Entomological impact of combined and separate use of indoor residual spraying and long-lasting insecticidal nets for malaria prevention in Adami Tullu district, South-Central Ethiopia

BY

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A Thesis submitted to the School of Graduate Studies of the Addis Ababa University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Biology (Insect Sciences)

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Declaration

I, Oljira Kenea Negasa Gulti, declare that this thesis is my original work, has not been presented for a degree in any other University, and that all sources of material used for the thesis have been duly acknowledged.

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ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

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Dedications

To my beloved family, particularly in loving memory of my father Kenea (Goolee) Negasa, my mother Desatu (Kabane) Fayisa, and my grandmother Birki (Yayi, Harare) Sendo.

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Acronyms

ACT	Artemisinine-based Combination Therapy
BBI	Bovine blood index
CB-LTC	Carbon dioxide baited light trap catch
CDC	Center for Disease Control and Prevention
CRT	Cluster randomized trial
SCP	Circumsporozoite protein
DDT	Dichloro-diphenyl-Trichloroethane
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
GLM	Generalized linear model
GME	Global Malaria Eradication
HBI	Human blood index
HBR	Human biting rate
HLC	Human landing catch
HSD	Host seeking density
IRD	Indoor resting density

IRR	Incidence Rate Ratio
IRS	Indoor residual spraying
ITN	Insecticide treated net
IVM	Integrated vector management
LLINs	Long-lasting insecticidal nets
LSM	Larval source management
LTC	Light trap catch
MOH	Ministry of Health
ORD	Outdoor resting density
PCR	Polymerase chain reaction
PIT	Outdoor artificial pit shelter
PSC	Pyrethrum spray catch
RDT	Rapid diagnostic test
RSE	Relative sampling efficiency
SSA	Sub-Saharan Africa
WHO	World Health Organizatio

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Abstract

Indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) are the key frontline malaria prevention interventions in Ethiopia. Both target *Anopheles arabiensis*, the sole primary malaria vector. Universal coverage of both interventions has been promoted and there is a growing demand in combination of interventions for malaria control and elimination. However, available evidence is contradictory wether the combined intervention is better than either IRS or LLINs alone. To investigate whether IRS and LLINs combination provides added protective impact on *An. arabiensis* compared to either IRS or LLINs alone, a cluster randomized controlled trial was carried out in Adami Tullu district, south-central Ethiopia. Villages were randomly allocated to four study arms: IRS+LLINs, IRS, LLINs, and control. All households in the IRS+LLINs and LLINs arms were provided with LLINs (PermaNet 2.0) free of charge. Households in the IRS+LLINs and IRS arms were applied with propoxur before the main malaria transmission season in 2014 and 2015. Adult mosquitoes were collected in randomly selected villages in each arm using CDC light trap catch (LTC) set close to a sleeping person, pyrethrum spray catch (PSC), and artificial pit shelter (PIT), for measuring host-seeking density (HSD), indoor resting density (IRD), and outdoor resting density (ORD) of the anophelines. Human landing catch (HLC) was performed in selected villages to monitor the impact of the interventions on local mosquito biting behaviours (biting location, time and host preference).

Collected anophelines were identified to species by use of standard morphological keys and additional use of molecular methods to separate sibling species of the *An. gambiae* complex. Enzyme-linked immunosorbent assay (ELISA) was used to detect malaria infections in mosquitoes and the sources of mosquito blood meals. Mean densities were compared using incidence rate ratio (IRR) calculated by negative binomial regression. Parity rate (percentage of

parous females) was also determined by ovarial dissection. Human blood index (HBI) was expressed as the proportion of mosquitoes with human blood divided by the total number of blood-fed mosquitoes tested.

A total of 1786 female anophelines of four species (*An. arabiensis*, *An. pharoensis*, *An. ziemanni* and *An. funestus* s.l.) were collected over two transmission seasons during the intervention period (2014-2015). *Anopheles* numbers were highest in the control arm (41.3% of total) followed by LLINs (25.4%), IRS (18.0%), and IRS+LLINs (15.8%). In most of the vector parameters estimated, the impact of IRS and LLINs combined and separate interventions were significantly higher in communities that recieved the interventions (in experimental groups) compared with untreated communities (control group). The mean HSD of *An. arabiensis* in the IRS+LLINs arm was similar to the IRS arm (0.03 vs. 0.03/ house/LTC/night) but lower than the LLINs arm (0.03 vs. 0.10/house/LTC/night, p=0.07) and so was the difference in IRD and ORD between the IRS+LLINs compared to the IRS arm. However, both IRD and ORD of *An. arabiensis* were higher in LLINs compared to IRS+LLINs ($p < 0.001$ for indoors). Parity rate of *An. arabiensis* were similar among the intervention arms. None of the 1786 samples of four species tested by ELISA was positive for *P. falciparum* and *P. vivax* CSP infection in all of the study arms. *Anopheles arabiensis* preferred mainly bovine and human hosts for blood meal sources with high HBI in the LLIN alone. Indoor resting habit of *An. arabiensis* was less impacted by LLINs alone intervention compared to IRS + LLINs or IRS alone.

In conclusion, the IRS+LLINs and the IRS alone each was similarly most effective against *An. arabiensis* as compared to the LLINs alone. The IRS+LLINs provided added impact on *An. arabiensis* compared to LLINs alone. The LLINs alone had poor impact on densities and human biting rates of *An. arabiensis* in this study setting.

Chapter 1. General Introduction

1.1. Global malaria transmission-An overview

Malaria remains one of the most severe public health problems worldwide. The estimated number of malaria cases globally in 2015 was 214 million (range: 149–303 million). In the same year, the disease killed about 438 000 people (range: 236 000–635 000). Most deaths in 2015 were in the WHO African Region (90%), followed by the WHO South-East Asia Region (7%) and the WHO Eastern Mediterranean Region (2%). In the sub-Saharan Africa (SSA), malaria was the fourth highest cause of death, accounting for 10% of child deaths in 2015 (WHO, 2015).

The bulk of the global malaria burden occurring in SSA is believed due in part to the presence of more efficient malaria vectors (Takken and Knols, 1999), predominant malaria parasites and local weather condition that enhance transmission and scarcity of resources and socio-economic instability which hinder effective malaria control efforts (Toure *et al*., 2004; Sogoba, 2007). The disease epidemics affect non-immune populations in many highland and semi-arid areas of the continent (Abeku, 2007).

Besides health, malaria also adversely impact wealth and economic development and contributes towards regional and national poverty. The ill-health and deaths due to malaria impedes socioeconomic development in multiple ways, including negative effects on fertility, population growth, saving and investment, worker productivity, causing school absenteeism, premature mortality and imposing medical cost on people (Malaney and Sachs, 2002).

However, recent reports indicated that the global burden of malaria is declining, mainly due to improved global malaria control strategies and scale-up and intensive use of malaria intervention

measures specifically insecticide treated nets and insecticide residual sprays (Kweka *et. al.*, 2013). Between 2000 and 2015, a substantial expansion of these malaria interventions contributed to a 60% decline in malaria mortality rates globally. In the WHO African Region, the malaria mortality rate in children under 5 years of age was reduced from 694 000 in 2000 (range: 569 000–901 000) to 292 000 in 2015 (range: 212 000–384 000) (WHO, 2015). Malaria control through long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) has resulted in dramatic decline in vector population (Bayoh *et al*. 2010, Mutuku *et al*. 2011) and in the number of clinical malaria cases (Bhattarai *et al*. 2007, Lee *et al*. 2010). These results have revived a growing global demand for sustainable scale-up of key malaria interventions for the disease elimination followed by gradual eradication (Kleinschmidt *et al*., 2009).

The main factors involved in malaria transmission are the parasite, the vector and the human host which interact with one another and also with their wider chemical, biological and physical environments (Silver, 2008). As per the most recent historical review of malaria (Coxa, 2010), in the process of malaria transmission, infection begins when (1) sporozoites, the infective stages, are injected by a mosquito and are carried around the body until they invade liver hepatocytes where (2) they undergo a phase of asexual multiplication (exoerythrocytic schizogony) resulting in the production of many uninucleate merozoites. These merozoites flood out into the blood and invade red blood cells where (3) they initiate a second phase of asexual multiplication (erythrocytic schizogony) resulting in the production of about 8-16 merozoites which invade new red blood cells. This process is repeated almost indefinitely and is responsible for the disease, malaria. As the infection progresses, some young merozoites develop into male and female gametocytes that circulate in the peripheral blood until they are (4) taken up by a female anopheline mosquito when it feeds. Within the mosquito (5) the gametocytes mature into male

and female gametes, fertilization occurs and a motile zygote (ookinete) is formed within the lumen of the mosquito gut, the beginning of a process known as sporogony. The ookinete penetrates the gut wall and becomes a conspicuous oocyst within which another phase of multiplication occurs resulting in the formation of sporozoites that migrate to the salivary glands of a mosquito and are injected when the mosquito feeds on a new host (Figure 1.1) (Cox, 2010).

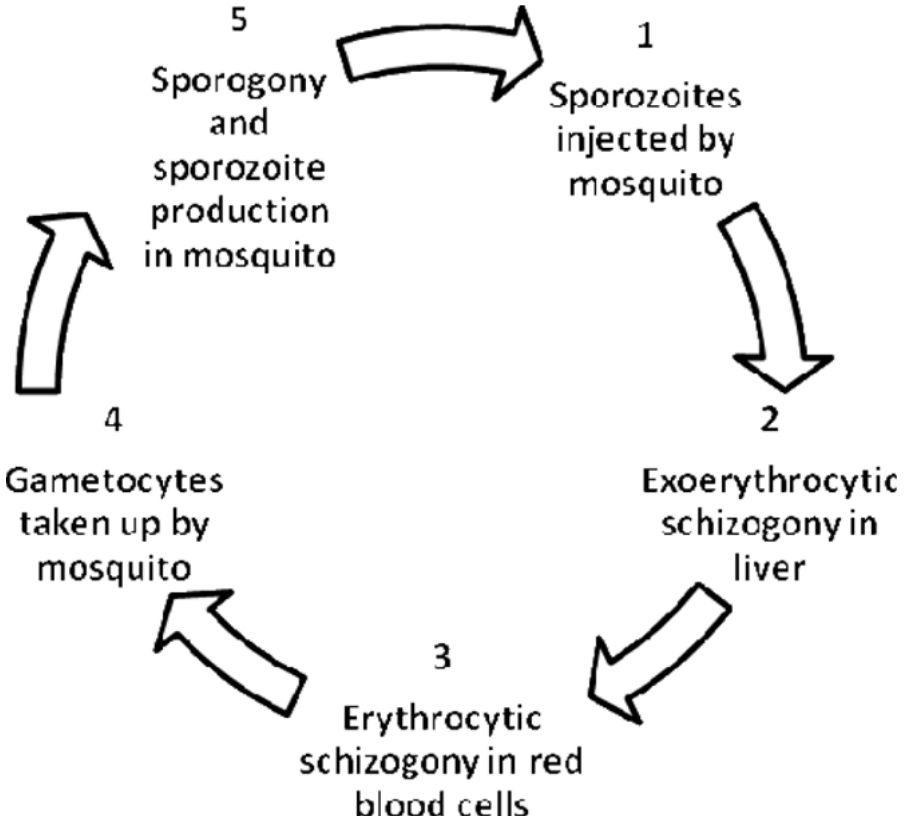

Figure 1.1: Schematic life cycle of malaria parasites, *Plasmodium* species (Source: Cox, 2010).

1.1.1. Malaria parasites

Human malaria is caused by protozoan parasites of the genus *Plasmodium*. It is caused by five species of *Plasmodium* that affect humans; *P. falciparum, P. vivax, P. ovale, P. malariae* and *P.*

knowlesi (Figtree *et al*., 2010). Of these, *P. falciparum* is responsible for severe malaria morbidity and mortality. This species is predominant in tropical Africa, eastern Asia, and the Amazon area (Arrow *et al*., 2004). *Plasmodium vivax* is less severe but more widespread, and the other three species are found much less frequently (WHO, 2011). Simian and human malaria parasites, including *P. knowlesi* and *P. malariae*, are often indistinguishable morphologically. The fifth species, (*P. knowlesi*), essentially a primate malaria, is now recognized as a cause of potentially fatal human malaria in forest areas of South East Asia (Figtree *et al*., 2010).

1.1.2. Malaria vectors

Human malaria is transmitted by female mosquitoes of the genus *Anopheles* from human to human. Approximately 490 species of *Anopheles* are known, of which 60 are indicated as vectors of malaria (Service, 2000). Recently, Sinka and colleagues (2010) documented 41 dominant vector species of human malaria worldwide. There are seven dominant *Anopheles* vectors of human malaria in Africa namely *An. gambiae s.s, An. arabiensis, An. funestus, An. merus, An. melas, An. moucheti* and *An. nili.* (Sinka *et al*., 2010). Of these, the first three are the principal malaria vectors. All but *An. funestus, An. moucheti* and *An. nili* are members of the *An. gambiae* complex (Coetzee *et al*., 2000). Some species are only locally important vectors, e.g. *An. melas* in western Africa, *An. merus* in eastern Africa, and *An. bwambae* in Uganda.

1.2. The malaria situation in Ethiopia

Malaria is endemic in Ethiopia with unstable and seasonal transmission. However, the disease is perennial in some lowland villages of the country particularly in those located in the vicinity of perennial rivers and other major drainage areas. More than three-quarter of the landmass (altitude < 2000 m) of the country is malarious, and about 68% of the total population is residing in areas

at risk of malaria infections (Negash *et al*., 2005; Ghebreyesus *et al*., 2006). Malaria epidemics have been recorded up to 2400-2500 meters above sea level (m.a.s.l.) (Senay and Verdin, 2005). The most devastating and well-documented malaria epidemic in Ethiopia was in 1958 when there were an estimated 3 million cases with 150000 reported deaths (Fontaine *et al*., 1961). Since then epidemics have occurred at intervals of five to eight years. The most recent epidemic occurred in 2003–2004 (Ghebreyesus *et al*., 2006) with focal epidemics as the commonest form. High malaria transmission intensity occurs as *An. arabiensis* increase during the wet season in the country. For this reason, malaria transmission peaks from September to December coinciding with the end of the major rainy season (Senay and Verdin, 2005). The seasonality and transmission intensity of malaria is also locally determined by water resource development activities such as irrigation and hydropower dams (Yohannes *et al*., 2005; Kibret *et al*. 2010; Yewhalaw *et al*., 2010). Irrigation and hydropower dam development not only enhance focal malaria transmission but also prolong the transmission season (Lautze *et al*., 2007; Jaleta *et al*., 2013).

Plasmodium falciparum and *P. vivax* are epidemiologically the most important human malaria parasites in Ethiopia, which account for around 60% and 40% of the all malaria cases in the country respectively (Ghebreyesus*et al*., 2006). *Anopheles arabiensis* is the primary malaria vector in Ethiopia (Abose *et al*., 1998) while *An. funestus* and *An. pharoensis* are considered secondary vectors (Ghebreyesus *et al*., 2006). *Anopheles nili* is believed to have a restricted importance in southwest Ethiopia (krafsur, 1977) while others such as *An. ziemanni* exhibit anthropohilic/zoophilic tendencies whose roles have not been aptly demonstrated yet.

Recently, there has been a decline in malaria prevalence in the country due to massive scale-up in malaria prevention and treatment, specifically the combination of mass distribution of LLINs to all children <5 years and nationwide distribution of Arthimisinin Combination Therapy (ACT) in

the public sector (Otten *et al*., 2009). For instance, in-patient malaria cases and deaths in children <5 years old in Ethiopia fell by 73% and 62%, respectively after the 2000 nationwide implementation of LLINs and ACT (Otten *et al*., 2009). Although a ten years retrospective analysis showed that the introduction of the current malaria control strategies in the country has led to a decrease in morbidity and mortality by malaria, malaria is still a major health problem (Alemu *et al*., 2012).

1.3. Rationale for the study

Vector control is the key intervention for global malaria control and elimination efforts. It is critical for the reduction and interruption of malaria transmission (WHO, 2015). The current core malaria vector control interventions are IRS and LLINs. IRS kills mosquitoes and reduces longevity when they rest on insecticide-sprayed surfaces inside houses or other structures, before and after feeding on occupants. LLINs reduce malaria parasite transmission mainly by killing or blocking mosquitoes that attempt to feed upon humans under net (WHO, 2014).

Both IRS and LLINs have been widely deployed jointly in the same community, at the same time, against the same vectors in national malaria control programmes. However, empirical evidence on the epidemiological impacts of IRS-LLINs combinations versus either method alone within operational malaria control settings have been limited and warrant basic and operational research (Okumu and Moore, 2011). Evidence from observational and cluster randomized trials on the effectiveness of combining these interventions are conflecting. Moreover, some cluster randomized trials compared epidemiological outcomes in communities receiving IRS plus LLINs versus those receiving LLINs alone but there were no trials comparing IRS plus LLINs versus IRS alone so far. Because evidence is needed to determine the effectiveness of combining IRS and LLINs in any transmission setting, countries that are already using both interventions in

combination should undertake an evaluation of the effectiveness of combining versus either LLINs or IRS alone (WHO, 2014).

In Ethiopia, IRS and LLINs have been used in combination in the same household or separately against *An. arabiensis* the sole primary malaria vector. However, entomological outcomes of such combination interventions are less known. Evaluation of IRS and LLINs combined trials elsewhere on *An. gambiae* s.l. cannot be extrapolated for *An. arabiensis* due to possible variations in mosquito biting behaviours, human activity behaviours and environmental settings. This necessitates evaluation and optimization of the impact of IRS and LLINs on local vector population. This study is therefore expected to provide information about the impact of IRS + LLINs as compared to either IRS or LLINs alone on *An. arabiensis*.

This study was aimed to answer the following research questions: Can the combined use of IRS and LLINs significantly reduce vector density, longevity, and entomological inoculation rate as compared to their separate use? Can IRS and LLINs co-application significantly impact on *An. arabiensis* biting, host preference and resting behaviors versus IRS or LLINs alone? Is CDC light trap catch as efficient as human landing catch for sampling *An. arabiensis*? Thus, the main hypothesis for the study is that the combined use of IRS and LLINs will significantly reduce vector density, longevity, sporozoite rate, human blood index and EIR as compared to either their separate use or the control group.

1.4. Objectives of the study

1.4.1. General objective

The overall objective of this study is to investigate whether the combined use of IRS and LLINs provides additional protection against malaria vectors (*An. arabiensis*) compared to either IRS or LLINs alone and generate evidence for enhanced and sound malaria vector control in Adami Tullu district, south-central Ethiopia.

1.4.2. Specific objectives

1. To provide pre-intervention baseline data about densities, biting activities, resting behaviour and host-preferences of local malaria vector populations in the study area

2. To evaluate relative mosquito sampling efficiency of light traps with and without yeast-generated carbon dioxide bait compared to human landing catches for effective monitoring of the impact of the interventions on local malaria vector mosquitoes

3. To assess the impact of IRS and LLINs combined versus separate interventions on *An. arabiensis* density, longevity and infectivity (entomologic inoculation rates)

4. To evaluate impact of the IRS and LLINs joint versus individual interventions on *An. arabiensis* biting, host-preference and resting behaviours and their significance in malaria vector control

Chapter 2. Literature Review

2.1. Malaria vector control interventions

2.1.1. Indoor residual spraying with insecticides

Indoor residual spraying (IRS) is the application of residual insecticide to potential malaria vector resting surfaces such as internal walls, eaves and ceilings of all houses or structures (including domestic animal shelters) where such vectors might come into contact with the insecticide (WHO, 2015). Control of adult female mosquitoes with IRS has been the most widely employed successful vector control method since the 1940s (Kager, 2002). The rationale for IRS is based on the behavior of those *Anopheles* species that rest on walls before or after biting humans (Kager, 2002). Indoor residual spraying contributed to the eradication of malaria in countries such as Brazil and Egypt in the 1930s and early 1940s and its wide use with dichlorodiphenyl trichloroethane (DDT) inspired the adoption of Global Malaria Eradication Program (GMEP) in 1955 (Kager, 2002). However, the goal of eradication proved elusive in most malaria-endemic countries in the tropics (Muturi *et al*., 2008). The use of IRS for vector control has continued to increase since 2006, particularly in the African Region where 78 million people, or 11% of the population at risk, were protected by IRS in 2010 (WHO, 2011). Worldwide, 116 million people were protected by IRS in 2014 (WHO, 2015). In West Africa, for years in which IRS was implemented, prevalence of malaria reduced by 71% compared to periods preceding the implementation of IRS (Edwin and Ernest, 2016).

In Ethiopia, IRS has been used for more than three decades (Abose *et al*., 1998). DDT has been the primary insecticide of choice for IRS in Ethiopia over many years, followed by a limited application of organophosphate, particularly malathion, as an alternative insecticide in the country until it was replaced by deltamethrin in 2009 (Yewhalaw *et al*., 2011).

IRS is usually carried out during June-July in targeted malarious villages to prevent potential malaria epidemics that can occur immediately after the rains when widespread and numerous mosquito breeding sites appear (MOH, 2003). As DDT has been in use for IRS since 1966 in Ethiopia (MOH, 2012), *An. arabiensis* has become resistant to DDT. The highest levels of DDT resistance in *An. arabiensis* was first recorded from Arba Minch in the South and Gambella in the West (Abose *et al*., 1998). DDT was in use until widespread vector resistance to DDT was first documented in 2007 (MOH, 2012). Resistance to deltamethrin and permethrin in *An. arabiensis* has been reported from different parts of the country (Abose *et al*., 1998; Balkew et al. 2010; Massebo *et al*., 2013). A recent report indicates that *An. arabiensis* showed increased resistance to insecticides belonging to the four major insecticides, namely DDT, malathion, deltamethrin and lambdacyhalothrin (Balkew *et al*., 2012).Therefore, the Ministry of Health has now decided to use carbamates for IRS (Balkew personal comm.).

2.1.2. Insecticide-treated nets/long-lasting insecticidal nets

In malaria control efforts, insecticide-treated nets (ITNs) have received considerable interest over the last two decades (WHO, 2008). Insecticide treated nets reduced malaria incidence by 50% compared to no use of ITN and by39% compared to the use of untreated bed net in areas of stable malaria (Lengeler 2004a). The incidence was reduced by 62% compared to no use of ITNs and by 43% compared to use of treated bed-nets in areas with unstable malaria (Lengeler 2004a). More than 100 trials in different settings worldwide have shown the protective impact of ITNs in reducing childhood and adult morbidity and mortality (Lengeler, 2004b).

In Africa, the need for large scale utilization of ITNs is well accepted and is viewed as the most efficacious and feasible intervention to prevent malaria morbidity and mortality (Steketee and

Campbell, 2010). LLINs, which are designed to protect people for up to 3-5 years of use, are now being prioritized over ordinary ITNs, which have a far shorter duration of insecticidal activity (WHO, 2011). In some areas LLINs are being distributed freely or heavily subsidized for pregnant women and children less than five years old (Roberts, 2007). LLINs are expected to have 3-5 years effective life time (WHO, 2011) but they may degrade before 3 years.

In Ethiopia, the use of ITNs was adopted in 1997-1998 with the support of WHO in selected malarious areas (Jima *et al*., 2005) and the distribution of LLINs was started in 2005. A Malaria Indicator Survey (MIS) done in 2007 showed that 65% of the households in malarious areas owned at least one LLINs, and 37% of them owned two or more LLINs (Jima *et al*., 2010). Likewise the MIS conducted in 2011, revealed that 55 % of surveyed households had at least one LLIN whereas the reported use by children under 5 years of age, during the night prior to the survey, within households with at least one net was 65% in 2011 (MOH, 2011). More than 20 million LLINs were distributed in Ethiopia between 2005 and 2007; a further 15 million were distributed in 2010 and 2011 to replace LLINs distributed previously (MOH, 2012). The type of LLINs distributed were Perma Net 2.0 and universal (100%) LLIN coverage with one LLIN per sleeping space on average was the current policy by the national malaria control program. The National Strategic Plan for malaria prevention and control in Ethiopia aims at scaling up LLINs coverage and utilization either in combination with IRS or separately (MOH, 2014).

2.1.3. Combination of IRS and LLIN: Current evidence

Nearly all malaria endemic countries in SSA have adopted ITNs, IRS or both (Okumu and Moore, 2011). Because of longer years of use (3-5 years), LLINs are currently being prioritized over ITNs (WHO, 2011) and universal coverage with LLIN or IRS has been promoted (WHO, 2012).

Either of IRS and LLINs is designed to be used independently in malaria vector interventions. However, IRS and LLINs have been recently deployed jointly in the same house in routine malaria control programmes.

However, the existing evidence about whether it is more effective to provide both interventions in combination relative to either intervention alone is inconsistent and contradictory. Some observational studies have shown that there was added protection conferred to those who received both interventions relative to those who received only one. For example, results from household surveys in Bioko, Equatorial Guinea, and Zambezia, Mozambique indicated that five out of eight previous studies reported a reduced risk of infection in those protected by both interventions compared with one intervention alone (Kleinschmidt *et al*., 2009). This study also found that in both places, the odds of contacting malaria were significantly lower for children living in houses with both IRS and ITNs, than for children living in houses with only IRS (Kleinschmidt *et al*., 2009). In Kenya, a non-randomized prospective cohort study on the combination of IRS and ITNs in comparison with ITNs alone by Hamel *et al.* (2011) found reduced incidence of *P. falciparum* from IRS and ITNs combination intervention as compared to ITNs alone. More recently Fullman and colleagues (2013) reported that children under the age of five living in households with both ITNs and IRS in medium and high transmission areas in SSA were at a significantly lower risk than those in ITNs houses alone.

On the other hand, some observational studies showed that IRS and LLINs combination interventions had no additional protection effect. For instance, in the highlands of Burundi, using insecticidal mosquito nets did not confer additional protective effect to spraying (Protopopoff *et al*., 2008). Moreover, evidence that IRS and LLINs combinations confer greater protection against malaria than either method alone is inconsistent and affected by the type of insecticide

used (Okumu *et al*., 2013). A recent mathematical evaluation of IRS and ITNs combinations by Okumu and colleagues (2013) showed that, where transmission is mediated primarily by *An. arabiensis*, adding IRS to high LLINs coverage provides only a modest incremental benefit when an organophosphate (pirimiphos methyl) is used; is redundant when a pyrethroid (lambda-cyhalothin) is used, or even regressive when DDT is used for the IRS.

These results may be subject to potential confounding and bias, because none of the observational studies described earlier (Protopopoff *et al*., 2008; Kleinschmidt *et al*., 2009; Hamel *et al*., 2011) randomized communities to receive either both interventions together or either intervention alone. Unlike observational studies, cluster randomized trials provide the best evidence for the effectiveness of such interventions and have been completed in three African countries namely in Benin, The Gambia and Tanzania to investigate whether the combination provides added protection compared to ITNs alone (WHO, 2014).

The Benin trial found no evidence of added protection from the IRS and LLINs combination as compared to LLIN alone (Corbel *et al*., 2012). However, WHO (2014) outlined some limitations of this trial as follows: 1) It had low statistical power with only seven clusters per study arm (compared to 25 and 35 in the Tanzania and Gambian trials, respectively); 2) Its reference arm had ITNs for targeted groups only, instead of universal coverage; and 3) Spraying was conducted at intervals considerably longer than the residual life of the insecticide used (bendiocarb) (WHO, 2014).

The trial in the Gambia compared LLINs in combination with IRS using DDT versus LLINs alone, and showed no evidence that the IRS offered increased protection compared to the use of LLINs alone (WHO, 2014). This was a large, well-conducted trial which found no evidence that

combining LLINs with IRS using DDT produced an advantage over very high usage of LLINs alone, if vectors are susceptible to the LLINs insecticide.

In a trial conducted in Muleba, Tanzania during 2011 and 2012, fifty study clusters were randomly assigned to either IRS plus universal coverage of ITNs or to universal coverage of ITNs alone. Results of this study showed strong evidence that combining IRS and LLINs give additional protection particularly during the peak of the transmission season, and during the residual period of the insecticide (West *et al*., 2014). However, in this study the LLINs usage reported was modest (between 36% and 53%). Whether this additional benefit would have been seen if net use had been at universal coverage level, is unknown. Furthermore, available data suggest some level of resistance in local vector populations to the insecticide used on nets (WHO, 2014). Furthermore, the Tanzanian trial targeted *An. gambiae* s.s. and trials targetting *An. arabiensis* are limited.

Overall, evidence from observational and cluster randomized trials on whether it is more beneficial to provide both interventions in combination relative to either intervention alone remain inconclusive. Since the cost of implementing both interventions would require considerable additional resources, it is important that such an approach is based on good evidence of additional protective efficacy and effectiveness (West *et al*., 2014). Evidence of likely impacts and costs of these interventions are crucial for optimal use of limited resources and to help national malaria control programmes and international funding agencies make smart decisions that are based on evidence.

As elsewhere in other malaria endemic and epidemic countries in Africa, Ethiopia has adopted both IRS and LLINS for malaria prevention. Ethiopia has demonstrated significant success in scaling up the two priority vector control interventions IRS and LLINs (MOH, 2011). The Federal Ministry of Health through donor support has been distributing LLINs to malaria-affected areas

starting end of August 2005. It plans that LLINs should be provided to households in malaria-endemic areas (areas below 2,000 m above sea level) and to cover 100% of households in malarious areas with at least one LLIN per sleeping space and at least 80% of people at risk of malaria use LLINs during 2011-2015 (MOH, 2012). Significant progress has also been made in scaling up IRS in epidemic-prone populations, with 6.5 million households sprayed in 2009 representing 55% of the target population (MOH, 2011).

Despite great interest and growing demand in combination of IRS and LLINs interventions for malaria control and ilimination in Ethiopia, evidence on the impact of the combination of these interventions versus either method alone on malaria transmission is limited. One published observational study reported that use of LLINs and DDT house spraying by the community of Aneno Shisho kebele in Adami Tulu Jido Kombolcha district resulted in a decline in the number of mosquitoes, and a reduction in malaria morbidity (Bekele *et al*., 2012). Notwithstanding these prior results, there is a critical need for cluster randomized trials to assess the impact of combinations of these interventions on malaria epidemiology in Ethiopia.

2.2. The impact of IRS and LLINs on malaria vector parameters

Indoor insecticide-based domiciliary interventions specifically IRS and LLINs are effective in Africa because they intentionally target the behavioural characteristics of the two major mosquito vectors, *Anopheles gambiae* s.s. and *Anopheles funestus* which are highly anthropophagic, endophilic and endophagic (Takken and Knols, 1999; Silver, 2008). LLINs work because these vectors generally feed at night when people are in bed and act as a physical barrier (preventing human mosquito contact and providing personal protection against malaria to the individual(s) using the nets). LLINs add a chemical barrier to the physical one as the insecticides have an excito-repellent

and lethal effect on the mosquitoes (Binka *et al*., 1998; Hawley *et al*., 2003). By reducing the vector population in this way, ITNs, when used by a majority of the target population, provide protection for all people in the community, including those who do not themselves sleep under nets (Hawley *et al*., 2003). Likewise, IRS works because these vectors rest inside houses after blood feeding, for this reason, spraying walls and ceilings of houses with residual insecticides kills and reduces the survival rate of indoor resting *Anopheles* mosquitoes and greatly reduce the chance of malaria transmission (Pates and Curtis, 2005).

Several studies have shown that IRS and LLINs can dramatically reduce malaria vector parameters including vector density, longevity, and infection prevalence of the mosquito species that primarily feed indoors on humans such as *An.gambiae* and *An. funestus* from sub-Saharan Africa (Boyoh *et al*., 2010; Russell *et al*., 2010; Killeen *et al*., 2011). Vector populations are sensitive to insecticidal spray and nets that are applied or used against them. For example, *An.gambiae s.l.* and *An. funestus* population density declined markedly in a randomized evaluation trial of permethrin-treated bed nets in treatment compared to control villages in western Kenya (Gimnig *et al*., 2003).

Similarly, Bayoh and colleagues (2010) reported that *An. gambiae* s.s decreased proportionately relative to *An. arabiensis*, and then declined to rarity coincident with increased bed net ownership as national bed net distribution programmes commenced in Western Nyanza Province of Kenya. The authors also observed that parity and malaria infection rates (EIRs) were lower in both species in communities with high bed net use as compared to communities with low bed use, but host choice did not vary within species in both communities (predominantly cattle for *An. arabiensis*, humans for *An. gambiae s.s.*).

Furthermore, in a rural Tanzania, the combined impact of longer-lasting insecticide treatments as well as high bed net coverage was associated with a 4.6-fold reduction in EIR, on top of the impact from the use of untreated nets alone. The scale-up of bed net use and subsequent insecticidal treatment has reduced the density of the anthropophagic, endophagic primary vector species, *An.gambiae s.s.*, by 79%. In contrast, the reduction in density of the zoophagic, exophagic sibling species *An. arabiensis* was only 38% (Russell *et al*., 2010).The scaling up of vector control by IRS and LLINs is an important driver affecting the dynamics and evolution of mosquito vectors (Kitau *et al*., 2012). Studies indicate that largest impact of IRS and LLINs were against the highly anthropophagic, endophagic primary vector; *An. gambiae* s.s, and *An. funestus*, leading to a shift in the sibling species composition of the *An. gambiae* complex (Russell *et al*., 2010; Kitau *et al*., 2012).

Although, the recent scale up of malaria vector control by both interventions has shown progressive decline in malaria morbidity and mortality in Ethiopia (Alemu *et al*., 2012; Hamusse *et al.*, 2012), entomological impacts of IRS and LLINs combined interventions have not been extensively studied.

2.3. The challenges to the effectiveness of IRS and LLINs

2.3.1. Insecticide resistance

The success of IRS and LLINs interventions depends on the continued effectiveness of the insecticides used (WHO, 2013). Currently, both IRS and LLINs are reliant on a severely restricted number of WHO recommended insecticides. In particular, only a single insecticide class, the pyrethroids are available for LLINs and only four classes of insecticides, including the pyrethroids are available for IRS. Thus, the challenge with IRS and LLINs is the spread of

resistance against pyrethroids and other insecticides in a number of malaria endemic countries (Awolola *et al*., 2002; WHO, 2013). This is of great concern since the pyrethroids among other insecticides, have many advantages; they are safe, highly active and with a long persistence (Kolaczinski and Curtis 2004).

In Ethiopia, insecticide resistance threatens the sustainability of IRS and LLINs based malaria control and elimination efforts. Substantial bodies of literature have shown insecticide resistance development by Ethiopian malaria mosquitoes against the existing insecticides (Abose *et al*., 1998; Yewhalaw *et al*., 2011; Balkew *et al*., 2012). The principal malaria vector in the country *An. arabiensis* showed increased resistance to insecticides belonging to the four major classes namely DDT, malathion, fenitrothion, primiphos-methyl, propoxur, bendiocarb, deltamethrin and lambdacyhalothrin (Balkew *et al*., 2012). However, the vector was susceptible to carbamate and resistant to prythroids in the present study area (Gari *et al*., 2016). Thus alternative insecticides are urgently needed.

2.3.2. Residual malaria transmission

Residual malaria transmission is defined as all forms of malaria transmission that persist after full universal coverage with effective IRS and/or LLINs has been achieved (Killeen, 2014). Because IRS and LLIN are indoor-based interventions and target endophilic and endophagic mosquitoes, residual malaria transmission may still continue being mediated by more exophilic, zoophilic and exophagic vectors that escape from contact with these core interventions. Exposure to mosquito bites outdoors and early at night could be other key challenges that threaten effectiveness of IRS and LLINs based on joint human and vector behaviours.

In malaria endemic countries, a combination of human and vector behaviours are responsible for malaria transmission. For example, people can be exposed to mosquito bites and malaria transmission during fishing activities, when they visit forest areas, when they look after their crops and cattle, when local vector species exhibit flexible behaviours that allow them to avoid IRS and LLINs interventions (Killeen, 2014; WHO, 2014). The effectiveness of IRS and LLINs interventions can be reduced by users exposure to mosquito bites based on human indoor and outdoor activity and vector biting behaviours which need to be investigated locally.

It is therefore hypothesized that even under full implementation of IRS and LLINs, residual malaria transmission will continue to occur, because, there is room for exophilic and exophagic vectors to escape from the interventions and maintain transmission (Durnez and Coosemans, 2013; Killeen, 2014). For these reasons, currently WHO strongly recommends to generate local evidence on the magnitude of the problem of residual transmission of malaria, including information on human and vector behaviour, and intervention effectiveness (WHO, 2014).

In Ethiopia, IRS and LLINs target *An. arabiensis*. However, *An. arabiensis* is more plastic in its behaviour exhibiting opportunistic feeding behavior in indoor-outdoor venues (Animut *et al*., 2013; Jaleta *et al*., 2016) and early night biting (Russel *et al*., 2011; Yohannes and Boelee, 2012). Currently Ethiopia is planning for malaria elimination and eventual eradication. In the drive to eliminate malaria, knowledge about residual malaria transmission and effectiveness of the existing intervention tools against local vectors plays a pivotal role. Monitoring and evaluating the operational impact of IRS and LLINs on *An. arabiensis* and other local malaria vectors need concern and action.

2.4. Mosquito sampling methods for monitoring vector interventions

Current malaria control and elimination efforts in Africa rely heavily on vector control with IRS and LLINs (WHO, 2013). In attempts to control malaria by attacking the vector with these interventions, it is important to measure the impact of such interventions on mosquitoes. This requires an appropriate method of sampling mosquitoes biting humans (Lines *et al*., 1991; Wong *et al*., 2013). The most direct way to do this is by the human landing catch (HLC) because mosquitoes are captured as they land and attempt to feed on collectors (Costantini *et al*., 1998). The HLC is the standard method for measuring exposure of humans to mosquito bites (WHO, 1975) and for estimating the human biting rate (HBR) which is a key determinant of the entomological inoculation rate (EIR), a measure of malaria transmission (Beier *et al*., 1999).

However, the HLC exposes collectors to potentially infectious mosquito bites and is expensive, labour intensive, requires highly trained collectors and difficult to supervise. Besides, results obtained by HLC can be biased due to natural human variations in attractiveness to mosquitoes (Lima *et al*., 2014; Briet *et al*., 2015). These issues limit the application of HLC particularly for monitoring the effectiveness of vector control interventions and necessitate the search for alternative methods.

Several mosquito sampling methods that do not require human exposure have been developed as alternative to HLC for estimating the HBR. For African malaria vectors, the main alternative mosquito sampling methods include the standard Centers for Disease Control light trap catches (LTC) placed beside human-occupied bed nets (Sudia and Chamberlain, 1962; Lines *et al*., 1991; Mbogo *et al*., 1993; Davis *et al*., 1995; Costantini *et al*., 1998), bed net traps (Methenge *et al*., 2002; Methenge *et al*., 2004; Methenge *et al*., 2005), tent traps (Govella *et al*., 2009; Govella *et*

al., 2011; Sikaala *et al*., 2013; Wong *et al*., 2013; Krajacich *et al*., 2014) and odour-baited traps (Dia *et al*., 2005; Owino, 2010).

A recent review by Briet *et al*. (2015) showed that LTC has been widely evaluated against HLC for collecting host-seeking vectors in several areas and is considered a safe and approximately equivalent alternative to HLC for measuring indoor exposure to mosquito bites and malaria transmission by African vectors. Light traps are affordable, easy to use and can be deployed large-scale and provide valuable entomological data of the impact of vector control interventions (Sikaala *et al*., 2013; Fornadel *et al*., 2010).

Another promising alternative to HLC is carbon dioxide-baited traps. Carbon dioxide (CO_2), a major constituent of vertebrate breath, is used to lure the host-seeking mosquitoes in to the vicinity of the trap (Mboera and Takken, 1997). Artificial sources of CO_2, specifically CO_2 from dry ice, industrial CO_2 released from pressurized gas cylinders or from propane are commonly used in mosquito traps (Kline, 2002; Jawara *et al*., 2009). However, adult mosquito surveillance in many rural areas is challenging due to lack of CO_2, either in the form of dry ice or compressed gas. In Japan, Saitoh *et al*. (2004) developed an easy and cheap method to produce CO_2 by using a yeast-sugar solution in plastic bottles and found that traps baited with yeast-generated CO_2 caught higher numbers of mosquitoes than unbaited traps. In Kenya Smallegange *et al*. (2010) found that traps baited with yeast-produced CO_2 caught significantly more *An.gambiae* mosquitoes than unbaited traps as well as traps baited with industrial CO_2. However studies on *An. arabiensis* as a target species is lacking.

Chapter 3. General materials and methods

3.1. Study area

The study was carried out in Adami Tullu part of Adami Tullu-Jiddo Kombolcha district, here after Adami Tullu district located in East Showa Zone, Oromia Regional State, south-central Ethiopia (Figure 3.1).

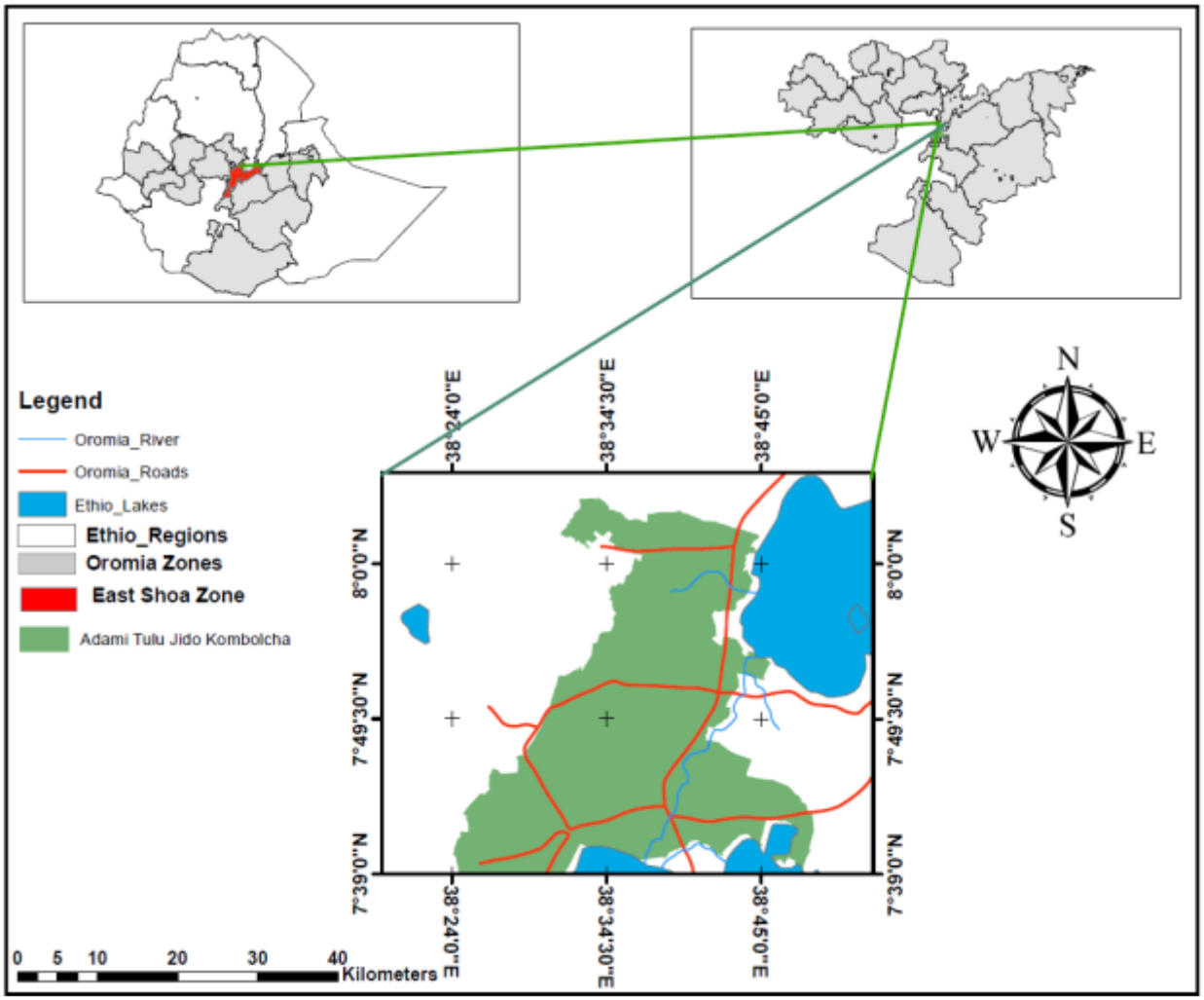

Figure 3.1: Map of Adami Tullu district and its location in Ethiopia

The study was conducted from 2013-2015 during June-November, the major malaria transmission seasons. The capital of the district, Batu (formerly Zeway) is located at 7°56′N 38°42′E. It is about 160 km south of Addis Ababa on the highway connecting Addis Ababa to Nairobi. The elevation of Batu is 1640 meters above sea level. Administratively, the district has 48 *Kebeles* (sub-districts) each with an average population size of about 1000 to 5000 people. Each *Kebele* is further divided into villages (gares) and each gare contains on average 170 people. The main topographic feature of the area is Lake Zeway which covers about 434 sqm areas and has a depth of 4m and a length of 25km (Abose *et al*., 1998). The lake supports agriculture and fishing, the main economic activities in the district. The area grows rain-fed maize and other cereal crops during the rainy season (usually June to October) and mainly vegetables such as onions, tomatoes, potatoes, green peppers, etc. by irrigation during the dry season (November to May) and the wet season aswell. The majority of the population in the district live in houses made of mud or cement walls and thatched or corrugated iron roofs. Local residents primarily depend on farming, livestock rearing, and fishing for subsistence. The total average annual rainfall was about 700 mm in 2013 and 2014, with peaks in July (250 mm) and August (220 mm). The mean minimum and maximum annual temperatures were 14.5^{o}C and 27.7^{o}C, respectively. More detailed description of the study area is given in the study protocol (Deressa *et al*., 2016).

Despite long history of control efforts, malaria remains one of the leading health problems in Adami Tullu district (Abose *et al*., 1998; Deressa *et al*., 2007; Bekele *et al*., 2012). Beside public health problems, malaria poses a significant economic burden on rural households and individuals both through health payment and person-days lost (Deressa *et al*., 2007). Malaria transmission is seasonal with recorded various degrees of epidemics (Deressa *et al*., 2007).The major malaria transmission occurs between September and December following the July-August heavy rainfalls

as elsewhere in the country. The main malaria hydrological area in the district is Lake Zeway that maintains malaria transmission by creating and sustaining numerous mosquito breeding sites specifically along the lake shorelines (Kenea *et al*., 2011). Mosquito abundance increases as the lake fills and extends to the nearby villages during the rainy seasons (July-November) and declines as the lake volume recedes during the following dry months. The area is semi-arid, rain-fed surface pools in the uphill villages are non-persistent. As a result, mosquito breeding sites are almost limited to the lake area even during the rainy season.

3.2. Study design and mosquito collections

This thesis consists of pre-intervention entomological studies, mosquito sampling method calibration study and intervention studies. In most part of this thesis entomological data collections were carried out in randomly selected villages and houses. A four arm community based cluster randomized controlled trial namely IRS+LLINs, IRS alone, LLINs alone and the control or routine arm was used in this vector control interventions.

Different adult mosquito sampling methods were used based on objectives of the study and mosquito behaviors. Centers for Disease Control light trap catches (LTC) was used for indoor host-seeking mosquito collection, indoor pyrethrum spray catch (PSC) and artificial outdoor pit shelters (PIT) were used for indoor and outdoor resting mosquito collections respectively. Human landing catch (HLC) was used to calibrate LTC and also to monitor biting activity levels of the local mosquitoes during pre-intervention and intervention studies.

Before routine mosquito collection, verbal consent was obtained from the household heads. The light traps were set indoors close to a sleeping person and allowed to operate from 6:00 PM to 6:00 AM. The traps were hung about 45cm above the floor near the feet of occupants protected by

LLINs provided by the Ministry of Health as part of the routine malaria control programme during pre-intervention study or by the project during the intervention study.

Before spraying the houses for PSC, all openings that could allow mosquito escaping (such as doors, windows, and holes on the walls) were closed and the entire floor was covered with white cloth. Spray sheet collection was performed by spraying an aerosol containing pyrethroids (Mobil®). A sprayed room was left closed for 10 minutes after which sheets were brought outside to inspect and collect knocked-down mosquitoes using forceps, Petridish and a torch light. Mosquitoes collected were also taken to the field laboratory for further processing.

Outdoor mosquito collections from PITs were performed concurrently with the PSC every morning from 6:00 AM to 9:00 AM. The PITs were dug under trees in a shady area in the compound of local houses. Each PIT was 1.5 m deep, 1.2 m x 1.2 m opening (Silver, 2008) with a round hallow cavities of about 15 cm width and 30 cm depth situated about 30-40 cm above the floor of the PIT (Bhatt *et al.*, 1989). To prevent mosquito escaping during collection, an untreated white mosquito net was stretched over the top of the pit and held by the collection team until a collector had collected all mosquitoes using hand held mouth aspirator, paper cups and torch light. Indoor and outdoor HLCs were performed by locally recruited volunteers based on WHO (1975) guideline. Volunteers were trained to collect landing mosquitoes as they arrive on their exposed feet before they bite them.

3.3. Mosquito processing and estimation of entomological indices

3.3.1. Mosquito species identification

At a field laboratory in Batu town, live mosquitoes were killed with chloroform. Mosquitoes were first sorted to culicines and anophelines. Adult female *Anopheles* mosquitoes were further identified by morphological criteria using identification keys (Verrone, 1962; Gilles and Coetzee, 1987) except for *Anopheles gambiae* which were kept for molecular identification.

For further processing, mosquito specimens were preserved individually in Eppendorff tubes containing silica gel and transported to Aklilu Lemma Institute of Pathobiology (AKLIP), Addis Ababa and stored at -20°C until processing. At AKLIP *An. gambiae* sibling species identification was carried out by polymerase chain reaction method (PCR) based on Scott *et al*. (1993). In brief, a leg was removed from each mosquito and mixed with 12.5 µl PCR master mix. The master mix contained 10x dNTPs, MgCl2 Solution, QD primer, UN Primer, GA primer, M primer, AR primer, deionized water and RTag. This solution was made in 0.2 ml PCR tube, which were centrifuged for 20s-20min at 16 K r.p.m. and amplified in a PCR machine. Finally, 5 µl PCR product loaded with 2 µl loading dye and 4 µl DNA ladder were electrophoresed through a 2% agarose-tris-borate-EDTA containing ethidium bromide gel (with 100 V and 150 mA power source) and visualized under UV light box.

3.3.2. Dissection of mosquitoes for parity determination

At the field laboratory, abdomens of unfed *Anopheles* were dissected for parity determination based on WHO (2013). Briefly, a fresh killed mosquito was placed on a microscope slide with a drop of physiological saline surrounding the posterior part of the abdomen. On the sixth or

seventh segment, a cut was made in the abdomen and the contents were pulled out gently. The ovaries were transferred to the slides and they were left to dry. The ovaries were then examined under a compound microscope (10x and 40x) to determine whether they were nulliparous or parous. The nulliparous condition is indicated by the tightly coiled endings to the tracheoles (skeins) while the parous female has uncoiled endings (WHO, 2013). The parity rate was estimated as the number of parous females divided by number of females examined multiplied 100 (WHO, 2013).

3.3.3. Determination of *P. falciparum* and *P. vivax* sporozoites rates

The head and thorax of each mosquito was carefully separated from the abdomen and tested for the presence of *P. falciparum* and *P. vivax* 210 and *P. vivax* 247 circumsporozoite protein (CSP) by direct enzyme-linked immunosorbent assay (ELISA) as described by Wirtz *et al*. (2007). Briefly, mosquitoes were ground individually in 50 µl boiled casein containing Igepal CA- 630 and a final volume brought to 250 µl with blocking buffer. 50 µl of the triturate was used in ELISA tests. Samples with green colour and with optical density (OD) values greater than two times the average OD of the negative controls were considered sporozoite positive. The sporozoite rate was estimated as the number of mosquitoes with sporozoites divided by number of females examined multiplied by100 (WHO, 2011).

3.3.4. Determination of human blood index

The sources of mosquito blood meals were determined using the direct ELISA procedure using human and bovine antibodies based on Beier *et al.* (1988) as follows. Abdomens of freshly fed mosquitoes were individually crushed in 50 µl phosphate buffered saline (PBS) solution (pH 7.4). Fifty microlitres of sample was added to each well in a 96-well microtitre plate, and incubated

overnight at room temperature. Each well was washed twice with PBS containing Tween-20 solution, and 50 µl host specific conjugate (either human or bovine) was added to each well and incubated for one hour. After one hour, each well was washed three times with a PBS–Tween-20 solution. Finally, 100 µl of peroxidase substrate was added to each well and after 30 minutes the absorbance at 405 nm was recorded with an ELISA plate reader. Each blood meal sample was considered positive if the absorbance value exceeded the mean plus three times the standard deviation of the four negative controls (from a laboratory colony of *An. arabiensis* adults not fed with blood). Positive controls contained human and bovine blood. The Human Blood Index (HBI) was calculated as the number of mosquitoes having fed on humans divided by the total number of mosquitoes tested and the bovine blood index (BBI) was also calculated as the number of mosquitoes having fed on bovine divided by the number of mosquitoes tested (WHO, 2011).

3.4. Ethical considerations

Ethical approval for the study was obtained from the Institutional Review Board of the College of Health Sciences at Addis Ababa University, the Ministry of Science and Technology in Ethiopia (Ref: 3.10/446/06), and the Regional Committee for Medical and Health Research Ethics, (Ref: 2013/986/REK Vest) Western Norway (Deressa *et al*., 2016). The protocol for the trial was registered at the Pan African Clinical Trials Registry under the registration number PACTR201411000882128. Verbal and written informed consent to take part in the study was obtained prior to the commencement of this study, from volunteers for landing catches who were older than 18 years of age and house owners using the local Afan Oromo language. For the human landing catches, a separate written informed consent describing the potential risks and benefits of the study was obtained from the volunteers. These volunteers were selected from the study village. To help minimize risk, data collectors for the human landing catches were provided

with an appropriate prophylactic drug (Malarone) before the collections. To our knowledge there are no reports on Malarone resistant *Plasmodium* parasites in Ethiopia. Fortunately, none of the mosquito collectors or householders was found parasite-positive during the study period.The participants were instructed that involvement in the study was voluntary, and that they had the right to withdraw at any time regardless of reason. Assurance was also given that a refusal to participate in this study would not affect their access to services at the health posts in the study villages in the community.

Chapter 4. Pre-intervention studies: *Anopheles* species composition, densities and biting activities in Adami Tullu district

4.1. Introduction

Pre-intervention entomological studies were carried out to provide basic information about local vector populations and behaviour for effective planning and implementation of IRS and LLINs combined and separate interventions in Adami Tullu district. The investigations were conducted during June to October 2013 and July to November 2014. This time coincides with the major malaria transmission season in Ethiopia, which is usually between September and November.

Anopheles arabiensis and *An. pharoensis* are the two most important anthropophagic malaria vector species in Adami Tullu area (Abose *et al*., 1998). Lake Zeway and Bulbula River, which drains out from the lake, are the key potential aquatic habitats for year round breeding of the vectors (Kenea *et al*., 2011). *Anopheles arabiensis* breeds in the lake-shore sun-lit pools and disperse to rain-fed pools within villages during wet season whereas, *An. pharoensis* breeds typically in vegetative swampy areas of the lake. Thus, in the villages close to the lake, *An. arabiensis* outnumber *An. pharoensis* during the wet season while the latter dominate the former in the dry season (Abose *et al*., 1998).

IRS and LLINs have been the primary vector interventions in the district (Bekele *et al*., 2012). IRS has been used for malaria vector control for more than three decades in the district (Abose *et al*., 1998). Insecticide-treated nets have been used since 2005. However, sufficient evidence is lacking on the impact of these interventions on local vector population and behavior in this district.

Notwithstanding previous findings regarding entomological impact of the interventions in Adami Tullu district and elsewhere in the country, empirical evidence about the impact of IRS and LLINs combination on local vector population and behavior were lacking particularly when it comes to entomological outcomes of vector control intervention trials. However vector intervention aimed at reducing malaria transmission requires baseline information upon which monitoring and evaluation of effectiveness of these control efforts can be assessed. Effective operation of IRS and LLINs require adequate information on local vector species, density, biting and resting behaviours (WHO, 2015). Therefore, in order to collect baseline data related to the existing vector species composition, density, longevity, infectivity, biting and host preference behavior, entomological studies were conducted in the study district before the implementation of the actual intervention trial.

4.2. Materials and methods

4.2.1 Study area

These pre-intervention studies were conducted in Adami Tullu district described in detail elsewhere (Deressa *et al*., 2016). Briefly, the size of the district is 1403 square kilometres (CSA, 2007) and administratively divided into 48 *kebeles* (sub-districts). Each *kebele* is further divided into *gares*, and each *gare* has an average of 35 households. The geographic location of the district and the selected *kebeles* for these studies are shown in Figure 4.1. In 2013 transmission season three *kebeles* namely Bochesa, Elka Chelemo and Gallo Raphe were randomly selected from all *kebeles* of the district using a computer generated list with the help of an expert. The whole village was considered to randomly select mosquito sampling houses. A total of 36 *gares* (twelve per *kebele*) from each of the three *kebeles* (Bochesa, Elka Chelemo and Gallo Raphe) were

randomly selected for adult mosquito collections. Households were selected at random from the 36 villages or *gares*.

Additionally human biting activities of local vectors were monitored during July to November 2014 major transmission season in Edo Kontola, a rural village near Batu (Zeway) town, the capital of the district. This village was selected based on past entomological studies (Abose *et al*., 1998) to investigate variations in biting patterns of anopheline species in the same locality and under similar environmental settings. Edo Kontola is situated along Lake Zeway on the main road from Addis Ababa to Hawassa between Abosa and Batu towns.

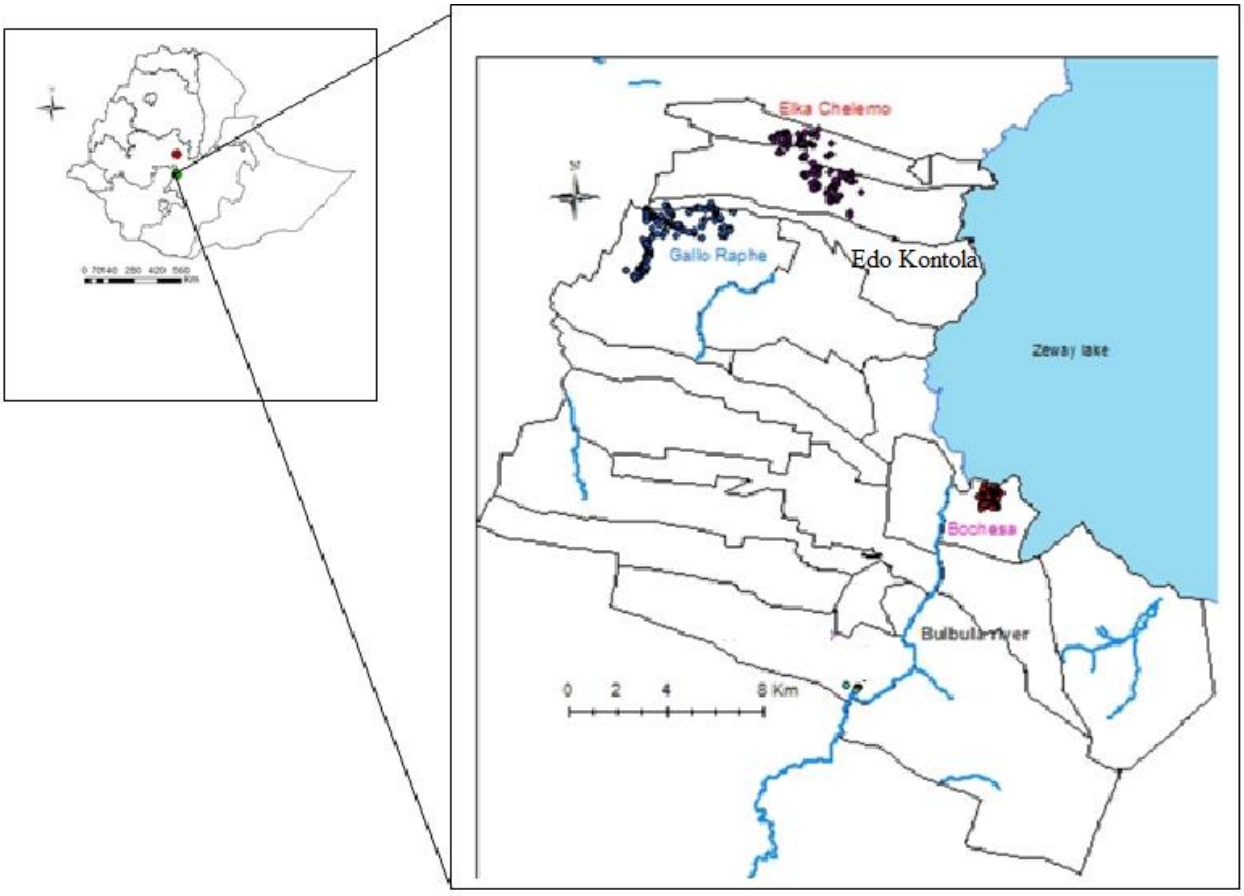

Figure 4.1: Geographical locations of Bochesa, Galo Raphe, Elka Chelemo and Edo Kontola villages, in Adami Tullu district, south-central Ethiopia

4.2.2. Mosquito collections

During the 2013 transmission season indoor and outdoor mosquito collections were undertaken every month using LTC, PSC and PIT as described under subheading 3.2. The LTCs were carried out in one house per *gare*. The PSC was carried out from one randomly selected house per *gare*, while pit shelter collections were performed from six pits per *kebele* in a total of 18 *gare*s.

The nocturnal biting habits of *Anopheles* species were monitored for 40 nights using HLC performed indoors and outdoors during July to November 2014, coinciding with the major malaria transmission season. The HLC, where human volunteers catch mosquitoes that land on their exposed body parts was used because it is the gold standard method for monitoring mosquitoes that bit humans (anthropophagic mosquitoes) and the number of mosquitoes caught by HLC can directly provide an estimate of mosquito-human biting activities (Lima *et al*., 2014; Briet *et al*., 2015).

Three houses close to the lakeshore were selected having similar size and design and with house owners agreeing to participate in the study. The houses were of traditional style with thatched conical-shaped roofs, circular floors and plastered walls. All houses had similar potential mosquito entry and exit points each having one door, eaves, and cracks in walls, but none of them had windows.

Each house in the village including the selected houses was located close to irrigation fields and within walking distance ($\leq$1km) from the lakeshore. It was also arranged in such way that the selected houses for HLC were free of cattle and human occupants on all collection nights to reduce the influence of alternative hosts. In addition, the houses were enrolled in the control arm

of the trial and neither treated by IRS nor received LLINs during the study period (Deressa *et al*., 2016).

The three houses were selected to reduce position bias driven by potential variations in indoor micro-climate such as indoor temperature, differences in mosquito entry points, mosquito density and proximity to animal shelter(s). Mosquito collections were performed in one house per night alternating each house for three consecutive nights per week. The collectors were rotated through the collection houses to compensate for any differences in attractiveness to mosquitoes and collecting abilities. Collections started in late July and ended in late November 2014 with intermittent collections in August and September.

Mosquito collections were conducted by volunteers who were selected from the local people and who gave their written consent. Mosquitoes were collected from 19:00 to 06:00hrs for 50 min each hour with 10 min rest for the volunteers. There were two collection shifts: one team of collectors worked from 19:00 to 24:00hrs followed by the second team from 24:00 to 06:00 hrs. Every hour, two volunteers rotated between indoor and outdoor positions and carried out the work to reduce position bias. Outdoor collectors were positioned within 10m from each study house. Each volunteer sat on a chair with the legs exposed from foot to knee and captured mosquitoes as soon as they land on the exposed legs before they commence feeding using a flashlight and mouth aspirator. Each hour's collection was kept separately in labeled paper cups. Supervisors were assigned to coordinate collection activities and watch volunteers not to fall sleep and bitten by mosquitoes over the study nights. The next morning, collected mosquitoes were identified to species and stored on silica gel for further analysis as described under 1.7.3.

4.2.3. Estimation of entomological parameters

Human biting rates (HBRs) for each *Anopheles* species were calculated as mean number of mosquitoes collected by HLC per person per night (m/p/n) separately for indoor and outdoor venues i.e. HBR=no. of mosquitoes collected/no. of nights/no. of collectors (Kabbale *et al*., 2013). The degree of endophagy was calculated as indoor $HBR_{19:00\rightarrow06:00hrs}$/(indoor $HBR_{19:00\rightarrow06:00hrs}$+ outdoor $HBR_{19:00\rightarrow06:00hrs}$). Exophagy was calculated as outdoor $HBR_{19:00\rightarrow06:00hrs}$/(outdoor $HBR_{19:00\rightarrow06:00hrs}$ + indoor $HBR_{19:00\rightarrow06:00hrs}$) (Govella *et al*., 2010).

The density of nocturnal biting was calculated as density of HBR during peak sleeping hours (hours starting 22:00 to 05:00) as follows (Govella *et al*., 2010): (indoor $HBR_{22:00\rightarrow05:00hrs}$ + outdoor $HBR_{22:00\rightarrow05:00hrs}$)/(indoor $HBR_{19:00\rightarrow06:00hrs}$+ Outdoor $HBR_{19:00\rightarrow06:00hrs}$).The nocturnal biting activities of each *Anopheles* species was expressed as mean number of each *Anopheles* species landing per person per hour separated by indoor and outdoor venues. Indoor and outdoor exposure to mosquito bites that took place early evening (19:00 to 22:00hrs), during night 22:00-05:00hrs) and early in the morning (05:00-06:00hr) were estimated as the number of mosquito catches by HLC either indoors or outdoors divided by number of indoor and outdoor combined catches by each species multiplied by 100. Parous rate was calculated as the total number of parous females for each species divided by the total number of mosquitoes dissected multiplied by 100. The man biting proportions of parous *An. arabiensis* that took place during the early evening, during the night, and during the early morning (assessed by HLC) were compared based on field observations and available literature. The human blood index (HBI) and bovine blood index (BBI) were calculated based on WHO guidlines (WHO, 2011).

4.2.4. Data analysis

Different statistical procedures were employed per entomological study as follows: Variation in adult mosquito host-seeking and resting density within and among the villages and study months were analysed using Kruskal-Wallis test. Comparisons of indoor and outdoor HLC data were done by Generalized Linear Models (GLM) with negative binomial distribution. The impact of the collection venues on mean anopheline biting density were therefore estimated by exponentiation of negative binomial regression coefficient, i.e. Incidence Rate Ratio (IRR). Results were considered significant at $P< 0.05$. Data were analyzed using the program SPSS version 20.0 (SPSS, Chicago, USA).

4.3. Results

4.3.1. *Anopheles* species composition and abundance

Overall 232 adult *Anopheles* mosquitoes were collected over the five months by LTC, PSC and PIT during 2013 transmission season (Table 4.1). The species composition was 71.1% *An. arabiensis,* 21.1% *An. pharoensis,* 5.2% *Anopheles ziemanni,* and 2.6% *Anopheles funestus s.l.*

Table 4.1: Species composition of adult *Anopheles* mosquitoes collected in Adami Tullu district, 2013 (Figures in parentheses indicate percentage)

Village	Species	Collection Method			Total
		LTC	PSC	PIT	
Bochesa	*An. arabiensis*	19 (14.7)	6 (9.2)	19 (50.0)	44 (19.0)
	An. pharoensis	12 (9.3)	2 (3.1)	0	14 (6.0)
Elka Chelemo	*An. arabiensis*	38 (29.5)	33 (50.8)	9 (23.7)	80 (34.5)
	An. pharoensis	30 (23.3)	3 (4.6)	0	33 (14.2)
	An. funestus	5 (3.9)	0	1 (2.6)	6 (2.6)
	An. ziemanni	9 (7.0)	0	3 (7.9)	12 (5.2)
Gallo Raphe	*An. arabiensis*	14 (10.9)	21 (32.3)	6 (15.8)	41 (17.7)
	An. pharoensis	2 (1.6)	0	0	2 (0.9)
Total *Anopheles*		129 (55.6)	65 (28.0)	38 (16.4)	232

The *Anopheles* abundance varied over the study months with a peak in September after the rainy season (Figure 4.2).The average monthly precipitation peaked in July and declined with low precipitation from August to October.

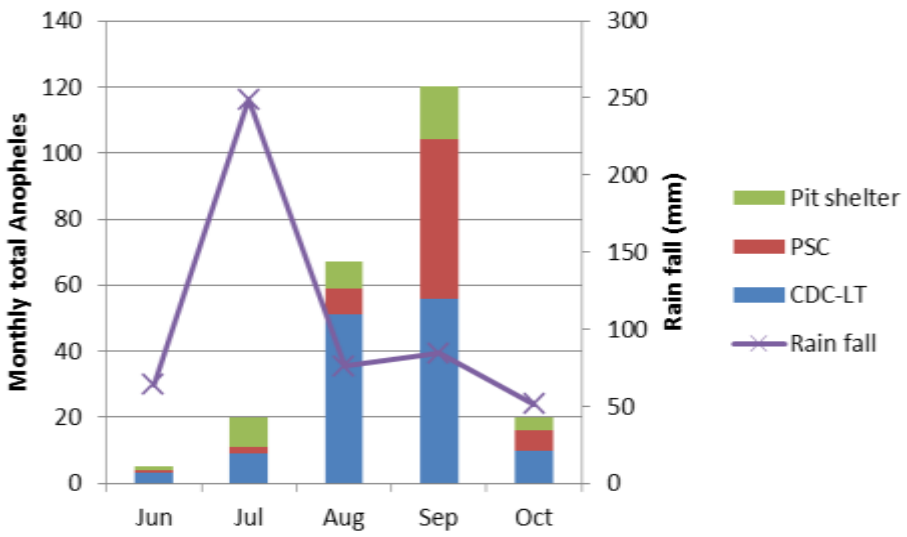

Figure 4.2: Monthly *Anopheles* abundance and average precipitation in Adami Tullu, 2013

4.3.2. Host-seeking and resting behaviour

The mean host-seeking density of *Anopheles* collected by LTC indoors was 0.7 *Anopheles* per LTC/night/house. The mean indoor resting density of *Anopheles* obtained by PSC was 0.4 *Anopheles* per house per day and the mean outdoor resting density collected from pit shelters was 0.4 *Anopheles* per pit shelter per day over the five months. The highest mosquito density was found in Elka Chellemo where there were significant differences between collection methods (Figure 4.3).

Anopheles abundance varied significantly between *kebeles* and *gares*. There were significant differences in abundance of anopheline mosquitoes between the three *kebeles* (Kruskal-Wallis test = 11.25, df = 2, P = 0.004) and between the 36 *gares* (Kruskal-Wallis test = 68.93, df = 35, P = 0.001). The same statistical test revealed that there were significant differences in host-seeking abundances (LTC) of *An. arabiensis* (P =0.025), *An. pharoensis* (P = 0.001) and *An. ziemanni* (P = 0.015) between *kebeles*. However, the indoor abundance (LTC) of *An. funestus* s.l was not

significantly different between *kebeles* (P = 0.458). No significant differences were detected between *kebeles* and *gares* in the abundance of indoor resting anophelines (PSC) (P > 0.05) and, outdoor resting anophelines (pit shelter) (P > 0.05).

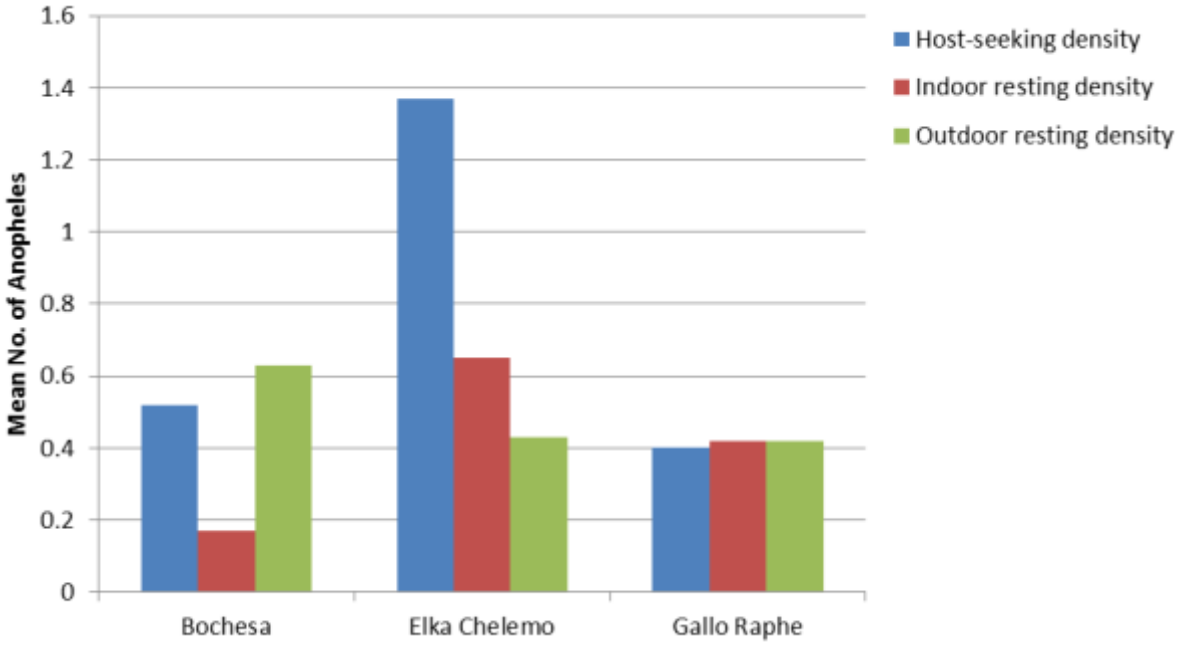

Figure 4.3: Mean indoor and outdoor density of *Anopheles* by village, Adami Tullu district

The average indoor host-seeking density, indoor resting density and outdoor resting density of *An. arabiensis* generally peaked in September and almost declined to zero in October (Figure 4.4).

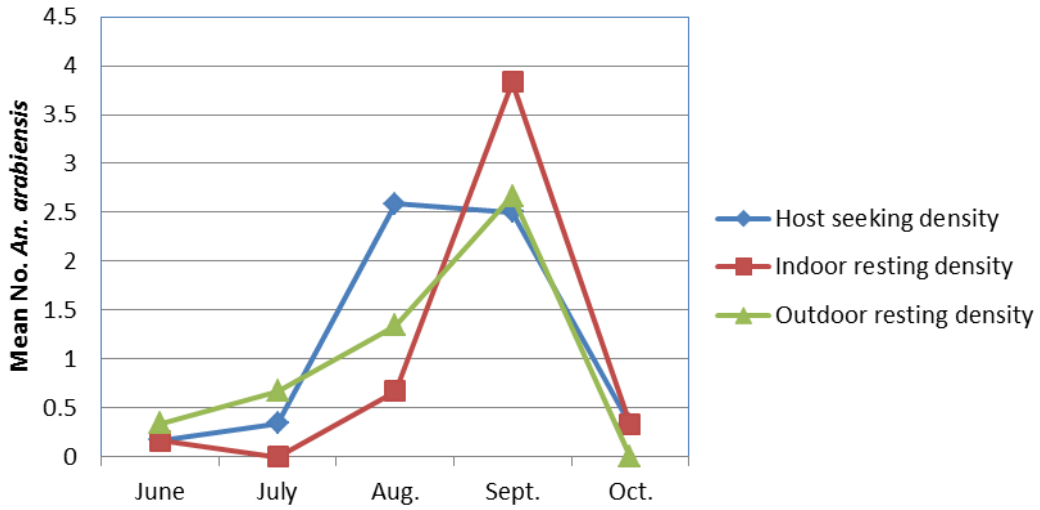

Figure 4.4: Overall average monthly host-seeking and resting densities of *An. arabiensis*, Adami Tullu district, 2013. Host-seeking density (LTC), indoor resting density (PSC), outdoor resting density (PIT)

4.3.3. Blood meal sources of the *Anopheles* mosquitoes

Of 107 freshly fed *Anopheles* tested, the overall human blood index (HBI) and bovine blood index (BBI) was 0.70 and 0.38, respectively (Table 4.2). The overall HBI and BBI for *An. arabiensis* was 0.69 and 0.39. *Anopheles arabiensis* preferred to feed more on humans (0.59) than bovine (0.29). The HBI was higher for *An. arabiensis* collected indoors (0.79) than for those collected outdoors (0.37). Inversely, BBI was higher for *An. arabiensis* caught outdoors (0.68) as compared to those collected indoors (0.27). All *An. pharoensis* females that had fed on humans were captured indoors. None of the indoor and outdoor collected *An. pharoensis* females had taken blood from bovines alone. *Anopheles ziemanni* fed more on bovine (BBI = 0.67) than human (HBI = 0.50).

Table 4.2: Blood meal sources of the *Anopheles* species collected by LTC, PSC, and PIT in Adami Tullu district.

Anopheles species	Collection venues	No. analyzed n(HBI)	Blood meals sources			
			Human n (HBI)	Bovine n (BBI)	Mixed n (MBI)	Unknown n
An. arabiensis	LTC	24 (0.75)	15 (0.63)	6 (0.25)	3 (0.13)	0
	PSC	48 (0.79)	35 (0.73)	10 (0.21)	3 (0.06)	0
	Pit shelter	19 (0.37)	4 (0.21)	10 (0.53)	3 (0.16)	2 (0.11)
	Total	91 (0.69) ¶	54 (0.59)	26 (0.29)	9 (0.09)	2 (0.02)
An. pharoensis	LTC	7 (1.00)	6 (0.86)	0	1 (0.14)	0
	PSC	2 (1.00)	2 (1.00)	0	0	0
	Total	9 (1.00) ¶	8 (0.89)	0	1 (0.11)	0
An. ziemanni	LTC	3 (0.67)	2 (0.67)	1 (0.33)	0	0
	Pit shelter	3 (0.33)	0	2 (0.67)	1 (0.33)	0
	Total	6 (0.50) ¶	2 (0.33)	3 (0.50)	1 (0.17)	0
An. funestus	Pit shelter	1	0	1	0	0
Overall *Anopheles*		107 (0.7) ¶	64 (0.60)	30 (0.30)	11(0.10)	2 (0.20)

When computing for human blood index (HBI) and bovine blood index (BBI), mixed blood meals were added to the number of human blood and bovine blood meals. Mixed blood meals = human + bovine, unknown blood meals are negative for both human and bovine antibodies, ¶ showsoverall HBI

4.3.4. Parity rate and longevity of the malaria vectors

The overall average age of *An. arabiensis* and *An. pharoensis* females was 14 days (range: 7-25 days) and 1.6 days (range: 0-6.3days), respectively (Table 4.3).

Table 4.3: Parity rates and longevity of *Anopheles* species caught by LTC in the Adami Tullu district, 2013

Kebeles	Species	Number of mosquitoes					
		Collected	Dissected	Parous	PR	P	Age (Days)
Bochesa	*An. arabiensis*	44	3	2	0.67	0.87	7
	An. pharoensis	14	5	1	0.20	0.58	1.8
Elka Chelemo	*An. arabiensis*	80	9	8	0.89	0.96	25
	An. pharoensis	33	18	11	0.61	0.85	6.3
Gallo Raphe	*An. arabiensis*	41	3	2	0.67	0.87	7
	An. pharoensis	2	2	0	0.00	0.00	0
Average for *An. arabiensis*		55	5	4	0.8	0.93	14
Average for *An. pharoensis*		16.33	25	4	0.16	0.54	1.6

PR is parity rate; P is probability of surviving one day

4.3.5. Number and proportion of human biting *Anopheles* species

During the 40 nights of human landing collections, a total of 3,408 adult female anopheline mosquitoes were captured (Table 4.4). *Anopheles ziemanni* was the predominant species (66.5%), followed by *An. arabiensis* (24.8%), *An. pharoensis* (6.8%) and *An. funestus* (*s.l.*) (1.8%). Overall, 76.6% (2,610) of the mosquitoes were captured outdoors and 24.4% (798) indoors.

Table 4.4: Total number and proportion of *Anopheles* species collected by human landing catches indoors and outdoors in Edokontola village, Ethiopia

Species	Indoor	Outdoor	Total
	n (%)	n (%)	n (%)
An. ziemanni	351 (15.5)	1916 (84.5)	2267 (66.5)
An. arabiensis	375 (44.4)	470 (55.6)	845 (24.8)
An. pharoensis	50 (21.5)	183 (78.5)	233 (6.8)
An. funestus s.l	22 (34.9)	41 (65.1)	63 (1.8)
Overall	798 (23.4)	2610 (76.6)	3408 (100)

4.3.6. Human biting rates by *Anopheles* species

The overall (indoor and outdoor combined) mean human biting rate (HBR) of *Anopheles* mosquitoes was 85.2 mosquitoes/person/night (m/p/n). The total (indoor and outdoor combined) mean HBRs for *An. ziemanni* was 56.7, *An. arabiensis* 21.1, *An. pharoensis* 5.8, and for *An. funestus* (*s.l.*) it was 1.6 m/p/n.

The overall mean outdoor anopheline human biting density (HBR) was 3.3 times higher than indoor (65.3 *vs* 19.9 m/p/n, (IRR: 3.3, 95% CI: 1.1–5.1, $P<0.001$). The mean HBRs of *An. ziemanni*, *An. pharoensis* and *An. funestus* (*s.l.*) collected outdoors were significantly higher than indoors for each species ($P< 0.05$, Figure 4.5). However, the mean outdoor HBR of *An. arabiensis* was similar to that indoors (11.8 vs 9.4 m/p/n, IRR: 1.3, 95% CI: 0.8–1.9, $P=0.335$).

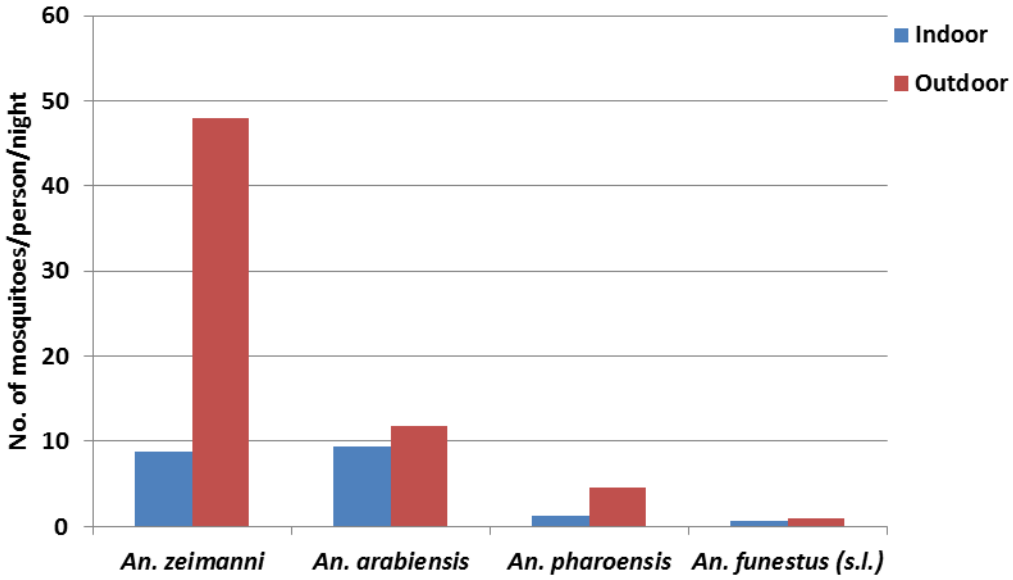

Figure 4.5: Indoor and outdoor human biting rates by *Anopheles* mosquitoes in south-central Ethiopia

4.3.7. Biting behaviours: endophagy, exophagy and nocturnality

The degree of endophagy and exophagy (indoor and outdoor feeding) is given in Table 4.5. Overall, the majority of anophelines (76.6%) exhibited exophagic (proportion of HBR outdoor) behaviour. The majority of *An. ziemanni* (84.5%), *An. pharoensis* (79.3%) and *An. funestus* (*s.l.*) (62.5%) were captured outdoors and were clearly exophagic. *Anopheles arabiensis* showed 55.7% and 44.3% exophagic and endophagic behaviours.

With respect to nocturnality, overall, 48.2% of anopheline biting occurred during peak sleeping hours (22:00 to 05:00hrs) as compared to when people were most likely awake (51.8%). None of the *Anopheles* species showed marked peak nocturnality (high nocturnal biting activities during peak sleeping hours). Similar proportion of *An. arabiensis*, *An. pharoensis* and *An. funestus*(*s.l.*) populations exhibited maximum human-biting activities during sleeping hours (50.0%) when

local people were potentially protected by LLINs and IRS as well as during non-sleeping hours (50.0%) when the local people were not protected (Table 4.5).

Table 4.5: Human biting rates (HBR; number of mosquitoes collected per person per night [95% confidence interval]), and feeding behaviors of *Anopheles* species in Edo Kontola village, Ethiopia.

Biting activities	*An. arabiensis*	*An. pharoensis*	*An. ziemanni*	*An. funestus* s.l.	*Total*
Indoor HBR (19:00-06:00)	9.4 (7.9-11.0)	1.2 (0.8-1.7)	8.8 (6.1-11.6)	0.6 (0.1-1.1)	20.0
Outdoor HBR (19:00-06:00)	11.8 (9.8-14.1)	4.6 (3.6-5.6)	47.9 (38.4-56.9)	1.0 (0.6- 1.5)	65.3
Nocturnal HBR (22:00-05:00)	11.2 (9.5-13.1)	2.5 (1.9-3.1)	26.5 (19.9-34.2)	0.9 (0.5-1.5)	41.1
Endophagy[1] (%)	44.3 (43.8-44.6)	20.7 (18.2-23.3)	15.5 (13.7-16.9)	37.5 (14.3-42.3)	23.4
Exophagy[2] (%)	55.7 (55.4-56.2)	79.3 (76.7-81.8)	84.5 (83.1-86.3)	62.5 (57.7-85.7)	76.6
Nocturnality[3](%)	52.8 (44.6-53.5)	43.1 (42.4-43.7)	46.7 (44.7-49.9)	56.3 (47.6-71.4)	48.2

[1]Proportion of indoor HBR between 19:00 and 06:00 hours.[2]Proportion of outdoor HBR between 19:00 and 06:00.[3]Proportion of HBR between 22:00 and 05:00 (during sleeping hours).

4.3.8. Human biting patterns of anopheline and potential exposure to malaria mosquitoes

The human biting activity of *An. arabiensis* was from dusk to dawn both outdoors and indoors with a single peak before midnight (21:00 to 22:00 hrs) outdoors followed by a general decline during the rest of the night. The indoor biting activity however showed two smaller peaks, one before midnight (20:00 to 21:00hrs) and a second peak around midnight (24:00 to 01:00 hrs) (Figure 4.6A).

All the other anophelines were also active throughout the night, but with differing peak periods of biting activities both outdoors and indoors. The outdoor biting activities of both *An. pharoensis* and *An. zeimani* were generally higher than indoors and both exhibited a pronounced unimodal

biting activities early in the evening (19:00 to 20:00 hrs) which declined progressively during the rest of the night (Figure 4.6B,C), while both species also followed the same pattern indoors, but with greatly reduced biting activities. On the other hand *An. funestus* s.l. appeared to show three peaks of biting activities outdoors of which two were the major ones: one before midnight (21:00 to 22:00hr) and another one early in the morning (5:00 to6:00 hr) between which was a smaller peak just after midnight (01:00 to 2:00 hr) (Figure 4.6D). The indoor biting activity on the other was bimodal with an early and smaller peak at 20:00 to 21:00 hrs and a major peak just before midnight (23:00 to 24:00 hr). Human biting activities of the main malaria vectors; *An. arabiensis* and *An. pharoensis* peaked early in the evening (before 22:00 hrs) before local people retire to bed and were generally higher outdoors than indoors (Figure 4.6A,B).

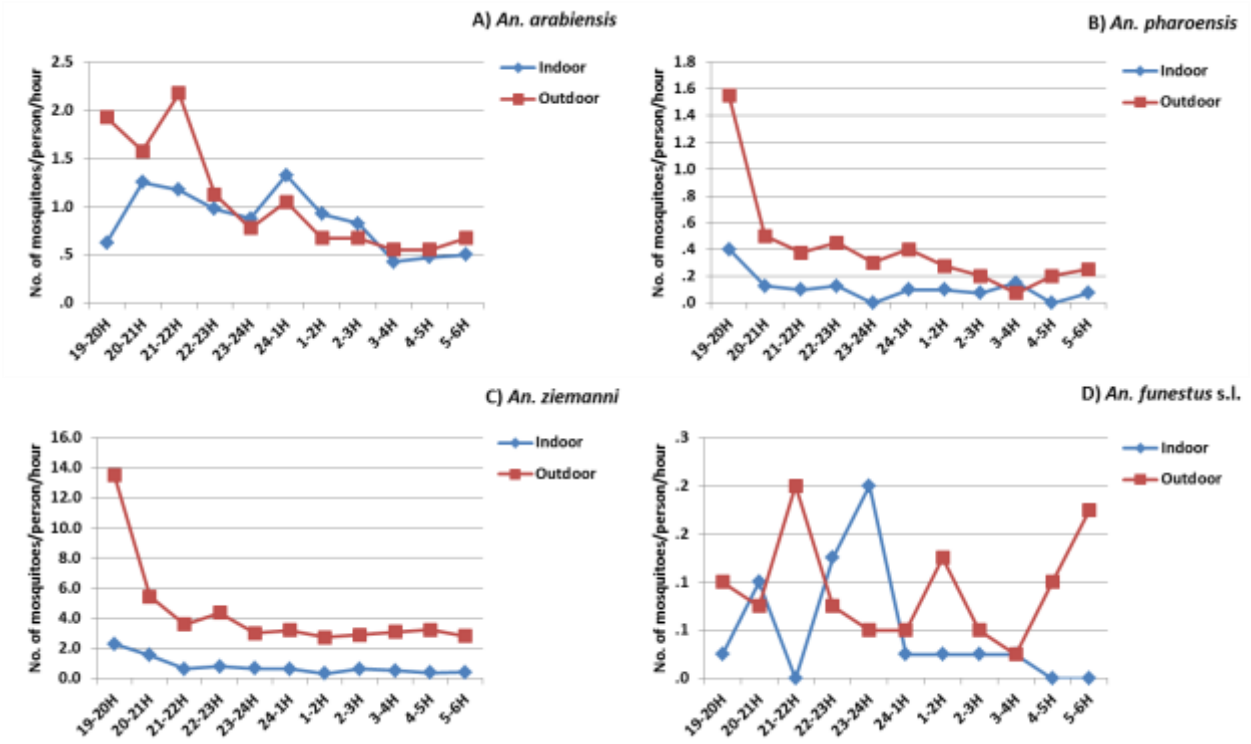

Figure 4.6: Human biting patterns of the *Anopheles* species in Edo Kontola, Ethiopia, 2014

Altogether, 27.6% of the major malaria vector, *An. arabiensis* bites took place during bedtime (22:00 to 05:00hrs) and might be potentially prevented by LLINs alone whereas 44.4% of this vector bites could be prevented by LLINs+IRS during the study period (Table 4.6). However, only 7.6% of the potential secondary vectors (i.e. *An. pharoensis, An. ziemanni* and *An. funestus* (*s.l.*)) bites occurred during bedtime (22:00 to 05:00hrs) and might be prevented by LLINs alone. Likewise, only 16.5% of these species bites might be prevented by IRS and LLINs combined interventions.

Table 4.6: Abundance of primary (*An. arabiensis*) and secondary (*An. pharoensis, An. ziemanni, An. funestus* s.l.) malaria vectors collected indoors and outdoors at different times of the night in Edo Kontola village, Ethiopia.

Anopheles species	Venue	Early evening (19:00-22:00) n (%)	Night (22:00-05:00) n (%)	Early morning (05:00-06:00) n (%)	Whole night (19:00-06:00) n (%)
Primary vector	Indoor	122 (14.4)	233 (27.6)	20 (2.4)	375 (44.4)
	Outdoor	227 (26.8)	216 (25.6)	27 (3.2)	470 (55.6)
Secondary vectors	Indoor	208 (8.1)	196 (7.6)	19 (0.7)	423 (16.5)
	Outdoor	1014 (39.6)	996 (38.8)	130 (5.1)	2140 (83.5)

Percentages were calculated as number of mosquito catches by HLC either indoor or outdoor divided by number of indoor and outdoor combined catches.

4.3.9. Man biting patterns of parous *An. arabiensis* population

In total 343 *An.arabiensis* were dissected to determine parity rates and man biting patterns of the parous population. The overall indoor parous rate of *An. arabiensis* was 70.6% and the corresponding outdoor parous rate was 67.5% (Table 4.7). The proportion of parous *An. arabiensis* population that showed indoor man biting activities during bedtimes (22:00 to

05:00hrs) when the local people were indoors and potentially protected by IRS and LLINs was 72.4%. Likewise 69.2% of parous *An. arabiensis* were collected while attempting to bite man before bedtimes (before 22:00hrs). The proportion of parous *An. arabiensis* (50.0%) caught biting people during early morning (05:00 to 6:00hr) was low compared to either before bedtime or during bedtime. The overall indoor parous rate of *An. arabiensis* was high (76.1%) in October and low (54.5%) in November. The corresponding outdoor parous rate was high (75.0%) in September and low (45.7%) in November. No ovarial dissection was carried out in July and August due to low mosquito density.

Table 4.7: Parity rates (in %) of *An. arabiensis* (no. parous/no. tested) collected by human landing collections indoors and outdoors at different times of the night during three months in Edo Kontola village, Ethiopia

Month	Venue	Time			Total
		Early evening (19:00-22:00)	Night (22:00-05:00)	Early morning (05:00-06:00)	Whole night (19:00-06:00)
September	Indoor	58.3 (7/12)	69.2 (9/13)	0.0 (0/0)	72.7 (16/22)
	Outdoor	40.0 (2/5)	84.6 (11/13)	100.0 (2/2)	75.0 (15/20)
October	Indoor	77.8 (28/36)	76.0 (38/50)	50.0 (1/2)	76.1 (67/88)
	Outdoor	71.8 (56/78)	73.3 (44/60)	57.1 (4/7)	71.7 (104/145)
November	Indoor	50.0 (10/20)	61.5 (8/13)	0.0 (0/0)	54.5 (18/33)
	Outdoor	62.5 (10/16)	33.3 (6/18)	0.0 (0/1)	45.7 (16/35)
Total	Indoor	69.2 (45/65)	72.4 (55/76)	50.0 (1/2)	70.6 (101/143)
	Outdoor	68.7 (68/99)	67.0 (61/91)	60.0 (6/10)	67.5 (135/200)

4.3.10. Sporozoite ELISA results

All collected mosquitoes (n = 232) were negative for *Plasmodium falciparum* and *P. vivax* circumsporozoite proteins (CSP) in 2013 transmission season. Similarly a total of 1,500 *An. ziemanni*, 800 *An. arabiensis*, 200 *An. pharoensis* and 60 *An. funestus* (*s.l.*) were tested for the presence of CSP of *P. falciparum* and *P. vivax*. However, none was found positive. For this reason, the entomological inoculation rate (EIR) could not be determined.

4.4. Discussion and conclusions

Four *Anopheles* species namely *An. arabiensis*, *An. pharoensis, An. ziemanni,* and *An. funestus* s.l. were found in this preliminary mosquito survey of which the first and the second were predominant species in Adami Tullu villages. These findings are consistent with Abose *et al.* (1998) who reported that *An. arabiensis* is primary malaria vector and *An. pharoensis* play secondary role in the area.

Results also showed that in Adami Tullu district, monthly average precipitation peaked in July and sharply declined with low precipitation from August to October whereas *Anopheles* abundance rose in September and sharply dropped in October. This was expected because *Anopheles* population dynamics and malaria transmission is driven by seasonal precipitation in Ethiopia (Senay and Verdin, 2005) and the mosquitoes proliferate in rain-fed residual pools after months of heavy rain in the country (Gebreyesus *et al*., 2006). *Anopheles arabiensis* populations expand during the wet seasons but excessive rainfall may flush out breeding pools (Charlwood *et al.*, 1995). Therefore, peak *Anopheles* abundance may not coincide with peak precipitation months.

Results indicate that the overall mosquito density captured by the different mosquito sampling methods was low compared to previous study in the area (Abose *et al*., 1998). The reason for the low *Anopheles* density could be, rapid scale-up and intensive use of vector intervention measures particularly ITNs and IRS in the country (Bekele *et al*., 2012) and elsewhere in eastern Africa (Charlwood *et al.*, 1995). Besides, global climatic changes particularly changes in hydrologic and climatic factors such as precipitation, humidity, temperature and wind (Kweka *et al*., 2013) may have adversely impacted *Anopheles* population controlling breeding and survival.

The other key potential reason for the low mosquito catches could be lack of efficient mosquito sampling tools (Kewaka *et al*., 2013). Efficient indoor and outdoor collection tools are required particularly for vectors such as *An. arabiensis* that have behavioral plasticity in host preferences (Lyimo and Ferguson, 2009) and shifts in peak biting time (Russel *et al*., 2011; Yohannes and Boelee, 2012); hence, there is a need to address the inefficient catching techniques. Because adult mosquitoes occur at a certain radius from their breeding sites, a district-wide random sampling of adult mosquitoes without referring to any mosquito breeding sites could also have a potential impact on the occurrence and abundance of mosquitoes, and needs to be revisited.

The overall HBI (0.69) for *An. arabiensis* was higher than BBI (0.38) for the same species. This finding contrasts prior studies that found a higher BBI for *An. arabiensis* than the HBI in the country (Massebo *et al*., 2013). However, the present finding is in line with kibret *et al*. (2010), which found a higher HBI for *An. arabiensis* compared to the BBI in the country. It should be noted that the present study used similar mosquito sampling methods as the previous study (Massebo *et al*., 2013), thus the potential influence of mosquito trapping on HBI is not expected. But the present finding was similar to the other study (kibret *et al*., 2010) that relied on the CDC light trap alone for mosquito collection, which is evidence that the trapping methods used did not

impact the HBI. The HBI for *An. arabiensis* was higher indoors (0.73) than outdoors (0.21), but the BBI was higher when collected outdoors (0.53) than indoors (0.21). These results are generally in agreement with prior studies, which observed the opportunistic feeding behaviour of *An. arabiensis* (Animute *et al*., 2013). *Anopheles pharoensis* showed anthropophilic and endophilic behaviour in the area, but more blood-fed females should be tested to reach such conclusions. Furthermore, the average longevity of *An. arabiensis* ranged from 7 to 25 days in the villages, thereby implying that the vector had a sufficient longevity for malaria transmission during the study period. For *An. pharoensis* females, the average life span was 1.6 days that ranged from 1.6 to 6.3 days in the study villages which was insufficient for malaria transmission during the study period.

Over 40 night follow-up of man biting pattern of *Anopheles* mosquitoes by HLC during pre-intervention period showed that the mosquitoes bite more frequently outdoors than indoors. Because, both IRS and LLINs are indoor based, high outdoor human biting rates imply directly high outdoor malaria transmission potential in the area. These findings compromise the efficacy and effectiveness of IRS and LLINs pointing to the necessity of outdoor vector intervention measures in the locality. The results were evident for the occurrence of residual malaria but the magnitude and impact of residual malaria transmission worth further investigation in the area and elsewhere in the country. *Anopheles ziemanni*, *An. pharoensis* and *An. funestus* s.l each exhibited more exophagic behaviour than endophagic behaviour. These results would be expected because; *An. ziemanni* and *An. pharoensis* are exophagic species in Ethiopia (Abose *et al*., 1998; Ghebreyesus *et al*., 2006; Krafsur, 1977; Taye *et al*., 2006) and elsewhere in Africa (Ijumba *et al*., 1990). But unlike the present findings, *An. funestus* s.l. was reported to be endophagic species in

Ethiopia (Krafsur, 1977) and the *An. funestus* s.l comprise a well known endophagic sibling species in other parts of Africa (Moiroux *et al*., 2012; Sougofara *et al*., 2014).

Unlike the other anopheline species, there were no significant differences in outdoor and indoor human biting rates of *An. arabiensis*. This indicates a high flexibility and plasticity of the vector with respect to indoor and outdoor feeding and potential host preferences. Previous studies show that *An. arabiensis* bite both indoors and outdoors (Abose *et al*., 1998; Krafsur, 1977; Taye *et al*., 2006). With respect to host preference, *An. arabiensis* has shown opportunistic feeding behaviour in Ethiopia (Animut *et al*., 2013), exhibiting either anthropophagic (Tirados *et al*., 2006; Gari *et al*., 2016; Seyeum *et al*., 2002) or zoophagic behavior (Massebo *et al*., 2013). This study did not look for host preferences because mosquito collections were done by HLC alone, which is an unsuitable method for blood meal source analysis.

Analysis of the biting patterns showed early-evening biting behaviour of *An. arabiensis* with the highest peak occurring before 22:00hrs indoors and outdoors at times when the local people are not protected by LLINs. We have observed that villagers, both children and adults, spend time outdoors performing various activities such as fishing, looking after their cattle and typically retire to bed after 22:00hr. Previous reports also indicated that the people retire to bed after 22:00hr (Abose *et al*., 1998). These human activities can increase exposure to mosquito bites. Previous studies in the same study area (Abose *et al*., 1998; Kibret *et al*., 2010) and elsewhere in the country (Yohannes and Boelee *et al*., 2012; Taye *et al*., 2016) have also recorded early biting behaviour of *An. arabiensis*. In contrast to the present results, some findings documented peak *An. arabiensis* man-biting activities after 23:00 hr (Taye *et al*., 2006). In short, the previous and the present results suggest that *An. arabiensis*'s behaviour is flexible and potentially opportunistic

in terms of host preference, and feeding and resting habits (Abose *et al*., 1998; Taye *et al*., 2006; Yohannes and Boelee *et al*., 2012; Massebo *et al*., 2013; Animut *et al*., 2013; Taye *et al*., 2016).

These flexible behaviours remain a key challenge for malaria control and elimination because the vector may be less vulnerable to IRS and LLINs, and as a result, may sustain malaria transmission. Although these behaviours are believed to be a consequence of long-term exposure to IRS and LLINs interventions in Ethiopia (Tirados *et al*., 2006; Yohannes and Boelee *et al*., 2012), evidence is still lacking. Sufficient historical and up-to-date evidence about the impact of insecticidal interventions on *An. arabiensis* population and behaviour is needed to suggest that the vector is showing behavioural adaptation or has consistent biting patterns in the country. These issues need special attention for malaria control and elimination efforts in the country.

The peak indoor and outdoor man-biting activities of *An. pharoensis* and *An. ziemanni* occurred during early hours of the evening and there has been no evidence of behavioural modifications or shifts. These results are in agreement with other studies undertaken in this area (Abose *et al*., 1998). *Anopheles funestus* s.l. did not show clear indoor and outdoor human biting patterns due to small numbers collected.

The overall indoor parity rate for *An. arabiensis* was 70.6% and is similar to earlier reports from the same area by Rishikesh (1966) who recorded a constant parity rate ranging from 65–70% for *An. gambiae* (*s.l.*) presumably *An. arabiensis*. With this parity rate, *An. arabiensis* lived long enough to maintain indoor malaria transmission. Results show that indoor parity rates of *An. arabiensis* were high at times when local people generally are asleep indoors and potentially under LLINs (Abose *et al*., 1998).This implies that IRS and LLINs have high potential intervention impact on indoor malaria transmission.

Results also show that all mosquito samples tested by ELISA (*n*=2,560) were negative for *P. falciparum* and *P. vivax* circumsporozoite protein infection. It is not uncommon to find sporozoite negative mosquito samples in areas with seasonal malaria transmission such as in this study area (Abose *et al.*, 1998). Low sporozoite infection rates have been repeatedly reported from the study area, for example, Rishikesh (1966) found 9 sporozoite positive mosquitoes (0.2%) out of 4,513 *An. gambiae* s.l. (*An. arabiensis*) dissected for salivary gland examination. Kibret *et al*. (2010) also found 0.6% and 1.2% *P. falciparum* sporozoite rates among 509 *An. pharoensis* and 424 *An. arabiensis*, respectively, collected by CDC light traps and tested by ELISA in an irrigated village in the proximity of Zeway Lake. In contrast, no sporozoite positive mosquitoes were detected in a non-irrigated village located relatively far from the lake (Kibret *et al*., 2010).The current malaria decline coinciding with the scale-up of vector interventions and malaria treatment measures in the country (Otten *et al*., 2009; Alemu *et al*., 2012) might have reduced malaria parasites in the mosquito population. Furthermore, it can be suggested that lack of large numbers of mosquito specimens due to low mosquito density in the area and lack of access to more sensitive sporozoite testing methods than ELISA (such as quantitative real-time PCR) to detect infective mosquitoes could be potential factors for the negative results.

In conclusion, the density of *An. arabiensis*, the main malaria vector in Ethiopia, varied within and among the villages over the study months. The vector had high human blood index (high human contact) and sufficient longevity for malaria transmission. Moreover, *Anopheles ziemanni*, *An. arabiensis*, *An. pharoensis* and *An. funestus* (*s.l.*) were found to be the human-biting species in the area, all with outdoor biting behaviours. A high proportion of parous *An. arabiensis* were collected during night times, when the local people are usually indoors and potentially protected by IRS and LLINs. These results suggest that: (i) early and outdoor biting behaviour of *An.*

arabiensis could compromise the effectiveness of IRS and LLINs and point to the need for complementary interventions, and (ii) IRS and LLINs still have an impact on indoor malaria transmission suggesting that application and adherence to these interventions need to be strengthened.This study provided preliminary information needed for effective implementation of the intervention trial against malaria in Ethiopia and results from this study will be used as a baseline for the trial.

Chapter 5. Mosquito sampling method calibration study: Comparison of light traps with and without yeast-generated carbondioxide bait versus human landing catch

5.1. Introduction

Anopheles arabiensis control intervention is primarily based on IRS and LLINs in Ethiopia either in combination or separately. An appropriate mosquito sampling method is required to monitor the impact of these interventions on *An. arabiensis* and other local *Anopheles* populations in the country. The human landing catch (HLC) is the standard reference method for measuring human exposure to mosquito bites (WHO, 1975; Lima *et al*., 2014; Briet *et al*., 2015). However, HLC is labour intensive, exposes collectors to infectious mosquito bites and is subjected to collector bias (Lima *et al*., 2014; Briet *et al*., 2015). These necessitate local calibration and application of alternative methods such as CDC light traps if they can be used as proxies instead of HLC to determine mosquito biting rates and hence entomological inoculation rates (EIR).

Although CDC light traps have been used in Africa to estimate EIR by locally calibrating against HLC and calculating a conversion factor (Lines *et al*., 1991; Fornadel *et al*., 2010), no such evaluation and determination of conversion factors have been carried out in Ethiopia so far. For example, Animut *et al*. (2013) estimated daily EIR for *An. arabiensis* in highland areas of south-central Ethiopia based on a conversion factor for LTC versus HLC of 1.91, determined for this species in Zambia (Fornadel *et al*., 2010). Likewise, Massebo *et al*. (2013) estimated annual EIR for the same species in Chano, south-western Ethiopia, using 1.605 a factor determined in Tanzania (Lines *et al*., 1991) similar to Drakeley *et al*. (2003). The efficiency of a collection method can vary according to the composition of the mosquito species present, mosquito

densities, availability of alternative hosts, and city lighting (Briet *et al*., 2015). Therefore, it is difficult to extrapolate a conversion factor from one local epidemiological situation to another. There is a critical need to evaluate the existing mosquito sampling methods against local vectors in the country.

5.2. Materials and methods

5.2.1. Study area

The study was conducted in Edo Kontola, in Adami Tullu district, central Ethiopia during July-November 2014, the major malaria transmission season. Description of this district is given in more details in the trial protocol (Deressa *et al*., 2016) including Edo Kontola. Edo Kontola is found next to Abine Germama in the north about 5 kilometers from Batu (Zeway) town along the main road from Addis Ababa to Hawassa (Figure 4.1). It is located at $7^0$58'N, $38^0$43'E. Elevation of the area is 1653m. a.s.l. There were about 2915 inhabitants in this village. This village was selected based on results from preliminary mosquito collections showing high numbers of mosquitoes compared to other sites (Gari *et al*., 2016). The mean minimum and maximum annual temperatures were 14.5^{o}C and 27.7^{o}C, respectively. The majority of the population in the village live in houses made of mud or cement walls and thatched or corrugated iron roofs. Local residents primarily depend on farming, livestock rearing, and fishing for subsistence from Lake Zeway.

5.2.2. Study design and mosquito collections

The experiment was conducted for 39 nights in a 3 × 3 Latin square randomized design replicated 13 times (cycles) in the period between July-November 2014 (Figure 5.1). Three mosquito sampling methods were used:1) Human landing catches (HLC), 2) CDC light trap beside a

human-occupied bed net (LTC) and 3) CDC light trap baited with yeast-generated CO2 (CB-LTC). Eight local volunteers were trained on how to collect mosquitoes. Three houses of approximately similar shape and design were selected.

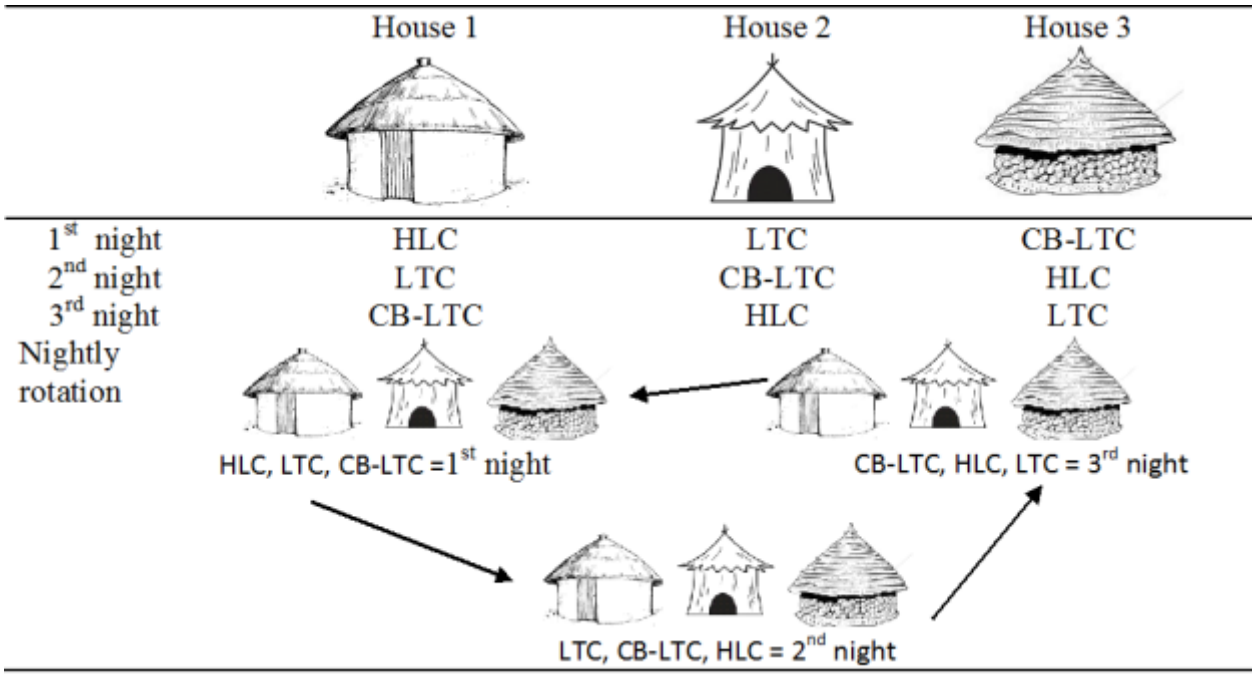

Figure 5.1: A 3 × 3 Latin square randomized design and rotational design for the three mosquito sampling methods for one round cycle of *Anopheles* collection in Edo Kontola, Ethiopia 2014

On each experimental night, four of the eight volunteers were allocated to one of the three houses to perform HLC, while the other four were assigned to sleep next to the traps individually under LLINs in indoor and outdoor venues of the other two houses. The sampling methods were rotated among the houses nightly for three consecutive nights per week. The traps operated overnight from 19:00 to 06:00. HLCs were performed for 50 minutes each hour with 10 minutes rest for the collectors. In a house assigned to HLC, four collectors conducted HLC in two rounds on each experimental night. During the first round, from 19:00 to 24:00, two collectors one indoor and the

other outdoor performed HLC. From 24:00 to 06:00, the other collectors took over and performed the same activities. Collectors sat on chairs indoors and outdoors with their legs exposed; the outdoor collector was positioned at least 10 m from the house. Using flashlights, collectors caught landing mosquitoes with a hand-held mouth aspirator and each hour's collection was kept separately in labeled paper cups.

Indoor and outdoor collectors changed venues at hourly interval during the 10 minutes break whereas the two groups of collectors changed for pre- and post-midnight shifts alternately each night, i. e. the group that collected during pre-midnight hours worked during the post-midnight period the next night and vice versa. The collectors worked during different times and sites to reduce the effects of a particular site and compensate individual differences in attractiveness to mosquitoes. At a house assigned to CDC light trap alone, two light traps, one indoor and the other outdoor, were hung at the feet of sleeping volunteers, who were protected by LLIN. Traps were generally positioned at 1.0m from the floor or ground, where the outdoor light trap was set 10 m from the outer wall of the house and on the opposite wall from where the indoor light trap was placed.

Two plastic bottles of each 2.5 liter volume were used to hold yeast-sugar solution for fermentation and production of CO_2 to be used in CB-LTC. This was made by mixing 17.5 g dry bakers' yeast and 250 g table sugar in 250 ml tap water (Saitoh *et al*., 2004) at ambient temperature one hour before the set up and operation of the traps on each experimental night. Silicon tubes each with 0.70 mm diameter were fitted through a hole drilled in the screw cap to release CO_2 to the vicinity of the light bulb of the trap. At a house assigned to this collection method, a CB-LTC was set indoors at the feet of a sleeping volunteer who was protected by a net similar to LTC alone as described above. The same procedure was applied for the outdoor CB-

LTC, which was placed at 10 m distance from the outer wall of the house. The collected mosquito specimens were processed as described under 3.3.1.

5.2.3. Data analysis

Data from the HLC were divided by 0.83, i.e. 50/60, to account for the fact that HLC was performed for only 50 minutes of each hour. The nightly number of mosquitoes (x) caught by each method was transformed to $\log10(x + 1)$, to normalize the distribution. Differences among sampling methods, collection venues (indoor/outdoor), dates of collection and mosquito species were evaluated by analysis of variance (ANOVA) and Tukey's Post-hoc test. To determine whether each of the alternative sampling methods was correlated with the reference method (HLC), Pearson correlation coefficients for relationships among log-transformed catches for each *Anopheles* species were used. The nightly mosquito catches for each *Anopheles* species in each alternative method were compared with those of the HLC by a simple linear regression analysis on log-transformed values (Altman and Bland, 1983).

The relative sampling efficiency (RSE) was measured as the ratio of the number of mosquito species caught by each alternative method to the number caught by the reference method (Altman and Bland, 1983). To test if the RSEs of LTC and CB-LTC were affected by mosquito density, the ratios of the numbers of mosquitoes in each alternative method to the number of mosquitoes in HLC (log (HLC + 1) - log (LTC + 1)), was plotted against the average mosquito abundance, calculated as [log (HLC + 1) + log (LTC + 1)]/2(Altman and Bland, 1983). Results were considered significant at $P < 0.05$. Mean log ratio and its antilog (geometric mean ratio) was used to estimate conversion factors between each of the alternative traps (LTC and CB-LTC) and the

reference method (HLC) for *Anopheles* species that showed consistent RSEs, i.e. that were not dependent on mosquito density (Lines *et al*., 1991).

5.3. Results

5.3.1. *Anopheles* abundance and density

Overall, 7606 *Anopheles* females were collected by the three sampling methods over the 39 trap nights (Table 5.1). Among these 5228 (68.7%) were *An. ziemanni*, 1153 (15.2%) *An. arabiensis*, 883 (11.6%) *An. funestus* s.l. and 342 (4.5%) *An. pharoensis*. HLC captured the highest number of anophelines, 3392 (44.6%), followed by CB-LTC 2150 (28.3%) and LTC 2064 (27.1%). Similarly, indoor catches by HLC, LTC and CB-LTC were 766 (35.2%), 726 (33.3%) and 685 (31.5%) respectively. The corresponding outdoor catches were 2626 (48.4%) by HLC, 1465 (27%) by CB-LTC and 1338 (24.6%) by LTC.

Anopheles arabiensis was most abundant in HLC (n = 833, 72.2%) and least in LTC (n = 140, 12.1%). Conversely, *Anopheles funestus* s.l. was most abundant in LTC (n = 416, 47.1%) and least in HLC (n = 60, 6.8%). All the *Anopheles* species were most frequent in HLC except *An. funestus* s.l. All species obtained by HLC were also collected by the other methods. Out of 234 mosquito sampling occasions over 39 nights (indoor and outdoor LTC, CB-LTC and HLC combined), there were five (2.1%) occasions without any *Anopheles* mosquitoes collected. Of the five zero catches, two occurred in outdoor CB-LTC, and one each in indoor LTC, outdoor LTC, and indoor CB-LTC, respectively. No zero catches occurred in indoor and outdoor HLC.

Table 5.1: Number and proportions of *Anopheles* species collected indoors (IN) and outdoors (OUT) by the different methods in Edo Kontola, Ethiopia 2014

Species	Venue	HLC		LTC		CB-LTC		Sum	
		n	%	n	%	n	%	n	%
An. arabiensis	IN	370	57.7	123	19.2	148	23.1	641	
	OUT	463	90.4	17	3.3	32	6.3	512	
	Total	**833**	**72.2**	**140**	**12.1**	**180**	**15.7**	**1153**	**15.2**
An.pharoensis	IN	44	31.6	60	43.2	35	25.2	139	
	OUT	180	88.7	14	6.9	9	4.4	203	
	Total	**224**	**65.5**	**74**	**21.6**	**44**	**12.9**	**342**	**4.5**
An. ziemanni	IN	330	36.9	323	36.1	241	26.9	894	
	OUT	1945	44.9	1111	25.6	1278	29.5	4334	
	Total	**2275**	**43.5**	**1434**	**27.4**	**1519**	**29.1**	**5228**	**68.7**
An. funestus s.l.	IN	22	4.4	220	43.7	261	51.9	503	
	OUT	38	10.0	196	51.6	146	38.4	380	
	Total	**60**	**6.8**	**416**	**47.1**	**407**	**46.1**	**883**	**11.6**
Total	IN	766	35.2	726	33.3	685	31.5	**2177**	**28.6**
	OUT	2626	48.4	1338	24.6	1465	27.0	**5429**	**71.4**
Overall *Anopheles*		3392	44.6	2064	27.1	2150	28.3	**7606**	**100.0**

Note: HLC: human landing catch, LTC: light trap catch and CB-LTC:CO_2 baited light trap catch.

The mean *Anopheles* mosquito catches/trap night for each species is given in Figure 5.2. The average density of female *Anopheles* collected by HLC was 52.4 (95% CI 39.9 - 66.2)

mosquitoes/man/night and the corresponding values of CB-LTC and LTC were 27.6 (95% CI 18.4 - 37.6) and 26.5 (95% CI 17.6 - 35.6) mosquitoes/trap/night, respectively.

There were statistically significant differences among the average number of *Anopheles* species captured by HLC ($F = 38.12$, $df = 3$, $p = 0.001$), LTC ($F = 11.17$ $df = 3$, $p = 0.001$) and CB-LTC ($F = 14.04$, $df = 3$, $p = 0.001$). Post-hoc analyses showed that HLC yielded significantly higher mean numbers of *An. ziemanni* ($F = 5.23$, $df = 2$, $p < 0.05$), *An. arabiensis* ($F = 60.14$, $df = 2$, $p < 0.001$) and *An. pharoensis* ($F = 36.26$, $df = 2$, $p < 0.001$) compared to either of the alternative methods. However, HLC caught significantly lower numbers of *An. funestus* s.l. than either LTC or CB-LTC ($F = 16.33$, $df = 2$, $p = 0.001$). Likewise, average mosquito catches by the three methods significantly varied between collection venues ($F = 14.98$, $df = 5$, $p = 0.001$). However, the average number of mosquito catches per house by HLC ($F = 0.417$, $df = 38$, $p > 0.05$), LTC ($F = 1.037$, $df = 38$, $p > 0.05$) and CB-LTC ($F = 1.23$, $df = 38$, $p > 0.05$) did not vary significantly by date of collection.

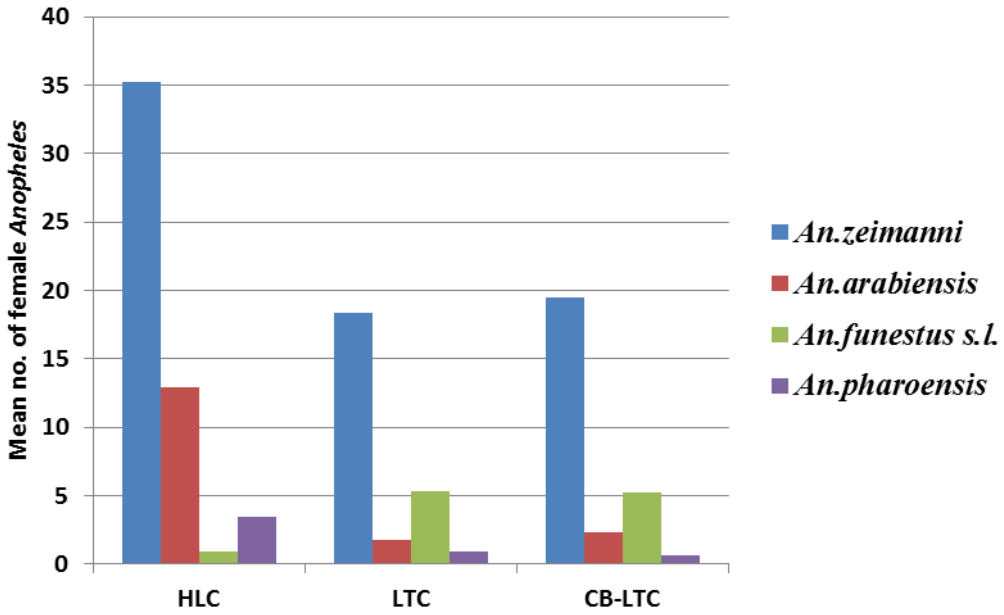

Figure 5.2: Mean number of female *Anopheles* species collected per person pernight, per LTC per night and per CB-LTC per night in Edo Kontola

5.3.2. Relative sampling efficiency (RSE) of the alternative traps versus human landing catch

Anopheles arabiensis. There was a weak positive correlation between indoor LTC and HLC for this species (r = 0.31) and the regression slope was not significantly different from zero (Table 5.2, Figure 5.3A), which means that the RSE of the indoor light traps were not dependent on mosquito density. The correlation between LTC and HLC for outdoor catches was positive and significant (r = 0.38), but the RSE of light traps was significantly dependent on outdoor abundance (Table 5.2, Figure 5.3B). Significant positive correlation (r = 0.49) was found between indoor CB-LTC and HLC; the RSE was not significantly dependent on mosquito density (Table 5.2, Figure 5.3C). However, for the outdoor CB-LTC and HLC, the regression slope was

significantly different from zero (Table 5.2, Figure 5.3D) meaning that RSE of outdoor CB-LTC was dependent on mosquito density.

Anopheles pharoensis. For this species, there were no significant correlations between LTC and HLC indoors or outdoors nor between CB-LTC and HLC indoors and outdoors (Table 5.2). The relative sampling efficiencies of indoor LTC and outdoor CB-LTC compared to HLC, respectively, were significantly dependent on mosquito density (Table 5.2, Figures 5.4A, 5.4D). However, the relative sampling efficiencies of outdoors LTC and indoor CB-LTC compared to HLC respectively did not depend on mosquito density (Table 5.2, Figures 5.4B, 5.4C).

Anopheles ziemanni. A positive significant correlation was found between LTC and HLC indoors ($r = 0.43$) as well as outdoors ($r = 0.78$). Similarly, there were positive and significant correlations between CB-LTC and HLC both indoors ($r = 0.63$) and outdoors ($r = 0.80$). None of the regression slopes were significantly different from zero (Table 5.2, Figures 5.5A-D), meaning that the relative sampling efficiencies were not dependent on mosquito density.

Anopheles funestus s.l. For this species complex, all the regression slopes were significantly different from zero, indicating that both LTC: HLC ratio and CB-LTC: HLC ratios were dependent on the mosquito density indoors and outdoors. That means both of the alternative traps were not consistent for sampling *An. funestus* s.l. (Table 5.2, Figures 5.6A-D).

Table 5.2: Correlation and regression analysis of log-transformed indoor (IN) and outdoor (OUT) HLC with either LTC or CB-LTC of *Anopheles* species in Edo Kontola, 2014.

Species	Alternate method vs. HLC	Venue	Correlation coefficient			Regression slope			
			n	r	p	b	95% C.I.	t	p
An. arabiensis	LTC	IN	39	0.308	0.056	0.073	-0.25-0.40	0.451	0.654
		OUT	39	0.378	< 0.05	-0.880	-1.20- -0.56	-5.565	<0.001
	CB-LTC	IN	39	0.493	0.001	-0.063	-0.33-0.20	-0.471	0.640
		OUT	39	0.288	0.076	-0.691	-0.91- -0.47	-6.393	<0.001
An. pharoensis	LTC	IN	39	-0.019	0.906	0.519	0.14-0.89	2.792	<0.05
		OUT	39	0.243	0.136	-0.352	-0.73-0.03	-1.839	0.074
	CB-LTC	IN	39	0.235	0.150	0.078	-0.27-0.43	0.443	0.660
		OUT	39	0.133	0.419	-0.622	-1.05- -0.18	-2.897	<0.05
An. ziemanni	LTC	IN	39	0.427	< 0.05	0.081	-0.20-0.36	0.581	0.565
		OUT	39	0.775	< 0.001	0.034	-0.12-0.19	0.442	0.661
	CB-LTC	IN	39	0.627	< 0.001	0.005	-0.21-0.22	0.048	0.962
		OUT	39	0.795	< 0.001	0.109	-0.03-0.25	1.544	0.131
An. funestus s.l	LTC	IN	39	-0.164	0.317	0.948	0.71-1.18	8.084	<0.001
		OUT	39	0.316	0.050	0.522	0.28-0.75	4.488	<0.001
	CB-LTC	IN	39	0.024	0.885	0.846	0.63-1.05	8.220	<0.001
		OUT	39	0.463	< 0.05	0.423	0.21-0.63	4.051	<0.001

Note: n = sample size, r = Pearson's correlation coefficient, b = regression slope, C.I. = confidence interval, t = t-test value, p = probability value. The correlation coefficients show the relationship between log(LTC + 1) and log(HLC + 1), log(CB-LTC + 1) and log(HLC + 1). The regression slopes are from regressing relative sampling efficiencies (log(LTC + 1) - log(HLC + 1)) on average abundance ([log(LTC + 1) + log(HLC + 1)]/2) and also (log(CB-LTC + 1) - log(HLC + 1)) on average abundance ([log(CB-LTC + 1) + log(HLC + 1)]/2).

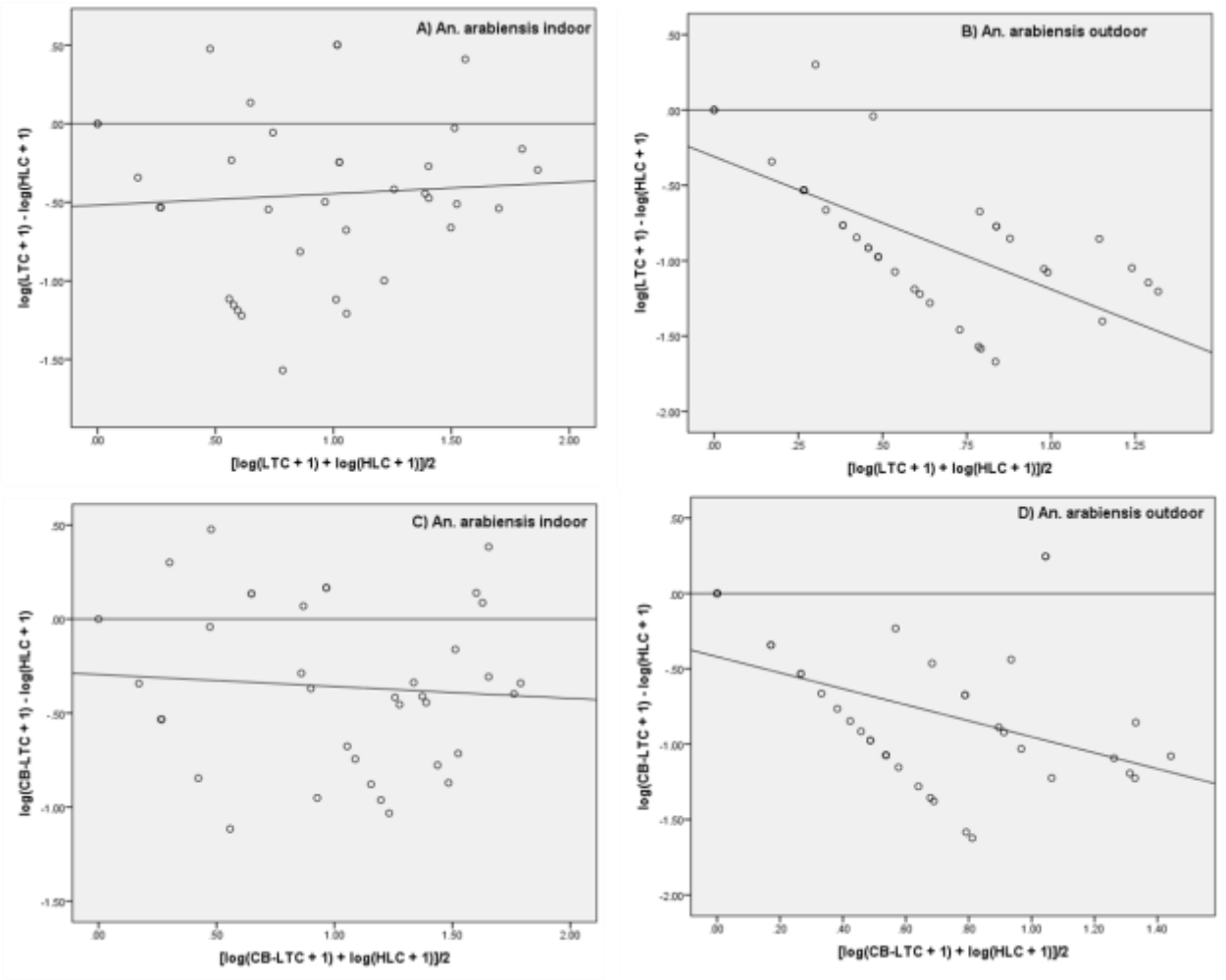

Figure 5.3: Relationship between RSE of indoor (A) and outdoor (B) LTC (upper panels), indoor (C) and outdoor (D) CB-LTC (lower panels) and abundance of *An. arabiensis*. RSE is the difference in the mosquito catches by either of the alternative methods and the human landing catch (y-axis). The mosquito abundance is the joint average of each alternative and the reference method (x-axis).

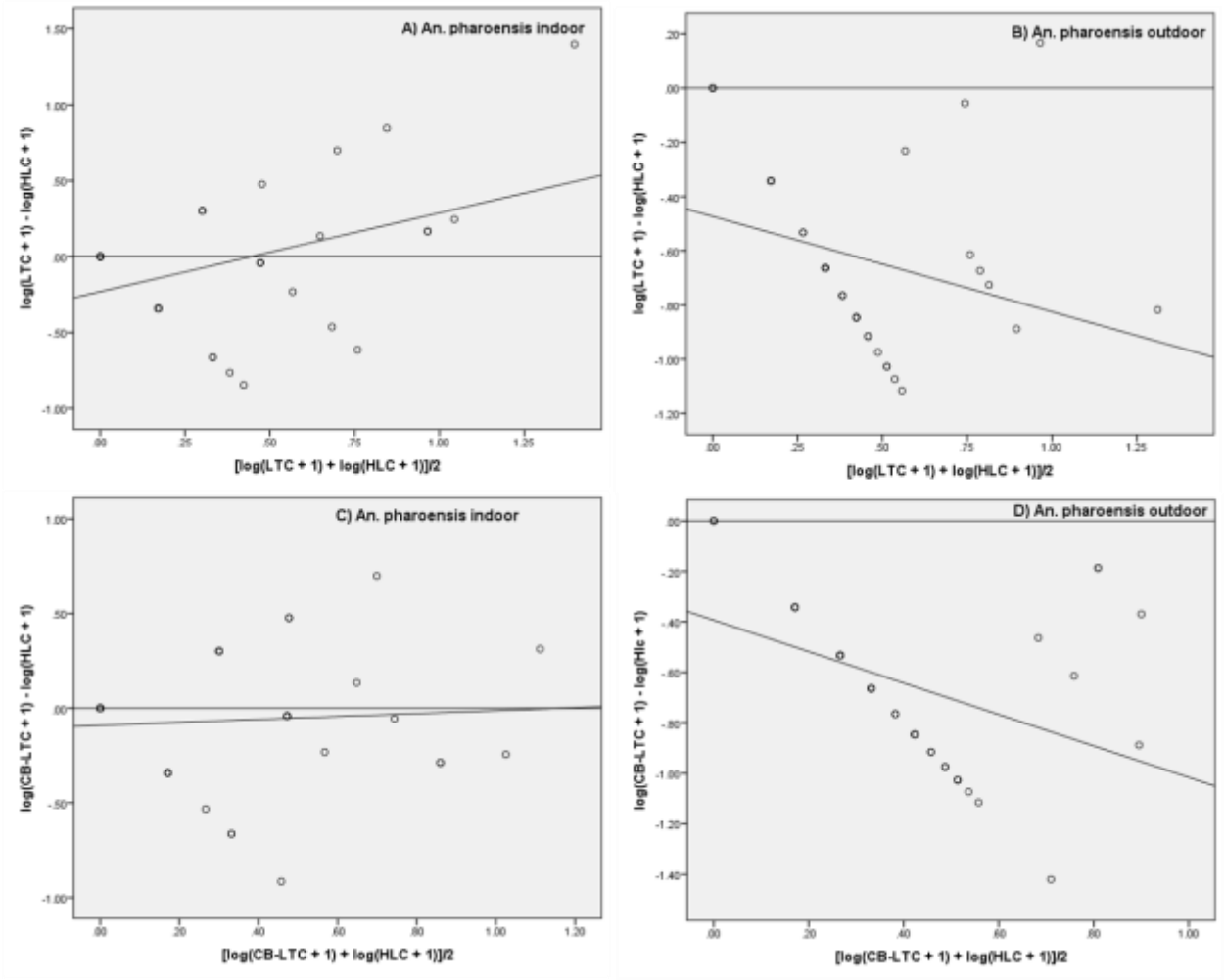

Figure 5.4: Relationship between RSE of indoor (A) and outdoor (B) LTC (upper panels) , indoor (C) and outdoor (D) CB-LTC (lower panels) and density of *An. pharoensis*. RSE is the difference in the mosquito catches by either of the alternative methods and the human landing catch (y-axis). The mosquito abundance is the joint average of each alternative and the reference method (x-axis).

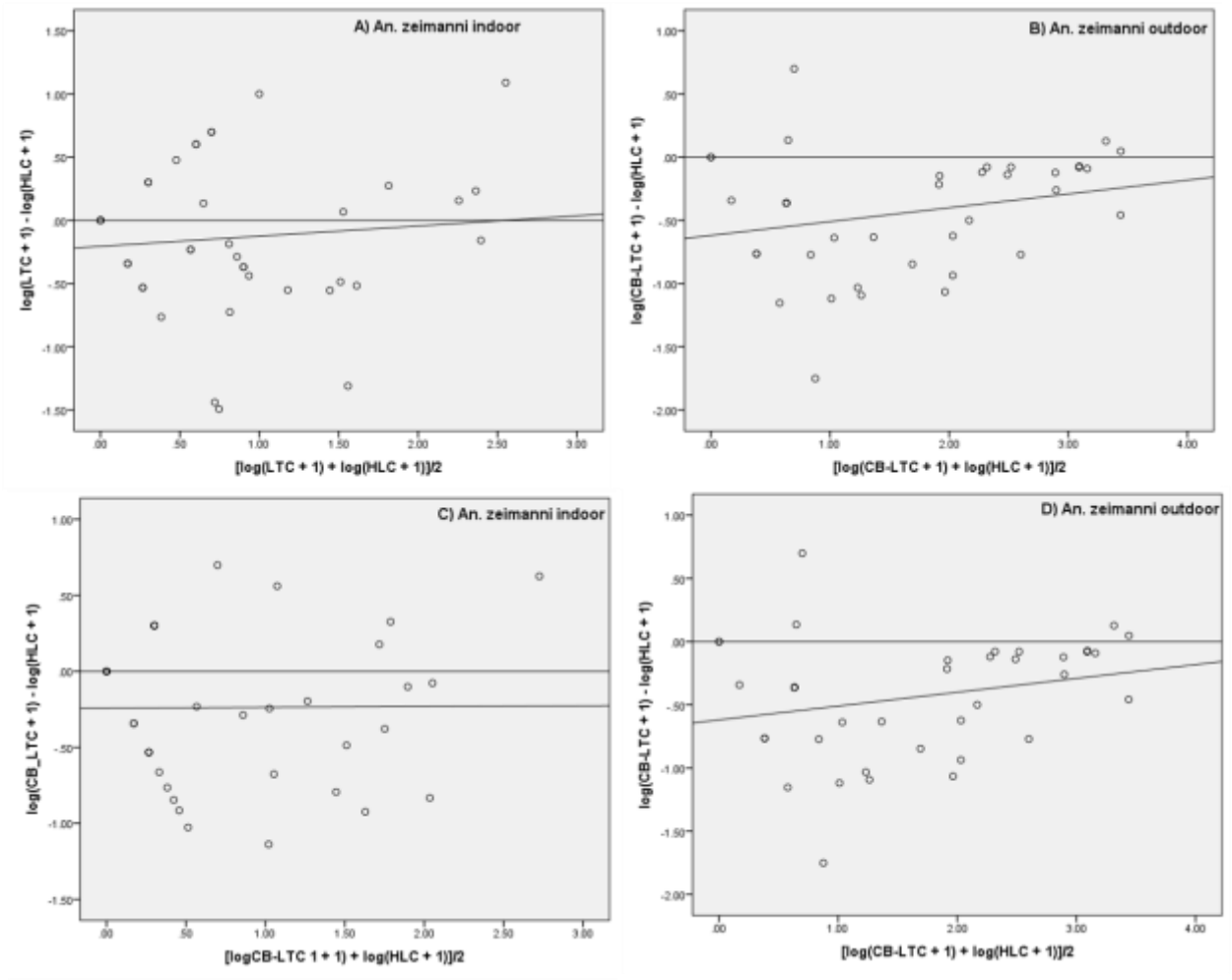

Figure 5.5: Relationship between RSE of indoor (A) and outdoor (B) LTC (upper panels), indoor (C) and outdoor (D) CB-LTC (lower panels) and abundance of *An. ziemanni*. RSE is the difference in the mosquito catches by either of the alternative methods and the HLC (y-axis). The mosquito abundance is the joint average of each alternative and the reference method (x-axis).

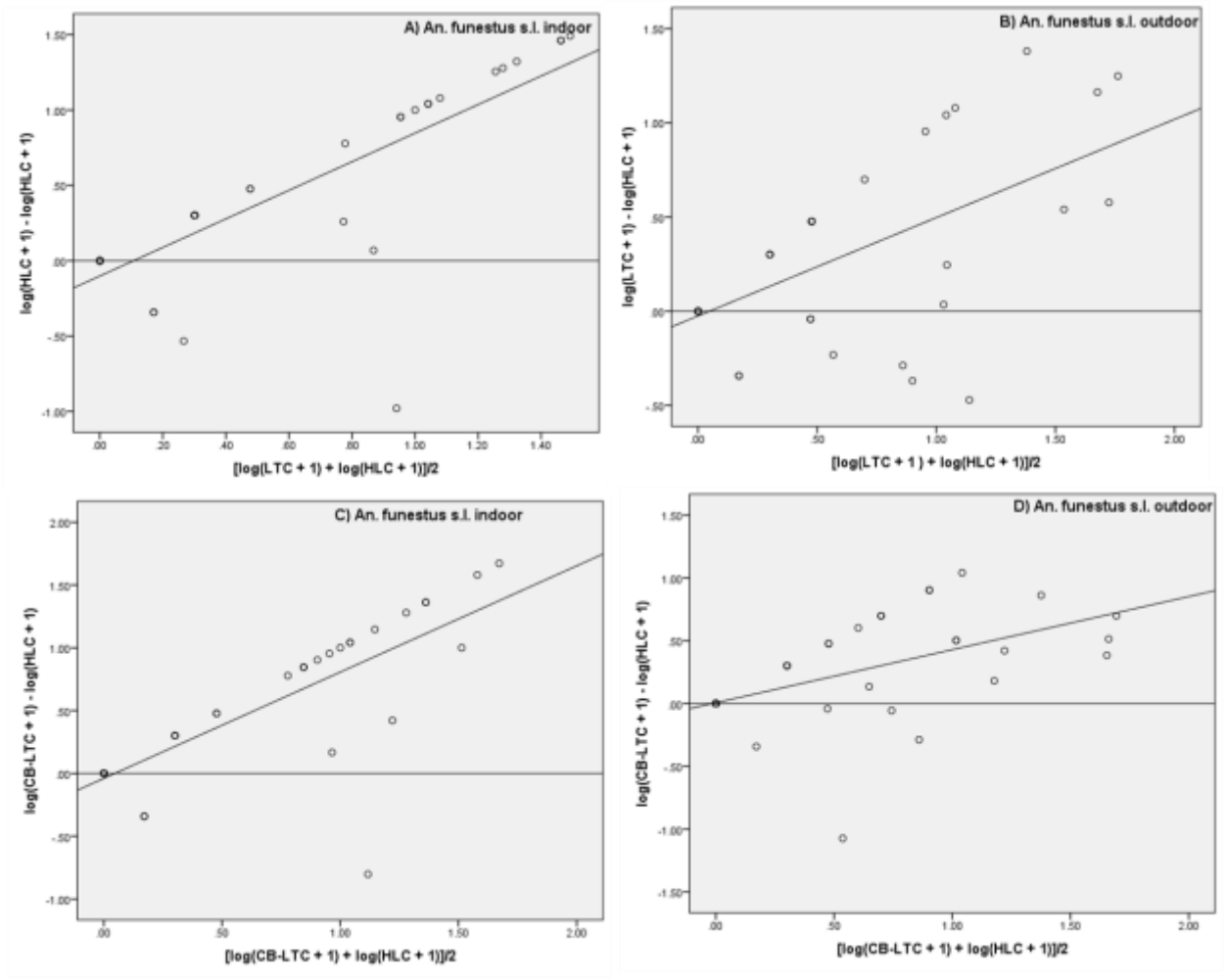

Figure 5.6: Relationship between RSE of indoor (A) and outdoor (B) LTC (upper panels), indoor (C) and outdoor (D) CB-LTC (lower panels) and abundance of *An. funestus* s. l.. RSE is the difference in the mosquito catches by either of the alternative methods and the HLC (y-axis). The mosquito abundance is the joint average of each alternative and the reference method (x-axis).

5.3.3. Conversion factors to estimate human biting rates

The mean log ratios of LTC and CB-LTC vs. HLC, respectively were negative (Table 5.3), meaning that the sampling efficiencies were lower than HLC and thus less efficient than HLC for

mosquito sampling in this setting. On average, the indoor LTC was 0.35 times that of indoor HLC and the indoor CB-LTC was 0.44 times that of HLC for *An. arabiensis*. For *An. pharoensis,* outdoor LTC caught on average 0.24 times that of outdoor HLC while indoor CB-LTC captured 0.86 times indoor HLC. However, for *An. ziemanni* on average, the catches from indoor and outdoor LTC were 0.73 and 0.39 times that from indoor and outdoor HLC, respectively. For the same species, on average indoor and outdoor CB-LTC captured 0.58 and 0.36 times the mosquito catch size of that of indoor and outdoor HLC, respectively.

Table 5.3: Mean log ratios and corresponding geometric mean ratios of alternative mosquito collection methods (light traps, LTC and CO_2-baited light traps, CB-LTC) against the reference method (human landing catches, HLC) for sampling *Anopheles* species in Edo Kontola, Ethiopia.

Species	Alternative method vs. HLC	Venue	Mean log ratio*	Standard error of the mean (S. E. M.)	Geometric mean ratio (GMR)	95% CI
An. arabiensis	LTC	IN	-0.4528	0.081	0.35	0.24-0.50
	CB-LTC	IN	-0.3578	0.067	0.44	0.33-0.59
An. pharoensis	LTC	OUT	-0.6281	0.052	0.24	0.18-0.29
	CB-LTC	IN	-0.0640	0.052	0.86	0.67-0.92
An. ziemanni	LTC	IN	-0.1341	0.095	0.73	0.48 -0.87
	LTC	OUT	-0.4098	0.074	0.39	0.27-0.54
	CB-LTC	IN	-0.2379	0.076	0.58	0.41-0.81
	CB-LTC	OUT	-0.4389	0.075	0.36	0.25-0.49

*Negative mean log ratio indicates that the efficiency of LTC and CB-LTC each were lower than HLC

5.3.4. Sampling efficiency of light traps with versus without CO2 bait

Mosquito sampling efficiency of LTC versus CB-LTC is given in Table 5.4. For *An. arabiensis*, there was a weak positive (r = 0.31) correlation between LTC and CB-LTC indoors but there was no significant correlation outdoors. The regression slopes were neither significantly different from zero indoors nor outdoors meaning that the RSEs were not dependent on indoor and outdoor *An. arabiensis* density in this setting. For *An. pharoensis*, the correlations between LTC and CB-LTC, both indoors and outdoors, were not significant. The RSEs of LTC and CB-LTC were significantly dependent on mosquito density both indoors and outdoors for this species. For *An. ziemanni*, a positive significant correlation was observed between LTC and CB-LTC indoors as well as outdoors and the RSEs were not dependent on mosquito density. Similarly, for *An. funestus* s.l., there were significant and positive correlation between LTC and CB-LTC indoors and outdoors and the relation was not density dependent.

Table 5.4: Correlation and regression analysis of log-transformed indoor and outdoor LTC with CB-LTC of *Anopheles* species in Edo Kontola, 2014.

		Correlation coefficient			Regression slope			
Species	Venue	n	r	p	b	95% C.I.	t	p
An. arabiensis	IN	39	0.311	0.054	0.287	-0.01-0.57	1.992	0.054
	OUT	39	-0.073	0.659	0.059	-0.44-0.56	0.237	0.814
An. pharoensis	IN	39	0.177	0.281	0.524	0.23-0.81	3.711	0.001
	OUT	39	-0.105	0.526	0.666	0.29-1.03	3.631	0.001
An. ziemanni	IN	39	0.643	<0.001	0.105	-0.09-0.31	1.056	0.298
	OUT	39	0.852	<0.001	-0.046	-0.17-0.08	-0.727	0.472
An. funestus s.l.	IN	39	0.525	0.001	0.082	-0.16-0.32	0.679	0.501
	OUT	39	0.507	0.001	0.179	-0.06-0.41	1.514	0.138

Note: n = sample size, r = Pearson's correlation coefficient, b = regression slope, C.I. = confidence interval, t = t-test value, p = probability value. The correlation coefficients show the relationship between log (LTC + 1) and log (CB-LTC + 1). The regression slopes are from regressing relative sampling efficiencies (log (CB-LTC + 1) - log (LTC + 1)) on average abundance ([log (CB-LTC + 1) + log (LTC + 1)]/2)

5.4. Discussion and conclusions

The ultimate aim of this study was to determine reliable conversion factors between either light trap (LTC) alone or light traps baited with yeast-generated carbon dioxide (CB-LTC) both set beside occupied long-lasting insecticidal nets against human landing (HLC) for entomological monitoring of the impact of malaria control interventions. The results showed that the HLC was the most efficient method compared to both LTC and CB-LTC for sampling the majority of the

Anopheles species including the major malaria vector, *An. arabiensis*. The results showed that despite lower relative *Anopheles* sampling efficiencies of both LTC and CB-LTC compared to HLC, they can be used as alternative to indoor HLC of *An. arabiensis*.

It was estimated that on average, indoor LTC caught 0.35 times the number of *An. arabiensis* as compared to indoor HLC. This implies that LTC is a less sensitive means to estimate indoor human biting activities of *An. arabiensis* compared to HLC. Despite lower efficiency, indoor LTC was comparable with that of HLC for *An. arabiensis* in the study area, because there was no significant tendency for the RSE of LTC to be affected by changes in *An. arabiensis* density. This finding was consistent with other studies (Lima *et al*., 2014; Mathenge *et al*., 2005; Govella *et al*., 2011; Dia *et al*., 2005; Overgaard *et al*., 2012; Okumu *et al*., 2008) which support that HLC is the most efficient sampling method for anthropophilic *Anopheles* mosquitoes and for routine monitoring of malaria vectors.

In contrast to these results, several studies (Lines *et al*., 1991; Costantini *et al*., 1998; Davis *et al*., 1995; Fornadel *et al*., 2010; Duo-quan *et al*., 2012; Kalima *et al*., 2014) showed higher sampling efficiency of LTC compared with HLC for different *Anopheles* species including *An. arabiensis*. This was particularly so in Ahero, Kenya (Mathenge *et al*., 2005) and in Macha, Zambia (Fornadel *et al*., 2010) where *An. arabiensis* was the sole *An. gambiae* sibling species. Similarly, in the present study *An. arabiensis* was the only member of *An. gambiae* complex. These observed differences in sampling efficiencies could be explained by local variations in host-seeking behaviours of *An. arabiensis* across Africa (Coetzee *et al*., 2000). Further possible reasons could be attributed to the crude nature of both sampling methods due to lack of operational standard procedures regarding trap placement, operation time, etc. in real world

settings (Briet *et al*., 2015). To make more valid comparisons these procedures should be standardized.

Moreover, the high efficiency of HLC versus LTC for sampling host-seeking *An. arabiensis* reflects the basic differences between the two methods. In the case of HLC, a cocktail of stimuli that attract host-seeking mosquitoes such as olfactory, visual cues, volatiles, body heat and humidity are present (Takken and Verhulst, 2013). Mosquitoes respond to such stimuli and can target the appropriate site for taking a blood meal. By contrast, LTC use mainly visual stimuli. Further, although the presence of human-occupied LLINs besides LTC is expected to augment the trap catches, the excito-repellent effect of the net might have decreased the efficiency of the LTC (Magbity *et al*., 2002), although some studies have shown that using LLINs have little or no impact on the efficiency of LTC (Govella *et al*., 2009; Kirby *et al*., 2008).

The present results also indicate that the correlation between LTC and HLC for outdoor *An. arabiensis* was statistically significant, but the relative sampling efficiency of LTC was significantly dependent on mosquito density. Such results were expected because *An. arabiensis* can have more diverse alternative hosts outdoors than indoors which might have diverted more host-seeking individuals from outdoor light traps to human and other animal hosts. *Anopheles arabiensis* is known to be flexible in host-preferences and indoor/outdoor feeding based on availability of domestic animals (Massebo *et al*., 2015). Furthermore, feeding behaviour of *An. arabiensis* can be influenced by indoor and outdoor availability of hosts (Ameneshewa and Service, 1996) and availability of cattle in the homestead (Seyoum *et al*., 2002; Tirados *et al*., 2006). There were plenty of cattle in Edo Kontola and this might be a potential cause for poor performance of the LTC in outdoor situations. Similar to the present results, some previous reports indicate that light traps were less efficient outdoors (Mboera, 2005). The present findings

therefore suggest that light traps may not be a reliable alternative to HLC for sampling *An. arabiensis* outdoors in this setting.

Results also revealed that CB-LTC was less efficient than HLC. It was estimated that on average the RSE of indoor CB-LTC was 0.44 times that of indoor HLC for *An. arabiensis*. The RSE of the trap was not significantly dependent on indoor density of *An. arabiensis*. This finding is in line with a study on *An. aquasalis* in Suriname that showed high efficiency of HLC compared to carbon dioxide baited traps (Hiwat *et al*., 2011).Yeast-produced CO_2 was originally developed to be compared with the standard and industrial mosquito attractants specifically CO_2 from dry ice, pressurized gas cylinders or propane as cheaper and more accessible alternative in remote localities (Saitoh *et al*., 2004). As a result, most existing evidence show the efficacy of traps baited with yeast generated CO_2 versus traps baited with the standard attractants (Smallegange *et al*., 2010; Saitoh *et al*., 2004; Oli and Jeffery, 2005, Obenauer *et al*., 2013). Smallegange *et al.* (2010) reported that traps baited with yeast-produced CO_2 caught similar number of *An. arabiensis* as traps baited with the standard industrial CO_2 and addition of human odour increased the trap catches. Based on this, it can be recommended that yeast generated CO_2 is a promising alternative to HLC and standard mosquito attractants for indoor collection of *An. arabiensis*. However, further studies are required to optimize the efficacy of CB-LTC, industrial CO_2 baited traps and the HLC in Ethiopia and elsewhere.

For the outdoor collection of the same species, there was consistent correlation between CB-LTC and HLC. However, the RSE of CB-LTC was significantly dependent on *An. arabiensis* density. This could be attributed to the diverse alternative hosts for *An. arabiensis* that compete its host-seeking attentions in outdoor settings. Further, outdoor environmental factors such as temperature, humidity and wind speed might affect fermentation of yeast-sugar solution and

hence the trap efficacy. In addition, persistence, flow rate and impact radius (attractive range) of the CO_2 volatile have not been optimized and warrant further study.

For *An. pharoensis*, although outdoor LTC captured 0.24 times that of outdoor HLC and indoor CB-LTC caught 0.86 times that of indoor HLC, the RSE of this species in indoor LTC and outdoor CB-LTC were affected by the mosquito density. These collection methods may not be appropriate for estimating reliable *An. pharoensis* human biting rates. This might be attributed to exophilic and zoophilic behaviour of this species (Abose *et al*., 1998).Thus, the presence of cattle in the surrounding area might have affected comparison of the sampling methods for this species. Therefore, further studies should consider animal baited traps to assess the impact of cattle on the efficacy of the sampling methods.

However, for indoor and outdoor *An. ziemanni* catches there were significantly consistent relationships between HLC and either of the two methods, respectively. It was estimated that on average, the efficiency of indoor LTC was 0.73 times that of indoor HLC and the corresponding outdoor LTC was 0.39 times that of outdoor HLC. The RSE of LTC was not significantly dependent on either indoor or outdoor *An. ziemanni* density. Based on these results it can be suggested that despite relatively low efficiency of LTC for collecting *An. ziemanni* indoors and outdoors, LTC can be used to determine reliable conversion factors for estimating human biting rates for this species. The RSE of HLC for collecting *An. ziemanni* indoors, shows endophagic and anthropophilic tendencies of this species. These results suggest that further studies should determine the vectorial role and potential public health importance of this species.

Likewise, the relationship between CB-LTC and HLC for indoor and outdoor *An. ziemanni* catches was statistically significant regardless of its density. On average, indoor CB-LTC yielded

0.58 times the number of *An. ziemanni* captured by indoor HLC, whereas outdoor CB-LTC caught 0.36 times the number of this species collected by outdoor HLC. Though *An. ziemanni* is known to feed predominantly on cattle in Ethiopia (Kibret *et al.*, 2010) the present results clearly showed its anthropophilic tendencies as captured by HLC. These contrast some studies (Dekker and Takken, 1998) which support that CO_2 attract more zoophilic and opportunistic anopheline species than anthropophilic ones, but shares the idea that CO_2 plays an important role in host seeking process of zoophilic, opportunistic and anthropophilic mosquito species (Smallenge *et al*., 2010).

For *An. funestus* s.l., there was no consistency between either LTC or CB-LTC with HLC indoors and outdoors. The mosquito sampling efficiency of both methods was significantly dependent on the mosquito density. This means that both methods may not be suitable for collection of host-seeking *An. funestus* s.l. as an alternative to HLC in this area. This is in contrast to some studies (Mathenge *et al*., 2005) that found consistent proportionality between LTC and HLC for *An. funestus* s. l. and the recent analyses (Briet *et al*., 2015) that showed that LTC were able to collect similar number of *An. funestus* s.l. with HLC in Africa. The differences might be attributed to geographical and ecological variations (Fornadel *et al*., 2010) coupled with variations in behaviour of the subspecies in the *An. funestus* group.

Finally, although the main objective of this study was to estimate the RSE of either LTC or CB-LTC against HLC and hence determine conversion factors for effective monitoring of the impact of IRS and LLINs interventions, the RSE of LTC against CB-LTC were also compared. The correlation between LTC and CB-LTC indoors for *An. arabiensis* was weakly positive regardless of mosquito density, but in outdoor venues there was no significant correlation for *An. arabiensis* catches regardless of the mosquito density. Based on these results it can be suggested that CB-

LTC does not substantially improve sampling of this major vector compared to LTC in this setting for both indoor and outdoor venues

In conclusion, mosquito collection efficiency of the sampling methods varied by *Anopheles* species. The HLC was more efficient than either of the alternative methods (LTC and CB-LTC) for sampling *An. arabiensis*, the major malaria vector in the study area. However, the RSEs of either of the two alternative methods were consistent and comparable with HLC for monitoring *An. arabiensis* indoors, but not outdoors. Therefore, CDC light traps with or without yeast-produced CO_2 represents an alternative to HLC for large scale indoor *An. arabiensis* surveillance and monitoring because of the various problems associated with using HLC. However, adding yeast-produced CO_2 to light traps does not seem to improve the sampling effectiveness of these traps in these settings.

Chapter 6. Impact of IRS and LLINs combined versus separate interventions on mosquito density, longevity and malaria infectivity

6.1. Introduction

In Ethiopia, IRS and LLIN are scaled-up and intensively implemented in combination or separately for malaria control interventions (MOH, 2014). Universal coverage of both interventions has been promoted and there is a growing demand in combinations of interventions for malaria control and elimination. However globally evidence is contradictory whether the combination intervention is better than their isolation (WHO, 2014). Both interventions primarily target *An. arabiensis* the sole major and widespread vector in the country. Nevertheless, entomological outcomes of vector control intervention trials that target *An. arabiensis* is lacking in Ethiopia. IRS and LLINs combined intervention trial results elsewhere in Africa on *An. gambiae* s.l. (Corbel *et al*., 2012; Protopopoff *et al*., 2015; Pinder *et al*., 2015) could not be specifically extrapolated because of locally variable environmental factors and unique bionomics of *An. arabiensis*. Evidence of such vector control interventions is important to help national malaria control programmes and international funding agencies to make sound decisions. Moreover, such evidence saves not only millions of lives due to improved effectiveness, but may also waste resources because costs of combined interventions are greater than costs of single interventions (West *et al*., 2014). Therefore, this study assessed the impact of IRS and LLINs combined intervention on host-seeking density, indoor resting density, parity rates (longevity) and infectivity of *An. arabiensis* compared to their individual interventions in Adami Tullu district, south-central Ethiopia.

6.2. Materials and methods

6.2.1. Study area

The study area is described in details in the published protocol (Deressa *et al*., 2016) and under subheading 3.1. in this thesis. Briefly, the study was carried out in 13 *kebeles* located within 5 km distance from Lake Zeway and Bulbula River in Adami Tullu district. Entomological collections were done in randomly selected clusters (villages) from the 13 *kebeles* (Figure 6.1).

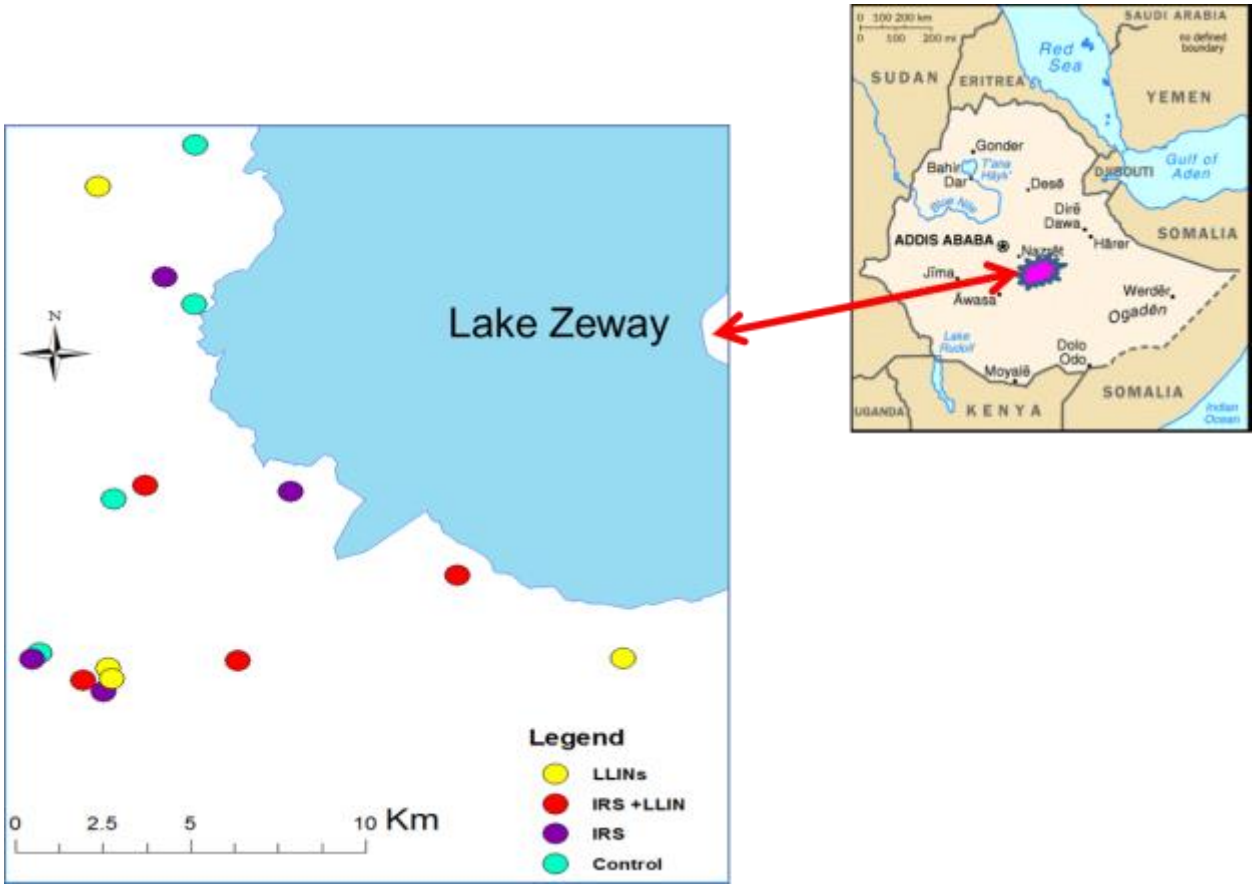

Figure 6.1: Distribution of village clusters in the study setting, Adami Tullu, 2014-2015

6.2.2. Study design, randomization and intervention

To assess the impact of IRS and LLINs combined and separate interventions on entomological outcomes, a cluster-randomized controlled trial (CRT) was carried out across rural clusters (villages or *gares*) of Adami Tullu district, during the 2014 and 2015 transmission seasons. In short, this study is a four arm CRT consisting of IRS, LLINs, IRS+LLINs, and a control.The target population were rural communities where both LLINs and IRS are prioritized for intervention. The target group for participation in both intervention and control arms of the trial were all people residing in the selected clusters. A cluster (village) is defined as a geographical division of a *kebele* which contains about 35 households, and this was the unit of randomization for the intervention. These clusters were selected from the 13 *kebeles* adjacent to Lake Zeway and Bulbula River in the study district based on the findings of a pre-intervention study and mapping of all *kebeles* in the district (Deressa *et al*., 2016).

An equal number of clusters were randomized to receive IRS+LLINs, IRS, LLINs, or control (control arm: the routine practice). The control arm of the trial was used for comparison and the communities received the routine practice of malaria prevention by the District Health Office (DHO) in collaboration with the project (Deressa *et al*., 2016). The control and intervention communities recieved early diagnosis and treatment through weekly home visit for malaria case detection free of charge. However, to the best of my knowledge, there were no vector interventions by IRS or LLINs or both over the two transmissions in the control arm provided by both the DHO and the project.

The 13 rural *kebeles* adjacent to Lake Zeway and Bulbula River were purposively included in this trial with the aim of getting more entomological data for the detailed investigation of impacts of the interventions on local vector bionomics. All villages located within 5 km radius from the main

breeding site in the selected *kebeles* were listed and used as the unit for randomization for this trial. From the 13 *Kebeles,* 44 clusters per arm for epidemiological and four clusters per arm for entomological studies were randomly selected. All villages and houses were numbered and then randomised to intervention and control arms of the trial following a computer-generated list using SPSS software. Due to the nature of the interventions, blinding of the study participants was not possible. Mosquito collector bias has been reduced using automated standard mosquito traps.

Following randomization, all households in the IRS+LLIN and LLIN arms of the trial received new LLINs free of charge provided by the project.The LLINs used for this trial were PermaNet 2.0 rectangular 100denier, purchased in June 2014 from Vestergaard Frandsen Group SA (Vestergaard Frandsen, Lausanne, Switzerland). PermaNet 2.0 is a factory-treated mosquito net manufactured with deltamethrin at a WHO approved LLIN containing 55 mg active ingredient per m^2 that is expected to retain its biological efficacy for a minimum of 20 standards WHO washes (Hwang *et al*., 2011). The life span of LLINs is about 3 years under field conditions (Hwang *et al*., 2011), which is sufficient to cover malaria transmission season in 2014-2015 trial years. The target households received light blue family size (160 cm width x 180 cm length x 150 cm height) models according to the number of LLINs recommended based on family size. The national malaria guidelines recommends one net for a family of 1-2 persons, two nets for a family of 3-5 persons, three nets for a family of 6-7 persons and four nets for a family of 8 and above people (MOH, 2012). Net use and retention at the household level was monitored and new nets were annually replaced for households that reported damaged or lost LLINs.

However, IRS with propoxur was applied in the IRS + LLIN and IRS arms of the trial in one spray round per year prior to the peak transmission season. Propoxur is an isopropoxy-phenyl methyl carbamate highly bioefficacious against mosquito vectors for 3-6 months at the dosage of

2 g/m^2 in the form of a water-dispersible powder. The residual activity of propoxur is sufficient to cover the main malaria season that take place from September-November. It was purchased in 2014 from the state-owned Adami Tullu Pesticide Processing Share Campany located in the study district. Propoxur 50% contains 2 g of active ingredient and packaged in 400 g/sachet. Two sachets (800 g) were mixed in 8 L of water. The IRS was conducted once a year according to the national spraying operation guidelines (MOH, 2012). IRS was deployed using 8L Hudson X-pertsprayer (HD Hudson Manufacturing Company, Chicago, IL USA).The interior walls and ceilings of each dwelling were sprayed with propoxur at 2 g/m^2.

6.2.3. Mosquito collections and processing

Exposure to malaria transmission was assessed by collecting vector mosquitoes in randomly selected villages and houses using light trap catch (LTC), Prythrum spray catch (PSC), and artificial outdoor pit shelter (PIT). These three collection methods were each carried out in 16 randomly selected clusters. LTC and PIT were each done in four clusters per study arm, one house per cluster whereas PSC was performed in four clusters per arm but four houses per cluster. LTC, PSC and PIT were used to monitor the impact of the interventions on host-seeking density (HSD), indoor resting density (IRD) and outdoor resting density (ORD) of malaria vectors respectively and their infection rates with malaria aswell. The operational procedures used for LTC, PSC and PIT have been described in section 3.2. In addition, HLC was employed in selected clusters to monitor the impact of IRS and LLINs interventions on local mosquito human biting rates as described under section 7.2.2.

Mosquito processing procedures followed for estimation of entomological indices particularly dissection of the female mosquito to obtain ovaries for parity determination and sporozoite assays

by direct ELISA for determination of *P. falciparum* and *P. vivax* sporozoites rates have been described under 3.3. For parity rate estimation, ovaries of unfed mosquitoes obtained by HLC from the study arms were dissected using WHO-recommended techniques (WHO, 2011; 2013) as briefly explained under 3.3 as well. The mosquitoes were selected for dissection from the study arms based on availability of fresh unfed mosquitoes convenient for ovary dissection from the study arms per the collection method. However sporozoite ELISA was carried out for most of the mosquito specimens obtained by LTC, PSC, PIT and HLC as described by Beier *et al*. (1987).

6.2.4. Data analysis

Mean mosquito density obtained by different sampling methods were compared among the study arms. Indoor host seeking mosquito density (HSD) was assessed by indoor LTC and calculated as the total number of mosquitoes of each species collected divided by the total number of light trap collection nights (mosquitoes/trap/night). Indoor resting density (IRD) was assessed by PSC and expressed as the total number of mosquitoes of each species divided by the number of houses and collection days (mosquitoes/house/day). Outdoor resting density (ORD) was assessed by PIT and calculated as the total number of each species divided by the number of pits and collection days (mosquitoes/pit/day).

Mean mosquito HBR obtained by LTC, PSC and HLC were also compared among the study arms. For indoor mosquito collections using LTC, the estimated HBR was calculated by dividing the total number of mosquitoes caught indoors using a conversion factor of 0.35 for *An. arabiensis*, representing species-specific relative efficiency to account for the lower efficiency of LTC relative to HLC (Chapter 5). The HBR for indoor LTC was not adjusted for the number of household inhabitants because it is considered proportionally representative of true adult exposure

(Lines *et al*., 1991). For PSC, the HBR (the number of biting mosquitoes per human-night) was also estimated by dividing the total number of blood-fed mosquitoes caught in PSC by the total number of human occupants who spent the night in the houses used for collection (WHO, 2011). For mosquito collections by HLC, the real HBR was directly calculated as the mean number of bites received per person per night of collection (b/p/n) (WHO, 2011).

Determination of mosquito parity rate relied on data obtained from HLC because, mosquitoes that were captured by PSC and PIT were mainly blood fed and gravid mosquitoes whereas mosquitoes caught by LTC contained low number of unfed mosquitoes convenient for ovary dissection. The parity rate was estimated as the number of parous females divided by number of females examined multiplied by 100 (WHO, 2013).

Mean *Anopheles* mosquito densities, parity rates and human biting rates collected by each mosquito sampling method was compared among study arms using negative binomial regression in Generalized Linear Models (GLMs). The impact of interventions on vector indices (vector parameters) were therefore estimated by exponentiation of negative binomial regression coefficient, i. e., Incidence Rate Ratio (IRR) at p-value < 0.05 significant level.

6.3. Results

6.3.1. *Anopheles* species abundance

Altogether 1786 female *Anopheles* of four species were collected over two transmission seasons, by three mosquito sampling methods (LTC, PSC and PIT) plus HLC which was performed during one transmission season (Table 6.1). *Anopheles pharoensis* was predominant (60.7%), followed by *An. arabiensis* (32.1%), *An. ziemanni* (6.9%) and *An. funestus* s.l. (0.3%). The highest number

of *Anopheles* mosquitoes were collected from the control arm (41.3%) followed by LLINs (25.4%), IRS (18.0%) and IRS + LLINs (15.8%) arms. Each of the *Anopheles* species was most frequently obtained from the control arm as compared to each of the intervention arm.

Anopheles arabiensis captured indoors by LTC was most abundant in the control arm (87.1%) and least in IRS (2.4%) and IRS + LLINs (2.4%) arms. However, by PSC, it was most frequently caught indoors from LLIN arm (53.1%) and least from IRS arm (3.7%). However, the number collected outdoors from LLIN arm (66.6%) was predominant compared to the number obtained from IRS (15.2%), control (12.1%) and IRS + LLINs (6.1%) arms. Using HLC, this species was most frequently collected indoors (53.3%) and outdoors (39.6%) in the control arm and least from indoors (7.2%) and outdoors (11.8%) in the IRS + LLINs arm.

In the indoor LTC, *An. pharoensis* was predominant in the control arm (81.2%) and least in the LLINs arm (0.5%). Conversely, using PSC, this species was most abundant in the LLINs arm (75.0%) and least in the control arm (25.0%) and it did not occur in the IRS and IRS + LLINs arms. In outdoor pit shelters, the occurrence of this species was similar in IRS and IRS + LLINs arms and so was its abundance in the control and LLINs arms. However, in HLC, indoor and outdoor abundance of this species was similar in the control and intervention clusters. Few specimens of *An. ziemanni* and *An. funestus* s.l. were caught using the LTC, PSC and PIT methods.

Table 6.1: Overall *Anopheles* mosquito species abundance in the study arms, 2014 and 2015

Method	Species	**Study arms**				**Total**
		Control	IRS	LLINs	IRS+LLIN	
LTC (Indoor)	*An. arabiensis*	108 (87.1)	3 (2.4)	10 (8.1)	3 (2.4)	124
	An. pharoensis	155 (81.2)	29 (15.2)	1 (0.5)	6 (3.1)	191
	An. ziemanni	9	0	0	0	9
	An. funestus s.l	4 (80.0)	0	1 (20.0)	0	5
PSC (Indoor)	*An. arabiensis*	27 (33.3)	3 (3.7)	43 (53.1)	8 (9.9)	81
	An. pharoensis	2 (25.0)	0	6 (75.0)	0	8
PIT (Outdoor)	*An. arabiensis*	4 (12.1)	5 (15.2)	22 (66.6)	2 (6.1)	33
	An. pharoensis	1	0	1	0	2
	An. funestus s.l	1	0	0	0	1
HLC (Indoor)	*An. arabiensis*	89 (53.3)	17 (10.2)	49 (29.3)	12 (7.2)	167
	An. pharoensis	105 (26.5)	98 (24.7)	99 (25.0)	94 (23.7)	396
	An. ziemanni	20 (37.0)	14 (25.9)	13 (24.1)	7 (12.9)	54
HLC (Outdoor)	*An. arabiensis*	67 (39.6)	25 (14.8)	57 (33.8)	20 (11.8)	169
	An. pharoensis	111 (22.8)	125 (25.7)	129 (26.5)	122 (25.0)	487
	An. ziemanni	35 (50.7)	3 (4.3)	23 (33.3)	8 (11.6)	69
Total *An. arabiensis*		295 (51.4)	53 (9.2)	181 (31.5)	45 (7.9)	574(32.1)
Total *An. pharoensis*		374 (34.5)	252 (23.2)	236 (21.8)	222 (20.5)	1084(60.7)
Total *An. ziemanni*		64 (52.0)	17 (13.8)	36 (29.3)	15 (12.2)	123 (6.9)
Total *An. funestus* s.l.		4 (80.0)	0	1 (20.0)	0	5 (0.3)
Overall anopheline		737 (41.3)	322 (18.0)	454 (25.4)	282 (15.8)	1786 (100)

6.3.2. Comparison of *Anopheles* densities among the study arms

6.3.2.1. Indoor host-seeking density

The mean indoor host seeking density (HSD) of *An. arabiensis* assessed by indoor LTC was 1.08, 0.03, 0.10 and 0.03 mosquitoes/trap/night in the control, IRS, LLINs, and IRS + LLINs arms respectively. The average indoor HSD of *An. pharoensis* in the LTC was 1.55, 0.29, 0.01 and 0.06 mosquitoes/trap/night in the control, IRS, LLINs, and IRS + LLINs arms respectively (Table 6.2). *Anopheles zeimanni* and *An. funestus* s.l.were rarely obtained indoors from the control arm and were completely absent in the intervention arms by LTCs. The mean HSDs of *An. arabiensis* and *An. pharoensis* were significantly lower in each of the intervention arm as compared to the control arm ($p < 0.001$, Figure 6.2, Table 6.2). However, the mean indoor HSD of *An. arabiensis* from LLINs arm was non-significantly higher compared to the IRS+LLINs or the IRS arm ($p = 0.07$, Figure 6.2, Table 6.3). There was no significant difference in mean indoor HSD of *An. pharoensis* between the LLINs and IRS+LLINs arms ($p > 0.05$). On the other hand**,** the impact of the IRS intervention on indoor HSD of *An. pharoensis* compared to the combined intervention was significantly different ($p < 0.05$) (Figure 6.2, Table 6.3).

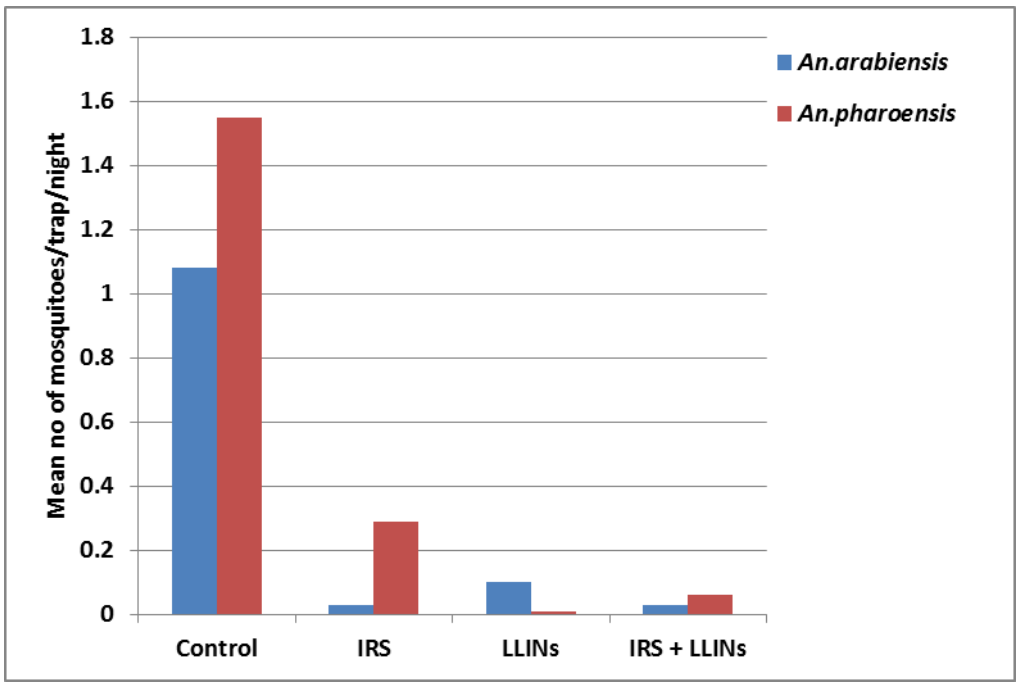

Figure 6.2: Mean indoor host seeking density of *An. arabiensis* and *An. pharoensis* collected by LTC from the study arms

6.3.2.2. Indoor and outdoor resting density of *An. arabiensis*

Indoor resting density (IRD) and outdoor resting density (ORD) of *An. arabiensis* collected by PSC and PIT respectively are shown in Figure 6.3. The mean IRD of *An. arabiensis* collected by PSC was 0.27, 0.03, 0.43 and 0.08 mosquitoes/house/day in the control, IRS, LLINs and IRS + LLIN arms respectively (Table 6.2). The mean ORD collected by PIT from the control, IRS, LLIN and IRS + LLINs arms were 0.11, 0.14, 0.61 and 0.05 mosquitoes/pit/day respectively. Compared to the control arm, the mean IRD of *An. arabiensis* was significantly lower in communities with either IRS used alone or in the combined arm ($P < 0.05$), but was similar to the LLINs arm ($p > 0.05$, Figure 6.3, Table 6.2). For ORD of the same vector however, there were no significant differences in mean densities between either IRS or combined arm compared to the

control arm (P > 0.05, Figure 6.3, Table 6.2). However, mean ORD in the LLINs arm was significantly higher compared to the control arm ($p < 0.05$).

Figure 6.3 and Table 6.3 compare indoor and outdoor resting densities of *An. arabiesis* among the intervention groups only. The impact of IRS versus combined use on either mean IRD or ORD was not significantly different ($p > 0.05$), but the combined intervention significantly reduced mean IRD and ORD of *An. arabiensis* compared to LLINs use alone ($p < 0.05$, Figure 6.3, Table 6.3). The catch size of *An. pharoensis*, *An. ziemanni* and *An. funestus* s.l. were nil in PSC and PIT in the study arms. Because of low mosquito catches IRD and ORD comparison were not done among the study arms for these species.

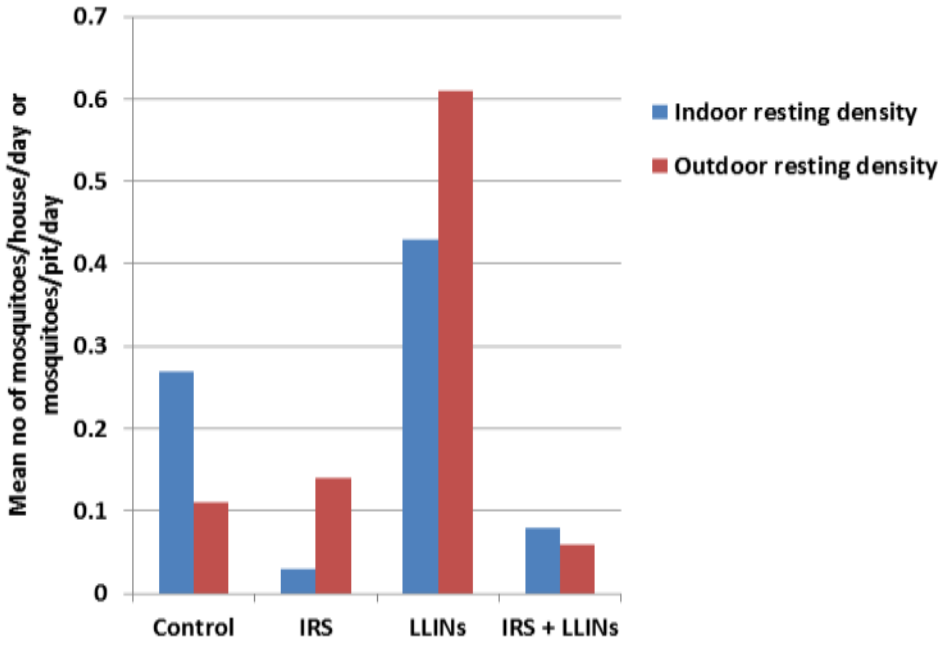

Figure 6.3: Impact of IRS and LLINs interventions on mean indoor and outdoor resting densities of *An. arabiensis* in Adami Tullu, Ethiopia

Table 6.2: Density and Incidence Rate Ratios (IRR) of intervention and control groups of *An. arabiensis* and *An. pharoensis* in Adami Tullu, Ethiopia. Control is the reference group, IRS-indoor residual spraying, LLINs-long-lasting insecticidal nets, LTC-light trap catch, PSC-Pyrethrum spray catch, PIT-Outdoor artificial pit shelter

Mosquito collection method and study arms	***Anopheles* species**			p-value
	Collection nights	Mean density (95%CI)	IRR (95% CI)	
LTC	***An. arabiensis***			
Control	400	1.08 (0.82-1.42)		
IRS	400	0.03 (0.01-0.09)	0.028 (0.008-0.090)	P < 0.001
LLINs	400	0.10 (0,05-0.19)	0.094 (0.046-0.187)	P < 0.001
IRS + LLINs	400	0.03 (0.09-0.09	0.028 (0.008-0.090)	P < 0.001
LTC	*An.pharoensis*			
Control	400	1.55 (1.21-1.99)		
IRS	400	0.29 (0.19-0.44)	0.187 (0.115-0.304)	P < 0.001
LLINs	400	0.01 (0.00-0,07)	0.006 (0.001-0.047)	P < 0.001
IRS + LLINs	400	0.06 (0.03-0.14)	0.039 (0.016-0.092)	P < 0.001
PSC	***An. arabiensis***			
Control	400	0.27 (0.18-0.41)		
IRS	400	0.03 (0.01-0.09)	0.111 (0.033-0.378)	P < 0.001
LLINs	400	0.43 (0.30-0.61)	1.592 (0.914-2.776)	0.101
IRS + LLINs	400	0.08 (0.04-0.16)	0.296 (0.128-0.683)	0.004
PIT	***An. arabiensis***			
Control	144	0.11 (0.04-0.31)		
IRS	144	0.14 (0.05-0.35)	1.249 (0.310-5.038)	0.754
LLINs	144	0.61 (0.36-1.04)	5.501 (1.723-17.566)	0.004
IRS + LLINs	144	0.06 (0.01-0.23)	0.500 (0.086-2.904)	0.440

Table 6.3: Density and Incidence Rate Ratios (IRR) of *An.arabiensis* and *An. pharoensis* among the intervention groups in Adami Tullu, Ethiopia. IRS + LLINs is the reference group, IRS-indoor residual spraying, LLINs-long-lasting insecticidal nets, LTC-light trap catch, PSC-Pyrethrum spray catch, PIT-artificial pit shelter

Mosquito collection method and study arms	***Anopheles* species**			
	Collection nights	Mean density (95%CI)	IRR (95% CI)	P value
LTC		***An. arabiensis***		
IRS + LLINs	300	0.03 (0.01-0.09)		
IRS	300	0.03 (0.01-0.09)	0.982 (0.197-5.073)	1.000
LLINs	300	0.10 (0.05-0.19)	3.333 (0.890-12.478)	0.074
LTC		***An. pharoensis***		
IRS + LLINs	300	0.06 (0.03-0.14)		
IRS	300	0.29 (0.19-0.44)	4.836 (1.923-12.146)	0.001
LLINs	300	0.01 (0.00-0.07)	0.167 (0.019-1.409)	0.100
PSC		***An. arabiensis***		
IRS + LLINs	300	0.08 (0.04-0.16)		
IRS	300	0.03 (0.01-0.09)	0.375 (0.097-1.455)	0.156
LLINs	300	0.43 (0.30-0.61)	5.376 (2.406-12.013)	P < 0.001
PIT		***An. arabiensis***		
IRS + LLINs	108	0.06 (0.01-0.23)		
IRS	108	0.14 (0.05-0.35)	2.497 (0.455-13.736)	0.292
LLINs	108	0.61 (0.36-1.04)	11.001 (2.406-50.249)	0.002

6.3.3. Parity rate of the malaria vectors

The overall outdoor and indoor parity rates of *An. arabiensis* from HLC were 57% (95%CI: 45%-71%) and 48% (95CI: 33%-61%). Neither indoor nor outdoor mean parity rates of *An. arabiensis* and *An. pharoensis* were significantly different among the study arms ($p > 0.05$).

6.3.4. Human biting rate

The mean HBR of *An. arabiensis*, as estimated using a conversion factor based on indoor LTC, was significantly higher in the control arm compared to each of intervention arms (Figure 6.4, Tables 6.4 and 6.5). However, the HBR in the IRS+LLIN arm was significantly lower than in the LLINs arm, but was not different from the IRS arm (Figure 6.4, Table 6.5). On the otherhand, there were no differences between arms in mean HBR of *An. arabiensis* as estimated using PSC (Figure 6.4, Table 6.4). The mean HBRs using these estimation methods for *An. pharoensis* and *An. ziemanni* were not calculated due to low density.

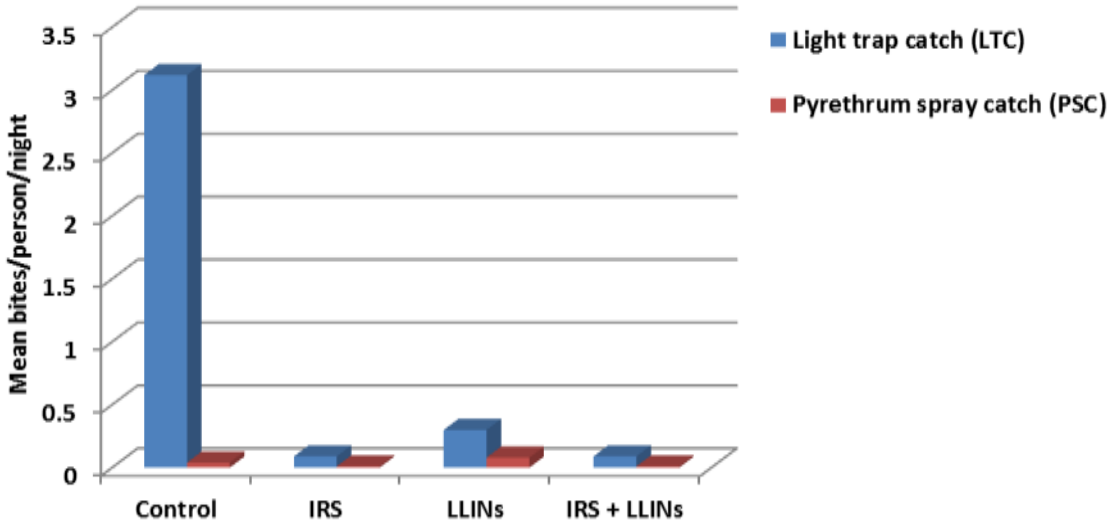

Figure 6.4: Human biting rates of *An. arabiensis* captured by LTC and PSC in Adami Tullu, Ethiopia

The mean nightly indoor HBR of *An. arabiensis* estimated by HLC was significantly higher in the control arm compared to the combined arm and the IRS arm, respectively and there were no significant differences between the IRS+LLINs and IRS arms. Nevertheless, the combined application had a significantly lower mean indoor HBR of this malaria vector compared to the LLINs arm (Figure 6.5 and Table 6.5). The outdoor HBR of *An. arabiensis* in the control arm was significantly higher than in the IRS and IRS+LLINs arms, respectively; but there were no differences between the IRS and IRS+LLIN arms (Table 6.4). Nevertheless, the combined application significantly lowered the mean outdoor HBR compared to LLINs alone. The mean indoor and outdoor HBRs of *An. pharoensis* were similar among the study arms (Figure 6.5).

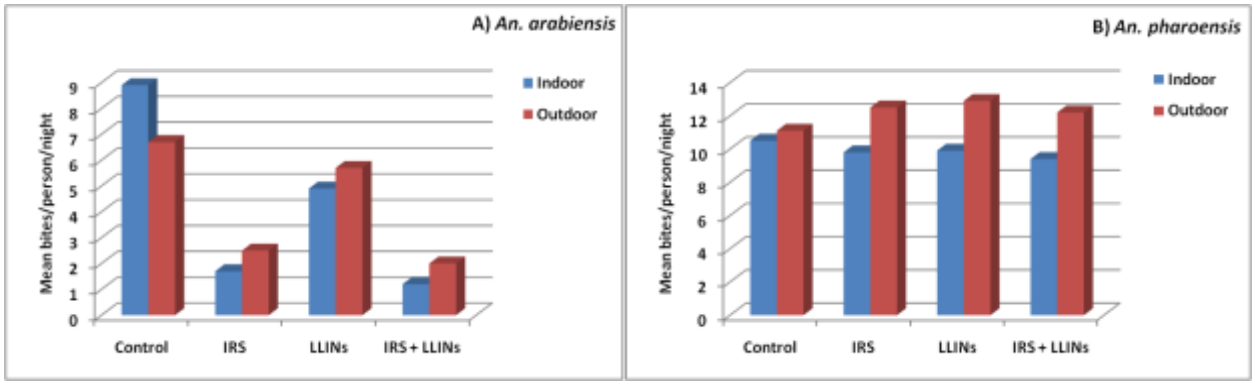

Figure 6.5: The impact of intervention on mean human biting rates of *An. arabiensis* (A) and *An. pharoensis* (B) in Adami Tullu, Ethiopia

Table 6.4: Human biting rates and Incidence Rate Ratios (IRR) of intervention and control groups of *An. arabiensis* in the Adami Tullu, Ethiopia. Control is the reference group, IRS-indoor residual spraying, LLINs-long-lasting insecticidal nets, LTC-light trap catch, PSC-Pyrethrum spray catch, PIT-artificial pit shelter, HLC-human landing catch

Mosquito collection method and study arms	***Anopheles* species**			
	Person-night catch	Mean density (95%CI)	IRR (95% CI)	P value
LTC indoor	***An. arabiensis***			
Control	400	3.12 (2.49=3.91)		
IRS	400	0.09 (0.05-0.18)	0.028 (0.014-0.059	P < 0.001
LLINs	400	0.30 (0.20-0.45)	0.096 (0.060-0.153)	P < 0.001
IRS + LLINs	400	0.09 (05-0.18)	0.028 (0.014-0.059)	P < 0.001
PSC indoor	***An.arabiensis***			
Control	400	0.04 (0.01-0.11)		
IRS	400	0.01 (0.00-0.07)	0.250 (0.027-6.835)	0.219
LLINs	400	0.08 (0.04-0.16)	1.999 (0.583-6.841)	0.270
IRS + LLINs	400	0.01 (0.00-0.07)	0.250 (0.021-6.835)	0.219
HLC Indoor	***An. arabiensis***			
Control	400	8.90 (4.63-17.11)		
IRS	400	1.70 (0.78-3.71)	0.191 (0.069-0.528)	0.001
LLINs	400	4.90 (2.48-9.67)	0.550 (0.214-1.415)	0.215
IRS + LLINs	400	1.20 (0.52-2.78)	0.135 (0.046-0.390)	P < 0.001
HLC outdoor	***An. arabiensis***			
Control	400	6.70 (3.45-13.02)		
IRS	400	2.50 (1.20-5.21)	0.373 (0.138-1.004)	0.051
LLINs	400	5.70 (2.91-11.16)	0.850 (0.330-2.188)	0.737
IRS + LLINs	400	2.00 (0.94-4.27)	0.298 (0.108-1.221)	0.019

Table 6.5: Human biting rates and Incidence Rate Ratios (IRR) of *An. arabiensis* among the intervention groups in Adami Tullu, Ethiopia. IRS+LLINs is the reference group, IRS-indoor residual spraying, LLINs-long-lasting insecticidal nets, LTC-light trap catch, HLC-human landing catch

Mosquito collection method and study arms	***Anopheles* species**			
	Person-night catch	Mean density (95%CI)	IRR (95% CI)	P value
LTC indoor	***An. arabiensis***			
IRS + LLINs	300	0.09 (0.05-0.18)		
IRS	300	0.09 (0.05-0.18)	0.985 (0.380-2.625)	1.000
LLINs	300	0.30 (0.20-0.45)	1.204 (0.409-1.999)	0.003
HLC indoor	***An.arabiensis***			
IRS + LLINs	30	1.20 (0.52-2.78)		
IRS	30	1.70 (0.78-3.71)	1.416 (0.450-4.459)	0.552
LLINs	30	4.90 (2.48-9.67)	4.083 (1.386-12.025)	0.011

6.3.5. Sporozoite rate and entomological inoculation rates

Altogether 1084 *An.* pharoensis, 574 *An. arabiensis*, 123 *An. ziemanni*, and five *An. funestus* s.l. collected from all the study arms were tested for the presence of CSP of *P. falciparum* and *P. vivax*. However none was found positive. For this reason, the entomological inoculation rate (EIR) which is the product of human biting rate and the sporozoite rate could not be determined.

6.4. Discussion and conclusions

The ultimate aim of this study was to examine the impact of IRS and LLINs individual versus combined interventions on malaria vector parameters specifically vector density, longevity, and infectivity. Results showed that mean indoor host seeking densities (HSD) of *An. arabiensis* and

An. pharoensis (using LTC) were significantly lower in communities that received IRS+LLINs, IRS and LLINs unlike those of the control group. This would be expected, because IRS and LLINs applied either individually or jointly kills and/or repels mosquitoes when they attempt to feed and rest indoors, so that vector survival and population densities are reduced in intervention arms. Significant reductions in mosquito density in intervention arms ensure that the interventions were effective. These findings were consistent with several studies (Binka *et al*., 1998; Hawley *et al*., 2003; Boyoh *et al*., 2010; Russell *et al*., 2010; Killeen *et al*., 2011) which support that IRS and LLINs intervention dramatically suppress density of malaria vector population by killing and/or deterring mosquitoes attempting to feed on humans and rest inside houses.

Although IRS and LLINs combined trials that compared the impact of intervention and non-intervention (control group) on *Anopheles* mosquitoes are lacking so far, the present results evident that the combined interventions was much better than without intervention in suppressing indoor HSDs of *An. arabiensis* and *An. pharoensis*. Results also showed that the mean human biting rate (HBR) of *An. arabiensis,* as estimated by a conversion factor from indoor LTC, was significantly lower in each of the intervention arms compared to the control arm. Likewise, the real HBR of *An. arabiensis* obtained by HLC was significantly lower in the IRS and IRS+LLINs arms each compared to the control arm. These results manifest that the IRS and LLINs combined interventions were more effective against *An. arabiensis* compared to non-intervention.

Similarly, mean indoor resting density (IRD) of *An. arabiensis* was significantly lower in either IRS or the combined arm each compared to the control arm. These results clearly show more effectiveness of IRS on IRD of *An. arabiensis* in both the IRS and IRS+LLINs arms unlike in the control arm and was also be expected because IRS primarily target indoor resting mosquitoes (WHO, 2014). However, compared to the control arm, mean IRD of *An. arabiensis* was similar to

the LLINs arm. This could be attributed to *An. arabiensis* resistance to LLINs (deltamethrin) (Balkew *et al*., 2012; Gari *et al*., 2016) or because LLINs mainly prevent blood feeding on occupant (Okumu and Moore, 2011) and hence have less impact on outdoor feeding but indoor resting *An. arabiensis*. As a result the effectiveness of LLINs alone against indoor resting *An. arabiensis* might be compromised to the extent that the LLINs arm resembles the control arm. In line with these results, there were no differences between control and LLINs arms in mean HBRs of *An. arabiensis* as estimated using PSC and HLC. These results show that LLINs alone was not sufficiently effective against *An. arabiensis*.

The present results also indicated that mean indoor HSD of *An. arabiensis* collected by LTC was lower in IRS+LLINs arm compared to LLIN arm but similar to the IRS arm. Likewise, mean indoor resting density (IRD) of *An. arabiensis* assessed by PSC was significantly lower in IRS+LLINs arm compared to LLINs arm, but similar to IRS arm. These results could be attributed to the potential basic differences in operational applications and efficacy between IRS and LLINs, feeding and resting behaviour of *An. arabiensis*. At household level, IRS was applied to all potential mosquito resting places in human dwellings unlike LLINs which were positioned at human sleeping spaces (often limited to bed-rooms) in this trial (Deressa *et al*., 2016). Therefore, relatively larger area-wide coverage of IRS in the combined and IRS alone might have equally suppressed densities of *An. arabiensis* in those arms. Lack of convenient space to hang more than one net were usually reported as key challenge that compromise the usable life of LLINs in this rural household settings and need to be considered. Householders in the combined arm might also interrupt using LLINs having their houses sprayed.

In addition, this vector prefers to feed on both human and bovine and rest both in human dwellings and in animal shelters (Seyoum *et al*., 2002; Massebo *et al*., 2013; Animut *et al*., 2013),

as tethering livestock in human dwellings is common in rural Africa including Ethiopia (Seyoum *et al*., 2002; Animut *et al*., 2013), IRS in both arms could equally suppress this vector population by hindering access to these hosts. These results suggest that IRS application alone is sufficiently powerful to significantly suppress indoor HSD and IRD of *An. arabiensis* as equally as IRS+LLINS but much greater than LLINs alone.

Beside application and operational coverage, there could be potential differences in efficacy and effectiveness between IRS and LLINs. IRS with insecticides potentially has more rapid mass killing impact on mosquitoes than LLINs. For example, IRS is commonly applied for the prevention of malaria epidemics even where residents already possess LLINs (Okumu and Moore, 2011). The efficacy of IRS applications is mainly due to repellency and toxicity to mosquitoes, whereas LLINs mainly inhibit blood feeding and kill mosquitoes (Okumu and Moore, 2011). Mass killing and repellency properties of IRS might have similarly reduced the number of indoor host seeking and resting *An. arabiensis* catches in the combined arm compared to the IRS arm.

The significant reduction in HSD and IRD of *An. arabiensis* in the villages that received IRS+LLINs compared to LLINs alone strongly suggest that unlike LLINs, IRS highly deterred or killed the mosquitoes. LLINs elicit either very low or no deterrence at all against susceptible African malaria vectors (Okumu and Moore, 2011). LLINs are effective mainly because they prevent blood feeding on occupants. As a result, in situations where mosquito vectors are highly flexible in host preference, biting and resting behaviour (e.g. *An. arabiensis*), high LLINs coverage and utilization alone may not dramatically reduce the vector density and malaria transmission. For example, high coverage of LLINs alone might not reduce the number of blood-feeding *An. arabiensis* that would normally settle on the walls because the vector might bite

outdoors and rest indoors or bite on domestic animals and rest indoors or might bite earlier before people retire to bed and rest indoors.

Evidence indicate that *An. arabiensis* has a marked peak biting activities occurring during early parts of the night well before most people retire to bed (chapter 3 and 6). Early at night and outdoor biting activities of this vector could compromise the efficacy of LLINs. This findings agree with the recent evidence that the added protective effect of IRS and LLINs will be dependent on the feeding and resting behaviour of a particular malaria vector (West *et al*., 2014).

Furthermore results revealed that mean HBR of *An. arabiensis* collected by LTC in the combined arm was significantly lower compared to the LLIN arm but non-significantly different compared to the IRS arm. This result clearly implicates that the impact of LLINs alone on decreasing HBR of *An. arabiensis* compared to IRS alone or compared to the combined intervention was minimal. This could be justified in connection with flexible biting behavior of the vector as described earlier. Exophagic and early biting behaviour of *An. arabiensis* has been reported as key challenge that negatively impact the efficacy and effectiveness of LLINs in Ethiopia (Yohannes and Boelee, 2011) and elsewhere in Africa (Kitau *et al*., 2012; Russel *et al*., 2011). Relatively high HBR in the LLINs arm further implicates potentially high entomological inoculation rate (EIR) and malaria risk unless malaria infection in mosquitoes in the area is zero. This is because EIR is directly proportional to HBR as it is the product of HBR and sprozoite rate (SR). When either HBR or SR is zero, EIR will be zero and there will be no malaria transmission ideally. Based on these findings, it can be suggested that high provision of LLINs alone is not sufficient to control *An. arabiensis* and necessitate complimentary interventions such as combining it with IRS or needs to consider other complementary intervention measures.

Similarly the impact of IRS and LLIN combined intervention versus IRS alone on mean indoor HBR of *An. arabiensis* collected by HLC was not significantly different. However the combined intervention significantly reduced mean indoor HBR of this malaria vector as compared to LLINs alone. These findings are consistent with the above discussion and further verify that LLIN alone had less impact on indoor HBR of *An. arabiensis* compared to either the combined intervention or IRS because HLC is standardized and direct measure for HBR and human exposure to mosquito bites (Lines *et al*., 1991). Now it can be recommended that integration of interventions are needed because high coverage of LLINs alone could not effectively protect *An. arabiensis* biting people.

Moreover, the difference in mean HBR of *An. arabiensis* collected by PSC were not significant different among the intervention arms. This could be attributed to low mosquito density captured by PSC. Besides the mean HBR was calculated by dividing the total number of blood-fed and half-gravid mosquitoes caught in PSC by the number of house occupant the night preceding the collection (Kipyab *et al*., 2013; WHO, 2011). Because equal number of houses were assigned to the study arms, the average number of occupant may be less likely to differ among the arms which might have resulted in similar HBR among the arms in low indoor mosquito density settings. It needs to be noted from the above discussion that the combined interventions significantly reduced IRD of this vector compared to LLIN alone but similar to IRS alone. This perhaps reveals the impact of the size of occupants on estimating HBR in this study. Estimating HBR from PSC as described above is an indirect method (WHO, 1975) and is not reliable for calculating HBR in vector control trials (Wilson *et al*., 2015). Therefore large mosquito number may be needed for more valid comparison of HBR in PSC among the study arms.

Mean outdoor resting density (ORD) of *An. arabiensis* collected from PIT in either IRS arm or the combined arm did not vary compared to the control arm. In the same way, the impact of IRS+LLINs versus IRS alone on mean outdoor HBR of *An. arabiensis* collected by HLC was similar. These results would be expected because IRS is mostly effective indoors and has less impact on outdoor resting and biting mosquitoes (Killeen, 2014). However, outdoor HBR of *An. arabiensis* estimated by HLC was significantly higher in the LLINs arm compared to either IRS arm or IRS+LLINs arm. This can be explained in terms of potentially more area-wide coverage and mass killing impact of IRS compared to LLINs as explained above.

Results also revealed that there was no significant difference in mean indoor HSD of *An. pharoensis* collected by LTC between IRS+LLIN and LLINs arms. Likewise, the differences in mean indoor HBRs of *An. pharoensis* collected by HLC among the study arms were non-significant. Similar HSD and HBR of *An. pharoensis* among the study arms could result due to the biting behaviour and flight range of this species. *Anopheles pharoensis* might be less affected by IRS and LLINs both of which are indoor-based interventions because; this species is typically exophagic and zoophagic species in Ethiopia (Krafsur, 1977; Abose *et al*., 1998; Seyoum *et al*., 2002; Taye *et al*., 2006). In addition, observation from the study area showed that *An. pharoensis* exhibited marked peak human biting activity outdoors early at night (19:00-20:00 hours) before the local people were indoors and retire to bed and potentially protected by IRS and LLINs (chapter 4 and 7). The other potential reason could be attributed to long flight range of this species. It has been observed that this species is larger in size compared to *An. arabiensis* and could disperse to further villages from Lake Zeway and Bulbula River. Therefore mixing of *An. pharoensis* among the study arms were likely within flight range of this species particularly along

the lake where the mosquitoes were collected by HLC. The study arms presumably shared the same breeding sites for this species leading to continual mixing of *An. pharoensis* population.

However, indoor HSD of *An. pharoensis* collected by LTC was significantly reduced in IRS+LLINs arm compared to the IRS arm. These differences could be driven by potential differences in live stock abundance between the intervention arms because this zoophilic species might be attracted to where cattle are accessible and abundant at night. Moreover, the differences in mean outdoor HBRs of *An. pharoensis* collected by HLC among the study arms were non-significant. This was expected because of exophagic and zoophagic behaviour of the vector as well.

Data obtained from HLC showed that mean parity rates of *An. arabiensis* and *An. pharoensis* did not vary significantly among the study arms. This might be caused by mixing of mosquito populations (contamination) between villages where HLCs were performed. Because villages for mosquito survey by HLC were chosen purposely based on where high vector density are likely along Lake Zeway, and not randomized across the study population, there was a potential for mixing of mosquitoes among study villages within flight range of the local vectors. Although flight ranges of the local vectors are not known, it has been estimated that most African malaria vectors are able to fly 4-5 km (Corbel *et al*., 2012). Furthermore, these vector populations were expected to come from the same breeding habitats (the lake shore). Despite these shortcomings, the overall mean parity rate of *An. arabiensis* estimated during this intervention study (48%) was low compared to its pre-intervention results (80%). These results suggest that propoxur might have produced high mortality of mosquitoes during the present intervention period. These results contrast The Gambian trial that found high parity rate for the target mosquitoes (77%) regardless

of no significant difference in vector density among IRS + LLIN and LLIN alone (Pinder *et al*., 2015).

Determination of parity rate relied on mosquitoes collected by HLC alone for reasons described above. Unfortunately, insufficient number of mosquitoes was found for parity rate comparison. Estimation of the impacts of IRS and LLINs combined versus separate interventions on local vector longevity was therefore influenced by low mosquito collection by LTC. Light trap catches are the standardised entomological data collection method for evidence-based vector control trials (Wilson *et al*., 2015). However, random mosquito sampling from the study area by LTC without referring to any breeding site is known to affect mosquito catch size during pre-intervention entomological studies (chapter 4).

Furthermore, results showed that none of the mosquitoes tested by ELISA was positive for *P. falciparum* or *P. vivax* circumsporozoite protein, a finding similar to the pre-intervention results from the study area (Gari *et al*., 2016) or earlier reports from the district (Rishikesh, 1966; Abose *et al*., 1998). Similarly, the most recent report from Seka district, south western Ethiopia found no-sprozoite positive in *An. gambiae* s.l. collected over one malaria transmission season from June to December 2012 (Taye *et al*., 2016). Likewise, none of *An. arabiensis* collected during two rainy seasons were found sporozoite positive while malaria cases continued to be seen in Macha, Zambia during two years after introduction of insecticide-treated nets (Fornadel *et al*., 2010). Additionally, none of *An. gambiae* s.s., *An. funestus* and *An. arabiensis* tested were positive for *P. falciparum* sporozoites over two peak transmission season in Zambia (Chanda *et al*., 2012).

The possible explanatins for negative sporozoite results in this study could be: 1) Impact of the present and past insecticidal vector control interventions in the area: Zeway area has been a

sentinel site for monitoring the impact of malaria control interventions, and different insecticides have been used since the time of Global Malaria Eradication (Deressa *et al*., 2016). Combined with this, the present scale-up and universal coverage of IRS and LLINs in the study area might have reduced malaria infected vector populations particularly from domestic venues (Bekele *et al*., 2013). These interventions might have also encouraging the vectors to feed more frequently on non-human hosts. 2) Malaria treatment impact in the area: The present scale-up of malaria diagnosing and treatment facilities in the area and elsewhere in the country have caused significant decline in malaria cases (Otten *et al*., 2009; Alemu *et al*., 2012). These might have also affected malaria parasite prevalence in humans and vector populations. 3) Abundance of deadend hosts in the study area: Zoophagic behaviour of *An. arabiensis* and *An. pharoensis* coupled with high abundance of cattle in the study area could enhance reduction in malaria transmission because the *Plasmodium* parasites that cause human malaria do not develop fully in cattle and other domesticated animals. Infective stages of the malaria parasite (sporozoites) injected in animals by malaria vectors, in the process of taking blood meals, reach a dead end in their development cycle (Ndenga *et al*., 2016).

Further explanation includes 4) Low density of malaria vectors and the disease incident in the area: Low density of malaria vectors and the disease episode prevalence in the area as evident by pre-intervention results (Gari *et al*., 2016) is a key implication for low malaria parasite density in mosquito and human population in the area. 5) Longevity of malaria vectors. Mosquito age structure determines parasite availability in vector population and malaria transmission because pre-mature death inhabits the development of malaria parasite to infective stage (Bugoro *et al*., 2011) and interrupt the parasite life cycle at large. Indoor parity rate of *An. arabiensis* (80%) was high during pre-intervention study but low (48%) during intervention period. This implicates that

the impact of intervention on longevity of the vector was high and hence caused low parasite load in the vector population. Despite negative sporozoite ELISA results, there was active malaria transmission taking place in the study area during the intervention period (Taye Gare personal com.). These implicate the need for more specialized equipments and techinques such as real time PCR for detection of sporzoite infected mosquitoes.

When compared with other trials elsewhere, the present results are in line with the recent trial evidence from Tanzania which support that combining IRS and LLINs have significant added impact on reducing malaria vector density as compared to LLINs alone (West *et al*., 2014; Protopopoff *et al*., 2015). However, the Tanzanian trial targeted on *An. gambiae* s.s and *An. arabiensis* the most important African malaria vectors and found no evidence for a reduction in *An. arabiensis* density between the combined and LLINs arms (Protopopoff *et al*., 2015) unlike the present study which focused on *An. arabiensis* and found significant differences between the two arms. Thus, the present results show that IRS+LLINs had added impact on *An. arabiensis* compared to LLINs alone. In addition, the results show that combining IRS and LLINs provides equal or comparable benefit in suppressing indoor HSD, IRD and HBRs of *An.arabiensis* compared to IRS application alone. To the best of my knowledge, this later finding could be the first evidence on *An. arabiensis*.

On the other hand, the present results contrast the recent two trials in Africa particularly in the Benin (Corbel *et al*., 2012) and The Gambian trial (Pinder *et al*., 2015) that found no significant difference in the density of vector mosquitoes captured by LTCs between IRS+LLINs compared to LLINs alone groups. The differences could be explained in terms of the target vector behaviour and insecticide used for interventions in the former compared to the present study. The Benin trial used bendiocarb (carbamate) and targeted *An. gambiae* s.s. and *An. funestus* while The Gambian

trial used DDT and targeted *An. gambiae* s.l. whereas the present study used propoxur (carbamate) and targeted *An. arabiensis.* Beside differences in IRS insecticides used, the previous studies mainly targeted on anthropophagic and endophagic primary vector species: *An.gambiae s.s.* which is more venerable to LLINs compared to the partial zoophagic and exophagic *An. arabiensis* which is less likely to be affected by LLINs. Okumu *et al.* (2013) suggested that the intervention impact of combining IRS and LLINs is affected by the type of insecticide used. Further potential reason could be due to some level of resistance in local vector populations to the insecticide used on nets and/or spray (WHO, 2014).

In conclusion, IRS+LLINs and IRS alone each had similarly most effective impact on densities and human biting rates of *An. arabiensis* whereas the LLINs alone had the least impact on the vector in this study setting. Results underscore that the combination intervention provided additional impact on densities and human biting rates of *An. arabiensis* compared to the LLINs alone. However results evident that the combination intervention had no additional significant impact on the vector compared to the IRS alone.

Chapter 7. Impact of IRS and LLINs combined and separate interventions on mosquito biting, host preferences and resting behaviors

7.1. Introduction

Mosquito biting activity, host preference and resting behaviors are important vector parameters that influence the role of malaria transmission because they determine the degree of anthropophily, the human biting rate and host location strategy (Pates and Curtis, 2005; Takken and Verhulst, 2012). The insecticides used for IRS and LLINs exert their impact on anopheline mosquitoes in a number of ways. These indoor insecticides may reduce vector survival and suppress vector population due to their toxic chemical action on the mosquitoes (Gimnig *et al*., 2003; Gatton *et al*., 2013). For this reason the large scale use of IRS or LLINs frequently results in a major reduction in the abundance of vectors often referred to as the mass community effect (Hawley *et al*., 2003).

The other key impact of IRS and LLINs on anopheline mosquitoes have been development of physiological and behavioral resistance. Physiological resistance involves biochemical mechanisms such as metabolic detoxification of insecticides, target site mutation and modifications in the insect cuticle or digestive tract linings that prevent or slow the absorption or penetration of insecticides (Liu, 2015). Behavioral resistance refers to any modification to mosquito behavior that facilitates avoidance or circumvention of insecticides (Gatton *et al*., 2013). Recent reviews have shown that IRS and LLINs can shift anopheline biting outdoors, to earlier in the evening, to alternate hosts or cause those that enter sleeping houses to exit more quickly (Pates and Curtis, 2005; Gatton *et al*., 2013).

Behaviours, such as preferential feeding on host species, resting outside human homes and early evening biting before people have gone to sleep may circumvent LLINs and IRS control interventions. Monitoring of the impact of these interventions on such traits on local mosquitoes is, therefore, important. Information of mosquito biting, host preference and resting behavior are important in designing appropriate malaria control interventions or reorient the existing interventions. The objective of this study was to monitor the impact of combining IRS and LLINs on anopheline biting, host preference and resting behaviors in Adami Tullu district, central Ethiopia.

7.2. Materials and methods

7.2.1. Study area

The study area is described in more details in the study protocol (Deressa *et al.*, 2016) and briefly under sub-headings 3.1. The geographical location of the study area is shown in Figure 6.1.

7.2.2. The study design and mosquito collections

This study is a four arm cluster randomized trial as explained earlier (Chapter 6). Mosquito human biting patterns were monitored by HLC alone, host preferences were estimated from blood meal source analysis assessed by LTC, PSC and PIT and resting habits were estimated from PSC carried out in the study arms. The procedures for mosquito collections by LTC, PSC and PIT have been described in more details under 3.2.

HLC was used due to lack of efficient mosquito sampling tools that can estimate mosquito vector biting activities correctly, particularly in outdoor venues. The alternative methods, particularly LTC is susceptible to theft outdoors and was found to be less efficient than HLC in the area

(Chapter 5). Furthermore, the PSC and PITs are not convenient to monitor the real human biting behaviours of malaria vectors.

For HLC, four houses of similar size and design (one house per arm) were selected purposely based on preliminary data (Gari *et al*., 2016) in high mosquito density areas along Lake Zeway. The four houses were also selected to reduce logistical constraints. At each house, five volunteers (one supervisor and four mosquito collectors) were recruited to perform landing collections on rotation. Two collectors, one indoor and the other outdoor, performed HLC from 18:00 to 24:00 and the other two collectors from 24:00 to 6:00 hours. The outdoor collectors were positioned at least 10 meters from the house. Collections were done for 50 minutes each hour with 10 minutes break for the collectors. During each break, indoor and outdoor collectors also changed venues to reduce the effects of a particular site and individual differences in mosquito attractiveness of the bait collectors. Collectors, with their legs exposed sat on chairs and caught mosquitoes landing on their exposed legs with a hand-held mouth aspirator using a flashlight to locate landing mosquitoes. The hourly collection was kept separately in labeled paper cups. . The protective measures that were undertaken for human volunteers have been described in section 3.4. Mosquitoes were collected once per week from August to October 2015. Mosquito collection was limited to these months due to the impact of precipitation and hence low occurrence of the mosquitoes as can be evident by the pilot study results (Gari *et al*., 2016).

Human behavioural survey was carried out, using closed and open ended questionnaires (Appendix 1). The purpose of doing this was to show the proportion of people potentially being exposed to mosquito bites before bed time i.e. during early parts of the night. This survey was also done to estimate association between the feeding habits of the vector versus habits of the local people. The sample size for this survey was determined as follows: Local people are

expected to be indoors 8 out of 12 night time hours, which is 66% of the time. The overall population size was estimated to be 30,800 people, i.e. 44 clusters in each of the four arms and 175 persons per cluster in 35 households (chapter 6). A sample size for frequency in a population was estimated using open source statistics for public health (http://www.openepi.com). With a hypothesized frequency of 66%, a precision of 5%, and a design effect of 2, the sample size was calculated to be 682 people. By dividing this number with an assumed average household number of 5 people, a total 136 households, 34 households per arm were selected. Households were selected by simple random sampling and the questionnaires were administered to five randomly selected family members.

7.2.3. Data analysis

Mosquito outdoor and indoor biting behaviours (degree of exophage and endophagy) and biting times were assessed using HLC alone. The endophagic rate was calculated as the ratio of indoor $HBR_{18:00\rightarrow06:00hrs}$/(indoor $HBR_{18:00\rightarrow06:00\ hrs}$+ outdoor $HBR_{18:00\rightarrow06:00hrs}$) while exophagic rate was calculated as outdoor $HBR_{18:00\rightarrow06:00hrs}$/(outdoor $HBR_{18:00\rightarrow06:00hrs}$ + indoor $HBR_{18:00\rightarrow06:00hrs}$) (Govella *et al*., 2010). Average endophagic and exophagic rates of each *Anopheles* species were compared among the study arms using Incidence Rate Ratio at 95%CI, $p<0.05$ significant level.

Mean mosquito bites per person per hour were compared among study arms using General Linear Model: Multivariate analysis. The differences in the time of retiring to bed for the duration of the night and getting up next morning between families, sex and age groups were compared using χ^2-square test. Host preference of the local mosquitoes was determined by mosquito blood meal analysis. The degree of human exposure to malaria mosquitoes expressed as human blood index (HBI) was calculated as the proportion of mosquitoes with human blood of total blood fed

females tested. Likewise, bovine blood index (BBI) was estimated as the proportion of mosquitoes with bovine blood of total blood fed females (WHO, 2011) and compared among the study arms. Mosquitoes with mixed human and bovine blood were not included in the calculaton of HBI and BBI.

Vector resting habit was estimated based on WHO (2011) formula as described below. An important index for vector resting habit is the proportion of mosquitoes that have taken a blood-meal on human and then rest indoors. One element of the success of IRS in interrupting transmission is its impact on the proportion of the vectors that rest on the sprayed surface before and after feeding on humans. The proportion of blood-meals taken on humans and followed by indoors resting is calculated as: *f = kHD/NPM, where: k = a correction value of 1.16, H = human blood index, D = indoor resting density (total number of females collected by PSC divided by number of houses), N = average number of persons per house (household size), P = duration of resting indoors after feeding, in days; P = 1 +(G/F), where G is the total number of half-gravid and gravid females collected by PSC, M = human biting rate and F is the number of freshly fed females collected by PSC.*

7.3. Results

7.3.1. Biting venues: Endophagy (indoor biting) and exophagy (outdoor biting)

The mean endophagic rates of *An. arabiensis* were significantly lower in the IRS+LLIN and IRS arm each compared to the control arm (Table 7.1). However, there was no significant difference in mean endophagic rate of *An. arabiensis* between the control and the LLINs arm. Likewise, the difference in mean endophagic rate of *An. pharoensis* between the control and each of the

intervention arms were not significant. On the other hand mean endophagic rate of *An. ziemanni* in each of the intervention arm was significantly lower than the control arm.

When comparing results among the three intervention groups only, the endophagic rate of *An. arabiensis* was significantly lower in the IRS+LLINs arm compared to the LLINs arm, but there were no significant differences between the IRS+LLINs and the IRS arms (Table 7.1). There were no differences in endophagic rates of *An. pharoensis* and *An. ziemanni* between IRS+LLINS and either of IRS alone or LLINs alone.

The mean exophagic rates of *An. arabiensis* and *An. ziemanni* were significantly lower in the IRS and IRS+LLINs arm each compared to the control arm (Table 7.2). However the difference in mean exophagic rates of these species in the LLIN arm relative to the control arm was non-significant. Likewise, the impact of the interventions on mean exophagic rate of *An. pharoensis* compared to the control was not significant. Compared to the combined arm, exophagic rates of *An. arabiensis*, *An. pharoensis* and *An. ziemanni* were similar either in LLIN or IRS arm (Table 7.2).

Table 7.1: The impact, as measured by the Incidence Rate Ratio (IRR), of LLINs and IRS interventions on mean endophagic rates of *Anopheles* species collected by HLC in Adami Tullu, Ethiopia.

Study arms	***Anopheles* species**			P-value
	Person-nights	Mean endophagic rate (95%CI)	IRR (95% CI)	
	An. arabiensis			
Control (Reference group)	480	0.8 (0.6-0.9)	1.0	
IRS	480	0.2 (0.0-0.3)	0.2 (0.1-0.3)	P < 0.001
LLINs	480	0.6 (0.4-0.8)	0.8 (0.7-1.0)	P > 0.05
IRS + LLINs	480	0.1 (-0.1-0.3)	0.2 (0.1-0.3)	P < 0.001
	An. pharoensis			
Control (Reference group)	480	1.2 (0.9-1.5)	1.0	
IRS	480	1.4 (1.1-1.6)	1.2 (0.8-1.8)	P > 0.05
LLINs	480	1.3 (1.0-1.6)	1.1 (0.7-1.6)	P > 0.05
IRS + LLINs	480	1.4 (1.1-1.6)	1.2 (0.8-1.8)	P > 0.05
	An. ziemanni			
Control (Reference group)	480	0.3 (0.2-0.4)	1.0	
IRS	480	0.1 (0.0-0.2)	0.8 (0.7-0.9)	P < 0.001
LLINs	480	0.1 (0.0-0.2)	0.8 (0.7-0.9)	P <0.05
IRS + LLINs	480	0.1 (0.0-0.2)	0.8 (0.7-0.9)	P < 0.001
	An. arabiensis			
IRS + LLINs (Reference)	360	0.1 (0.0-0.3)	1.0	
IRS	360	0.2 (0.0-0.3)	1.0 (0.8-1.2)	P > 0.05
LLINs	360	0.6 (0.4-0.7)	0.4 (0.3-0.8)	P < 0.05
	An. pharoensis			
IRS + LLINs (Reference)	360	1.4 (1.1-1.7)	1.0	
IRS	360	1.4 (1.1-1.7)	1.0 (0.7-1.5)	P > 0.05
LLINs	360	1.3 (1.0-1.6)	0.9 (0.6-1.3)	P > 0.05

Table 7.2: The impact, as measured by the Incidence Rate Ratio (IRR), of LLIN and IRS interventions on mean exophagic rates of *Anopheles* species collected by HLC in Adami Tullu, Ethiopia.

Study arms	***Anopheles* species**			
	Person-night s	Mean exophagic rate (95%CI)	IRR (95% CI)	P-value
	An. arabiensis			
Control (Reference group)	480	0.6 (0.5-0.8)	1.0	
IRS	480	0.2 (0.1-0.4)	0.7 (0.5-0.8)	P < 0.001
LLINs	480	0.4 (0.3-0.6)	0.8 (0.6-1.1)	P > 0.05
IRS + LLINs	480	0.2 (0.0-0.3)	0.6 (0.5-0.8)	P <0.001
	An. pharoensis			
Control (Reference group)	480	1.3 (1.0-1.5)	1.0	
IRS	480	1.3 (1.1-1.6)	1.0 (0.7-1.5)	P >0.05
LLINs	480	1.2 (1.0-1.4)	0.9 (0.6-4.5)	P >0.05
IRS + LLINs	480	1.3 (1.0-1.5)	0.9 (0.7-1.3)	P >0.05
	An. ziemanni			
Control (Reference group)	480	0.3 (0.2-0.4)	1.0	
IRS	480	0.04 (0.02-0.1)	0.1 (0.1-0.2)	P<0.001
LLINs	480	0.2 (0.1-0.3)	0.7 (0.4-1.3)	p> 0.05
IRS + LLINs	480	0.1 (0.04-0.2)	0.3 (0.1-0.6)	P < 0.05
	An. arabiensis			
IRS + LLINs	360	0.1 (0.0-0.2)	1.0	
IRS	360	0.1 (0.0-0.2)	1.5 (0.6-3.2)	P > 0.05
LLINs	360	0.1 (0.0-0.2)	1.5 (0.6-3.5)	p > 0.05
	An. pharoensis			
IRS + LLINs	360	1.3 (0.2-1.0)	1.0	
IRS	360	1.3 (1.0-1.7)	1.0 (0.7-1.5)	P > 0.05
LLINs	360	1.2 (0.9-1.5)	0.9 (0.7-1.3)	P > 0.05

7.3.2. Biting times of the *Anopheles* species

Biting by all of the *Anopheles* species began early in the evening (18:00 hr) indoors and outdoors and mosquitoes continued to be active until day break (06:00hr) in all of the study arms (Figure 7.1). Peak indoor biting by *An. arabiensis* was observed between 23:00-24:00, 21:00-22:00, 20:00-21:00 and 19:00-20:00hrs in the control, LLINs, IRS and IRS+LLINs arms respectively (Figure 7.1A-B). Peak outdoor biting by *An. arabiensis* also occurred between 21:00-22:00hrs in the control arm whereas its outdoor peak biting activities in the intervention arms happened after midnight (after 24:00hrs). Indoor biting activities of *An. arabiensis* were lower in each of the intervention arm compared to the control arm. Biting activity of *An. arabiensis* in the IRS+LLINs arm was similar to the IRS arm but was not-significantly lower compared to LLINs arm. The corresponding outdoor biting activities of this vector did not show significant difference among the study arms in time.

Peak indoor biting by *An. pharoensis* was observed between 18:00-19:00hrs in all of the study arms. Likewise outdoor peak biting by *An. pharoensis* also mainly occurred before 24:00hr. However, neither indoor nor outdoor biting activities of this species were significantly different among the study arms in time (Figures 7.1C and D).

High peak biting by *An. ziemanni* took place between 19:00 and 20:00hrs indoors and 18:00 and 19:00hrs outdoors in the control arm. Biting activities of *An. ziemanni* was low in the combined arm and IRS arm each compared to the control arm both indoors and outdoors (Figure 7.1E and F). Intervention impact of LLINs alone on *An. arabiensis* and *An. ziemanni* temporal biting activities were minimal compared to the other two interventions.

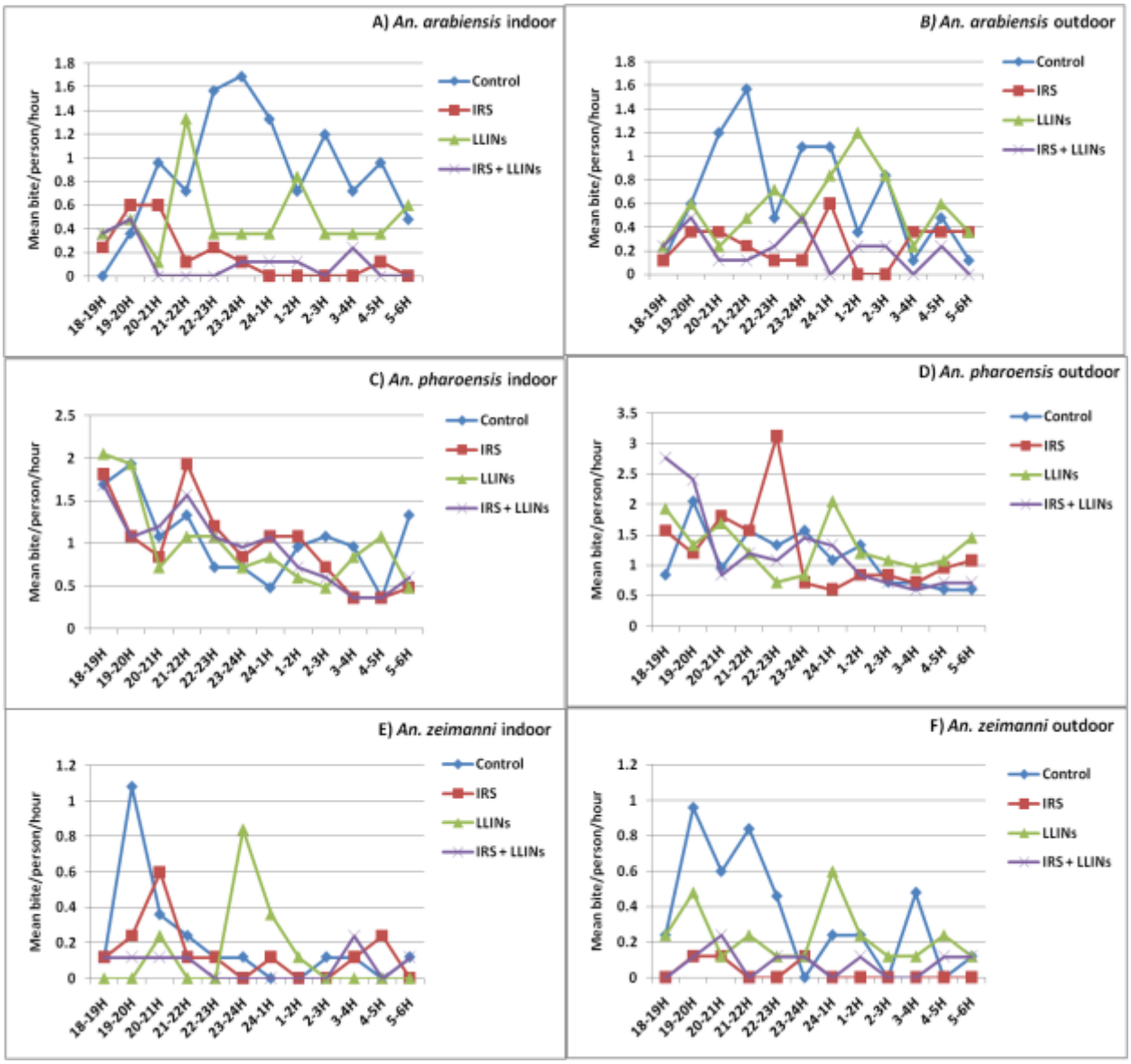

Figure 7.1: Indoor and outdoor nocturnal biting activities of *An. arabiensis* (7.1A &B), *An. pharoensis* (7.1C&D) and *An. ziemanni* (7.1E &F) in the study arms, Adami Tullu, Ethiopia

Potential exposure of the local people to mosqiuito bites before or after bed times were assessed using questianiares (Appendix 1). Cumulatively 82.0% of the local family members retired to bed after 21:00 hour during the study period whereas 18.0% of them were in bed before 21:00 hour (Appendix 2). Next morning, cumulatively 83.3% of these people got up from their sleep after 6:00 hour and only 16.7% of them got up before 6:00 hour. High proportion of the house

occupants (approximately 80.0%) were awake up to 22:00hrs and had high potential exposure to *An. arabiensis* biting activities than those that retired to bed before 22:00hrs (Chapter 4, Figures 7.1A and B). The proportion of people potentially exposed to mosquito bites between 18:00-23:00hrs was 79.9% (range 69.5% - 99.1%). The cumulative proportion of the local family members that awake from their sleep after 6:00hr in the morning was also estimated to be more than 80% and had less potential exposure to *An. arabiensis* bites in the early morning after bed times (Chapter 4, Figure 7.1A).

The time at which the family members of the study population usually went to sleep for the duration of the night was significantly different but the time at which they usually got up in the morning was similar. The majority (72.7%) of father respondents reported that they usually went to sleep between 20:00-22:00 hour and the remaining 27.0 % of them reported that they usually went to sleep after 22:00hrs. The majority of these fathers (80.0%) usually got up after 6:00 hour in the morning and 20.0% of them awake earlier (before 06:00hr). Whereas 78.0% and 84.6% of mothers reported that they usually went to sleep after 22:00 hours for the duration of the night and got up after 6:00 hour in the morning. The remaining 20.0% and 15.4% of mothers went to bed and got up earlier respectively. The higher proportion of children also reported that they went to sleep between 21:00-22:00 hours and got up between 6:00-7:00 hours at similar times.

7.3. Blood feeding and host preferences

The impact of the interventions on anthropophily was assessed by comparing the HBI of engorged *Anopheles* species collected by LTC, PSC and PIT (Table 7.3). The overall number of blood fed *An. arabiensis* collected by LTC, PSC and PIT was high in the control and LLINs arms (48/107=44.8%) each compared to either the IRS+LLINs arm (7/107=6.5%) or the IRS arm

(4/107=3.7%). Of 107 blood fed *An. arabiensis* tested, the overall blood origin of this species collected by LTC, PSC and PIT were 62 (57.9%) from bovine, 36 (33.6%) from human, five (4.8%) not identified and four (3.7%) from both bovine and human. The overall HBI (0.16) of *An. arabiensis* was high in the control arm compared to each of the intervention arm. However bovine blood index (BBI) of *An. arabiensis* expressed as the proportion of mosquitoes with bovine blood out of total tested, was high in the LLINs arm (0.26) compared to the other study arms. Both HBI and BBI of *An. arabiensis* were similar in the IRS arm relative to IRS+LLINs arm. Number of blood fed *An. arabiensis* and hence HBI and BBI of this species were low in the IRS and IRS+LLINs arms.

Out of 46 blood fed *An. arabiensis* collected indoors by LTC, 35 (76.1%) were fed in the control arm followed by 7 (15.2%) in the LLINs arm and 2 (4.3%) in each of the IRS and IRS+LLINS arms. The overall HBI of *An. arabiensis* collected by LTC in the study arms was 0.41 whereas its BBI was 0.46. Number of blood fed *An. arabiensis* obtained by LTC and hence its HBI and BBI were high in the control arm compared to each of the intervention arms. However, the number of blood fed *An. arabiensis* tested and its HBIs were low in the intervention arms.

A total of 42 blood-fed indoor resting *An. arabiensis* were captured by PSC from the study arms. Blood fed *An. arabiensis* captured by PSC was high (73.8%) in the LLINs arm and low in the control arm (19.1%), IRS+LLINs arm (4.8%) and the IRS arm (2.4%).The overall estimated HBI (0.36) of this species collected by PSC was lower than its BBI (0.57). Both HBI (0.24) and BBI (0.43) of *An. arabiensis* were high in the LLINs arm compared to each of the study arms. The number of blood fed *An. arabiensis* collected from IRS, IRS+LLINs and the control arms by PSC was low for valid comparison of HBI and BBI among the groups.

Outdoor collections of *An. arabiensis* from PIT caught 19 blood-fed *An. arabiensis* that comprised 10 (52.6%) from LLINs arm, five (26.3%) from the control arm, three (15.8%) from the IRS+LLINs arm and one (5.3%) from the IRS arm. Overall 19 blood-fed outdoor resting *An. arabiensis* collected by PIT from the study arms did not take blood from human except two vectors one from the LLIN arm and the other from the control arm. The overall BBI of this vector was high (0.89) compared to HBI (0.11). From the total catches of *An. arabiensis* by PIT, none of them took blood from other hosts than either bovine or human host. Outdoor resting *An. arabiensis* collected from PIT showed more preference to bovine than human host.

Overall 41 blood fed *An. pharoensis* that comprised 80.5% in the control arm, 12.2% in the IRS arm, 4.9% in the IRS+LLINs arm and 2.4% in the LLINs arm were collected by LTC. Among the 41 blood-fed *An. pharoensis*, 30 (73.2%) females fed on bovine, eight (19.5%) fed on human, two (4.9%) fed on other host and only one (2.4%) fed mixed blood from bovine and human hosts. Both the HBI and BBI of this species were high in the control arm compared to each of the intervention arms.The estimated HBI (0.19) of this species was much lower than BBI (0.73). The number of blood fed *An. pharoensis* and its HBIs obtained from each of the intervention arms were low and so was for its BBI.

Table 7.3: Blood meal sources of *Anopheles* species collected by LTC, PSC and PIT from the study arms. Human blood index (HBI) and bovine blood index (BBI) are given in bracket

Species & collection method	Study arms	Blood fed n (%)	Human n (HBI)	Bovine n (BBI)	Mixed	Other host
An. arabiensis (LTC)	Control	35 (76.1)	13 (0.28)	17 (0.37)	3 (0.07)	2 (0.04)
	IRS	2 (4.3)	0 (0.00)	2 (0.04)	0 (0.00)	0
	LLINs	7 (15.2)	5 (0.12)	1 (0.02)	1 (0.02)	0
	IRS + LLINs	2 (4.3)	1 (0.02)	1 (0.02)	0 (0.00)	0
	Total	**46 (100.0)**	**19 (0.41)**	**21 (0.46)**	**4 (0.09)**	**2 (0.04)**
An. arabiensis (PSC)	Control	8 (19.1)	3 (0.07)	5 (0.12)	0	0
	IRS	1 (2.4)	0 (0.00)	1 (0.02)	0	0
	LLINs	31 (73.8)	10 (0.24)	18 (0.43)	0	3
	IRS + LLINs	2 (4.8)	2 (0.05)	0 (0.00)	0	0
	Total	**42 (100.0)**	**15 (0.36)**	**24 (0.57)**	**0**	**3 (0.07)**
An. arabiensis (PIT)	Control	5 (26.3)	1	4 (0.21)	0	0
	IRS	1 (5.3)	0	1 (0.05)	0	0
	LLINs	10 (52.6)	1	9 (0.47)	0	0
	IRS + LLINs	3 (15.8)	0	3 (0.16)	0	0
	Total	**19 (100.0)**	**2 (0.11)**	**17 (0.89)**	**0**	**0**
An. arabiensis (Total)	Control	48 (44.8)	17 (0.16)	26 (0.24)	3 (0.03)	2 (0.02)
	IRS	4 (3.7)	0 (0.00)	4 (0.04)	0 (0.00)	0 (0.00)
	LLINs	48 (44.8)	16 (0.15)	28 (0.26)	1 (0.01)	3 (0.03)
	IRS + LLINs	7 (6.5)	3 (0.03)	4 (0.04)	0 (0.00)	0 (0.00)
	Overall	**107 (100)**	**36 (33.6)**	**62 (57.9)**	**4 (3.7)**	**5 (4.8)**
An. pharoensis(LTC)	Control	33 (80.5)	7 (0.17)	24 (0.59)	1	1
	IRS	5 (12.2)	1 (0.02)	4 (0.10)	0	0
	LLINs	1(2.4)	0	0 (0.00)	0	1
	IRS + LLINs	2 (4.9)	0	2 (0.05)	0	0
	Overall	**41 (100.0)**	**8 (19.5)**	**30 (73.2)**	**1 (2.4)**	**2 (4.9)**

7.3.4. Resting habits of *Anopheles arabiensis*

The proportion of this species that frequently fed on human and rested indoors was high in the LLINs arm compared to each of the other study arms (Table 7.4). Indoor resting proportion of the vector population that had fed on human in the IRS alone was low relative to the other three study arms.

Table 7.4: The resting habit of *An. arabiensis,* i.e. the proportion of mosquitoes having fed on human blood and then resting indoors.

Vector parameters	Control	IRS	LLINs	IRS + LLINs
Total number of females collected	27	3	43	8
D (indoor resting density)	27/16 = 1.69	3/16 = 0.19	43/16 = 2.69	8/16 = 0.50
Number of blood-fed females	11	1	30	8
HBI (human blood index)	0.07	0.00	0.24	0.05
Number of half-gravid females	0	1	4	0
Number of gravid females	0	0	0	0
P (indoor resting post-feeding)	1.00	2.00	1.13	1.00
M (human biting rate or b/p/n)	0.04	0.01	0.08	0.01
N (Average number of occupants)	4.34	5.56	5.14	5.30
Resting habit	**0.79**	**0.00**	**1.61**	**0.54**

7.4. Discussion and conclusions

Monitoring malaria vector feeding patterns, host preferences and resting habits in time will provide useful information on the success of vector control interventions (Wamae *et al.*, 2015). This study was undertaken to monitor the impact of IRS and LLINs combined and separate

interventions on local mosquito biting (biting venues, time), host preference and resting behaviours. The mean endophagic and exophagic rates of *An. arabiensis* were significantly lower in the IRS and IRS+LLINs arm each compared to the control arm. This was expected because the IRS and LLINs interventions suppress *An. arabiensis* population due to their toxic chemical action on the mosquitoes (Gimnig *et al*., 2003; Gatton *et al*., 2013). Significant decrease on endophagic rate of this species in IRS+LLINs and IRS arms could also had an impact on exophagic rates of this mosquitoes because these interventions have mass killing effect indoors and outdoors (Hawley *et al*., 2003). However mean endophagic rate of *An. arabiensis* in the control arm was similar to the LLINs arm.This could be explained by early biting activities of the vector (Yohannes and Boelee, 2012), insecticide resistance development by the vector against deltametrin (Gari *et al*., 2016) and lack of adherence to LLINs use by the target community all of which could compromise protective effectiveness of LLINs.

Results also showed that mean endophagic rate of *An. arabiensis* in the combined arm was similar to the IRS arm. However endophagic rate of this vector were significantly lower in the combined and IRS arm each compared to the LLINs arm. These findings concur with previous reports (Kitau *et al.* 2012) and confirm that LLINs alone could not sufficiently address spacial and temporal biting behaviour of this species.

However, the difference in mean endophagic and exophagic rates of *An. pharoensis* in the control arm was not significant compared to each of the intervention arm and between the intervention arms.This might happen due to conatmination or spill over effects as all *An. pharoensis* mainly breeds in permanant swampy habitats along Lake Zeway where HLC were performed in the study arms as explained in chapter 6. Moreover, *An. pharoensis* has been frequently reported as exophagic and zoophagic species in the area (Abose *et al*., 1998; Gari *et al*., 2016) and may be

less influenced by the current indoor-based insecticidal interventions. Compared to the combined arm, exophagic rates of *An. arabiensis, An. pharoensis* and *An. ziemanni* were similar either in LLINs or IRS arm. This implies that *An. arabiensis, An. pharoensis* and *An. ziemanni* appears to be less impacted by IRS and LLINs outdoors.

This study also revealed that the local vectors were actively feeding on human throughout the study night from 18:00 to 6:00 hour. Whole-night HLC of *An. arabiensis, An. pharoensis* and *An. ziemanni* showed biting rhythms which were significantly similar in the combined arm compared to either IRS or LLINs arm indoors and outdoors. These could be due to similar early night increased feeding habits of the three *Anopheles* species in this locality. The marked peak biting activities (bite before 22:00 hours) of these species observed in this study agrees with pre-intervention results in the area (chapter 4). Whether these enhanced early biting activities of these species were driven by the impact of IRS and LLINs or low night temperature is unknown and need further investigations.

Human sleeping time survey results showed that more than 80% of the local family members retired to bed after 21:00 hour and got up from their sleep after 6:00 hour the next morning during the study period. These imply that the distinct peak biting activities of the vectors in the early hours of the evening coincides with human outdoor and before bed time activity behaviours. These potential exposure to mosquito bites in outdoor venues and before bed time could compromise the efficacy of both IRS and LLINs. These may jeopardize the overall intervention impact of IRS and LLINs. It should be noted that the early night biting activities of these species observed might not be the consequence of the current intervention, rather it may be a pre-existing behaviour as these results are similar with the pre-intervention results (chapter 4) and with other

studies in the country (Abose *et al*., 1998; Kibret *et al*., 2010; Yohannes and Boelee 2011; Taye *et al*., 2016).

The overall proportion of blood fed *An. arabiensis* collected by LTC, PSC and PIT were very low in the IRS+LLINs and IRS compared to the control arm and so were the HBI and BBI of this species. These results demonstrate operational effectiveness of the interventions and suggest that combing IRS and LLINs had additive impact on suppressing blood feeding mosquitoes. However, the proportion of blood fed *An. arabiensis* collected in the LLINs arm compared to the control arm was similarly high and thus indicates that LLINs alone was not sufficiently effective against *An. arabiensis* in this study setting.

Bovine and human hosts remain the major blood meal sources of *An. arabiensis* in the study area. This finding agrees with previous studies in the area (Abose *et al*., 1998; Kibret *et al*., 2010; Gari *et al*., 2016; Kenea *et al*., 2016) and elsewhere in the country (Massebo *et al.* 2013; Animut *et al*., 2013; Yewalaw *et al.* 2014; Taye *et al.* 2016). In this study, the overall BBI of this species was higher than HBI. This could be partly attributed to the impact of the current intervention because IRS and LLIN reduce human-vector contact and blood feeding success of the vector from human host (Takken and Verulst, 2012) and might have diverted the mosquitoes to bovine. Another possible explanation includes differences in availability and accessibility of the two hosts to the vector population. This study was conducted within 5 km distance from Lake Zeway and Bulbula River (Deressa *et al*., 2016) where a cattle raising is the major activity and where cattle were more abundant in the district due to availability of wet land and pasture. These cattle were kept in open shelters close to human dwellings at night and were accessible to mosquito exposure compared to human host who are more protected indoors. This might favour the mosquitoes to feed more on cattle than human host.

The host choice of opportunistic mosquitoes such as *An. arabiensis* is often determined by the host species that is most abundant or readily available. Host availability and accessibility are key factors that influence *An. arabiensis* host species preferences (Ameneshewa and Service, 1996; Lyimo and Ferguson, 2009). *Anopheles arabiensis* may readily switch to other hosts in high IRS and LLINs coverage settings as human hosts are more protected (Takken and Verhulst, 2012).

Results also showed that the HBI and BBI of *An. arabiensis* were significantly lower in the combined and IRS arm each compared to the LLINs arm. These could be explained in terms of area-wide coverage of IRS relative to LLINs alone at household level as described in chapter 6. Application of IRS into the whole interior walls and ceilings of houses directly protect both human and domestic animals living in that house and reduce their exposure to mosquito bites. A sizeable number of the rural inhabitants were observed tethering livestock inside residential houses, thus, application of IRS directly impact on vectors such as *An. arabiensis* that feed mainly on human and bovine hosts. The key implication of higher HBI and BBI of *An. arabiensis* in the LLINs arm is that net alone is not sufficient to address the biting behaviour of *An. arabiensis* that could take place before bed times and/or outdoors when the local people are not in the LLINs. Therefore, complimentary interventions are required for effective control of *An. arabiensis*.

Results also showed that the proportion of blood fed *An. pharoensis* collected in each of the intervention arm was low compared to the control arm. This was expected because the interventional impact of IRS and LLINs suppress blood feeding success of the mosquitoes. *Anopheles pharoensis* preferred to feed most frequently on bovine than human host both in the control arm and each of the intervention arms. *Anopheles pharoensis* was reported to be more zoophagic and exophagic species in the area (Abose *et al.*, 1998; Kibret *et al.*, 2010) similar to the current pre-intervention results (chapter 4).

In this study comparison of the impact of interventions on host preferences has shown that the target vectors were affected by the size of blood fed mosquito catches. The numbers of blood fed mosquitoes were too small, particularly in the IRS and IRS+LLINs arms to compare HBI among the intervention arms. The main reasons for low blood fed mosquito catches in these arms were: 1) Collection by LTC, PSC and PIT were randomized over the entire study area and hence might have affected the adequate collection of mosquito samples in Adami Tullu. 2) The impact of the interventions itself. Both IRS and LLINs adversely affect vector-host contact and blood feeding success of the mosquitoes.

High proportion of *An. arabiensis* that fed on human was estimated to rest indoors in the LLINs arm compared to the other study arms. This might be attributed to the early biting activities of the vector before the local people retire to bed. It could also be due to the impact of capturing some fraction of exophagic but endophilic *An. arabiensis* by PSC. Resting behaviour of *An. arabiensis* appears to be relatively plastic with considerable potential for variation between endophilly and exophilly (Paaijmans and Thomas, 2011). The most recent study on the impact of housing conduction on resting density of *Anopheles* mosquitoes near to the study area noted that *An. arabiensis* were more abundant inside residential houses than outdoors in pit shelters (Animut *et al*., 2013). This results suggest that there is a possibility for outdoor biting proportion of this vector population to rest indoors as they are attracted by indoor temperature (Paaijmans and Thomas, 2011) and odours of humans and cattle coming from the houses (Tirados *et al*., 2011).

In addition, the tradition of cooking, sleeping and tethering livestock inside houses could also contribute to the indoor abundance of this mosquito by increasing indoor temperature and providing access to blood meal sources (Animut *et al*., 2013). This calls for the need of window entry and exit traps to estimate biting location of *An. arabiensis* for evidence based vector control

interventions in this area and elsewhere in the country. Housing conditions such as presence of open eaves, and absence of window were reported to enhance indoor resting density of *An. arabiensis* (Animut *et al*., 2013). During the present study, house entry and exit behaviour of this vector and the impact of housing conditions on the vector population and behaviour were not assessed. Therefore the influence of these factors on resting habit of *An. arabiensis* warrant further investigation with respect to LLINs ownership and utilization in the study area.

Furthermore, it is possible that high proportion of *An. arabiensis* that fed on human could rest indoors because the house occupants were exposed to the vector bites. Perhaps LLINs are used improperly or the vector might be feeding through the nets. In this connection, the researcher has frequently observed local people using the distributed LLINs for other purposes such as for window curtain, table cloth, for holding and threshing of crops during harvest, and for transporting vegetables to the local markets. Therefore indoor biting and resting behaviour of the vector will need to be monitored along side with LLINs ownership and utilization during transmission season.

However, indoor resting habit of this vector was relatively low in the IRS and combined arm. This was expected because IRS prevents mosquitoes resting in houses (WHO, 2011). These results contrast the Tanzanian trial that found no additional impact of combining IRS and LLINs on indoor resting density of *An. arabiensis* (Protopopoff *et al*., 2015). The authors justified that *An. arabiensis* was less endophilic and as a result, might have reduced contact with insecticides on the walls. However, *An. arabiensis* were more endophilic vectors compared to the other *Anopheles* species in Ethiopia (Abose *et al*., 1998; Massebo *et al*., 2015) and this behaviour of the vector might have reduced indoor resting due to the impact of IRS in both the combined arm and the IRS arm. Furthermore, *An. arabiensis* is responsible for outdoor and indoor malaria

transmission in Ethiopia unlike in Tanzania where several reports indicate that *An. arabiensis* had little contribution to indoor malaria transmission and considered the principal vector responsible for residual transmission (Russel *et al*., 2011; Okumu *et al*., 2013).

The proportion of *An. arabiensis* that fed on humans and rested outdoors in this study was almost nil in all of the study arms. This is an evident that the interventions might have less impact on outdoor resting fraction of the vector population. It is also possible that the vector might have rested in other outdoor resting sites such as tree holes, animal shelters (Abose *et al*., 1998). Given a large portion of the mosquito gonotrophic cycle (blood-feeding, egg maturation and oviposition, which are repeated several times throughout adult life) is spent resting (Paaijmans and Thomas, 2011), outdoor resting sites of *An. arabiensis* need to be targeted and monitored with appropriate outdoor mosquito sampling methods in the area for successful implementation of outdoor control interventions. As far as is known, the pioneer vector intervention trials in Benin, Tanzanian and The Gambian did not analyse the impact of combining IRS and LLINs on indoor and outdoor resting habits of *An. arabiensis*. Thus, published evidence was lacking to compare the impact of the intervention on resting behaviour of the vector with similar trials elsewhere.

In conclusion, the combination and IRS alone intervention each had most significant impact on endophagic *An. arabiensis* population compared to LLINs alone. Nevertheless, there were no significant difference between IRS+LLINs and either IRS or LLINs alone on exophagic population of this vector. The vectors fed mainly in the early hours of the evening on cattle and human hosts with high HBI and BBI in the LLINs arm. High proportion of *An. arabiensis* that fed on human was estimated to rest indoors in the LLINs alone compared to IRS alone or IRS+LLINs.

Chapter 8. Conclusions and recommendations

8.1. Conclusions

This study involved evaluation of indoor residual spraying (IRS) with propoxure and deltametrin based long-lasting insecticidal nets (LLINs) combined versus separate interventions against *Anopheles arabiensis*, the sole primary malaria vector in Ethiopia. Based on the study results subsequent conclusions were drawn. Conclusions were mainly drawn based on the impact of the interventions on the vector host-seeking density (HSD), indoor resting density (IRD), outdoor resting density (ORD), human biting rates (HBRs), host-preference and resting habit of the vector as a proxy for malaria transmission. This is because sporozoite infections in mosquitoes were found to be negative for all mosquito specimen tested. As a result, malaria entomological inoculation rate (EIR), which is the product of sporozoite rate and human biting rate was not estimated and compared among the study groups in this study. This study therefore reports impact of the interventions on vector abundance (density) rather than infectivity.

Results underscore that indoor HSD of *An. arabiensis,* as estimated by LTC was significantly higher in none-exposed communities (the control) as compared with communities exposed to the IRS, LLINs and IRS+LLINs interventions (i.e. HSD of *An. arabiensis* in the control communities > each of the intervention communities). Among the three interventions, IRS+LLINs and the IRS alone intervention each was similarly most effective against HSD of *An. arabiensis* each compared to the LLINs alone intervention (i.e. HSD of *An. arabiensis* in (IRS+LLINs) =IRS < LLINs). This implies that combining IRS with LLINs provided improved significant impact on HSD of *An. arabiensis* compared to LLINs alone. However, the combination intervention did not provide significant difference on HSD of the vector as compared to the IRS alone.

Indoor resting density (IRD) of *An. arabiensis* assessed by PSC was significantly lower in communities exposed to the combined intervention and the IRS alone each compared to the control communities (i.e. IRD of *An. arabiensis* in the (IRS+LLINs) =IRS < the control). However, the difference in IRD of the vector in communities that exposed to LLINs alone compared to communities without vector intervention (control) was not significant (i.e. IRD of *An. arabiensis* in LLINs=Control). The combined intervention and the IRS alone each provided similarly better protection against IRD of *An. arabiensis* compared to LLINs alone, (i.e. IRD of the vector in (IRS+LLINs) = IRS < LLINs). This implies that the LLINs alone intervention had the least impact on IRD of the vector in this study setting.

Results also show that human biting rate (HBR) of *An. arabiensis*, as estimated using a conversion factor, from indoor LTC was significantly lowered in communities exposed to the IRS, LLINs and IRS+LLINs interventions compared to the control communities (i.e. HBR of *An. arabiensis* in the intervention communities < contol). However, communities in the IRS + LLINs intervention and IRS alone each was similarly least exposed to the vector bites as compared to communities in the LLINs alone (i.e. HBR of *An. arabiensis* in (IRS+LLINs) = IRS < LLINs). This suggests that there were no significant difference between the combination and the IRS alone interventions on HBR of the vector. However, the LLINs alone provided the least intervention impact on HBR of the vector.

Parity rate was expressed as the proportion of parous females and was used to estimate mosquito survival rate or longevity among study arms. Neither indoor nor outdoor parity rate of *An. arabiensis* and *An. pharoensis* collected by human landing catches (HLC) significantly varied among the study arms (control=IRS+LLINs=IRS=LLINs). Evaluation of the impact of the

interventions on the mosquito parity rate therefore might be influenced by low mosquito collections in LTC in the study setting.

As described above, the impact of the different interventions on EIR were unknown because none of the mosquito samples tested by ELISA was positive for *P. falciparum* and *P. vivax* circumsporozoite proteins. The impact of the interventions on EIRs was unknown and warrant further study.

IRS alone or the addition of IRS to LLINs provided significant intervention impact on indoor biting (endophagic) and outdoor biting (exophagic) populations of *An. arabiensis* estimated by HLC compared to the control group (i.e. mosquito endophagic or exophagic rate in control arm > (IRS+LLINs)=IRS). However, the impact of LLINs alone on indoor biting (endophagic) *An. arabiensis* was similar to the control group. Thus, it can be suggested that LLINs need to be combined with IRS to significantly suppress endophagic *An. arabiensis* population. The IRS+LLINs and IRS alone each had significant intervention impact on indoor biting (endophagic) *An. arabiensis* compared to LLINs alone (i.e. endophagic rate of the vector in (IRS+LLINs)=IRS < LLINs). The IRS alone and the combined intervention each provided better intervention against indoor bitting population of *An. arabiensis*. Whereas, the LLINs alone provided the least impact on this population.The impact of IRS and LLINs combined and separate interventions on outdoor biting (exophagic) population of *An. arabiensis* were not significantly different (i.e. mosquito exophagic rate in IRS=LLINs=IRS+LLINs). This suggests that complementary vector intervention measures are needed to address outdoor biting behaviour of this vector.

In all study arms, *An. arabiensis* was actively biting indoors and outdoors throughout the night with an early night biting peak before local people retire to bed. Early indoor and outdoor biting

could potentially retard the effectiveness of IRS and LLINs. *Anopheles arabiensis*, *An. pharoensis* and *An. ziemanni* had biting rhythms which were similar in the IRS+LLINs compared to either IRS or LLINs indoors and outdoors. Early at night and outdoor biting behavior of the local vectors implicate the occurrence of potential residual malaria transmission in the study setting.

Results underscore that the overall proportion of blood fed *An. arabiensis* assessed by LTC, PSC and PIT were low in IRS+LLINs and IRS arm each compared to either the control or LLINs arm (i.e. blood fed mosquito in control=LLINs > (IRS+LLINs)=IRS). *Anopheles arabiensis* preferred mainly human and bovine hosts for blood meal sources. The human blood index (HBI) expressed as the proportion of mosquitoes with human blood and bovine blood index (BBI), the proportion of mosquitoes with bovine blood for *An. arabiensis* was high in the control and the LLINs arms each compared to either the IRS or IRS+LLINs arm.

The impact of IRS+LLINs intervention and IRS alone on indoor resting habit of *An. arabiensis* was high relative to the LLIN alone or the control arm (i.e. proportion of indoor resting *An. arabiensis* in control=LLINs > (IRS+LLINs) =IRS).

8.2. Recommendations

In operational vector control interventions, IRS application should be strengthened as it has prompt and more powerful impact on *An. arabiensis* indoors than LLINs. Application of IRS should be carried out in late July and early August before proliferation of this vector population in September and October. Furthermore cost-effectiveness of deploying IRS and its durability need to be evaluated at local operational settings.

The impact of LLINs alone on *An. arabiensis* biting behavior was insufficient and least effective compared to all other interventions considered in this study. The use of LLINs against local malaria vectors needs concern. Consistent and proper use of LLINs needs attention because LLINs are effective if users are effective as well. The use of LLINs in integrated vector management (IVM) should be consolidated through formulation of appropriate net use policy for the target community and the public at large. In particular, implimentation and adherence to the LLINs interventions need special concern, attention and action by the target communities in malarious areas. The community should be sensitized through information, education and communication campaigns for effective LLINs utilization and adherence. Behavioral change communication and education should be strengthened for operational use of IRS and LLINs during the malaria transmission season.

Combining IRS with LLINs provided improved intervention impact on *An. arabiensis* compared to LLINs alone. Therefore, the combined intervention should be encouraged based on cost-effectiveness analysis of single intervention versus double intervention in rural Ethiopian settings with respect to limited resources.

No single best vector control intervention can be duly recommendable against *An. arabiensis*. IRS and LLINs combined or separate interventions even under high coverage and utilization settings could not completely control *An. arabiensis* that feed outdoors, on other hosts, early at night and that bite indoor and run outdoor. This warrant enhanced implementation of complimentary vector intervention strategies such as larval source management, Ivermectin treated cattle, to address this flexible feeding behavior of the vector. Effective IRS application against malaria vectors depends on whether target mosquitoes rest indoors (endophilic behaviour), optimum effectiveness of LLINs depends on vectors biting at hours when most people are in bed and proper utilization. Therefore, continuous entomological monitoring and evaluation of the operational impact of IRS and LLINs interventions should be strengthened. Susceptability of local mosquitoes to the insecticides used in IRS and LLINs need special attention at malaria sentinel sites to generate up-to-date evidence for effective malaria vector interventions in Ethiopia.

National malaria control programs should consider implementing IRS in combination with LLINs in high malaria risk areas to inhance malaria reduction, compensate for loss of effectiveness of LLINs due to improper use and low coverage, to catchup early biting activities of *An. arabiensis* and for management of insecticide resistance as well. As we drive to malaria elimination and eventual eradication, combination of interventions or improved malaria interventions are urgently needed. Thus, the use of IRS and LLINs for vector control interventions should be strengthened as IVM strategy.

The impact of residual malaria transmission driven by outdoor biting, early biting behaviors of *An. arabiensis* need special concern and action. Additional complementary interventions are required to control malaria transmission encountered before bed-time and outdoors.

The results of this study indicate that there are many research gaps that may be prsued for future studies. In particular, flight range and survival rates of the local vectors should be thoroughly studied. Future research could focus on the impact of IRS and LLINs interventions on infectivty (EIRs) rather than vector abundance alone. The impact of cattle ownership, ivermectin treated cattle and chicken keeping on malaria transmission in comparson to the conventional insecticidal vector interventions (IRS and LLINs) need to be considered. The impact of vector interventions on mosquito biting venues should be monitored using additional sampling methods such as window entry and exit traps. Moreover malaria vector research and development should focus on innovative approaches that have profound effect on malaria such as transmission blocking vaccine or that effectively target outdoor malaria transmission such as ivermectin treated cattle, and/or that can complement these indoor based insecticidal interevntions such as improved housing, etc. Assessment of malaria transmission potential of *An. ziemanni* and *An. funestus* s.l. warrant special considerations. Efficient methods for sampling *An. arabiensis* outdoors need further research.

In the end, the present trial design has potential limitations. Selection bias was minimised by random selection of village clusters and households for LTC, PSC and PIT. However, the villages for the HLC were selected for convenience, being chosen for high mosquito density and accessibility of similar types of houses for this work. In addition, study investigator and mosquito collectors could not be totally masked to the interventions arms. Furthermore, spillover of mosquito population was reduced by equal randomization of the cluster villages. Nevertheless, the village clusters enrolled in the study was within 5 km distance from Lake Zeway and Bulbula River. Thus, mixing of mosquito population between villages might be likely within flight range of the mosquitoes coming from common water sources as boundaries of sstudy arms were not buffered.

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Appendices

Appendix 1: Survey of human sleeping time and night activity behaviors in south-central Ethiopia

House number:_____Date:_____

S.No.	Family (01--Head, 02--Spouse, 03--son, 04--daughter)	First name	Gender (1…Male, 2…Female)	Age (years), if greater than 1 year	Age (months) if less than 1 year	What time did you go to sleep for the duration of the night? (01--18-19, 02--19-20, 03--20-21, 04---21-22, 05---after 22 hour)	What time do you usually get up in the morning? (01--4-5, 02--5-6, 03--6-7, 04---7-8, 05---after 8 hour)
	1	2	3	4	5	6	7
1							
2							
3							
4							
5							
6							

I thank you so much for your time!

Appendix 2: Sleeping and getup time of household members in the study area, Adami Tulu, Ethiopia 2015

Variables	Respondents								Total	
	Father		Mother		Son		Daughter			
	n	%	n	%	n	%	n	%	n	%
When did you sleep the previous night?										
Between 18-19	0	0.0	0	0.0	5	2.0	1	0.6	6	**0.9**
19-20	0	0.0	1	0.8	27	10.6	15	9.1	43	**6.3**
20-21	4	3.0	3	2.3	35	13.8	32	19.5	74	**10.9**
21-22	92	69.7	25	18.9	157	61.8	77	47.0	351	**51.5**
After 22 hour	36	27.3	103	78.0	30	11.8	39	23.8	208	**30.5**
Total	**132**	**100.0**	**132**	**100.0**	**254**	**100.0**	**164**	**100.0**	**682**	**100.0**
When did you get up this morning?										
Between 4-5	0	0.0	0	0.0	4	1.6	1	0.6	5	**0.7**
5-6	27	19.9	21	15.4	36	14.5	15	9.3	99	**14.5**
6-7	87	64.0	86	63.2	144	58.1	113	69.8	430	**63.0**
7-8	18	13.2	24	17.6	61	24.6	33	20.4	136	**19.9**
After 8 hour	4	2.9	5	3.7	3	1.2	0	0.0	12	**1.8**
Total	**136**	**100.0**	**136**	**100.0**	**248**	**100.0**	**162**	**100.00**	**682**	**100.0**