.3.3 Community-based mosquito-trapping scheme

In order to assess the effectiveness of CB surveillance of adult mosquito populations, one of the three CHWs in each cluster (specifically the one with 45 households to survey) had additional training in basic entomology. One exception was at Luangwa High School (cluster 4) where two out of the three CHWs were engaged in conducting entomological surveillance of adult mosquito populations and one covered 45 while the other 60 households in the surveys of infection among the human residents. Fifteen houses per cluster for mosquito trapping were selected semi-arbitrarily to be well distributed across the cluster, with the exception of Luangwa High School where this figure was doubled to 30 due to the involvement of an additional CHW in mosquito trapping. Therefore, the targeted number of trapping nights per house per month was one. The cluster, village, and household codes, and household owner name for each household were recorded for all 299 households where CB surveys of mosquitoes were conducted. A consistent date of the month for mosquito trapping using the LT and ITT at each house was pre-agreed with each household head. The LTs were placed inside the house on the foot end of an occupied sleeping space already covered with LLIN at a height of approximately 1.5 m above the floor whilst an adult male from the same household occupied an ITT placed immediately outside, approximately 5 m away from the house where the LT was installed. The only occasion volunteers were replaced was in cases of illness, resignation or unreliability. Due to the inconvenience of the bulkiness of the ITT (Chaki, Mlacha et al. 2012;

Sikaala, Killeen et al. 2013) CHWs were provided with spare parts to maintain their bicycles to facilitate transport of the traps from one household to another during the study period. Mosquito traps were set up in the evenings and captured mosquitoes were collected by aspiration as early as was convenient the next morning. CHWs were trained to sort mosquitoes to genus level by eye, to store them over silica, and to keep it desiccated. *Anopheles* specimens were stored individually in 1.5 ml microcentrifuge tubes while culicines were pooled in ziplock bags. Based on this crude morphological classification, the numbers of mosquitoes caught were recorded on a simple form by the CHW.

A team from the centralized National Malaria Control Centre (NMCC) entomological team collected the mosquito samples from all of the clusters once per month and delivered them to the central laboratory at the NMCC in Lusaka. At the central laboratory, anopheline mosquito samples were subjected to further morphological identification (Gillies and Coetzee 1987) and the data entered into an Excel sheet. Then the *An. gambiae* complex and *An. funestus* group were taken to the molecular laboratory for further analysis and long-term storage. CB mosquito trapping was conducted continuously from January 2011 to March 2013 in Luangwa and from April 2011 to March 2013 in Nyimba district.

3.3.4 Quality assurance surveys of the community-based trapping

In order to assess the validity of the CB trapping schemes using the LTs and ITTs, a QA team was assembled towards the end of the study to determine how closely the numbers of mosquitoes caught by the CB staff mirrored those of carefully conducted surveys by specialist technicians in the approximately the same time and place. This team was recruited selectively from among the most experienced CHWs who were involved during the previous trap efficacy study in Chisobe village of Luangwa district. None of these team members had any other responsibilities within this particular study and were supervised by a technical team of trained entomologists from the central level at NMCC.

To validate the CB trapping schemes, the QA team visited the same households that the CB team had placed their traps a day or two earlier. The trapping efficacy of LT and ITT applied by the QA team, and their efficiency and effectiveness as applied by the CHWs, were compared with the gold standard human landing catches technique (HLC) (Silver and Service 2008) conducted by one male adult volunteer indoors and another outdoors. As described above, every month on a date that was pre-agreed with the household owner, the CB team placed the LT indoors and ITT outdoors, and then at the next household on the schedule the following day. The QA team followed this sequence but delayed by a day or two to enable them have at least two houses to re-survey that the CHW had surveyed no more than three days previously. The QA team conducted HLC indoors and outdoors in one of the two houses while the other was surveyed with LT indoors and ITT outdoors. During this process a QA team member slept in the

ITT. On the following day, the pair of participants conducting HLCs would remain in the same house but would apply the LT and ITT methods, while the other pair also stayed in the same house as the previous night but applied HLC. Therefore, each cluster was visited for at least one night by the QA team with a lag of only a day or two after CB catches in or around the same houses. The only exception was cluster 10 which the QA team never visited because the households were closely situated to those of cluster 11. Therefore only one of the two clusters was sampled for convenience. A specific form was used to record the data including the cluster name, village name, household code, household owner name, date, and trapping method. All trapping methods were applied by the QA teams between 19:00 and 07:00 hours. The CHWs were informed in advance about the QA team so that they could conveniently get consent from the household owners for the additional days of mosquito collections.

For QA surveys, samples of mosquitoes were collected and morphologically differentiated to genus level individually in the field. Female *Anopheles* mosquitoes were further separated, recorded and preserved individually in microcentrifuge tubes over desiccated silica gel. All males were recorded and discarded.

3.3.5 Mosquito processing in the laboratory

Further morphological identification of *Anopheles* to species group or complex (Gillies and Coetzee 1987) was conducted at the NMCC main laboratory. Female *Anopheles* samples were

processed for detection of circumsporozoite protein ELISA (Burkot, Williams et al. 1984), including confirmation following boiling of the head-thorax homogenates to prevent false-positives (Durnez, Van Bortel et al. 2011), and polymerase chain reaction (PCR) identification of species within the *An. gambiae* complex (Scott, Brogdon et al. 1993) or *An. funestus* group (Koekemoer, Lochouart et al. 1999).

3.3.6 Data analysis

Data were entered using Microsoft Excel 2007 and analysed using R statistical analysis software version 2.15.1, augmented with lattice, matrix and lme4 packages. To estimate the relative trapping efficiency of the different trapping schemes, a generalized linear mixed model (GLMM) was fitted using the number of mosquitoes of a given taxon as the Poisson-distributed dependent variable and trapping scheme as a categorical independent variables with five levels (CB-LT, CB-ITT, QA-HLC, QA-LT and QA-ITT). In order to account for spatial and temporal heterogeneity, as well as for over dispersion, date, as well as households nested within sub-villages were treated as random effects. To ensure full comparability, data from the CB surveys collected more than seven days before or after a survey by the QA team in the same cluster were excluded from this analysis, so this comparison relates only to selected observations from the last three months of the study when both surveys were operational and overlapped in space and time.

Further, in estimating how *An. funestus* abundance predicts malaria infection risk among the human population, a GLMM was fitted with R statistical software augmented as above, with RDT results as the binomial dependent variable while the base 10 logarithm of the mean *An. funestus* catch per LT for each cluster, estimated from the Poisson model described above, was included as a continuous independent variable. Note however, that to obtain specific estimates of the mean catches of *An. funestus* at each cluster, the model described above has to be modified so that cluster was treated as a categorical variable, rather than a random effect, and no intercept was included so that those estimates would be absolute rather than relative to an arbitrary reference group. Age categories of RDT-tested participants and date were treated as random effects and, to avoid any confounding effects household clustering would have on the *An. funestus* catch estimates, individual households were included nested within clusters as random effects. Data selected for this analysis of the dependence of malaria infection risk upon vector densities were restricted to the period from the onset of the study in January 2011 to September 2011 to avoid any confounding effects that the introduction of IRS in October and November 2011 would have on the densities or infection prevalence.

3.3.7 Cost analysis

This QA exercise was conducted for only the three final months of the study (February to April, 2013) in 13 of the 14 clusters, each of which was visited at least once using motorized transport provided to the QA team for that period. The government employed technical team members and the driver received their normal *per diems* during this period, which were ZMW500 (\$95.6)

and ZMW300 (\$57.4) per night, respectively. The cost incurred also included vehicle fuel, maintenance and depreciation (purchase cost of \$15,000 depreciated to an expected value of \$2,500 when disposed of by tender after five years of use) as well as the daily remuneration of the CHWs at the rate of ZMW100 (\$19.1) per night of execution of the QA exercise. Whenever QA was conducted in clusters in Nyimba district, accommodation costs were also paid for the CHWs in the QA team because it was impractical for the team to return to their home in Chisobe village (Luangwa) on a daily basis due to the long distance between the districts and the bad terrain between clusters during the rainy season. The CB CHWs received a minimal monthly incentive in form of the monthly remuneration of ZMW350 (\$66.9) agreed upon at the start of the study. In addition to this incentive, the additional costs incurred included provision of field supplies and having their bicycles repaired in order to facilitate their ease of movement and carrying of the traps to the selected households where the trapping surveys took place.

In estimating these costs, the approximated amount of time and efforts spent on each trapping scheme was also factored in the total expenditure to calculate the cost per sampling night as well as collecting a single specimen of *An. funestus*. Consideration was restricted to *An. funestus* because it is overwhelmingly the most important vector mediating malaria transmission in this part of Zambia (Seyoum, Sikaala et al. 2012; Sikaala, Killeen et al. 2013).

3.4 Results

3.4.1 Species composition and abundances

A total of 20,683 female mosquitoes were collected by both the CB and QA sampling schemes in the 3,174 trap nights (Table 3.4.1). Morphological identification showed that the *An. funestus* group and *An. gambiae* complex comprised 34.5% (n = 7,127) and 3.3% (n = 685) respectively, while other anophelines and the culicines mosquitoes comprised 3.2% (n = 661) and 59.0% (n = 12,210) respectively, of the total. Of the 596 specimens that were initially identified as members of the *An. funestus* group by routine morphology, and then also successfully identified to species by PCR, 96.5% (n = 575) were confirmed to be *An. funestus*, with the remainder being *Anopheles rivulorum* (1.8%, n = 11) and *Anopheles lesoni* (1.7%, n = 10), respectively. Densities of the *An. funestus* group, as determined by routine morphological classification can therefore be considered quite reliable of *An. funestus* as a species. All the other anophelines were morphologically identified as *Anopheles coustani* (34.0%; n = 225), *Anopheles pretoriensis* (22.5%; n = 149), *Anopheles rufipes* (19.1%; n = 126), *Anopheles squamosis* (13.2%; n = 87), *Anopheles implexus* (11.0%; n = 73) and one (0.2%) *Anopheles maculipalpis*.

Of the total 550 *An. funestus* s.l. that were tested for circumsporozoite ELISA, only 23 *An. funestus* were detected with *Plasmodium falciparum* sporozoites in their salivary glands, corresponding to a sporozoite rate of 4.2%. This sporozoite infection prevalence is considerably higher than that previously reported from Chisobe (Sikaala, Killeen et al. 2013), presumably

because the period and geographical scope of sampling were far larger and also possibly because levels of insecticide resistance in the area may have increased. The abundance of *An. funestus* s.s., reported here across both districts is approximately consistent with previous studies at one of the clusters in Chisobe (Seyoum, Sikaala et al. 2012; Sikaala, Killeen et al. 2013) and confirms that it is the predominant species sustaining malaria transmission in this part of Zambia.

Table 3.4.1: Total and unadjusted mean catches of malaria vectors and other mosquito species by community-based and quality assured sampling schemes

	Quality assurance				Community- based	
Trapping method:	HLC indoor	HLC outdoor	LT	ITT	LT	ITT
Person trap-nights	20	20	20	317	3171	2195
Number of houses sampled	20	20	20	20	505	432
Mean trap-nights per surveyed house	1.0	1.0	1.0	1.0	6.3	5.1
Mean trap-nights per cluster	1.5	1.5	1.5	1.5	226.6	156.8
<i>Anopheles funestus</i>	174	149	66	46	5,827	865
<i>Anopheles quadriannulatus</i>	10	2	0	0	613	60
Other anophelines	9	26	0	0	591	35
<i>Culex</i> species	426	394	94	82	9,548	1,666
<u>Mean catch of female mosquitoes</u>						
<i>Anopheles funestus</i>	8.7	7.5	3.3	2.3	1.8	0.4
<i>Anopheles quadriannulatus</i>	0.5	0.1	0.0	0.0	0.0	0.0
Other Anophelines	0.5	1.3	0.0	0.0	0.2	0.0
<i>Culex</i> species	21.3	19.7	4.7	4.1	3.0	0.8

3.4.2 Sampling intensity and total catches of community-based trapping

There was some inconsistency in the number of trap-nights of sampling by the CB trapping schemes over the 28 months of mosquito collections in all the clusters in both districts and the scheduled target sampling intensity was only occasionally achieved in Luangwa and never in Nyimba (Figure 3.2). It was only in February 2011 and April 2012 when trap-nights in Luangwa district exceeded the average of 105 trap-nights that had been expected to be attained per month per cluster. Nevertheless, adequate sampling to measure mean mosquito densities was sustained throughout the study. Interestingly, it appears that more trap-nights were conducted during the wet seasons when the CHWs observed increased abundance of *An. funestus* and *Culex* species (Figure 3.2). The overall numbers of person trap-nights conducted by the CB surveys were >100 greater than the QA surveys (Table 3.4.1), not only because the former had far greater numbers of staff operating, each of whom sampled with slightly greater frequency, but also because these were conducted over a much longer period of 28 months while the QA were restricted to the last three months of the study.

3.4.3 Comparison of community-based and quality assurance mosquito trapping surveys

Summaries of the mean number of trap-nights of sampling per household and per cluster surveyed, mean catches and relative rates of capture for each taxon in times and places when both the CB and QA surveys were operational are shown in Table 3.4.2. The total numbers of person trap-nights and mean number of trap-nights per sampled cluster completed by the CB scheme were far higher (Table 3.4.2), despite the fact that inclusion of this data was restricted

to within a week before or after a QA survey in the same cluster, simply because the frequency of sampling with a single, centralized QA team was limited by the practical logistical limitations described in background.

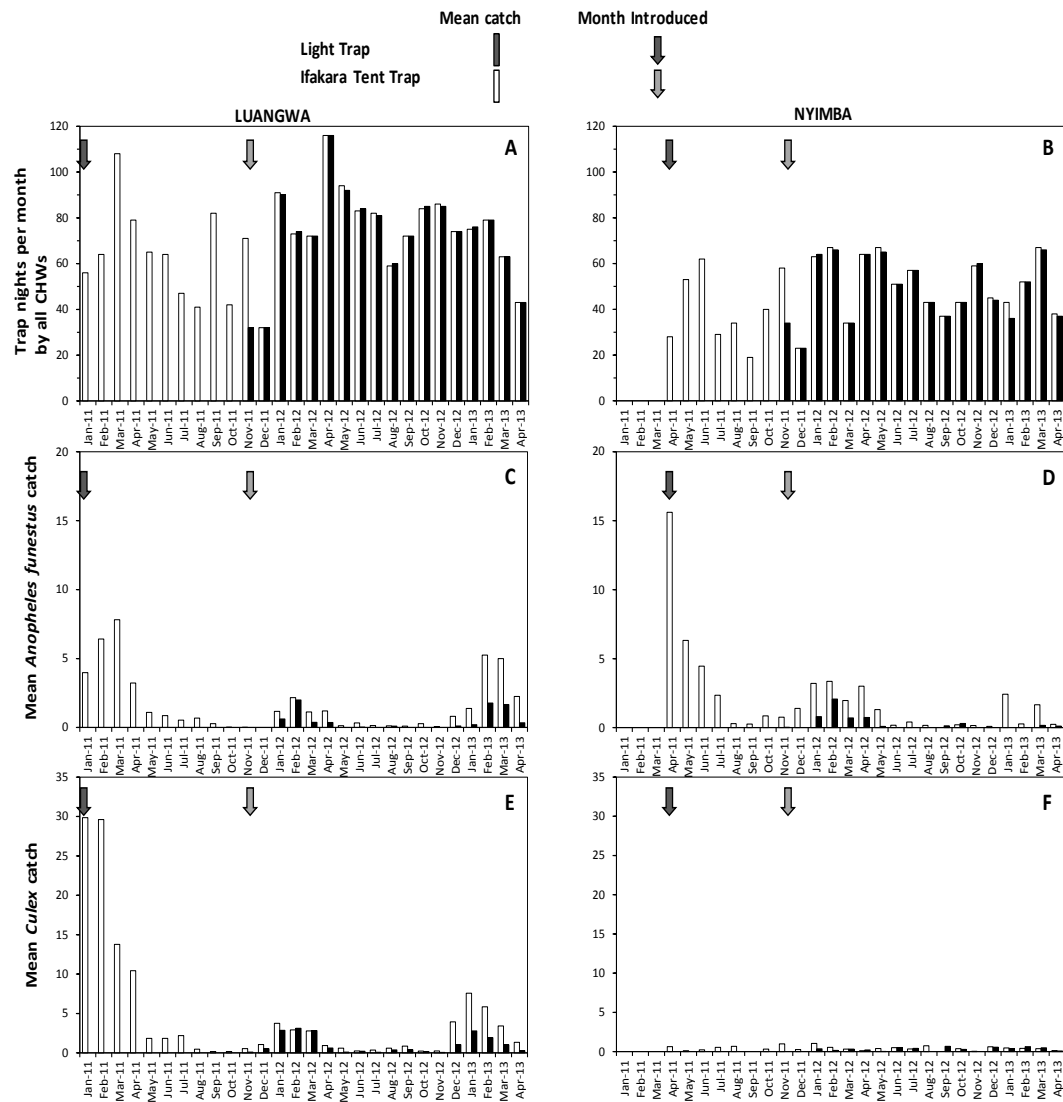


Figure 3.2: Monthly trap-nights of community-based trapping schemes in the 14 clusters (A and B) and mean catches of *Anopheles funestus* (C and D) and *Culex* (E and F) in Luangwa and Nyimba districts

For *An. funestus*, relative rate of capture per trap-night of the CB-LT was only 13% when compared with the indoor HLC, while that of CB-ITT was <3% (Table 3.4.2). However, comparing the CB-LT and the CB-ITT sampling methods with their application through the QA scheme, their relative capture rate per night of trapping was estimated to be 39% (relative rate (RR) [95% confidence interval (CI)] = 0.130 [0.079, 0.212]; $P < 0.001$) and 9% (RR [95% CI] = 0.022 [0.012, 0.041]; $P < 0.001$), respectively. Combined QA surveys with LT and ITT neither captured any *Anopheles quadriannulatus* nor any other anophelines in the three months these were conducted over. The CB-LT captured more *An. quadriannulatus* than any other method, including QA-HLC, but overall numbers of this mosquito were so low that this difference was not significant (Table 3.4.2). Overall, CB trapping with either LT or ITT exhibited relatively low rates of capture compared with QA surveys of HLC and even with the same trapping methods when conducted simultaneously (Table 3.4.2). Comparing the mean catches of *An. funestus* and culicines by both the LTs and the ITT CBs, where paired with their QA counterparts, there appeared to be weak associations for both species of mosquitoes (figure 3.3).

Using the mean *An. funestus* trap catches (M_t) by CB application of LT and ITT, as well as their relative capture rates compared with indoor HLC (λ_t), as estimated by GLMM (Table 3.4.2) and the sporozoite prevalence estimate (S) described in the second paragraph of the results section, entomologic inoculation rates (EIR_t) for each of the two traps of 68.6 and 70.1 infectious bites per unprotected non-net user were calculated ($EIR_t = M_t \times S \times 365 / \lambda_t$) assuming that the vast bulk of exposure of unprotected humans occurs indoors in this setting (Seyoum, Sikaala et al. 2012).

Table 3.4.2: Relative sampling sensitivity of community-based trapping scheme using CDC Light Traps and Ifakara Tent Traps to capture mosquitoes compared with quality assured catches when both operated simultaneously as estimated by generalized linear mixed models

	Quality assurance				Community-based	
Trapping method	HLC indoor	HLC outdoor	LT	ITT	LT	ITT
Person trap-nights	20	20	20	20	82	82
Number of houses sampled	20	20	20	20	76	76
Number of clusters surveyed	13	13	13	13	13	13
Mean trap-nights per surveyed house	1.0	1.0	1.0	1.0	1.1	1.1
Mean trap-nights per cluster	1.5	1.5	1.5	1.5	6.2	16.2
<u>Total catch of female mosquitoes</u>						
<i>Anopheles funestus</i>	174	149	66	46	126	24
<i>Anopheles quadriannulatus</i>	10	2	0	0	63	9
Other anophelines	9	26	0	0	36	1
<i>Culex</i> species	426	394	94	82	224	112
<u>Mean catch [95% confidence interval]</u>						
<i>Anopheles funestus</i>	4.507	3.860	1.498	1.047	0.584	0.101
	[2.115,9.604]	[1.807,8.244]	[0.680,3.300]	[0.468, 2.343]	[0.284, 1.201]	[0.045, .227]
<i>Anopheles quadriannulatus</i>	0.097	0.019	0	0	0.184	0.026
	[0.025, 0.383]	[0.003, 0.127]	[NE]	[NE]	[0.086, 0.394]	[0.010, 0.070]
Other anophelines	0.005	0.014	0	0	0.016	0.000
	[0.001, 0.046]	[0.002, 0.124]	[NE]	[NE]	[0.004, 0.071]	[0.000, 0.005]
<i>Culex</i> species	11.941	11.044	1.743	1.374	0.305	0.146
	[5.186, 27.494]	[4.795, 25.439]	[0.771, 3.943]	[0.604, 3.126]	[0.145, 0.642]	[0.069, 0.312]

Relative rate of capture [95% confidence interval]						
<i>Anopheles funestus</i>	1.00	0.856 [0.688, 1.065]	0.332*** [0.185, 0.596]	0.232*** [0.127, 0.426]	0.130*** [0.079, 0.212]	0.022*** [0.012, 0.041]
<i>Anopheles quadriannulatus</i>	1.00	0.200* [0.042, 0.959]	0 [NE]	0 [NE]	1.885 [0.497, 7.153]	0.266 [0.061, 1.157]
Other anophelines	1.00	2.889** [1.343, 6.213]	0 [NE]	0 [NE]	3.215 [0.355, 29.131]	0.085 [0.004, 1.740]
<i>Culex</i> species	1.00	0.925 [0.807, 1.061]	0.146*** [0.075, 0.283]	0.115*** [0.059, 0.224]	0.026*** [0.014, 0.047]	0.012*** [0.007, 0.023]

* P<0.05 , **P<0.01, ***P<0.001

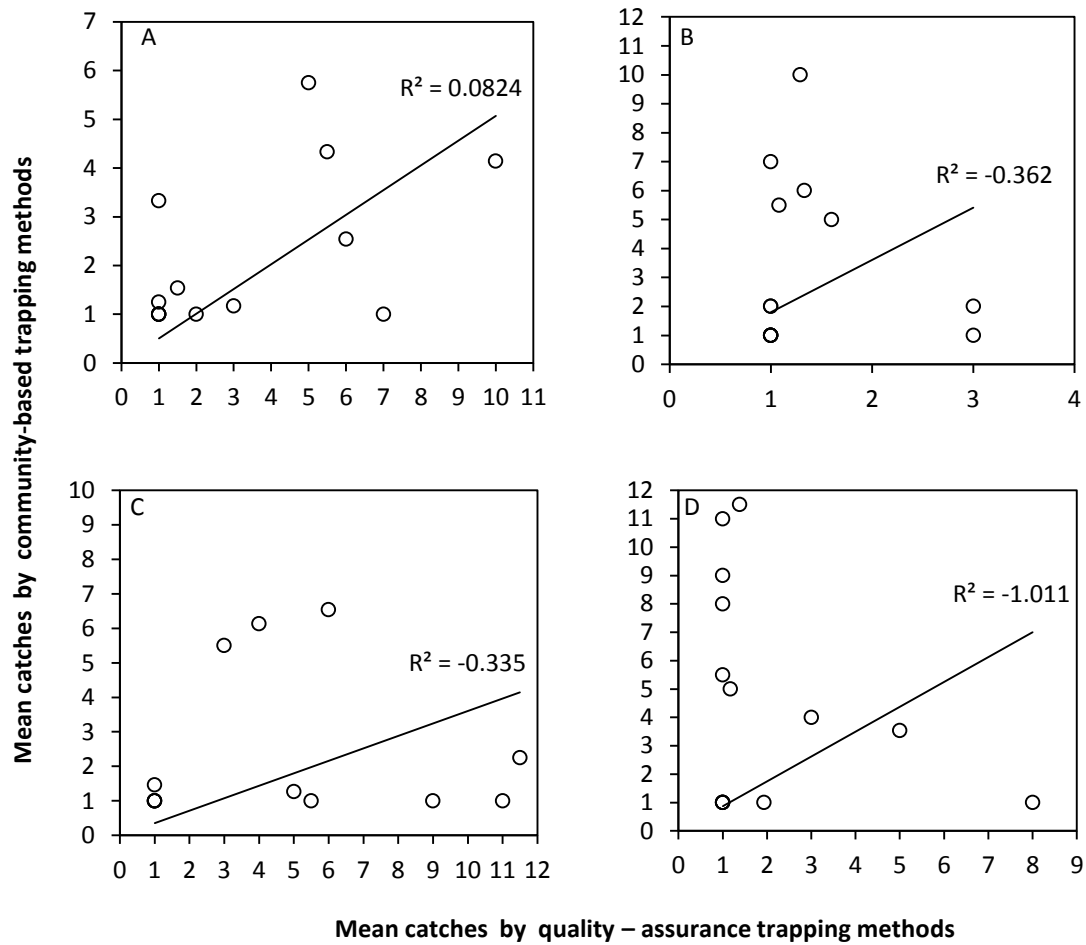


Figure 3.3: Pairwise comparisons, with each point representing the mean catches of *An. funestus* with by Light traps (A), Ifakara tent trap (B) and mean catches of *Culicines* with Light traps (C) and Ifakara tent trap (D) during the same time window.

3.4.4 Cost effectiveness of community-based and quality assurance surveys for capturing *Anopheles funestus*

Results for the HLC placed indoors and outdoors were combined and considered as a single trapping method. Cost per sampling night was lowest for CB-LT, followed by CB-ITT, and then far more distantly by the HLC, ITT and then LT QA surveys, which were all approximately an order of magnitude more expensive than either CB approach (Table 3.4.3). Cost per specimen of *An. funestus* captured was by far the lowest for CB-LT, followed by the potentially hazardous QA-HLC and then CB-ITT which were approximately five and seven times less cost effective, respectively, and then QA-LT and QA-ITT which were both at least an order of magnitude less cost effective than either CB method or QA-HLC (Table 3.4.3).

Table 3.4.3: Crude estimates of the costs per sampling scheme per trap-night and per *Anopheles funestus* caught for the three months when community-based sampling was validated with quality assured sampling schemes

Estimated parameter	Units	Quality assured			Community-based	
		QA-HLC	QA-LT	QA-ITT	CB-LT	CB-ITT
Number of samples	Person-night	40	20	20	249	243
Numbers caught	Number of <i>An. funestus</i>	526	41	32	637	156
Mean caught	Number of <i>An. funestus</i> per person-night	13.2	2.1	1.6	2.6	0.6
Personal costs ^a	\$(ZMW)	2,180(11,401.4)	1,520(7,949.6)	1,076(5,627.5)	2509.4(13,124.2)	2,939.4(15,373.1)
Per diem costs ^b	\$(ZMW)	414(2,165.2)	1,243(6,500.9)	1,243(6,500.9)	621(3,247.8)	621(3,247.8)
Trap depreciation costs	\$(ZMW)	0(0)	87.5(457.6)	125(653.8)	87.5(457.6)	125(653.8)
Transport costs ^a	\$(ZMW)	225(1,176.8)	225(1,176.8)	225(1,176.8)	0(0)	0(0)
Vehicle maintenance costs ^c	\$(ZMW)	212(1,108.8)	211(1,108.8)	212(1,108.8)	71(371.3)	71(371.3)
Vehicle depreciation cost ^d	\$(ZMW)	2,500(13,075)	2,500(13,075)	2,500(13,075)	0(0)	0(0)
Bicycle repair costs ^c	\$(ZMW)	0(0)	0(0)	0(0)	94(491.6)	611(3,195.5)
Bicycle depreciation costs ^d	\$(ZMW)	0(0)	0(0)	0(0)	5(26.2)	5(26.2)
Total expenditure	\$(ZMW)	5,531(28,927.1)	5,788(30,268.6)	5,381(28,142.6)	3,388(17,718.7)	4,372(22,867.7)
Cost per person-night of sampling	\$(ZMW)	138.3(723.2)	289.4(1,513.4)	269.1(1,407.1)	13.6(71.2)	18.0(94.1)
Cost per specimen of <i>An. funestus</i> caught	\$(ZMW)	10.5(55)	141.2(738.3)	168.2(879.5)	5.3(27.8)	28.0(146.6)

^a Cost estimates were based on the approximated time and efforts spent on each trapping method

^b Assumptions made on the salaries paid and *per diem* to the central level teams during their visits

^c Estimated cost incurred for maintaining the equipment for transporting or visiting the trapping schemes per location

^d Monthly depreciation costs calculated when both trapping schemes where operational for three months

\$ - US dollar

ZWK - Zambian Kwacha

Note: 1\$ ≈ ZMK 5.23 which was the average exchange during the midpoint year of 2012

3.4.5 Epidemiological relevance of community-based surveys of *Anopheles funestus*

Figure 3.4 shows how the time-trends of malaria parasitaemia over the course of this period approximately follow those for the mean *An. funestus* catch by the CB entomological surveys. Consistent with previous studies in the area (Keating, Miller et al. 2009) parasite rates were generally much lower in Luangwa than in Nyimba district, with the least transmission recorded in the southernmost corner of the study area, at or near the district capital in Luangwa Boma, and these spatial trends in malaria parasitaemia were clearly associated with *An. funestus* density (Figure 3.5).

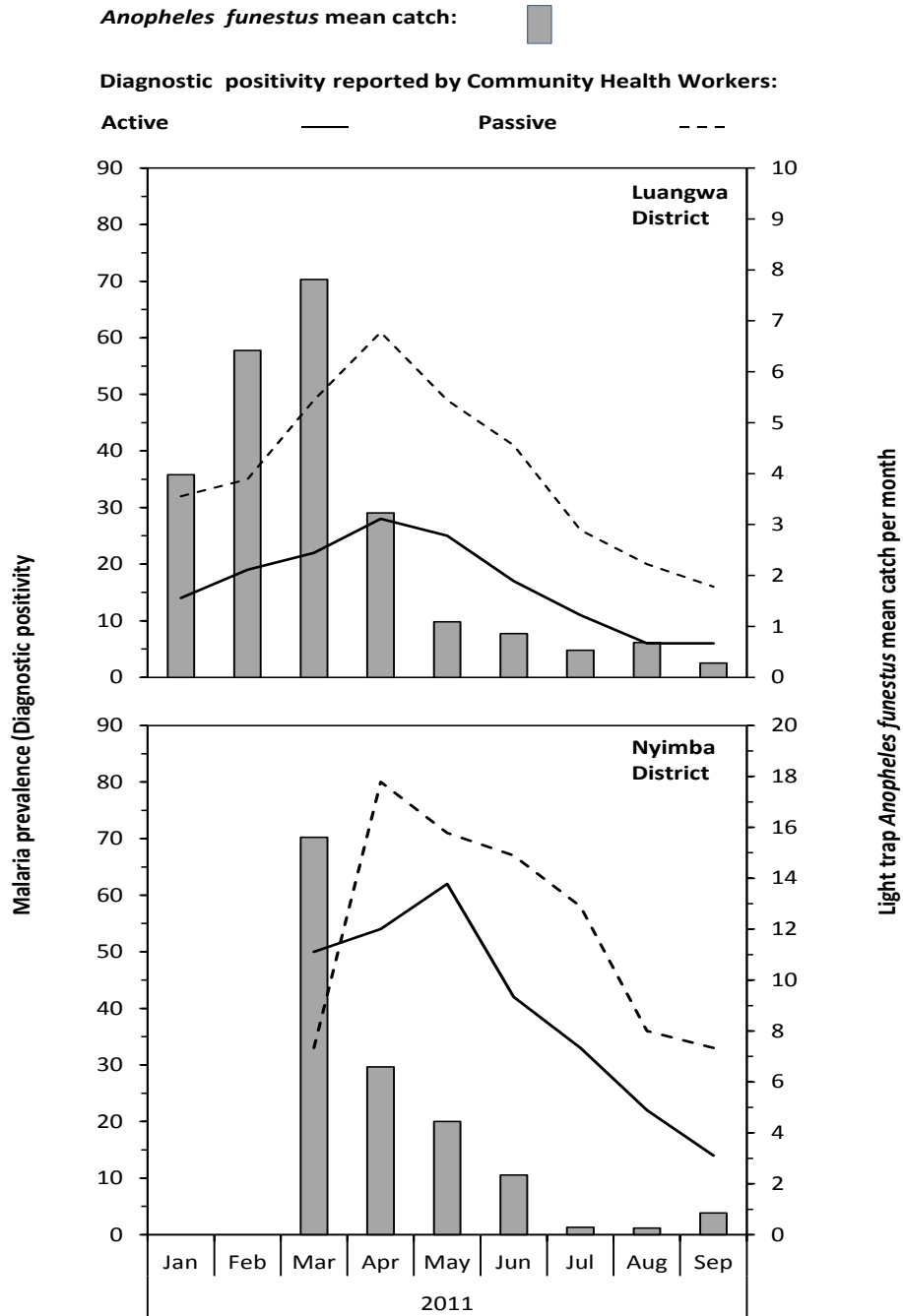


Figure 3.4: Temporal variations of *Anopheles funestus* mean catches by light traps and the malaria diagnostic positivity among human residents from January to September 2011 in Luangwa and Nyimba districts

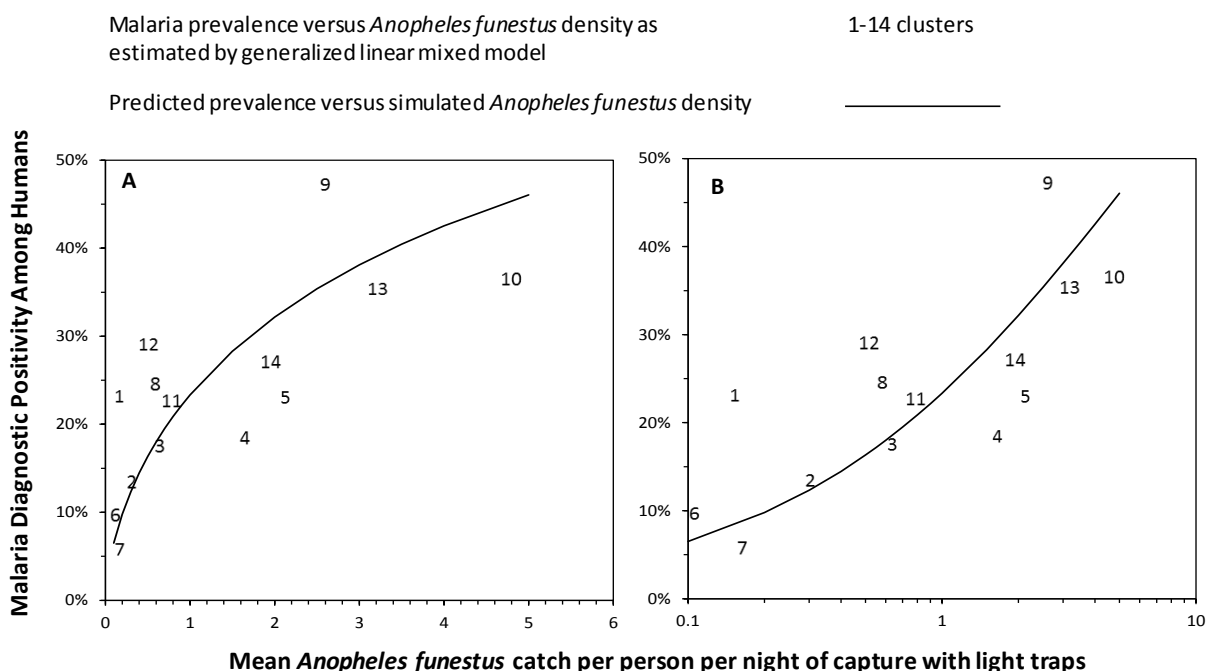


Figure 3.5: Relationship between malaria diagnostic positivity among human residents and mean catches of *Anopheles funestus* per trap night of capture with light traps in each cluster, plotted with a standard (A) and logarithmic (B) horizontal axis.

3.5 Discussion

The CB trapping schemes proved to be far more practical, effective and cost effective for trapping large numbers of *An. funestus* because the higher frequency and overall numbers of mosquito samples collected within each population cluster captures temporal trends with far greater resolution and precision than conventional surveys by centralized teams, as exemplified by the QA surveys described herein. Familiarity of the CHWs with the communities and the collection sites enables convenient, repeated, high frequency trapping in each cluster simply because the CHWs live where they work. Overall, CB trapping with either LT or ITT exhibited

relative low rates of capture per night of sampling compared with HLC, or even with the same trapping methods, implemented by the QA team. While a weak association between trapping schemes for numbers of *An. funestus* captured was obvious (Figure 3.3), this is typical of such comparisons because of the highly variable and aggregated nature of mosquito trapping data generally (Govella, Chaki et al. 2009; Sikulu, Govella et al. 2009; Govella, Chaki et al. 2011; Chaki, Mlacha et al. 2012; Overgaard, Saebo et al. 2012; Kilama, Smith et al. 2014). Nevertheless, CB trapping schemes caught far more mosquito of all taxa simply because these procedures allowed for more intensive sampling of each cluster in terms of trap-nights conducted over the whole period of the study. While longitudinal surveillance CB trapping scheme may not be as sensitive as the gold standard HLC in terms of estimating the absolute biting densities of host-seeking vectors, such assessments merely reflect the efficiency of the trapping method rather than the effectiveness of the system through which they are applied. When evaluated in terms of effectiveness it does appear to represent a far more affordable option for routine vector population dynamics monitoring at programmatic level that yields far more spatial and especially temporal resolution than is otherwise possible to obtain.

It is notable that the estimated efficacy of relative sampling sensitivity of both the LT and ITT compared to HLC, as all these techniques were applied by the QA team while they moved around these two districts during this effectiveness evaluation (Table 3.4.2), were approximately 5- and 4-fold lower than the equivalent measurement obtained by the same team in a single pair of neighbouring villages during the preceding efficacy study (Table 2.3.1). While the GLMMs fitted in both table 3.4.2, and in table 2.3.1 from the preceding efficacy

study, did account for the effects of both spatial and temporal resolution by including both date and location as random effects, these studies were conducted over very different temporal and spatial sampling frames. While a re-analysis of the data in tables 3.4.2 and 2.3.1 was attempted using only data restricted to the same subset of two villages common to both studies, and at the same time of the year from February to March, this yielded only 20 and 120 trap nights of observations from these studies, respectively. A reliable direct comparison, controlling for location and time of year, was therefore not possible. In any case, these inconsistencies between efficacy measurements in the two studies is not entirely surprising given the known imprecision and erratic sampling efficacy of LT (Overgaard et al., 2012; Wong et al., 2013; Govella et al., 2009). These inconsistencies are also of secondary relevance to the main objectives of this study, namely to evaluate the effectiveness, costs and epidemiologic relevance of these mosquito traps as applied under conditions representative of normal programmatic use by CB staff. The relative sampling sensitivity of both the LT and ITT as applied by CB personnel, relative to HLC in QA surveys, was indeed lower than when these same traps were applied by the QA team. However, this is unsurprising because effectiveness under conditions of routine programmatic use is, by definition (Glasgow, Lichtenstein et al. 2003), expected to be lower than efficacy under controlled conditions of optimal use by carefully supervised specialist technicians.

The only previous study to have validated the affordability, accuracy and epidemiological relevance of a CB trapping system relates to a municipal-scale platform for monitoring and evaluation the impact of an urban larviciding programme where *An. gambiae* s.s. is

predominately present (Chaki, Mlacha et al. 2012). The findings reported here further provide evidence of the applicability of the CB trapping schemes in a transmissions system where local vectorial capacity is dominated by *An. funestus*. Unlike the preceding example from an urban Tanzanian setting which necessarily relied on the locally designed and effective ITT (Chaki, Mlacha et al. 2012), this study demonstrates for the first time how solar-recharged LT can be practically applied by CB staff to yield vector density data that predict malaria risk infection in 14 clusters distributed across >14,000 sq km of an isolated part of Zambia (Figures 3.4 and 3.5). The sampled clusters were far too widely distributed across these two districts for the QA team to visit more than once or twice every three months and these same logistical limitations are likely to apply to any centralized QA surveillance system with finite human and financial resources, especially if attempting to monitor vector populations on larger provincial or national scales. While others (Mouatcho et al. 2007, Sharp et al. 2007) used trapping schemes to evaluate large-scale intervention progress, none conducted QA or cost estimates incurred under conditions comparable with programmatic operational conditions. The observations reported here complement these findings and provide another encouraging example of how much can be achieved by imparting basic entomological skills to non-specialist community-based staff and availing them with minimal resources to monitor vector population dynamics and how it responds to control in their own communities.

Like all studies, this evaluation had limitations that merit careful consideration. The QA validation exercise was only carried out for three months during the rainy season so it can only be assumed, rather than proven, that these comparisons are representative of CHW and trap

performance throughout the study. Furthermore, the CHWs were informed approximately one day in advance that the QA team would be coming to visit the cluster so it is possible that they conducted a small proportion of their trapping in that interim period more carefully than they normally would. Future studies of CB trapping schemes, especially those evaluating prototype systems operating at larger scales, should therefore incorporate continuous, randomized and unannounced, if not necessarily as intensive, QA surveys. It was also observed that the CHWs often conducted lower numbers of trap nights of sampling during the dry season when the catches were lowest because they thought it unnecessary to continue collecting even when the catches were often zero. It may therefore be necessary to sensitize CB staff to the critical importance of measuring the low but non-zero vector densities that occur in the dry season, especially in the context of any pre-elimination scenario where supplementary mass drug administration, mass screen and treat, or vector control measures are specifically introduced and evaluated as interventions to achieve termination of local transmission. It may also be useful to introduce on-site support and supervision by locally-employed environmental health technicians, to ensure that the quantity and quality of data that is validated through external QA by NMCC staff are optimized through on-site quality control.

3.6 Conclusions

Despite these study limitations, the prototype CB mosquito trapping scheme evaluated here clearly has considerable potential for improvement and scale-up. It is therefore recommended that future operational studies are undertaken to adapt, optimize and evaluate CB trapping schemes for monitoring mosquito population dynamics at nationally representative scales so that the influence regarding physiological and phenotypic traits as determinants of success, limitations and failures of vector population control can be assessed continuously, indefinitely and sustainably.

INCREMENTAL IMPACT UPON MALARIA TRANSMISSION OF SUPPLEMENTING PYRETHROID-IMPREGNATED LONG-LASTING INSECTICIDAL NETS WITH INDOOR RESIDUAL SPRAYING USING PYRETHROIDS OR THE ORGANOPHOSPHATE PIRIMIPHOSMETHYL

This paper is in preparation for peer review submission to a journal

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4.0 Abstract

Background

LLINs and IRS are the most widely accepted and applied malaria vector control methods. However, evidence that incremental impact is achieved when they are combined remains limited and inconsistent.

Methodology

Fourteen population clusters of approximately 1000 residents in Zambia's Luangwa and Nyimba districts, which had high pre-existing usage rates (81.7%) of pyrethroid-impregnated long-lasting insecticidal nets (LLINs) were quasi-randomly assigned to receive IRS with either of two pyrethroids, namely Deltamethrin (Wettable granules (WG)) and Lambda-cyhalothrin (Capsule suspension (CS)), with an emulsifiable concentrate (EC) or CS formulation of the organophosphate pirimiphosmethyl, or with no supplementary vector control measure. Diagnostic positivity of patients tested for malaria by community health workers in these clusters was surveyed longitudinally over pre and post-treatment periods spanning 29 months over which the treatments were allocated and re-allocated in advance of 3 sequential rainy seasons.

Results

Supplementation of LLINs with pirimiphosmethyl CS offered the greatest initial level of protection against malaria in the first 3 months of application (incremental protective efficacy (IPE) [95% confidence interval (CI)] = 0.63 [CI 0.57,0.69], $P < 0.001$), followed by

lambdacyhalothrin (IPE [95%CI] = 0.31 [0.10,0.47], P=0.006) and pirimiphosmethyl (IPE, 0.23 [CI 0.15,0.31], P<0.001) and then by deltamethrin (IPE [95%CI] = 0.19 [-0.01,0.35], P=0.064). Neither pyrethroid formulation provided protection beyond 3 months after spraying, but the protection provided by both pirimiphosmethyl formulations persisted undiminished for longer periods: 6 months for CS and 12 months for EC. The CS formulation of PM provided greater protection than the combined pyrethroid IRS formulations throughout its effective life IPE [95%CI] = 0.79 [0.75, 0.83] over 6 months. The EC formulation of PM provided incremental protection for the first three months (IPE [95%CI] = 0.23 [0.15, 0.31]) that was approximately equivalent to the two pyrethroid formulations (lambdacyhalothrin, IPE [95%CI] = 0.31 [0.10, 0.47] and deltamethrin, IPE [95%CI] = 0.19 [-0.01, 0.35]) but the additional protection provided by the former, apparently lasted an entire year.

Conclusion

Where universal coverage targets for LLINs utilization has been achieved, supplementing LLINs with IRS using pyrethroids may reduce malaria transmission below levels achieved by LLIN use alone, even in settings where pyrethroid resistance occurs in the vector population. However, far greater reduction of transmission can be achieved under such conditions by supplementing LLINs with IRS using non-pyrethroid insecticide classes such as organophosphates so this is a viable approach to mitigating and managing pyrethroid resistance.

4.1 Background

Long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are the two first-choice malaria vector controls available globally (WHO, 2013) because they can achieve massive (Koella, Sorensen et al. 1998) community-wide impact upon malaria transmission, even at partial coverage (Killeen, Smith et al. 2007). This is possible because many of the world's most potent vector species prefer people as a source of blood and must feed several times upon humans inside houses before they are old enough for infectious sporozoite-stage malaria parasites to have fully developed within them (Koella, Sorensen et al. 1998). While IRS and LLINs decrease exposure of directly protected humans to infected vectors and *vice versa*, through contact irritancy or spatial repellency, most of the impact of LLINs and IRS upon human transmission exposure and parasitaemia, results from community-level suppression of vector population density and infection prevalence, achieved by reducing their longevity through lethal exposure to their toxic active ingredients (Smith and Webley 1968; Lines, Myamba et al. 1987). The success of these modes of action are influenced by the choice, dosage and formulation of insecticide utilized, as well as its coverage and mode of application, combined with the behavioural and physiological susceptibility of the targeted vector species (Dezulueta, Cullen et al. 1963; Grieco, Achee et al. 2007; White, Conteh et al. 2011).

Compared to IRS, LLINs coverage is much higher in most endemic countries (Lengeler 2004; Pluess, Tanser et al. 2010) due to their flexibility of delivery mechanism and cheaper costs of implementation (Bhatia, Fox-Rushby et al. 2004). Also, while most African vector populations

predominantly feed indoors, at night (Huho, Killeen et al. 2012), they may not rest on the walls after a blood meal or rest for a period insufficient to pick up a lethal dose of the active insecticide (Pates and Curtis 2005). However, for LLINs to be fully effective they require deliberate active participation of individuals to use them consistently and appropriately, in addition to them being regularly replaced and kept in good repair (Rehman, Coleman et al. 2011; Mejía, Teklehaimanot et al. 2013). In contrast, IRS requires only initial consent by the community to have their houses sprayed and compliance with not painting or plastering over the sprayed walls for the expected duration of efficacy of the insecticide used. Additionally, a major advantage of IRS over LLINs is simply that the treated surfaces are rarely in direct contact with occupants of protected houses so the safety requirements for active ingredients that may be used are far less stringent and a much wider variety of active ingredients can therefore be used (Rehman, Coleman et al. 2011). The evidence on the effects of combining IRS and LLINs varies, with some studies suggesting an incremental benefit of using both interventions (Kleinschmidt, Schwabe et al. 2009; Fullman, Burstein et al. 2013), while others suggest that IRS adds no incremental impact relative to LLINs alone and/or vice versa (Curtis, Maxwell et al. 1998; Corbel, Akogbeto et al. 2012), that LLINs alone have greater impact than IRS (Curtis 1999; Mnzava, Dlamini et al. 1999) and others again indicate that the contrary is true (Misra, Webber et al. 1999; Mabaso, Sharp et al. 2004). These diverse comparisons between IRS and LLINs are based on a variety of outcome measures which include impacts on vector densities or entomological inoculation rates, as well as prevalence, incidence or diagnostic positivity of parasitaemia among humans, as well as the relevant costs of providing such protection (Curtis,

Maxwell et al. 1998 ; Curtis 1999; Misra, Webber et al. 1999; Conteh, Sharp et al. 2004; Mabaso, Sharp et al. 2004).

Currently there are four classes of insecticides approved for use in IRS formats: organochlorines, organophosphates, carbamates and pyrethroids (Najera 2001), but only the latter are considered safe enough for use in LLINs. The wide-scale deployment of pyrethroids in both LLIN and IRS formats has undoubtedly exerted considerable selection pressure upon vector populations, resulting in the rapid and widespread emergence of physiological resistance to these active ingredients which may negatively influence the efficacy of LLINs in particular (WHO 1976; WHO 1992; Hargreaves, Koekemoer et al. 2000; Curtis, Jana-Kara et al. 2003). As a consequence, the WHO recommends a reduction in use of pyrethroids for IRS, particularly in areas where LLIN deployment has been scaled up to reach high coverage (WHO 1976; WHO 1992). Furthermore, IRS application of multiple insecticides from different classes, ideally with complementary modes of action and non-overlapping resistance mechanisms, in rotations or mosaics is recommended as the optimal means of insecticide resistance management in the short-to-medium term (WHO 2012). Unfortunately, the utilization of organochlorines for IRS, particularly DDT, has been discouraged and scaled down due to concerns about potentially negative environmental effects associated with their use (Eskenazi, Chevrier et al. 2009). The remaining recommended formulations of organophosphates and carbamates have not been extensively used in IRS programs due to their comparatively high cost and relatively short residual periods of approximately 2 to 6 months (Najera 2001), which necessitates spraying

more than once in areas with protracted transmission seasons or perennial transmission. Fortunately, new formulations of the organophosphate pirimiphosmethyl (PM) have recently reformulated been brought to market for public health use that appear to offer increased and prolonged efficacy, notably against pyrethroid-resistant vectors (Rowland, Boko et al. 2013; Tangena, Adiamoh et al. 2013).

Given the substantial additional cost of supplementing LLINs with IRS, especially with such expensive new insecticides, and the persisting controversy about whether incremental protection against malaria is accrued, it is important to directly evaluate such combinations at community-level with epidemiological primary outcomes and explanatory entomological secondary outcomes in representative malaria-endemic settings. Thus, the overall aim of the study was therefore to evaluate the incremental impact of supplementary vector control with IRS upon malaria transmission by the widespread and highly efficient African vector *An. funestus* in a study area with relatively high usage rates of pyrethroid-impregnated LLINs, using either, one of two different formulations of pyrethroids, or one of two different formulations of the new PM organophosphate.

4.2 Methods

4.2.1 Study area

The study was conducted in the predominantly rural districts of Luangwa and Nyimba, located in Lusaka and Eastern provinces, respectively, of the Republic of Zambia (Figure 4.1). These districts have perennial transmission of *Plasmodium falciparum*, with the overwhelmingly predominant vector being *Anopheles funestus* Giles, which mediates a mean entomological inoculation rate (EIR) for non-users of LLINs of approximately 70 infectious bites per unprotected person per year (Sikaala, Chinula et al. 2014). The district of Luangwa (3,468 km²) is located 350-500 meters above sea level 325 km south-east of Lusaka, the capital city of Zambia. It has a population of approximately 27,560 residents, with an annual growth rate of 2.9% (CSO 2011). The main economic activities in the district are fishing and agriculture. Nyimba is a larger district (10,943 km²), with an approximate population of 108,637 inhabitants and an annual growth rate of 3.4 % (CSO 2011). The district is located 400-1200 meters above sea level, 350 kilometres east of Lusaka. Agriculture is the predominant economic activity in Nyimba district.

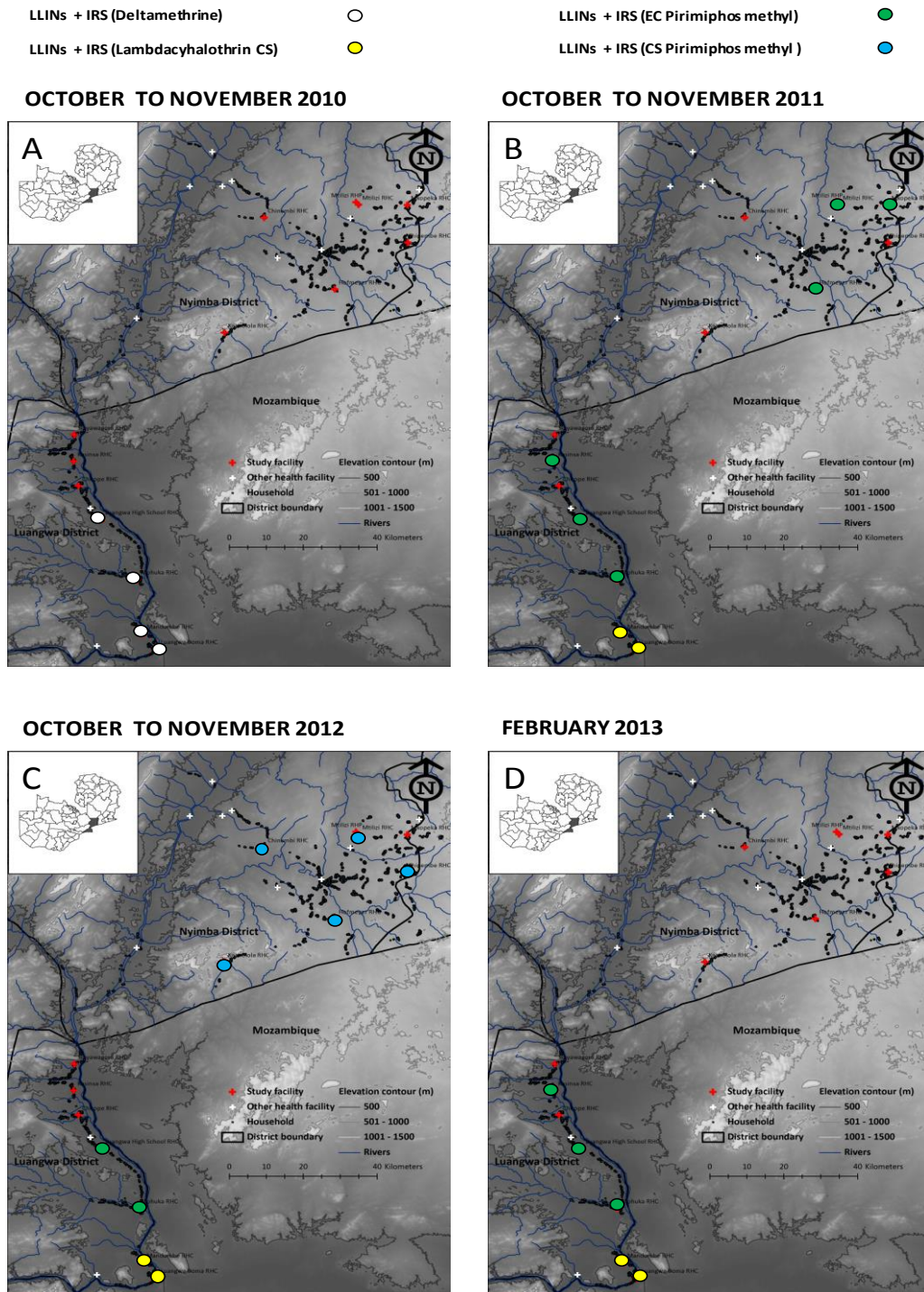


Figure 4.1: Map indicating location of health facilities and associated catchment populations enrolled in the study, with allocation of IRS treatments per cluster and year

4.2.2 Study design

In each district, 7 clusters of approximately 165 households were selected and enrolled in the study to participate in longitudinal parasite surveys (Hamainza, Moonga et al. 2014). Of these, 15 households in each cluster were selected and enrolled at the discretion of the CHW, so that they were geographically distributed across the cluster, for participation in monthly entomological observations, with the exception of Luangwa High School where 30 household were enrolled. Both parasitological and entomological assessments were conducted continuously from January 2011 to March 2013 in Luangwa and from April 2011 to March 2013 in Nyimba district in all clusters. The pyrethroid Deltamethrin (Wetable granule (WG) formulation) was sprayed in all consenting households at the four southernmost clusters in Luangwa in October 2010, immediately before that year's rainy season and initiation of this study. During the study period, 3 other selected IRS insecticide treatments (capsule suspension) (CS) formulation of the pyrethroid lambdacyhalothrin, as well as the emulsifiable concentrate (EC) and CS formulations of the organophosphate pirimiphosmethyl) were randomly allocated to clusters in advance of each rainy season. In practice this randomized allocation was not strictly adhered to by the implementation agencies in the two districts (District Medical Office) (DMO) in Luangwa and Abt Associates under the supervision of the DMO in Nyimba), thus resulting in a quasi-randomised study design, described cartographically in (Figure 4.1). The parasitological and entomological surveys were conducted by paid community health workers (CHWs) as previously described in detail (Hamainza, Moonga et al. 2014) and summarized below. In the south of Luangwa district, between October and November 2010, clusters 4, 5, 6 and 7 received pyrethroid-based IRS with deltamethrin (K-Othrine WG® 250, Bayer

Environmental Science, South Africa) as described in Figure 4.1A. Subsequently, the organophosphate pirimiphosmethyl was introduced as an alternative insecticide for IRS in a response to detection of resistance to pyrethroids in the primary vector, *An. funestus* in Luangwa district (Chanda, Hemingway et al. 2011; Seyoum, Sikaala et al. 2012; Sikaala, Killeen et al. 2013). The only formulation of pirimiphosmethyl that was available at the time was the relatively short-lived emulsifiable concentrate (EC) formulation (Actellic® EC, Syngenta Crop Protection AG, Switzerland). This formulation was sprayed during the months of October and November 2011, in clusters 2, 4, 5 in Luangwa and 9, 11, 13 in Nyimba, while IRS with pyrethroid lambdacyhalothrin (Icon® 10 Capsule Suspension (CS) formulation, Syngenta Crop Protection AG, Switzerland) was applied in only two of the four clusters in the south of Luangwa district which had been sprayed with deltamethrin the previous year, specifically in clusters 6 and 7 (Figure 4.1B). The following year, in November 2012, the longer-lasting microencapsulated formulation of pirimiphosmethyl (Actellic® 300CS, Syngenta Crop Protection AG, South Africa) was applied in clusters 8, 9, 10, 12 and 14, all of which were in Nyimba district (Figure 4.1C). In February 2013, IRS in Luangwa district was implemented with PM-EC in clusters 2, 4 and 5 while clusters 6 and 7 received the CS formulation of lambdacyhalothrin (Figure 4.1D).

4.2.3 Parasitological surveys of human infection

Active monthly parasitological surveys were coupled with questionnaires recording clinical symptoms of illness, as well as access and utilization of preventive measures such as LLINs, IRS

and intermittent preventive therapy (IPT), between January 2011 and May 2013, spanning a period of approximately 29 months, as described in detail previously (Hamainza, Moonga et al. 2014). These surveys were conducted by paid CHWs who made active monthly visits to households that consented to participate in the study. In between active visits, study participants who developed symptoms were encouraged to seek care through passively offered diagnosis and treatment services, either from the CHWs at their place of residence or at the nearest health facility (HF). The rapid diagnostic test used in the study was manufactured by ICT Diagnostics to detect circulating *P. falciparum* histidine-rich protein-2 antigen (ICT Malaria P.f. cassette test). All participants that were found positive for antigenemia, which was presumed equivalent to infection, were treated with Artemether-Lumefantrine as per National Malaria Diagnosis and Treatment Policy (NMCC 2010). In both the active and passive visits, all participants found to be negative for malaria infection but febrile or had any other complaints were referred to the nearest HF.

4.2.4 Mosquito densities, species identification

The monthly mosquito collections were conducted by paid Community Health Workers (CHWs) using Centres for Disease Control and Prevention light traps (LT) and Ifakara Tent Traps (ITT) between January 2010 and April 2013, spanning a period of approximately 28 months, as described in detail previously (Sikaala, Chinula et al. 2014). In each consenting household, the LTs were placed at the foot end of an occupied sleeping space covered with an LLIN, hanging approximately 1.5m above the floor. An ITT was placed immediately outside, approximately

5meters away from the house where the LT was installed and was occupied by an adult male volunteer from the same household. All the mosquito traps were set up in the evenings and collection of the captured mosquitoes was done in the early morning by aspiration. All the collected mosquitoes were initially sorted in the field to genus level, by the CHWs, based on crude taxonomic features and then stored over silica until they were collected monthly and transported to a central laboratory at the NMCC for further detailed examination. Additional morphological identification of *Anopheles* to species group or complex (Gillies and Coetzee 1987) was conducted at the central laboratory of the NMCC in Lusaka. Polymerase Chain Reaction (PCR) for the identification of species within the *An. funestus* group (Koekemoer, Lochouarn et al. 1999) or *An. gambiae* complex (Scott, Brogdon et al. 1993) were conducted on selected samples in the NMCC laboratory.

4.2.5 Vector susceptibility to different classes of insecticides

A team of trained entomological technicians from the NMCC periodically collected samples from the study sites to ascertain the susceptibility of the mosquitoes to different classes of insecticides, as background descriptive data to support appropriate interpretation of apparent impacts of various supplementary IRS treatments upon the vector population. In Luangwa district, mosquitoes were collected from cluster 2 from 2010 to 2013. However, in Nyimba district, collections were done over 3 three years in different clusters (Cluster 14 in 2011, cluster 9 in 2012 and cluster 13 in 2013). Adult mosquitoes were either collected while attacking humans by human landing catch (HLC) or by using pack aspirators for the indoors wall

resting mosquitoes. These were collected in cups covered with a netting material and placed in cooler box for transportation to the NMCC insectary where individual female *Anopheles funestus* mosquitoes were allowed to feed on mouse blood so they could lay eggs that were then reared into F1 generation mosquitoes. Standard World Health Organization (WHO) susceptibility tests using insecticide-impregnated papers with discriminatory dosages of two pyrethroids (Deltamethrin 0.05%, and Lambda-cyhalothrin 0.05%), a carbamate (Bendiocarb 0.1%), an organophosphate (Malathion 0.4%) and an organochlorine (DDT 4%) were carried out on 2 to 5 day old F1 *An. funestus* mosquitoes. Control papers were impregnated with oil as directed by the WHO protocol (WHO 2013). Knock down and mortality rates after 1 hour and 24 hour post exposure periods were recorded.

4.2.6 Indoor-outdoor distribution of human exposure to *An. funestus* bites.

To estimate proportions of human exposure to *An. funestus* bites and malaria transmission that occurs indoors and outdoors, HLCs were conducted both indoors and outdoors by a team of trained entomological technicians from the NMCC in Lusaka and these were complemented by cross-sectional questionnaire surveys of when residents went indoors for the night, went to sleep, awoke in the morning and left the house in the morning, as previously described in detail (Seyoum, Sikaala et al. 2012), again as background descriptive data to support appropriate interpretation of apparent impacts of various supplementary IRS treatments upon the vector population and malaria transmission. Trained CHWs conducted HLC from 6pm to 6am the next morning, with the exception of the previously described 2010 studies where the starting time

was 7pm and finishing at 7am. The 2010 and 2011 HLC surveys were conducted in cluster 4 (Chisobe and Nyamumba villages of Luangwa district) and as part of a trap effectiveness study (Sikaala, Killeen et al. 2013), while those conducted in 2012 and 2013 were part of the quality assurance surveys conducted in 13 clusters as part of a subsequent effectiveness assessment for a community-based trapping scheme (Sikaala, Chinula et al. 2014). Mosquitoes were collected for 45 minutes per hour to allow a 15 minutes break for rest and refreshment to the collectors. Each hourly collection was labelled and kept for identifications to genus and species as described above. The proportion of time residents spent whilst outdoors and indoors, as well as asleep in bed, was estimated directly from answers to questionnaires during a cross-sectional household survey in April 2010 in Luangwa district, in which people indicated the time they usually went indoors and when they went to the bed as well as when they arose in the morning and when they left their houses in (Seyoum, Sikaala et al. 2012).

4.2.7 Data management and statistical analysis

The CHW malaria register data describing RDT results associated questionnaire responses were double entered into Excel[®], verified, reconciled and then cleaned following descriptive frequency analysis of the distributions of values for each variable. All entomological data were single entered, verified and cleaned prior to analysis. All statistical analyses were accomplished using SPSS version 20 (IBM) and R version 2.14.1, augmented with the lattice, Matrix and LME4 packages.

4.2.8 Incremental protection of humans against malaria infection risk by IRS treatments

Previous analyses of these data collected by CHWs have demonstrated that diagnostic positivity (DP) for malaria infection, expressed as the proportion of RDT-tested individuals who were found to be positive, was an extremely powerful indicator of malaria risk that allowed numerous important epidemiological phenomena to be clearly illustrated (Hamainza, Moonga et al. 2014). It also proved to be more a consistent and robust indicator of geographic and temporal variation than absolute numbers of malaria infections detected, presumably because variations in CHW service utilization rates, as well as RDT and ACT availability, occur in both the nominator and denominator of DP (Hamainza, Killeen et al. 2014) and was therefore treated as the primary epidemiological outcome used for statistical analysis of the effects of various IRS treatments, rather than incidence in terms of detected events per number of participants per unit time.

Four sequential time period categories, based on the integer number of months since the most recent spray round was completed were created for all the IRS treatments: 1 to 3 months, 4 to 6 months and 7 to 12 months since before the last spray round started, as well as a fifth category combining areas that had not yet received spraying during the study period and those for which the last spray round began more than 13 months, which was treated as the reference value. Generalized linear mixed models (GLMMs) were fitted to evaluate the association between observed malaria infection risk among human residents and the various IRS treatments applied. Malaria infection status was treated as the binary dependent, with use of

an LLIN, having slept in a house that had been treated with IRS in the previous 6 months and the categorised cluster-wide IRS treatments as the independent variables of primary interest. Age category (<1, 1 to 4, 5 to 10, 11 to 14, 15 to 24, 25 to 44 and >45 years of age), sex, season (hot and wet from December to April, cool and dry from May to August, and hot and dry from September to November), number of previous RDT tests conducted per individual and geographical location (cluster) were also included as independent variables of secondary interest (all categorical except for number of RDT tests) while random effects to capture variance associated with other variables of no direct interest were also included in the model (the individual identity number nested within the CHW catchment nested within the study cluster, as well as date of participant contact). Further, in order to test for and quantify incremental impact of PM IRS as a supplement to LLINs, relative to LLINs supplemented with pyrethroid-based IRS, both pyrethroid formulations were represented by a single treatment variable, coding the same periods of months since before spraying. Similarly, in order to test for and quantify the incremental impact of the CS formulation of PM, relative to the EC formulation of the same active ingredient, as well as the two pyrethroid formulations, an additional variable was created which combined any previous treatment with any of the latter three formulations in the reference group. In all cases, incremental protective efficacy (IPE) was calculated as the complement of the odds ratio (OR) estimated directly by these GLMMs (1-OR).

4.2.9.1 Incremental protection of humans against human exposure to mosquito bites and malaria parasite inoculation by IRS treatments

The effect of different IRS treatment regimens on densities of *An. funestus* species were estimated by fitting GLMMs where *An. funestus* densities were treated as a dependent variable with a Poisson distribution. In order to account for variance in mosquito densities by location, identities for households where nested within those villages and then nested within clusters as random effects. Similarly, nightly temporal variance in vector density was accounted for by including date as an additional random effect. While the presence or absence of open eaves is a simple binary independent variable with only two levels, it was nevertheless treated as a random effect because it is not of direct interest to this analysis so it was also treated as a random effect so that the other independent variables represent values for the mean of all houses with and without open eaves, rather than the mean for a reference condition, presumably absence of eaves. The different IRS treatment regimens were coded in terms of time period since the last round of IRS application began, exactly as described above for the epidemiological primary outcomes, so that these treatments could be included as categorical independent variables with which to detect and quantify impact upon these entomological secondary outcomes. The relative rate (RR) at which mosquitoes were captured, was calculated as estimated directly by these GLMMs. The incremental protective efficacy (IPE) was calculated as the complement of the relative rate (RR) estimated directly by these GLMMs (1-RR). Unfortunately, efforts to develop laboratory capacity for determining sporozoite infection status by enzyme-linked immunosorbent assay at NMCC were unsuccessful so neither

sporozoite prevalence nor entomological inoculation rate could be assessed as additional entomological secondary outcomes.

4.2.9.2 Physiological resistance to insecticides

Insecticide susceptibility assays were conducted on 2 – 5 days old F1 generation *An. funestus* as described by the WHO standard protocol (WHO 1998) using papers impregnated with Deltamethrin (0.05%), Lambdacyhalothrin (0.05%), Bendiocarb (0.1%), Malathion (0.1%) or DDT (4%).

In order to test for time trends in physiological resistance of *An. funestus* to pyrethroids and carbamates over time, survival status of mosquitoes exposed to these insecticides in standard WHO protocols (WHO, 1998) was treated as the binary outcome variable in GLMMs with, year as a continuous covariate, and a unique identification code for each experimental replicate as a random effect. The data were stratified into subsets on the basis of the insecticide class with separate models fitted for the carbamate (Bendiocarb), and the combined pyrethroids (Deltamethrin and Lambdacyhalothrin). The model of resistance time trends for the two pyrethroids, the identities of these two insecticides within this class were included as a categorical independent variable. No such model was fitted for either the organochlorine (DDT) or the organophosphate (Malathion) because no resistance to either insecticide was apparent.

4.2.9.3 Proportions of human exposure to *An. funestus* bites occurring indoors and outdoors

The distribution of human exposure to *An. funestus* bites, and presumably malaria transmission, across different times of the night and across indoor and outdoor compartments of their living environment was calculated by weighting HLC measurements of indoor and outdoor biting rates for each hour of the night by the estimated proportion of humans indoors and outdoors during that time period, exactly as previously described (Seyoum, Sikaala et al. 2012). These estimates of human exposure distribution across indoor and outdoor environments were calculated and presented graphically for both users and non-users of LLINs, so that the proportions of human exposure that occur indoors in the presence ($\pi_{i,n}$) and absence (π_i) of a protective LLIN could be quantified and visualized.

4.2.9.4 Protection of human participants and ethical approval

Prior to the study, community sensitization was conducted and permission obtained from the local community leadership. Informed consent was obtained from all study participants during all surveys and spraying activities. The study team ensured that all treatment and diagnostic protocols were adhered to and that patients requiring malaria treatment received it promptly or were referred to the nearest health facility. All standard safety protocols were adhered to during the process of IRS as per national guidelines. Ethical approval was obtained from the University of Zambia, Biomedical Research Ethics Committee (Reference 004-05-09) and the Research Ethics Committee of the Liverpool School of Tropical Medicine (Approval 09.60). Authority to conduct and publish the study was also obtained from the Ministry of Health in Lusaka, Zambia.

4.3 Results

4.3.1 Characteristics of study participants

A total population of 25354 people centred on HFs in the 14 clusters participated in the study and were followed up for a period of 29 months in Luangwa and 26 months in Nyimba, starting from January 2011 and April 2011, respectively. Out of these participants, 29% (7412) were children under the age of 5 but DP peaked in older children between the age of 5 and 10. The overall cluster populations ranged from 1158 to 3429. A total of 31974 malaria infections (21.7% DP) were identified, which translates into an incidence of 9 infections per 100 person years. The study population reported a relatively high average rate of LLIN utilization of 81.7% of questionnaire responses over the course of the study indicating that the respondent had slept under an LLIN the previous night, while 39.2% of participant questionnaire responses indicated that the respondent's house had been treated by IRS in the last six months. During the same period, overall mean DP by cluster across all age groups and other potential stratification criteria ranged from 6.4% to 41.9% (mean = 24.5%) with the lowest being in the southern urban cluster and the highest in the northern rural cluster (Table 4.3.1). The potential confounding effect of LLIN ownership was excluded from the model because it had no significant effect ($p>0.05$) on DP (Table 4.3.1).

Table 4.3.1: Association of malaria infection status with age, sex, LLINs, IRS, number of tests conducted per participant, geographical location, season and IRS insecticide used^{i ii}

Category	DP% ^a	n/N ^b (I)	OR[95%CI] ^c	p ^d
Overall	21.7	31974 /147257(25354)	0.13 [0.08,0.21]	<0.001
Age				
<1	14.2	501 /3535 (1735)	1.26[1.09,1.45]	0.001
1-4	24.0	6127 /25505 (5677)	2.75[2.54,2.98]	<0.001
5-10		10066 /36779 (7608)	3.62[3.35,3.91]	<0.001
11-14	27.4	4892 /18840 (4746)	3.36[3.09,3.65]	<0.001
15-24	26.0	4491 /22077 (5685)	2.04[1.88,2.22]	<0.001
25-44	20.3	4028 /27044 (5807)	1.24[1.14,1.34]	<0.001
≥45	14.9	1796 /13027 (2903)	1[NA]	NA
Sex				
Male	23.3	16068 /79208 (12008)	1[NA]	NA
Female	20.3	15750 /67567 (13228)	0.86[0.83,0.90]	<0.001
Interventions				
LLINs	20.0	20613/103149 (20706)	0.89[0.85,0.93]	<0.001
IRS	17.4	7568 /43560 (9926)	0.87[0.82,0.93]	<0.001
Number of tests conducted per participant	21.7 ^e	31974 /147257(25354)	0.97[0.97,0.98]	<0.001
Type of visit				
Passive	43.4	6416 /14785 (8922)	1[NA]	NA
Active	19.2	25281 /131359 (22055)	0.29[0.28,0.31]	<0.001
Clusters				
Luangwa district				
Sinyawagora RHC	19.7	1314 /6655 (1959)	2.86[1.65,4.97]	<0.001
Kasinsa RHC	16.7	2232 /13402 (3429)	4.67[2.78,7.84]	<0.001
Chitope RHC	19.6	3419 /17463 (1215)	2.92[2.04,4.17]	<0.001
Luangwa High School RHC	16.5	2854 /17320 (1158)	7.37[4.31,12.61]	<0.001
Mphuka RHC	24.9	2981 /11957 (2147)	7.37[4.31,12.61]	<0.001
Mandombe RHC	10.3	1386 /13508 (1805)	1.54[0.89,2.66]	0.119
Luangwa Boma RHC	6.4	839 /13161 (2033) 166	1[NA]	NA
Nyimba district				
Kacholola RHC	26.7	3108 /11654 (1166)	7.50[4.75,11.84]	<0.001
Hofmeyer RHC	41.9	2601 /6214 (2120)	15.81[10.20,24.52]	<0.001
Mtilizi RHC	37.6	2238 /5949 (2024)	12.35[7.72,19.76]	<0.001

Mtilizi RHP	25.3	2478 /9788 (3379)	13.49[8.45,21.56]	<0.001
Chinambi RHC	31.9	1740 /5463 (1741)	9.16[5.79,14.48]	<0.001
Mkopeka RHC	32.8	2761 /8413 (1311)	14.22[8.55,23.63]	<0.001
Chipembe RHC	32.1	2023 /6310 (1916)	13.54[8.03,22.84]	<0.001
Season				
Hot & wet (Dec – April)	25.3	18283 /72217 (20243)	4.20[3.67,4.81]	<0.001
Cool & dry (May - Aug)	23.9	11216 /46860 (16513)	3.25[2.80,3.76]	<0.001
Hot & dry (Sept – Nov)	8.7	2444/ 27983 (12590)	1[NA]	NA
Insecticide applied for IRS				
<i>Deltamethrine</i>				
1-3 months since last spray	13.3	322 /2419 (2166)	0.81[0.65,1.01]	0.064
4-6 months since last spray	23.8	2411 /10150 (4231)	1.07[0.94,1.23]	0.295
7-12 months since last spray	13.6	2128/15640 (4434)	1.16[1.03,1.30]	0.013
Never sprayed and >13 months since last spray	22.8	27083/118899 (23233)	1[NA]	NA
<i>Lambdacyhalothrin</i>				
1-3 months since last spray	4.7	145/3102 (1526)	0.69[0.53,0.90]	0.006
4-6 months since last spray	9.4	207/2199 (1264)	1.26[1.01,1.57]	0.042
7-12 months since last spray	4.5	157/3508 (1469)	0.94[0.74,1.21]	0.653
Never sprayed and >13 months since last spray	22.7	31435/138299 (24931)	1[NA]	NA
<i>Primiphosmethyl EC</i>				
1-3 months since last spray	18.9	1922/10194 (5527)	0.77[0.69,0.85]	<0.001
4-6 months since last spray	28.8	2666/9259 (5926)	0.64[0.58,0.71]	<0.001
7-12 months since last spray	16.0	1793/11184 (5760)	0.63[0.56,0.71]	<0.001
Never sprayed and >13 months since last spray	21.9	25563/116471 (22311)	1[NA]	NA
<i>Primiphosmethyl CS</i>				
1-3 months since last spray	13.0	468/3590 (2675)	0.37[0.31,0.43]	<0.001
4-6 months since last spray	30.6	1386/4536 (3349)	0.24[0.21,0.27]	<0.001
7-12 months since last spray	49.5	95/192 (191)	1.35[0.85,2.15]	0.204
Never sprayed and >13 months since last spray	21.6	29995/138790 (24588)	1[NA]	NA

ⁱ a – Diagnostic positivity, b – (n – Number RDT positive, N – Total number), I – number of individuals that participated, c – odds ratio with 95% confidence intervals, d – p-value, e - RDT determined diagnostic positivity at first, NA –Not applicable /reference group

ii The association of malaria infection with age, sex, use of LLINs, use of IRS, geographical location (cluster), number of tests conducted per participant, season and insecticide used in IRS was determined using GLMM; with observed malaria RDT determined status as a binary dependent outcome with the independent categories of age, sex, access and use of LLINs or IRS, insecticide used in IRS, number of tests conducted per participant and seasons. The models included date and participant nested within CHW catchment nested within geographical location (cluster) as random effects except for one in which cluster was treated as a categorical variable to determine the effects of each cluster. The final model consisted of age, sex, access and use of LLINs or IRS, insecticide used in IRS, season, number of tests conducted per participant and geographical location as the determinants of malaria infection.

A descriptive comparison of summarized data restricted to the period 1 to 6 months post spraying demonstrates variability among study clusters in not only in IRS coverage (Range = 0% to 100%, mean= 29.4%) but also LLIN use (Range = 6.6% to 100%, mean= 68.2%) and diagnostic positivity (Range = 2.99% to 61.9%, mean= 25.4%) (Table 4.3.2). Further analysis using Pearson's correlation, revealed a positive but weak association ($R^2=0.31$) between IRS coverage and LLIN use, suggesting that as IRS coverage increases, so does LLIN use. However, this does not necessarily imply any causal relationship and factors which affect delivery (e.g. accessibility) and acceptance (e.g. attitudes towards malaria or mosquitoes) may well be similar for both of these vector control measures. However, there was no obvious and clear-cut effect of any particular IRS treatment in this crude descriptive comparison (Table 4.3.2).

Table 4.3.2: IRS coverage, LLIN utilization and diagnostic positivity between 1 and 6 months post implementation of IRS, broken down by survey cluster.

Period	Cluster	IRS Status	IRS Coverage % (n/N)	LLINs use % (n/N)	Diagnostic positivity % (n/N)
October 2010 to March 2011	1	None	0.0 (0/1577)	65.1 (1025/1575)	24.7 (372/1508)
	2	None	0.0 (0/827)	98.0 (2485/2537)	20.9 (559/2676)
	3	None	0.0 (0/2182)	49.0 (1492/3046)	26.9 (809/3006)
	4	Deltamethrin WG	72.0 (1920/2667)	82.3 (2194/2667)	33.2 (825/2489)
	5	Deltamethrin WG	92.4 (1362/1474)	95.8 (1412/1474)	27.5 (396/1439)
	6	Deltamethrin WG	95.3 (2750/2886)	95.4 (2744/2876)	11.9 (338/2845)
	7	Deltamethrin WG	98.5 (2358/2395)	99.7 (2467/2474)	6.0 (144/2415)
	8	None	0.0 (0/424)	6.6 (28/426)	55.7 (202/363)
	9	None	0.0 (0/7)	36.4 (4/11)	36.4 (4/11)
	10	None	0.0 (0/343)	39.7 (136/343)	50.7 (172/339)
	11	None	0.0 (0/134)	32.1 (43/134)	51.3 (60/117)
	12	None	0.0 (0/49)	79.6 (39/49)	61.9 (26/42)
	13	None	0.0 (0/221)	30.8 (68/221)	60.0 (120/200)
	14	None	0.0 (0/77)	41.6 (32/77)	52.4 (33/63)
October 2011 to March 2012	1	None	0.0 (0/1105)	87.4 (1063/1217)	9.5 (95/998)
	2	Pirimiphosmethyl EC	10.3 (245/2380)	75.8 (2235/2948)	8.5 (280/3292)
	3	None	0.0 (0/2593)	33.1 (1253/3790)	10.8 (436/4033)
	4	Pirimiphosmethyl EC	57.9 (2194/3788)	92.9 (3541/3811)	5.9 (217/3708)
	5	Pirimiphosmethyl EC	57.6 (1952/3392)	97.7 (3388/3469)	18.2 (624/3436)
	6	Lambdacyhalothrin CS	80.7 (1128/1398)	80.2 (1139/1421)	5.2 (76/1457)
	7	Lambdacyhalothrin CS	81.8 (2317/2833)	99.9 (3107/3109)	4.2 (130/3111)
	8	None	0.0 (0/3918)	75.4 (2952/2917)	29.9 (974/3261)
	9	Pirimiphosmethyl EC	33.5 (541/1613)	51.95 (838/1613)	46.6 (684/1467)
	10	None	0.0 (0/1466)	55.4 (812/1466)	35.4 (444/1254)

October 2012 to March 2013	11	Pirimiphosmethyl EC	1.8 (74/4117)	60.1 (2474/4117)	30.2 (941/3112)
	12	None	0.0 (0/1546)	59.7 (925/1549)	33.9 (514/1517)
	13	Pirimiphosmethyl EC	16.8 (407/2422)	45.9 (1111/2422)	27 (5.033/1974)
	14	None	0.0 (0/1964)	52.1 (1023/1964)	41.3 (786/1904)
	1	None	0.0 (0/464)	100.0 (464/464)	14.4 (150/1039)
	2	None	0.0 (0/1076)	99.8 (1074/1076)	11.9 (126/1061)
	3	None	0.0 (0/1915)	99.1 (1897/1915)	14.1 (282/2004)
	4	Pirimiphosmethyl EC	100 (1170/1170)	100.0 (2632/2632)	10.8 (314/2908)
	5	Pirimiphosmethyl EC	100 (1387/1387)	99.7 (1725/1730)	27.3 (456/1673)
	6	Lambdacyhalothrin CS	100 (348/348)	100.0 (1386/1386)	3.8 (57/1505)
	7	Lambdacyhalothrin CS	88.8 (645/726)	100.0 (1123/1123)	2.99 (33/1105)
	8	Pirimiphosmethyl CS	0.7 (18/2439)	28.5(696/2439)	9.0 (209/2321)
	9	Pirimiphosmethyl CS	73.7 (1386/1881)	48.5 (913/1881)	23.5 (366/1561)
	10	Pirimiphosmethyl CS	0.7 (10/1379)	85.1 (1174/1379)	27.1 (363/1341)
	11	None	0.0 (0/3440)	16.5 (566/3440)	11.9 (300/2531)
	12	Pirimiphosmethyl CS	30.3 (346/1143)	82.2 (940/1143)	21.7 (246/1132)
	13	None	0.0 (0/2433)	46.4 (1128/2433)	30.6 (666/2180)
	14	Pirimiphosmethyl CS	40.4 (574/1421)	38.1 (541/1421)	16.99 (221/1301)

The close associations of DP for *P. falciparum* malaria infection and *An. funestus* density, clinical symptoms of illness, and a variety of other factors of this setting are described in detail elsewhere based on the first year of data collection (Hamainza, Moonga et al. 2014). The detailed profile of the study participants, and their survey contacts over the course of the entire study, are summarized in the context of the study design in Figure 4.2.

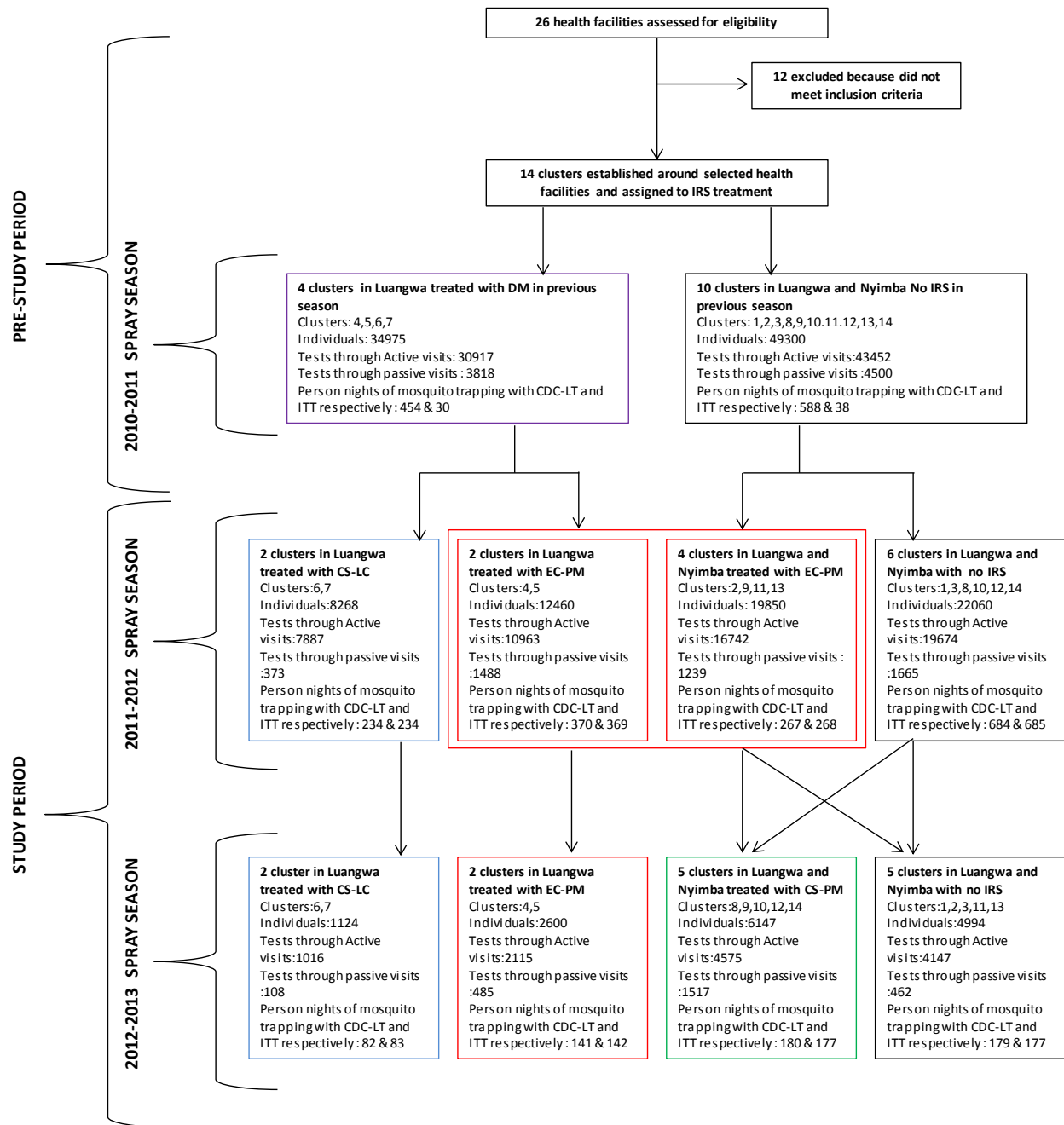


Figure 4.2: Study profile indicating treatments provided to each cluster with associated timelines, populations surveyed and person nights of mosquito trapping

4.3.2 Magnitude and duration of incremental impact of IRS treatments as supplements to LLINs upon human risk of infection with malaria

Reported coverage of deltamethrin WG, lambdacyhalothrin CS, pirimiphosmethyl EC, and pirimiphosmethyl CS, by respondents within the first 3 months after their application in clusters to which they were assigned were 82% (2132/2599), 61% (2068/3384), 53% (5909/11078) and 69% (2716/3913), respectively. As illustrated in Figure 4.3, Primiphosmethyl CS conferred the strongest initial incremental protection in the first 3 months after application (Incremental protective efficacy (IPE) [95% Confidence interval (CI)= 0.63 [0.57, 0.69], $P < 0.001$), relative to LLINs alone, followed by the CS formulation of Lambdacyhalothrin (IPE [95%CI] = 0.31 [0.10, 0.47] , $P = 0.006$), the EC formulation of primiphosmethyl (IPE [95%CI] = 0.23 [0.15, 0.31], $P < 0.001$) and the WG formulation of Deltamethrin (IPE [95%CI] = 0.19 [-0.01, 0.35], $P = 0.064$). However, neither pyrethroid formulation provided any incremental protection beyond 3 months post-application, while the incremental protection provided by CS and EC formulations of Pirimiphosmethyl persisted undiminished for 6 and 12 months respectively (Figure 4.3).

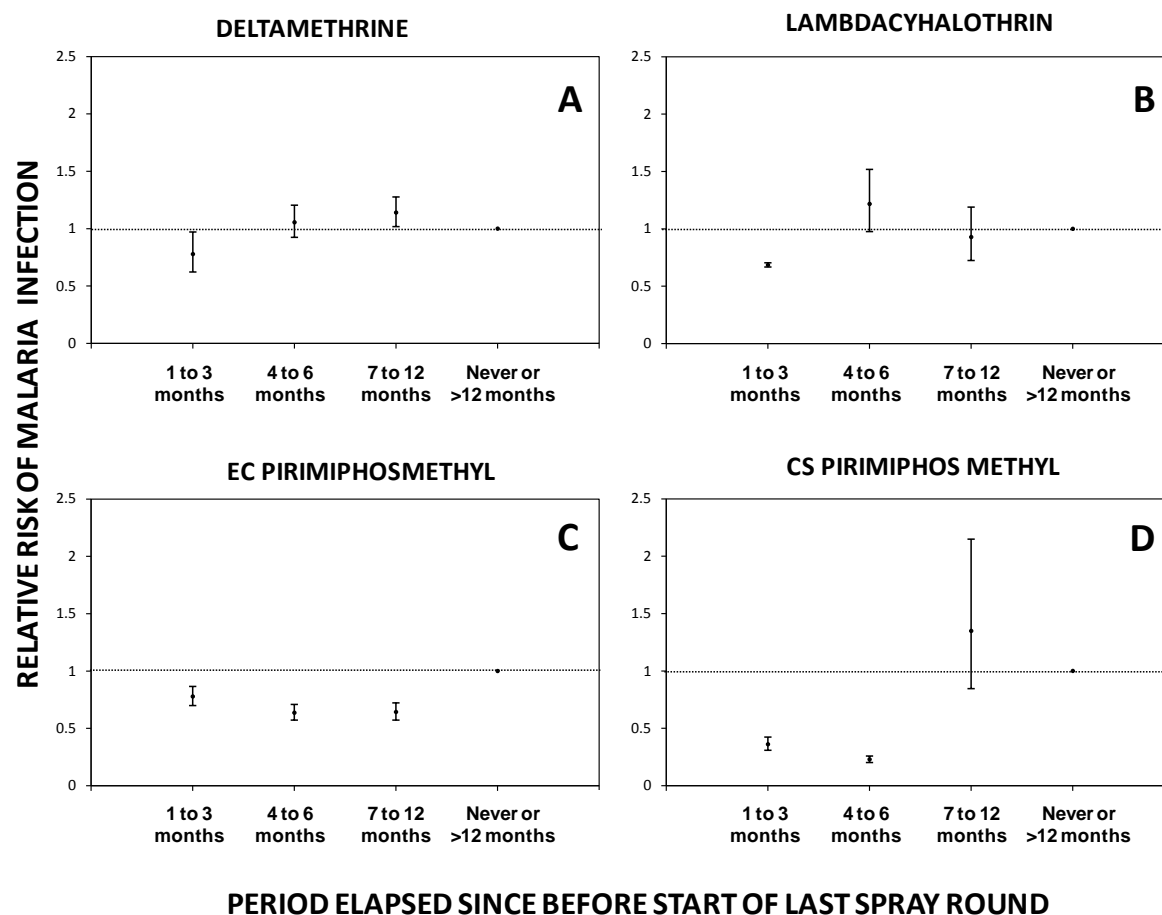


Figure 4.3: The incremental protective efficacy of each of the four IRS treatments on diagnostic positivity for *Plasmodium falciparum* malaria infection over several time periods since the last spray round began, relative to clusters that has either never been sprayed or had last been sprayed >12 months ago (reference group).

In the first three months after spraying, IRS with the CS formulation of pirimiphosmethyl offered greater protection against malaria infection than IRS with pyrethroids IPE [95%CI] = 0.51 [0.38, 0.62], $P < 0.001$ for LLINs + IRS with PM-CS compared to LLINs + IRS in all clusters treated with either DM-WG or LC-CS but not PM-EC ($P < 0.001$). The incremental protection against malaria infection by IRS with both PM formulations outlasted both pyrethroid formulations so that they both offered greater protection from 4 to 6 months post-application

IPE [95%CI] = 0.79 [0.75, 0.83], $P < 0.001$ for LLINs + IRS with PM-CS and IPE [95%CI] = 0.42 [0.33, 0.48], $P < 0.001$ for LLINs + IRS with PM-EC, compared to LLINs + IRS with either DM-WG or LC-CS) (Figure 4.4).

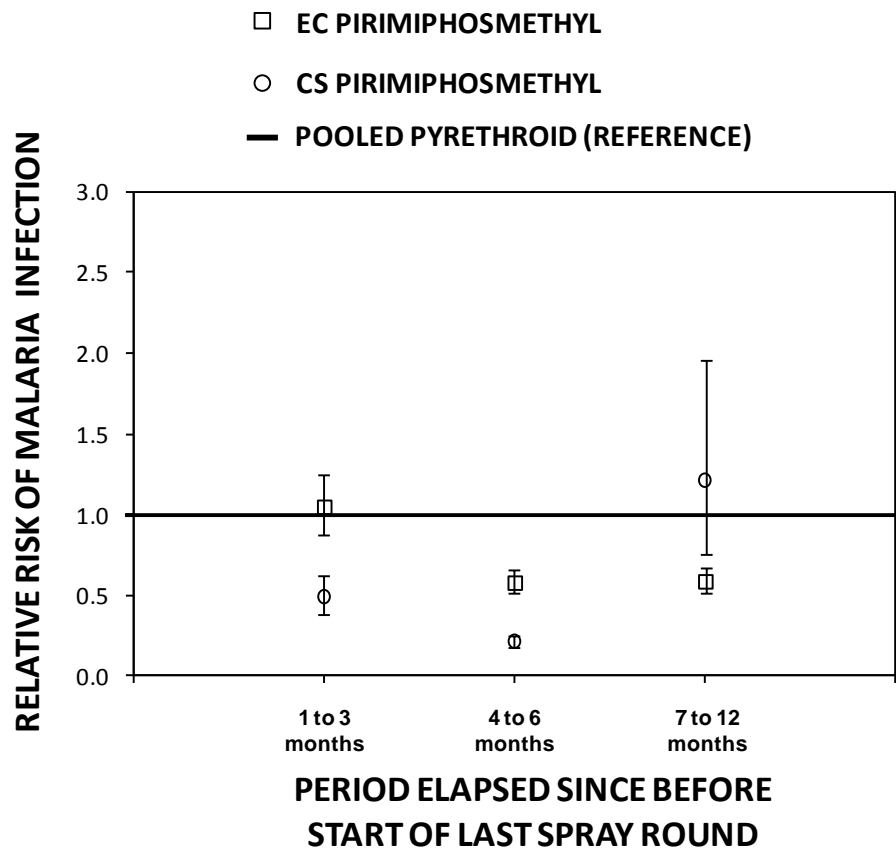


Figure 4.4: The incremental protective efficacy of primiphosmethy EC and CS IRS treatments on diagnostic positivity for *Plasmodium falciparum* malaria infection over several time periods since the last spray round began, relative to clusters that have been sprayed with either deltamethrin and /or lambdacyhalothrin (reference group)

Beyond 6 months post-application, LLINs plus IRS with PM-CS provided no apparent incremental protection relative to LLINs alone ($P=0.204$), much less LLINs + IRS with pyrethroids ($P=0.432$). However, LLINs + PM-EC continued to provide incremental protection relative to not only LLINs alone (Figure 4.4), but also relative to all other IRS+LLIN treatments (IPE [95%CI] = 0.41 [0.34, 0.48] , $P<0.001$). When the duration of efficacy of PM-EC was examined in further detail by breaking down the third post-spray time period into two halves, it was clear that it lasted approximately a full year because similar levels of incremental protection was confirmed for both the 7 to 9 month post-spray period (IPE [95%CI] = 0.32 [0.22, 0.40], $P<0.001$) and the 10 to 12 month post-spray period (IPE [95%CI] = 0.42 [0.31, 0.52], $P<0.001$).

Comparing these two IRS formulations of PM with each other as supplements to LLINs, the CS formulation confers greater protection than the EC formulation, (IPE [95%CI] = 53.6 [0.43, 0.66] %, $P<0.001$ from 1 to 3 months post-application and 0.64 [0.57, 0.69], $P<0.001$ from 4 to 6 months post-application for the contrast between LLINs + PM-EC *versus* the LLIN + PM-CS as the reference group) (Figure 4.5). However, once the incremental benefit of supplementing LLINs with IRS using PM-CS waned after 6 months, IRS using PM-EC proved statistically superior to all other IRS formulations as supplements to LLINs for a further 6 months, including the CS formulation of the same active ingredient (IPE [95%CI] =0.52 [0.21, 0.70], $P<0.001$ for the contrast between LLINs + PM-EC *versus* LLIN + PM-CS as a reference group between 7 and 12 months post application) (Figure 4.5).

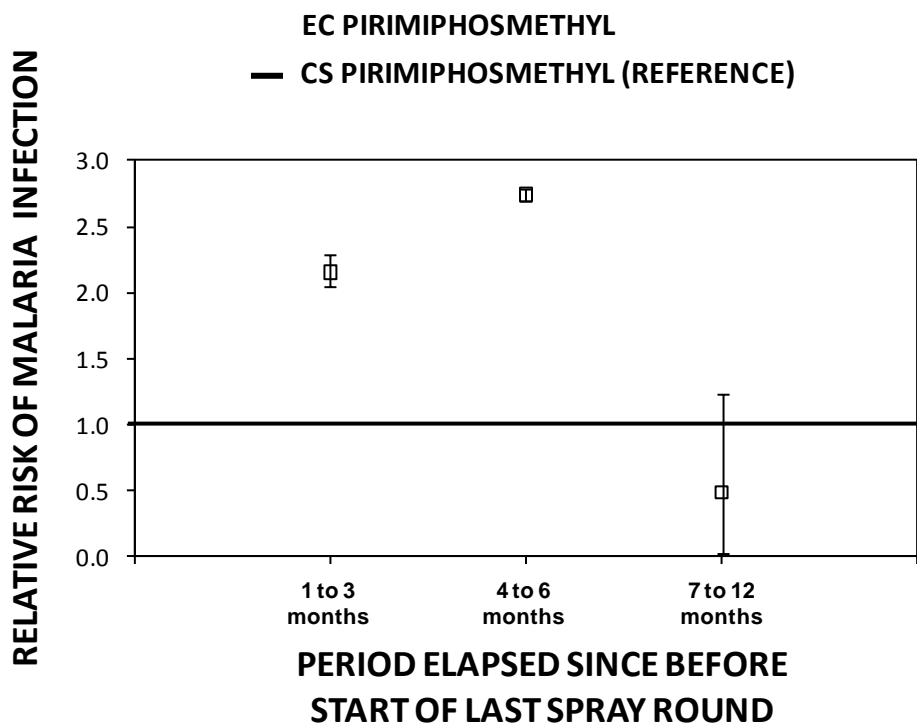


Figure 4.5: The incremental protective efficacy of pirimiphosmethyl EC IRS treatment on diagnostic positivity for *Plasmodium falciparum* malaria infection over several time periods since the last spray round began, relative to clusters that have been sprayed with pirimiphosmethyl CS (reference group)

4.3.3 Magnitude and duration of incremental impact of IRS treatments as supplements to LLINs upon human risk of exposure to bites of *An. funestus*

Detailed description of the local mosquito fauna in the study area (Sikaala, Chinula et al. 2014) showed that 34.5% of all mosquitoes caught over the course of the study were identified morphologically as members of the *An. funestus* group, of which 96.5% (575/596) of those which were successfully amplified by PCR were confirmed to be *An. funestus* Giles. Densities of the *An. funestus* group, as determined by routine morphological classification can therefore be considered quite reliable as of *An. funestus*, the abundance of which is consistent with previous studies in this area (Seyoum, Sikaala et al. 2012; Sikaala, Killeen et al. 2013) indicating it as the overwhelmingly dominant vector of malaria in these two districts of Zambia. Therefore, subsequently in this report we refer to all mosquitoes caught from the *An. funestus* group as the nominate species in the strict sense.

The relative rates and the mean catches of *Anopheles funestus* per IRS treatment are presented in Table 4.3.3. Relative to the times and places that had never been sprayed, or sprayed or had been sprayed >12 months ago, there were no obvious differences in the densities of *An. funestus* during the first three months post-spraying for both pyrethroid formulations (DM-WG (IPE [95%CI] = 0.01 [-0.56,0.37], P=0.103) and LC-CS (IPE [95%CI] = -0.03 [-0.88,0.44], P=0.195) and PM-EC (IPE [95%CI] = -0.04 [-0.30,0.17], P=0.103) (Figure 4.6, Table 4.3.3). However, where PM-CS was applied, mosquito densities were dramatically reduced during the same period of three months immediately after spraying (IPE [95%CI] =0.93[0.87, 0.97], P<0.001). Between the

fourth and the sixth month after spraying with DM-WG, there was an apparent, but presumably spurious, three-fold increase in *An. funestus* densities while LC-CS, PM-EC and PM-CS achieved 5, 3 and 71-fold reductions, respectively (Table 4.3.3). However, from the seventh to twelfth months after spraying, DM-WG and PM-EC had no obvious effect on the *An. funestus* densities while insufficient data was available to examine the incremental impact of LC-CS or PM-CS.

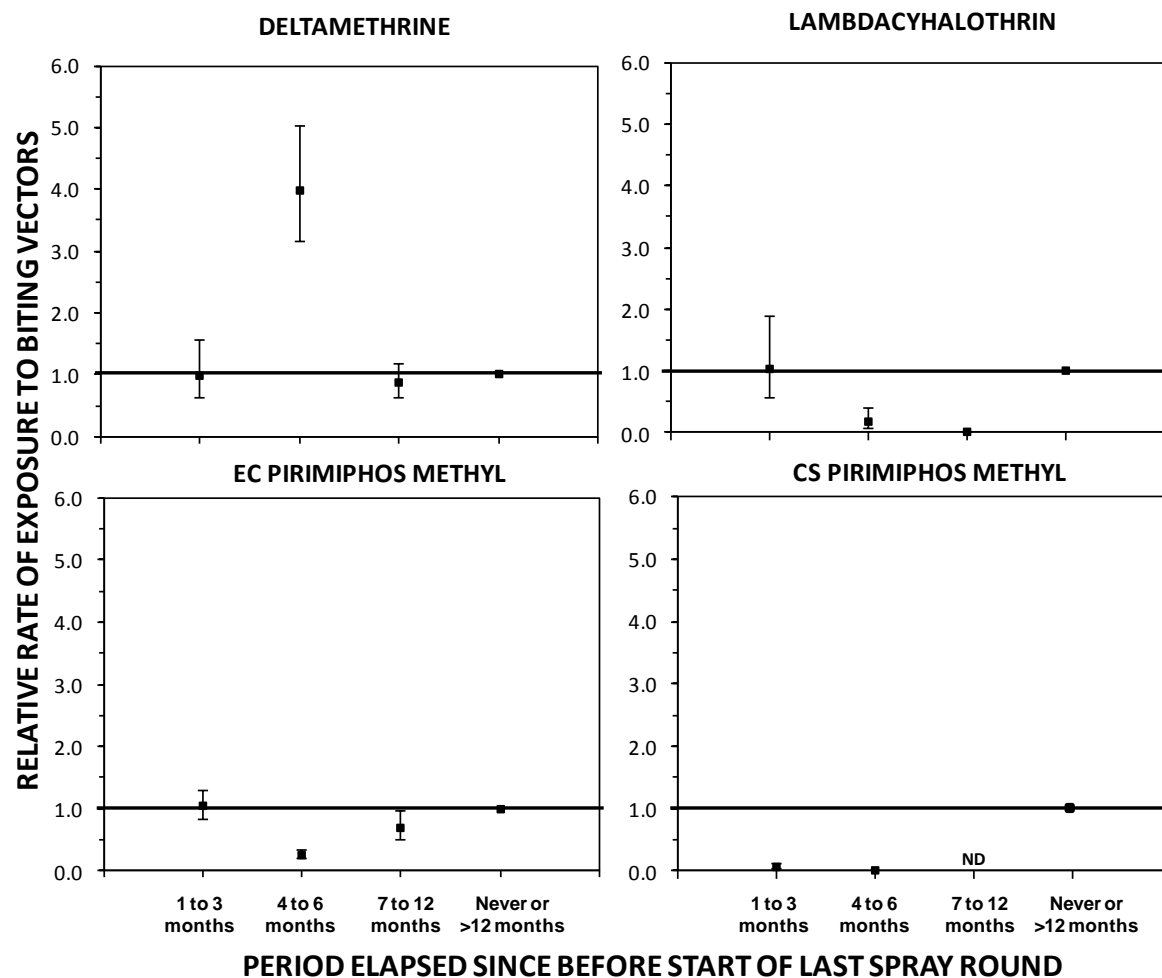


Figure 4.6: The incremental protective efficacy of each of the four IRS treatments against *An. funestus* bites over several time periods since the last spray round began, relative to clusters that has either never been sprayed or had last been sprayed >12 months ago (reference group)

Table 4.3.3. Association of *Anopheles funestus* densities with different IRS insecticides supplementing LLINs upon months before, during and when not spraying

Indoor residual spraying Insecticide treatment regimen applied	Absolute numbers caught	Mean catches ^a [95% Confidence Interval (CI)]	Relative rates of <i>An. funestus</i> densities (RR) ^b [95% CI]	P
Deltamethrin WG)				
1-3 months since last spray	73	0.112[0.641, 0.371]	0.99 [0.63, 1.56]	0.897
4-6 months since last spray	1229	0.641[0.371, 1.109]	3.98 [3.15, 5.04]	<0.001
7-12 months since last spray	134	0.111[0.062, 0.199]	0.86 [0.64, 1.17]	0.067
>12 months since last spray or never	1186	0.189[0.113, 0.317]	1[NA] ^c	NA
Lambdacyhalothrin CS				
1-3 months since last spray	20	0.191[0.090, 0.405]	1.03[0.56, 1.88]	0.805
4-6 months since last spray	6	0.055[0.022, 0.141]	0.17[0.08, 0.39]	<0.001
7-12 months since last spray	0	NE ^e	NE	0.972
>12 months since last spray or never	182	0.198[0.121]	1[NA] ^c	NA
Pirimiphosmethyl EC				
1-3 months since last spray	478	0.234[0.131, 0.417]	1.04[0.83, 1.30]	0.786
4-6 months since last spray	346	0.055[0.030, 0.098]	0.25[0.20, 0.33]	<0.001
7-12 months since last spray	160	0.159[0.086, 0.293]	0.69[0.50, 0.95]	0.151
>12 months since last spray or never	2823	0.234[0.131, 0.417]	1[NA] ^c	NA
Pirimiphosmethyl CS				
1-3 months since last spray	14	0.021[0.009, 0.047]	0.07[0.04, 0.13]	<0.001
4-6 months since last spray	70	0.004[0.002, 0.008]	0.02[0.01, 0.02]	<0.001
7-12 months since last spray	NA ^d	NA ^d	NA ^d	NA ^d
>12 months since last spray or never	2087	0.253[0.152, 0.422]	1[NA] ^c	NA

^{a & b} The effect of different IRS treatment regimens on the mean catches of *An. funestus* species where estimated by fitting generalized linear mixed models (GLMMs) with *An. funestus* catches treated as dependent variables. The households where nested within villages which were also nested within the clusters, these together with date were treated as random effects , while the different IRS treatment regimens were categorized as independent variables. A Poisson distribution with no intercept was used to estimate the mean catches while an intercept was included in estimating the RR .

^c Treated as the reference group

^d No spraying was conducted therefore and no data available for estimates

4.3.4 Background observations of insecticide resistance and human exposure profiles for local *Anopheles funestus* populations

From the outset of the study, *An. funestus* exhibited high levels of resistance to both pyrethroids against which they were tested, and resistance level generally increased over the course of the study ($P < 0.001$). Alarming rates of resistance to the carbamate bendiocarb were also observed but these did not increase over the course of the study ($P = 0.565$). During this same period, there was no evidence of Malathion or DDT resistance detected in the mosquito populations (Figure 4.7).

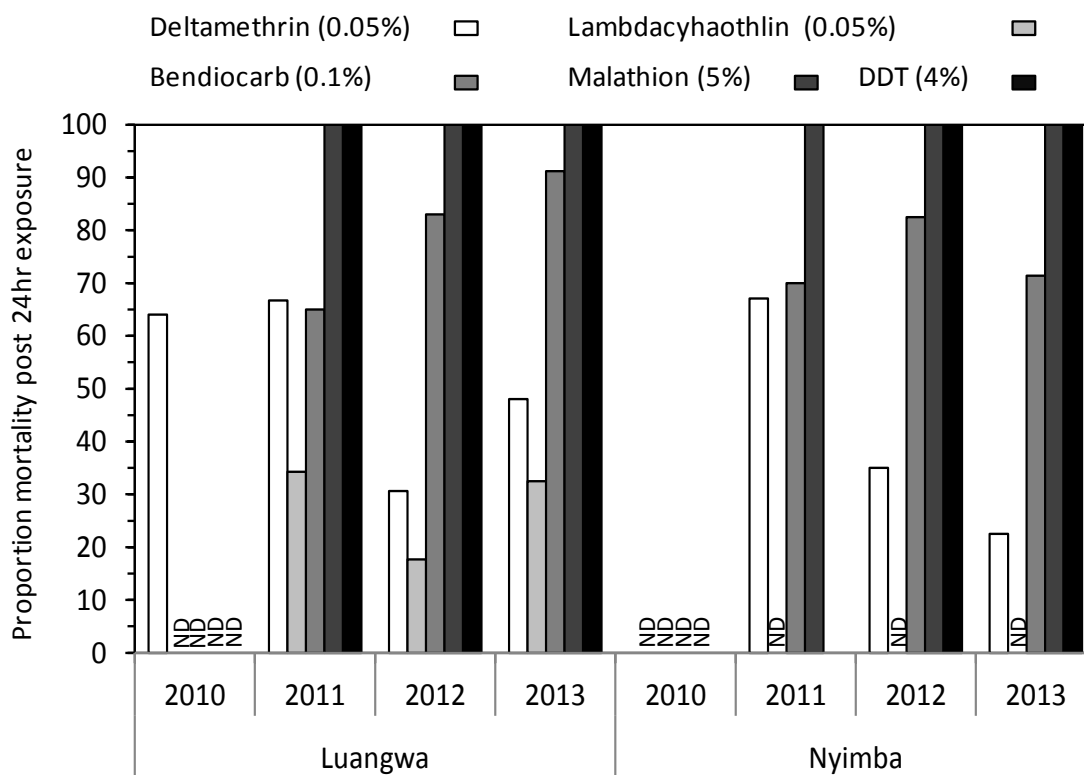


Figure 4.7: Insecticide resistance profile of *Anopheles funestus* in the study site from 2010 to 2013.

Throughout the study period, humans lacking LLINs were exposed to far more bites by *An. funestus* indoors during the late hours of the night up to the early morning hours (Figure 4.7), consistent with the known behaviour of *An. funestus* across the continent (Gillies and Coetzee 1987; Huho, Briet et al. 2013). The vast majority potential exposure to bites by this dominant vector occurred indoors at times when most individuals are asleep (Figure 4.7). Even for those using an LLIN to prevent most indoor transmission, most residual human exposure to *An. funestus* bites, and presumably malaria transmission, occurred indoors, increased gradually from 57% in 2010 to 71% by 2013 (Figure 4.7).

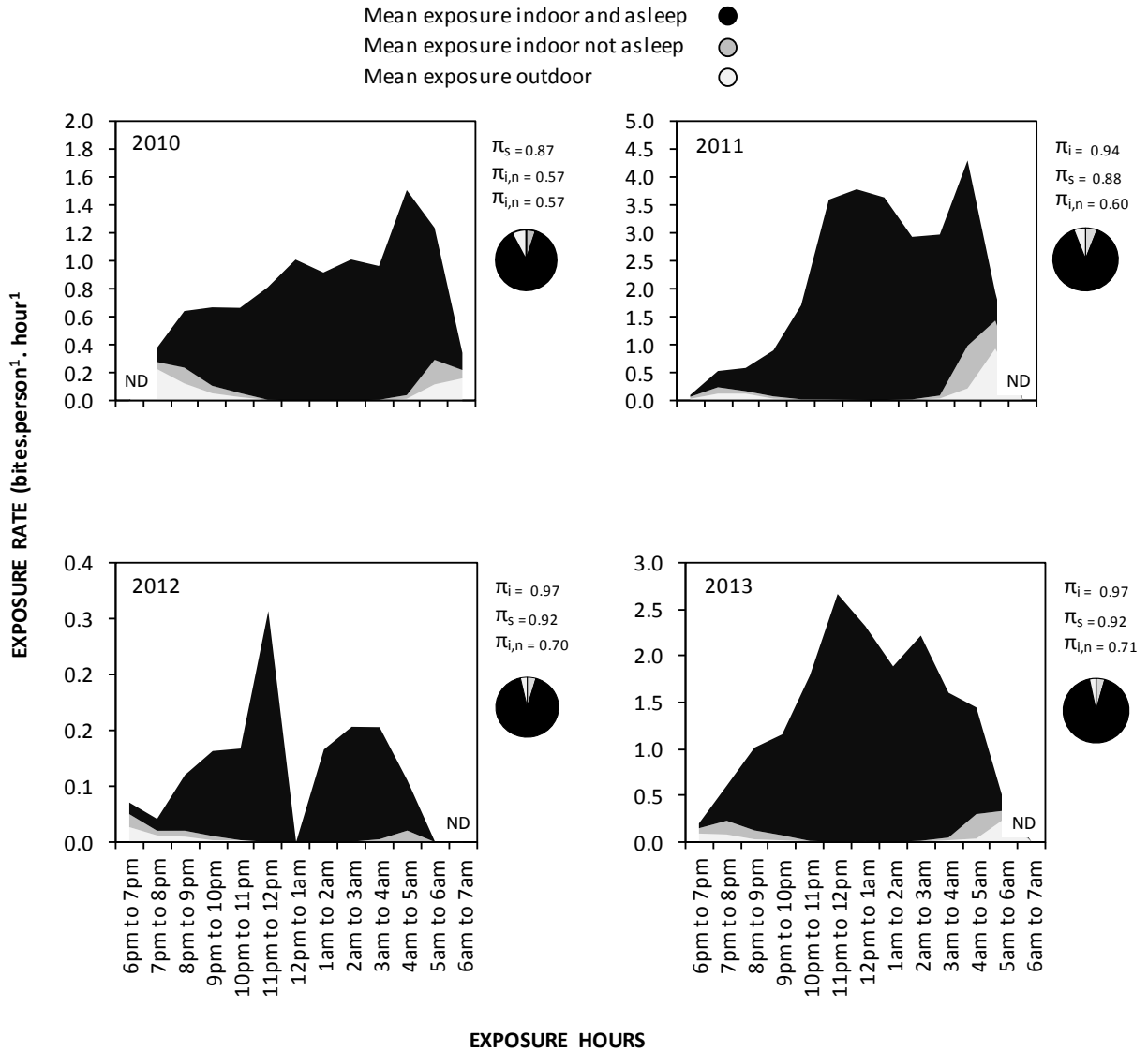


Figure 4.8: Mean exposure of humans to *Anopheles funestus* bites when they are indoors or outdoors. Where π_i is the average proportion of human exposure to bites of *Anopheles funestus* population which occurs indoors in the absence of any protective measure, π_s is the average proportion of human exposure to bites of *Anopheles funestus* population which occurs indoors when individuals are asleep in the absence of any protective measure and $\pi_{i,n}$ is the average proportion of residual human exposure for users of net which occurs indoors.

4.4 Discussion

In this setting of high LLIN utilization (>80%), even the modest (53 to 82%) coverage achieved with supplementary IRS conferred an incremental protection against malaria parasite infection through reduced vector population density, human exposure to bites and, presumably, to sporozoite inoculations. Overall supplementing of LLINs with IRS using PM-CS gave the greatest apparent protection against malaria risk, which lasted for a full 6 months, while IRS with PM-EC conferred less dramatic protection that was comparable with pyrethroids but apparently lasted for one full year. Neither of the two pyrethroid formulations exhibited any incremental protective effect for more than 3 months but it is notable that LC-CS conferred an apparently greater protective effect than DM-WG. These observations that quasi-randomly assigned IRS treatments conferred additional protection when provided as a supplement to LLIN utilization are consistent with a variety of other observational studies (Kleinschmidt, Schwabe et al. 2009; Kim, Fedak et al. 2012), as well as more recent randomized controlled studies (West, Protopopoff et al. 2014). The high level of incremental impacts observed, despite sometimes mediocre coverage, with IRS are actually entirely consistent with the predictions of process-explicit models used to support the policy switch to universal coverage for both LLINs and IRS (WHO 2007 position statement), especially for a very anthropophagic mosquito like *An. funestus* (Killeen, Smith et al. 2007), which is even more anthropophagic than the *An. gambiae* species (Killeen, McKenzie et al. 2001) used as an example mosquito in that simulation paper.

The high protective effect of PM-CS is also evident in the low densities of *An. funestus* caught in that group. The modest and short-lived protective effect of the two pyrethroid formulations, DM-WG and LC-CS most probably a result of the emergence of resistance to pyrethroids in the *An. funestus* population present in this study area, consistent with evidence from Benin in west Africa that the protective effect of these insecticide formulations can be dramatically reduced to as little as a month by physiological resistance, even where these specific formulations have a residual activity against susceptible insectary-reared mosquitoes for up to 6 months (Rowland, Boko et al. 2013). While this rapid loss of incremental protection towards malaria elimination with pyrethroid-based supplementary IRS is of obvious and very direct concern (Wondji, Coleman et al. 2012; Haji, Khatib et al. 2013; Wang, Xia et al. 2013), the encouraging results obtained with IRS using PM, the CS formulation in particular, provide further evidence that pyrethroid resistance may be mitigated and managed in areas of high LLIN coverage using IRS (Corbel, Akogbeto et al. 2012; West, Protopopoff et al. 2014), or alternatively impregnating wall linings (Djenontin, Chandre et al. 2010; Ngufor, Tchicaya et al. 2014), with non-pyrethroids selected on the basis of standard WHO susceptibility assays. These observations are therefore consistent with similar recent reports from several distinct settings across Africa (Hunt, Edwardes et al. 2010; N'Guessan, Boko et al. 2010; Akogbeto, Padonou et al. 2011; West, Protopopoff et al. 2014) and can be readily rationalized on the basis of the combined observations of strong resistance to pyrethroids, complete susceptibility to organophosphates, and strong tendency to feed, and presumably rest indoors among the local *An. funestus* population.

It was expected that PM-CS would be the most persistent because this microencapsulated formulation is known to confer residual longevity for 6 months (Rowland, Boko et al. 2013; Oxborough, Kitau et al. 2014) as confirmed here. However, it was surprising that PM-EC had the longest longevity on these surfaces, apparently lasting 12 months after spraying, contrary to other studies suggesting that PM-EC is ineffective on mud surfaces (Oxborough, Kitau et al. 2014) and WHO estimates of a residual effect of only 3 months (WHO 2013) but is consistent with one other recent study (Fuseini, Ebsworth et al. 2011). While it is possible to speculate that the persistence of PM-EC may have resulted from an initial absorption into the porous mud walls in most of the houses in the study areas, followed by slow subsequent release, it is also possible that this is simply the result of a spurious model fit to data from such a limited number of treated clusters with considerable inter-cluster variation in malaria risk level and seasonality, as presumably occurred for DM-WG which is highly unlikely to have really increased malaria transmission (Figure 4.3, Table 4.3.1) or vector density (Figure 4.6, Table 4.3.3). The observation that impact of both PM formulations and LC-CS upon vector density was greatest between 4 and 6 months after spraying suggests that maximum impact upon the vector population required sustained impact upon several generations of mosquitoes, well into the peak rainy season when they would be expected to grow exponentially and improve in reproductive fitness as the availability of larval habitat rapidly increases (Briet 2002; Russell, Lwetoijera et al. 2013).

Of course, there are several substantive limitations to this study, the most obvious of which are that it was not registered in advance as a randomized control trial and that deviations from the original randomization plan resulted in only a quasi-randomised design in practice. An additional considerable limitation arising from dependency on delivery of supplementary IRS through routine programmatic implementation mechanisms was the lack of consistent availability of a single, optimal formulation of a single pyrethroid or a single formulation of PM, so the study was unfortunately fragmented into more treatment arms with smaller numbers of assigned clusters per spray round than originally planned. Also, delays and limitations in the availability of PM formulations in the final year of the study resulted in a mismatch in the timing of application of PM-CS in Nyimba (November 2012), PM-EC, and LC-CS in Luangwa (Both February 2013). While the community-based nature of both the parasitological and entomological surveys, with only modest supervision and quality assurance, does leave some uncertainties about the data quality, recent detailed analyses of these primary (Hamainza, Moonga et al. 2014) (Hamainza B, Killeen GF, Kamuliwo M, Bennet A and Yukich J, personnel communication) and secondary outcomes (Sikaala, Chinula et al. 2014) provide reassuring confirmation of their epidemiological relevance and discriminative power. While this study did not explicitly or comprehensively track the distinct costs of IRS and LLINs, these costs may be assumed to be incurred largely independently of each other because of their distinct delivery methods, and have already been evaluated in detail across a variety of settings by other authors (Goodman, Mnzava et al. 2001; Guyatt, Kinnear et al. 2002; Bhatia, Fox-Rushby et al. 2004; Conteh, Sharp et al. 2004).

4.5 Conclusions

Despite these study limitations, the results presented here do provide substantial evidence that (1) supplementing pyrethroid-based LLINs with pyrethroid-based IRS confers some, albeit short-lived, incremental protection against malaria infection relative to LLINs alone, and (2) Replacing pyrethroids with an alternative insecticide class, in this case a long-last CS formulation of the organophosphate PM, as the active ingredient for supplementary IRS confers considerably enhanced protection, relative to IRS with pyrethroids. Supplementing LLINs with IRS using non-pyrethroids therefore appears to be efficacious for mitigating the immediate epidemiological consequences of vector population resistance to pyrethroids, and the observed impact on *An. funestus* densities suggest it may also be a valuable option for managing such resistance traits, ideally by using mosaics, rotations or combinations of complementary active ingredients (WHO 2012). Of course the primary limitation to the realization of such insecticide resistance management and mitigation plans in practice are (1) the availability of more efficacious, affordable and diverse insecticide formulations (Vontas, Moore et al. 2014), (2) increased financing for malaria vector control generally (WHO 2014), and (3) more cost-effective methods for targeting insecticides to vector populations so that both the biological resource coverage (Kiware, Chitnis et al. 2012; Killeen, Seyoum et al. 2013) and mortality rates arising from exposure to their active ingredients are maximized (Elliott 1972; Kitau, Oxborough et al. 2012; Okumu, Kiware et al. 2013; Okumu, Mbeyela et al. 2013; Killeen and Chitnis 2014).

CHAPTER FIVE

GENERAL DISCUSSION AND CONCLUSION

5.0 General discussion

There has been overwhelmingly global support to provide the National Malaria Control Programmes (NMCPs) of African countries with the necessary technical skills to monitor and evaluate progress achieved upon implementation of malaria interventions. However, complete elimination of malaria transmission and occurrences of resurgences have both been reported, historically and also more recently (Mendis, Rietveld et al. 2009; Feachem, Phillips et al. 2010; Najera, Gonzalez-Silva et al. 2011; Cohen, Smith et al. 2012; Smith, Cohen et al. 2013; WHO 2013). The inability of a given vector control measure to completely eliminate malaria transmission can be explained by either a vector population that exhibits inherent, stable, pre-existing behavioural traits that make it resilient to control (Figure 1.4A). While a vector population may also exhibit emerging behavioural or physiological resistance that allows it to recover from initial suppression, resulting in a rebound of malaria transmission to baseline pre-intervention levels (Figure 1.4B). While the former is characterized by some sustained but incomplete levels of impact of a sustained programme, the latter is characterized by outright failure of a programme despite sustained implementation practice, resulting in rebounding vector populations and malaria transmission (Figure 1.4). Therefore the current arsenal to tackle such vectors' populations requires programmatic monitoring systems that are sustainable and affordable.

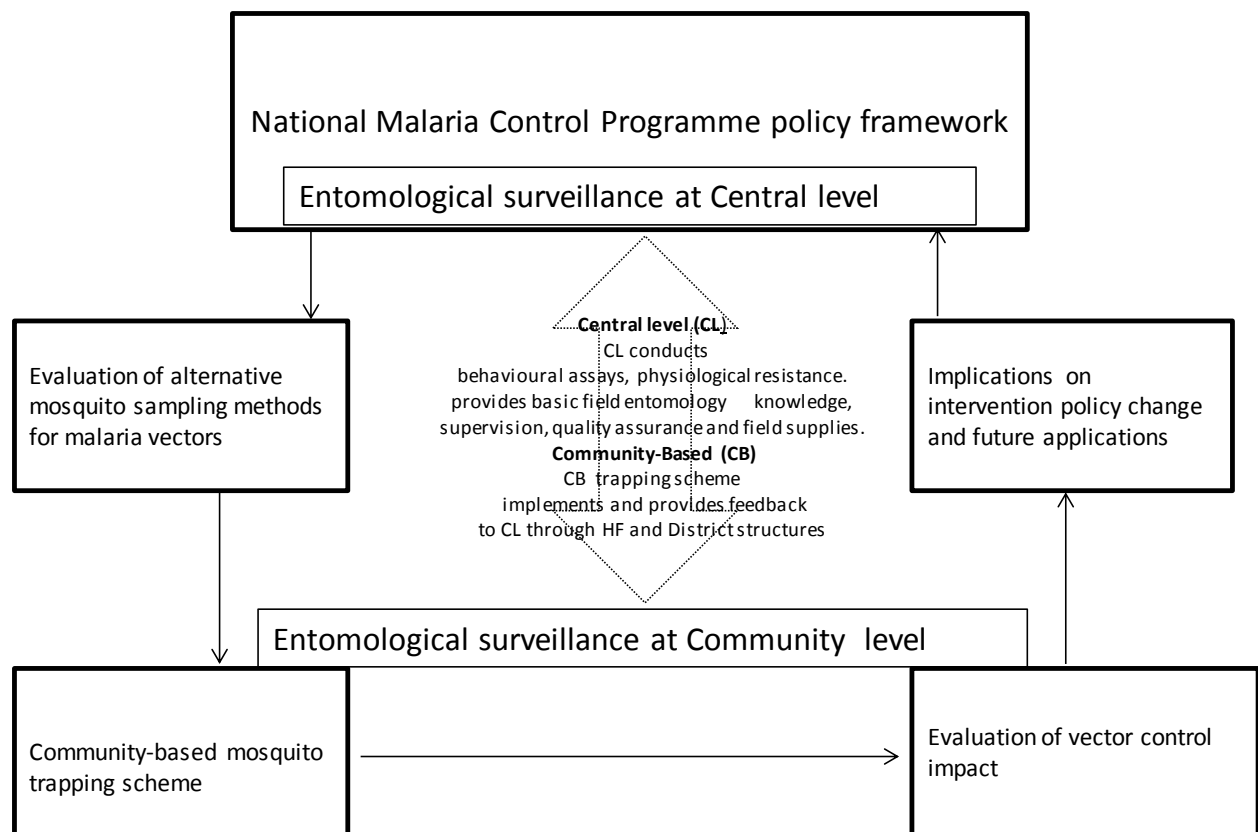


Figure 5.1: Roles and relationships between the central programme and the community-based surveillance system for sustained monitoring of vector population dynamics and implications on intervention efforts.

5.1 Calibrated exposure-free entomological sampling methods: Options for monitoring local vector population dynamics

As described in chapter 2, LTs and the ITT are the only two alternative exposure-free methods that had comparable sensitivities with HLC indoors to capture *An. funestus* in the study site. Recently, these methods have shown varying degrees of efficiencies across several different settings in sub-Saharan Africa (Govella, Chaki et al. 2009; Sikulu, Govella et al. 2009; Govella, Chaki et al. 2011; Ndiath, Mazenot et al. 2011; Overgaard, Saebo et al.

2012; Wong, Bayoh et al. 2013). Estimated efficacy of sensitivity of LTs relative to HLC for sampling *An. funestus* in this setting were inconsistent when compared between chapter 2 (direct efficacy study) and chapter 3 (efficacy estimates obtained from quality assurance data). While chapter 2 demonstrated a higher sensitivity of LTs than HLC (RR [95% CI] = 1.532 [1.441, 1.628], $P < 0.001$), the opposite (RR [95% CI] = 0.332 [0.185, 0.596], $P < 0.001$) was evident in the subsequent study described in chapter 3. Such inconsistency is, however, understandable because the former was conducted within the small geographic area of a single sampling cluster, whilst the latter was conducted across a much wider set of sampling points 14 clusters. Furthermore, such erratic sensitivity estimated are consistent with similar studies where efficacy was evaluated twice at a single location over different time periods (Govella, Chaki et al. 2009) or across multiple locations at the same time (Overgaard, Saebo et al. 2012; Wong, Bayoh et al. 2013). While these disparities appear discouraging across these sites, it is reassuring that they did prove adequate for evaluating the impact of adult-targeted vector control interventions (Chapter 4), and LTs have been used reliably to quantify impact on vector populations across various settings (Curtis, Maxwell et al. 1998; Russell, Lwetoijera et al. 2010; Tangena, Adiamoh et al. 2013; West, Protopopoff et al. 2014).

It is also worth noting that, even though HLC is considered the golden standard method for trapping mosquitoes attempting to bite humans (Service 1977; WHO 1992), its reliability is still questionable simply because catchers with exposed lower limbs operating in the dark over the course of a long night are difficult to execute and standardize. However, in spite of the limitations of LTs, chapters 3 and 4 demonstrate that they are clearly adequate for

detecting major temporal, spatial and intervention-induced variations in vector density. It is also apparent that new exposure-free tools for outdoor sampling of host-seeking vectors are required. While it has long been known that LTs have very poor sensitivity outdoors (Silver and Service 2008), it is also apparent that the same is true of alternatives such as the RBs described here (Table 2.3.1) and a variety of other outdoor resting traps evaluated elsewhere (Killeen, Kiware et al. 2014). While the ITT exhibited encouraging levels of sensitivity for capturing *An. funestus* in this study site, it remains unclear whether it is an indoor or outdoor sampling method considering that it exhibited a lower efficiency, in comparison with HLC, for catching exophilic mosquito species (Chapter 2). Furthermore, as suggested in Chapter 2 and by others (Wong, Bayoh et al. 2013), there is need to identify the blood meal source of engorged mosquitoes to establish (1) whether this trap is actually exposure-free and, (2) whether it also acts as an alternative outdoor resting place for mosquitoes undergoing digestion and gestation.

Despite these limitations, as well as their practical disadvantages (maintenance of moving parts and electrical power requirements of LTs, as well as bulkiness of the ITT which makes it difficult to move around), these two trapping methods are both clearly useful for CB vector surveillance as respectively demonstrated by this study (Chapter 3), as well as others elsewhere with WET in rural Zambia (Chanda, Hemingway et al. 2011) and in with ITTs urban Tanzania (Chaki, Mlacha et al. 2012).

5.2 Potential for implementing a community-based sampling framework at national scale to monitor population dynamics

The results in Chapter 2 show that exposure-free sampling methods in LTs and ITT can be used by community-based staff living at sentinel sites distributed across a geographical scale spanning > 14,000 km² of rural south-east Zambia, in an eco-system where transmission is mediated by *An. funestus*. Such sampling tools are viable primarily because the CHW who operates LTs and ITTs are not exposed to increased risk of malaria or other mosquito-borne pathogens, as would be the case with HLC.

In the Zambian context, CHWs are an integral part of the primary health care structure (Harvey, Jennings et al. 2008; Chanda, Hamainza et al. 2011; Hamainza, Moonga et al. 2014). They are trained in basic disease prevention methods, diagnosis, treatment of simple ailments and are generally selected by the community members they serve (Hamainza, Moonga et al. 2014). Furthermore, upon qualifying from the training package of basic primary health care, they are given a modest monthly allowance as a motivation. One of the major advantages of scaling up CB surveillance systems to a national scale is because it is driven at local level with personnel who are residents within and familiar with the community surrounding the health facility (HF) they operate from (Chapter 3). Elsewhere in the tropics, community members have been used to scale-up malaria control activities, without compromising the quality of the health services they are already providing (Yasuoka, Poudel et al. 2012). Furthermore, this study demonstrates that CHWs were also able to incorporate entomological and parasitological surveillance systems into their daily

working schedule, so that these activities were seen as part of their normal work supervised by the HFs they operate from and report to.

Therefore it is envisaged that HFs can further be strengthened in human resource by training CHWs in basic entomological collections so that data collection at those levels can be generated in real time. It is also encouraging to note that HFs are under the district medical office, so supervision of the CB entomological platforms can be incorporated within the routine supervisory visits conducted by district management teams, so that this additional activity does not stand alone as a purely vertical programme controlled exclusively from the CL. Furthermore, the cost per sampling framework and per specimen of *An. funestus* has been quantified in Chapter 3 and therefore this could serve as a basis for districts and the CL estimate the costs and jointly plan budgets for sustaining such surveillance schemes. However because malaria transmission is heterogeneous (Smith, Dushoff et al. 2005), caution needs to be taken when extrapolating these estimates in epidemiological zones with different vectors with varying vectorial capacities. For example, the cost of sampling *An. arabiensis* which exhibits different behavioural traits may be different from that for *An. funestus*.

While cost implications are important in estimating an effective surveillance platform at all levels, sustaining and documenting adequate quality of the data generated by CHWs is essential to securing confidence in this evidence for guiding programmatic selection and implementation of interventions. The quality of a wide variety of healthcare services

provided by CHWs have evaluated by a number of studies, many of which indicate they perform simple routine tasks more effectively and consistently than trained professional health workers but evaluations of surveillance for larval-stage mosquitoes by CHW have this far proven discouraging (Vanek, Shoo et al. 2006; Chaki, Govella et al. 2009; Chaki, Dongus et al. 2011). However, adult vector population surveillance is a pre-requisite to effective, evidence-based implementation of CB malaria vector control (Mukabana, Kannady et al. 2006; Chaki, Kannady et al. 2014) and the evidence presented in chapters 3 and 4 indicate that continuous, longitudinal CB mosquito trapping systems might well provide authentic and adequate data so long as they are well structured, supervised and quality-assured by an independent, technically-expert team managed centrally at national level.

5.3 Incorporating routine continuous quality assurance and quality control into systems for longitudinal surveillance of vector population dynamics

A study conducted in urban Tanzania (Chaki, Mlacha et al. 2012) as well as this study (Chapter 3), have both provided evidence that at community-based, longitudinal entomological surveillance of trends in vector population densities is practical, cost effective, predictive of malaria risk infections, and can be quality assured to validate the data it produces.

While QA was conducted in this study, there still remained three fundamental limitations in this study that will require addressing before scaling up to national scale: (1) QA was only conducted at the end of the study for only three months; (2) participants had been informed

a day or 2 before about the QA team coming to assess their work, and (3) the spatial distribution of clusters meant that it was not feasible to conduct QA across such a wide geographic area as frequently as projected (Chapter 3). So there is needed to establish a pool of technical expertise at the central level of the NMCC that can routinely conduct QA at a much greater scale than was the case here. Firstly, this requires investing in training personnel within the government structures, to such a level that they are able to programmatically sustain these surveillance platforms (Mukabana et al. 2006, Shiff et al. 2011, Killeen, 2014). Currently, the staffing of the NMCC with technically trained entomologists is so minimal that we depend on part-time technicians employed on a casual basis, who inevitably depart for other permanent job opportunities that offer security or/and a clear career progression path. Secondly, the roles of the NMCC, districts and HFs need to be well defined and incorporated within existing structures so that day-to-day logistical operations are devolved to the district and HF level, while the necessary supporting technical expertise remains preserved within a specialized central team employed by the NMCC and its national partner institutions (Figure 5.1). Therefore, the NMCC intends to further strengthen the central team entomological surveillance system to meet the challenges of planning, securing resources for, and then scaling up CB surveillance of the impact of vector control interventions upon mosquito populations. This team of expert technicians can then independently and objectively conduct frequent, random, and un-announced QA visits, at multiple sentinel sites distributed across large geographic scales. By using the same sampling methods as those used by the CHW, these two sources of data can be directly compared, so that the quality of the CB data stream is validated a means to inform programmatic selection of optimal intervention application (Govella, Chaki et al. 2013).

The NMCC currently has 6 sentinel sites designated for the monitoring of physiological resistance, to provide evidence for guiding the selection of active ingredients for programmatic implementation of IRS. However, these sites are currently confined to areas with high malaria prevalence (Figure 1.5) where scale-up of IRS has been prioritized and funded. However, such surveillance data can be equally relevant to management of LLINs and any additional vector control interventions that may be required in the future (Killeen 2014; WHO 2014). Therefore, a future national CB longitudinal entomological surveillance should include a larger but manageable number of sentinel sites (See Figure 5.2 for an example with 12 sentinel sites) that are geographically more widely-distributed and are representative of all major transmission systems in the country (Figure 1.5).

This work suggests that such mosquito trapping sentinel clusters could be established within the catchment of selected health facilities with carefully-managed and consistently-reported diagnostic services that are routinely quality assured so that the epidemiological data is linked to the entomological observations. Passively collected HF data within entomological surveillance sentinel sites could also be supplemented by paid CHW providing test and treat services through regular, active household visits (Hamainza, Moonga et al. 2014), ideally reporting in real time using mobile phone platforms (Hamainza, Killeen et al. 2014) .

Each catchment cluster could be managed by a local environmental health technician (EHT) in charge of supporting and supervising one or more local CHWs responsible for the day to day collections of samples in their assigned areas, as described above and chapter 3. While an independent specialized team employed centrally by the NMCC would need to focus on conducting regular but randomly scheduled unannounced QA, the EHTs could incorporate much more frequent and purposeful quality control as just one part of their numerous daily activities. In addition to collating data and specimens from CHWs, as well as passing these on to the NMCC for detailed analysis, their presence as resident health extension workers on the ground allows them support and supervise the CHWs operationally. Specifically, they are ideally placed to check whether stipulated trapping procedures are consistently adhered to, such as hanging of LTs beside occupied bed nets within houses, and ensure adequate quality and timeliness of data recording, entry and reporting, as well as taxonomic identification of mosquitoes.

The cost per person-night of QA trapping at 14 clusters with a single CHW each, spread across a study area of approximately 14,000 sq km, were \$289 and \$269 for LT and ITT, respectively. The costs of routine CB mosquito trapping at sentinel sites distributed across the entire country would probably be similar those incurred here (\$ 2,388 per cluster per year) but with the additional cost of quality control by EHTs (currently \$ 7,628 Per annum per full time equivalent) also included., However, the QA of surveillance sites distributed across the entire country would undoubtedly be greater than those incurred in these two districts (\$ 5,138 per cluster per year) because of the far greater costs of travel, and perhaps the need to engage more than one QA team. For now, the potential benefits and costs of

these entomological surveillance innovations, as adapted to such conditions of national scale up remain a matter for speculation for now. Ultimately, they will need to be rigorously evaluated and compared under full-scale programmatic conditions of routine application before they are convincingly accepted as robust, with a sustainable place in the repertoire of national entomological and epidemiological surveillance platforms.

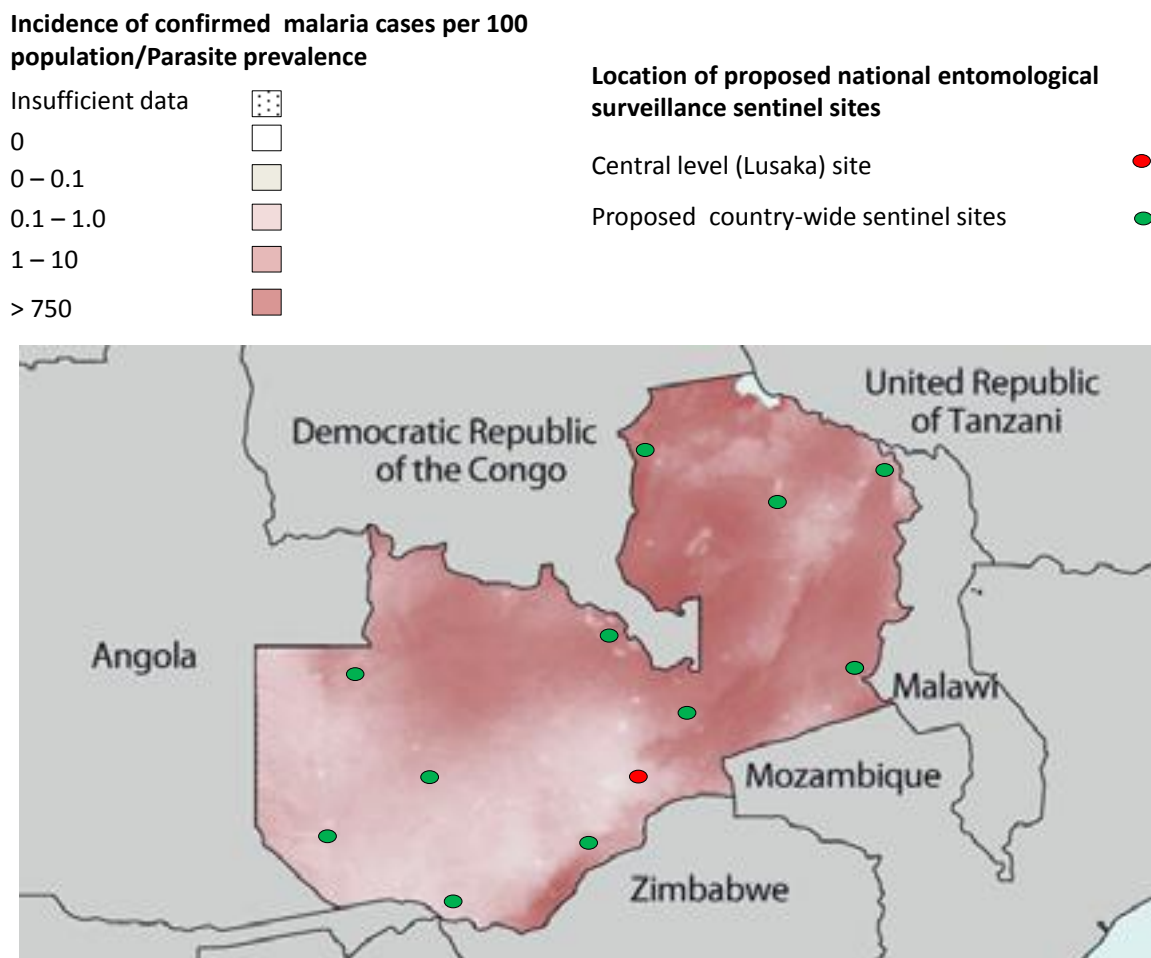


Figure 5.2: Proposed sentinel sites for supplementing conventional, annual entomological surveys of physiological resistance with community-based longitudinal of malaria vector population density and infection prevalence trends, as well as epidemiological surveillance of trends in human infection burden.

5.4 Monitoring mosquito behaviours so that vector control impacts and limitations can be understood and addressed: Current practice, methodological hurdles and future potential

There was a clearly significant incremental benefit upon diagnostic positivity (Table 4.3.1 and figure 4.3) among the human population and associated declines in the densities of *An. funestus* (Table 4.3.3 and figure 4.6) when LLINs are supplemented with pirimiphos methyl in this study area where this vector had high pyrethroid resistance (Chapter 4). Currently, malaria control programmes have prioritized monitoring vector populations for the emergence of physiological insecticide resistance to enable effective management of these dangerous traits using rotations or mosaics of active ingredients (WHO 2012). Both physiologically resistant and behavioural resilient or resistant vector populations can have dramatic impact upon programme implementation (Figure 1.4) that clearly needs to be understood as a dynamic phenomenon by programmes responsible for controlling or eliminating malaria transmission. Therefore in this context, both attributes of vector populations need to be monitored through a robust longitudinal surveillance systems.

Established procedures for monitoring vector population insecticide resistance are typically applied on annual surveys cycles, practiced by specialized centrally-managed teams of technicians. Wild adult indoor-resting mosquitoes are collected in selected houses, transported whilst fed on 10% sucrose solution to the insectary at the NMCC, blood-fed upon mice, and then allowed to lay eggs which are reared to provide F1 generation adults, all of which are then subjected to the standardized WHO protocol for susceptibility assays (WHO 1998). Through partnership with implementing partners and international research

institutes, Zambia has been monitoring resistance profiles of vector populations across the country over the last decade (Chanda, Coleman et al. 2012; Thomsen, Strode et al. 2014).

While physiological resistance mosquitoes clearly needs to be monitored as the most important single entomological indicator used by control programmes, vector population behavioural attributes also need to be considered, including those arising as a result of the application of insecticides. For instance, mosquito vectors are known to avoid insecticide contact by entering and exiting houses without exposure to active ingredients on the sprayed surface wall (Elliott 1972; Kitau, Oxborough et al. 2012; Okumu, Kiware et al. 2013; Okumu, Mbeyela et al. 2013). Vectors such as *An. arabiensis* in Africa, as well as *An. darlingi*, *An. punctimacula* and *An. nunezovari* in Latin America, exit rapidly without resting so that fatal levels of contact exposure to the insecticide is avoided (Killeen 2014). Such behavioural traits may also be induced or exaggerated by repellent or irritant actions of the active ingredients used for IRS or LLINs or other insecticidal measures. More so, vectors such as *An. arabiensis* that also feed upon animals often prefer to feed upon humans when they are outside the protective reach of IRS and LLINs and fully exposed by biting outdoor at dawn and dusk. It is therefore imperative that such populations are monitored to continually monitor and optimize the impact of LLINs, IRS and any further supplemental vector control tools, such as personal protection of humans with spatial repellents to control zoophagic or insecticide treatment of livestock to suppress populations of zoophagic mosquitoes. Continuous monitoring of the proportion of vector blood meals obtained from humans and from livestock, as well as the proportion of human exposure that occurs indoors or while sleeping may be invaluable for selecting optimal control measures for

specific vector species based on these metrics of their behaviours (Killeen, Seyoum et al. 2014).

While the proportions of blood meals mosquitoes obtain from humans and alternative animal hosts can be readily measured with simple, well-established immunoassay of blood meals from recently-fed specimens of mosquitoes (Garrett-Jones 1964; Garrett-Jones 1980), reliable techniques for direct detection of feeding attempts upon humans or animals outdoors do not yet exist. Unfortunately, all existing exposure-free methods for trapping host-seeking mosquitoes, are only effective for collecting mosquitoes attempting to feed upon humans whilst indoors, and are generally unreliable for measuring outdoor biting rates upon humans or animals. They therefore cannot be used to quantify the proportion of human-vector exposure that occurs whilst outdoors. The proportions of human exposure to mosquitoes that occurs indoors and outdoors can therefore only be estimated using the HLC method thus far. While the commercially-manufactured electrocuting grids recently evaluated in Tanzania yielded encouragingly similar results to HLC (Majambere, Massue et al. 2013), this technology will require further adaptation and then validations before it can be reliably used to measure the nocturnal distribution of human exposure. For now, such important behavioural metrics of where and when humans are exposed to malaria transmission rely on the hazardous HLC method, which requires constant drug prophylaxis against malaria (Gimnig, Walker et al. 2013) at the very least so these activities cannot be safely devolved to CHWs lacking careful, constant supervision and clinical support. Fortunately, the working conditions under which centrally-managed QA of the longitudinal CB surveys of vector population dynamics are conducted do satisfy these safety

requirements. Figure 4.8 demonstrates exactly how such data obtained from HLC conducted through QA activities can be used to provide invaluable data about the behavioural interactions between humans and mosquitoes, to help explain the extent and limitations of vector control impacts.

5.5. Overall conclusions

The findings of this study provide sufficient evidence to suggest that CB surveillance systems, using carefully-assessed trapping tools, could be applied longitudinally to monitor vector population dynamics so that the impact of insecticide-based vector control interventions on malaria transmission can be assessed. However, further improvements to improve this system are suggested and evaluation of such adaptations as applied at nationally representative scales, remain a subject for future research.

For example, all existing alternatives to HLC that allow exposure-free trapping of mosquitoes are unreliable for estimating human-vector interactions outdoors. Therefore future work may require developing tools comparable to HLC in estimating human-vector interaction indoors and, more crucially, outdoors (Majambere, Massue et al. 2013) across a wide range of vector systems in sub-Saharan Africa. Once these have been developed, they will also need to be standardized and validated across a wide variety of scenarios: Inconsistencies in the application of existing sampling methodologies and statistical analyses across a range of different transmission systems in the current literature mean that reports can only be interpreted in a site-specific manner, and are not generalizable across

the diverse spectrum of settings in Africa (Kelly-Hope and McKenzie 2009). Future research will need to take holistic approaches to designing and evaluating of alternative sampling methods that are comparable to the golden standard HLC which can be applied safely and generalizable across a wide range of transmission contexts across national scales. It should be noted, however, that regardless of how well such traps function, centralized laboratory services and analytical capacity will be required to estimate EIR, rather than merely mosquito density so entomological monitoring of transmission intensity *per se* cannot be entire devolved to community or even district level.

Even though these CB platforms and QA systems for monitoring vector population density trends are clearly feasible to implement in this rural Zambia study site and elsewhere in urban Tanzania (Chaki, Mlacha et al. 2012), further systematic improvements are suggested: (1) QA needs be conducted regularly and routinely by the expert team from the central level and quality controlled by the district and HF staff, so that CHWs adhere to the specified procedures; (2) In addition to the standard data paper entry formats, CHWs can be provided with simple, tailor-made pictorial reference material for ease of identification so that mosquitoes can be separated at genus level with addition differentiation to species complex of group for *An. gambiae* s.l. and *An. funestus* s.l., respectively, while others of far lesser medical importance in Zambia are simply entered as either culicines or other Anophilines. While such a system will require further training and continuous re-training, the future benefits of such investments will entail that the small number of professionally-qualified staff NMCC will be only need to conduct QA, laboratory testing, advanced data analyses, well as conducting specialized operational research; (3) In order to receive data in real time,

the content of all the paper forms can be electronically transmitted through mobile phone networks, as was conducted successfully for human infection test results conducted by CHWs in this study site (Hamainza, Killeen et al. 2014; Hamainza, Moonga et al. 2014) and elsewhere in Zambia (Kamanga, Moono et al. 2010). While these mobile platforms to collect epidemiological data reported weekly or monthly, the suggested system for future epidemiological and entomological surveillance systems will ideally require remission of data once entry is complete on the particular day of sampling, so that it also works as a quality control tool to help supervising EHTs keep track of whether activities are conducted as planned.

All such ambitions will require further human, financial and logistical resource investments. As earlier suggested (section 5.3) a motivated, strong team with expertise in entomological and epidemiological surveillance at NMCC and supporting national institutions will be a prerequisite to the success and sustainability of such programmes (Mukabana, Kannady et al. 2006; Shiff, Thuma et al. 2011). Trained laboratory and field scientists will also be required to conduct the day to day supervision and train districts and CHWs in the complementary longitudinal CB monitoring surveillance systems. While most entomological surveillance activities in African countries are currently financed, and indeed executed by external partners, it is essential for the governments to increase their financial support to their own NMCPs, so that home-grown operational research and surveillance provides the scientific evidence for cost-effective application of interventions. Increased domestic funding is also essential to strengthen the sustainability, responsiveness and local ownership of such programmes, especially now that the goal of malaria elimination is increasingly emphasized.

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