Studies on the Spatial Ecology of Malaria and the Impact of Mass Trapping of *Anopheles* Mosquitoes on Malaria Transmission in Southern Ethiopia

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This is to certify that the thesis prepared by Yared Debebe Desta, entitled: ***Studies on the Spatial Ecology of Malaria and the Impact of Mass Trapping of Anopheles Mosquitoes on Malaria Transmission in Southern Ethiopia*** and submitted in fulfilment of the Requirements for the Degree of Doctor of Philosophy in Biology (Insect Science) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abstract

Studies on the Spatial Ecology of Malaria and the Impact of Mass Trapping of* Anopheles *Mosquitoes on Malaria Transmission in Southern Ethiopia

Yared Debebe

Addis Ababa University, 2020

The sustainability of current indoor vector control methods is challenged by the emergence of insecticide resistance in malaria mosquito populations and the behavioural shift of vectors, resulting in increased outdoor biting activities. As a result, there is a dire need for novel vector control tools, which complement the existing strategies, particularly targeting the outdoor-active mosquitoes. Effective control of mosquitoes outdoors can be achieved through in-depth understandings of their spatial and behavioural ecology.

The effect of landscape elements on the resting site selection of the outdoor *Anopheles* mosquito population was assessed. Fine-scale characterization of landscape factors within 10 m radius from resting clay pots was conducted and their association with the number of resting anophelines was determined. Canopy cover, distance from the nearest focal house, and land cover type significantly influenced the aggregation of resting mosquitoes. Canopy cover was the strongest predictor for both the number and presence of *Anopheles* mosquitoes in the clay pots. Female *Anopheles* were most frequently found resting in the pots placed within the banana plantations, and at sampling points that were ≥75 m from the focal house.

To identify the factors underlying hotspots for higher vector densities and malaria incidence, and associated landscape features, monthly entomological monitoring, and four-stage repeated seasonal malaria prevalence surveys were conducted in two rural villages in southern Ethiopia. Moreover, characterization of the landscape features in and around every household of the study villages was conducted. Spatial analyses using Getis-Ord Gi* statistics were used to identify hotspots for malaria incidence, as well as malaria vector density and associated sporozoite prevalence. The result from the regression models revealed that household occupancy, location and housing conditions were the main

predictors of vector density, entomological inoculation rate, and malaria incidence. The spatial analyses revealed that statistically significant hotspots for malaria vector densities and *Plasmodium*-infected individuals were identified at village edges.

The impact of mass trapping of *Anopheles* mosquitoes using odour-baited traps was assessed in a controlled before-and-after study design in two rural villages of Southern Ethiopia. Baseline monthly entomological and seasonal cross-sectional malaria prevalence surveys were conducted in the two villages for a year. Then, mass trapping was implemented in one of the villages immediately before the beginning of the rainy season, while the monthly entomological monitoring and the seasonal malaria prevalence surveys continued in both villages for nine months, throughout the long and short rainy seasons. The impact of the mass trapping was then assessed by computing the relative reduction of entomological indices and malaria incidence in the intervention village in a seasonal comparison with the control village. The mass trapping resulted in a significant reduction in the population of the primary malaria vector in the area, *An. arabiensis* and the associated entomological indices (the human biting-, sporozoite-, and entomological inoculation rates) in the intervention compared to the control village. This resulted in a relative reduction of malaria incidence by 61 %, 44 %, and 49 % in the long rain, short rain, and dry seasons, respectively.

Keywords: *Anopheles*, Exophilic, Hotspot, Malaria incidence, Mass trapping, Relative reduction

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"ሁሉ አመክሩ ወዘሠናየ አጽንዑ"

1 ተሰሎንቄ 5፡21

"Prove all things; hold fast that which is good"

1 Thessalonians 5:21

Dedication

To my beloved father, Debebe Desta, who is the inspiration in every walk of life and my academic career

Table of Contents

List of Figures

List of Tables

Acronyms

AHD	Acetoxy Hexadecanolide
AL	Antennal Lobe
ATSB	Attractive Toxic Sugar Bait
Bti	*Bacillus thuringiensis* var *israelensis*
Ca	Circa
cAMP	cyclic Adenosine Monophosphate
CDC	Center for Disease Control and Prevention
CSP	Circumsporozoite protein
DAG	Diacylglycerol
DDT	Dichlorodiphenyltrichloroethane
EIR	Entomological inoculation rate
GDP	Gross Domestic Product
GR	Gustatory Receptor
IRD	Indoor resting density
IP3	Inositol 1,4,5 triphosphate
IR	Ionotropic Receptor
IRS	Indoor Residual Spray

ITNs	Insecticide Treated Nets
IVM	Integrated Vector Management
HBR	Human-biting rate
LLINs	Long Lasting Insecticidal Nets
MoH	Ministry of Health
OBP	Odorant Binding Protein
OR	Odorant Receptor
Orco	Odorant Receptor Co-receptor
ORN	Olfactory Receptor Neuron
PSC	Pyrethrum Spray Catches
RBC	Red Blood Cells
SR	Sporozoite rate
SSA	sub-Saharan Africa
WHO	World Health Organization

Chapter 1

General Introduction

1.1. Global trends in the burden of malaria

Malaria is the most prevalent vector-borne disease transmitted by mosquitoes and exerts devastating public health and socioeconomic obstacles in the developing world (Gallup and Sachs, 2001; Basili and Belloc, 2015, Campbell-Lendrum *et al.,* 2015). Protozoan parasites in the genus *Plasmodium* are responsible for causing malaria in different vertebrate taxa, including primates, rodents, reptiles, and birds (Outlaw and Ricklefts, 2011). Four *Plasmodium* species cause malaria exclusively in humans; *Plasmodium falciparum*, *P. vivax*, *P. ovale,* and *P. malariae* (Vangapandu *et al.,* 2007). The recently discovered zoonotic species, *P. knowlesi* in southeast Asia (Antinori *et al.,* 2013) and *P. simium* (Brasil *et al.,* 2017) in Brazil, also cause malaria in humans. These parasites are transmitted through infectious bites of female mosquitoes in the genus *Anopheles* (Muriu *et al.,* 2008).

Perhaps half of everyone who ever lived on earth had died of malaria (Whitfield, 2002) and hundreds of thousands of people are still dying of this disease every year. In 2018 alone, a total of 228 million malaria cases was reported globally, and approximately 405,000 people died (WHO, 2019). The malaria burden is highly concentrated in sub-Saharan Africa (SSA) where 94% of the deaths occur (WHO, 2019). Along with prevailing favourable ecological conditions (Kiszewski and Teklehaimanot, 2004), socioeconomic

factors contribute to the intensity of malaria disease burden in this region (Collins and Paskewitz, 1995). The higher prevalence of *P. falciparum*, and wider geographical distribution of some of the most efficient malaria vectors, such as *Anopheles gambiae sensu lato* and *An. funestus*, are significant factors that contribute to the higher rate of mortality and morbidity in SSA (Kiszewski *et al.,* 2004; Nkumama *et al.,* 2017).

Malaria was eradicated from most European, American, and Asian countries during the 1950's eradication campaign (Najera *et al.,* 2011). However, in the past few decades, the re-emergence of malaria has been confirmed in different regions of the world where it had been declared as eliminated or eradicated, including e.g. Russia and some provinces of China (Gao *et al.,* 2012; Mironova *et al.,* 2020). Expansion of malaria, due to the global climate change, into previously non-affected areas of malaria-endemic countries, is the latest challenge complicating the global malaria prevention and control effort (Hay *et al.,* 2002; Chaves and Koenraadt, 2010). In Ethiopia, reports have shown that malaria has expanded into the highlands where people lack protective immunity (Woyessa *et al.,* 2013) increasing the risk of epidemics. Water-related development projects such as irrigation schemes and hydroelectric power dams are also increasing the risk of malaria transmission in the country (Jaleta *et al.,* 2013; Kibret *et al.,* 2014).

Malaria is also an impediment to the economic growth of many tropical and sub-tropical countries and has been called a disease of poverty since it is widely distributed in developing countries (Gallup and Sachs, 2001). Malaria negatively affects the economic growth of a country either directly, through personal and government expenditure for vector control and case management, or indirectly, through absenteeism of working power

from the economy due to illness (Sachs and Malaney, 2002). For instance, in Ethiopia, the direct cost budgeted to malaria prevention and control between the years 2011 and 2015 was about 180 million USD per annum (MOH, 2011). At the household level, in a single malaria episode, at least 5 USD is spent in seeking treatment and covering other indirect expenses (Hailu *et al.,* 2017). Furthermore, work absenteeism due to taking care of a sick child negatively affects the economy of the household, especially those relying on the earnings of women (Chima *et al.,* 2003; Singh *et al.,* 2019). The impact of malaria on the gross domestic product (GDP) of malaria-endemic areas with *P. falciparum* (e.g. the SSA) had an average GDP growth of six times less than those with no *P. falciparum* in the years between 1965 and 1990 (Gallup and Sachs, 2001; Sachs and Malaney, 2002).

The adoption and implementation of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) as the frontline malaria vector control strategies have brought a significant decline in malaria cases and mortality in many SSA countries and other malarious regions of the world. For example, malaria-related deaths have significantly reduced globally and in SSA by 29 % and 31 %, respectively, between the years 2010 and 2015 (WHO, 2016). However, malaria is still a serious disease of public health importance in many of these places. The annual global malaria cases have increased by eight million in 2018 from the previous year (WHO, 2019), alerting the scientific and public health communities that a more consolidated prevention and control effort is needed.

Despite the effectiveness of LLINs and IRS, their long-term utilization has resulted in the replacement of primary indoor biting mosquitoes, like *An. gambiae sensu stricto*, with the more versatile species, *An. arabiensis*, which feeds and rests both indoors and outdoors

(Bayoh *et al.,* 2010; Govella and Ferguson, 2012). These indoor vector control interventions are also reported to have diverted endophagic mosquitoes to seek blood meals outdoors and exhibit trophic deviation to non-human hosts enabling them to maintain higher population densities (Pappa *et al.,* 2011; Reddy *et al.,* 2011; Russell *et al.,* 2011; Moiroux *et al.,* 2012). Changes in the peak biting time of *Anopheles* vectors has also been observed (Yohannes and Boelee, 2011; Moiroux *et al.,* 2012; Sougoufara *et al.,* 2014). These shifts in biting and resting behaviour have resulted in an increased rate of human-vector contact outdoors early in the evening before people retire to bed under LLINs, indoors. This scenario has increased residual malaria transmission, the current global challenge that needs immediate attention to achieve the objectives of a "malaria-free world" by 2030 (Yohannes and Boelee, 2011; WHO, 2015a), which at the moment seems less likely as the progress being made by many countries in eliminating malaria has installed recently (WHO, 2019).

1.2. Rationale of the study

Malaria elimination and eradication cannot be achieved through the currently implemented vector control strategies. Novel vector control tools need to be added to complement existing strategies. Development of novel vector control tools targeting the vector populations exhibiting shifts in biting and resting behaviour is essential to minimize the burden of malaria to a level, which no longer creates a public health problem in the developing world. Currently, efforts are being made to develop novel vector control methods, among which control efforts targeting the odour-mediated behaviour of malaria

vectors has the potential to be used as a complementary weapon in the arsenal of strategies available in the fight against malaria.

An in-depth understanding of the chemical ecology of malaria vectors is a steppingstone for the development of odour-based control methods. There is a plethora of studies conducted to understand the role of chemical cues in resource location by malaria vectors. The resources required by a mosquito include sugar meals (Foster and Hancock, 1994), blood meals (Takken and Knols, 1999), and oviposition sites (Eneh *et al.,* 2016; Wondwosen *et al.,* 2016; 2017; 2018; Asmare *et al.,* 2017). The chemical cues from these resources can be identified, optimized and developed into attractive blends which can be used to manipulate mosquito behaviour, enabling mass trapping and killing of mosquitoes (Kline, 2007; Homan *et al.,* 2016). However, studies aimed at evaluating the efficacy of attractive semiochemical blends in reducing malaria prevalence and vector population density are scarce. In one such intervention study, the effect of mass trapping of *Anopheles* mosquitoes using odour-baited traps, Homan *et al.* (2016) demonstrated a significant reduction of malaria cases in intervention areas as compared with non-intervention areas.

We hypothesize that the combined use of attractants and other integrated vector management (IVM) tools can provide community-wide malaria protection, as the attractant used to mass trap mosquitoes will reduce human-vector interaction and at the same time reduce vector density. A mass-trapping system could also serve as an important remedy for the problems associated with behavioural and physiological resistance of mosquitoes to most available insecticides. The use of odour-baited traps in malaria vector control particularly targeting the outdoor population requires an increased knowledge of the spatial

ecology of the vector species (Zhu *et al.,* 2017). Moreover, it is important to determine the ecological factors that dictate the spatial heterogeneity in malaria vector densities and malaria incidence within local landscapes. This in turn allows for targeted vector control approach by optimizing the positions of the odour-baited traps within the landscape addressing the heterogenous distributions of the vectors (Crepeau *et al.,* 2013). In this study, we have characterized the landscape elements that affect the resting site selection of exophilic anopheline populations, identified the environmental factors that influence the heterogenous distribution of malaria vectors and incidence at village and household levels. Furthermore, we have evaluated the effect of the combined use of IVM tools with mass trapping of *Anopheles* mosquitoes using odour-baited traps on malaria transmission intensity in southern Ethiopia.

1.3. Hypotheses

- Outdoor resting site location by exophilic *Anopheles* mosquitoes depends on different environmental features within the landscape.
- The heterogeneity in the distribution of malaria vectors and the resulting risk of malaria exposure can be explained by the theoretical framework underlying resource seeking in phytophagous insects.
- Mass trapping of *Anopheles* mosquitoes using odour-baited traps in integrated vector management in low-to-intermediate transmission settings will reduce the density of the vector population and malaria transmission significantly.

1.4. Objectives of the study

1.4.1. General objective

This study aimed to assess the spatial ecology of malaria and evaluate the effect of odour-based mass trapping of *Anopheles* mosquitoes, and demonstrate the complementarity of outdoor vector control to conventional integrated vector management to reduce malaria burden in southern Ethiopia.

1.4.2. Specific objectives

This project specifically intends:

- To identify landscape characters that dictate outdoor resting site selection of exophilic *Anopheles* mosquitoes.
- To explain the spatial heterogeneity of malaria vector densities and the resulting malaria incidence with established ecological theories underlying resource seeking in phytophagous insects.
- To generate baseline preintervention entomological and parasitological data in a control and an intervention village.
- To assess the impact of mass trapping of *Anopheles* mosquitoes on malaria prevalence and entomological indices such as population density, infectivity and entomological inoculation rates in the intervention village.

Chapter 2

Literature Review

2.1. Distribution of malaria parasites

The global malaria burden is mainly caused by *P. falciparum* and *P. vivax*, which account for more than 95% of malaria cases in the world (Vangapandu *et al.,* 2007). However, more than 90% of malaria-related deaths are caused by the most pathogenic of all the species, *P. falciparum* (Snow, 2015). The burden is higher in some regions of the world partly due to the variations in the geographical distributions of *Plasmodium* parasites. Infections caused by *P. falciparum* are common in sub-Saharan Africa (SSA), East Asia, and South America, as well as in Oceania (Gething *et al.,* 2011). In contrast, *P. vivax* is the causative species primarily in South East Asia, Central Asia, Central America, and is also common in Oceania and South America (Gething *et al.,* 2012). *Plasmodium ovale* is highly prevalent in West Africa and the Far East (Collins and Jeffery, 2005), whereas *P. malariae* is found in most endemic areas such as SSA, having similar geographical distribution as *P. falciparum* (Collins and Jeffery, 2007). *Plasmodium knowlesi* has a limited geographical distribution confined to the forest areas of Southeast Asia (Antinori *et al.,* 2013).

2.2. Transmission cycle of malaria parasites

Malaria parasites exhibit a heteroxenous life cycle, with alternation of generations between the human host and the mosquito vectors (Figure 2.1). The cycle begins when an infected

mosquito injects the infective stage of the parasite, sporozoites, into the bloodstream of an uninfected human (Cowman *et al.,* 2012). The sporozoites then migrate to the liver shortly where they invade the liver cells, the hepatocytes (Soulard *et al.,* 2015) leading to an asymptomatic asexual multiplication stage called the pre-erythrocytic cycle, resulting in tens of thousands of daughter merozoites (Vaughan *et al.,* 2008). The merozoites are then released into the bloodstream invading the red blood cells (RBCs) and undergo asexual multiplication (erythrocytic cycle) producing a greater number of merozoites within 24-72 hours depending on the species (Antinori *et al.,* 2012; Cowman *et al.,* 2012). The merozoites then rupture the RBC and invade other RBCs and the cycle continues multiple times until controlled by either the immune system or antimalarial drugs (Crompton *et al.,* 2014). Some of the merozoites mature into male and female gametocytes, which will be picked up by the female *Anopheles* mosquitoes during a blood meal (Talman *et al.,* 2004).

In the mosquito's midgut, the sporogonic cycle begins with the maturation of the female and male gametocytes into female and male gametes, respectively, followed by fertilization (Simonetti, 1996). The resulting zygote will develop into a slowly motile ookinete which later develops into an oocyte after penetrating the midgut membrane (Beier, 1998; Aly *et al.,* 2009). The oocytes will then multiply asexually and produce sporozoites, which are released into the hemocoel of the mosquito (Simonetti, 1996). The sporozoites then migrate to and invade the salivary glands where they will be injected into a healthy individual during a blood meal completing the cycle (Beier, 1998).

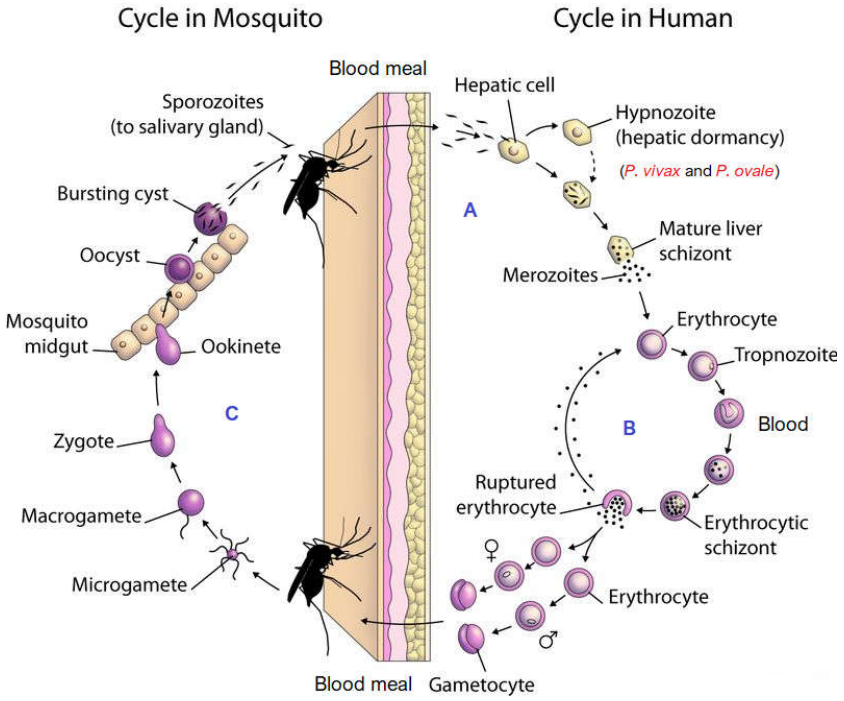


Figure 2.1. The life cycle of *Plasmodium* parasites. The pre-erythrocytic, the erythrocytic, and the sporogonic cycles are represented by letters A, B, and C, respectively. (Modified from http://ocw.jhsph.edu/).

2.3. Malaria vectors

2.3.1. Major Afro-tropical malaria vectors

A total of around 3500 mosquito species have been described globally (Rueda, 2008). Of these, 537 described species belong to the genus *Anopheles* (Harbach, 2013), consisting of species that vector both malaria and filariasis (Derua *et al.,* 2015). Only 70 anopheline species have been incriminated as vectors of malaria, out of which 41 species are considered as primary vectors (Sinka *et al.,* 2012). Two species complexes, the *An. gambiae* complex and *An. funestus* complex, are the primary malaria vectors responsible

for the transmission of malaria in SSA (Stevenson and Norris, 2017). However, other *Anopheles* species are important vectors at local scales due to their restricted geographical distributions (Afrane *et al.,* 2016).

The *An. gambiae* species complex is composed of nine sibling species; *An. gambiae s.s* (hereafter *An. gambiae*), *An. coluzzii*, *An. arabiensis*, *An. melas*, *An. merus*, *An. bwambae*, *An. quadriannulatus*, *An. amharicus* (Coetzee *et al.,* 2013) and the most recently described species, *An. fontenillei* (Barron *et al.,* 2019). *An. gambiae, An. coluzzii* and *An. arabiensis* are the primary malaria vectors in the species complex in SSA (Fahmy *et al.,* 2015). These are sympatric species in most of SSA but display different behaviour and ecological requirements (Stevenson and Norris, 2017). The first two species are highly anthropophilic, endophagic, endophilic, and highly susceptible to *P. falciparum* (Simard *et al.,* 2009) while *An. arabiensis*, besides its high vector competence, displays more plasticity in terms of its feeding and resting behaviour making its control more difficult using indoor antivector interventions (Sinka *et al.,* 2010).

Anopheles melas and *An. merus* are marine breeders distributed along the coastal areas of West Africa and eastern and southern Africa, respectively (Wiebe *et al.,* 2017). *Anopheles bwambae* is the least common species in the complex recorded only in limited locations in Uganda (Besansky *et al.,* 2006). *Anopheles quadriannulatus* and *An. amharicus* (formerly called *An. quadriannulatus* A and *An. quadriannulatus* B, respectively) are the only members in the complex that do not take part in human malaria transmission as they feed mainly on animals (Hunt *et al.,* 1998; Coetzee *et al.,* 2013). The newest member in the complex, *An. fontenillei,* was described very recently in Gabon with very few individuals,

the details of its ecology, behaviour and its role in malaria transmission is yet to be investigated (Barron *et al.,* 2019).

The *Anopheles funestus* species complex group is also a dominant malaria vector in many of the SSA countries, consisting of many morphologically similar but molecularly distinct species that coexist with the *An. gambiae* species complex (Choi *et al.,* 2012). The species include nine formerly identified species; *An. aruni*, *An. brucei, An. confusus, An. funestus*, *An. fuscivenosus, An. leesoni, An. parensis, An. rivulorum, An. vaneedeni* (Gillies and Coetzee, 1987) and two recently identified species; *An. funestus*-like (Spillings *et al.,* 2009), and *An. rivulorum*-like (Cohuet *et al.,* 2003). Among these species, *An. funestus s.s.* is the prime malaria vector due to its anthropophilic nature, vector competence, higher abundance, and wider geographical distribution (Coetzee and Fontenille, 2004).

2.3.2. Other important malaria vectors in Africa

In addition to *An. gambiae s.l.* and *An. funestus s.s.* there are other dominant malaria vectors such as *An. moucheti* and *An. nilli s.l.* responsible for significant levels of malaria transmission in parts of the SSA (Sinka *et al.,* 2010). These two species/species complexes are considered as neglected species with lack of knowledge on their biology, ecology and behaviour (Antonio-Nkondjio and Simard, 2013). *Anopheles moucheti* is widely distributed in the forested areas of tropical Africa particularly in the western and central regions where it plays significant role of malaria transmission (Antonio-Nkondjio *et al.,* 2002). There exists morphologically and behaviourally distinction among the populations of *An. moucheti* with three different morphological forms; *An. moucheti moucheti*, *An.*

moucheti nigeriensis and *An. moucheti bervoetsi* (Fontenille and Simard, 2004). *Anopheles nill* species complex consists of four species namely *An. nili s.s*, *An. carnevalei*, *An. somalicus* and *An. ovengensis*, majorly found in tropical Africa with preference to humid savannas areas (Fontenille and Simard, 2004; Osse *et al.,* 2019).

2.3.3. Malaria vectors in Ethiopia

In Ethiopia, 46 anopheline species and subspecies have been recorded (Kyalo *et al.,* 2017). However, only a few species are incriminated as vectors of malaria to date (Massebo *et al.,* 2013a). *Anopheles arabiensis* and *An. amharicus* are the only members of the *An. gambiae* species complex reported to date (Hunt *et al.,* 1998). *Anopheles arabiensis* is the primary vector of malaria having a wider geographical distribution, whereas *An. amharicus* has a very limited geographical distribution, is predominantly zoophagic, and thus plays no role in malaria transmission (Hunt *et al.,* 1998; Coetzee *et al.,* 2013). Other species such as *An. funestus s.l*., *An. pharoensis* and *An. nilli* take part in the transmission of malaria at local scales (Taye *et al.,* 1996). Recently, Carter *et al.* (2018) made the first report on the presence of the malaria mosquito, *An. stephensi*, in Eastern Ethiopia (formerly restricted to the Indian sub-continent), which could aggravate the malaria burden in the country.

2.4. Mosquito behaviour

Adult female *Anopheles* mosquitoes display a wide array of behaviours at each step in their gonotrophic cycle. These behaviours are important for the survival, fitness, and perpetuation of the species. Understanding these behaviours has paramount importance in finding out how to manipulate the behaviours and come up with appropriate vector control

strategies targeting them. Interventions targeting each stage in the life cycle need to consider how the mosquitoes behave in locating and utilizing resources from the environment.

2.4.1. Sugar feeding behaviour

During emergence, adult mosquitoes usually have a very low energy reserve, which needs to be compensated for within one- or two-days post-emergence (Foster and Takken, 2004). The origin of their first energetic meal depends on the mosquito species. Most mosquito species are obligatory sugar feeders that feed on different host plants to obtain sugar, as an energy source for survival, fitness, fecundity, vertebrate host-seeking, and flight (Foster and Hancock, 1994). The sugars from host plants are readily available as floral and extrafloral nectarines, honeydew and decaying fruits, saps, etc (Gu *et al.,* 2011). Some mosquito species, like *An. gambiae* and *Aedes aegypti,* are opportunistic feeders in which they may or may not consume sugars depending on the availability of vertebrate hosts (Foster and Takken, 2004).

Sugar feeding behaviour in mosquitoes is mostly mediated by volatile compounds, such as cyclic or bicyclic monoterpenes (Jhumur *et al.,* 2008) These compounds dictate the orientation and attraction of mosquitoes to host plants (Takken and Knols, 1999). *Anopheles* mosquitoes exhibit preference for some sugars, like sucrose and a mixture of glucose and fructose, the most frequent sugars in nectars, over others (Kessler *et al.,* 2013; Kessler *et al.,* 2015). *Anopheles gambiae* that fed on the preferred sugars had prolonged longevity (Kessler *et al.,* 2015). In contrast, mosquitoes feeding on less preferred sugars,

like glucose alone (Kessler *et al.,* 2015), and those that are deprived of any sugar (Straif and Beier, 1996), exhibit higher frequencies of blood-feeding to compensate for their sugar requirements, a behaviour that may facilitate the risk of malaria transmission (Afrane *et al.,* 2012).

2.4.2. Mating behaviour

Mating is a crucial stage in the reproductive biology of mosquitoes, which involves the insemination of females by males once in their lifetime after adult emergence (Clements, 1992). However, mating behaviour has not received sufficient attention to date (Benelli and Mehlhorn, 2016). Mating in most mosquito species takes place in swarms formed by males just after sunset (Achinko *et al.,* 2016). Females are attracted to the swarm drawn by visual, acoustic, and chemical cues (Tripet *et al.,* 2004; Diabate *et al.,* 2011; Diabate and Tripet, 2015). Mating usually occurs after females had their first meal, which could be either sugar or blood depending on the species, to replenish their energy reserve for movement (Takken *et al.,* 2006). Understanding the swarming and mating behaviour of mosquitoes is crucial for novel vector control approaches to increase the efficacy of swarm bomb spray (Diabate and Tripet, 2015), genetically modified mosquitoes, and the sterile insect technique (Achinko *et al.,* 2016).

2.4.3. Host-seeking behaviour

Blood feeding is an obligatory activity for anautogenous mosquitoes as the blood meal provides important nutrients for egg maturation (Takken, 1991). These mosquitoes have well-developed host locating mechanisms, mainly using their chemical senses. A

combination of environmental factors, including chemical, visual, and thermal cues dictate how mosquitoes find their preferred hosts (Raji and DeGennaro, 2017). The physical cues appear to be more important at closer distances, while vision and chemical cues appear to dictate the behavioural decisions of host-seeking mosquitoes at larger distances (Takken, 1991). Anthropophilic *Anopheles* species such as *An. gambiae* are more attracted to human body odours and thus predominantly feed on humans, whereas opportunistic species like *An. arabiensis* has a wider range of host preference (Busula *et al.,* 2015).

2.4.4. Resting behaviour

Following successful feeding on host animals, *Anopheles* mosquitoes need to select suitable resting sites for blood meal digestion and egg maturation (Service, 2012). Resting behaviour of *Anopheles* mosquitoes varies from species to species. Some species, like *An. gambiae* and *An. funestus*, are endophilic while others prefer resting outdoors (Charlwood *et al.,* 2018). *Anopheles arabiensis*, on the other hand, shows plasticity in resting site choosing both indoors and outdoors depending on the availability of blood meal sources (Mahande *et al.,* 2007). Endophilic species rest on the walls, ceilings, under furniture, or any suitable places pre- or post-blood meal (Rozendaal, 1997). Exophilic mosquitoes rest on vegetation, eaves, under bridges, cattle sheds, clay pots, and holes on the ground (Forattini *et al.,* 1993). However, their resting site choice is highly influenced by the landscape elements particularly the micro-climate and the physical environment surrounding the resting sites (Paaijmans and Thomas, 2011). Shaded areas are preferred resting sites as they provide microhabitats with suitable temperature and humidity (Forattini *et al.,* 1993; Paaijmans and Thomas, 2011), protecting the resting mosquitoes from desiccation (Chown *et al.,* 2011).

2.4.5. Oviposition behaviour

Oviposition site selection is a crucial stage in the life cycle of mosquitoes that affects the fitness and survival of the progeny (Bentley and Day, 1989). Therefore, mosquitoes have to make sure that they select suitable breeding grounds for their developing offspring. Different biological, chemical and physical factors are responsible for guiding gravid mosquitoes to select oviposition sites (Day, 2016). These environmental factors may attract, stimulate, repel, or deter the mosquitoes to or from the potential oviposition substrates (Dethier *et al.,* 1960).

Odour plumes given off from oviposition sites play a major role in informing the suitability of the habitat for successful hatching of the eggs and the survival of the developing larvae (Bentley and Day, 1989). By sensing these odorous volatiles, mosquitoes can detect the presence of potential predators, competitors, conspecifics, larval foods, etc (Afify and Galizia, 2015) that help them decide whether to oviposit or avoid the aquatic habitat.

Mosquitoes also make use of volatiles released from the aquatic stages of conspecifics, either as oviposition stimulant or deterrent (Day, 2016). Ogbunugafor and Sumba (2008) indicated that *An. gambiae* preferred to oviposit in breeding sites with a lower larval density of conspecifics over those with no conspecific larvae. Moreover, Munga *et al.* (2006) demonstrated that *An. gambiae* prefer to lay eggs in oviposition sites with a lower density of conspecific larval as compared with habitats having a high larval density. Both studies indicated that mosquitoes prefer optimal conditions to lay their eggs, and are guided by chemical cues from the immatures of conspecifics. The presence of conspecific larvae at low density could indicate the suitability of the breeding habitat. The presence of higher

numbers of larvae in the breeding habitat on the other hand could indicate the probable depletion of resources due to competition (Ogbunugafor and Sumba, 2008), signalling the unsuitable conditions of the oviposition site to gravid mosquitoes (Suh et *al.,* 2016). Mosquitoes may also use cues from other species in different genera to select oviposition sites; Wachira *et al.* (2010) indicated that *An. gambiae* preferred oviposition sites with a low density of *Culex quinquefasciatus* larvae*,* while a higher density deterred egg-laying.

2.5. Olfaction in mosquitoes

2.5.1. Olfactory structures of mosquitoes

Mosquitoes predominantly rely on their olfactory system to interact with the chemical environment surrounding them (Hansson and Stensmyr, 2011). The olfactory system of mosquitoes is composed of hair-like cuticular extensions called sensilla, which are found on the antennae, maxillary palpi, and at the tip of the proboscis called the labella (Carey and Carlson, 2011) [Figure 2.3A]. The sensilla are either single or double-walled and contain odour-sensitive cells called the olfactory receptor neurons (ORNs), which are surrounded by different categories of supportive cells (Jacquin-Joly and Merlin, 2004) [Figure 2.3B]. The ORNs are the smallest functional units of the olfactory system, where chemical stimuli are translated into electrical impulses (Zwiebel and Takken, 2004). The dendrites of the ORNs are bathed within the sensillum fluid, containing the odorant-binding proteins (OBPs). The OBPs are produced by the accessory cells in the sensilla (de Bruyne and Baker, 2008). On the dendrites of the ORNs found membrane proteins called olfactory receptors, which are selective and sensitive to the incoming odorants (Fleischer *et al.,* 2018).

Three different kinds of olfactory receptor proteins are recognized on the dendrites of the ORNs: odorant receptors (ORs), ionotropic receptors (IRs), and gustatory receptors (GRs). These receptors are specialized in detecting different classes of odour molecules. For instance, ORs are sensitive to aromatic compounds, terpenes, and aliphatic compounds (de Fouchier *et al.,* 2017), whereas IRs detect acids and amines (Pitts *et al.,* 2017). The GRs, on the other hand, are sensitive to CO_2, 1-octen-3-ol (Lu *et al.,* 2007), and diethyltoluamide (DEET) (Sanford *et al.,* 2013).

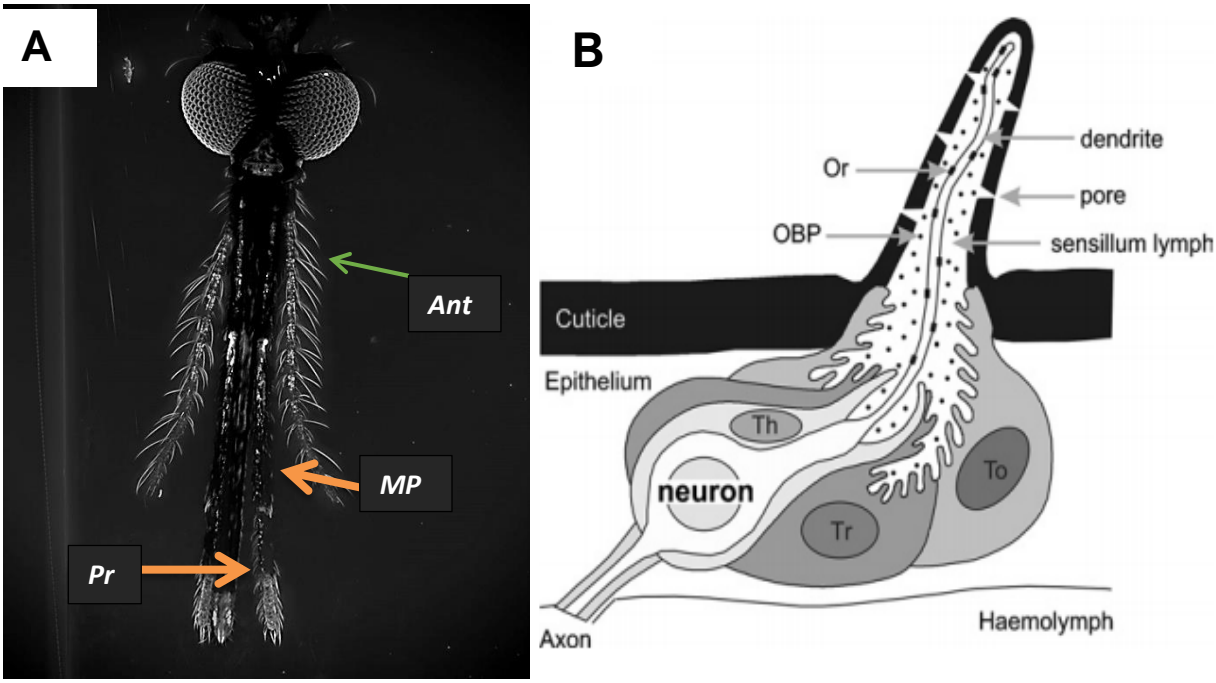


Figure 2.2. (A) Structures of the female *Anopheles gambiae* housing olfactory sensilla; Ant: Antennae; MP: Maxillary palps; Pr: Proboscis (modified from Shen, 2017). (B) Fine structure of a sensillum housing the olfactory receptor neurons and accessory cells; Th: thecogen; To: tormogen; and Tr: tricogen. The odorant receptors (Ors) are expressed in the dendritic membrane of the olfactory receptor neurons, while the odorant-binding proteins (OBPs) float in the sensillum lymph (Source: Jacquin-Joly and Merlin, 2004).

2.5.2. Mechanism of odour detection in mosquitoes

Odour detection and transduction in mosquitoes begins when odorant molecules enter into the sensillum lymph. Odorants from the source will dissolve in the environment and enter into the sensillum via the sensillum pore (Hansson and Stensmyr, 2011). The hydrophobic odorant will then bind to the OBPs in the sensillum lymph forming a hydrophilic complex. The odorant-OBP complex will then be transported to the receptors on the dendrites of ORNs, where transduction of chemical stimuli into electrical impulses takes place (Pellegrino and Nakagawa, 2009). The odorant-OBP complex will then be degraded by odorant degrading enzymes freeing the OBP for new incoming odorants (Younus *et al.,* 2017).

Two models are proposed for how the odorant molecules bind to the ORs and generate ORN action potentials, as illustrated in Figure 2.4. The first model, i.e. the odorant-gated ion channel model, proposes that the binding of the odorant to the OR results in the formation of an ion channel between the OR and Orco resulting in the influx of cations, thereby causing depolarization and hyperpolarization of the ORN (Sato *et al.,* 2008). As a result, an electrical impulse will be generated, and transported, via the axon of the ORN, to the primary olfactory center in the brain, the antennal lobe (AL), resulting in physiological and behavioural responses (Jacquin-Joly and Merlin, 2004). The second model is the classical model of insect olfaction mechanism, i.e. the G-protein mediated signalling pathway, which is historically widely accepted. In this model, the binding of the odorant to the OR elicits a series of chemical reactions resulting in the formation of secondary messenger molecules such as inositol 1,4,5 triphosphate (IP3), diacylglycerol

(DAG) and cyclic adenosine monophosphate (cAMP). These molecules will, in turn, activate the opening and closing of the membrane ion channel via Orco, resulting in the generation of action potentials (Wicher *et al.,* 2008). Figure 2.4 illustrates the schematic representation of the two models.

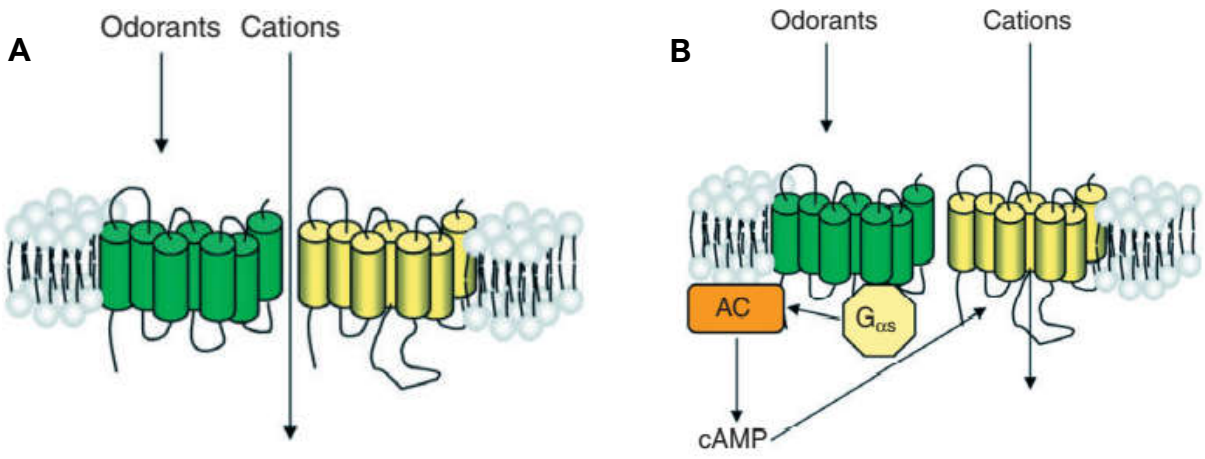


Figure 2.3. Models of signal transduction mechanisms in insect olfactory sensory neurons. (A) The odorant-gated ion channel model predicts that the binding of odorants to an OR (green) will induce the formation of an ion channel between the ORs and Orco (yellow) allowing the influx of cations. (B) The G-protein mediated signalling pathway predicts that binding of odorants to ORs elicits a cascade of chemical reactions in the cytosol resulting in the generation of secondary messenger molecules, which mediates the opening and closing of ion channels within the Orco. (Source: Pellegrino and Nakagawa, 2009).

2.6. Malaria vector control: a historical perspective

Malaria is one of the most ancient and deadliest infectious diseases affecting humanity. The disease is assumed to be older than 4700 years as historical records of a malaria-like febrile disease have been found in Chinese scriptures from about 2700 BC. Written pieces of evidence have also been found in Egyptian literature, witnessing the presence of malaria before 4000 years (Coll-Seck, 2010). The cause of malaria, however, was mysterious until the discovery of the infectious agents, the *Plasmodium* parasites, and the agents of transmission, the *Anopheles* mosquitoes, by Charles Louis Alphonse Laveran and Ronald Ross, respectively, at the end of the nineteenth century (Capanna, 2006). Following these discoveries, vector control methods, particularly larval source management was the most feasible and reliable means in preventing the disease, since effective drugs were not available as treatment options until the 1940s. Different vector control options such as screening of houses, larviciding, environmental management of breeding habitats, and application of oil or Paris green (copper (II) acetate triarsenite) to breeding places were also frequently used (Rozendaal, 1997; WHO, 2005).

The discoveries of the powerful insecticide, dichlorodiphenyltrichloroethane (DDT), and the effective drug, chloroquine, in the 1940s were major steps forward in the efforts made to control malaria and other vector-borne diseases (Bruce-Chwatt, 1985; Najera *et al.,* 2011). For its strong residual effect, DDT was embraced in the malaria control programs of many countries right after the discovery (Sougoufara *et al.,* 2017). Before the middle of the nineteen-century, almost every corner of the globe had suffered from malaria (Mendis *et al.,* 2009) (Figure 2.2). However, following these discoveries, there was a glimmer of

hope in controlling the disease in many of the malarious areas of the world through the global malaria eradication campaign launched by the world health organization (WHO) (Bruce-Chwatt, 1985; Sougoufara *et al.,* 2017). The campaign was successful in eradicating the disease from many endemic regions of the world such as the Americas, Europe, Southeast Asia, and the Indian sub-continent in the 1950s and 1960s (Bruce-Chwatt, 1987). As a result, the global malaria distribution had narrowed down majorly to the tropical and sub-tropical countries (Mendis *et al.,* 2009). Despite the efforts made, these "success" stories were not eternal. Malaria had re-emerged in some of the "malaria freed" areas due to the emergence of insecticide resistance in the mosquito vectors against DDT (Raghavendra *et al.,* 2011) coupled with the appearance of *Plasmodium* parasite populations resistant to chloroquine (Bloland, 2001).

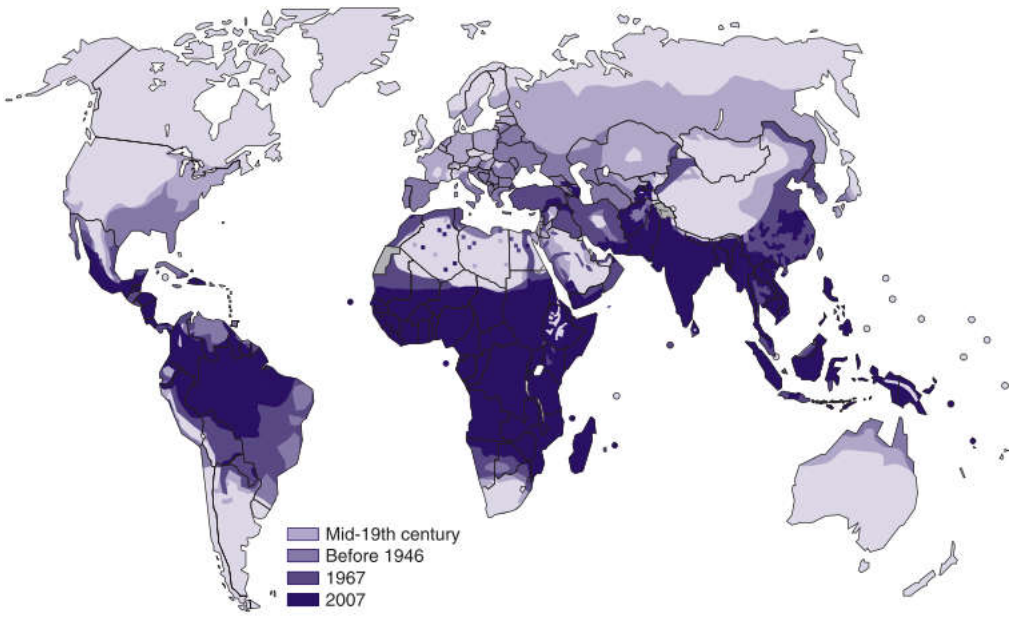


Figure 2.4. Global spatial and temporal distribution of malaria risks from the mid-nineteen century to the present (Source: Mendis *et al.,* 2009).

2.7. Conventional mosquito vector control strategies

2.7.1. Larval source management

Larval source management was the primary vector control method before the advent of strong chemical insecticides such as DDT to control adult mosquitoes (WHO, 2013). This control method includes all practices used to interrupt the life cycle, by preventing the completion of development of the aquatic stages of the mosquitoes (Tusting *et al.,* 2013). Currently, there is an increased interest in using larval source management to complement LLINs and indoor residual spraying (IRS) in association with the increased residual malaria transmission (WHO, 2012a). Integrating larval source management in malaria vector control effort is also important for minimizing the use of harmful synthetic insecticides, thereby reducing the undesired effect on the environment and resistance selection pressure on the vector population (Fillinger and Lindsay, 2011).

Malaria vector control via larval source management can be achieved by using any of the following four methods singly or in combination; habitat modification, habitat manipulation, biocontrol agents, or larviciding (Thwing *et al.,* 2011).

2.7.1.1. Habitat modification and manipulation

Habitat modification is a complete alteration of the landscape structure, targeting the removal of mosquito breeding sites through activities such as draining, filling, land reclamation, and levelling (Thwing *et al.,* 2011). This physical transformation of the landscape is permanent and provides a long-lasting control option, making the area devoid

of suitable breeding sites for mosquito vectors (WHO, 2013). However, it often needs regular maintenance to make the control effort sustainable (WHO, 1982). On the other hand, habitat manipulation involves the reduction of larval breeding habitats through various recurrent activities that result in temporary changes of the aquatic environment to reduce the number of emerging adult mosquitoes (WHO, 2013). These activities include regular drying or draining of streams and ponds, avoiding or covering man-made water containers, and changing the chemical nature of water bodies, e.g., through a change in salinity (Walker and Lynch, 2007).

Management of vegetation within and surrounding the breeding sites could also be considered as environmental manipulation, as the presence or absence of shade could favour or deter mosquitoes to lay their eggs in the breeding habitat depending on the vector species (Rafatjah, 1988; Baird, 2017). It has also been reported that some breeding-site associated grass species could act as attractants and stimulants of oviposition in some anopheline species (Asmare *et al.,* 2017). Thus, regular clearing of these grasses could be of great importance in making the breeding sites less favourable for the ovipositing mosquitoes.

2.7.1.2. Biological control

Biological control takes the form of the introduction of natural enemies of the aquatic stages of mosquitoes into the breeding habitats to reduce the survival of the larvae and pupae and the number of emerging adults, either through direct feeding or through inflicting disease (Bukhari *et al.,* 2013; Benelli *et al.,* 2016). The biocontrol agents

commonly employed in this approach represent various taxonomic groups encompassing vertebrates, invertebrates, and microbes (Kamareddine, 2012).

Vertebrates include different species of larvivorous fishes that feed on the mosquito larvae (Bukhari *et al.,* 2013). The predatory fish, *Gambusia affinis*, is one of the most widely used fish species for the biological control of mosquito larvae (Walton, 2007), a method that dates back to the first quarter of the twentieth century (Hildebrand, 1919). This species has been introduced to various parts of the world from its native place, southeastern United States (Rauchenberger, 1989), and has been used in controlling mosquitoes in New Ireland (Holland, 1933), India (Menon and Rajagopalan, 1978), Kenya (Imbahale *et al.,* 2011) and many other places where malaria is common (Haas and Pal, 1984). *Poecilia reticulata* is another commonly used fish species in the control of malaria mosquitoes (Sharma and Sharma, 1989; Sabatinelli *et al.,* 1991; Kusumawathie *et al.,* 2006; 2008). However, several other fish species such as *Oreochromis niloticus* (Howard *et al.,* 2007), *Aphanius dispar* (Fletcher *et al.,* 1992), and other hundreds of species (Walshe *et al.,* 2013) have been used as biocontrol agents against mosquitoes.

2.7.1.3. Entomopathogenic fungi

Several pathogenic fungal species have been tried as biological control agents against mosquitoes. Species in the genus *Metarhizium* and *Beauveria* are the most widely investigated entomopathogenic fungi, with promising efficacy against malaria mosquito populations. (Scholte *et al.,* 2003; Blanford *et al.,* 2005; Bukhari *et al.,* 2011). One important advantage of fungal pesticides is that they attack mosquitoes simply through

contact, as opposed to bacterial toxins that need to be ingested (Thomas and Read, 2007; Thwing *et al.,* 2011). This, in turn, makes it easy to prepare formulations that can effectively work both indoors and outdoors with a simpler delivery system (Farenhorst *et al.,* 2008; Kamareddine, 2012), with a relatively long duration of the desired effect (Mnyone *et al.,* 2010). Entomopathogenic fungi are also promising biocontrol agents targeting insecticide-resistant vector populations and may be considered in resistance management (Howard *et al.,* 2010).

2.7.1.4. Larviciding

Larviciding is the treatment of mosquito breeding habitats by using either chemical or biological insecticides resulting in mortality of the developing larvae (Thwing *et al.,* 2011). Besides killing the larvae, the longevity of larvicide resistant adults that emerge from larvicide-treated breeding habitats is significantly reduced (Liu and Gourley, 2013). However, this method is only effective when the breeding sites are relatively small and clearly defined (Walker and Lynch, 2007), since the treatment of breeding habitats covering larger geographical area may not be cost-wise feasible (Fillinger and Lindsay, 2011). Therefore, understanding the breeding habit and the habitat of the vector species at the local ecological context has paramount importance for optimizing the effectiveness of larviciding (Walker and Lynch, 2007).

Larvicidal agents could be obtained either from biological sources or chemicals of natural or synthetic origins (Antonio-Nkondjio *et al.,* 2018). The biological larvicides are endotoxins obtained from different bacterial species (Kamareddine, 2012). Two species,

namely *Bacillus thuringiensis* var *israelensis* (*Bti*) and *B. sphaericus*, are commonly used in mosquito control (Poopathi and Tyagi, 2004). Biological larvicides are superior over the synthetic ones due to their limited or no negative direct impact on the environment (Kamareddine, 2012). However, due to their lower residual effect, frequent application is required to obtain the desired effect (Lacey, 2007). The use of different formulations of *Bti* along with other integrated vector control strategies has brought a significant reduction of vector population densities, as well as malaria incidence and prevalence in some African countries (Fillinger *et al.,* 2009; Geissbuhler *et al.,* 2009; Tchicaya *et al.,* 2009).

Chemical larvicides, of natural or synthetic origin, are another class of larvicides with a direct killing effect on the aquatic stages of mosquitoes (Raghavendra *et al.,* 2011). A wide range of chemical larvicides, including organochlorines, organophosphate-derived compounds, paris green (copper acetoarsenite), synthetic pyrethroids, insect growth regulators, as well as petroleum oils and surface films, are commonly used in the control of malaria and other mosquito-borne diseases (Antonio-Nkondjio *et al.,* 2018). Some of these chemicals were the front-line vector control methods that helped many countries in the eradication of malaria and other mosquito-borne diseases between the 1900s and 1940s (Gubler, 1998). One of the most notable stories was the eradication of accidentally introduced Afro-tropical malaria vector, *An. gambiae*, from Brazil in the 1930s using paris green (Soper, 1966). Paris green was also used in the eradication of *An. gambiae* from Egypt in 1945 (Soper, 1966).

Despite their effectiveness in mosquito larvae control, chemical larvicides persist in the environment longer and exert an undesired effect on the environment (WHO, 2006a). As a

result, organophosphate, carbamates, and pyrethroids with lower residual effect and are safer to non-target organisms are recommended, and are now being used instead of those with a strong residual effect such as DDT (Rozendaal, 1997). The use of pyrethroids as larvicides, however, is no longer recommended so as not to facilitate resistance, as they are the only class of insecticides used for the treatment of insecticide-treated nets (ITNs) (Raghavendra *et al.,* 2011; WHO, 2012b).

2.7.2. Adult mosquito control

2.7.2.1. The use of bed nets

The use of ITNs and LLINs has brought a significant reduction of the global malaria prevalence in the past decade (Bhatt *et al.,* 2015). For instance, a 50% reduction of malaria cases has been recorded in communities with marked utilization of ITNs compared with no use in malaria-endemic areas (Lengeler, 2004). Insecticide-treated nets have averted almost three-quarters of falciparum malaria between the years of 2000 and 2015 in Africa (Bhatt *et al.,* 2015).

The nets are predominantly used for personal protection and to minimize human-vector contact by targeting endophagic and endophilic mosquito populations (Gnanguenon *et al.,* 2013), either by killing the ones landing on the net or through its excito-repellency effect repelling them out of the house (Lengeler, 2004). The use of ITNs and LLINs, however, is not effective in preventing malaria in areas where the vector species bite outdoors and before people retire to bed (Steinhardt *et al.,* 2017). Despite their effectiveness indoors, many people complain about the discomfort with using ITNs due to poor air circulation,

unpleasant smell of insecticides, and shape of the nets that are not compatible with the house structures in rural villages of many African countries (Birhanu *et al.,* 2015; Manu *et al.,* 2017). These factors, along with others, could make people not to adhere to use the nets regularly.

ITNs are treated conventionally by dipping them in pyrethroids and require recurrent treatment with the insecticides. Alternatively, insecticides may be incorporated in the fabrics during the manufacturing process (LLINs), and can then be used for about three years (Randriamaherijaona *et al.,* 2017). The emergence of pyrethroid-resistant mosquito populations (Ranson *et al.,* 2011), and the irrational use of the nets for other purposes such as fishing (McLean *et al.,* 2014; Short *et al.,* 2018), nursing seeds (Ntonifor and Veyufambom, 2016), granary protection, crop protection and other purposes (Honjo *et al.,* 2013) are the major bottlenecks in the use of ITNs and LLINs for malaria control.

In Ethiopia, concerted efforts of malaria prevention and control were re-launched since 2005, which have resulted in a significant reduction in malaria-related sickness and deaths (MoH, 2014). Vector control activities mainly through mass distribution of ITNs were among the major contributors to the reduction of malaria burden in the country (Gari and Lindtjorn, 2018). The malaria indicator survey (MIS, 2007) indicated that 65 % of the surveyed households had at least one ITN in 2007. However, this ownership of at least one LLINs remains the same in 2015 (MIS, 2015) showing universal coverage of LLINs is yet to be achieved (Solomon *et al.,* 2019). Lack of persistent utilization (Solomon *et al.,* 2019) and misuse of LLINs for purposes other than mosquito control (Doda *et al.,* 2018) are hurdles that need to be overcome.

2.7.2.2. Indoor residual spraying

The world health organization defines IRS as *"the application of long-acting chemical insecticides on the walls and roofs of all houses and domestic animal shelters in a given area, to kill the adult vector mosquitoes that land and rest on these surfaces*" (WHO, 2006b). IRS has been used since the 1940s after the discovery of DDT and was the major mosquito control approach in the "malaria-eradication" era during the 1950s (Ramirez *et al.,* 2009). The method substantially helped many countries in Europe, Asia, and the Americas in eradicating malaria (Ramirez *et al.,* 2009). However, SSA lagged from the rest of the world in implementing IRS due to political and socio-economic factors though there were some uncoordinated efforts made (WHO, 2006b). As a result, malaria continued being a disease of priority of public health importance in the region.

Since the year 2006, there has been a growing interest in the SSA to fully implement IRS as one of the major components of integrated vector management (IVM) in combination with ITNs (WHO, 2010). Only 1% of the people of SSA were protected from malaria by IRS in 2005 (WHO, 2006c) and this figure reached around 10% in 2009 (WHO, 2010). However, there was a significant decline in the implementation of IRS in the following years, and reached coverage of only around 4.5% in 2018 (WHO, 2019), due to the need of shifting to more expensive insecticides as pyrethroid-resistant vector populations are becoming more common (WHO, 2019).

Indoor residual spraying has proven effective against anthropophilic species such as *An. gambiae* that bites and rests indoors (Killeen *et al.,* 2014). It kills mosquitoes before the

malaria parasite completes its sporogonic cycle and reaches the infective stage, and also reduces the density of mosquitoes (WHO, 2006b) thereby reducing malaria transmission. Indoor residual spraying has also some excito-repellent effect providing some protection against mosquito bites (Skarbinski *et al.,* 2012). Various insecticides from different classes are used in the IRS. However, the effectiveness of this approach is endangered as mosquitoes have developed resistance to most of the insecticides, almost everywhere in SSA (Ranson and Lissenden, 2016). As a result, IRS is used in combination with other IVM strategies to make sure that the vector control effort is successful (Kleinschmidt *et al.,* 2009; Fullman *et al.,* 2013).

2.7.3. Integrated vector management

Integrated vector management is *a rational decision-making process to optimize the use of resources for effective, ecologically sound, and sustainable control of vector-borne disease* (WHO, 2004). IVM is an approach directed to the sustainable reduction of vector populations, thereby minimizing the burden of one or more vector-borne diseases (WHO, 2012c) by combining two or more chemical and/or non-chemical based interventions to optimize the vector control effort (van den Berg and Takken, 2007). To be effective, IVM should be tailor-made, and consider the local eco-epidemiological context of the implementation area (Towson *et al.,* 2005), emphasizing the dynamics of local vector populations and their role in malaria transmission (van den Berg and Takken, 2007). The engagement of all stakeholders including health personnel, the local people, policymakers, and political leaders at the lowest administrative levels is crucial in executing IVM approaches (Beier *et al.,* 2008).

IVM has been successful in alleviating the malaria burden in different African countries. One of the most notable success stories of IVM is the Zambian experience. In Zambia, intensive operational research on local vector species, scaling up of ITNs and IRS, along with larviciding and environmental management, with strong political commitment and capacity building, brought a significant reduction in vector population density and malaria cases (Chanda *et al.,* 2008). Other countries such as Kenya (Fillinger *et al.,* 2009) and Tanzania (Geissbuhler *et al.,* 2009) have successfully implemented IVM and have been able to minimize malaria prevalence at least at the local scale.

2.8. Spatial ecology of malaria

The success of IVM interventions in malaria prevention and control as well as elimination programmes could be achieved through a thorough understanding of the spatial ecology of vectors and parasites at local scales (Carter *et al.,* 2000). Heterogeneity of the landscape at smaller spatial scales contributes to the uneven distribution and clustering of malaria vectors at sub-village or household levels (Kaindoa *et al*., 2016) resulting in elevated malaria risks and transmission intensity (Kabaghe *et al.,* 2018). These higher malaria transmission foci which are referred to as hotspots are assumed to be good starting points for targeted intervention to alleviate malaria disease burden (Durnez *et al.,* 2018). The hotspots usually are characterized by suitable abiotic and biotic environmental conditions supporting a higher density of the vectors (Bousema *et al.,* 2012). The proximity of these areas to perennial water sources (Zhou *et al.,* 2012) and host aggregation (Kaindoa *et al*., 2016) are among the many local environmental conditions making the hotspots a good clustering points for the vectors.

2.9. Semiochemicals as novel malaria vector control strategies

2.9.1. Targeting host-seeking mosquitoes

The host-seeking behaviour of mosquitoes has received much attention from the scientific community as blood-feeding is directly related to disease transmission (Zwiebel and Takken, 2004). Investigations, targeting the chemical ecology of blood meal seeking mosquitoes are paving the way to developing novel mosquito control strategies complementing the existing methods. Two approaches are currently underway: 1) development of host-seeking attractants using volatile compounds derived from preferred mosquito hosts (Constantini *et al.,* 2001; Okumu *et al.,* 2010) and 2) development of novel spatial repellents from non-preferred host volatile compounds (Mwangi *et al.,* 2008; Jaleta *et al.,* 2016).

2.9.1.1. Attractants

Several attractive chemical compounds have been isolated and characterized from hosts of different mosquito species. These chemicals are either found in most of the vertebrate hosts or are specific to the host species (Kline, 2007). Amongst these chemicals, CO_2 is a generic kairomone shared by all vertebrate hosts and known to be attractive to mosquitoes alone or in synergy with other volatile compounds (Dekker and Takken, 1998). Other host-derived compounds include 1-octen-3-ol, lactic acid, ammonia, and carboxylic acids (Sukumaran, 2016). Several hundred other skin emanations from humans and other vertebrates have been isolated, with some tested for their attractiveness to different mosquito species (Bernier *et al.,* 2000; Syed and Leal, 2009; Verhulst *et al.,* 2018; Raji *et al.,* 2019).

However, the application of these attractive compounds in the IVM of malaria vectors is non-existent.

2.9.1.2. Repellents

Novel spatial repellents could be developed from non-preferred hosts once the host breadth of the vector species under target is defined. In pioneering work, Gikonyo *et al.* (2003) analysed the behavioural response of tsetse flies to host odours isolated from its non-preferred host, waterbuck. The authors indicated that waterbuck specific odour blends repelled tsetse flies in a choice assay. Currently, spatial repellents are developed from these odour blends and are being used in the control of nagana (Saini *et al.,* 2017). The possibility of developing repellents from non-preferred host against *Anopheles* mosquitoes was confirmed for the first time by Jaleta *et al.* (2016). Following host preference studies of *An. arabiensis* and confirmation of chickens as the non-preferred host for the species, Jaleta *et al.* (2016) isolated chicken specific compounds, which were repelling host-seeking mosquitoes under field conditions. These findings could be the stepping stone for further investigations in the search for novel spatial repellents.

2.9.2. Attractive toxic sugar baits

The use of attractive toxic sugar baits (ATSB) is a relatively newer concept, which applies the lure and kill principles to control mosquito vectors. It consists of an attractant, usually floral scents or decaying fruits, sugar, and a toxicant. The sugar is used to induce feeding and along with the toxicant is ingested resulting in mortality of the mosquitoes (Beier *et*

al., 2012). The toxicant integrated could be chemical insecticides, insect growth regulators, or biopesticides (Fiorenzano *et al.,* 2017).

Attractive toxic sugar baits are proven effective in controlling sugar feeding mosquitoes of both sexes, indoors and outdoors (Qualls *et al.,* 2015). A field trial conducted in Mali by Muller *et al.* (2010) confirmed that the use of ATSB could be used along with other vector control strategies to suppress vector populations significantly. The authors demonstrated a 90% reduction in the population density of *An. gambiae s.l* in areas treated with ATSB as compared with the population density before treatment. Qualls *et al.* (2015) also indicated that indoor applications of ATSB were effective in the reduction of indoor feeding *An. gambiae s.l* by 90% post-treatment. These findings suggest that ATSB could be a promising complement to existing malaria vector control tools.

2.9.3. Oviposition semiochemicals

Investigations directed toward the possible use of semiochemicals targeting gravid mosquitoes have not received attention until recently, with the number of new publications on the isolation and characterization of semiochemicals that mediate oviposition in mosquitoes increasing. The first semiochemical identified in eliciting oviposition in mosquitoes was the *Culex* oviposition pheromone, erythro-6-acetoxy-5-hexadecanolide (AHD), isolated from *Cx*. *quinquefasciatus* (Laurence and Pickett, 1982). The pheromone is commercially available and has been integrated with other vector control methods such as biological larvicides, and proven successful (Schorkopf *et al.,* 2016). Since the discovery of AHD, many chemical compounds that attract, stimulate, deter, or repel mosquitoes from

selecting oviposition sites have been identified. Oviposition attractants such as *p*-cresol from wood infusions (Bentley *et al.,* 1979), and repellents such as isobutyric, butyric, isovaleric, and hexanoic acids from fermenting plants (Hwang *et al.,* 1980) have been identified.

Studies have also been conducted to identify semiochemicals that mediate oviposition behaviour in *Anopheles* mosquitoes. The first oviposition attractant for *An. gambiae* and *An. arabiensis* is cedrol, which was isolated from two fungal species (Eneh *et al.,* 2016). Different semiochemicals that attract gravid malaria mosquitoes have been isolated from vegetation (Wondwosen *et al.,* 2016; 2017; 2018; Asmare *et al.,* 2017), microbial origins (Sumba *et al.,* 2004; Huang *et al.,* 2006; Lindh *et al.,* 2008) and water containing conspecific larvae (McCrae, 1984).

2.9.4. Cattle urine as a mosquito attractant

Some insect taxa are known to feed on the urine of higher animals to compensate for the lack of important nutrients in their regular diet (Molleman, 2010). Feeding on fresh cattle urine has also recently been demonstrated in malaria mosquitoes, *An. arabiensis*, in which nitrogenous compounds were used as a source of energy for flight and reproductive fitness (Dawit, 2018). Cattle urine was also found to be an important resource locating cue for hematophagous insects of public and veterinary importance, including biting midges (Isberg *et al.,* 2016; 2017) and mosquitoes (Mahande *et al.,* 2010; Kweka *et al.,* 2011; Dawit, 2018).

In-depth characterization of the chemical profile of cattle urine and the electrophysiological and behavioural responses of malaria mosquitoes was conducted by Dawit (2018), who demonstrated that host-seeking and blood-fed *An. arabiensis* were differentially attracted to the compounds in fresh cattle urine. This was also further demonstrated under field condition, where a synthetic odour blend from 24 h aged cattle urine was demonstrated to attract *An. arabiensis* with different physiological states (Dawit, 2018). From an IVM perspective, these findings suggest that synthetic odour-blends can be used to complement the conventional vector control tools.

Chapter 3

Shady Business: Understanding the Spatial Ecology of Exophilic *Anopheles* Mosquitoes

3.1. Introduction

Current interventions targeting indoor malaria vectors, particularly the use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), have been a cornerstone of the significant decline in malaria morbidity and mortality during the early part of this century (Cibulskis *et al.,* 2016). Malaria-related deaths have declined by more than half in sub-Saharan Africa between 2000 and 2015 (Cibulskis *et al.,* 2016; WHO, 2015b). The sustainability of these interventions is, however, threatened due to increased vector resistance to available insecticides (Ranson *et al.,* 2010; WHO, 2014; Sougoufara *et al.,* 2017), and the change in mosquito biting behaviour to seeking blood meals outdoors (Reddy *et al.,* 2011; Russell *et al.,* 2011; Moiroux *et al.,* 2012), with some populations shifting the time of biting activity from late night to early evening (Yohannes and Boelee, 2011; Moiroux *et al.,* 2012; Sougoufara *et al.,* 2014). These behavioural changes favour residual malaria transmission, presenting a major roadblock to further reduce malaria prevalence and enhance the sustainability of malaria vector control (Durnez and Coosemans, 2013).

Whilst the current strategy of IRS and LLINs control has made great strides against malaria, it is estimated that the number of malaria cases in Africa increases by approximately 11 million every year due to increased outdoor transmission (Sherrard-Smith *et al.,* 2019). Outdoor interventions directed against adult mosquitoes are lacking (Govella and Ferguson, 2012), and an increased understanding of the ecology and behaviour of exophilic malaria vectors is needed to improve the sustainability of existing control strategies. Besides, this may further act as a guide for the deployment of appropriate outdoor monitoring and control tools (Killeen *et al.,* 2017).

The sustainability of existing IVM tools should be actively maintained, and enhanced by the addition of novel interventions, particularly vector control strategies targeting adult anophelines outdoors (Govella and Ferguson, 2012; Zhu *et al.,* 2017). Early studies by Gillies (1954a, b) revealed that *Anopheles gambiae*, the primary malaria vector, predominantly rested indoors, but with a small proportion of mosquitoes found to be resting in shady zones at some distance from human habitation. In the interim, changes in the biting patterns of several mosquito species have arisen, whereby a far greater proportion of female *Anopheles* species are found to both feed and rest outdoors (Reddy *et al.,* 2011; Russell *et al.,* 2011; Yohannes and Boelee, 2011; Moiroux *et al.,* 2012; Sougoufara *et al.,* 2014). Additionally, the habitat has undergone considerable changes, populations of humans are denser, and the agricultural environment is more intensely farmed with greater use of irrigation (Güneralp *et al.,* 2017). In view of the known changes in mosquito feeding behaviour and the habitat, few recent studies describing the outdoor resting behaviour of mosquitoes have been conducted (e.g. Burkett-Cadena *et al.,* 2008), which may be partly due to the large effort required to catch mosquitoes outdoors as opposed to indoors

(Service, 1993). Existing knowledge builds extensively on the foundation of the work of Gillies (1954a) who studied the resting site selection of *An. gambiae s. l.* and *An. funestus s. l.* in natural and artificial resting sites. More recent studies in *Anopheles* mosquitoes showed that these mosquitoes choose outdoor resting micro-habitats based on several different environmental factors within the landscape at a fine spatial scale (Paaijmans and Thomas, 2011). Moreover, a number of studies have associated landscape characters with the distribution or aggregation of exophilic mosquitoes (Forattini *et al.,* 1993; Overgaard *et al.,* 2003; Paaijmans and Thomas, 2011; Burkett-Cadena *et al.,* 2013). These studies have indicated that different physical and biological components of the environment are important factors affecting mosquito ecology, with habitat type (Burkett-Cadena *et al.,* 2013), land cover (Overgaard *et al.,* 2003), shade (Forattini *et al.,* 1993), microclimate (Paaijmans and Thomas, 2011) and the availability of blood meal hosts (Burkett-Cadena *et al.,* 2013) being positively associated with the adult distribution of exophilic mosquito species.

Outdoor monitoring and control tools can be used alone, or to augment other IVM strategies, to alleviate the malaria burden. It is, however, essential to fully understand the behaviour of exophilic populations to make the best use of both existing and novel tools. This study was conducted to explore the resting habitat selection behaviour of *Anopheles* mosquitoes outdoors and identify landscape characteristics associated with the resting sites, which can later be used to optimize the positioning of traps in the landscape around human habitations.

3.2. Materials and Methods

3.2.1. Description of the study area

The study was conducted in southern Ethiopia in Arba Minch Zuria district of the Gamo-Gofa zone near a village called Sile (5°53′24′′N, 37°29′24′′E). The study site is 517 km south of Addis Ababa, the capital city of Ethiopia, and 17 km south of the city of Arba Minch, the capital of the Gamo-Gofa zone (Figure 3.1). The area is characterized by a bimodal rainy season, with a long rainy period between April and July/August and a short rainy season between September and November. This study was conducted between September 2016 and June 2017. The annual rainfall ranges from 900 to 1300 mm, and the average annual temperature is 25 to 36 °C. Banana is the main commercial crop in the area and covers approximately half of the landmass. Maize is cultivated predominantly for subsistence and makes up approximately 20 % of the land used. The presence of abundant irrigation canals in the study area and its proximity to Lake Chamo creates suitable breeding sites for malaria vectors, making it one of the areas with the highest malaria transmission in the Gamo-Gofa zone (based on personal communication with the district health officer). Livestock rearing, including both cattle and small ruminants, is a major activity in the area and provides potential blood meal sources for mosquitoes.

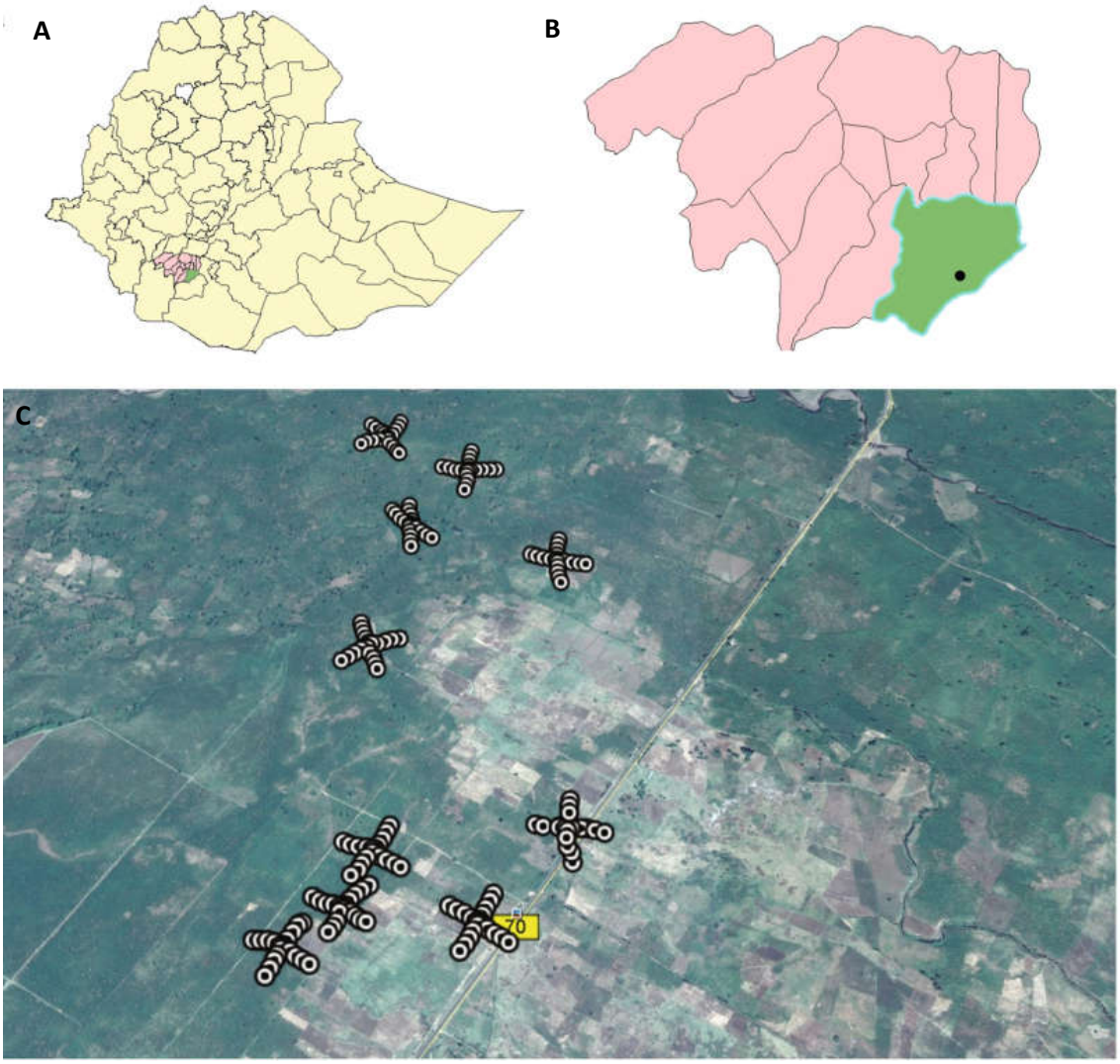


Figure 3.1. Maps showing **A**: district map of Ethiopia indicating the Gamo-Gofa zone; **B**: the Gamo-Gofa zone indicating Arba Minch Zuria district; and **C**: the study area with the sampling points.

3.2.2. Study design and mosquito collection

In order to identify the environmental factors affecting outdoor resting site selection by *Anopheles* mosquitoes, resting clay pots (Figure 3.2AB) were used to collect adult mosquitoes. The clay pots were spherical and made to our specifications by local potters.

The pots had an opening of approximately 15 cm, a depth of 40 cm and a capacity to hold ca. 10 l. A 2 cm hole was made at the bottom of the pots to avoid rainwater accumulation and discourage potential theft.

Ten isolated, inhabited houses, located a minimum of 200 m apart, were selected for the study. The selected houses had mud-plastered walls with grass-thatched roofs. Twenty clay pots were placed in a criss-cross pattern, with the house at the center, and single pots being placed at 5 m, 25 m, 50 m, 75 m and 100 m away from the house in each of the four directions (Figure 3.2A). The hillside of the village was used to orient the position of the pots (Figure 3.1).

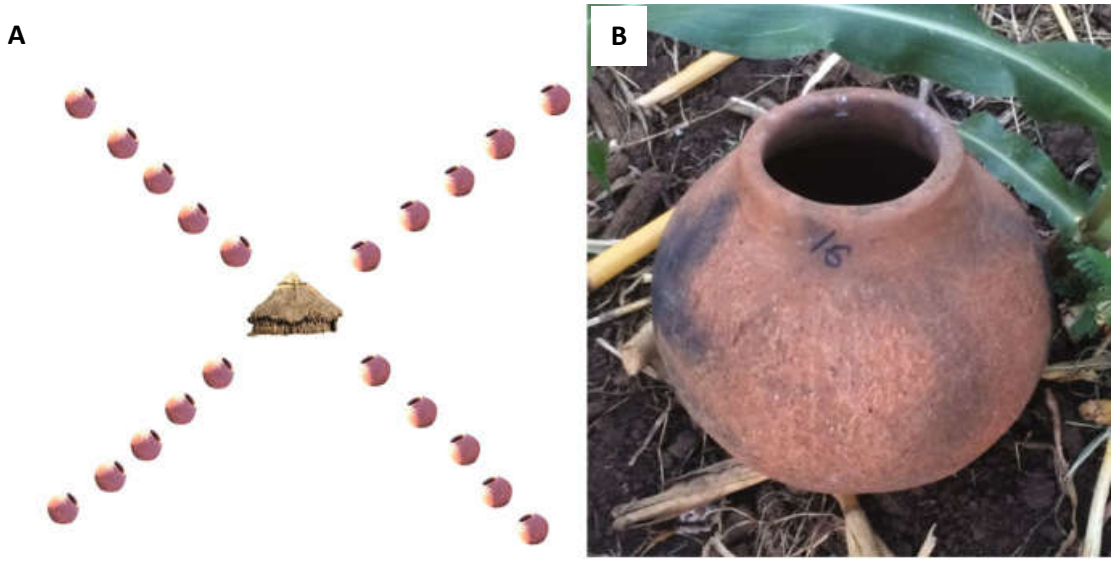


Figure 3.2. Schematic representation of the clay pot arrangement for collecting outdoor resting *Anopheles* mosquitoes **(A)** and a resting clay pot **(B)**.

3.2.3. Environmental variables

Landscape characteristics were determined within a 10 m radius from each sampling points: 1) the distance of the sampling point from the nearest house with potential blood meal sources; 2) the number of potential breeding sites; 3) the land cover type and the percentage canopy cover, and 4) the relative percentage of ground (grasses and other herbs) and tall (shrubs and trees) vegetation. The geographical location of each sampling point and the houses were recorded using a handheld global positioning system (GPS) instrument.

3.2.4. Mosquito sampling

Sampling of mosquitoes was conducted in the morning between 06:00 and 09:00. During collection, a mosquito cage (BugDorm 32.5 cm x 32.5 cm x 32.5 cm) was placed over the opening of the clay pot, and by gently lifting and shaking the pot, as well as blowing air through the small opening at the bottom of the pot, the resting mosquitoes were encouraged into the cage. Then, the mosquitoes were aspirated from the cage, knocked down using ethyl acetate, and transported to the field laboratory.

3.2.5. Mosquito processing and identification

The collected mosquitoes were counted and sorted according to genus, species or species complex and sex. Female mosquitoes were morphologically identified to species following Verrone (1962) and Gillies and Coetzee (1987) and subsequently categorized according to their abdominal status as unfed, blood-fed, semi-gravid, or gravid following the categories

defined by the WHO (1975). Female *Anopheles* mosquitoes, provisionally identified as *Anopheles gambiae s.l.*, were individually preserved in 1.5 ml Eppendorf tubes containing silica gel and stored at ambient temperature for subsequent molecular identification to sibling species.

3.2.6. Identification of sibling species of *Anopheles gambiae* using PCR

Molecular identification of female *Anopheles* mosquitoes belonging to the *An. gambiae* complex was conducted using species-specific polymerase chain reaction (PCR) following Scott *et al.* (1993). Briefly, a leg was removed from each mosquito and mixed with PCR master mix containing 10x dNTPs, $MgCl_2$ Solution, DNA polymerase, buffer solutions, universal forward primer (GCTGCGAGTTGTAGAGATGCG), species-specific primers (AR-3T R with sequence GTGTTAAGTGTCCTTCTCCgTC for *An. arabiensis* and QD-3T R with sequence GCATGTCCACCAACGTAAAtCC for *An. amharicus*). The mixture was then centrifuged for 20 s at 16 krpm and amplified in a thermocycler (PTC-100™ Programmable Thermocycler, MJ Research, Inc., USA) with the following PCR cycle conditions: [95 °C/5 min] × 1 cycle; [95 °C/30s, 50 °C/30s, 72 °C/30s] × 30 cycles; [72 °C/5 min] × 1 cycle; 4 °C hold]. The PCR product (5 µl) was loaded with 2 µl loading dye and 4 µl DNA ladder was electrophoresed through a 2 % agarose-tris-borate-EDTA containing ethidium bromide gel (using a 100 V and 150 mA power source) and visualized under UV lightbox. The fragment sizes were compared with that of known *Anopheles* species, e.g. 315 base pairs (bp) for *An. arabiensis* and 153 bp for *An. amharicus.*

3.3. Data analysis

Data analysis was conducted using R statistical software version 3.4.1 and JMP® version 10.0.0. (SAS Institute Inc., Cary, NC, USA). As the response variable was an over-dispersed count data with unequal mean and variance, and due to the excess number of zero captures, a zero-inflated negative binomial regression with log link function was used to model the effect of environmental factors on the number of outdoor resting *Anopheles* mosquitoes caught. Before conducting the regression analysis, a multiple correlation analysis was conducted to assess multicollinearity among the continuous predictor variables. Since canopy cover was positively correlated with the percentage of tall vegetation within a 10 m radius of the sampling points, the percentage of tall vegetation was removed from the subsequent model. The non-parametric Kruskal-Wallis was followed by Wilcoxon pairwise comparison post hoc test with Bonferroni correction to compare the number of mosquitoes between the categories: land cover, shading, and distance from the focal house. Binomial logistic regression was conducted to predict the probability of catching at least a single *Anopheles* mosquito in the clay pots, followed by a backward selection of non-significant independent variables to model the count and binary outcomes.

3.4. Results

3.4.1. Exophilic mosquito abundance and physiological condition

Surveillance of resting *Anopheles* mosquitoes was conducted in a rural Ethiopian setting (Figure 3.1) using clay pots as artificial resting sites (Figure 3.2). A total of 420 *Anopheles* mosquitoes (353 females and 67 males) were caught in the clay pots. Three *Anopheles* species/species complexes were collected, of which *An. gambiae s. l.* was the most abundant species with 370 (88.1 %) mosquitoes, followed by *An. pharoensis* consisting of 49 individuals (11.67 %) and *Anopheles tenebrosus* with a single individual (0.23 %). Molecular identification of *An. gambiae s. l.* using PCR was conducted on 63 individuals (17 %); all were identified mosquitoes as *Anopheles arabiensis*. The physiological state of female anophelines collected from each of the land cover types demonstrated that the highest proportions caught were semi-gravid, followed by unfed (Figure 3.3).

3.4.2. Effect of landscape elements on mosquitoes caught

The association between the number of *Anopheles* mosquitoes caught and the landscape characteristics, within a 10 m radius from each sampling point, was modelled using zero-inflated negative binomial regression (log-likelihood = -264.8; df = 13; theta = 1.19) for females (log-likelihood = -110.8; df = 13; theta = 1.48) and for males; (Table 3.1).

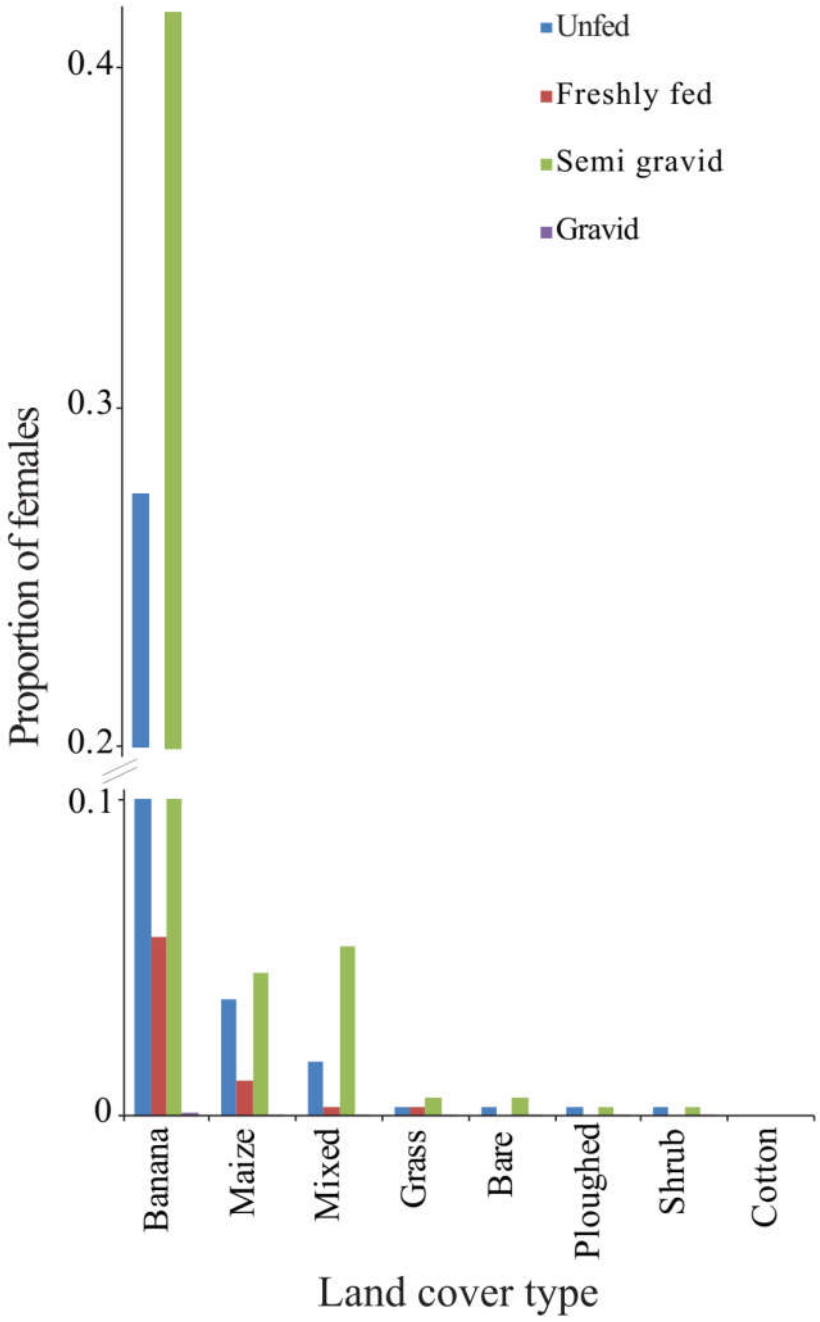


Figure 3.3. The proportion of different physiological states of *Anopheles* mosquitoes caught in clay pots distributed amongst different land cover types.

Table 3.1. Results obtained from zero-inflated negative binomial regression on the association between the number of *Anopheles* mosquitoes collected in the resting clay pots and landscape characteristics within a 10 m radius of the sampling points.

Variables	Females		Males	
	Estimate	**Pr(>|z|)**	**Estimate**	**Pr(>|z|)**
Count model coefficients (negbin with log link)				
(Intercept)	-1.3478	0.0326*	-1.3673	0.2419
Distance to nearest dwelling (m)	0.0132	0.0086**	0.0053	0.4724
Breeding sites (present)	-0.2112	0.8520	-1.3583	0.5030
Number of breeding sites	0.4284	0.6704	0.6199	0.6883
Percent canopy cover	0.0232	0.0004***	0.0199	0.2034
Percent ground vegetation	0.0110	0.2401	-0.0379	0.0814
Zero-inflation model coefficients (binomial with logit link)				
(Intercept)	1.5594	0.0685	2.1742	0.144
Distance to nearest dwelling (m)	-0.0030	0.7128	0.0171	0.326
Breeding sites (present)	-0.7048	0.7523	3.1967	0.549
Number of breeding sites	0.0956	0.9616	-3.3128	0.487
Percent canopy cover	-0.0283	0.0014**	-0.0444	0.098
Percent ground vegetation	0.0096	0.4803	0.0021	0.960

*p < 0.05, **p < 0.01, ***p <0.001

Backward selection of non-significant independent variables indicated that percent canopy cover ($P < 0.001$) and distance of sampling points from the nearest dwelling ($P < 0.01$) significantly affected the number of female *Anopheles* mosquitoes caught in the resting clay pots, as indicated from the count model coefficients in the model (Table 3.2). Both variables are the dominant characteristics of the banana-dominated land cover, where the highest *Anopheles* density was recorded. The result from the zero-inflation model also indicated that the odds of having an excess number of zeroes decreased with increasing percent canopy coverage and distance of sampling points from the focal house (Table 3.2).

In contrast, none of the predictor variables from either the count or the zero-inflation models significantly affected the number of male *Anopheles* caught (Table 3.2).

Table 3.2. The association of landscape characteristics within a 10 m radius of the sampling points with the number of *Anopheles* mosquitoes caught in resting clay pots, as shown by zero-inflated negative binomial regression, followed by a stepwise backward selection of non-significant independent variables.

Variables	Estimate	Std. Error	z value	Pr(>|z|)
Females				
Count model coefficients (negbin with log link)				
(Intercept)	-1.2435	0.6274	-1.982	0.04747*
Distance to nearest dwelling (m)	0.0136	0.0052	2.599	0.0094**
Percent canopy cover	0.0238	0.0068	3.483	0.0005***
Zero-inflation model coefficients (binomial with logit link)				
(Intercept)	1.3602	0.6274	1.640	0.1010
Distance to nearest dwelling (m)	-0.0016	0.0087	-0.185	0.8530
Percent canopy cover	-0.0279	0.0094	-2.963	0.0030**
Males				
Count model coefficients (negbin with log link)				
(Intercept)	-1.4289	1.2516	-1.142	0.2536
Percent canopy cover	0.0215	0.0151	1.420	0.1555
Percent ground vegetation	-0.0386	0.0231	-1.672	0.0946
Zero-inflation model coefficients (binomial with logit link)				
(Intercept)	2.5915	1.5563	1.665	0.0959
Percent canopy cover	-0.0432	0.0290	-1.490	0.1361
Percent ground vegetation	0.0075	0.0503	0.149	0.8819

*p < 0.05, **p < 0.01, ***p <0.001

3.4.3. Effect of land cover, shade, and distance from focal houses on exophilic *Anopheles* mosquitoes caught

The number of mosquitoes caught in the resting clay pots was compared among land cover types, as well as shading and distance categories from the focal houses. The analysis indicated that land cover type affected the number of both female ($P < 0.0001$) and male anophelines ($P = 0.02$) caught. Most of the mosquitoes were recorded in banana-dominated land cover for both sexes (Figure 3.4).

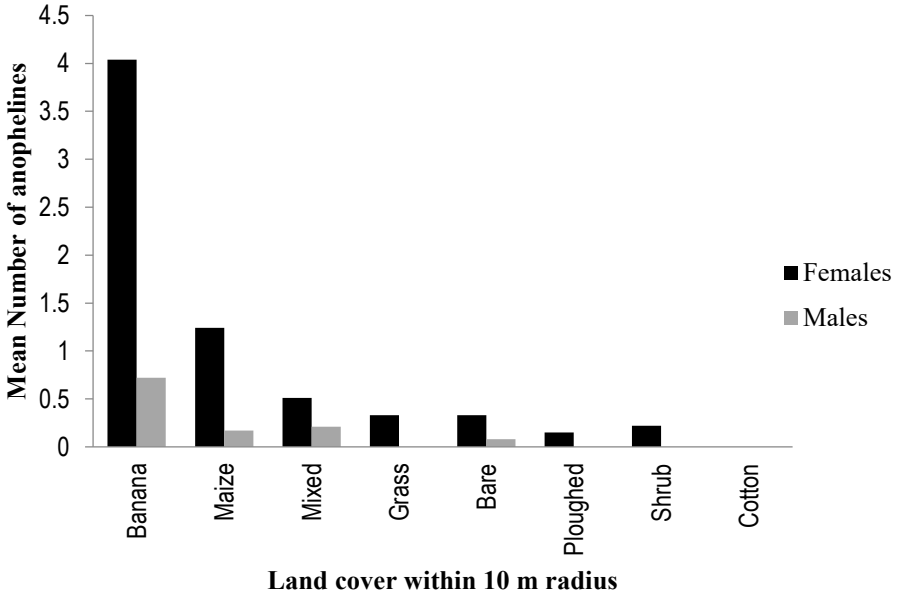


Figure 3.4. The mean number of *Anopheles* mosquitoes caught in the resting clay pots in different land cover types.

Shading also had a significant positive effect on the number of both females ($P < 0.0001$) and males ($P < 0.0001$). Clay pots placed in fully shaded areas caught a higher number of *Anopheles* mosquitoes than those positioned in partially shaded or non-shaded areas (Table 3.3). The number of *Anopheles* caught at a distance of 5 m, 25 m, 50 m, 75 m, or 100 m

radius from the focal houses was also compared revealing that the number of female *Anopheles* mosquitoes was higher at distances farther away from the focal house ($P < 0.05$). However, the distance of sampling points from the focal house had no significant effect on the number of male *Anopheles* caught ($P > 0.05$) (Table 3.3).

Table 3.3. The association of categorical variables within 10 m radius of the sampling points with the number of *Anopheles* mosquitoes caught in resting clay pots, as shown by the Kruskal-Wallis test followed by the Wilcoxon pairwise comparison method.

Category	Number	Density of Mosquitoes (Number/category)	
		Males	Females
Land cover			
Banana	69	0.72^{a}	4.04^{a}
Bare	12	0.08^{b}	0.33^{b}
Cotton	5	0.00^{b}	0.00^{b}
Grass	12	0.00^{b}	0.33^{b}
Maize	29	0.17^{ab}	1.24^{b}
Mixed	51	0.21^{ab}	0.51^{b}
Ploughed	13	0.00^{b}	0.15^{b}
Shrub	9	0.00^{b}	0.22^{b}
P-value		0.002	0.000
Shading			
Open	85	0.04^{a}	0.14^{a}
Partial	41	0.12^{a}	1.29^{b}
Shaded	74	0.80^{b}	3.89^{c}
P-value		0.000	0.000
Distance category			
Within 5 m	40	0.3	0.32^{a}
Within 25 m	40	0.13	0.73^{ac}
Within 50 m	40	0.25	2.15^{bc}
Within 75 m	40	0.57	2.55^{b}
Within 100 m	40	0.43	3.08^{b}
P-value		0.429	0.0115

^{abc}: values within each category in the same column, followed by the same letter are not significantly different ($P > 0.05$).

The probability of catching at least a single *Anopheles* mosquito in the resting clay pots increased with an increasing percentage of canopy cover ($P < 0.0001$). The rest of the environmental factors had no significant effect on the probability of catching at least one *Anopheles* mosquito ($P > 0.05$). The model showing the effect of all predictor variables on the number of mosquitoes caught is indicated in Table 3.4, and Table 3.5 shows the model after removing the non-significant predictor variables. The estimated probability of catching at least one single anopheline in relation to canopy coverage is indicated in Figure 3.5.

Table 3.4. Results obtained from binary logistic regression on the association between the presence or absence of *Anopheles* mosquitoes and landscape characteristics within a 10 m radius of the sampling points.

Variables	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	-2.6140	1.139	-2.295	0.0217 *
Distance to nearest dwelling (m)	0.0003	0.006	0.068	0.9456
Breeding sites (present)	-0.0732	2.034	-0.036	0.9713
Number of breeding sites	0.4235	1.907	0.222	0.8242
Percent canopy cover	0.0321	0.008	4.154	0.0000 ***
Land cover (banana)	1.0090	1.185	0.851	0.3945
Land cover (bare)	0.7281	1.295	0.562	0.5740
Land cover (grass)	1.2520	1.562	0.802	0.4226
Land cover (maize)	0.2452	1.195	0.205	0.8375
Land cover (mixed)	0.4082	1.165	0.350	0.7260
Land cover (ploughed)	0.4134	1.342	0.308	0.7580
Percent ground vegetation	-0.0136	0.014	-0.970	0.3318

*p < 0.05, **p < 0.01, ***p <0.001

Table 3.5. The effect of percentage canopy cover within a 10 m radius of the sampling points on the probability of catching at least one *Anopheles* mosquito in resting clay pots, as shown by binary logistic regression, followed by a stepwise backward selection of non-significant independent variables.

Variables	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-2.3495	0.3633	-6.466	0.0011***
Percent canopy cover	0.0377	0.0059	6.389	0.0000***

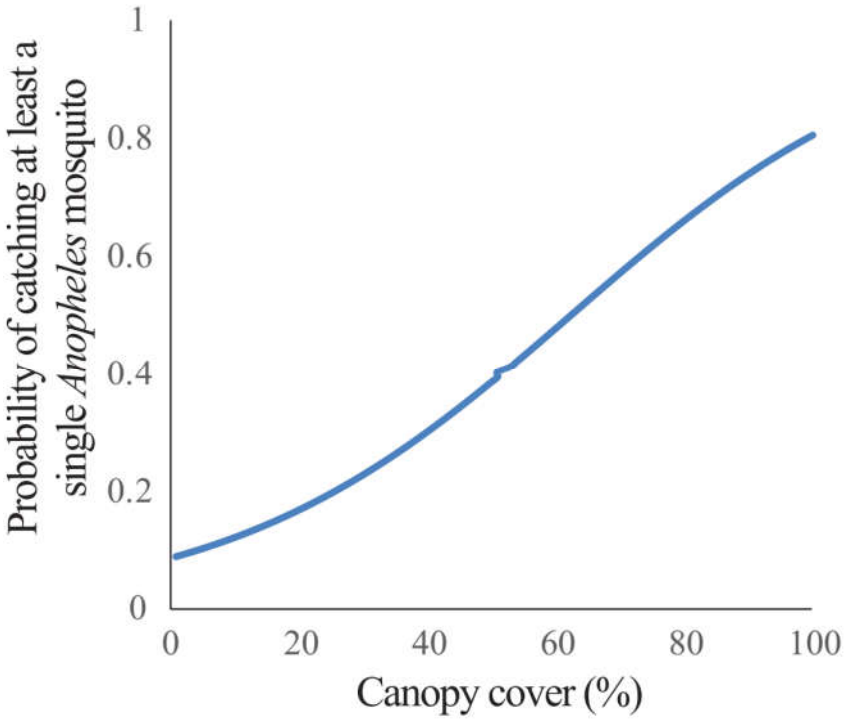


Figure 3.5. Estimated probability of catching at least one *Anopheles* mosquito in relation to percent canopy coverage.

3.5. Discussion

This study found that the distance of the sampling points from the focal house, the percentage of canopy cover, as well as the land cover characteristics are important landscape predictor variables influencing the resting site selection of exophilic female *Anopheles* mosquitoes, particularly *An. arabiensis*. Similarly, canopy and land cover are important factors for male *Anopheles*. This study reveals that female *Anopheles* mosquitoes fly 50-100 m away from their blood-feeding environment, to rest in favoured habitats, primarily banana plantations, but also maize fields, which provide optimal shade cover for both males and females. This knowledge is an important step in understanding movement patterns of *Anopheles* mosquitoes and provides a foundation for further studies on the development of intervention strategies that can complement IRS and ITNs.

Among the significant explanatory variables in our study, shade is the strongest driver of the distribution of exophilic female *Anopheles* mosquitoes in the landscape, in line with previous studies on other mosquito species (Gilles, 1954a; Ibry and Apperson, 1992; Githeko *et al.,* 1996). The two *Anopheles* species in this study share a preference for shaded resting sites with other *Anopheles* species in different geographical locations throughout the tropical and subtropical regions of the world (reviewed by Gillies, 1991). This preference for shaded areas has been linked to the avoidance of excess water loss, as dehydration negatively influences mosquito physiology, survival, and fitness (Benoit *et al.,* 2010; Chown *et al.,* 2011).

Despite a lack of a statistically significant difference with other land cover types, the maize-dominated areas caught the second-highest number of *Anopheles*. It is noteworthy that

maize cultivations have been shown, in this and other regions of eastern Africa, to harbour a large number of resting mosquitoes (*personal observation*). This is likely due to the fact that maize provides relatively high levels of shade, up to 2 m in height, in comparison with the other land cover classes, where mosquito abundance was found to be low or non-existent. Moreover, it has been shown that there is a direct link between the breeding sites and malaria prevalence during maize and other cereal crop irrigated cultivation (Ye-Ebiyo *et al.,* 2000; Ye-Ebiyo *et al.,* 2003; Kebede *et al.,* 2005; Wondwosen *et al.,* 2017). The main driver for this is the maize pollen, which provides an important food source for mosquito larvae increasing the chance of survivorship and higher pupation rate (Kivuyo *et al.,* 2014). The adults that emerge from well-nourished larvae are larger, less susceptible to chemical insecticides, show increased biting frequency, have longer blood meal duration and longevity; all of these biological traits positively contribute to the vectorial capacity of the adult mosquitoes (Ye-Ebiyo *et al.,* 2000; Araujo *et al.,* 2012; Oliver and Brooke, 2013 Kivuyo *et al.,* 2014).

The distance of the sampling points from the nearest house had a positive effect on the number of female *Anopheles* mosquitoes caught, with catches being higher further away from the house. One likely explanation of this is that sampling clay pots placed near the houses had fewer mosquitoes due to the recurrent disturbance by human and livestock activities. Furthermore, canopy cover, as the strongest predictor variable, is associated with dense banana cultivation, which is usually located further away from the houses. Thus, female mosquitoes may be motivated to fly a long distance to reach a shaded refuge. This is in line with previous research, which studied the spatial movement pattern of mosquitoes from the edge of a forest into the interior (Mendez *et al.,* 2001). Mendez *et al.* (2001),

demonstrated that mosquitoes aggregated 100 m and 200 m from the forest edge, leaving the high disturbance, low shade area. One of the pioneer works in understanding the outdoor resting behaviour of *Anopheles* mosquitoes was conducted by Gillies (1954a). The author studied the outdoor resting behaviour of *An. gambiae s. l.* by using artificially constructed resting boxes placed at different distances from residential houses. The results indicated that resting boxes placed at distant positions caught a higher number of *An. gambiae s. l.* than resting boxes placed near the houses. However, most of the resting *An. gambiae s. l.* mosquitoes were caught indoors. The findings of the present study are in partial agreement with the work of Gillies (1954a), finding that outdoor resting *An. arabiensis* also prefer heavily shaded resting sites providing an optimal microclimate for blood meal digestion.

Previous studies aimed at modelling the effect of landscape characteristics on the distribution of mosquitoes have used a relatively large spatial scale of up to 1000 m to analyse the position of mosquitoes in the landscape (Moncayo *et al.,* 2000; Diuk-Wasser *et al.,* 2006). Findings presented in this study show that fine-scale spatial heterogeneity of landscape structures affects the distribution or aggregation of *Anopheles* mosquitoes, in line with studies on *Cx pipiens estuans* (Trawinski and Mackay, 2010). Here, the landscape characters are shown to be important drivers of movement patterns and resting site selection of exophilic mosquitoes. In this era of the uncertain sustainability of two major vector control strategies, IRS and ITNs, the search for novel vector control options, particularly targeting outdoor populations, is of great importance. Knowledge of the mosquito ecology is critical for further studies intended to develop novel monitoring and control tools that work for outdoor feeding and resting *Anopheles* populations.

Chapter 4

Living on the Edge: Malaria Hotspots Explained from the Perspective of Ecological Theory Underlying Insect Foraging

4.1. Introduction

Malaria prevention and control strategies have resulted in a remarkable reduction of malaria mortality and morbidity throughout most of sub-Saharan Africa over the past two decades ((Haakenstad *et al*., 2019). The implementation of *e.g.*, indoor residual spraying, long-lasting insecticide treated bed nets and rapid detection tests for malaria parasites, however, are not sufficient to eliminate malaria at a local level (Dhiman, 2019). Climatic factors and transmission seasons are considered important drivers of malaria epidemiology for implementing integrated vector management (IVM) tools over wider geographic areas, *e.g*., wards, counties, and countries (Alimi *et al.,* 2015). To develop a more robust and targeted intervention approach, which considers heterogeneity at the local level, however, there is a need to consider the eco-epidemiological settings of malaria transmission over much finer geographical scales (Peterson *et al.,* 2009; Bannister-Tyrrell *et al.,* 2018; Durnez *et al.,* 2018).

Fine scale spatial heterogeneity of malaria incidence and vector density has been demonstrated in areas with different transmission magnitudes in many malaria-endemic countries (Ye *et al.,* 2007; Bousema *et al.,* 2010; Sissoko *et al.,* 2015). Pockets of high

malaria incidence, hotspots, have been described at the village level (Rulisa *et al.,* 2013; Seyoum *et al.,* 2017; Durnez *et al.,* 2018). Hotspots constitute the major reservoir for persistent malaria transmission and are associated with higher vector density, sporozoite prevalence and malaria incidence than neighbouring areas (Sissoko *et al.,* 2015; Kabaghe *et al.,* 2018). Malaria transmission in these hotspots has been suggested to be primarily driven by local environmental conditions, *e.g.*, proximity to vector breeding sites (Staedke *et al.,* 2003; Zhou *et al.,* 2012; Rulisa *et al.,* 2013), vegetation cover (Debebe *et al.,* 2018; Hawkes *et al.,* 2019), housing structure and condition (Ye *et al.,* 2006; Wanzirah *et al.,* 2015; Ondiba *et al.,* 2018), bed net use (Plucinski *et al.,* 2014; Levitz *et al.,* 2018) , and household occupancy (Kaindoa *et al*., 2016). To date, however, there is no consensus as to which factors underlie the formation of hotspots at the village level, and the identification of these is crucial to attain the Sustainable Development Goal of zero malaria incidence level in a given area (Bousema *et al.,* 2012; WHO, 2015a).

Female mosquitoes, like many insects, seek a range of different resources over the course of their lifespan, and the trade-off between the benefits of *e.g.*, sugar meals and blood meals are critical to the overall fitness of a female seeking to gain such resources (Ma and Roitberg, 2008). Moreover, these trade-offs can be modulated with time and physiological state, *e.g.*, as the female ages, the likelihood of taking a blood meal over that of a sugar meal increases (Ma and Roitberg, 2008; Omondi *et al.,* 2019). Resources are not evenly distributed within space and time, and the theoretical framework describing the observed spatial heterogeneity of blood feeding resources in the mosquito landscape is currently lacking. However, there is a plethora of theories based on insect-plant interactions that may be adapted for this purpose (Agrawal *et al.,* 2006). Jones (1992) emphasized that search

outcomes in herbivorous insects are dependent on behavioural patterns within species, which can be broadly categorized into the resource concentration hypothesis (Root, 1973) or the edge effect (Cromartie, 1975). The resource concentration hypothesis postulates that searching individuals are likely to locate and remain in stands with a high host density, and the edge effect favours resources being more extensively used in sparse patches, and importantly more on the edge than in the centre of those patches. The edge is defined as the abrupt transition between habitats differing in quality, affecting the performance of individuals (Lidicker and Peterson, 1999). A patch can be defined as a relatively homogenous nonlinear area that differs from its surroundings (Forman, 1995) and for mosquitoes, we may regard the assembly of blood hosts in a community, either at the household or village level, as a patch, and the homes placed on the outskirts of a community as on the edge of that patch.

In this study, we examine the distribution of indoor resting and host-seeking mosquitoes, and the resulting spatial occurrence of malaria, within village communities to assess whether residents associated with individual households are more likely to be exposed to blood feeding by mosquitoes within village communities. Spatial clustering and distribution of mosquito activity and malaria incidence, as well as the distribution of sporozoite-infected vectors, were mapped, and hotspots/coldspots of malaria incidence and vector densities identified in two rural villages in southern Ethiopia. To identify the direct and indirect factors, *e.g.*, household location and occupant density, underlying these hotspots/coldspots, we created and tested local landscape models. Local environmental conditions were categorized and their influence on mosquito density and malaria incidence

was determined. We conclude that an increased understanding of vector ecology and biology is required to identify novel strategies that can complement current IVM.

4.2. Materials and Methods

4.2.1. Description of the study areas

The study was conducted in two rural villages located near to Arba Minch, ca. 500 km south of Addis Ababa. Abulo (6º03'48"N, 37º35'30"E) is located 6 km northeast of Arba Minch and is an isolated village, more than 2 km away from the nearest settlement and main road, with 134 clustered households and a total population of ca. 900. The village is situated 1.5 km from Lake Abaya, and is well-drained by irrigation canals, with an adjacent river. Magge (5º51'49"N, 37º29'32"E) is located 22 km south of Arba Minch. The total population of this isolated (> 2 km away from nearest settlement and main road) and clustered village is ca. 700 residents. The village is situated 1.7 km from Lake Chamo. The houses in both villages are roofed with either grass thatch or corrugated iron sheets and have mud walls. Residents of both villages have similar socio-economic status with noticeable poverty. Banana plantations, livestock rearing, fishing and subsistent farming are the common economic activities sustaining the livelihood of the residents. In addition to the economic benefit, keeping cattle and other domestic animals is a traditional heritage exercised by the majority of the residents. Farmlands are usually located at the outskirts of the villages, where maize is predominantly harvested. Malaria is a common problem in both villages. The presence of perennial water and the proximity of both villages to lakes

make both villages suitable for proliferation of mosquito vectors throughout most of the year (Fig. 4.1AB).

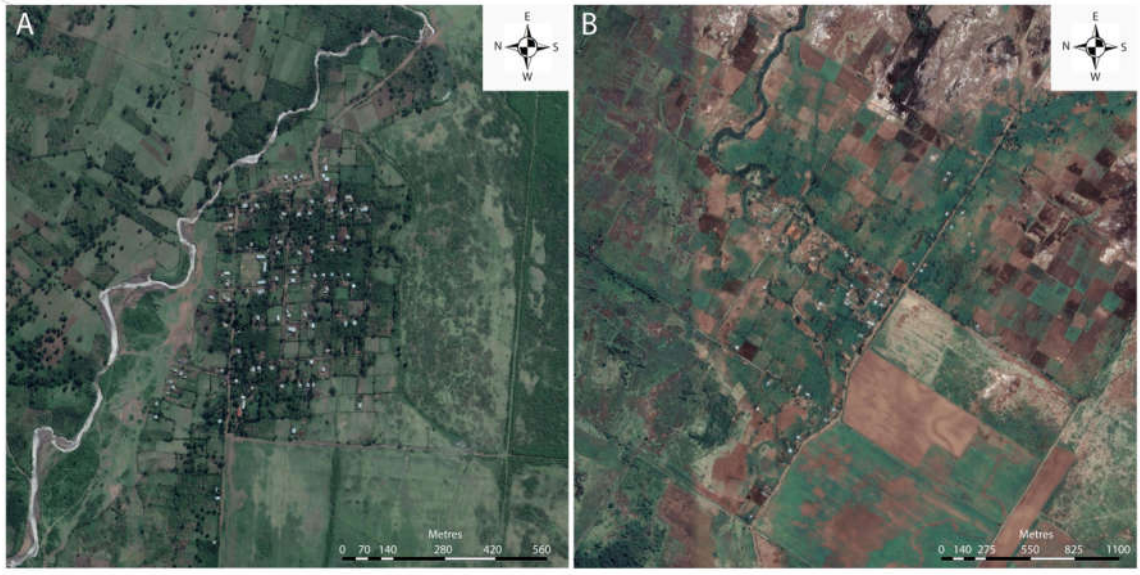


Figure 4.1. Aerial images of the two the study villages, Abulo (A) and Magge (B). The two rural villages are situated within similar agricultural landscapes. Aerial images are taken at different times of the year, which is seen in the difference in crop cover in the two villages. The scale bars indicate distance (m).

4.2.2. Characterization of environmental factors

In order to identify potential environmental risk factors underlying the clustering of malaria vectors, with and without sporozoite infection, as well as of malaria-infected people, at household and village levels, and a survey of each house and the surrounding environment was conducted and characterized. The location of each house was georeferenced using a global positioning system (GPS; Garmin eTrex 10, Garmin International Inc, USA). Data on the location of the house in the village; housing condition (type, construction material,

presence or absence of eaves, door and window condition, roofing and wall materials and condition); the number of residents; cooking habits (inside or outside houses); bed net use; presence or absence of alternate hosts outdoors; and presence of mosquito breeding sites within 50 m in the two study villages were recorded.

4.2.3. Sampling of *Anopheles* mosquitoes

Monthly sampling of malaria mosquitoes was conducted in the two study villages from January 2018 to March 2019. A systematic random sampling approach was used to select 30 houses in each village for the subsequent sampling of malaria mosquitoes. Ten of the houses were used for indoor collections with Centres for Disease Control and Prevention (CDC) light traps (BioQuip Products, Inc, CA, USA), while ten other houses were selected for pyrethroid spray collections (PSC; Mobile® insecticide, Fujian Quanzhou Gaoke Daily Chemical Manufacturing Co Ltd, China). In addition, ten houses were used for outdoor collections using CDC light traps. To optimize spatially balanced sampling, the two study villages were each divided into three blocks, comprising two at the edges and one at the center. Six houses from the two edge blocks and four houses from the central block were randomly selected in each study village. The selected houses were similar in structure and construction materials. In the case in which a house was different in structure, the neighbouring house with similar features was chosen.

The indoor CDC light traps were hung next to the feet of an occupant, sleeping under an insecticide-treated bed net, approximately one meter above the ground, following the protocol outlined by the WHO (1975), while outdoor CDC traps were hung next to the

houses approximately 50 cm above the ground. The traps were operated from 6:00 PM to 6:00 AM and the indoor traps collected over three alternate nights, resulting in 30 CDC trap nights per village per month, while the outdoor traps were operated once a month. The PSC collections were conducted once a month according to the WHO specifications WHO (1975). Before spraying, occupants were requested to leave the house. Utensils used for food, drinking water and clothes were taken out of the houses, and openings in the walls and doors were covered with cloth in order to prevent the mosquitoes from escaping during spraying. The floor of the house was covered with white sheets. Knocked down female *Anopheles* mosquitoes were then collected and preserved individually in microfuge tubes (1.5 ml) containing silica gel desiccant.

The collected mosquitoes from all sampling methods were sorted according to their physiological status, as unfed, blood-fed, semi-gravid or gravid, and morphologically identified into species using standard keys (Verrone, 1962; Gillies and Coetzee, 1987). All female *Anopheles* mosquitoes were individually preserved in labeled tubes containing silica gel and stored at room temperature, until further processing. Mosquitoes in the *An. gambiae* species complex were considered as *An. arabiensis*, since no other species in the complex has been recently recorded in the area (Massebo *et al.,* 2013b; Debebe *et al.,* 2018).

4.2.4. Detection of *Plasmodium* sporozoites in *Anopheles* mosquitoes

All female *An. arabiensis*, *An. pharoensis,* and *An. ziemanni* caught in indoor CDC light traps were analysed for the presence of *Plasmodium falciparum* and *P. vivax* circumsporozoite proteins (CSP). This was done using enzyme-linked immunosorbent assay (ELISA) following the procedure outlined by Wirtz *et al.* (2007). *Plasmodium falciparum*, *P. vivax*-210, and *P. vivax*-247 capture monoclonal antibodies (mAb) (50 µl each) were coated onto three separate 96-well microtiter plates, incubated at room temperature for 30 min, and the contents aspirated. Then 200 µl of blocking buffer (0.5 % Casein from bovine -Sigma, and 0.1N NaOH in PBS, pH 7.4) was added to each well of the plates and incubated at room temperature for 1 h. Dried heads and thoraces of the preserved female *Anopheles* mosquitoes were separated from the abdomen and individually homogenized in 50 µl of grinding buffer (blocking buffer containing Igepal CA-630 - Sigma) in a 200:1 ratio. The grinding pestle was then rinsed twice with 100 µl grinding buffer, for a final homogenate volume of 250 µl. After 1 h of incubation, the blocking buffer was aspirated from the plates and 50 µl of each mosquito triturate was added to a well in each of three test plates. A CSP positive sample and laboratory-bred *An. arabiensis* was used as positive and negative controls, respectively. After 2 h of incubation, the well contents were emptied and the wells washed two times with 200 µl PBS-Tween 20 - Sigma (2000:1) and then 50 µl aliquots of homologous peroxidase-conjugated mAb were added to each well. The plates were incubated for 1 h and the wells emptied and washed three times with PBS-Tween 20 and then 100 µl 2,2'-Azino-bis [3-

ethylbenzothiazoline-6-sulfonic acid] (ABTS peroxidase substrate- Roche Diagnostics GmbH, Mannheim, DE) was added per well and incubated for 30 min.

Results were determined visually and by measuring the optical density at 405 nm using an ELISA plate reader (Thermo Fischer Scientific, Vantaa, Finland). Green samples with optical density values of greater than two times the average optical density of the negative controls were considered sporozoite positive.

4.2.5. Cross-sectional parasitological studies for malaria parasites

Four phase cross-sectional studies were conducted in each village, in January (dry season), August (long-rainy season), November 2018 (Short-rainy season) and March 2019 (dry season). The participating households were randomly selected and then visited. Informed consent was obtained from study participants and parents of children involved in the study prior to taking blood samples. The geographic location of each house visited was recorded using a GPS.

4.2.5.1. Sample size determination

The sample size required for the cross-sectional parasitological examination was determined based on a prior study on the prevalence of malaria in the district (Loha and Lindtjorn, 2012), which reported that the overall malaria prevalence was 24.3 %. Assuming the expected prevalence of 24.3 %, with a marginal error of 5 % and a 95 % confidence interval, together with population sizes of 897 for Abulo and 701 for Magge villages, at least 215 people for Abulo and 201 people for Magge were assessed during each study visit. To determine this number, the finite population formula was used: $n' = [NZ^2 P (1-P)]$

/ d^2 (N-1) + Z^2P (1-P), where n' is the sample size; N is the population size; Z is the Z statistic for a level of confidence; P is the expected proportion of people carrying *Plasmodium* parasites; and d is the level of confidence (Naing *et al.,* 2006). After calculating the minimum sample size required, about 15 % of contingency was added, which resulted the final sample size of 241 for Abulo and 232 for Magge.

4.2.5.2. Blood smear preparation and microscopic examination

Thick and thin blood smears were prepared on clean microscopic slides, by puncturing the 'ring finger' of the study participants with a sterile lancet, following WHO standard guidelines (WHO, 1991). Rapid diagnostic test (RDT) kits were also used for the immediate detection and treatment of positive cases. Individual information about the study participant was recorded and the slide was labelled and coded. The slides were allowed to air dry and the thin smear was fixed with absolute methanol (Sigma). The dried slide was then stained with 10% Giemsa for 10 min and assessed for *Plasmodium* parasites by two experienced microscopists (WHO, 1991). During microscopy, a slide was considered negative if no parasite was observed in 100 fields with a magnification of 100 times. When found positive in the thick smear, the identity of the parasites was confirmed from the thin smear.

4.2.5.3. Ethical consideration

A research permit was obtained from the institutional review board of the College of Natural and Computational Sciences, Addis Ababa University (CNSDO/284/08/2016), and Arba Minch Zuria District Health Office (AZWHO/1163/2). Informed consent was obtained from the study participants and parents of children involved in the study before taking blood samples. All malaria positive cases were treated according to the Ethiopian Ministry of Health Guidelines (MoH, 2004). In the case of falciparum malaria, Coartem was administered, whereas vivax malaria was treated with chloroquine phosphate. Subjects were excluded from the study if they had taken antimalarial drugs within two weeks before the study period, did not give consent, or were not permanent residents of the study villages.

4.2.6. Environmental risk factor analysis

The effect of housing structure and other environmental factors on the density of indoor *Anopheles*, as well as the prevalence of sporozoites, was modelled using negative binomial regression of the generalized linear model (GLM; JMP Pro version 13 SAS Institute Inc., Cary, NC, USA). In contrast, repeated measures generalized linear mixed model (GLMM) was used to model the effect of housing structure and local environmental factors on the number of *Plasmodium*-positive individuals. To determine the overall effect of the environmental variables on the overall density of indoor mosquitoes, sporozoite-infected mosquitoes and malaria prevalence, the regression analysis was first conducted by pooling the data from both study villages. This model determined that the village was a non-significant factor, and therefore subsequent analyses were conducted at the village level.

The regression analysis was first conducted using all the predictor environmental factors for each village to generate the overall models. Selection of the final model was based on the Akaike's information criterion (AIC) in which the most parsimonious model with the lowest AIC value. Stepwise backward selection and removal of the independent variables with the highest p-values from the overall model were conducted until the AIC value of the model was minimized. The entomological inoculation rate (EIR) was estimated from the CDC indoor captures using the formula: 1.605 × (number of circumsporozoite-positive ELISA results from CDC light trap / number of mosquitoes tested) × (number of mosquitoes collected from CDC light trap / number of catches) × 365 days (Drakeley *et al*., 2003).

4.2.7. Spatial analysis

To assess the local spatial autocorrelation of malaria vectors and *Plasmodium*-infected individuals, the Getis-Ord Gi statistics was used. The Getis-Ord Gi statistics is effective in identifying pockets of high and low incidence points referred to as hotspots and coldspots, respectively (Getis and Ord, 1992). Hotspots and coldspots for the density of mosquito vectors and malaria cases were identified, and statistical significance was determined at Gi^* P values of 0.05 or less. Sporozoite-infected mosquitoes and the annual EIR were mapped based on their respective value. Graduated symbols and bars indicate the variation in the number of sporozoite-positive mosquitoes and the annual EIR, respectively, among the houses. ArcGIS (v. 10.3, ESRI, USA) was used to produce all the maps.

4.3. Results

4.3.1. Environmental factors differentially affect the density of indoor *Anopheles*

A total of 4154 *Anopheles* mosquitoes, belonging to eight species, were captured and collected indoors using CDC light traps and PSC, respectively (Table 4.1). Approximately 98 % of the mosquitoes were identified as *An. arabiensis*, *An. pharoensis* and *An. ziemanni* (Table 4.1). For the analysis, data for these three species were pooled, as the number of *An. pharoensis* and *An. ziemanni* were relatively low, and the other species removed. The overall regression analysis demonstrated that none of the environmental variables significantly affected the indoor density of these species (Appendix 4.1), likely due to the inherent high variation when comparing between villages. As a result, subsequent analyses were conducted at the village level, which revealed that the environmental variables differentially affected the indoor mosquito density. In Abulo, the overall GLM analysis revealed that the density of mosquitoes indoors was significantly affected by the household size, bed net use and the interaction of bed net use and house location in the village (Appendix 4.2) with an AIC value of 258. Backward elimination of non-significant variables resulted in the final simplest model, with a reduced AIC value of 227, indicating that household size, bed net use and the interaction of bed net use and house location in the village significantly affected the indoor density of *Anopheles* mosquitoes (Table 4.2).

In Magge, the overall GLM analysis demonstrated that the housing conditions, including door, wall and roof condition, household size, bed net use, and the interaction between bed net use and location of the house in the village significantly affected the indoor density of mosquitoes (Appendix 4.3; AIC= 282). The final model (AIC= 234) revealed that there was significant effects of wall and roof condition, as well as significant interaction between bed net use and location of the house in the village. This interaction demonstrates that the density of indoor *Anopheles* mosquitoes is higher in houses that did not use bed nets properly and are located at the edge of the village (Table 4.2).

Table 4.1. The species of *Anopheles* and the total number of mosquitoes caught in indoor and outdoor CDC light traps and collected following pyrethrum spray applications.

***Anopheles* species**	**Abulo**			**Magge**			**Total**
	CDC light traps		PSC (N)	CDC light traps		PSC (N)	
	Indoor (N)	Outdoor (N)		Indoor (N)	Outdoor (N)		
An. arabiensis	787	108	761	1286	145	388	**3475**
An. pharoensis	381	135	3	226	73	0	**818**
An. ziemanni	120	86	0	102	115	1	**424**
An. demeilloni	19	28	19	21	3	13	**103**
An. pretoriensis	20	39	0	3	1	0	**63**
An. tenebrosus	2	3	0	0	0	0	**5**
An. garnhami	1	0	0	0	0	0	**1**
An. squamous	1	0	0	0	0	0	**1**
An. cinereus	0	1	0	0	0	0	**1**
An. natalensis	0	3	0	0	0	0	**3**
Total	**1331**	**403**	**783**	**1638**	**337**	**402**	**4894**

4.3.2. Environmental factors do not affect the density of sporozoite-infected vectors

Out of the 2898 mosquitoes caught in the indoor CDC traps in both villages, 83 were found to be sporozoite positive. The number of sporozoite-infected mosquitoes was not significantly affected by any of the environmental factors included in the regression models, both at the overall and village levels.

Table 4.2. Statistical summary of the effect of environmental variables on the density of indoor mosquitoes at the village level following stepwise backward selection and removal of non-significant independent variables.

Variable	Estimate	Std Error	Wald χ^2	Prob > χ^2
Abulo				
Intercept	2.70	0.65	17.21	<0.0001
House location in the village (center/edge)	0.45	0.48	0.89	0.34
Household size (No of occupants)	0.19	0.079	5.55	0.019
Net use (proper use/no use)	1.20	0.39	9.60	0.0019
Net use * House location in the village	-1.81	0.64	7.93	0.0049
Magge				
Intercept	4.31	0.91	22.54	<0.0001
Wall condition (poor/good)	1.60	0.49	10.68	0.0011
House location in the village (center/edge)	0.70	0.72	0.93	0.34
Net use (proper use/no use)	-0.59	0.71	0.71	0.40
Net use * House location in the village	2.30	0.98	5.27	0.022
Roof condition (poor/good)	-1.47	0.73	4.04	0.045

4.3.3. Environmental factors affect malaria incidence

Blood samples were taken from the general population with a diverse age distribution. The majority of blood samples were taken from children between the ages of 5 and 15 years (55 %). Teenagers and adults of ages 16 and above (25 %), children aged 1 to 4 years (19

%), and infants less than one year old made up the rest. Out of the 1894 persons sampled, a total of 64 individuals were found to be infected with *Plasmodium* parasites during the repeated cross-sectional surveys in both villages. The overall prevalence of malaria in Abulo was 3.53 % (34/964) and 3.23 % (30/930) in Magge. The overall GLMM model revealed that the number of malaria cases in the two village s combined was significantly affected by the interaction of house location and household size (GLMM; $F = 5.50$; $P = 0.021$), with people living in houses located at the edge of the villages, with a higher number of occupants (Fig. 4.2AB), being more likely to become infected with malaria. Separate analyses provided further support for these findings in both villages. In Abulo, when all of the housing and environmental conditions were included in the GLMM model, household size was found to significantly affect the number of *Plasmodium*-infected individuals (AIC = 267; Appendix 4.4). Stepwise backward elimination of independent variables with the highest P-value resulted in the final model, in which house location in the village and household size both affected malaria prevalence resulting in AIC value of 218 (Table 3). In Magge, only the location of the house in the village in both the overall (AIC = 259; Appendix 4.5) and the final model (AIC = 230; Table 4.3) was found to be a significant predictor of malaria prevalence.

Table 4. 3. Statistical summary of the effect of environmental variables on the incidence of malaria at the village level, following stepwise backward selection and removal of non-significant independent variables.

Variable	Estimate	Std Error	t Ratio	Prob>|t|
Abulo				
Intercept	-0.018	0.099	-0.19	0.85
Household size (No of occupants)	0.047	0.015	3.04	0.0029
Breeding site within 50 m radius (present/absent)	-0.086	0.062	-1.38	0.17
House location in the village (center/edge)	-0.082	0.041	-2.02	0.045
Magge				
Intercept	0.20	0.043	4.54	<0.0001
House location in the village (center/edge)	-0.14	0.043	-3.18	0.0018

4.3.4. Spatial clustering of mosquitoes

From a total of 750 sampling nights in each village, 2517 and 2377 mosquitoes were caught/collected indoors and outdoors in Abulo and Magge (Fig. 4.1), respectively (see Table 4.1 above). Clustering analyses revealed that the three most abundant *Anopheles* species aggregated in and around a few houses located close to one another near the edge of each village (Fig. 4.2A-D). In Abulo, one hotspot was identified at the eastern edge (Gi^* $Z \geq 1.96$, Gi^* $P \leq 0.05$), whereas the clustering of mosquitoes in other parts of the village was not statistically significant (Gi^* $Z < 1.96$, Gi^* $P > 0.05$; Fig. 4.2C). Similarly, in Magge, one statistically significant (Gi^* $Z \geq 1.96$, Gi^* $P \leq 0.05$), and one nearly significant hotspot (Gi^* $Z < 1.96$, Gi^* $P < 0.1$) were identified at the northern and eastern edges of the village, respectively, in which the majority (52.6 %) of the mosquitoes were collected (Fig. 4.2D). In contrast, a coldspot was detected in the centre of Magge (Gi^* $Z > 1.96$, Gi^* $P < 0.05$; Fig. 4.2D).

4.3.5. Local spatial clustering of malaria incidence

Malaria incidence indicated the presence of high and low malaria risk areas, hotspots and coldspots, respectively, in both villages. In Abulo, two hotspots were identified (Gi^* $Z > 1.96$, Gi^* $P < 0.05$), which accounted for 35.3 % of the malaria incidence, and were located at opposite ends at the edge of the village, along with a close to a significant hotspot at the southwestern edge of the village (Gi^* $Z < 1.96$, Gi^* $P < 0.1$; Fig. 1G). A close to significant coldspot (Gi^* $Z < 1.96$, Gi^* $P < 0.1$) around the center of the village was detected in Abulo (Fig. 1G). Similar to Abulo, a higher incidence of malaria was found clustered at the remote edge of the village in Magge (Gi^* $Z > 1.96$, Gi^* $P < 0.05$), with a coldspot clustered at the center of the village (Gi^* $Z < 1.96$, Gi^* $P < 0.05$; Fig. 1H).

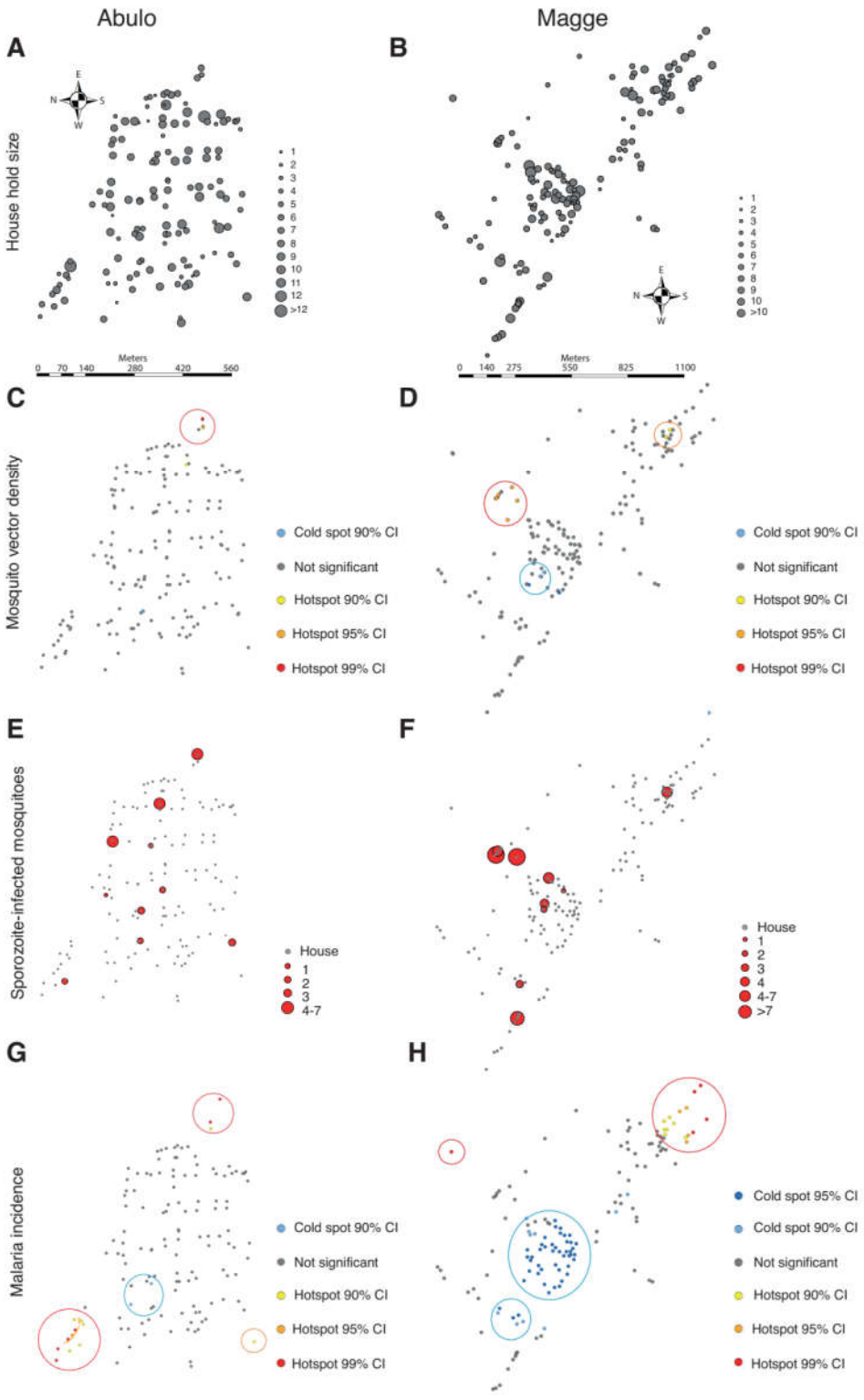


Figure 4.2. Living on the edge increases malaria incidence. The distribution of households and occupant density in Abulo (A) and Magge (B), in which the size of the circles indicate the number of inhabitants in each household. The scale bars indicate distance (m). Clustering of malaria mosquitoes generated from hotspot analyses in Abulo (C) and Magge (D) is shown. Cold- and hotspots are indicated with 90 %, 95 % and 99 % confidence intervals (CI). Please note the single coldspot in Abulo indicated by an arrow. The distribution and abundance of sporozoite-infected mosquitoes is mapped for Abulo (E) and Magge (F). The size of the circles indicates the number of sporozoite-infected mosquitoes. The clustering of malaria infected people and people with lower risk of getting the infection is revealed by the hotspot analyses for Abulo (G) and Magge (H). The coloured rings indicate the different significance levels of hot- and coldspots, with 90 %, 95 % and 99 % CI.

4.3.6. Distribution of sporozoite-infected vectors

While spatial clustering analysis is not possible for this variable, there being only ten locations per village rather than the 30 required, the geographical location of the 83 sporozoite-infected mosquitoes was mapped for Abulo (Fig. 4.2E) and Magge (Fig. 4.2F). While the majority of the sporozoite-infected mosquitoes were caught in houses at the edge of the villages (Fig. 4.2EF), the proportion of sporozoite-infected mosquitoes at each sampling point was dependent on the density of the vectors, as indicated by the different entomological inoculation rates (EIR) in houses located at the edge and the centre of the villages (Fig. 3). In Abulo, the chance of receiving an infectious bite doubled in houses located at the edge compared to those at the centre: occupants of houses at the village edge experienced the risk of 50.23 infectious bites per person per year (ib/p/y) as opposed to those at the centre who risk 25.78 ib/p/y. Similarly, in Magge, the likelihood of an occupant receiving an infectious bite at the village edge was three times higher (82.44 ib/p/y) at the edge compared to the centre (26.21 ib/p/y).

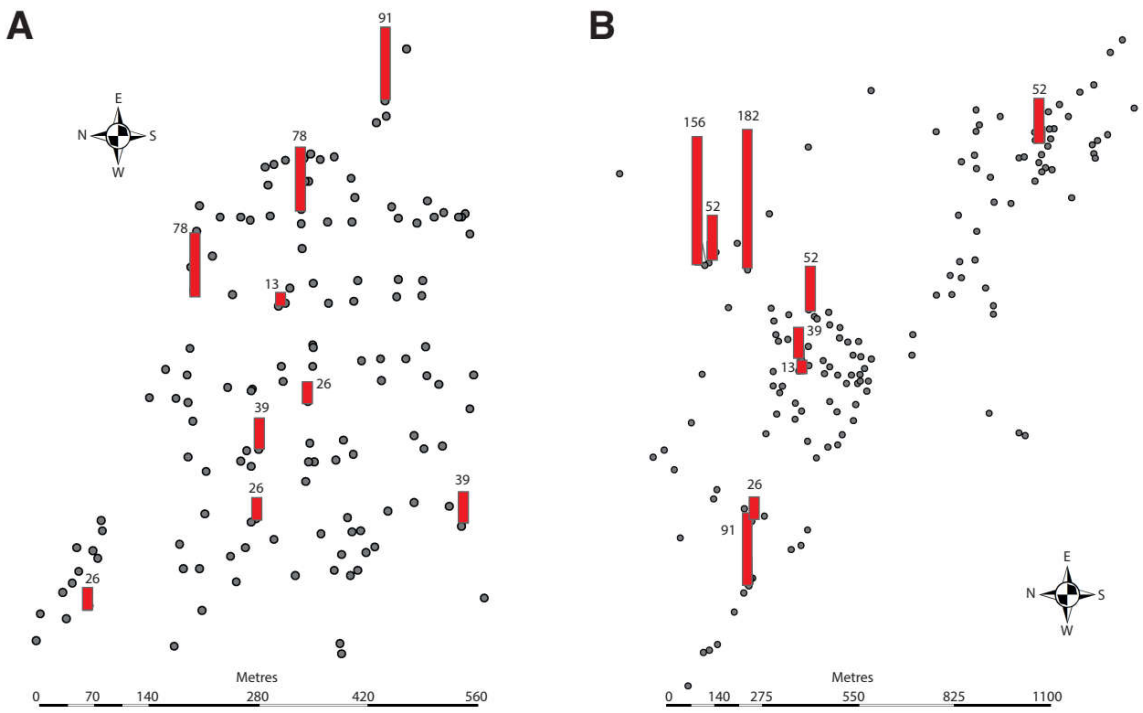


Figure 4.3. Map showing the number of infectious bites an individual receives per year (the red bars) and the households in the study areas (the grey dots). The scale bars indicate distance (m).

4.4. Discussion

Insect-plant interactions provide the theoretical framework by which vector density and malaria prevalence can be modelled in a heterogeneous local landscape. By testing local landscape models within two rural villages in southern Ethiopia, we identify household size and location within the village as the main predictors of vector density and malaria prevalence, supporting the resource concentration hypothesis and edge effect, respectively (Root, 1973; Cromartie, 1975). By extending the analysis to sporozoites, we further identify the edge of the villages as at increased risk for obtaining malaria through enhanced EIR. The efficacy and ability to implement malaria interventions based on the identification of hotspots is controversial, and has been said to rely heavily on the ease of hotspot identification, persistence and the level of landscape heterogeneity (Bousema *et al.,* 2012; 2016; Platt *et al.,* 2018). We argue that hotspot analysis coupled with landscape models based on direct and indirect effects on mosquito density, and in the light of established ecological frameworks, can inform malaria intervention strategies at the local level using generalizable criteria. Thus, we may avoid time-consuming and low sensitivity methods of identifying people with increased exposure to infectious mosquitoes.

Through the regression models, hotspot analyses and distribution of EIRs in two rural villages over fifteen consecutive months, we identify the edge of the villages as areas of increased risk for malaria as a consequence of high, overlapping populations of both mosquitoes and people. In contrast, people at the centre of the villages experience a reduced risk of exposure. This effect is likely not due to heterogeneity in the quality within the patch, in terms of blood meals, but rather to the heterogeneity associated with the risk

connected with foraging in the centre or at the edge of the patch (Lidicker and Peterson 1999). While the limited number of studies investigating malaria hotspots at the within-village level did not consider the mosquito density and household location, these still demonstrate a clear impact of the edge effect on the spatial clustering of malaria incidence (Zhou *et al.,* 2012; Rulisa *et al.,* 2013; Durnez *et al.,* 2018). Although entomological detection of hotspots may currently be logistically unattractive and hampered by poorly standardized sampling strategies, the implementation of standardized entomological sampling strategies, including CDC light traps and PSC, which are based on the knowledge of the edge effect and the resource concentration hypothesis on vector activity, can provide a robust indicator of malaria hotspots.

The host-seeking behaviour of female mosquitoes is influenced by various factors, including the dispersal ability of the mosquitoes, as well as the availability, density and distribution of hosts (Charlwood and Alecrim, 1989; Takken *et al.,* 1998; Thiemann *et al.,* 2011; Cummins *et al.,* 2012). Host-seeking mosquitoes are known to leave their resting sites, situated either in tall vegetation at some distance from the households, or in the agricultural landscape surrounding the village, to gain access to their blood hosts (Gillies, 1954a,b; Debebe *et al.,* 2018; Jansson *et al.,* 2020). Household occupancy is known to influence indoor vector densities, and thereby affect mosquito dispersal, and by extension, the distribution of human biting risk and malaria transmission within communities (Port *et al.,* 1980; Kirby *et al.,* 2008; Kaindoa *et al,* 2016). Moreover, the overall directional movement of mosquitoes within villages is influenced by the spatial distribution and demographic composition of households in these villages (Thomas *et al.,* 2013). As a result, households with high occupancy may form pockets (patches) of high transmission

of mosquito-borne diseases (Bannister-Tyrrell *et al.,* 2017), as demonstrated in this study. However, the fine-scale and within-village clustering of vector densities in this study does not appear to overlap with the clusters of houses with high occupancy, except at the village edge. In fact, houses with high occupancy within the centre of both study villages are coldspots for malaria incidence and vector presence, indicating that the indirect effect of the location can mitigate the direct effect of household occupancy. Thus, census data alone does not accurately reflect malaria vector hotspots, as previously suggested (Kaindoa *et al.,* 2016). Regardless of the difference in topography between Abulo and Magge, this study demonstrates that the same fine-scale landscape features modulate the spatial relationships underlying malaria transmission.

In this study, we identify the landscape features that are involved in forming vector and malaria hotspots within villages. We propose that maintaining or scaling up conventional vector control and malaria interventions, in combination with the targeted intensification of these, or other novel controls, in predicted hotspots, can be implemented to reduce malaria incidence. Previously, the implementation of hotspot-targeted vector control strategies was seen as highly labour intensive with variable outcomes Bousema *et al.,* 2016). With the implementation of the general rules identified in this study, there would a minimal increase in labour and cost associated with the treatment of predicted hotspots, in addition to the current maintenance control efforts.

Chapter 5

Evaluation of an Expanded Integrated Vector Management Intervention: Mass Trapping of Malaria Vectors using Odour-baited Traps

5.1. Introduction

The reduction in the malaria burden over the past fifteen years is threatened by a reversal, as we are currently witnessing an increase in the number of global malaria cases (WHO, 2019). Development of insecticide resistance (Ranson and Lissenden, 2016), behavioural change of the vector population (Sokhna *et al.,* 2013; Thomsen *et al.,* 2017), reduced use of bed nets (Honjo *et al.,* 2013; McLean *et al.,* 2014; Ntonifor and Veyufambom, 2016), as well as the reduced acceptability of indoor residual spraying (IRS) by communities and the demotivation of governments to spend money on expensive insecticides for IRS (Stelmach *et al.,* 2018; Tangena *et al.,* 2020), are among the factors contributing to the recent rise of global malaria cases. Besides, malaria transmission in the outdoor environment is increasing exacerbating the situation and thus needs to be addressed through the use of interventions that target the outdoor vector populations (Govella and Ferguson, 2012; Benelli and Beier, 2017). Odour-based mosquito control approaches have the potential to complement the existing IVM interventions (Ignell and Hill, 2020), strengthening the ongoing malaria control efforts and pave the way for malaria elimination.

Compounds, and blends thereof, which attract different physiological states of malaria vectors have been isolated and characterized. The sources of these compounds range from human body odour and breath (Constantini *et al.,* 2001; Mukabana *et al.,* 2004), body odour of preferred host species (Abong'o *et al.,* 2018; Verhulst *et al.,* 2018; Bakker *et al.,* 2020), floral scents (Nyasembe *et al.,* 2012; 2018) and oviposition substrates (Okal *et al.,* 2013; Lindh *et al.,* 2015; Eneh *et al.,* 2016; Asmare *et al.,* 2017). Synthetic odour blends have been developed, optimized and evaluated for their capacity to attract malaria mosquitoes (Smallegange *et al.,* 2005; Mukabana *et al.,* 2012; Wondwosen *et al.,* 2016, 2017, 2018). The versatility of existing compounds and blends, however, are restricted in their use in IVM, as these either require carbon dioxide for potency, or target a limited number of mosquito vector species (van Loon *et al.,* 2015; Wooding *et al.,* 2020). That said, mass trapping of mosquitoes using odour-baited traps with a synthetic human odour blend has proven effective in reducing mosquito vector densities and malaria prevalence in Kenya (Homan *et al*., 2016). Homan *et al.* (2016), however, concluded that the future lures for mass trapping need to be improved to increase both the number and diversity of trap captures.

Building on research by Mahande *et al.* (2010) and Kweka *et al.* (2011), Dawit (2018) demonstrated that cattle urine attracts host-seeking, blood-fed, and gravid *An. arabiensis*, which feed directly on cattle urine to supplement their requirement for nitrogenous compounds to supply nutrients for survival, flight, and reproduction. Dawit (2018) further identified a synthetic odour blend based on 24 h aged cattle urine, which attracts and traps wild *An. arabiensis*. Thus, feeding on cattle urine could be an important evolutionary adaptation that mosquitoes underwent in the absence of potential hosts as compensation

for blood meals. Such compensatory feeding on vertebrate excretion is common in other insect taxa, in which nitrogen and salt are acquired to enhance survival and reproduction (Molleman, 2010).

This study aimed to evaluate the impact of mass trapping, using traps baited with a slow-release formulation of the synthetic cattle urine odour blend, on the vector population and malaria transmission under operational conditions in southern Ethiopia. For this purpose, monthly entomological and repeated cross-sectional malaria prevalence surveys were conducted to generate baseline information in two rural villages for fifteen consecutive months. Then mass trapping of mosquitoes was commenced in one of the villages, while the entomological monitoring and malaria prevalence surveys continued in both villages. Intervention impact was assessed by calculating relevant entomological and parasitological parameters in the intervention village compared with the control village.

5.2. Materials and Methods

5.2.1. Description of the study areas

The study was conducted in the villages, *Abulo* (6º03'48"N, 37º35'30"E) and *Magge* (5º51'49"N, 37º29'32"E), in the Arba Minch Zuria district, Gamo Zone. A detailed explanation of the study sites is described in chapter 4. *Abulo* was the intervention village, where the mass trapping of *Anopheles* mosquitoes using odour-baited traps was conducted, and *Magge* served as the control.

5.2.2. Study design

Using a controlled before-and-after study design, the intervention impact of mass trapping on malaria transmission in *Abulo* (intervention) and *Magge* (control) was assessed. Monthly entomological monitoring and repeated cross-sectional malaria prevalence surveys were conducted in the control and intervention villages from January 2018 to December 2019, to generate data on the entomological and parasitological profiles of malaria in the villages, both before and during the intervention, as described below (5.2.3-5.2.6; Figure 5.1).

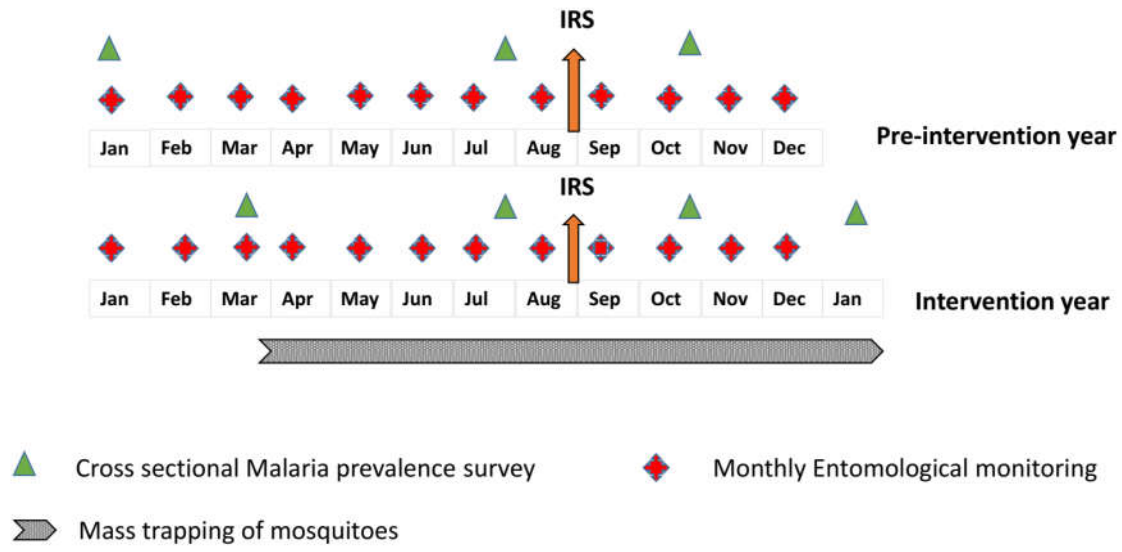


Figure 5.1. Schematic representation showing the timeframe for entomological monitoring, cross-sectional malaria prevalence surveys, and the commencement of the mass trapping.

Mass trapping of malaria mosquitoes commenced in the intervention village in April 2019. In preparation for the intervention, 50 houses from a total of 134 were selected for trap placement in *Abulo* in January 2019. Thereafter, 20 W solar panels (Zhejiang Perlight Solar

Co., Ltd., Zhejiang, China) were installed on the roof of each house, with a controller (Qingdao Skywise Technology Co., Ltd., Qingdao, China) preventing overcharging of the batteries, installed indoors, connecting the solar panel to a 12V 18 Ah 20 h^{-1} battery (Future Green Technology Co., Ltd., Qingdao, China). The Suna traps (Biogents AG, Regensburg, DE) were installed outdoors, 20 cm above the ground, next to the wall, away from doors and windows of the house, and shaded by the roof (Figure 5.2). The Suna traps were enhanced with lures made of low-density polyethylene sachets (100 mm × 100 mm × 0.1 mm) containing the synthetic cattle odour attractants (2.5 g) loaded onto high-density polyethylene pellets (2.5 g) under vacuum (Biogents AG). The traps were operated on a daily basis and the sachets were replaced every 3-4 weeks to ensure the continuous and consistent release of the attractant over this time. To assess the efficacy of the mass trapping, and monitor the monthly population of mosquitoes affected by mass trapping, sampling of mosquitoes from the suna traps was conducted for five consecutive nights every month.

Figure 5.2. Solar panel installed on the roof of a house (A) and installation of the odour-baited trap (B). Picture Credit (A: Yared Debebe and B: Prof. Rickard Ignell).

5.2.3. Sampling of *Anopheles* mosquitoes

Monthly sampling of *Anopheles* mosquitoes in the intervention and control villages were conducted for two years from January 2018 to December 2019. Indoor host-seeking anophelines were sampled using CDC light traps (BioQuip Products, Inc, CA, USA), while indoor resting mosquitoes were knocked down using pyrethrum sprays (Mobile®, Fujian Quanzhou Gaoke Daily Chemical Manufacturing Co Ltd, China). Besides, the activity of the outdoor *Anopheles* population was monitored using CDC light traps. See chapter 4 section 4.2.3 for detailed sampling procedures.

5.2.4. Mosquito processing and identification

Morphological identification of the collected *Anopheles* mosquitoes was conducted using standard keys (Verrone, 1962; Gillies and Coetzee, 1987). The identified female *Anopheles* mosquitoes were further sorted according to their physiological state as unfed, fed, semi-gravid, or gravid following WHO (1975). All *An. gambiae s.l.* were considered as *An. arabiensis*, as no other member of the species complex have been previously recorded in the area (Massebo *et al.,* 2013b; Debebe *et al.,* 2018).

5.2.5. Malaria parasite circumsporozoite protein detection in mosquitoes

The presence of *P. falciparum* and *P. vivax* circumsporozoite proteins (CSPs) using enzyme-linked immunosorbent assay (ELISA) was conducted following the procedure outlined by Wirtz *et al.* (2007). All *An. gambiae s.l.*, *An. pharoensis,* and *An. ziemanni* from the indoor CDC light trap collections were checked for the CSPs. See chapter 4 section 4.2.4 for detailed ELISA procedure.

5.2.6. Cross-sectional parasitological study

A total of seven cross-sectional surveys were conducted in both the intervention and control villages throughout the study period; four in the pre-intervention period and three during the intervention period (see Figure 5.1 above). The detailed sampling procedure, sample size determination, blood film preparation, treatment of infected individuals, and ethical issues are presented in chapter 4 sections 4.2.5 and 4.2.6.

5.2.7. Outcome variables

Malaria prevalence, the human-biting rate (HBR) and the entomological inoculation rate (EIR) were the primary outcomes in measuring the intervention impacts, whereas the density of host-seeking and resting mosquitoes were the secondary outcomes. Malaria prevalence per 1000 people was determined by dividing the number of *Plasmodium*-infected individuals by the total number of people tested multiplied by 1000. The HBR was computed by dividing the total number of *Anopheles* species to the total trap nights from the CDC indoor collections following Lines *et al.* (1991). Indoor resting density (IRD) from the PSC was determined by dividing the total number of *Anopheles* species by the number of houses and collection days (Oljira *et al.,* 2019). The sporozoite rate (SR) was determined by dividing the number of sporozoite positive mosquitoes by the number of mosquitoes tested (Beier, 2002). The EIR was estimated from the CDC indoor captures using the formula: 1.605 × (no. circumsporozoite-positive ELISA results from CDC light trap/no. mosquitoes tested) × (no. mosquitoes collected from CDC light trap/no. catches) (Drakeley *et al*., 2003).

5.3. Data analysis

Binary logistic regression was used to predict the probability of an individual being infected with malaria in the intervention and control villages during the pre-intervention and intervention periods. Following the regression analysis, *posthoc* tests were conducted to test for variation in pair-wise seasonal malaria prevalence in the control and intervention villages pre- and post-intervention. A generalized linear mixed model (GLMM) was used to determine the variation in the mean number of *Anopheles* mosquitoes from all collection methods by considering seasons and villages as fixed factors, and the individual sampling houses as a random factor. Negative binomial regression was used to assess the incidence rate ratios of the HBR, SR and EIR in the intervention village using the control village as reference category (JMP Pro version 13 SAS Institute Inc., Cary, NC, USA). The impact of the intervention was assessed by calculating the relative percentage of reduction for the primary and secondary outcomes in the intervention village before and after the commencement of the intervention (Grimshaw *et al.,* 2000). The intervention impact was assessed for the two major malaria transmission seasons (the long rain and the short rain). The relative percent reduction of a given parameter was computed using the formula developed by Mulla *et al*. (1971) as % reduction = $100(T_2/T_1 \times C_1/C_2)$-100, where T_1 is the parameter in the intervention village during pre-intervention; T_2 is the parameter in the intervention village during intervention; C_1 is the parameter in the control village during pre-intervention, and C_2 is the parameter in the control village during the intervention. C_1 and C_2 were used as correction factors for each parameter of interest for the intervention village between pre-intervention and intervention.

5.4. Results

5.4.1. Malaria prevalence

A total of 3228 blood smears were assessed for the presence of *Plasmodium* parasites during the seven seasonal parasitological surveys in the two villages. The result from the binary logistic regression revealed that the probability of a person becoming infected with malaria in the intervention and control villages was not statistically different ($\chi^2 = 0.13$, OR = 0.91, P = 0.71) during pre-intervention, while the probability significantly differed during the intervention ($\chi^2 = 27.47$, OR = 2.53, $P < 0.0001$). Pairwise seasonal comparison between the intervention and control villages during pre-intervention revealed that malaria prevalence was not statistically different between seasons (Figure 5.3). However, during the intervention, the proportion of people infected with *Plasmodium* parasites in the control village was significantly higher than that in the intervention village during the long rainy season ($\chi^2 = 27.14$, $P < 0.0001$; Figure 5.3). As a whole, mass trapping in the intervention village resulted in a relative reduction of malaria prevalence by 64 % in the long rains and 44 % in the short rains. Besides, malaria prevalence has reduced by 49 % during the dry season as compared to the control village (Table 5.1).

Table 5.1. Efficacy of the mass trapping of *Anopheles* mosquitoes on seasonal malaria prevalence per 1000 people in the intervention village in reference to the control village.

Season	**Malaria infection**	**Pre-intervention prevalence**		**Intervention prevalence**		**% Reduction**
		Int. village	Con. village	Int. village	Con. village	
Long rain	*P. falciparum*	12.50	26.21	41.32	211.21	59 %
	P. vivax	20.83	17.47	24.80	17.24	-21 %
	Mixed	0	0	8.26	21.55	----
	Total	33.33	43.67	74.38	250	61 %
Short rain	*P. falciparum*	46.41	41.10	50.00	64.04	31 %
	P. vivax	21.10	22.83	31.82	59.11	42 %
	Mixed	0	0	0	14.78	----
	Total	67.51	63.93	81.82	137.93	44 %
Dry season	*P. falciparum*	13.00	9.17	52.63	66.67	44 %
	P. vivax	8.66	9.17	21.93	57.14	59 %
	Mixed	0	0	0	0	----
	Total	21.65	18.35	74.56	123.81	49 %

Int. village: Intervention village Con. village: Control village

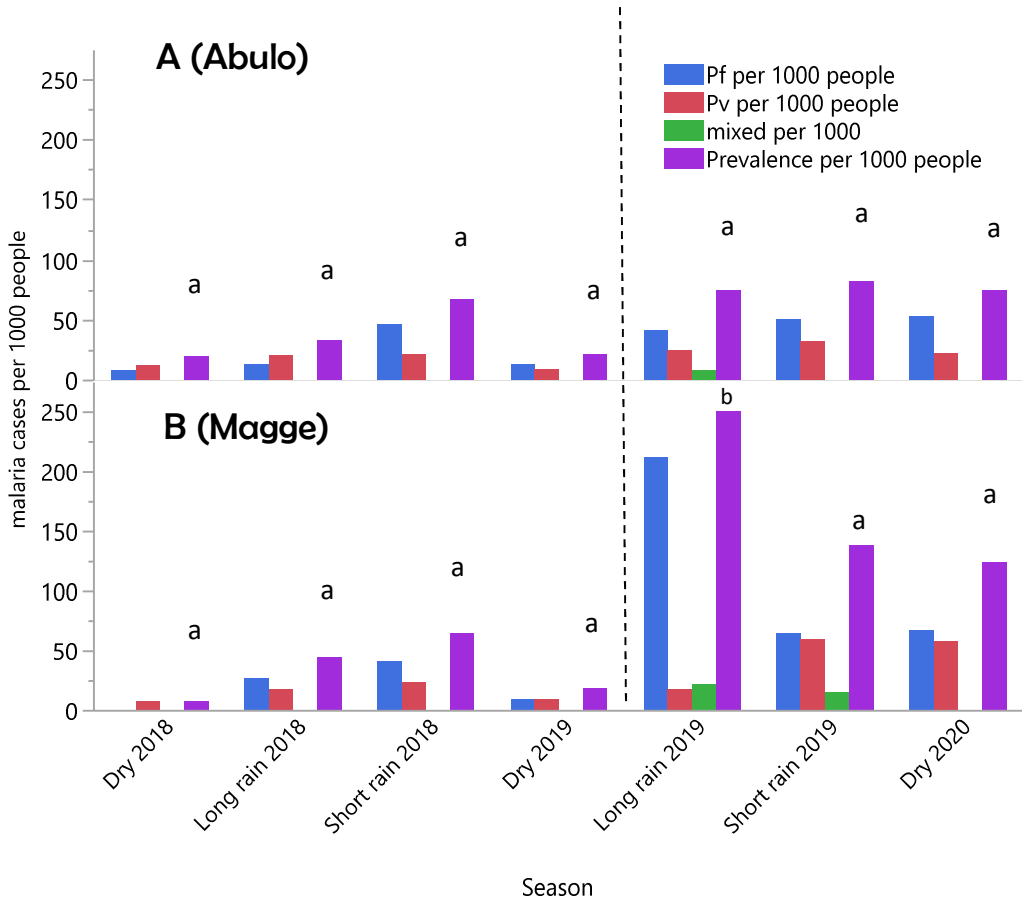


Figure 5.3. Prevalence of malaria per 1000 people, before and after the mass trapping intervention, in the intervention (A) and control (B) villages. The dashed line indicates the time when the odour-baited traps were introduced in the intervention village. Pairwise seasonal comparisons of malaria prevalence between villages are indicated with lower case letters. Different letters indicate significant differences in variation ($P \leq 0.05$) in malaria prevalence between villages during that season.

5.4.2. Abundance and diversity of *Anopheles* mosquitoes

During the two years of entomological monitoring, a total of 9198 *Anopheles* belonging to ten species were captured and/or collected in the study villages. *Anopheles arabiensis* was the most abundant species (75.9 %), followed by *An. pharoensis* (12.7 %) and *An. ziemanni* (9.1 %). The other species included *An. demeilloni*, *An. pretoriensis*, *An. tenebrosus*, *An. garnhami*, *An. squamous*, *An. natalensis* and *An. cinereus* constituted a total of 2.3 % (Table 5.2). The total number of *Anopheles* mosquitoes captured/collected in the intervention village was 2518 (3.36 mosquitoes/trap/nights) during pre-intervention and 1321 (2.94 mosquitoes/trap/nights) during the intervention, whereas 2375 (3.17 mosquitoes/trap/nights) and 2984 (6.63 mosquitoes/trap/nights) *Anopheles* were captured in the control village during the pre-intervention and intervention periods, respectively (Table 5.2). In the nine months of mass trapping, *An. arabiensis* was the most abundant *Anopheles* species in the odour baited traps, which captured a total of 3708 *Anopheles* mosquitoes belonging to four species: *Anopheles arabiensis* (88.9 %), *An. pharoensis* (9.1 %), *An. ziemanni* (1.9 %) and *An. demeilloni* (0.1 %). Besides, 3047 *Culex* mosquitoes were captured. (see Appendix 5.1 for the number and physiological states of mosquitoes captured in the traps).

The overall abundance of *Anopheles* mosquitoes from all catches between the intervention and control villages was not statistically different during pre-intervention (GLMM: $F = 0.004$, $df_n = 1$, $df_d = 58$, $P = 0.95$) whereas a significantly lower number of *Anopheles* were caught in the intervention village during the intervention (GLMM: $F = 8.8412$, $df_n = 1$, $df_d = 58$, $P = 0.004$). The mean number of *Anopheles* mosquitoes between preintervention

and intervention periods was not statistically different in the intervention village (GLMM: $F = 0.3604$, $df_n = 1$, $df_d = 689$, $P = 0.55$). In contrast, the mean overall number of *Anopheles* mosquitoes significantly increased in the control village during the intervention compared to the pre-intervention period (GLMM: $F = 28.9293$, $df_n = 1$, $df_d = 689$, $P < 0.0001$). Comparison of mosquito between indoor and outdoor CDC light trap catches revealed that the mean number of *Anopheles* mosquitoes collected was similar during the pre-intervention (GLMM: $F = 2.5379$, $df_n = 1$, $df_d = 18$, $P = 0.13$) and intervention (GLMM: $F = 2.313$, $df_n = 1$, $df_d = 18$, $P = 0.15$) periods in the intervention village.

Table 5.2. Species diversity and abundance of adult female *Anopheles* mosquitoes in the intervention and control villages during pre-intervention and intervention.

***Anopheles* species**	***Abulo***						***Magge***					
	CDC light traps				**PSC**		**CDC light traps**				**PSC**	
	Indoors		**Outdoors**				**Indoors**		**Outdoors**			
	PI	**INT**	**PI**	**INT**	**PI**	**INT**	**PI**	**INT**	**PI**	**INT**	**PI**	**INT**
An. arabiensis	788	429	108	112	761	307	1286	1424	145	351	388	882
An. pharoensis	381	105	135	77	3	1	225	66	73	102	0	0
An. ziemanni	120	128	86	143	0	0	94	95	113	56	1	0
An. demeilloni	19	13	28	4	19	2	21	5	13	1	13	1
An. pretoriensis	20	0	39	0	0	0	3	0	0	0	0	1
An. garnhami	1	0	0	0	0	0	0	0	0	0	0	0
An. tenebrosus	2	0	3	0	0	0	0	0	0	0	0	0
An. squamous	1	0	0	0	0	0	0	0	0	0	0	0
An. cinereus	0	0	1	0	0	0	0	0	0	0	0	0
An. natalensis	0	0	3	0	0	0	0	0	0	0	0	0

PI: Pre-intervention period INT: Intervention period PSC: Pyrethrum spray catch

5.4.3. Intervention impact on seasonal entomological factors

During the pre-intervention long rainy season, the mean number of indoor host-seeking *An. arabiensis* in *Abulo* (3.18 mosquitoes/trap/night) was not significantly different from that in *Magge* (5.63 mosquitoes/trap/night) (GLMM: F = 1.1479, $df_n = 1$, $df_d = 18$, P = 0.30). Similarly, during the short rainy season, the average indoor host-seeking density was not significantly different between *Magge* (3.67 mosquitoes/trap/night) and *Abulo* (1.38 mosquitoes/trap/night) (GLMM: F = 2.2036, $df_n = 1$, $df_d = 18$, P = 0.16). However, during the long rainy season of the intervention period, the mean indoor host-seeking density of *An. arabiensis* was significantly higher in the non-intervention community of *Magge* (6.94 mosquitoes/trap/night), compared with *Abulo,* where the intervention had been operating (2 mosquitoes/trap/night) (GLMM: F = 9.6853, $df_n = 1$, $df_d = 18$, P = 0.006). Similarly, during the short rainy season of the intervention, the indoor host-seeking density in *Magge* (3.19 mosquitoes/trap/night) was significantly higher than *Abulo* (1.07 mosquitoes/trap/night) (GLMM: F = 5.5697, $df_n = 1$, $df_d = 18$, P = 0.03). Thus, in both the long and short rainy seasons of the intervention, ca. 3 times more indoor host-seeking density of *An. arabiensis* was captured in *Magge* than *Abulo* (Figure 5.4). The relative reduction of indoor host-seeking density of *An. arabiensis*, taking into account both time (pre- and post-intervention) and location (village), was 48.9 % and 10 % during the long and short rainy seasons, respectively (Table 5.3).

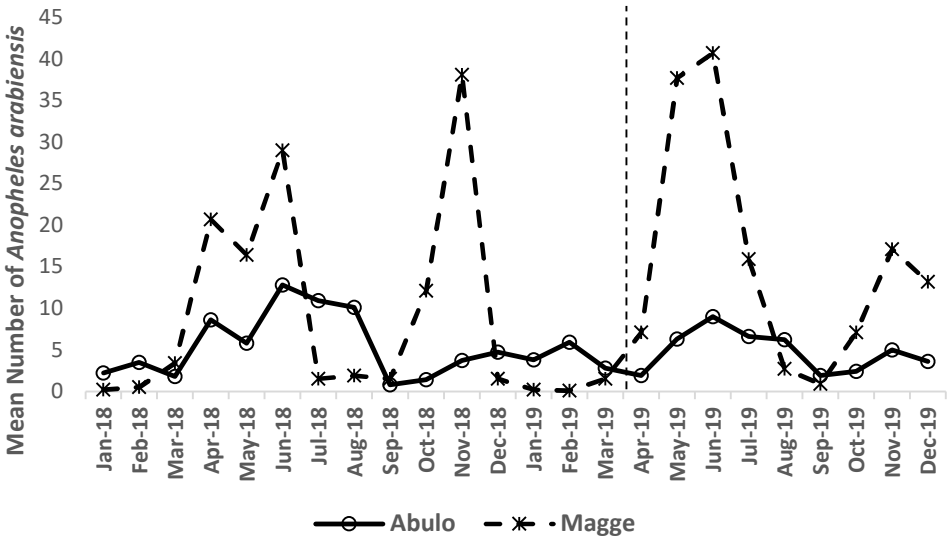


Figure 5.4. The mean number of indoor host-seeking *Anopheles arabiensis* collected by CDC light traps during the pre-intervention and following the implementation of mass trapping. The dashed-line indicates the time when the odour-baited traps were introduced in the intervention village.

Table 5.3. Efficacy of the mass trapping of *Anopheles* mosquitoes on the indoor and outdoor density of *Anopheles* mosquitoes during the major malaria transmission seasons.

Season	**collection method**	***Anopheles* spp**	**Pre-intervention density**		**Intervention density**		**% Reduc.**
			Int. village	Con. village	Int. village	Con. village	
Long rains	CDC Indoors	*An. arabiensis*	3.18	5.63	2.00	6.94	49.0
		An. pharoensis	1.39	0.52	0.38	0.15	5.23
		An. ziemanni	0.24	0.33	0.31	0.05	-752.5
	CDC Outdoors	*An. arabiensis*	1.8	2.65	1.56	5.02	54.2
		An. pharoensis	1.58	0.28	0.6	0.96	88.9
		An. ziemanni	0.73	1.18	0.52	0.12	-600.5
	PSC	*An. arabiensis*	7.75	6.18	4.08	14.78	78.0
Short rains	CDC Indoors	*An. arabiensis*	1.38	3.67	1.08	3.45	16.7
		An. pharoensis	1	1.02	0.4	0.38	-7.4
		An. ziemanni	0.53	0.37	0.68	0.84	43.5
	CDC Outdoors	*An. arabiensis*	0.34	0.64	0.85	2.5	36
		An. pharoensis	0.9	1.16	1.18	1.35	-12.7
		An. ziemanni	1	1.2	2.93	1.25	-181.3
	PSC	*An. arabiensis*	3.02	1.18	2.58	3.58	71.8

Int. village: Intervention village; Con. village: Control village; % Reduc: % Reduction

The pre-intervention outdoor *An. arabiensis* density was not significantly different between the two villages during the long (GLMM: F = 1.3103, df_n = 1, df_d = 18, P = 0.27) and short (GLMM: F = 2.1160, df_n = 1, df_d = 18, P = 0.16) rainy seasons (Figure 5.5). However, the density significantly increased in Magge during both the long (GLMM: F = 9.3903, df_n = 1, df_d = 18, P = 0.006) and short (GLMM: F = 5.5974, df_n = 1, df_d = 18, P = 0.03) rains of the intervention. Thus, the relative reduction of the outdoor *An. arabiensis* population density in the intervention village was 54.2 % and 36 % during the long and short rainy seasons, respectively, relative to the control village (see Table 5.3 above).

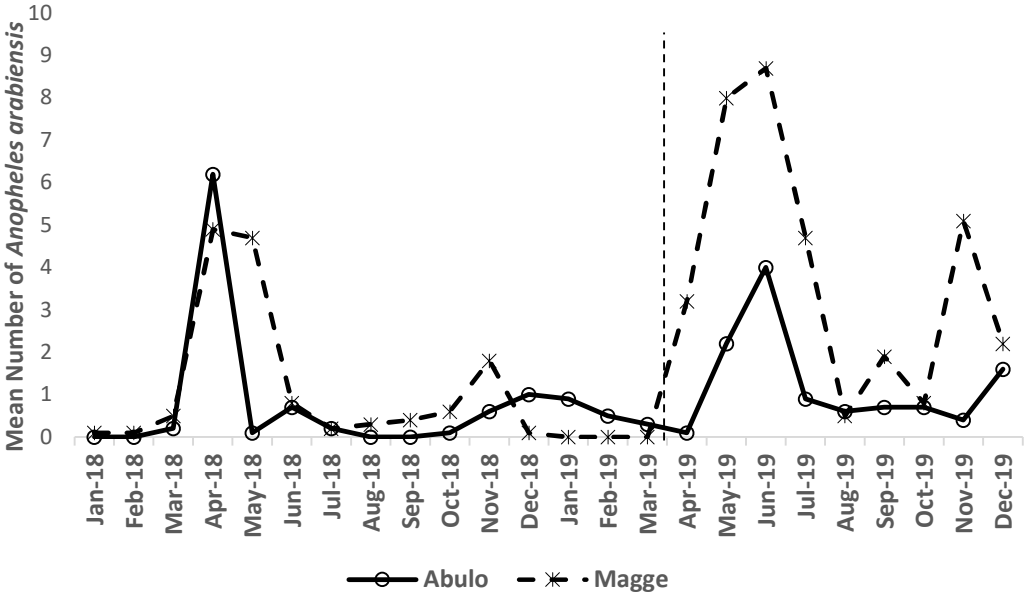


Figure 5.5. The mean number of *Anopheles arabiensis* collected by CDC light traps outdoors during the pre-intervention and following the implementation of mass trapping. The dashed-line indicates the time when the odour-baited traps were introduced in the intervention village.

During pre-intervention, the average IRD of *An. arabiensis*, as assessed by PSC, was not significantly different between the control and the intervention villages during both the long (GLMM: F = 0.1283, df_n = 1, df_d = 18, P = 0.72) and short (GLMM: F = 2.4094, df_n = 1, df_d = 18, P = 0.14) rainy seasons. However, the mean IRD of *An. arabiensis* significantly increased during the long rainy season of the intervention year in the control village as compared to the intervention village (GLMM: F = 4.6397, df_n = 1, df_d = 18, P = 0.04; Figure 5.6). As a result, the relative percent of reduction in IRD in the intervention village, compared to the control village, was 78 % in the long rainy seasons (Table 5.3). In contrast, there was no significant difference between the IRD of *An. arabiensis* in the control and intervention villages during short rainy season of the intervention period (GLMM: F = 0.104, df_n = 1, df_d = 18, P = 0.75). However, the relative percent of reduction in IRD in the intervention village, compared to the control village, was 72 % in the short rainy seasons (see Table 5.3 above).

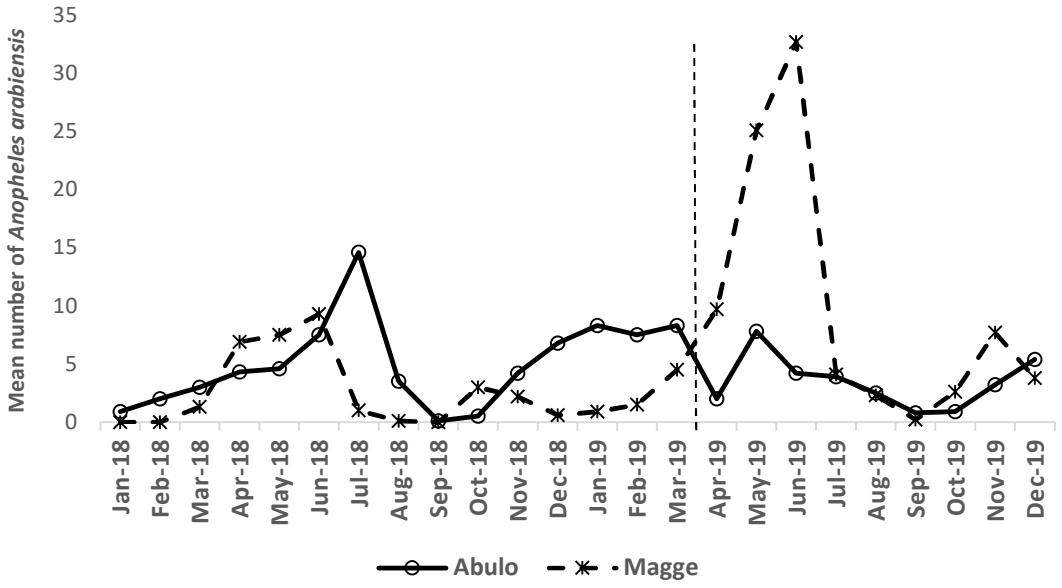


Figure 5.6. The mean number of indoor resting *Anopheles arabiensis* collected by PSC during the pre-intervention and following the implementation of mass trapping. The dashed-line indicates the time when the odour-baited traps were introduced in the intervention village.

Pre-intervention, during the long rainy season, the density of indoor host-seeking *An. pharoensis* was not significantly higher in *Abulo* (1.39 mosquitoes/trap/night) than *Magge* (0.52 mosquitoes/trap/night) (GLMM: F = 1.5067, df_n = 1, df_d = 18, P = 0.24). Moreover, the variation during the short rainy season was not statistically significant (GLMM: F = 0.0007, df_n = 1, df_d = 18, P = 0.97) as 1 and 1.02 mosquitoes/trap/night were captured in *Abulo* and *Magge*, respectively (Figure 5.7). Furthermore, a comparison of the indoor host-seeking density of *An. pharoensis* between *Abulo* and *Magge* was not statistically different, neither during the long (GLMM: F = 2.1382, df_n = 1, df_d = 18, P = 0.16) nor the short (GLMM: F = 0.1550, df_n = 1, df_d = 18, P = 0.7) rainy seasons of the intervention period (Figure 5.7; Table 5.3).

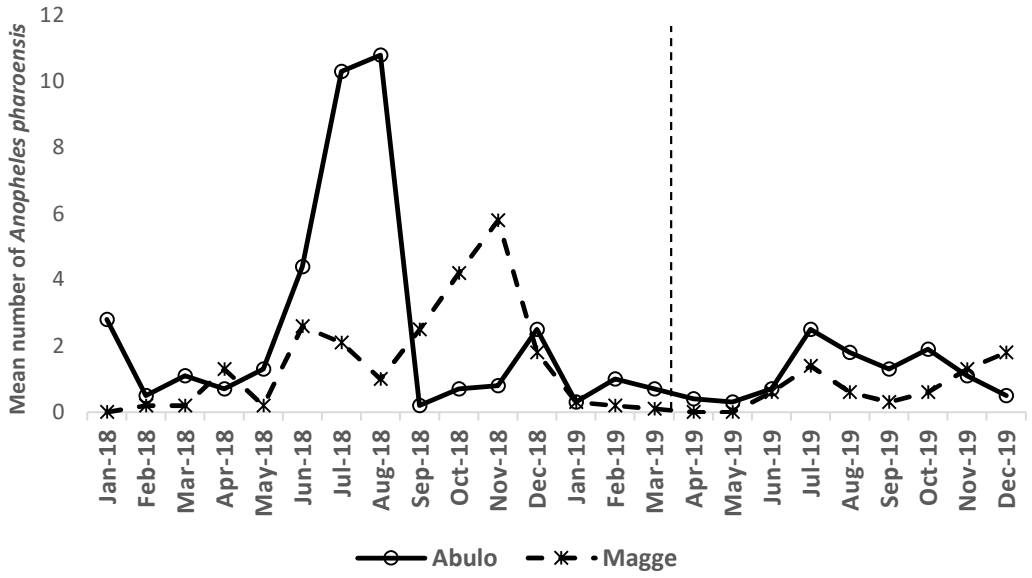


Figure 5.7. The mean number of indoor host-seeking *Anopheles pharoensis* collected by CDC light traps during the pre-intervention and following the implementation of mass trapping. The dashed-line indicates the time when the odour-baited traps were introduced in the intervention village.

Comparison of the outdoor population revealed that the density of *An. pharoensis* during the pre-intervention period was significantly higher in *Abulo* than *Magge* (GLMM: F = 9.6190, $df_n = 1$, $df_d = 18$, P = 0.006) during the long rainy season, while the variation during the short rainy season was not statistically significant (GLMM: F = 0.2604, $df_n = 1$, $df_d = 18$, P = 0.62). However, during the intervention period, the indoor host-seeking density of *An. pharoensis* did not significantly differ between *Abulo* and *Magge* during both the long (GLMM: F = 1.0658, $df_n = 1$, $df_d = 18$, P = 0.32) and short (GLMM: F = 0.0892, $df_n = 1$, $df_d = 18$, P = 0.77) rainy seasons (Figure 5.8; Table 5.3).

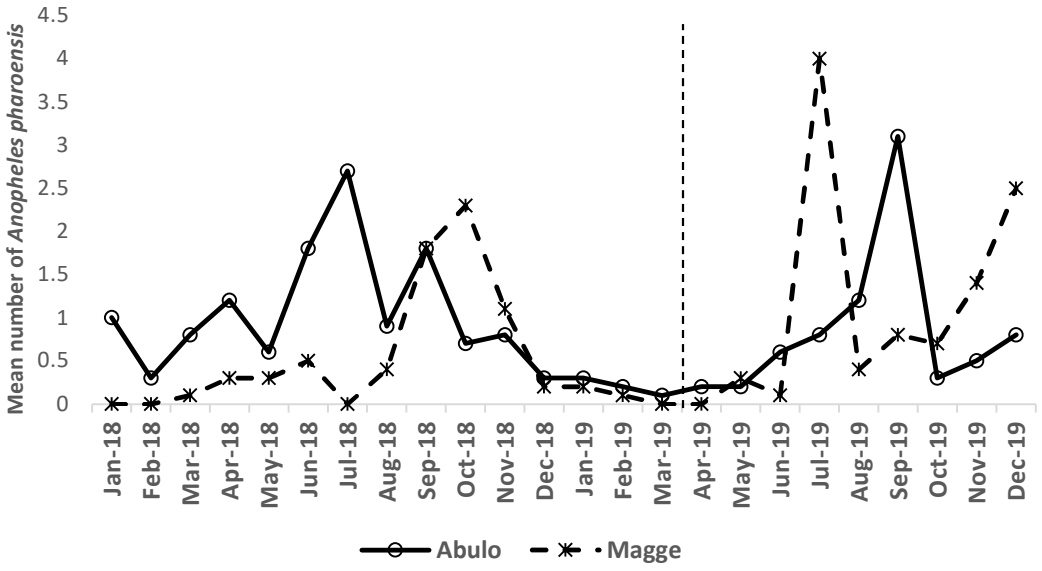


Figure 5.8. The mean number of *Anopheles pharoensis* collected by CDC light traps outdoors during the pre-intervention and following the implementation of mass trapping. The dashed-line indicates the time when the odour-baited traps were introduced in the intervention village.

The seasonal abundance of indoor host-seeking *An. ziemanni* did not differ significantly between the two villages during neither the long ($F = 0.0158$, $df_n = 1$, $df_d = 18$, $P = 0.9$) nor the short ($F = 0.6350$, $df_n = 1$, $df_d = 18$, $P = 0.44$) rains in the pre-intervention period. However, the mean number of *An. ziemanni* caught in the intervention village was significantly higher than the control during the long rains ($F = 6.0811$, $df_n = 1$, $df_d = 18$, $P = 0.02$) of the intervention period. In contrast, the mean number of *An. ziemanni* was not statistically different between the two villages during the short rain season of the intervention period ($F = 0.0340$, $df_n = 1$, $df_d = 18$, $P = 0.86$, Figure 5.9; Table 5.3).

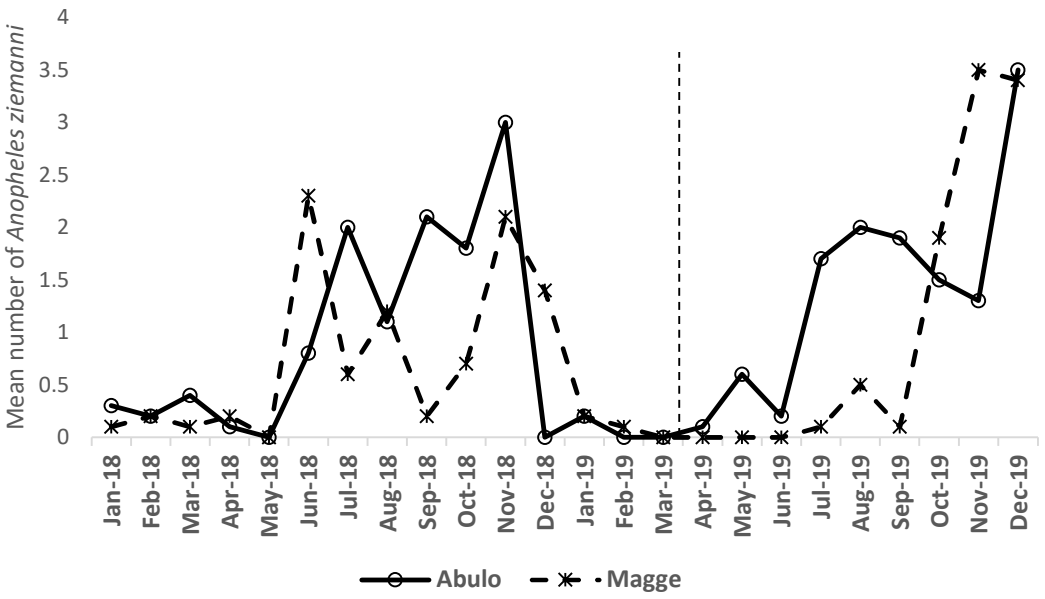


Figure 5.9. The mean number of indoor host-seeking *Anopheles ziemanni* collected by CDC light traps during the pre-intervention and following the implementation of mass trapping. The dashed-line indicates the time when the odour-baited traps were introduced in the intervention village.

Moreover, the average number of *An. ziemanni* caught outdoors was not statistically different during the long ($F = 0.9448$, $df_n = 1$, $df_d = 18$, $P = 0.34$) and short ($F = 0.3358$, $df_n = 1$, $df_d = 18$, $P = 0.57$) rains of the pre-intervention period. While the mean number of *An. ziemanni* significantly increased in *Abulo* during the long rainy season of the intervention period ($F = 11.6883$, $df_n = 1$, $df_d = 18$, $P = 0.003$, Figure 5.10; Table 5.3), these results are inconclusive due to the generally low number of mosquitoes (≤ 1 mosquito/trap/night) caught (see Table 5.3 above).

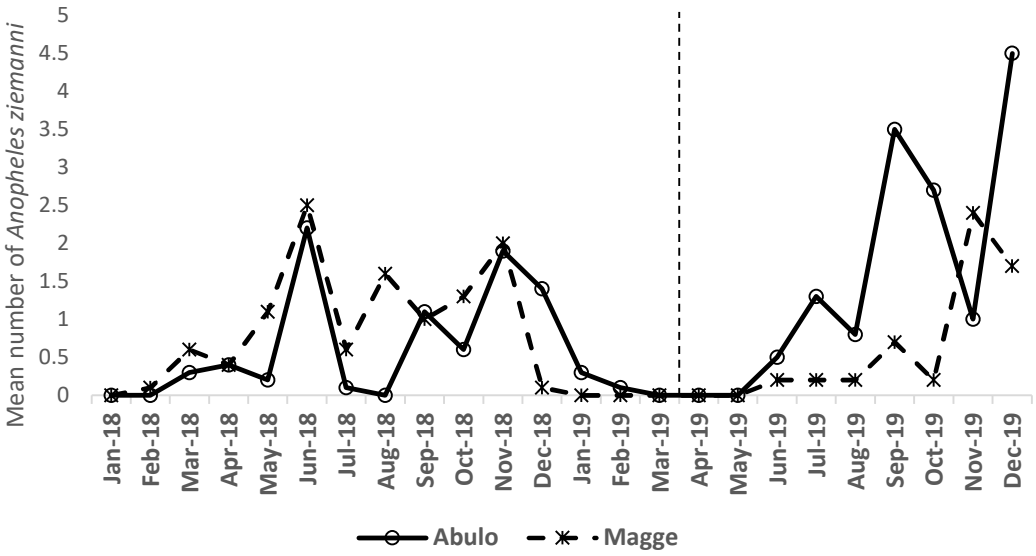


Figure 5.10. The mean number of *Anopheles ziemanni* collected by CDC light traps outdoors during the pre-intervention and following the implementation of mass trapping. The dashed-line indicates the time when the odour-baited traps were introduced in the intervention village.

5.4.4. Intervention impact on human-biting-, sporozoite- and entomological inoculation rates

The mean daily HBR for *An. arabiensis*, *An. pharoensis* and *An. ziemanni* were determined from the CDC light trap indoor collections for the entire study period and compared between seasons pre- and post-onset of intervention (Figure 5.11). The daily HBR of *An. arabiensis* significantly reduced following the introduction of the odour-baited traps in the intervention village both during the long (GLM: $\chi^2 = 17.75$, IRR = 0.29, $P < 0.0001$) and short (GLM: $\chi^2 = 6.18$, IRR = 0.34, $P = 0.01$) rainy seasons (Table 5.4).

To determine the SRs and the resulting EIR, a total of 5153 *Anopheles* mosquitoes collected from the CDC light trap indoor collections throughout the study period were assessed for

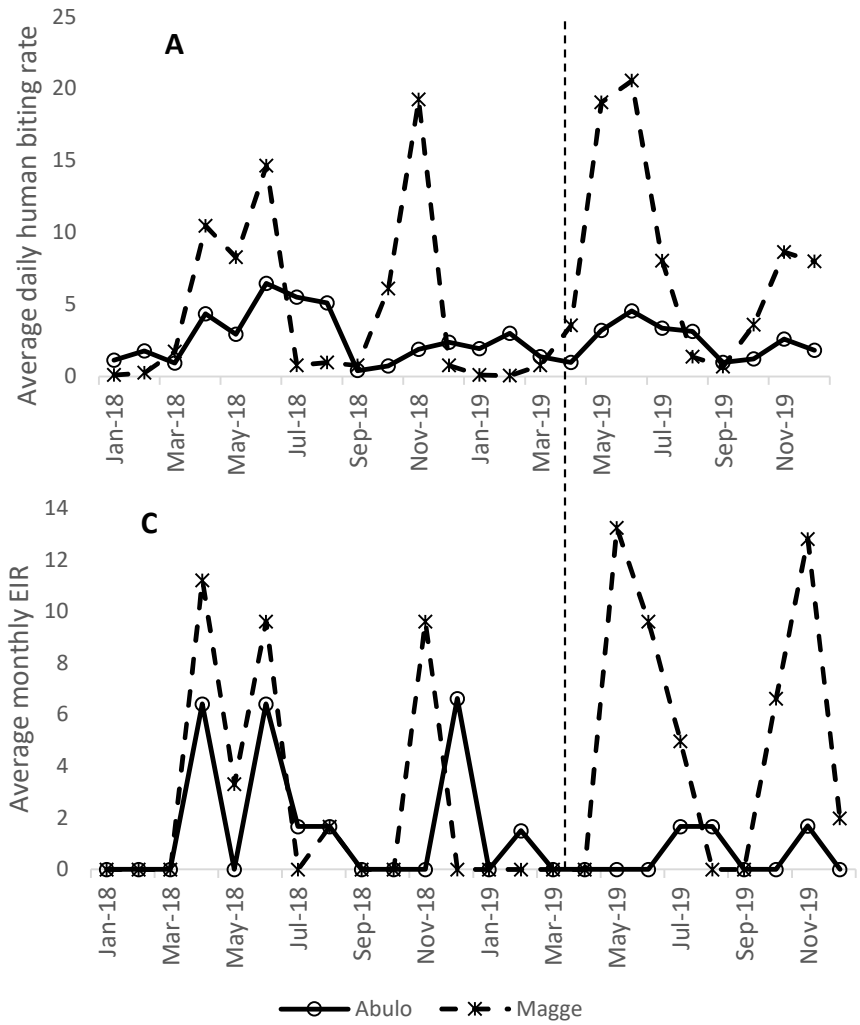
Plasmodium sporozoites, resulting in 151 positive samples. While the results from the regression analysis revealed that the SR and daily EIR of *An. arabiensis* in the intervention village had reduced after the introduction of the odour-baited traps, this reduction was not statistically significant (Table 5.4; Figure 5.11).

Table 5.4. Incidence rate ratios of the daily human-biting rate, sporozoite rate and daily entomological inoculation rate of *Anopheles arabiensis* during the long and short rainy seasons, pre- and post-onset of intervention. The control village was used as the reference category.

Study period	Season	Entomological indices	Village	Mean (95 %CI)	IRR	P-value
Pre-intervention	Long rains	HBR	Control	8.53 (-0.72-17.0)		
			Intervention	4.82 (2.38-7.4)	0.57	0.04
		SR	Control	0.022 (-0.006-0.04)		
			Intervention	0.024 (-0.01-0.06)	1.1	0.96
		EIR	Control	0.2 (-0.08-0.48)		
			Intervention	0.12 (-0.06-0.30)	0.6	0.76
	Short rains	HBR	Control	5.56 (-4.34-15.48)		
			Intervention	2.10 (-0.23-4.41)	0.38	0.07
		SR	Control	0.013 (-0.014-0.04)		
			Intervention	0.024 (-0.02-0.07)	1.85	0.94
		EIR	Control	0.072 (-0.06-0.17)		
			Intervention	0.05 (-0.09-0.25)	0.70	0.89
Intervention	Long rains	HBR	Control	10.52 (-0.46-21.48)		
			Intervention	3.03 (1.42-4.63)	0.29	< 0.0001
		SR	Control	0.016 (-0.002-0.02)		
			Intervention	0.006 (-0.004-0.02)	0.57	0.93
		EIR	Control	0.17 (-0.05-0.42)		
			Intervention	0.02 (-0.01-0.06)	0.12	0.51
	Short rains	HBR	Control	5.23 (-0.78-11.23)		
			Intervention	1.64 (0.36-0.50)	0.31	0.01
		SR	Control	0.034 (-0.02-0.07)		
			Intervention	0.008 (-0.01-0.02)	0.2	0.82
		EIR	Control	0.18 (-0.13-0.48)		
			Intervention	0.013 (-0.03-0.06)	0.07	0.56

HBR: daily human-biting rate; SR: sporozoite rate; EIR: entomological inoculation rate

However, the relative reduction in the seasonal EIR in the intervention village with respect to the control was 78 % and 88 % during the long and short rainy seasons, respectively (Table 5.5). Thus, a person in the intervention village was predicted to receive 3.26 and 1.7 infectious bites per season during the long and short rainy seasons, respectively, whereas in the control villages, the seasonal EIR were 26 and 21 infectious bites in the long and short rainy season, respectively (Table 5.5). The seasonal EIR of *An. pharoensis* was minimal in both villages due to the lower number of mosquito- catches and lower sporozoite infectivity rate (Appendix 5.2). Moreover, the daily HBR, SR and the resulting daily EIR of *An. ziemanni* were not statistically different between the control and intervention villages in both the long and short rainy seasons of the intervention (Appendix 5.3). However, the seasonal EIR of *An. ziemanni* increased in the intervention village during the long and short rains in contrast to the control (Table 5.5). The incidence rate ratio of the daily HBR, SR and daily EIR of *An. ziemanni* is indicated in Appendix 5.4.



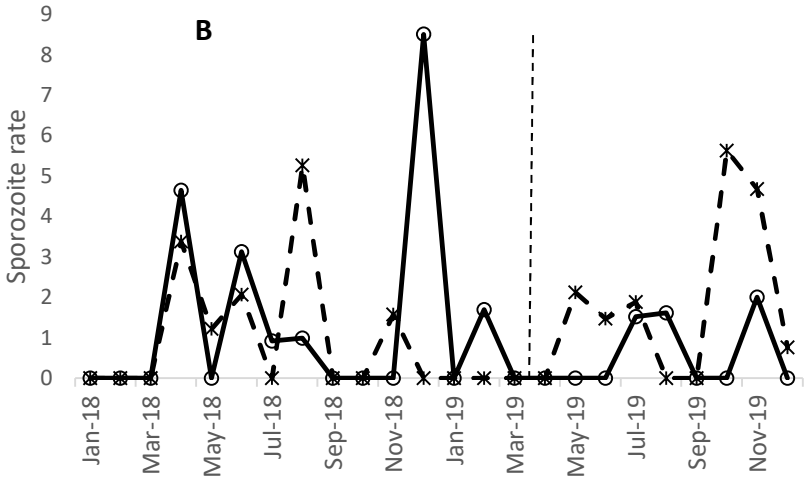


Figure 5.11. The daily human-biting **(A)**, sporozoite **(B)** and monthly entomological inoculation rates **(C)** of *Anopheles arabiensis*. The dashed line represents the time when the intervention commenced.

Table 5.5. Efficacy of mass trapping of *Anopheles* mosquitoes on entomological indices during the major malaria transmission seasons.

Season	*Anopheles* spp	Entomological indices	Pre-intervention		Intervention		% Reduction
			Int. village	Con. village	Int. village	Con. village	
Long rains	*An. arabiensis*	HBR	4.82	8.53	3.03	10.52	49.0
		SR	0.024	0.022	0.006	0.016	59.90
		daily EIR	0.12	0.20	0.022	0.17	78.7
		Seasonal EIR	14.11	22.89	3.26	26.01	79.7
	An. pharoensis	HBR	2.11	0.79	0.58	0.23	5.58
		SR	0.006	0.016	0.035	0	-483.33
		EIR	0.013	0.013	0.021	0	-61.54
		Seasonal EIR	1.57	1.57	3.21	0	-102.59
	An. ziemanni	HBR	0.36	0.5	0.47	0.08	-716.00
		SR	0.17	0.15	0.20	0.14	-26.70
		daily EIR	0.065	0.064	0.10	0.01	-884.62
		Seasonal EIR	7.93	7.81	15.3	1.53	-884.87
Short rains	*An. arabiensis*	HBR	2.10	5.56	1.64	5.23	16.9
		SR	0.024	0.013	0.008	0.034	87.25
		daily EIR	0.053	0.072	0.014	0.17	88.31
		Seasonal EIR	8.11	11.02	1.71	21.23	89.1
	An. pharoensis	HBR	1.52	1.55	0.61	0.58	-7.25
		SR	0.02	0	0.042	0.024	-110.00
		daily EIR	0.032	0	0.027	0.013	15.63
		Seasonal EIR	4.89	0	3.29	1.59	32.72
	An. ziemanni	HBR	0.80	0.56	1.03	1.27	43.2
		SR	0.095	0.30	0.16	0.075	-582.67
		daily EIR	0.081	0.18	0.16	0.089	-339.38
		Seasonal EIR	12.39	27.69	21.35	10.86	-339.30

Int. village: Intervention village; Con. Village: Control village; HBR: daily human-biting rate; SR: sporozoite rate; EIR: entomological inoculation rate

5.5. Discussion

The use of outdoor mosquito vector control tools along with the established indoor interventions has the capacity to enhance malaria control. In the present study, we have demonstrated the complementarity of an attractant-driven malaria vector control system with the IVM interventions, LLINs and IRS, in reducing malaria transmission, as revealed by a controlled before-and-after study conducted in two rural villages of southern Ethiopia. The mass trapping of malaria mosquitoes using odour-baited traps significantly suppressed the density of the primary malaria vector, *An. arabiensis*, in the intervention village, which resulted in a relative reduction of malaria incidence, when compared with the control village. Mass trapping using the odour-baited traps is, therefore, a viable additional tool in the arsenal for fighting malaria.

There is currently a lack of tools targeting malaria vectors outdoors, which can be used either alone or in combination with other IVM approaches. Controlling malaria mosquitoes using an attractant-driven system that targets a wide range of physiological states, however, has the potential to minimize the likelihood of malaria occurrence, as demonstrated in this study. The mass trapping of *Anopheles* mosquitoes with an odour-baited attractant resulted in the suppression of the primary malaria vector density thereby reducing the malaria incidence. While previous mass trapping of malaria vectors has been proven effective in reducing the prevalence of malaria on Rusinga Island, Kenya, following the significant suppression of the *An. funestus* population (Homan *et al.,* 2016), this study demonstrated a higher efficacy in terms of reduced SR and the resulting EIR. This increase in efficacy is likely related to the fact that the active lure in this study attracts both host-seeking and

mosquitoes that have taken a blood meal. Similar effects have been demonstrated after the implementation of conventional vector control tools such as IRS (Steinhardt *et al.,* 2013; Tukei *et al.,* 2017; Abong'o *et al.,* 2020), and larval source management using microbial larvicides (Majambere *et al.,* 2007; Fillinger *et al.,* 2009; Geissbuhler *et al.,* 2009; Dambach *et al.,* 2019).

The use of LLINs in mosquito control has brought a significant decline of malaria morbidity and mortality, predominantly by reducing the population density as well as being a physical barrier between the human host and the mosquito vector (Kabbale *et al.,* 2014; Zhou *et al.,* 2016; Pryce *et al.,* 2018). Mass trapping of malaria mosquitoes likely operates in a similar manner as LLINs, since both suppress the vector population (Mutuku *et al.,* 2011; Homan *et al.,* 2016; Staedke *et al.,* 2020). While mass trapping of malaria mosquitoes is a recent concept (Homan *et al.,* 2016), it is a common practice employed during the long-term management of pest populations damaging crops (Steiner, 1952; Silverstein *et al.,* 1968; Madsen and Carty, 1979; Zhang *et al.,* 2002) and in the eradication of invasive species disrupting local ecosystems (Knipling, 1983; Koyama *et al.,* 1984; Douce *et al.,* 1994; Soroker *et al.,* 2005). Mass trapping of malaria vectors reduces the population density and the HBR, the direct measure of the frequency of human-vector interaction, and the resulting likelihood of getting malaria infectious bites (Homan *et al.,* 2016). The demonstrated substantial decline in these important entomological indices correlate with lower malaria incidence in different malaria-endemic areas of the world (Degefa *et al.,* 2015; Killeen *et al.,* 2019; Musiime *et al.,* 2019).

The findings observed in this study confirm the impact of mass trapping of malaria mosquitoes, particularly *An. arabiensis*, in reducing the likelihood of malaria occurrence despite the rise of malaria morbidity in the district during the intervention period. The overall malaria prevalence in the district over the two years before the onset of the intervention was 17 % in 2017 and 18 % in 2018, as confirmed by microscopy and RDT examinations as reported by the district health office (Annual report of district health office). However, during the intervention year, malaria prevalence in the district increased to 35 %. This was also demonstrated in both the control and the intervention villages from the seasonal malaria prevalence surveys of this study, where the overall annual malaria prevalence in the control village was four times higher during the intervention period than the pre-intervention. Similarly, the intervention village had approximately 1.8 times more malaria cases during the intervention period than the pre-intervention. Despite the increase in the intervention village, the mass trapping of malaria mosquitoes has resulted in a significant reduction of malaria prevalence when compared to the control village. This finding is in agreement with Homan *et al.* (2016) who demonstrated that the combined use of mass trapping and LLINs resulted in a significant relative reduction of the density of *An. funestus* and the associated malaria prevalence in intervention areas as opposed to the non-intervened clusters.

In conclusion, this study demonstrated that mass trapping of malaria mosquitoes is a viable option to augment current IVM methods in efforts to prevent and control malaria. Mass trapping was effective in reducing the density of the main malaria vector, *An. arabiensis*, and associated entomological indices, including HBR, SR and EIR, resulting in a reduced likelihood of an individual obtaining infectious bites. The mass trapping with the odour-

blend is environmentally safe and easy to use and does not require the active participation of professionals, as household members can operate the traps by themselves. However, to further elucidate the efficacy of this control tool for use in wide-ranging implementation strategies, large scale mass trapping trials in combination with conventional IVM methods are required. Additional components should also be tested to determine the flexibility of this tool, e.g. in a push-pull system by adding a push component, spatial repellent.

Chapter 6

General Discussion, conclusions and Recommendations

6.1. Discussion

The search for novel vector control tools and new antimalarial drugs is required for malaria elimination, as the existing methods are not sufficient. The sustainability of LLINs and IRS is uncertain due to the development of insecticide resistance of mosquito vectors, making these vector control methods less effective (Ranson and Lissenden, 2016). In addition, higher outdoor mosquito feeding activity is being observed (Russell *et al.,* 2011; Moiroux *et al.,* 2012), resulting in increased malaria transmission outdoors (Sherrard-Smith *et al.,* 2019). As a result, a search for novel vector control tools targeting the outdoor population is urgent. In this study, the complementarity of odour-baited traps with conventional vector control methods (LLINs and IRS) was assessed in reducing malaria transmission intensity.

The need to control the outdoor malaria vector population is crucial to alleviate malaria mortality and morbidity. As a result, in-depth understandings of the spatial and behavioural ecology of the outdoor vector population in the heterogeneous landscape is essential to optimize the control effort. This study identified the landscape elements that partly regulate the active search of resting sites by exophilic *Anopheles* mosquitoes. Exophilic *Anopheles* mosquitoes preferred resting sites in banana dominated landcover class. Shade was indicated as a strong environmental driver in outdoor resting site selection by the *Anopheles* population as observed in previous studies (Forattini *et al.,* 1993; Paaijmans and Thomas, 2011). Shaded areas tend to provide suitable micro-climatic conditions, such as optimum

temperature and humidity, for resting mosquitoes (Paaijmans and Thomas, 2011), particularly for those which are engorged and aerodynamically challenged.

Furthermore, this study explored the factors regulating localized aggregation of resource-seeking malaria vectors in a heterogeneous landscape at the village level. Ecological theories underlying resource seeking in phytophagous insects were adopted to explain the spatial heterogeneity of malaria vector densities and the resulting malaria incidence. Higher densities of malaria mosquitoes were observed in houses located at the periphery of the villages and with a higher number of occupants (Zhou *et al.,* 2012; Kaindoa *et al.,* 2016, supporting the ecological theories, the resource concentration hypothesis (Root, 1973) and the edge effect (Cromartie, 1975), thus indicating that malaria mosquitoes tend to seek blood meal in houses with higher occupancy located at the village edges. Residents at the village edges are exposed to a higher number of infectious mosquito bites per year and higher malaria incidence than the houses at the center (Kabaghe *et al.,* 2018).

In this study mass trapping of malaria mosquitoes using odour-baited traps deployed outdoors, in conjunction with the current control strategies (LLINs and IRS), reduced the risk of contracting malaria, demonstrating its potential as an additional tool in the vector control arsenal. The effectiveness of mass trapping of mosquitoes in reducing malaria vector densities and malaria prevalence was demonstrated in a previous study by Homan *et al.* (2016). The odour-baited traps significantly suppressed the *An. arabiensis* population of different physiological states, resulting in the reduction of the malaria prevalence in the intervention village by 61 %, 44 %, and 49 % in the long rains, short rains, and dry seasons, respectively, compared to the control village. This study further demonstrated in the

reduction of entomological indices, including HBR, SR and EIR, in the intervention village, compared to the control village.

6.2. Conclusions

- The findings of this study revealed that landscape elements play important role in resting site selection by exophilic *Anopheles* mosquitoes.
- Landcover type, shade, and distance of resting sites from the nearest dwelling are important environmental factors associated with the resting sites of exophilic *Anopheles* mosquitoes.
- The observed fine-scale spatial heterogeneity in mosquito vector densities and the resulting malaria incidence is correlated with some environmental factors such as household size, location, housing conditions, etc.
- Hotspots of higher malaria vector densities and *Plasmodium*-infected individuals were identified at the edges of the villages.
- This study demonstrated the complementarity of outdoor odour-baited traps with indoor vector control methods (LLINs and IRS) in reducing malaria vector density and malaria prevalence.
- The density of *An. arabiensis* and malaria prevalence were significantly reduced in the village where the odour-baited traps were used along with LLINs and IRS as opposed to the control village where LLINs and IRS were the vector control methods implemented.

- Odour-baited traps are viable options of vector control particularly targeting the outdoor mosquito population enhancing the sustainability of the indoor vector control tools.

6.3. Recommendations

- In-depth investigations on the ecology of outdoor mosquito vector populations need to be conducted prior to the implementation of outdoor interventions, such as the use of odour-based technologies.
- Increased vector control efforts should be implemented in predicted malaria hotspots at local scales while maintaining IVM interventions throughout a targeted area to reduce malaria incidence in endemic and residual malaria settings.
- Large scale randomized control trials (RCT), of mass trapping of mosquitoes using the odour-blend alone or in combination with other established vector control methods, or through a push-pull system by adding a push component need to be conducted in different eco-epidemiological settings.
- Studies on the social acceptability of the odour-baited mass trapping technology need to be conducted.

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Appendices

Appendix 4.1: Statistical summary of the effect of housing conditions and local environmental variables on the density of indoor mosquitoes in Abulo and Magge.

Variable	Wald Chi-Square	Prob > Chi-Square
House location in the village (center/edge)	2.20	0.14
Net use (proper use/no use)	0.53	0.46
Wall condition (good/poor)	0.41	0.52
Livestock ownership (yes/no)	0.37	0.54
Household size (No of occupants)	0.12	0.73
Cooking inside houses (yes/no)	0.11	0.74
Breeding site within 50 m radius (present/absent)	0.073	0.79
Door (curtain/wood/metal)	1.020	0.79
Village	0.026	0.87
Door condition (good/poor)	0.0091	0.92
Eaves (present/absent)	0.00032	0.99
Roof condition (good/poor)	0.00022	0.99

Appendix 4.2: Statistical summary of the effect of housing conditions and local environmental variables on the density of indoor *Anopheles* mosquitoes in Abulo.

Variable	Wald χ^2	Prob > χ^2
Net use (proper use/no use)	12.02	0.0005
Net use * House location in the village	11.07	0.0009
Household size (No of occupants)	4.37	0.037
Door material (curtain/wood/metal)	3.36	0.067
House location in the village (center/edge)	2.51	0.11
Wall condition (good/poor)	1.32	0.25
Livestock ownership (yes/no)	1.07	0.30
Household size * House location in the village	1.01	0.31
Door condition (good/poor)	0.91	0.34

Appendix 4.3: Statistical summary of the effect of housing conditions and local environmental variables on the density of indoor *Anopheles* mosquitoes in Magge.

Variable	Wald χ^2	Prob > χ^2
Wall condition (good/poor)	20.13	<0.0001
Net use * House location in the village	17.61	<0.0001
Roof condition (good/poor)	12.06	0.0005
Household size (No of occupants)	7.41	0.0065
Net use (proper use/no use)	6.69	0.0097
Door condition (good/poor)	5.01	0.025
Breeding site within 50 m radius (present/absent)	2.69	0.10
No of residents * House location in the village	1.19	0.28
House location in the village (center/edge)	0.32	0.57
Cooking inside houses (yes/no)	0.20	0.65

Appendix 4.4: Statistical summary of the effect of housing conditions and local environmental variables on the number of malaria cases in Abulo.

Variable	F Ratio	Prob > F
House shape (square/circle)	0.038	0.96
Wall material (wood/mud/painted)	0.18	0.84
Wall condition (good/poor)	0.17	0.68
Door material (curtain/wood/metal)	2.67	0.074
Door condition (good/poor)	0.46	0.71
Number of windows	0.027	0.87
Roof material (grass/metal)	0.013	0.91
Roof condition (good/poor)	0.0076	0.93
Presence or absence of eaves	0.32	0.57
Livestock ownership (yes/no)	0.023	0.88
Cooking inside or outside of houses	0.39	0.53
Breeding site within 50 m radius (present/absent)	2.65	0.11
Household size (No of occupants)	6.70	0.011
House location in the village (center/edge)	1.07	0.30
Net use (proper use/no use)	0.15	0.70
Net use * House location in the village	0.13	0.72

Appendix 4.5: Statistical summary of the effect of housing conditions and local environmental variables on the number of malaria cases in Magge.

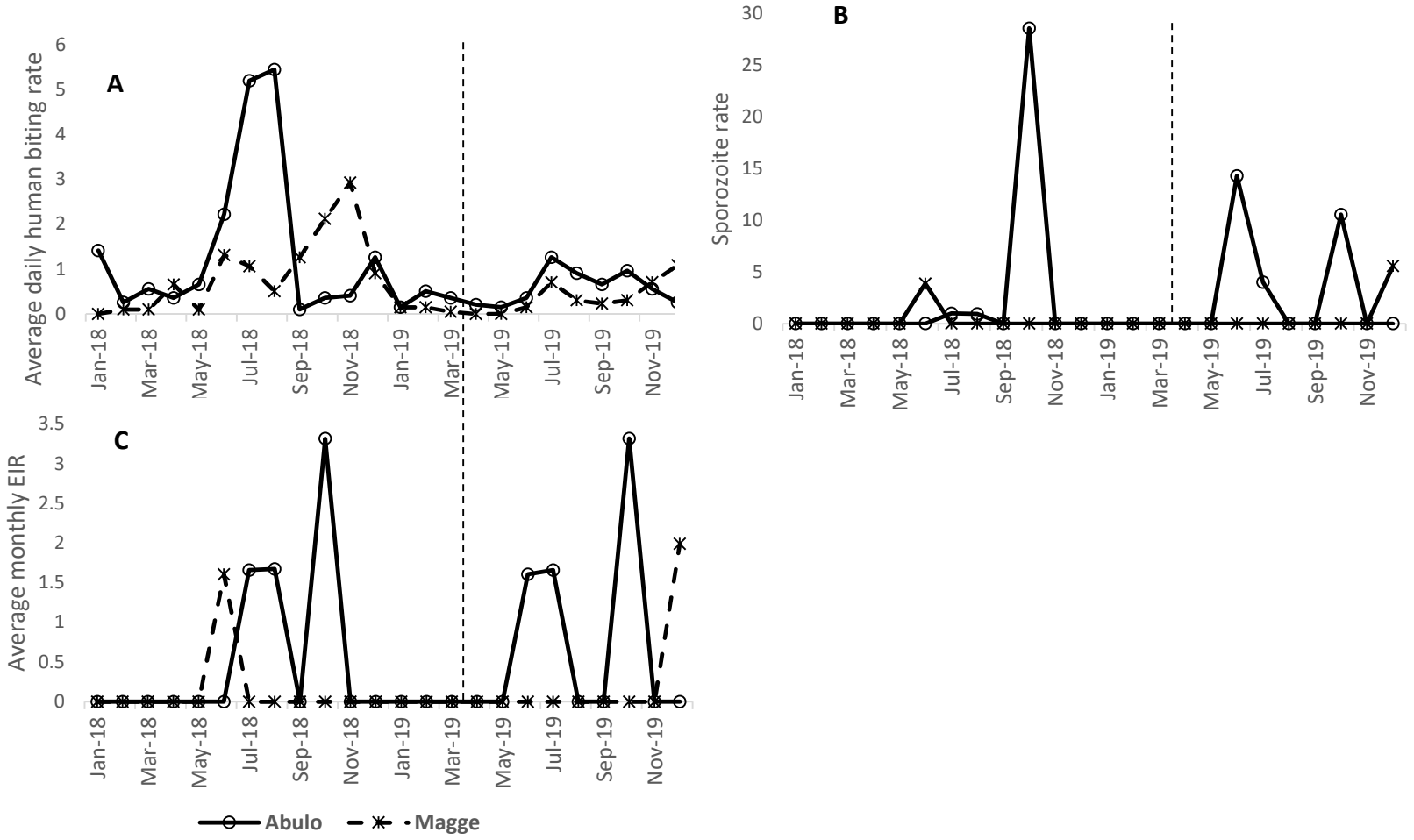
Variable	F Ratio	Prob > F
House shape (square/circle)	0.33	0.72
Wall material (wood/mud/painted)	0.58	0.45
Wall condition (good/poor)	2.53	0.11
Door material (curtain/wood/metal)	0.35	0.79
Door condition (good/poor)	2.85	0.062
Number of windows	0.39	0.53
Window coverage (none/metal/wood)	1.01	0.39
Roof condition (good/poor)	2.88	0.092
Presence or absence of eaves	0.50	0.48
Livestock ownership (yes/no)	3.12	0.080
Cooking inside or outside of houses	1.34	0.25
Breeding site within 50 m radius (present/absent)	0.39	0.54
Household size (No of occupants)	1.40	0.24
House location in the village (center/edge)	5.75	0.018
Household size * House location in the village	1.61	0.21
Net use (proper use/no use)	1.22	0.27
Net use * House location in the village	0.048	0.83

Appendix 5.1. Diversity and abundance of mosquito species with different physiological states collected in the odour-baited traps.

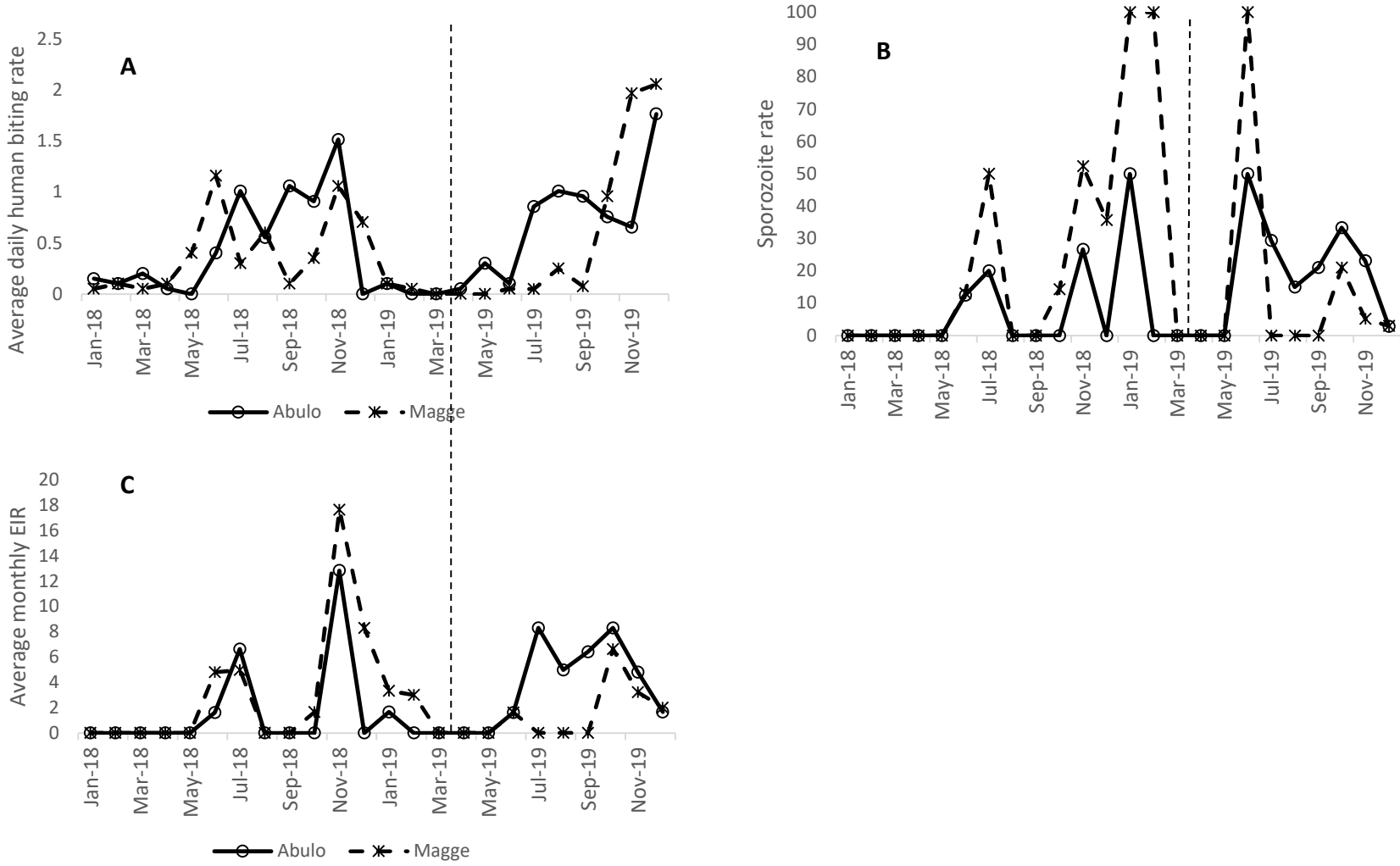
Mosquito spp.	Physiological state				Total
	HS	BF	SG	GR	
An. arabiensis	2972	314	3	8	3297
An. pharoensis	330	7	0	1	338
An. ziemanni	64	6	0	0	70
An. demeilloni	1	2	0	0	3
Culex spp.	2826	221	0	0	3047

HS: host-seeking BF: Blood-fed SG: semi-gravid GR: gravid

Appendix 5.2. The daily human-biting **(A)**, sporozoite **(B)** and monthly entomological inoculation rates **(C)** of *Anopheles pharoensis*. The dashed line represents the time when the intervention commenced.



Appendix 5.3. The daily human-biting **(A)**, sporozoite **(B)** and monthly entomological inoculation rates **(C)** of *Anopheles ziemanni*. The dashed line represents the time when the intervention commenced.



Appendix 5.4. Incidence rate ratios of the daily human-biting rate, sporozoite rate and daily entomological inoculation rate of *Anopheles ziemanni* during the long and short rainy seasons, pre- and post-onset of intervention. The control village was used as the reference category.

Study period	Season	Entomological indices	Village	Mean (95 %CI)	IRR	P-value
Pre-intervention	Long rains	HBR	Control	0.5 (-0.25-1.23)		
			Intervention	0.36 (-0.37-1.11)	0.72	0.79
		SR	Control	0.15 (-0.22-0.53)		
			Intervention	0.17 (-0.08-0.24)	1.13	0.77
		EIR	Control	0.075 (-0.07-0.23)		
			Intervention	0.06 (-0.09-0.23)	0.8	0.95
	Short rains	HBR	Control	0.56 (0.11-1.02)		
			Intervention	0.80 (0.1-1.51)	1.43	0.65
		SR	Control	0.3 (-0.08-0.49)		
			Intervention	0.09 (-0.09-0.2)	0.3	0.55
		EIR	Control	0.17 (-0.13-0.5)		
			Intervention	0.07 (-0.15-0.32)	0.41	0.7
Intervention	Long rains	HBR	Control	0.08 (-0.06-0.02)		
			Intervention	0.47 (-0.08-1.01)	5.87	0.31
		SR	Control	0.14 (-0.36-0.76)		
			Intervention	0.2 (-0.07-0.45)	1.6	0.96
		EIR	Control	0.01 (-0.02-0.04)		
			Intervention	0.09 (-0.05-0.24)	9.0	0.63
	Short rains	HBR	Control	1.27 (-0.23-2.76)		
			Intervention	1.03 (0.23-1.84)	0.81	0.84
		SR	Control	0.075 (-0.36-0.76)		
			Intervention	0.16 (-0.001-0.4)	2.13	0.82
		EIR	Control	0.095 (-0.05-0.24)		
			Intervention	0.16 (0.03-0.32)	1.68	0.77

HBR: daily human-biting rate; SR: sporozoite rate; EIR: entomological inoculation rate

Declaration

I, the undersigned, declare that this doctoral thesis is my original work and has not been presented in any other University, College or Institution, seeking for a similar degree or other purposes. All source of materials used for the thesis have been duly acknowledged.

Name: Yared Debebe Desta

Signature: _____

Date: _____

Place: _____

This doctoral dissertation has been submitted for examination with my approval as a university advisor.

Dr. Habte Tekie,

Signature: _____

Date: _____