Addis Ababa University

Graduate Programme

College of Natural Sciences

Department of Zoological Sciences

Ecology and Distribution Pattern of *Anopheles* Mosquitoes and Malaria Transmission in Ghibe River Basin, southwestern Ethiopia

BY

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

Addis Ababa, Ethiopia

May, 2017

Declaration

I, Dejene Getachew Bekele, 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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Dedication

This work is dedicated to Almighty God, Who helped me throughout my study.

Acknowledgements

First and for most, I would like to express my heartfelt thanks to Dr. Habte Tekie who was with me throughout my study, his encouragement, prompt response to all my request, tolerance, academic and technical support. I would also like to appreciate his motivation on field work that enabled me to get immense experience and hardworking inspiration. I would like to thank Dr. Teshome Gebre-Michael, Aklilu Lemma Institute of Pathobiology (ALIPB), for his unlimited advice, suggestions, unreserved encouragements, constructive comments and academic support. I would like to sincerely thank Dr. Meshesha Balkew (ALIPB) for his encouragement, scientific guidance on laboratory activities, and unreserved scientific comments throughout my study. I sincerely thank Dr. Irish R. Seth for his material support and scientific comments.

I sincerely thank Prof. Abebe Getahun, Chair, Department of Zoological Sciences for his encouragement and assistance in management issues. I acknowledge ALIPB for all the supports provided to conduct my laboratory activities. Department of Zoological Sciences, Addis Ababa University is acknowledged for providing me financial support. Dire Dawa University is acknowledged for sponsoring me throughout my study.

My heartfelt thank to Dr. Oljira Kenea and Dr. Essayas Aklilu for their support in mosquitoes identification and processing of laboratory activities; Debele Abera for mapping the study area; Wossen Sysay and Selamawit Yaregal for their assistance in laboratory activities and Bereket Taphano for his support in mosquito collection. I would like to thank National Meteorological Agency for their support in providing meteorological data of the study area. Personnel and employee of Ghibe farm enterprise particularly Ato Zenebe Getachew and Melkamu Mihret for providing resting house and a space for mosquito processing on the field. I am thankful to the Abeshge district health office personnel particularly Mohammed Jemal and Petros for providing

malaria prevalence data of the study area, Abeshge Woreda Agricultural Development Office for providing domestic animals data, administrative personnel and all residents of Ghibe and Darge study sites.

All friends and relatives who encouraged me during my study are duly acknowledged.

Lastly but not list, my wife Mestawot Kebede and my son Fedhesa Dejene are acknowledged for their understanding, encouragement, patient and being with me throughout my study period.

Abbreviations and Acronyms

ACT	artemisinin-based combination therapy
ALIPB	Aklilu Lemma Institute of Pathobiology
BBI	Bovine blood index
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CSP	Circumsporozoite protein
DDT	dichlorodiphenyltrichloroethane
DEET	*N*,*N*-diethyl-*3*- methylbenzamide or diethyl-*m*-toluamide
EIR	Entomological inoculation rate
ELISA	Enzyme-linked immunosorbent assay
FMoH	Federal Ministry of Health
GMEP	Global Malaria Eradication Program
HBI	Human blood index
HMIS	health management and information system
IRS	indoor residual spraying
ITN	insecticide-treated mosquito net
LLINs	long-lasting insecticidal nets
LSM	larval source management
masl	meter above sea level
PBS	Phosphate-buffered saline
PSC	Pyrethrum spray catch
PR	parity rate
RDT	rapid diagnostic test

s.l.	sensu lato
SP	sulfadoxine-pyrimethamine
s.s.	sensu stricto
SSA	sub-Saharan Africa
WHO	World Health Organization

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Abstract

Malaria is a leading public health problem in Ethiopia. Though malaria prevalence showed high reduction in many parts of the country, its transmission is likely to be more and more focal with some areas still having substantial risk of malaria. There was paucity of information on the malaria vectors and malaria transmission pattern in the study area. So that, the objective of this study was to investigate the species composition and monthly distribution of *Anopheles* mosquitoes, their blood meal sources, sporozoite infection rates and level of insecticide susceptibility in the Ghibe River basin, southwestern Ethiopia. Study on *Anopheles* mosquitoes distribution and malaria transmission was conducted in two study sites. Longitudinal larval and adult *Anopheles* mosquitoes collections were carried out monthly from November 2014 to October 2016. Late instar larvae were morphologically identified. Habitats containing *Anopheles* larvae were identified and their environmental variables were recorded. Adult *Anopheles* mosquitoes were collected from selected houses near to or far from the river with CDC light traps, pyrethrum spray catches, pit shelter and mouth aspirator. Adult female *Anopheles* mosquitoes were morphologically identified and their parity rate, blood meal sources and malaria infections were analysed using Enzyme-linked immunosorbent assay (ELISA). *Anpheles* larvae and pupae were collected from their breeding sites, allowed to emerge into adult, morphologically identified, and exposed to insecticides using WHO discriminating concentrations. Awarness of the communities towards malaria prevention and control were also investigated. Multiple regression analysis was used to identify the most predictor larval habitat environmental variables with the occurrence of *Anopheles* larvae. Human blood index (HBI) was calculated as the ratio of blood-fed mosquitoes that had fed on human to the total tested expressed in percent. Sprorozoite rate was expressed as the proportion of mosquitoes with

sprorozoite to the number of mosquitoes tested. Insecticide susceptibility was determined based on mean mortality of the four replicates after 24 hour exposure. In total, 9,277 larvae of *Anopheles* mosquitoes were sampled. Mean *Anopheles* larval densities were higher in the pools in drying river beds (35.2 ± 7.9 larvae/dip), borrow pits (14.3 ± 8.6 larvae/dip) and pools at river edges (13.0 ± 2.1 larvae/dip) in Darge, and in borrow pits (11.2 ± 6.3 larvae/dip) and rain pools (11.9 ± 5.1 larvae/dip) in Ghibe. From totally identified *Anopheles* mosquitoes larvae more than 95% of them were *An. gambiae* s.l. Temperature at the time of collection (p = 0.03) and emergent vegetation (p = 0.003) were the best predictors of *Anopheles* larval density. In total, 2,669 adult female *Anopheles* mosquitoes were collected and morphologically identified. In Ghibe, mean density of *An. gambiae* s.l. (=*An. arabiensis*) using CDC light trap was higher outdoors (1.8/CDC light trap/night) as compared to indoors (0.7/CDC light trap/night) but in Darge 0.125/CDC light trap/night indoor and 0.07/CDC light trap/night outdoor. In Ghibe, the HBI for *An. arabiensis* was 58.0% from CDC light trap collections indoors and 16.0% outdoors but in Darge it was 14.0% indoors and 13.0% outdoors. Overall sporozoite rates of *An. arabiensis* for *P. vivax* and *P. falciparum* were 0.06% each. *Anopheles arabiensis* developed insecticide resistance to all the insecticide classes tested with, except fenitrotion. Study on awareness showed, there was knowledge gap in the communities towards the way malaria is transmitted. In conclusion, *An. arabiensis* is the predominant species of malaria vector in the study area. Malaria prevalence and vectors infection rates were very low. However, the outdoor abundance and the development of resistance to the major insecticides need to look for alternative control options in addition to LLINs and IRS.

Keywords: *Anopheles gambiae* s.l., CDC light trap, Darge, Ghibe, human blood index, larval habitat, sporozoite rate.

Chapter 1. General Introduction

1.1 Epidemiology of malaria

Malaria is a vector borne infectious disease that can cause major health problem globally. It is a leading cause of morbidity and mortality in many countries (WHO, 2013a). Globally malaria killed 995,000 in 1980, 1,817,000 in 2004, and 1,238,000 in 2010 (Murray *et al*., 2012). Even though malaria prevalence has shown reduction in many countries, it infected 212 million and killed 429,000 people globally in 2015. Among these, most cases (90%) and deaths (92%) have occurred in the WHO African Region (WHO, 2016). In the same year among all global deaths due to malaria, 70% occurred in children under 5 years of age (WHO, 2016).

Malaria is mostly distributed in the tropical and subtropical regions of the world. These areas often coincide with the regions where low income countries predominates (Gallup and Sachs, 2001). Global epidemiology of malaria varies depending on factors related to available *Plasmodium* species in a given area, the susceptibility of the pathogen to antimalarial drugs, the availability of important malaria vectors, climate and other environmental factors, and the level of acquired immunity and behaviour of infected people (Bloland, 2001; Autino *et al*., 2012). Malaria endemic countries of the world are indicated in figure 1.1 (WHO, 2016).

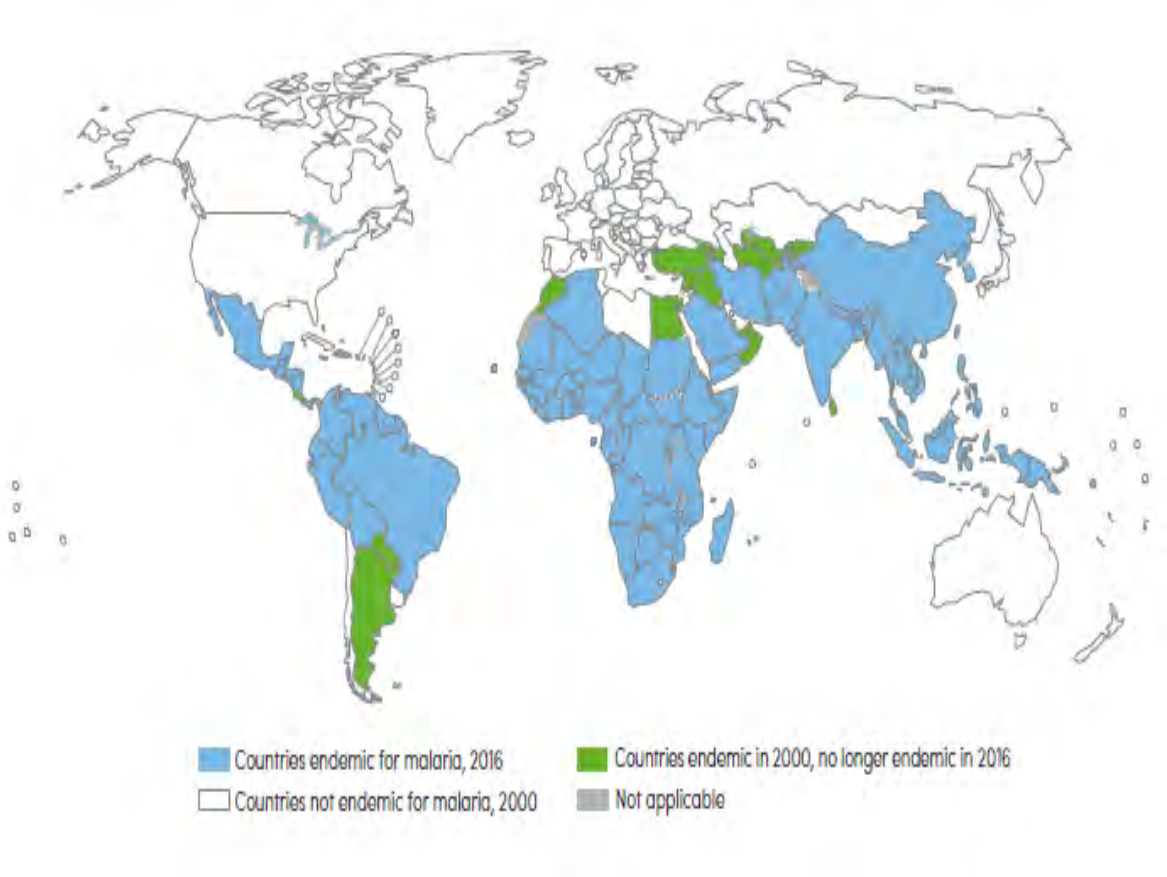


Figure 1.1 Countries endemic for malaria in 2000 and 2016 (Source: WHO, 2016)

People living in highlands and semi-arid regions are mostly affected by frequent malaria epidemics. In the highlands most malaria epidemics occur as a short and annual transmission that affect people living in the area while in semi arid areas, epidemics are caused by irregular rainfall that favour the development of malaria vectors (Abeku, 2007). There is also variation in susceptibility to infection with malaria among people. Mostly malaria attack people who lack or reduced immunity and repeatedly unexposed to disease causing agents. These include pregnant women and non-immune visitors to malarious areas (Bloland, 2001), people living in highland fringes and unstable transmission settings (Brooker *et al*., 2004), and younger children (Carneiro *et al*., 2010).

Malaria is caused by infection of red blood cells with protozoan parasites of the genus *Plasmodium* inoculated into the human host by a feeding female *Anopheles* mosquito. *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* are identified plasmodia species that cause human malaria in different parts of the world (WHO, 2015a). These differ in their geographical distribution, structure, clinical features, and potential for development of resistance to antimalarial drugs. Among these, *P. falciparum* is the deadliest than others (WHO, 2016).

The world malaria epidemiology is classified as of no risk, unstable risk and stable risk of *P. falciparum* transmission based on *P. falciparum* annual parasite incidence. In unstable and stable malaria transmission areas, less than one and at least one clinical case per 10, 000 population per year, respectively should be recorded (Noor *et al*., 2014). It was estimated that, 1.13 billion and 1.44 billion people lived in areas of unstable and stable *P. falciparum* transmission risks, respectively in 2010. Populations at stable risk were located in Africa (52% of the global total) or Central, South and East Asia (46%), and the remaining smaller proportion in the Americas (Gething *et al*., 2011). In unstable malaria transmission areas, the intensity of malaria transmission varies greatly by season and year, and over relatively small distances (WHO, 2015a).

In stable malaria regions, transmission occurs every year at high levels and malaria transmission can be either perennial or seasonal (Mouchet *et al*., 1998). In this region, partial immunity to clinical disease and a reduced risk of developing severe malaria are acquired in early childhood (WHO, 2015a). The highest levels (99% of the global area) of *P. falciparum* transmission risk occur in Africa. This highly diverse endemicity leads to the requirement of different interventions that enhance disease control efficacy (Gething *et al*., 2011).

Plasmodium vivax occurs within 95 countries in tropical, sub-tropical and temperate regions. Worldwide it was estimated that 2.85 billion people lived at risk of *P. vivax* malaria transmission in 2009, the majority of whom are in the tropical belt of Central and South East Asia (Guerra *et al*., 2010). In 2015, *P. vivax* malaria affected 13.8 million people globally and the vast majority of the cases (74%) occurred in the WHO South-East Asian Region. Out of the total infections occurred by *P. vivax*, about 80% of its cases occurred in Ethiopia, India and Pakistan (WHO, 2015b). Although its distribution is wide spread in Africa, the high frequency of Duffy negativity among African populations makes many individuals resistant to infection with *P. vivax* (Gallup and Sachs, 2001). As a result, *P. vivax* infection is rare in Western and Central sub-Saharan Africa population (Autino *et al*., 2012). However, there are different studies (Ryan *et al*., 2006, Culleton *et al*., 2009) that could explain how *P. vivax* can infect these people even if they have Duffy negative phenotype on their red blood cells. Even though it is highly neglected, malaria caused by *P. vivax* could be potentially dangerous and causes highly prevalent infections (Baird, 2007).

Malaria caused by *Plasmodium ovale* is common in sub-Saharan Africa (SSA) and on some islands of the western Pacific. Like that of the *P. vivax*, some parasites of *P. ovale* have delayed development as quiescent liver stage forms (hypnozoites) (Collins and Jeffery, 2005). It has two variants i. e. *P. ovale curtisi* and *P. ovale wallikeri* which may occur together and widespread in Africa (Oguike *et al*., 2011). *Plasmodium malariae* is also distributed in SSA and on the islands of the western Pacific, and Southeast Asia and Indonesia. The lower number of merozoites produced, the extended developmental cycle, the preference of the parasite to develop in older erythrocytes, its difficulty of identification using microscopy, and the earlier development of

immunity by the human host may result in low number of *P. malariae* parasite identification (Collins and Jeffery, 2007).

Plasmodium knowlesi is distributed only in the forest areas of South East Asia Region (Autino *et al*., 2012; Ahmed and Cox-Singh, 2015). This species of malaria parasite was identified from patients in Malaysia (Singh *et al.,* 2004) and Thailand (Jongwutiwes *et al*., 2004). Countries like Indonesia, parts of the Philippines, Cambodia, Thailand, Myanmar and Vietnam can have the potential for the *P. knowlesi* malaria transmission due to the availability of the known hosts and vectors (Moyes *et al*., 2014). Long-tailed and pig-tailed macaques (*Macaca fascicularis* and *M. nemestrina,* respectively) monkeys are the natural hosts for *P. knowlesi* (Luchavez *et al*., 2008) and transmitted by *Anopheles balabacensis* mosquitoes (White, 2008). During microscopic blood examination, its morphology create problem for its correct identification and was incorrectly identified either as *P. falciparum* or *P. malariae* (Jongwutiwes *et al*., 2004; Ahmed and Cox-Singh, 2015). It is speculated as the incidence of *P. knowlesi* infection in humans may not be new and was not as rare as previously thought (Joveen-Neoh *et al*., 2011). Clear understanding of its geographical distribution is very important and its transmission will continue after the human malarias have been eliminated due to its difficulty to eliminate this parasite from its reservoir hosts (Ahmed and Cox-Singh, 2015).

1.2 Malaria in Africa

In Africa, malaria transmission occurs from the southern border of the Sahara (20°N) to South Africa and Namibia (30°S) and is endemic at altitude below about 2000m (Mouchet *et al*., 1998), though the upper altitudinal limit for malaria transmission is difficult to define (Lindsay and Martens, 1998).

In Africa, malaria causes huge burden and about 74% of its population live in highly malaria endemic areas (WHO, 2006a). In many African countries, malaria remains a major public health threat and has its own impact on economic development of the countries (Snow *et al*., 2012). In African highlands, all age groups were affected during malaria epidemics but in malaria endemic lowland areas, young children and pregnant women are more affected (Lindsay and Martens, 1998).

In SSA, malaria morbidity and mortality is high in children under 5 years and pregnant women. In this region of Africa, malaria is highly endemic and unstable (WHO, 2006a). Malaria prevalence in SSA has impact on the global malaria toll. In this region, malaria killed 394,000 people in 2015 and out of these 74% of deaths were in children aged less than 5 years (WHO, 2016).

At the end of the 1980s and into the 1990s, malaria incidence began to rise reaching, in some areas, pre-1940s levels by the late 1990s. However, starting from 2000 incidence of malaria declined in many parts of Africa (Snow *et al*., 2012). Hence, the population living in areas of highest transmission intensity (hyperendemic and holoendemic) was reduced to 183.5 million in 2010 from that of 218.6 million in 2000. Generally, in 2010, 217.6 million people in the malaria-endemic countries of Africa lived in areas where endemicity had reduced by at least one endemic class from that of 2000 (Noor *et al*., 2014). This reduction in malaria toll is due to massive increase in investment in malaria control interventions. Although control interventions play a great role, it was thought that human settlement, climate, human behavior and factors affecting malaria vectors should not be undermined (Snow *et al*., 2012).

1.3 Malaria in Ethiopia

Malaria is a leading public health problem in Ethiopia where about 68% and 27% of the population live at risk and high risk of malaria transmission areas, respectively. In 2015, 2.8 million cases and 4,900 malaria attributed deaths were reported (WHO, 2016). It is estimated that, about 75% of the total area of the country (FMoH, 2007), and around 80% of the districts (woredas) are considered malarious (The Carter Center, 2012). In Ethiopia, malaria may occur in the low lands and also extend to highlands at altitudes about 2,200 meters above sea level (masl) (Woyessa *et al*., 2012). Though malaria prevalence showed high reduction in many parts of malarious areas of the country, its transmission is likely to be more and more focal with some areas still having substantial risk of malaria transmission (Jima *et al*., 2010).

In Ethiopia, malaria transmission is unstable and seasonal, and there are also recurrent malaria epidemics (The Carter Center, 2012). In most malaria endemic areas of the country, seasonal transmission of malaria occurs after the main rainy season (June to August) and light rains (March and April). Thus, peak malaria transmission mostly occurs after the main rainy seasons, between September and December (FMoH, 2007).

Previous experiences showed that, malaria epidemics occur every five to eight years in the country the magnitude depending on specific geographic characteristics. In the highlands, it occurs as a result of abnormal increase in minimum temperature while in the lowlands rainfall might play a significant role (Abeku *et al*., 2003). This variation in disease transmission may depend on altitude, rainfall and population movement (The Carter Center, 2012).

In Ethiopia, malaria is caused by *P*. *falciparum* and *P. vivax* which account about 60% and 40% of transmission, respectively (WHO, 2015b). However, these figures are not consistent in

different studies in the malaria endemic areas of the country. The variation may occur due to lack of efficient equipment for diagnosis of low density of malaria parasite in the blood and/or differences based on season of transmission (Tesfaye *et al*., 2011, Santana-Morales *et al*., 2012; Tadesse *et al*., 2015). The coexistence of these two species of *Plasmodium* makes malaria elimination efforts complicated in Ethiopia (Tadesse *et al*., 2015). Hence, correct species identification is required for prompt treatment that can save lives (Mekonnen *et al*., 2014). Though low in prevalence, *P. ovale* and *P. malariae* were also recorded in the country (Armstrong, 1969).

1.4 Rationale for the study

Malaria control policies and strategies through vector control involve reducing human-vector contact and protect individuals from malaria vectors bite and lower malaria transmission intensity at the community level by reducing the average lifespan of the local mosquito population (WHO, 2013b). In some situations, transmission of malaria can continue even when universal coverage of vector control interventions targeting indoor resting and biting mosquitoes has been achieved. This might be due to, changing behavior of malaria vectors from biting indoors to outdoors, from biting during the night time to early in the evening or through biting non-human blood meal sources (WHO, 2015b).

Entomological and parasitological studies are important in providing information on the intensity of malaria transmission of the area, and the behavior and habitats of the specific vector species. Entomological studies are important for identification of important malaria vectors, behavior and habitats of vector species, change in vector species composition, rates of infection and susceptibility to insecticides (WHO, 2011).

In Ethiopia, malaria transmission is unstable and seasonal, and more and more localized to specific areas. Hence, identification of the breeding sites of immature stages of *Anopheles* mosquitoes and the most important malaria vector species, the adult blood meal sources and insecticide susceptibility of the predominant malaria vectors to mostly used insecticides for indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) in a specific area is vital for the implementation of available malaria vector control interventions. In addition, identifying the knowledge and perception of the community living in malaria endemic areas with respect to malaria transmission and its control interventions is very important for malaria prevention and proper utilization of available malaria control interventions.

This study aimed to answer the following questions: What is the most abundant *Anopheles* species at larval stage and do the different species of *Anopheles* larvae prefer to breed in different larval habitats? What is the species composition and blood meal sources of adult female *Anopheles* mosquitoes? What is the status of malaria prevalence, parity rate, EIR and insecticide susceptibility of the most predominant malaria vector? What is the level of awarness of the community towards malaria prevention and control interventions?

1.5 Objectives of the study

1.5.1 General Objective

The overall objective of this study was to investigate the species composition and monthly distribution of *Anopheles* mosquitoes, their blood meal sources, sporozoite infection rates and level of insecticide susceptibility for integrated malaria vector management in the Ghibe River basin, southwestern Ethiopia.

1.5.2 Specific objectives

1. To determine *Anopheles* larval species composition, breeding habitats characterization and productivity in the study area
2. To investigate indoor and outdoor densities and blood meal sources of *Anopheles* mosquitoes in the study area
3. To evaluate malaria prevalence, sporozoite infection rate, entomological inoculation rate, longevity and insecticide susceptibility of *Anopheles* mosquitoes in the study area
4. To investigate awareness of the communities towards malaria prevention and its control interventions in the study area

Chapter 2. Literature review

2.1 Predominant *Anopheles* vectors of malaria in Africa

Appropriate species identification of malaria vectors and understanding of their distribution and biology is very important for implementation of effective malaria control (Coetzee, 2004). Hay *et al*. (2010) reviewed that, about 462 formally named and additionally 50 unnamed *Anopheles* species were recorded globally. Out of these *Anopheles* mosquitoes, 70 of them are competent vectors of human malaria of which 52 of them are dominant vector species. In Africa, the major malaria vectors include *An. gambiae*, *An. arabiensis*, and *An. funestus* (Mouchet *et al*., 1998).

2.1.1 *Anopheles gambiae* complex

Species complexes are those groups which are morphologically unidentifiable species that may vary in their behaviour and vectorial capacity. *Anopheles gambiae* complexes are the most efficient vectors of human malaria. These species complexes are reported from most parts of Africa, Saudi Arabia and Yemen. *Anopheles gambiae* sensu lato (s.l.) complex include *An. gambiae* sensu stricto (s.s.) (composed of the S molecular form *An. gambiae* and M molecular form *An. coluzzii*, respectively), *An. arabiensis*, *An. quadriannulatus*, *An. amharicus, An. bwambae, An. merus* and *An. melas* (Coetzee *et al*., 2000; Coetzee *et al*., 2013).

Anopheles gambiae s.s. and *An. arabiensis*, the two most efficient vectors of human malaria of the complex, have wide distribution and are sympatric over large areas. However, they differ in behaviour, seasonal abundance, and levels of vectorial capacity (White, 1974; Coluzzi, 1984). *Anopheles gambiae* s.s. is predominant in humid areas conducive to mosquito longevity and

rapid extrinsic parasite development while, *An. arabiensis* is common in arid and sparsely vegetated ecology where breeding places suitable to be scattered and temporary (White, 1974; Coluzzi *et al*., 1979). *Anopheles gambiae* s.s. has predominantly endophilic and anthropophilic behavior while *An. arabiensis* has a wide range of feeding and resting behaviour. *Anopheles arabiensis* may feed and rest indoor, feed outdoor and rest indoor but in the presence of available host and suitable resting sites outdoor, they have predominantly exophagic and exophilic behavior (White, 1974; Coluzzi *et al*., 1979). Both species mostly breed in shallow, open, and sunlit freshwater and share similar larval ecology (Coluzzi *et al*., 1979).

Anopheles melas and *An. merus* are distributed in West and East African coasts, respectively. They breed in habitat with high salt concentrated water. These species have zoophagic and exophilic behavior (Coluzzi, 1979; 1984). However, they also feed on human in the absence of alternative blood meal sources (White, 1974).

Anopheles bwambae breed only in pools of mineral springs in the Semliki Forest in Uganda. This species feed outdoor on non-human hosts (Coluzzi, 1979; 1984). *Anopheles quadriannulatus* are highly zoophilic (Coluzzi, 1984), in some cases they bite human both indoor and outdoor when closer to human habitation (White, 1974; Seyoum *et al*., 2012). *Anopheles quadriannulatus* species B was identified from southwestern Ethiopia (Hunt *et al*., 1998) and recently named as *An. amharicus* (Coetzee *et al*., 2013).

2.1.2 *Anopheles funestus*

Anopheles funestus was identified as a vector that played a primary role in malaria transmission in the Kenyan southern coastal area and it was the predominant species throughout the dry

season (Mbogo *et al*., 2003). They bite both indoors and outdoors depending on the presence of human host (Seyoum *et al*., 2012).

Coetzee and Fontenille (2004) reviewed that, *An. funestus* complex consists of 9 species which are morphologically very similar in the adult stage. Except *An. funestus*, other members of the *An. funestus* groups are mainly zoophilic. *Anopheles funestus*, *An. vaneedeni*, *An. rivulorum*, *An. leesoni* and *An. parensis* species were identified from 11 African countries using cocktail polymerase chain reaction (PCR) assay (Weeto *et al*., 2004). The distribution of the most efficient African malaria vectors; *An. gambiae*, *An. arabiensis* and *An. funestus* is shown in figure 2.1 (Sinka *et al*., 2012).

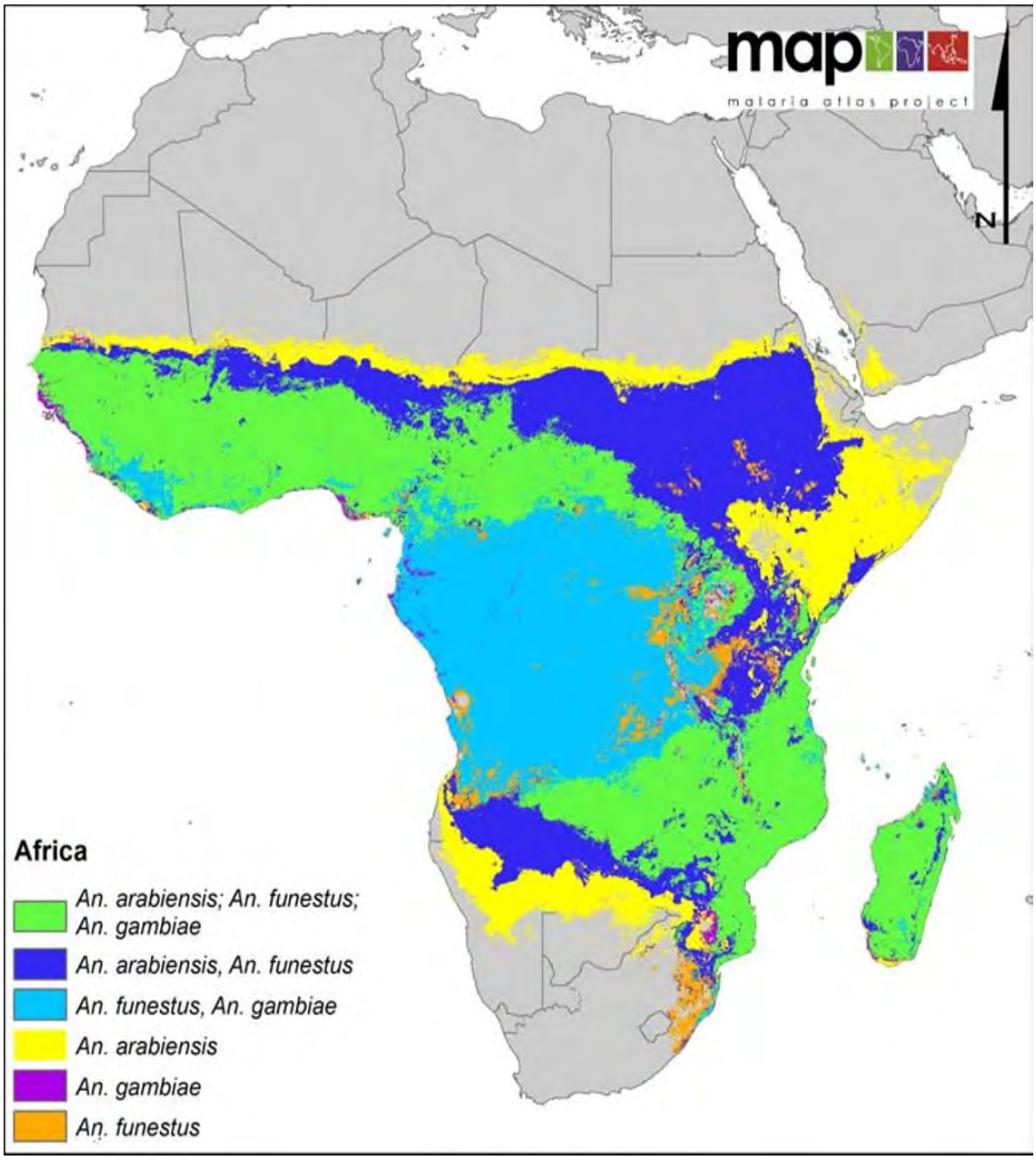


Figure 2.1 The distribution of the three most dominant malaria vectors in Africa (Source: Sinka *et al*., 2012)

2.2 Malaria vectors in Ethiopia

In Ethiopia, *An. arabiensis* is considered as the principal malaria vector, and *An. funestus, An. pharoensis* and *An. nili* as secondary vectors of malaria (Nyanjom *et al*., 2003; FMoH, 2007). As studies conducted in different parts of the country showed, *An. gambiae* s.l. (most likely *An. arabiensis*) is the predominant malaria vector species in Ethiopia. This species of malaria vector is common in low altitude areas with low humidity or arid areas (Ribeiro *et al*., 1996; Animut *et al*., 2013; Massebo *et al*., 2013).

In addition to *An. arabiensis*, other species: *An. pharoensis*, *An. christyi*, *An. cinereus*, *An. demeilloni* were also identified with varying abundance (Animut *et al*., 2013). A study conducted in houses of Sille village, 20km south of Arba Minch (southern Ethiopia) showed that, *Anopheles gambiae* s.l. (= *An. arabiensis*) was the principal species collected and *An. funestus*, *An. nili* and *An. pharoensis* were also collected in very low densities (Ribeiro *et al*., 1996).

2.3 Malaria vector control methods

Malaria control and elimination programmes need planned and targeted implementation of appropriate malaria control interventions. Most practiced malaria control interventions include diagnosis and treatment of infected individuals with appropriate drugs, vector control interventions (IRS, insecticide treated bed nets (ITNs), larviciding, and environmental modification) and political and social mobilization (Breman and Brandling-Bennett, 2011; WHO, 2013a). Though the goal of malaria eradication during Global Malaria Eradication Program (GMEP) campaign conducted from 1955-1969 was not achieved, malaria eradication program is also advocated again by Bill and Melinda Gates foundation in recent years. However,

many scholars argue that eradication of malaria could not be feasible with existing tools (Roberts and Enserink, 2007).

For malaria control and elimination, WHO's Global Malaria Programme recommends, diagnosis and treatment of malaria cases with effective drugs and full coverage of ITNs and IRS to populations at risk of malaria (WHO, 2006b). Hence, clear understanding of human settlement pattern, *Anopheles* larval habitat distribution, the important malaria vector species of the area and their biting and resting behavior, and malaria transmission pattern are very important for the implementation of malaria control interventions to a specific area (Smith *et al*., 2007; Russell *et al*., 2011).

Currently, malaria vectors control interventions focus on attacking the vector mosquitoes using environmental management and larviciding that used to target the immature stages (Russell *et al*., 2013) or LLINs and IRS which mostly target adult stages to control indoor feeding and resting mosquito vectors (WHO, 2012a; Russell *et al*., 2013). For effective implementation of vector control interventions, understanding of the vector species distribution dynamics in the area is very important (Meyrowitsch *et al*., 2011; Russell *et al*., 2016), to target only where and when intervention is needed (WHO, 2006b).

2.3.1 Control interventions targeting larval stages

Mosquito larval control methods consist of larval source management (LSM), larviciding and biological control. Though this intervention has been implemented in many countries, its impact on malaria control intervention is not well understood (WHO, 2015b). As a result of control measures that targeted immature stages through source reduction were used as successful GMEP in the United States, Europe, and the Middle East, malaria was eradicated from large parts of

these regions before control measures targeted the adult stages were properly implemented (Kitron and Spielman, 1989).

For the effectiveness of malaria control interventions, combining habitat-based interventions with other control programs reduce mosquito populations to a minimum, because when the number of larval habitats reduced, adult population which could be involved in malaria transmission will be reduced in number (Fillinger *et al*., 2008; Gu *et al*., 2008). The costs for larval management using larvicides depend on the types of aquatic habitat, local vector ecology, population density, and impact of other malaria control intervention on malaria transmission (Worrall and Fillinger, 2011). Unlike control interventions targeting the adult stages, LSM might support malaria control with other control interventions by attacking not only the indoor mosquito populations but also those outdoor biting and/or resting malaria vectors which are less likely affected by LLINs and IRS (Worrall and Fillinger, 2011). LSM requires correct identification of the larval habitat and involvement of local community participation to reduce the costs incurred for the manpower and larvicides applied (Fillinger and Lindsay, 2011).

2.3.2 Adult control interventions used to interrupt biting and resting

2.3.2.1 Application of personal repellents

DEET (also known as *N,N*-diethyl-*3*- methylbenzamide or diethyl-*m*-toluamide) which has been the main active ingredient in repellent formulations since its discovery in 1954 (Frances *et al*., 2005), is likely to remain one of the most cost-efficient and safe options for repellent-based vector control campaigns (Costantini *et al*., 2004). Its application might be effective in a short term disease transmission interventions like epidemics and temporary conditions where disease may occur to people not previously exposed to infection (Rowland *et al*., 2004).

Repellents are very costly to use at the community level to protect vector borne diseases due to their short persistence (Costantini *et al*., 2004). Application of repellents might also be more effective when the environmental condition is too hot or in areas where vectors bite early in the evenings that might not be convenient to protect vectors bite by sleeping under insecticide treated materials. Hence, use of repellents requires health education and self-discipline for its proper application when and where it is required (Rowland *et al*., 2004).

2.3.2.2 Insecticide treated bed nets and indoor residual spraying

ITNs and LLINs use as personal protection from mosquitoes bite through repelling and/or killing the mosquitoes seeking blood meals from human while sleeping at night (Atieli *et al*., 2011). While, IRS is "the application of long-acting chemical insecticides on the walls and roofs of all houses and domestic animal shelters in a given area, in order to kill the adult vector mosquitoes that land and rest on these surfaces" (WHO, 2006b). IRS played a great role in reducing the global malaria burden during the GMEP (1955-1969) and with other interventions it was used to eradicate malaria from Europe, the former USSR, and several countries of Asia and the Caribbean (WHO, 2006b).

The resting and feeding behavior of malaria vectors is very important for their control using IRS and ITNs/LLINs (Russell *et al*., 2016). These control interventions are effective for endophagic, endophilic and anthropophilic *Anopheles* mosquitoes (Griffin *et al*., 2010; Killeen *et al*., 2013) which is the characteristics of the most efficient African malaria vectors (Lindblade, 2013). ITNs would be most effective for endophagic vectors that feed primarily late at night but IRS against primarily endophilic vectors that rest inside houses after blood feeding (Gimnig *et al*., 2016). When ITNs are used, in addition to direct killing effect of the anthropophilic mosquitoes, they

inhibit blood feeding, partially due to the excito-repellency property of bed nets, which could result in mortality through deprivation of blood (Bayoh *et al*., 2010). Even after application of these control interventions, mosquitoes may reappear in the areas previously they were eliminated by changing their behavior to feeding on human outdoor or may feed on other available alternative hosts (Killeen *et al*., 2013) or biting early in the evening (Bayoh *et al*., 2014).

In Africa, recent declines in malaria-related morbidity and mortality resulted partly because of the widespread scale-up of ITNs and IRS (Lindblade, 2013). However, factors like behavior of the mosquitoes which bite early in the evening before people goes to bed, resting outdoors, and feeding on other available alternative non-human hosts, lack of proper utilization of nets, declining insecticide concentration, and holes or tears in nets, the decomposition of insecticides sprayed in houses within few weeks or months could have impact on the effectiveness of control interventions targeting mosquitoes entering houses (Gatton *et al*., 2013; Lindblade, 2013).

2.4 Factors that influence malaria transmission

According to Zhou *et al*. (2004) model, climatic conditions, topography, human settlement pattern, land use and drug resistance play a crucial role in initiating a malaria epidemic. Some of the factors that affect malaria transmission and malaria vectors survival were discussed below.

2.4.1 Ambient temperature and rainfall

Ambient temperature plays a key role in determining the suitability of local environments for transmission of human malaria (Huang *et al*., 2011). Maximum values of temperature for transmission potential of mosquito vector is in the range of 29-33°C (Martens *et al*., 1997), though Mordecai *et al*. (2013) described transmission peak at 25°C.

The length of the larval mosquito cycle to complete its development in its breeding habitat and the sporogonic cycle of the parasite in the adult mosquito vector depends on the local temperature (Mouchet *et al*., 1998; Zhou *et al*., 2004). Temperature also affects the length of the gonotrophic cycle which in turn affects the vectorial capacity of a mosquito vector (Lardeux *et al*., 2008).

Rainfall changes cause the temporal change in mosquito abundance by increasing the number and productivity of breeding sites (Mouchet *et al*., 1998). Rainfall is recognized as one of the major environmental factors influencing variability in malaria transmission in warm semi-arid and desert-fringe areas (Grover-Kopec *et al*., 2005). Epidemics may occur after excessive rains, usually with a lag-time of several weeks (Grover-Kopec *et al*., 2005). This time is required for receding of water to provide suitable breeding sites, time for development of adult, and parasite development in the vector (Devi and Jauhari, 2006).

Rainfall often leads to formation of small puddles that serve as mosquito larval breeding sites. Rainfall also increases humidity, which enhanced mosquito survival (Huang *et al*., 2011). If the amount of rainfall is low, it will evaporate or infiltrate over a shorter time. Conversely, with higher amounts of rainfall, some water remains long enough for the vector to complete its development cycle (Yé *et al*., 2007).

2.4.2 Vegetation cover and land use pattern

Vegetation cover and land use pattern affect the formation of larval habitat and survival and distribution of adult *Anopheles* mosquitoes. The average daily water temperature was increased by 3-3.5°C in unshaded, open habitats compared with those shaded habitats (Tuno *et al*., 2005). Tree canopies reduce the water temperature of larval habitats by reducing the amount of solar

radiation reaching the larval habitats (Zhou *et al*., 2007). It also affects the availability of mosquito resting sites (Githeko *et al*., 2000). Houses located in deforested areas showed a 1.2-1.8°C higher average temperature than those in the forested areas (Afrane *et al*., 2005).

Land use changes can cause the environmental conditions to be more favorable for the development and reproduction of malaria vector mosquitoes and the parasites (Zhou *et al*., 2004). Temperature increases because of land cover and land use changes may accelerate the sporogonic development rate of *Plasmodium* parasites and consequently enhance the vectorial capacity of malaria vectors (Afrane *et al*., 2005).

2.4.3 Socioeconomic factors and human behavior

Though malaria is considered as disease of the poor, it may also leads to poverty. If a person lives in a malaria endemic area and infected with malaria, there are additional costs incurred for the diagnosis, treatment, and vector control and even a sick person could not avail himself on his duty due to illness. Hence, it is suggested to consider the malaria-poverty interplay bidirectional (Sachs and Malaney, 2002). Communities who have settled in rural areas have been affected by their economic level, because most of their time could be elapsed on travel to the urban areas for purchase of materials from markets. Indirectly, these populations may be affected with disease burden (Linard *et al*., 2012). If there is economic development, intensified and improved health systems with well equipped diagnostic tools and medication will be increased (Snow, 2015). Malaria was eliminated from the United States and southern European countries during the GMEP due to the economic development to implement intensified malaria control interventions (Sachs and Malaney, 2002; Snow, 2015).

Poverty has direct association with malaria risk. This could be attributed to lack of access to antimalarial drugs, non-use of vector control measures, construction of less quality houses, less access to education, and distance from health facilities (Yadav *et al*., 2014). When the socio-economic status of the community increases, the quality of house construction design and the material used for construction could also be improved, which can reduces the abundance of malaria vectors inside houses. In areas such as some development activities like mining where environmental change may favor malaria vectors, people with low economic status may settle and construct poor houses and poor drainage creating conducive grounds for malaria transmission (Dery *et al*., 2015).

The design of constructed houses, the materials it is made of, and the location of house within the village affect the abundance and species composition of malaria vectors (Smith *et al*., 1995). If the house is well constructed and possesses window and door screening materials it may protect the mosquitoes from biting humans and reduce malaria transmission (Sachs and Malaney, 2002).

Household distance to health facilities affects malaria treatment seeking bahaviour of the house heads, because those households close to the health facilities visit the health service providers timely with reduced transportation cost than those located at far distance. People with good education level are more likely adhere to health education like bed net retreatment and mosquitoes larval control interventions. Individuals with a higher income have a positive response to public health intervention for many tropical diseases, including malaria (Lowassa *et al*., 2012).

2.5 Development of insecticide resistance in malaria vectors

Changes in the insecticide target site and increases in the rate of insecticide metabolism are the major insecticide resistance mechanisms in malaria vector mosquitoes (Ranson *et al*., 2011). The volume and frequency of applications of insecticides and the characteristics of insect vector species are the determinant factors for the development of insecticide resistance. Repeated and high quantity insecticides application to farmlands to control agricultural pests could induce insecticide resistance to malaria vectors through washing by water runoff during rainy seasons to the larval breeding habitats (Dabiré *et al*., 2008; Edi *et al*., 2012; Nkya *et al*., 2014). This was pronounced by seasonal variation of insecticide susceptibility by malaria vectors based on the amount of insecticide applied on agricultural farmlands in areas where IRS was not applied (Ranson *et al*., 2009). In addition, insecticide resistance could also be caused by insecticides applied for the control intervention tools (IRS and LLINs) used to control malaria vectors (Padonou *et al*., 2012).

Resistance to DDT was observed for the first time in Greece on a major malaria vector species *An. sacharovi* in 1951 after wide application from 1946-1950 for house spraying program (Livadas and Georgopoulos, 1953). Since then, resistance has been recorded in all the four classes of insecticides that has been used for IRS and ITNs in major African malaria vectors species *An. gambiae* s.l. and *An. funestus* (Coetzee, 2004; Kelly-Hope *et al*., 2008), and to pyrethroid insecticides in most parts of Africa (Hemingway *et al*., 2016). In areas where pyrethroid resistance development has been recorded, it reduces the efficacy of control interventions like ITNs and IRS (N'Guessan *et al*., 2007). Hence, to prevent the development of insecticide resistance, regular monitoring of insecticide resistance development is very important (Ranson *et al*., 2011). However, assessing resistance on the efficacy of ITNs is difficult due to

poorly understood relation between insecticide content, net usage, net integrity, and net efficacy. As a result, it may have impact on the registered results on reducing malaria in Africa (Edi *et al*., 2012).

Multiple insecticide resistance to both metabolic and target site resistance was recorded to *An. gambiae* s.l. which could have a great threat for the control of malaria vectors based on IRS and ITNs (Nwane *et al*., 2013). In urban areas where agricultural activities were performed, large amount of insecticides might be released into the environment which could serve as a source for the development of multiple insecticide resistance (Corbel *et al*., 2007). It is surprising that, insecticide resistance to carbamates and/or organophosphates was recorded in The Gambia in the areas where these insecticides were not used for public health (Opondo *et al.,* 2016).

In general, the major malaria vectors in Africa are *An. arabiensis*, *An. gambiae*, *An. coluzzii* and *An. funestus*. They are distributed in different parts of the continent with varying proportion. Malaria morbidity can be the combined result of the precence of the predominant local malaria vectors, the *Plasmodium* species available, integrated control strategies and the awareness of the communities living in the specific area. So that, understanding the local malaria vectors and malaria transmition status is very important for effective utilization of available control methods.

Chapter 3. *Anopheles* larval species composition and characterization of breeding habitats in the Ghibe River basin, southwestern Ethiopia

3.1 Introduction

In 2015, globally malaria affected 212 million and killed 429,000 people and most cases and deaths occurred in the WHO African Region in children under 5 years of age (WHO, 2016). In Ethiopia, it is estimated that about 75% of the total area of the country is malarious (FMoH and WHO, 2007). About 58.3 million people lived in areas at risk of malaria in 2013 (FMoH, 2014) and in 2015 it was estimated that, malaria infected 2.8 million and killed 4,900 people in Ethiopia (WHO, 2016). Malaria transmission is seasonal and mostly occurs at the end of the main rainy season, from June to August and small rains from March to April (FMoH and WHO, 2007; Yeshiwondim *et al*., 2009).

As in most malaria endemic countries, the most commonly used malaria vector control interventions in Ethiopia are application of IRS and utilization of LLINs, which target the adult stages of malaria vectors (WHO, 2016). Due to the development of insecticide resistance by the major malaria vector, *An. arabiensis* (Yeshiwondim *et al*., 2009; Balkew *et al*., 2010) and drug resistance by the *Plasmodium* species (Schunk *et al*., 2006; Ketema *et al*., 2011) implementation of integrated control interventions that could target the larval stage could become very important. In addition to adult vector control, malaria transmission can be reduced by suppressing larval densities using appropriate methods based on the type of their breeding habitats (Shililu *et al*., 2003; Liu *et al*., 2012).

Anopheles arabiensis larvae are more abundant in sunlit, small and temporary habitats with low emergent plants and canopy cover. These habitats may not favor the development of predators and competitors which may feed upon the mosquito larvae (Mwangangi *et al*., 2006; Mereta *et al*., 2013). Such type of habitats may also dry out due to evaporation before the larvae complete their development. However, mosquito larvae like *An. arabiensis* were observed colonizing larval habitats within two days after the rains (Mwangangi *et al*., 2008).

For the implementation of malaria vector control interventions, understanding the spatial and temporal distribution patterns of *Anopheles* species is very important (Shililu *et al*., 2003; Liu *et al*., 2012). This requires, clear identification and knowledge of larval habitat ecology and larval population dynamics by a thorough understanding of the factors affecting larval abundance (Mala and Irungu, 2011). Control of immature stages of malaria vectors can be advantageous because the larvae are concentrated, relatively immobile, and occupy minimal habitat areas compared with adults that can rapidly disperse over large areas (Floore, 2006). Thus, knowledge of vector densities and species composition is required to design effective vector control programs (Mwangangi *et al*., 2007; Kweka *et al*., 2011; Tabbabi *et al*., 2015). This can be done through understanding the characteristics of larval habitat (Mwangangi *et al*., 2007; Muturi *et al*., 2008). Integrated malaria vector control management and characterization of the major malaria vectors habitat is important (Walker and Lynch, 2007). Thus, the main objective of this study was to determine the species composition and abundance of *Anopheles* larvae, and to describe mosquito larval habitats at Ghibe and Darge study sites in Ghibe River basin, southwestern Ethiopia.

3.2 Material and Methods

3.2.1 Description of the study area

The study was conducted at Ghibe and Darge kebele (administrative unit with approximately 2,000 to 5,000 populations) located along Ghibe River basin in southwestern Ethiopia in Southern Nations Nationalities and Peoples Regional State, Guraghe Zone, Abeshge district. The zonal and Abeshge district town (Wolkite) is located at 158km southwest of Addis Ababa. Ghibe study site is a rural area [8°14'N, 37°33'E, altitude 1080-1134 masl] located 30 km south of Wolkite near the Ghibe River. The area has an annual average rainfall of 625mm (National Meteorological Agency, unpublished report). Acacia trees and savannah grassland dominate the vegetation of the area. In 2016, the study site had 420 households with 2,167 total inhabitants of whom 1,105 were male and 1,062 were female (Abeshge district health office, unpublished report). There is a government owned health post and one clinic owned by the Ethiopian seed enterprise.

Darge study site is a rural area (8°24'N, 37°31'E, altitude 1500-1800 masl) located at 42 km west of Wolkite and 52km from Ghibe study site at the outskirts of Darge town. The Darge River is available near by Darge study site and crosses Darge town. It serves as one of the tributaries of the Ghibe River in the Ghibe River valley. There is a health post and a health center in the study area. In 2016, Darge study site had 731 households with 3,518 inhabitants, of these 1,724 male and 1,794 female (Abeshge district health office, unpublished report). The study area has annual average rainfall of 1,022mm (National Meteorological Agency, unpublished report). The study area was selected due to its malaria endemicity based on information obtained during preliminary survey from Abeshge woreda health office that was reported from heath centers and

health posts and also due to the presence of perennial rivers near the study sites which might serve as *Anopheles* larval breeding habitat.

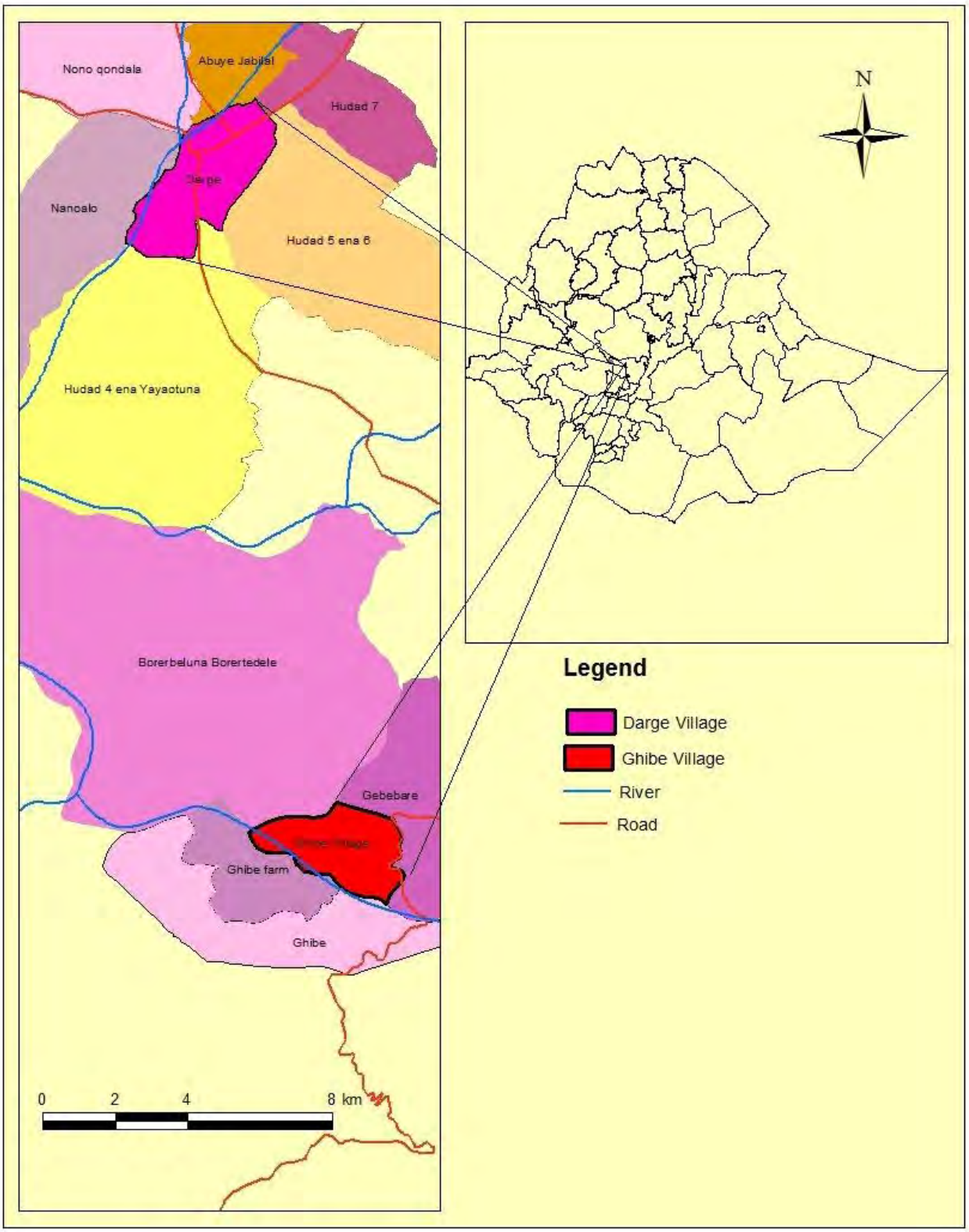


Figure 3.1 Geographical locations of Ghibe and Darge study sites, in Ghibe River basin, southwestern Ethiopia

3.2.2 Mosquito larval sampling and processing

Longitudinal larval collections were carried out in each of the study site every month over a 24-month period (November 2014-October 2016). In each month, mosquito larvae were surveyed in each natural habitat that contained water. During sampling, if *Anopheles* larvae were available, 3-15 dips were taken using a standard dipper (350 ml capacity, BioQuip Products, Inc. California, USA) depending on the size of each larval habitat at intervals along the edge, with a greater sampling effort in areas of low mosquito density. For small habitats like hoof prints, several sites were pooled to get appropriate larval sampling (Mereta *et al*., 2013). The water was collected in a white plastic tray and carefully observed for the presence of *Anopheles* larvae. Sampling was always done in the morning (09:00-12:00) or in the afternoon (14:00-17:00) for about 30 minute at each larval habitat.

All *Anopheles* larvae were sorted from culicine larvae and counted and recorded. Larval density was determined by taking the average number of mosquito larvae from the total dips taken at specific habitat. Early stages *Anopheles* larvae were discarded but late stage larvae were killed in hot water and immediately preserved in vials containing 70% ethanol and transported to laboratory for species identification.

3.2.3 Identification of *Anopheles* mosquito larvae

Late instar larvae which were collected from different habitats were transported to the Insect Vectors and Entomopathogen Research laboratory, Department of Zoological Sciences, Addis Ababa University. A drop of Hoyer's mounting medium was placed on a clean microscopic glass slide. Each larval specimen was mounted on a slide, covered with cover slip and allowed to dry

and identified morphologically using the identification key of Gillies and Coetzee (1987) under a compound microscope.

3.2.4 Characterization of larval habitats

Habitats containing *Anopheles* larvae were identified. Environmental variables like habitat perimeter, water depth, direct sunlight, water still or flowing, water temperature at the time of sampling, water pH, water turbidity, distance to the nearest house, vegetation coverage, permanence of the habitat, presence of algae, surface debris coverage, emergent plant coverage, habitat type and substrate type were recorded for each habitat. Water depth of a habitat was measured from different places depending on size of the habitat using a meter stick of one meter long and the average depth was taken. Distance to the nearest homestead was measured using a tape measure for less than 100 m and estimated if more than 100 m. Distance was categorized into 4 classes: 1) ≤100 m, 2) 101 to 200 m, 3) 201 to 300 m, 4) 301 to 400 m. Surface debris, presence of algae and emergent plant coverage were determined based on visual observation. Vegetation cover was visually observed and expressed as open (no vegetation), tree (for the presence of large tree within a range of 10-15 meters where shade and foliages could reach), and shrub (woody plants smaller than a tree forming like a bush). Habitat perimeter was measured using tape measure and classified < 10m, 10-100m and >100m. Habitat stability was expressed in terms of the length of time the habitat contained water after the rain. A habitat was considered temporary if it held water for 2 weeks or less and permanent if it held water for more than 2 weeks after rain. Turbidity was measured by placing water samples in glass test tubes and holding them against a white background, and categorized into 3 levels: low, medium, and highly turbid (Minakawa *et al*., 1999). Light intensity was visually categorized as sunlit if the

habitat received full sunlight that could occur throughout the day otherwise described as shaded. The substrate type was categorized as mud, stone (if the pool was lined with stones that were large in size (rocky) generally larger than 10cm in diameter) and gravel (when the stones were small in size but larger than sand). Water temperature was recorded using water thermometer at the time of collection and pH was measured using pH indicator paper (Kenea *et al*., 2011). Rainfall of the study area during the study period was obtained from National Meteorological Agency (unpublished report).

3.2.5 Data analysis

Descriptive analysis was used to compare the larval breeding habitats and number of immature *Anopheles* mosquitoes sampled. Correlation analysis was used to investigate the relationship between pH, temperature and water depth to the *Anopheles* larval density. *Anopheles* larval density was determined as the number of *Anopheles* larvae divided by the number of dips taken from each larval habitat. Larval density was log transformed $\log_{10}(x + 1)$ to improve the normality of distribution. Multiple regression analysis was used to identify the most predictor environmental variables with the occurrence of *Anopheles* larvae. One-way analysis of variance (ANOVA) was used to compare the differences in larval density between habitat types. When significant differences were observed, the means were separated by Tukey HSD (honest significant difference) test. Mann-Whitney *U* test was used to compare variables with two groups; presence of algae (presence or absence), habitat permanency (temporary or permanent), surface debris (present or absent), intensity of light (sunlit or shaded), and water movement (still or slow flowing). Kruskal-Wallis H test was used to compare variables with more than two groups; water turbidity, water perimeter, distance to the nearest house, canopy cover, emergent plant coverage, habitat type, and substrate type. These non-parametric tests were used to

compare habitat characteristics with the most abundant *Anopheles* larvae. Data were analyzed using IBM SPSS statistical for Windows (IBM corp., Armonk, NY), version 20.0. Values were considered significantly different if $p< 0.05$ for all the tests.

3.3 Results

3.3.1 *Anopheles* species composition and larval habitats

3.3.1.1 *Anopheles* larvae species composition

Anopheles mosquitoes identified from each study site is shown in Table 3.1. In total, 3,485 late instar *Anopheles* mosquito larvae were morphologically identified belonging to 10 species. From the total *Anopheles* larval species, *An. gambiae* s.l., *An. christyi*, *An. phareonsis* and *An. pretoriensis* were identified from Darge and Ghibe study sites. *Anopheles coustani*, *An. nili*, *An. concolor* and *An. ardensis* were identified in Darge study site only while *An. rivulorum* and *An. demeilloni* were identified in Ghibe study site only. *Anopheles gambiae* s.l. constituted 97.8% and *An. pharoensis* 1.3% of all identified larvae in Darge. In Ghibe, 90.6% were *An. gambiae* s.l. and 7.9% were *An. christyi*.

Table 3.1 Total number of *Anopheles* larvae identified from Ghibe and Darge study sites (November 2014-October 2016)

Anopheles larvae identified	Study site			
	Darge		Ghibe	
	No.	%	No.	%
An. gambiae s.l.	2317	97.8	1012	90.6
An. pharoensis	30	1.3	9	0.8
An. christyi	13	0.5	88	7.9
An. rivulorum	-	-	6	0.5
An. demeilloni	-	-	1	0.1
An. pretoriensis	1	0.0	1	0.1
An. coustani	3	0.1	-	-
An. nili	1	0.0	-	-
An. concolor	2	0.1	-	-
An. ardensis	1	0.0	-	-
Total	**2368**	**100**	**1117**	**100**

3.3.1.2 *Anopheles* larval productivity in different habitat types

The results of monthly larval sampling and the types of larval habitats that were productive in the study area are presented in Table 3.2. Eight habitat types were identified in Darge, including borrow pits, hoof prints, rain pools, pools at river edges, pools in the bed of drying river, rock pools, tire tracks and swamps (Figure 3.2). All these types of habitats were also identified in Ghibe except pools in the bed of drying river. In both study sites, the most frequently encountered larval habitats were pools at river edges (Darge, n = 38 and Ghibe, n = 24), rain pools (Darge, n = 24), and tire tracks (Ghibe, n = 17). *Anopheles* larvae were collected from a total of 167 larval habitats (96 sites in Darge and 71 sites in Ghibe).

In total, 9,277 larvae of *Anopheles* mosquitoes and 494 pupae were sampled and the result was shown in Table 3.2. At Darge, large densities of immature *Anopheles* mosquitoes were collected from pools in the drying river beds (mean density of 35.2 larvae/dip and 3.5 pupae per dip) and the lowest densities were in swamps (mean density of 2.1 larvae/dip and 0.3 pupae/dip). At Ghibe, larvae were densely populated in rain pools, borrow pits and pools at river edges with 11.9, 11.2 and 7.9 larvae/dip and pupae were from borrow pits (0.5 pupae/dip) and the lowest densities of larvae were sampled from the swamp.

Table 3.2 Density of different stages of *Anopheles* larvae in different habitat types in Darge and Ghibe study sites (November 2014 to October 2016)

Study site	Habitat type (n)	Total no. of dips	Total larval count	No. larvae/dip (Mean± se)	Total pupal count	No. pupae/dip (Mean ± se)
Darge	Borrow pit (3)	16	228	14.3 ±8.6	8	0.5 ± 0.1
	Hoof print (8)	52	286	5.5 ± 1.2	8	0.2 ± 0.1
	Rain pool (24)	146	806	5.5 ± 1.5	84	0.6 ± 0.2
	Pools at river edge (38)	231	3063	13.0 ± 2.1	148	0.7 ± 0.2
	Pools in drying river beds (4)	20	704	35.2 ± 7.9	70	3.5 ± 0.8
	Rock pool (7)	42	271	6.5 ± 3.4	27	0.6 ± 0.3
	Tire track (7)	47	300	6.4 ± 3.2	22	0.5 ± 0.2
	Swamp (5)	43	92	2.1 ± 0.2	13	0.3 ± 0.1
	Total	**597**	**5750**	**9.6**	**380**	**0.6**
Ghibe	Borrow pit (4)	42	471	11.2 ± 6.3	23	0.5 ± 0.4
	Hoof print (9)	83	328	3.9 ± 0.6	9	0.1 ± 0
	Rain pool (12)	67	800	11.9± 5.1	6	0.1 ± 0
	Pools at river edge (24)	145	1146	7.9 ± 1.9	48	0.3 ± 0.1
	Rock pool (4)	20	38	1.9 ± 0.5	0	0 ±0
	Tire track (17)	138	737	5.3 ± 2.5	28	0.2 ±0.1
	Swamp (1)	15	7	0.5 ± 0.0	0	0 ±0
	Total	**510**	**3527**	**6.9**	**114**	**0.2**

n = number of larval habitat surveyed, se = standard error of the mean, values in bold for mean larval and pual density indicate mean larval or pupal density of each study site

Figure 3.2 *Anopheles* larval breeding habitats: A) Rain pool B) Borrow pit C) Pools at river edge D) Pools in drying river beds E) Rock pool F) Tire track G) Hoof print H) Swamp

3.3.1.3 Abundance of *Anopheles* species larvae in breeding sites

Anopheles species composition and their preferred habitats in each study site are depicted in Table 3.3. *Anopheles gambiae* s.l. was identified from all types of larval habitats but *An. christyi* were from pools at river edge, rain pools, rock pools and tire tracks, and *An. pharoensis* were from swamps, borrow pits, pools at river edge and rain pools. In Darge study site, except swamps which had the largest proportion of *An. pharoensis* (52.6%), in all other larval habitats almost all the identified larvae were *An. gambiae* s.l. In Ghibe, almost all the identified larvae from hoof prints and tire tracks were *An. gambiae* s.l.

Table 3.3 *Anopheles* mosquito larval species composition in different habitats in the study area (November 2014- October 2016) (values in parenthesis indicate % of total specimens identified in each habitat type)

Larval species	Habitat type								
	Swamps	Borrow pits	Hoof prints	Pools at river edge	Rain pool	Pools in drying river beds	Rock pool	Tire track	**Total**
Darge									
An. gambiae s.l.	20 (35.1)	151(100)	67 (100)	1134 (99.3)	362 (99.7)	298 (100)	129 (99.2)	156 (99.4)	**2317**
An. christyi	-	-	-	11 (0.9)	-	-	1(0.8)	1 (0.6)	**13**
An. pharoensis	30 (52.6)	-	-	-	-	-	-	-	**30**
An. coustani	3 (5.3)	-	-	-	-	-	-	-	**3**
An. concolor	2 (3.5)	-	-	-	-	-	-	-	**2**
An. pretoriensis	-	-	-	-	1 (0.3)	-	-	-	**1**
An. nili	1 (1.8)	-	-	-	-	-	-	-	**1**
An. ardensis	1 (1.8)	-	-	-	-	-	-	-	**1**
Total	**57 (100)**	**151 (100)**	**67 (100)**	**1145 (100)**	**363 (100)**	**298 (100)**	**130 (100)**	**157 (100)**	**2368**
Ghibe									
An. gambiae s.l.	2 (66.7)	88 (96.7)	179 (100)	381 (80.9)	107 (92.2)	*NA*	-	255 (99.6)	**1012**
An. christyi	-	-	-	81 (17.2)	5 (4.3)	*NA*	1 (100)	1 (0.4)	**88**
An. pharoensis	1 (33.3)	3 (3.3)	-	2 (0.4)	3 (2.6)	*NA*	-	-	**9**
An. rivulorum	-	-	-	6 (1.3)	-	*NA*	-	-	**6**
An. pretoriensis	-	-	-	-	1 (0.9)	*NA*	-	-	**1**
An. demeilloni	-	-	-	1(0.2)	-	*NA*	-	-	**1**
Total	**3 (100)**	**91 (100)**	**179 (100)**	**471 (100)**	**116 (100)**	***NA***	**1 (100)**	**256**	**1117**

Log transformed mean larval densities showed, *Anopheles* larvae were higher in the pools in drying river beds (mean density of 1.5 ± 0.1 se) and pools at river edges (mean density of 0.9 ± 0.1 se) and least in swamps (mean density of 0.2 ± 0.1 se) (Table 3.4). There was a statistically significant difference between groups as determined by one-way ANOVA ($F_{7,159}$ = 4.8, p < 0.001). Tukey HSD post hoc test revealed that the density of larvae in borrow pits, pools at river edges, hoof prints, rain pools, rock pools, swamps and tire tracks was not significantly different (p > 0.05). There was no statistically significant difference between pools at river edges and pools in drying river beds (p > 0.05).

Table 3.4 Log10 transformed density of *Anopheles* larvae in different larval habitat types from all study sites (November 2014- October 2016)

Habitat type	Number of *Anopheles* larvae per dip (log transformed) (Mean ± se)
Borrow pit	0.7 ± 0.3[a]
Hoof print	0.5 ± 0.1[a]
Rain pool	0.7 ± 0.1[a]
Pools at river edge	0.9 ± 0.1[a,b]
Pools in drying river beds	1.5 ± 0.1[b]
Rock pool	0.4 ± 0.1[a]
Swamp	0.2 ± 0.1[a]
Tire track	0.5 ± 0.1[a]

se = standard error of the mean, different letters show results with significant difference

3.3.2 Association between *Anopheles* larval density and habitat variables

Multiple regression model showed that, temperature at the time of larval collection (p = 0.03), and emergent vegetation (p = 0.003) were the best predictors of *Anopheles* larval density in the habitats (Table 3.5). The F-ratio in the ANOVA table showed that, the independent variables were statistically significantly predict the dependent variables, $F_{15, 151} = 3.7$, $p < .001$, $R^2 = 0.266$.

Table 3.5 Multiple regression analysis showing the key predicting larval habitat environmental factors for *Anopheles* larvae density

Variable	B	95% C.I. for B		*t*	Sig
		Lower	Upper		
Depth	0.006	-0.002	0.013	1.549	0.123
Temperature	0.027	0.003	0.052	2.197	0.03
pH	0.176	-0.015	0.367	1.824	0.07
Perimeter	-0.046	-0.237	0.145	-0.475	0.636
Turbidity	-0.08	-0.178	0.018	-1.606	0.11
Distance to nearest house	-0.071	-0.163	0.022	-1.509	0.134
Canopy cover	-0.068	-0.234	0.099	-0.799	0.426
Surface debris	-0.133	-0.318	0.051	-1.429	0.155
Algae	0.114	-0.069	0.297	1.232	0.22
Emergent plants	-0.093	-0.153	-0.033	-3.071	0.003
Habitat type	-0.026	-0.066	0.014	-1.278	0.203
Substrate type	-0.027	-0.177	0.124	-0.353	0.725
Intensity of light	0.185	-0.251	0.622	0.839	0.403
Water current	-0.584	-1.302	0.134	-1.606	0.11
Habitat permanence	0.103	-0.104	0.31	0.986	0.326
Constant	-2.301	-3.913	-0.689	-2.82	0.005

CI = confidence intervals, B = the unstandardized coefficient value,

Sig = significant at $p < 0.05$

In Darge, during the months of March 2015 to April 2015 and February 2016 to March 2016, *Anopheles* larvae density were high, but were least in August 2015 and between the months of

April-July 2016. In Ghibe study site, the largest proportion of *Anopheles* larval density were in November 2014 and May 2016 but were least between October 2015 and December 2015 and in June 2016 and July 2016 (Figure 3.3). The study further showed that, at Ghibe *Anopheles* larvae monthly density were slightly significantly positively correlated with rainfall (r = 0.43, p = 0.036). At Darge, rainfall was not significantly correlated with larval density (r = -0.052, p = 0.808).

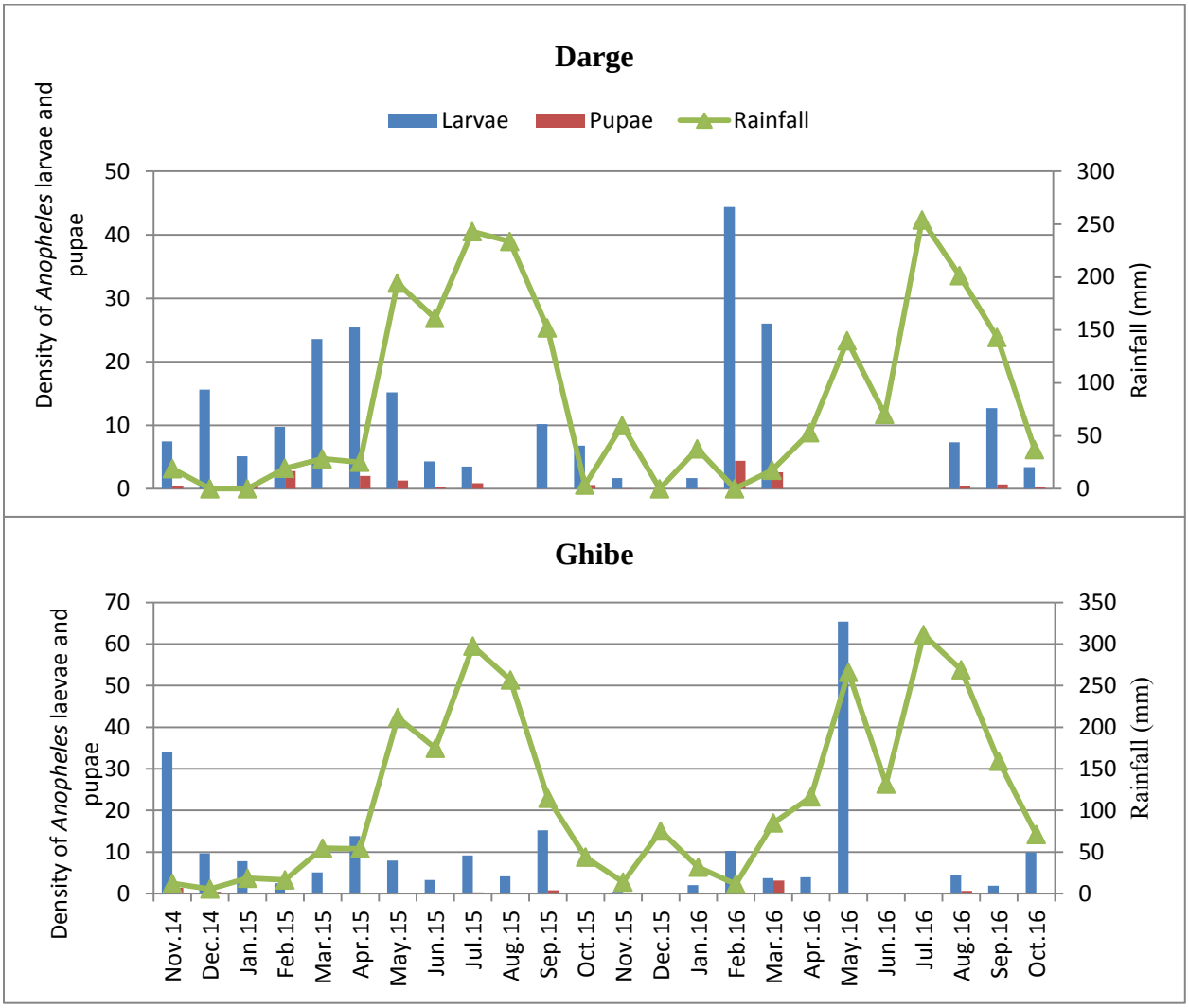


Figure 3.3 Monthly density of *Anopheles* larvae and pupae in the two study sites over the 24-months sampling period (November 2014 to October 2016)

There was slight correlation between water pH and that of *Anopheles* mosquitoes larval density (r = 0.187, p = 0.016) (Table 3.6). Water temperature was also slightly positively correlated with the *Anopheles* mosquito larval density (r = 0.163, p = 0.035). However, there was no association between the depth of the larval habitat and *Anopheles* larval density (r = 0.069, p = 0.376).

Table 3.6 Correlation of some larval habitat characteristics with the average *Anopheles* larval density sampled

Physico- chemical parameter	Mean ± SD	Correlation coefficient (r)	p value
Water pH	7.19 ± 0.42	0.187*	0.016
Water Temperature	28.49 ± 3.24	0.163*	0.035
Water depth	13.49 ± 11.70	0.069	0.376

*Correlation is significant at the 0.05 level, SD = standard deviation

From the total identified *Anopheles* larvae species, only *An. gambiae* s.l., *An. christyi* and *An. pharoensis* late instars were used for the analysis of environmental variables (Appendix 3.1). Mann-Whitney U test showed that, the length of habitat stability has no significant difference for *An. gambiae* s.l. (U = 3137, p = 0.455) but permanent habitats were preferred by *An. christyi* (U = 2960.5, p = 0.019) and *An. pharoensis* (U = 3115, p = 0.047). The presence or absence of algae on larval habitat had no impact on the larval density of *An. gambiae* s.l., *An. christyi* and *An. pharoensis* (p > 0.05). Habitat lacking surface debris favour the development of *An. gambiae* s.l. (U = 2117, p = 0.009) and *An. christyi* (U = 2267, p = 0.00) but no significant association with *An. pharoensis*.

Anopheles gambiae s.l. prefered sunlit habitats than shaded habitats ($U = 228$, $p = 0.028$) while *An. christyi* and *An. pharoensis* were found with no significant difference ($p > 0.05$) in sunlit and shaded habitats. A Kruskal-Wallis H test showed that, *An. pharoensis* larvae were highly abundant in habitats with perimeter greater than one hundred meter $\chi^2 = 68.7$, df = 2, $p < 0.001$ but *An. gambiae* s.l. and *An. christyi* were available more in smaller habitat though there was no significant difference ($p > 0.05$). *Anopheles* mosquitoes were found breed in clean and turbid water with no significant difference ($P > 0.05$). Larval habitat distance to the nearest house showed that, *An. gambiae* s.l. found most abundant in habitats closer to human habitations ($\chi^2 = 10.6$, df = 3, $p = 0.014$) while *An. christyi* densities were highest in habitats far (between 301 and 400m) from houses ($\chi^2 = 24.9$, df = 3, $p < 0.001$). *Anopheles gambiae* s.l. densities were significantly higher in habitats without vegetation (trees or bushes) near larval habitat ($\chi^2 = 8.0$, df = 2, p = 0.019) but *An. pharoensis* densities were significantly higher in habitats with the presence of vegetation ($\chi^2 = 9.5$, df = 2, $p = 0.009$) but there was no significant difference for *An. christyi* densities ($p > 0.05$).

Anopheles gambiae s.l. larval densities were significantly higher in habitats without emergent plants within the larval habitat ($\chi^2 = 8.7$, df = 3, $p = 0.034$) (Appendix 3.1) but *An. pharoensis* larval abundance was higher in habitats with grass and weeds available together ($\chi^2 = 11.8$, df = 2, $p = 0.008$). *Anopheles christyi* larvae were significantly more abundant in habitats with the substrates that were lined with large stones ($\chi^2 = 21.1$, df = 2, $p = 0.001$).

3.4 Discussion and Conclusions

This study examined the *Anopheles* mosquito species composition and identified and characterized their larval habitats in two localities in the Ghibe River basin, southwestern

Ethiopia. *Anopheles gambiae* s.l. was the primary *Anopheles* mosquito found in all larval habitats except in swamps. Similarly *An. gambiae* s.l. bred in almost a wide variety of sites that available in Mbita, a rural town in western Kenya (Fillinger *et al*., 2004). In line with our study, *An. gambiae* s.l. has been found to be the most abundant species in stream edges in Eritrea (Shililu *et al*., 2003) and southern Ethiopia (Gone *et al*., 2014) and along the river edge during the dry and small rainy season in Rift Valley, central Ethiopia (Kenea *et al*., 2011).

Certain breeding sites, such as rain pools and tire tracks were formed after rains while other like habitats at the pools at river edges were formed after rivers receded following rainy seasons. During the rainy season water accumulated in pockets and served as larval breeding site as rain pools and tire tracks. However, these small water bodies could not persist for long period if there is no rain (Sogoba *et al*., 2007). On the other hand, during the time of rain the amount of rivers increase in size and breeding sites may not be formed at the edge of the rivers (Konradsen *et al*., 1998; Imbahale *et al*., 2011; Mutero *et al*., 2000).

In Darge, during an extended period without rain, before the completely drying-out of the river, there was the collection of stagnant water in the river beds that served as larval breeding habitats. This corroborates with other studies that showed drying streams (Kenea *et al*., 2011; Dida *et al*., 2015), drying river (Sogoba *et al*., 2007) and habitats at river fringes (Imbahale *et al*., 2011) supported the greatest numbers of mosquito larvae during the dry season. Hence, such type of habitats should be considered during larval control interventions (Fillinger *et al*., 2004) because targeting these habitats during the dry seasons reduces costs of control interventions and considerable impact on malaria transmission in surrounding areas and subsequent rainy season (Sogoba *et al*., 2007; Worrall and Fillinger, 2011).

The highest proportions of *Anopheles* larvae and pupae were collected from borrow pits, pools in drying river beds and pools at river edges. Borrow pits were dug by humans for house plastering at Darge and ditches that were used to protect the cultivated crops from hippopotamuses at Ghibe. Mosquitoes larval survival and development depends on biological and physicochemical properties of the habitats (Edillo *et al*., 2004), the stability of habitats for longer periods (Mala and Irungu, 2011), oviposition behavior of gravid females (Gimnig *et al*., 2001), and cannibalism and predation (Koennraadt and Takken, 2003; Munga *et al*., 2006).

Larval breeding habitats such as hoof prints, rock pools and tire tracks in both study sites and rain pool in Darge sudy site had lower larval densities than other breeding habitat types. This corroborates a study conducted in the Butajira area where *Anopheles* larvae were not available in hoof-prints and most of temporary rain pools (Animut *et al*., 2012). This could be due to temporary behavior of the habitats which may dry rapidly after rains (Afrane *et al*., 2012) and such habitats rarely lasted more than five days to enable the larval stages to complete their development to emerge into an adult (Mala and Irungu, 2011). It may also result from the period of larval sampling which was done on monthly base and larvae might not have been missed if it was done on a weekly or fortnightly basis (Afrane *et al*., 2012). Reduced food availability and growth rate may have resulted in mosquito competition and predation that may lead to lower mosquito density (Mwangangi *et al*., 2006). On the other hand, temporary water bodies such as drainage canals, hoof prints, rain pools and tire tracks were identified as the most productive habitats in two studies (Mala and Irungu, 2011; Imbahale *et al*., 2011) but erosion pits and habitats along the river fringe were identified as less important mosquito breeding habitats (Imbahale *et al*., 2011). This might be related with frequent occurrence of rain in their study area which supports the availability of water in these temporary habitats (Mala and Irungu, 2011).

Most of these habitats had dried up within few weeks after the end of the rainy season so that few mosquito larvae could be found (Imbahale *et al*., 2011).

The present study showed least density of *Anopheles* larvae were sampled in swamps as compared to other larval breeding habitats. Sattler *et al*. (2005) stated that, *Anopheles* larvae were less likely to be present in swamps and if present they were present in low densities and such type of habitats should not be ignored. During control interventions it should be noted that, larger habitats with low larval density may have greater importance in productivity than smaller habitats with high larval density (Gu and Novak, 2005). This type of habitats might support large number of macroinvertebrate predators and competitors which may affect the development of *Anopheles* mosquitoes. The longer development time of *Anopheles* larvae to complete its larval stage in such type of habitats increases their chance to be preyed upon (Mereta *et al*., 2013; Munga *et al*., 2006). In swamps though *An. gambiae* s.l. were identified, *An. pharoensis* occupied the larger proportion of larvae identified as has been found elsewhere (Kenea *et al*., 2011).

Habitats near the edges of rivers were not available when the river level rose as a result of rainfall in upstream areas and in case of the Ghibe River when there was also released water from Gilgel Gibe I reservoir. When there was intense rainfall, the amount of sampled larvae was reduced. A study in Eritrea showed that, *Anopheles* larval densities were negatively correlated with rainfall (Shililu *et al*., 2007). This might happen as the result of larval flushing and mortality due to heavy rainfall (Kweka *et al*., 2011; Imbahale *et al*., 2011; Afrane *et al*., 2012). Larval flushing might be related with rain drops or related to runoff that created small temporary streams to the larval habitats after rains. As a result, rainfall decreases the larval density in a breeding site (Paaijmans *et al*., 2007).

As multiple regression analysis indicated, temperature at the time of sampling and emergent vegetation were the most important variables for *Anopheles* mosquito larval density. *Anopheles gambiae* s.l. was significantly associated with habitats that had smaller perimeter, sunlit, low vegetation cover, and lack of emergent plants. This corroborates with the studies conducted elsewhere (Gimnig *et al*., 2001; Kenea *et al*., 2011). In habitats with lower water temperature, *Anopheles* larval development period elongated and increases the chance of larvae to be predated (Tuno *et al*., 2005). In contrast to our study it was shown that, habitats contained growing grass had more *Anopheles* larvae than without vegetation in the larval habitats (Imbahale *et al*., 2011). Habitats that have less vegetation cover and that may not occur throughout the year costs less for the application of larval source management as a control of mosquito larvae. Combining such intervention with LLINs might provide higher reduction in new malaria infections than using nets alone (Worrall and Fillinger, 2011).

Anopheles pharoensis was sampled more from habitats that were permanent with large perimeter, presence of trees and emergent plants that contain weeds and grasses. Study by Kenea *et al*. (2011) showed that, higher densities of *An. pharoensis* larvae were sampled from permanent lakeshore habitats with vegetation and algal mats.

Generally, *Anopheles* mosquito larval density was not significantly associated with water pH, water temperature, water turbidity, algal content, and larval habitat depth. The mean water temperature was 28.5 ± 3.2°C which may have at a range of suitable water temperature for the *Anopheles* larvae to survive and develop into an adult as was stated by Bayoh and Lindsay (2003). Larval habitat depth was measured only from those habitats which contain *Anopheles* larvae so that most of the habitats were not very deep with few exceptions that reached 65cm deep and this might not lead to variation in suitability for mosquito larval breeding. *Anopheles*

gambiae s.l. larvae were more abundant in habitats closer to human habitation as compared to those habitats far from houses. It was stated that, gravid mosquitoes prefer to lay eggs in habitats closer to human habitation to conserve energy lost flying long distances in search of oviposition sites (Mwangangi *et al*., 2010).

There were a few limitations in this study. Larval samplings were conducted on monthly basis so that mosquito larvae might be missed during the study period to show the whole picture of the species abundance and larval habitats productivity. Water temperature of the larval habitat was recorded only during larval sampling time and was not measured at different times of the day. On the other hand, environmental variables including detailed analysis of water chemistry, mosquito predators and competitors to know the most predictor of larval habitat characteristics were not included in the study.

Anopheles gambiae s.l. which is considered as the main malaria vector in Ethiopia was the most abundant larval species in the study area. This species of mosquito larvae were available both during the dry and rainy seasons. The rivers found along this study area serve as refugia during the dry seasons and enable the malaria vectors to sustain throughout the year. While planning for malaria control program that incorporate larval control interventions, both dry and rainy seasons should be considered. However, dry season habitats became limited, and application of control interventions during this season might be more effective.

Chapter 4. Species composition, seasonal density and blood meal sources of *Anopheles* mosquitoes in Ghibe River basin, southwestern Ethiopia

4.1 Introduction

Malaria has an overwhelming impact on people's health and livelihoods with an estimated 212 million cases and 429,000 deaths globally in 2015 and about 99% of these deaths had been because of *P. falciparum*. Globally, about 70% of deaths had been in children aged less than 5 years (WHO, 2016). In Ethiopia, malaria is the major public health problem with variable transmission and occurrence. Malaria transmission is seasonal and shows variation in its endemicity in the country due to large diversity in altitude, rainfall and population movement. However, relatively long transmission season exists in the western lowland regions, river basins, valleys, and irrigation schemes (FMoH, 2016).

In Ethiopia, approximately 68% of the population lives in malaria endemic regions. It was estimated that 2.8 million cases and 4,900 deaths occurred because of malaria in 2015. The massive proportion of malaria was caused by *P. falciparum* (64%) and *P. vivax* (36%) (WHO, 2016; FMoH, 2016) but, this percentage might not be constant because of higher degree of seasonal variation in *Plasmodia* species (Woyessa *et al*., 2004; Abeku, 2007; Alemu *et al*., 2012; Sena *et al*., 2014). These Plasmodia species found solely in humans so that to sustain local transmission, *Anopheles* mosquitoes should be physiologically competent, survive long enough for complete sporogonic development of the pathogen and feed at least occasionally on people (Killeen, 2014). Lowland areas are endemic to malaria while highlands and highland fringe areas are prone to epidemics associated with a month of unusually high minimum temperature together with lack of immunity in populations (Abeku *et al*., 2003).

Anopheles arabiensis is the principal malaria vector in Ethiopia and *An. pharoensis*, *An. funestus*, and *An. nili* are considered as secondary vectors (FMoH, 2007; WHO, 2016). Understanding the biting and resting habits of *Anopheles* mosquitoes is essential for the implementation of effective vector control interventions (Breman and Brandling-Bennett, 2011). *Anopheles arabiensis* exhibits a zoophagic and anthropophagic habit and rests both indoors and outdoors with variable proportions in specific settings depending on the availability and location of alternative hosts (Faye *et al*., 1997; Tirados *et al*., 2006; Muriu *et al*., 2008; Massebo *et al*., 2013; Mayagaya *et al*., 2015) and land use pattern (Muriu *et al*., 2008). This species is flexible and bites humans both indoors and outdoors (Kenea *et al*., 2016).

In Ethiopia, malaria vector control heavily relies on IRS and LLINs. Larval control through environmental management and chemical larviciding are applied in selected areas (FMoH, 2016). LLINs and IRS are intradomiciliary-based control measures effective for vectors that closely depend on human for feeding and resting inside houses. The species of malaria vectors having such type of habits are small in number but play a great role for world's malaria burden (Killeen, 2014).

To achieve maximum impact on malaria elimination using vector control interventions that focus on the adult stages, it requires full understanding of where and when persons are most exposed to the bites of vectors. In the presence of even low number of outdoor feeding and resting *Anopheles* mosquitoes, which could not be targeted with those control interventions, result in failure of elimination. Even, the presence of these insecticide based interventions may lead in the shift of the biting behavior of the vectors from indoor to outdoor, from human to animal and to early before bed time or late at night (Govella and Ferguson, 2012; Moiroux *et al*., 2012; Mwangangi *et al*., 2013; Killeen, 2014). A shift from human to animal might have its own

advantage in reducing malaria transmission via reducing the human-vector contact being animals are the dead-end hosts for the common human malaria parasites (Mwangangi *et al*., 2013). In rural Africa, vectors biting outdoor and at instances while the people not asleep pose a greater impact on malaria transmission, because humans wakeup early in the morning and stay outdoors at night for agricultural and household activities (Moiroux *et al*., 2012).

A shift in the sibling species composition was also observed after long-term application of ITNs from extremely anthropophagic and endophagic *An. gambiae* s.s. to the zoophagic and adaptable *An. arabiensis* (Russell *et al*., 2011). Even, *An. funestus* was observed biting on human indoors between 07:00 and 11:00 in broad daylight once most of the active population were occupied with different tasks (Sougoufara *et al*., 2014). Less efficient malaria vectors that feed mostly on animals could also be capable of sustaining endemic malaria transmission even though they feed solely seldom upon humans. It is often difficult to control this type of *Anopheles* mosquitoes using LLINs and IRS because of their preference to feed at dusk and dawn when people are outdoors in addition to their behavior of feeding on animals (Russell *et al*., 2011; Killeen, 2014).

To achieve malaria eradication program, it needs both raising the current control methods and also the development of novel interventions to interrupt transmission. The development of new tools to include into this management interventions needs understanding of the biology of important malaria vectors (The malERA Consultative Group on Vector Control, 2011). Understanding the resting and feeding behaviour of *Anopheles* mosquitoes is extremely vital with regard to the efficacy of control measures. Hence, the objective of this study was to identify the *Anopheles* mosquito species and to evaluate their blood meal source using different collection methods in the Ghibe River basin, southwestern Ethiopia.

4.2 Material and Methods

4.2.1 Description of the study area

The study was conducted in Ghibe and Darge study sites located within the Ghibe River basins, southwestern Ethiopia. Description of the study area is presented in Chapter 3, section 3.2.1 above. In each study site, there are perennial rivers. In Ghibe, the majority of houses were built with thatched roof and wooden walls plastered with mud while in Darge, they were constructed with thatched roofs or corrugated roofs and wooden walls plastered with mud. The average number of occupants per house in Ghibe and Darge were 5.2 and 4.8, respectively. The proportions of cattle owned by the residents were much higher in Ghibe than in Darge. Human to cattle ratio in Darge and Ghibe were 1.8 and 0.4, respectively (Table 4.1) (Abeshge District Agricultural Development Office, unpublished data). There was application of IRS on yearly bases before the onset of the main rainy season. The residents own LLINs (PermaNet® 2.0) distributed free of charge by the Ministry of Health.

Table 4.1 Number of humans and domestic animals in the study area

Study site	Human	Cattle	Goat	Sheep	Donkey	Chicken
Darge	3518	1974	327	499	569	1976
Ghibe	2167	5040	2100	740	500	2520

LLINs and IRS distribution data in both the study sites is depicted in Table 4.2 (Abeshge district health office, unpublished report).

Table 4.2 LLINs and IRS distribution in Ghibe and Darge study sites, 2011-2016

	Study site	2011	2012	2013	2014	2015	2016
LLINs	Ghibe	350	-	750	50	-	1193
	Darge	450	-	1371	-	-	1936
IRS	Ghibe	707	628	503	634	717	678
	Darge	2434	974	1113	961	905	1068

LLINs = no. distributed, IRS = no. of houses sprayed

4.2.2 Adult mosquito collections

Adult *Anopheles* mosquitoes were collected monthly using CDC light traps, pyrethrum spray catches, and pit shelter from each study site from January 2015 to February 2016 and April 2016 to October 2016 over 21 months. Houses were first stratified into near to (<500m) and far from (>500m) Ghibe and Darge Rivers at Ghibe and Darge study site, respectively. Sixteen houses (eight houses near to the river and eight houses far from the river) were selected at random for Centre for Disease Control and Prevention (CDC) light traps (BioQuip products, Inc. CA 90220, USA) for collection of host seeking mosquitoes indoors and outdoors. In each house, light traps were set at 1-1.5m above the ground close to the foot of an occupant sleeping under LLIN (Mboera *et al*., 1998). Outdoor collections using CDC light traps were conducted starting from June 2015. Light traps were hanged near houses or in cattle sheds if cattle were available around homesteads. Traps were set for 12 hours (18:00 in the evening to 7:00 in the morning) for two sequential nights each month for a total of 1,184 trap-nights of which 672 for indoor and 512 outdoor trap nights in each study site.

Indoor pyrethrum spray collections (PSC) and pit shelters were used to collect indoor resting and outdoor resting mosquitoes, respectively (Schunk *et al*., 2006). Ten houses (five houses near and five houses far from the river) were selected for spray collection for a total of 210 spray-days in each study site. Before the application of insecticides, all food items, drinking water and domestic animals were moved out from the house. All holes and eaves were closed with pieces of cloth. White sheet of cloth was spread covering all the floor and furniture within the room where people slept the previous night. Baygon aerosol (prallethrin 0.10% w/w, permethrin 0.10% w/w, Saudi Johnson Co. Ltd, Sc Johnson & Son, Inc, USA) was sprayed starting from the outside near the eaves of roof and within the house. Then the door/s and window/s were closed and after ten minutes, the sheet was moved out and knocked down mosquitoes were collected using forceps (WHO, 2011). All collections were made in the morning (6:30 - 9:00). Four pit shelters (two near to the river and two far away from the river) were prepared under shade in households' backyard in each study site. Each shelter had the dimensions of 1.5 m deep, 1 m wide and 1.2 m long. At a distance of 0.5 m from the bottom of the pit one or two cavities about 30 cm deep were dug out horizontally from each of the four sides (Silver, 2008). Mosquitoes were collected from the small cavities and from the wall of the pit itself using hand held torch and mouth aspirator from 6:30-9:30 in the morning.

Mosquitoes were also collected from the walls and roofs inside homes and cattle sheds by aspiration using hand held mouth aspirator and battery operated torch in the morning from 7:00 - 9:00. In Ghibe, livestock were kept in separate enclosure outdoors or also constructed thatched roof and wall shelters. In Darge, it was common to keep livestock in houses with humans at night.

4.2.3 Mosquito identification

Female *Anopheles* mosquitoes were counted and sorted to abdominal conditions as unfed, freshly fed, half gravid and gravid, and morphologically identified under stereomicroscope using morphological identification keys of Gillies and Coetzee (1987). The abdomens of freshly blood fed *Anopheles* mosquitoes were used for blood meal analysis.

4.2.4 Blood meal analysis

Freshly blood fed *Anopheles* mosquitoes were cut transversally between the thorax and abdomen beneath a dissecting microscope, x10-20 magnification powers for the identification of blood meal sources using enzyme-linked immunosorbent assay (ELISA) following the procedure described by Beier *et al*. (1988). ELISA was carried out to test the presence of human or bovine blood in the mosquitoes. The abdomen of each mosquito was placed separately in a labeled eppendorf tube containing 50µl phosphate-buffered saline (PBS, pH 7.4) and was ground with a pestle. The samples were diluted 1:50 dilution with PBS. Nintysix well polyvinyl microtiter plates were used for the ELISA and 50µl each mosquito triturates were coated per well. Blood meals were tested for humans and bovines in order that, one plate for human blood and one plate for bovine blood tests were run simultaneously. On the same plate, 50µl samples of positive control human antisera for human and bovine antisera for bovine were added to a single well each. Additionally, four negative controls of unfed female *An. arabiensis* mosquitoes from ALIPB were used for each plate and handled following the procedure stated above. Then, the plates were covered and incubated overnight at room temperature.

After incubation, the plates were washed twice with PBS-Tween-20. Fifty microliter of each host-specific conjugate [anti-human IgG conjugated in peroxidase diluted 1:2,000 in 0.5% boiled

casein (BC) containing 0.025% Tween-20; anti-bovine IgG conjugated in phosphatase diluted 1:250 in BC-Tween-20] were added to each well on the human and bovine microtiter plates, respectively. The plates were incubated for 1 hr at room temperature. After incubation, wells were washed thrice with PBS-Tween-20 and 100 µl of peroxidase substrate [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS] were added to each well and incubated. After 30 and 60 minutes, plates were visually observed for the formation of dark green reaction. Samples absorbances were also read with an ELISA plate reader after 60 minutes. Samples were considered positive if absorbance values exceed the mean plus three standard deviations of four negative controls.

4.2.5 Data analysis

Monthly *Anopheles* mosquitoes' abundance in different collection methods was expressed with descriptive statistics using percentage. *Anopheles arabiensis* monthly average density using CDC light trap, PSC and pit shelter was expressed as the total number of mosquitoes collected per total monthly catches for each sampling methods. Correlation analysis was used to estimate the association between *An. arabiensis* densities with rainfall. Chi-square test was used to compare the *Anopheles* mosquitoes' abundance using CDC light trap between near and far houses from the river and between indoor and outdoor catches. Pit shelter and PSC were not included in this analysis because *An. arabiensis* was the only species identified in these collection methods.

The human blood index (HBI) and bovine blood index (BBI) were calculated as the ratio of blood-fed mosquitoes that had fed on human and cattle, respectively to the total tested. Mixed blood meal source was the ratio of blood-fed mosquitoes that had fed both human and bovine to the total tested represented in percent. Unknown blood meal source was the ratio of blood fed

mosquitoes that their blood sources were not known to the total tested and expressed in percent (Muriu *et al*., 2008). Forage ratio of the *Anopheles* mosquitoes was not determined due to the availability of kebeles adjacent to the study sites within the flight range of the mosquitoes. Chi-square test was used to compare the differences in the HBI and BBI between indoor and outdoor collected *An. arabiensis*. Data were analyzed using IBM SPSS statistical for Windows (IBM corp., Armonk, NY), version 20.0. Values were considered significantly different if $p < 0.05$ for all the tests.

4.2.6 Ethical considerations

Approval to conduct the study was obtained from the ethical committees of College of Natural Sciences Institutional Ethics Review Board, Addis Ababa University (Reference No. CNSDO/213/07/15). Consent was also obtained from Abeshge district (woreda) administration and the manager of each study site after the purpose of the investigation was explained to them. Verbal consent was obtained from each household head of selected houses where mosquitoes were sampled from their houses or their premises in each study site.

4.3 Results

4.3.1 Species composition of *Anopheles* mosquitoes

Species composition of *Anopheles* mosquitoes are depicted in Table 4.3. Microscopic examination of 2,669 female adult *Anopheles* mosquitoes collected with different methods revealed the presence of 13 species in the study area. *Anopheles gambiae* s.l., *An. coustani*, *An. pretoriensis*, *An. demeilloni*, *An. rupucolus*, *An. christyi*, *An. pharoensis*, *An. nili* and *An.*

rivulorum were identified in both Ghibe and Darge study sites where as *An. tenebrosus*, *An. ardensis*, *An. natalensis* and *An. zeimanni* were recored only in Ghibe.

Most of the collected and identified adult *Anopheles* mosquitoes (91.6%, n = 2,446) were from Ghibe study site. *Anopheles gambiae* s.l. (= *An. arabiensis* Balkew *et al*., 2010; Demissew *et al*., 2016) were the predominant species in both the study sites (87.9% in Ghibe and 67.7% in Darge) followed by *An. coustani* (Ghibe = 5.4%, Darge = 14.3%) (Table 4.3).

All of the *Anopheles* species identified were represented in CDC light trap collections. Only *An. arabiensis* mosquitoes were collected by PSC and hand collections with mouth aspirators from inside houses. *Anopheles arabiensis* and *An. pretoriensis* were collected from pit shelter outdoors while *An. arabiensis* and *An. coustani* were collected using mouth aspirators from cattle shed. All species identified were collected from indoors and outdoors except *An. tenebrosus*, *An. natalensis*, *An. ziemanni* and *An. nili* which were sampled only from outdoors. About 69% of *Anopheles* mosquitoes were collected using CDC light trap (Table 4.3).

Table 4.3 *Anopheles* species composition in different collection methods in the two study sites (January 2015 to October 2016)

Study site	Species	Indoor					Outdoor					Total (%)
		CDC		PSC		MA inside houses	CDC		Pit shelter		MA cattle Shed	
		Near (%)	Far (%)	Near (%)	Far (%)		Near (%)	Far (%)	Near (%)	Far (%)		
Ghibe	*An. arabiensis*	390(92.9)	47(74.6)	35(100)	1(100)	119(100)	807(83.4)	113(56.8)	131(99.2)	12(100)	495(99.6)	2150(87.9)
	An. coustani	6(1.4)	12(19.1)	-	-	-	67(6.9)	44(22.1)	-	-	1(0.2)	130(5.4)
	An. pretoriensis	4(1.0)	-	-	-	-	27(2.8)	14(7.0)	1(0.8)	-	-	46(1.9)
	An. demeilloni	10(2.4)	1(1.6)	-	-	-	12(1.2)	3(1.5)	-	-	-	26(1.1)
	An. rupicolus	3(0.7)	-	-	-	-	16(1.7)	8(4.0)	-	-	-	27(1.1)
	An. christyi	1(0.2)	-	-	-	-	10(1.0)	8(4.0)	-	-	-	19(0.8)
	An. pharoensis	5(1.2)	1(1.6)	-	-	-	11(1.1)	1(0.5)	-	-	-	18(0.7)
	An. tenebrosus	-	-	-	-	-	12(1.2)	5(2.5)	-	-	-	17(0.7)
	An. ardensis	-	2(3.2)	-	-	-	3(0.3)	1(0.5)	-	-	-	6(0.2)
	An. natalensis	-	-	-	-	-	-	1(0.5)	-	-	-	1(0.1)
	An. ziemanni	-	-	-	-	-	1(0.1)	-	-	-	-	1(0.1)
	An. nili	-	-	-	-	-	2(0.2)	-	-	-	-	2(0.1)
	An. rivulorum	1(0.2)	-	-	-	-	-	-	-	-	1(0.2)	2(0.1)
	Unidentified	-	-	-	-	-	-	1(0.5)	-	-	-	1(0.1)
	Total	**420(100)**	**63(100)**	**35(100)**	**1(100)**	**119(100)**	**968(100)**	**199(100)**	**132(100)**	**12(100)**	**497(100)**	**2446(100)**
Darge	*An. arabiensis*	60(84.5)	24(96.0)	2(100)	1(100)	-	20(26.7)	28(90.3)	9(100)	5(100)	2(50)	151(67.7)
	An. coustani	3(4.2)	-	-	-	-	25(33.3)	2(6.5)	-	-	2(50)	32(14.3)
	An. demeilloni	5(7.1)	-	-	-	-	8(10.7)	-	-	-	-	13(5.8)
	An. rupicolus	-	1(4.0)	-	-	-	12(16.0)	-	-	-	-	13(5.8)
	An. nili	-	-	-	-	-	6(8.0)	-	-	-	-	6(2.7)
	An. pretoriensis	1(1.4)	-	-	-	-	2(2.7)	-	-	-	-	3(1.3)
	An. rivulorum	1(1.4)	-	-	-	-	-	1(3.2)	-	-	-	2(0.9)
	An. christyi	1(1.4)	-	-	-	-	1(1.3)	-	-	-	-	2(0.9)
	An. pharoensis	-	-	-	-	-	1(1.3)	-	-	-	-	1(0.4)
	Total	**71(100)**	**25(100)**	**2(100)**	**1(100)**	**-**	**75(100)**	**31(100)**	**9(100)**	**5(100)**	**4(100)**	**223(100)**

PSC = pyrethrum spray sheet collection, MA = mouth aspirator, Far = houses located > 500 meter from the river, Near = houses < 500 meter from the river.

In Ghibe, mean density of *An. arabiensis* using CDC light trap was higher outdoors (1.8/ CDC light trap/night) as compared to indoors (0.7/CDC light trap/night) (Figure 4.1). The largest proportions of monthly densities of *An. arabiensis* using CDC light trap indoors were 4.1/CDC light trap/night and in outdoor catches were 6.7/CDC light trap/night. The density of this species in PSC was higher in August 2016 (2/house/day) and in November 2015 (14.8/pit shelter/day) using pit shelter. *Anopheles arabiensis* density was lower in Darge as compared to Ghibe.

In Darge, mean density of *An. arabiensis* was 0.125/CDC light trap/night indoors and 0.07/CDC light trap/night outdoors. The largest proportions of monthly densities of *An. arabiensis* using CDC light trap indoors were 0.69/CDC light trap/night and in outdoor catches were 0.41/CDC light trap/night. However, it was 0.01/house/day using PSC and 0.67/pit shelter/day using pit shelter. Higher mean density was 0.7/CDC light trap/night indoor in August 2016 and 0.4/CDC light trap/night outdoor in July 2015 and August 2016 (Figure 4.1). *Anopheles arabiensis* density was not significantly correlated with rainfall ($p > 0.05$).

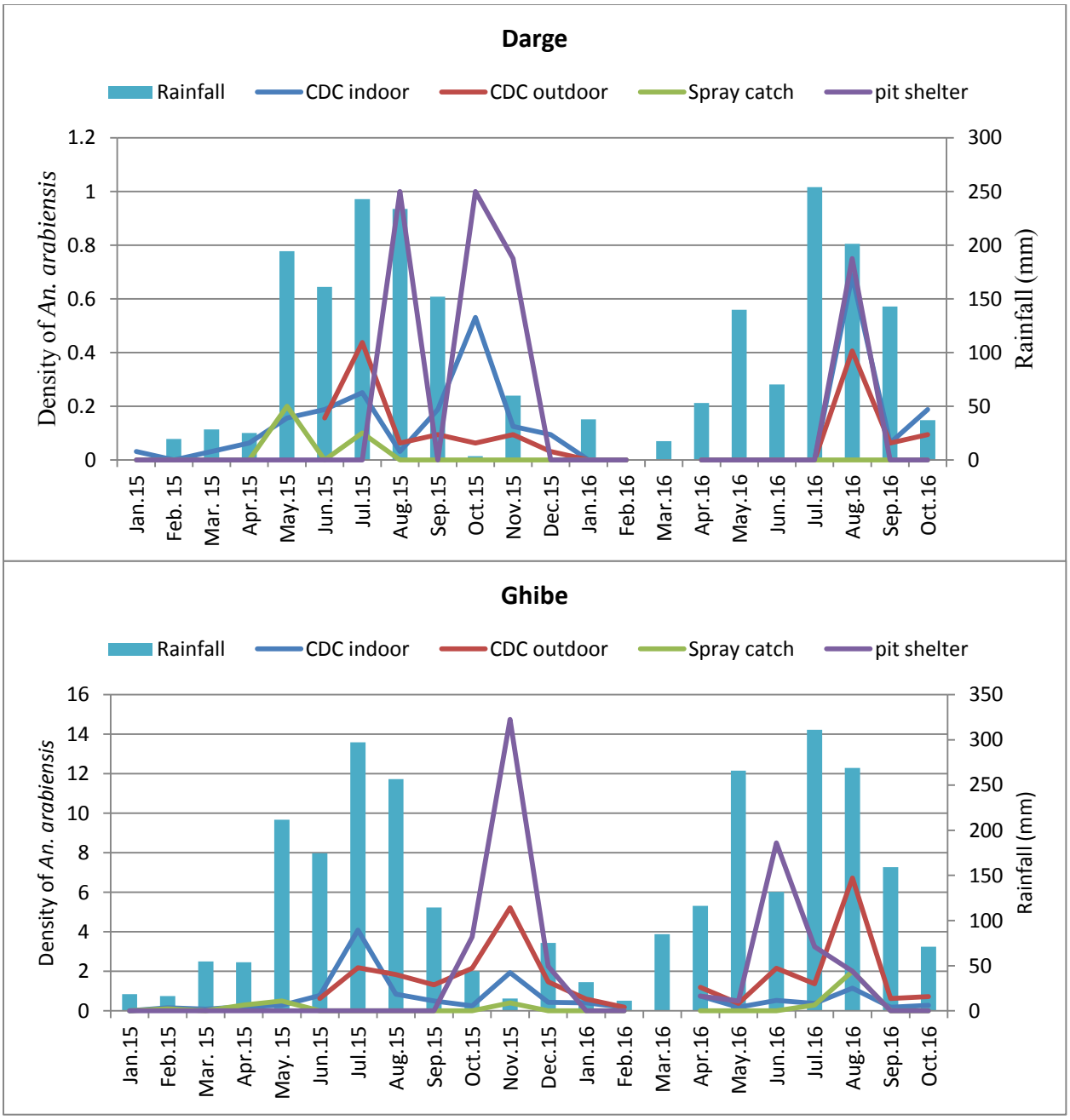


Figure 4.1 Association between monthly density of *An. arabiensis* and rainfall (in mm) in the Ghibe and Darge study sites of Ghibe River basin, southwestern Ethiopia (January 2015 – October 2016)

Analysis to compare *Anopheles* mosquitoes densities from near and far houses from the river was based on data collected using CDC light trap from June 2015 to October 2016 due to the start of

outdoor collection in June 2015 and the result is presented in Table 4.4. *Anopheles ardensis*, *An. natalensis*, *An. ziemanni*, *An. nili*, *An. rivulorum* and unidentified species were excluded from this analysis because of their low number. In Ghibe, the remaining *Anopheles* mosquitoes were 1,157 (72.0%) outdoors and 450 (28.0%) indoors (Table 4.4) and there was significant difference between indoor and outdoor catches ($\chi^2 = 49.037$, df = 7, $p < 0.001$). *Anopheles* caught in houses closer to the river were 1,350 (84.0%) and in houses located far from the river were 257 (16.0%) and the difference was significant ($\chi^2 = 113.038$, df = 7, $p < 0.001$). In Darge, 101 (54.6%) *Anopheles* mosquitoes from outdoors and 84 (45.4%) from indoors were sampled (Table 4.4) and there was statistically significant difference between outdoor and indoor catches ($\chi^2 = 39.902$, df = 4, $p < 0.001$). *Anopheles* mosquitoes were 127 (68.6%) in houses near to the river and 58 (31.4%) far from the river. There was significant difference between near and far houses from the river ($\chi^2 = 30.759$, df = 4, $p < 0.001$).

Table 4.4 Comparison of *Anopheles* abundance from CDC light trap collections indoor/outdoor and in houses near/ far from rivers in the study sites of Ghibe River basin, southwestern Ethiopia (June 2015 to October 2016)

Study site	Species	CDC light trap catch indoor			CDC light trap catch outdoor		
		Near (%)	Far (%)	**Total**	Near (%)	Far (%)	**Total**
Ghibe	*An. arabiensis*	367 (89.1)	45 (10.9)	**412**	805 (87.5)	115 (12.5)	**920**
	An. christyi	1 (100)	-	**1**	10 (55.6)	8 (44.4)	**18**
	An. demeilloni	10 (90.9)	1 (9.1)	**11**	12 (80.0)	3 (20.0)	**15**
	An. pretoriensis	1 (100)	-	**1**	27 (65.9)	14 (34.1)	**41**
	An. coustani	7 (36.8)	12 (63.2)	**19**	66 (60.0)	44 (40.0)	**110**
	An. pharoensis	2 (66.7)	1 (33.3)	**3**	11 (91.7)	1 (8.3)	**12**
	An. rupicolus	3 (100)	-	**3**	16 (66.7)	8 (33.3)	**24**
	An. tenebrosus	-	-	-	12 (70.6)	5 (29.4)	**17**
	Total	**391 (86.9)**	**59 (13.1)**	**450**	**959 (82.9)**	**198 (17.1)**	**1157**
Darge	*An. arabiensis*	48 (64.0)	27 (36.0)	**75**	20 (41.7)	28 (58.3)	**48**
	An. demeilloni	5 (100)	-	**5**	8 (100)	-	**8**
	An. coustani	3 (100)	-	**3**	25 (92.6)	2(7.4)	**27**
	An. rupicolus	-	1 (100)	**1**	12 (100)	-	**12**
	An. nili	-	-	-	6 (100)	-	**6**
	Total	**56 (66.7)**	**28 (33.3)**	**84**	**71 (70.3)**	**30 (29.7)**	**101**

4.3.2 Blood feeding status of adult *Anopheles* mosquitoes

The abdominal condition of female *Anopheles* mosquitoes collected is depicted in Table 4.5. With the exception of 87 (3.3%) *Anopheles* mosquitoes, the abdominal conditions of all were classified into unfed, freshly fed, half gravid and gravid. The proportion of blood fed mosquitoes

varied between the collection methods used. From mosquitoes collected using CDC light trap, among abdominal conditions identified 67.7% (n = 1,195) were unfed and only 22.5% (n = 398) were blood fed. From PSC there were no unfed and 51.3% (n = 20) were blood fed but 57.3% (n = 355) of resting *Anopheles* mosquitoes collected with mouth aspirator were blood fed and only 3.9% (n = 24) were unfed. Catches from pit shelter showed, 38.0% (n = 60) were blood fed but lower proportion (12.7%, n = 20) of unfed mosquitoes were collected.

Table 4.5 Abdominal status of *Anopheles* mosquitoes collected by different methods in the study sites of Ghibe River basin, southwestern Ethiopia (January 2015 to October 2016)

Species	CDC					PSC			Pit shelter				Mouth aspirator				**Total**
identified	UF	FF	HG	GR	UN	FF	HG	GR	UF	FF	HG	GR	UF	FF	HG	GR	
An. arabiensis	1060	218	71	79	61	20	12	7	19	60	51	27	24	351	138	103	**2301**
An. coustani	79	72	-	1	10		-	-	-	-	-	-	-	3	-	-	**162**
An. pretoriensis	8	35	1	2	2	-	-	-	1	-	-	-	-	-	-	-	**49**
An. rupicolus	15	22	2	-	1	-	-	-	-	-	-	-	-	-	-	-	**40**
An. demeilloni	14	18	2	2	3	-	-	-	-	-	-	-	-	-	-	-	**39**
An. christyi	3	14	-	1	3	-	-	-	-	-	-	-	-	-	-	-	**21**
An. pharoensis	3	7	4	5	-	-	-	-	-	-	-	-	-	-	-	-	**19**
An. tenebrosus	6	9	-	1	1	-	-	-	-	-	-	-	-	-	-	-	**17**
An. nili	2	3	-	-	3	-	-	-	-	-	-	-	-	-	-	-	**8**
An. ardensis	2	1	-	1	2	-	-	-	-	-	-	-	-	-	-	-	**6**
An. rivulorum	2	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	**4**
An. ziemanni	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	**1**
An. natalensis	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**
Unidentified	1	-	-	-		-	-	-	-	-	-	-	-	-	-	-	**1**
Total	**1195**	**398**	**80**	**92**	**87**	**20**	**12**	**7**	**20**	**60**	**51**	**27**	**24**	**355**	**138**	**103**	**2669**

PSC = Pyrethrum spray sheet collection, MA = mouth aspirator, UF = unfed, FF = fresh fed, HF = half gravid, GR = gravid, UN = abdominal status unknown.

4.3.3 Blood meal sources and blood indices

In total, 832 *Anopheles* mosquitoes were tested for identification of their blood meal sources and the result is shown in Table 4.6. Human blood was identified from *An. arabiensis*, *An. coustani*, *An. demeilloni* and *An. nili* and no other species of *Anopheles* mosquitoes were identified fed on human. Human and cattle mixed blood was detected from *An. arabiensis* and *An. demeilloni*. More than 35% of the blood meal sources of *An. arabiensis* collected outdoors using CDC light traps were not known (Table 4.6).

In Ghibe, the HBI for *An. arabiensis* was 58.0% from CDC light trap collections indoors and 16.0% outdoors. The BBI from CDC light trap collections in indoors were 14.0% and in outdoors were 47.0%. There was significant difference between indoor and outdoor catches in their blood meal sources ($\chi^2 = 42.134$, df = 3, $p < 0.001$). The HBI was 75.0% from PSC. Mixed blood (human and bovine) source for *An. arabiensis* using CDC light trap indoors, CDC light trap outdoor and PSC were 4.4%, 1.3% and 5.0%, respectively.

In Darge, the HBI of *An. arabiensis* from CDC light trap collections indoors and outdoors was 14.0% and 13.0%, respectively while the BBI was higher both indoors (57.0%) and outdoors (50.0%) than that of HBI. However, there was no significant difference between HBI or BBI of *An. arabiensis* caught indoors and outdoors ($\chi^2 = 0.216$, df = 2, $p = 0.898$).

Table 4.6 Blood meal origins of *Anopheles* mosquitoes collected using CDC light trap and PSC in two study sites in the Ghibe River basin (January 2015 – October 2016)

Study site and	CDC indoor					CDC outdoor					PSC				
species	n	HBI	BBI	Mix	Un	n	HBI	BBI	Mix	Un	n	HBI	BBI	Mix	Un
Ghibe															
An. arabiensis	113	58.0	14.0	4.4	23.0	76	16.0	47.0	1.3	35.5	20	75.0	10.0	5	10
An. pretoriensis	3	-	67.0	-	33.0	29	-	97.0	-	3.5	-	-	-	-	-
An. pharoensis	1	-	100	-	-	6	-	83.0	-	16.7	-	-	-	-	-
An. coustani	3	33.0	67.0	-	-	52	-	98.0	-	1.9	-	-	-	-	-
An. tenebrosus	-	-	-	-	-	9	-	100	-	-	-	-	-	-	-
An. demeilloni	4	-	100	-	-	9	-	78.0	11.1	11.1	-	-	-	-	-
An. ardensis	-	-	-	-	-	1	-	100	-	-	-	-	-	-	-
An. christyi	-	-	-	-	-	13	-	100	-	-	-	-	-	-	-
An. natalensis	-	-	-	-	-	1	-	100	-	-	-	-	-	-	-
An. rupicolus	1	-	100	-	-	12	-	100	-	-	-	-	-	-	-
Darge															
An. arabiensis	21	14.0	57.0	-	28.6	8	13.0	50.0	-	37.5	-	-	-	-	-
An. pretoriensis	1	-	100	-	-	2	-	100	-	-	-	-	-	-	-
An. rivulorum	1	-	100	-	-	-	-	-	-	-	-	-	-	-	-
An. coustani	2	50.0	-	-	50	12	-	92.0	-	8.3	-	-	-	-	-
An. demeilloni	3	-	100	-	-	1	-	100	-	-	-	-	-	-	-
An. christyi	-	-	-	-	-	1	-	100	-	-	-	-	-	-	-
An. rupicolus	1	-	100	-	-	8		100	-	-	-	-	-	-	-
An. nili	-	-	-	-	-	3	33.0	67.0	-	-	-	-	-	-	-

PSC = pyrethrum spray sheet collection, n = number tested, HBI = human blood index in %, BBI = bovine blood index in %, Mix = human and bovine mixed blood expressed in %, Un = unidentified blood meal in %

In Ghibe, the BBI of *An. arabiensis* collected from pit shelter and mouth aspirated from cattle sheds were 83.0% and 68.0%, respectively and the HBI using mouth aspirator from human dwellings was 74.0% (Table 4.7). Though the proportion was low, human blood fed and mixed blood fed mosquitoes were also identified from pit shelter and cattle sheds. In Darge, the tested mosquitoes collected from cattle sheds were all fed on bovine.

Table 4.7 Blood meal origins of *An. arabiensis* mosquitoes collected using pit shelter and mouth aspirator in Ghibe study site, Ghibe River basin (January 2015 - October 2016)

Collection method	n	HBI	BBI	Mixed	UN
Pit shelter	60	3.0	83.0	1.7	11.7
MA human house	58	74.0	14.0	-	12.1
MA cattle shed	291	2.0	68.0	0.7	29.6

MA = mouth aspirator, n = number tested, HBI = human blood index in %, BBI = bovine blood index in %, Mix = human and bovine mixed blood expressed in %, Un = unidentified blood meal in %

4.4 Discussion and conclusions

Anopheles arabiensis was the predominant species in the study area followed by *An. coustani*. *Anopheles arabiensis* was collected both from indoors and outdoors with the largest proportions as compared to other species. Previous study by Tekie (1989) in the area also showed *An. gambiae* s.l. was the predominant species. In line with our study, *An. arabiensis* was the most abundant species in different studies conducted in the country (Massebo *et al*., 2013; Animut *et al*., 2013; Gari *et al*., 2016). Unlike our study, *An. ziemanni* was the predominant species followed by *An. arabiensis* in Edo Kontola village, south-central Ethiopia and this difference

might be due to variation in available larval breeding sites which support the development of the former species (Kenea *et al*., 2016).

More number of *Anopheles* mosquitoes were sampled from outdoors using CDC light trap, pit shelter and mouth aspirator as compared to collections made indoors. Study using human landing catch in Ghibe Horticulture Development Farm showed *An. gambiae* s.l. bite human both indoors and outdoors (Tekie, 1989). Though there was no significant difference in outdoor and indoor human biting rates of *An. arabiensis*, higher numbers of overall *Anopheles* mosquitoes were collected outdoors than indoors using human landing catches (Tirados *et al*., 2006; Kenea *et al*., 2016). This outdoor abundance of *Anopheles* mosquitoes might be because of females which fed indoors might left houses immediately after feeding (Tirados *et al*., 2006), diverted to outdoor biting due to availability of cattle outside houses (Tirados *et al*., 2011; Mayagaya *et al*., 2015), due to vector control interventions targeted indoor biting and resting mosquitoes that induced exophagic and late biting behavior (Russell *et al*., 2011; Moiroux *et al*., 2012; Mwangangi *et al*., 2013; Kenea *et al*., 2016), or rapid exit after entering houses (Killeen, 2014). The presence of malaria vectors that bite outdoors at dusk and dawn may respond poorly to control interventions that targeted inside houses so that additional tools which target outdoor biting mosquitoes are required to complement ITNs and IRS (Russell *et al*., 2011). The availability of cattle outdoor might enhance outdoor catches using CDC light trap, thus extending the host-seeking flight period which in turn increases the chance of getting caught in the trap (Githeko *et al*., 1994). In outdoor collections using CDC light trap, *Anopheles* mosquitoes might be better sampled when the trap could be baited with CO_2 than unbaited (Sriwichai *et al*., 2015).

Low densities of *Anopheles* mosquitoes were collected from Darge as compared to Ghibe. This might be due to difference in the type of house construction materials (Zhou *et al*., 2007; Kirby

et al., 2008), variation in proportions of cattle owned by the residents (Mayagaya *et al*., 2015), and altitudinal variation between the two study sites (Animut *et al*., 2013). *Anopheles arabiensis* density was not significantly correlated with rainfall. This might be due to the spray of houses with insecticides before the onset of the main rain or the larval habitat could be affected due to heavy rain or formed after rains. Our study also showed that, *Anopheles* mosquitoes were more abundant in houses closer to the river (within 500 m distance) than in houses far away from the river. In agreement with our results, a study in western Kenya highlands (Zhou *et al*., 2007) and Iguhu Village, western Kenya (Li *et al*., 2008) showed that, houses located closer to the vector habitats had a significantly higher distribution of adult mosquitoes than those farther away. Residents near to the major vector-breeding sites were also observed more affected with malaria in urban Uganda (Staedke *et al*., 2003) and Adama town, in Ethiopia (Peterson *et al*., 2009) as compared to far from breeding sites.

Most of the *Anopheles* mosquitoes were collected using CDC light traps. This corroborates with other studies that, fewer mosquito vectors were captured in resting collections than in host seeking collections (Tirados *et al*., 2006; Sikaala *et al*., 2013; Mayagaya *et al*., 2015). Among *Anopheles* mosquitoes collected using CDC light trap the large proportions were unfed. This might be because mosquitoes were caught while searching for their blood meals before they took blood. In line with our study, in northwestern Thailand (Sriwichai *et al*., 2015) and The Gambia (Jawara *et al*., 2008) most of the collected *Anopheles* mosquitoes using CDC light trap were unfed. High proportions of fed *Anopheles* mosquitoes were sampled in artificial resting places (Sikaala *et al*., 2013). This might be because fed mosquitoes have low flight activities and rest most during their gestation time (Githeko *et al*., 1994; Sikaala *et al*., 2013). If mosquitoes fed indoors, they might rest indoors for one or two nights till the blood meal is digested and eggs are

developed and can be targeted by application of indoor vector control intervention (Killeen, 2014).

Our study showed that, in Ghibe HBI was higher from mosquitoes collected indoors from human dwellings while BBI was higher from outdoor collections. Our result was in agreement with the study of Hadis *et al*. (1997) and Massebo *et al*. (2015). As opposed to our result, the HBI of *An. arabiensis* collected indoors and outdoors were not different, but higher BBI among indoor than outdoor collected samples (Muriu *et al*., 2008) and mosquitoes collected outdoors had higher HBI than those collected indoors (Bashar *et al*., 2012). It was suggested that, people might be bitten more frequently outdoors, or indoor-biting mosquitoes do not remain inside and instead exit houses after feeding (Tirados *et al*., 2006; Bashar *et al*., 2012) or might also bite in the early-evening indoors and outdoors at times when the local people were not protected by LLINs (Yohannes *et al*., 2012; Kenea *et al*., 2016). However in Darge, there was no significant difference between BBI in CDC light trap set indoors and outdoors. It was observed that, people share houses with cattle in Darge so that, mosquitoes may bite cattle indoors. The human biting habit of *An. arabiensis* depends on host availability (Faye *et al*., 1997).

From the total *An. arabiensis* mosquitoes tested for blood meal source, 25.3% were not fed on human or bovine. This might be they fed on other animal sources like ovine and donkeys which were available in the study area. Bovine blood fed mosquitoes were identified from indoor catches and this might be their outdoor feeding on cattle was interrupted and enter houses for further blood feeding or to rest indoors (Hadis *et al*., 1997; Muriu *et al*., 2008; Tirados *et al*., 2011). Availability of alternative hosts like cattle can significantly affect the resting and feeding preference of *Anopheles* mosquitoes at the household level (Massebo *et al*., 2015; Mayagaya *et al*., 2015). Study in costal Kenya showed that, the primary malaria vectors have shifted from

feeding on human to animal and it coincides with mass distribution of LLINs in the area (Mwangangi *et al*., 2013). Animals were considered as dead end of the malaria pathogens (Mwangangi *et al*., 2013) and serve as zooprophylaxis for zoophagic vectors like *An. arabiensis* (Bugoro *et al*., 2011). Hence, in areas where *An. arabiensis* which has zooghagic behavior is the major malaria vector, use of cattle treated with insecticides could be helpful for their control (Habtewold *et al*., 2004; Franco *et al*., 2014). New control tools that target mosquitoes biting outdoor and early at night before people went to bed are urgently required to control malaria in combination with the existing control interventions (Govella and Ferguson, 2012).

The limitations of our study were; the study could not incorporate window exit trap to study the indoor feeding and resting behavior of *Anopheles* mosquitoes as they leave the houses after feeding and/or resting. For determination of blood meal sources of *Anopheles* mosquitoes in the study area, they were tested only for identifying human or bovine blood sources which might missed other animals that serve as alternative blood meal sources.

In conclusion, *Anopheles arabiensis*, which is considered as the main malaria vector in Ethiopia, was the predominant species in the study area. This species of mosquitoes were collected both indoors and outdoors. Blood meal analysis showed they fed both on human and bovines. *Anopheles* mosquitoes were abundant in houses located closer to the river as compared to houses far away from the river. In determining malaria risks, vector control strategies targeted to houses closer to the river could have great importance in malaria reduction. It might be difficult to control malaria with the current vector control interventions due to the availability of malaria vectors both indoors and outdoors. Thus, it needs looking for additional measures which will reduce their numbers outdoors.

Chapter 5. Malaria prevalence, entomological inoculation rate and insecticide susceptibility of *An. arabiensis* from two study sites in Ghibe River basin, southwestern Ethiopia

5.1 Introduction

In Ethiopia, malaria transmission is seasonal and highly variable at micro-geographical scales where transmission is likely to be more and more focal, with some areas being relatively free and others having substantial risk of malaria (Abeku *et al*., 2003; Jima *et al*., 2010). An abnormal increase in minimum temperature and lack of immunity by the community in highlands and highland fringe areas, and monthly total rainfall in the lowlands significantly affect malaria transmission in the country (Abeku *et al*., 2003; Alemu *et al*., 2011). Though recurrent malaria epidemic occurs in the highlands and highland fringe areas, the highest malaria morbidity occur after the main rainy seasons from June to September. *Plasmodium falciparum* and *P. vivax* are the two predominant malaria species which result in large proportions of malaria cases and deaths in the country (Abeku *et al*., 2003; Alemu *et al*., 2011; 2012; Woyessa *et al*., 2013).

Though the number of malaria cases and deaths declined after scale-up of deployment of artemisinin based combination therapy (ACT), IRS and wide distribution of LLINs (Jima *et al*., 2010; Aregawi *et al*., 2014), retrospective studies in the past showed that the number of malaria cases and deaths fluctuated over the years in Ethiopia. This variability was suggested to be due to climatic variables, variation in population immunity, local ecological factors, host and vector characteristics, and social and economic determinants (Alemu *et al*., 2011; 2012), inconsistency in using LLINs (Jima *et al*. 2010) and population movement, settlement from non-malarious areas to malarious areas, and urbanization (Deressa *et al*., 2006).

Anopheles mosquitoes transmit malaria to human once they were infected with malaria pathogens and release sporozoite stage when they tend to take blood. Even a single infectious mosquito can cause human malaria infection and life threatening disease (Beier *et al*., 1999; Churcher *et al*., 2015). There is variation among people to be infected with malaria due to variation in attraction to *Anopheles* mosquitoes. Some people have a higher probability to be infected with malaria and more likely to remain infected if prevalence in the overall population falls (Churcher *et al*., 2015).

Anopheles arabiensis, a member of the *An. gambiae* complex, is the primary malaria vector in Ethiopia, with *An. funestus, An. pharoensis* and *An. nili* considered as secondary vectors (WHO, 2016). Indoor malaria vector control interventions like LLINs and IRS target *Anopheles* mosquitoes that tend to bite and rest indoors. In the presence of these interventions, host-seeking mosquitoes might be diverted to seek blood meals in outdoor venues and might feed on non-human hosts (Pappa *et al*., 2011; Moreno *et al*., 2015). The effectiveness of these tools should be evaluated continuously for the effective control of malaria. This could be done using entomological inoculation rate (EIR) which is the direct measurement of intensity of malaria parasite transmission by *Anopheles* vectors depending on human biting rate and sprorozoite rate of malaria vectors (Shaukat *et al*., 2010).

Factors such as expansion of irrigation scheme (Kibret *et al*., 2010; 2014), construction of large dams (Kibret *et al*., 2017), asymptomatic subpatent *P. falciparum* and *P. vivax* malaria carriage in low endemic or pre-elimination transmission settings (Tadesse *et al*., 2015), insecticide resistance (Balkew *et al*., 2010; Yewhalaw *et al*., 2011; Massebo *et al*., 2013) and drug resistance (Schunk *et al*., 2006; Ketema *et al*., 2011) might pose problems in control and eventual elimination of malaria in Ethiopia. This study aimed to examine the prevalence of

malaria based on case reports, the EIR and insecticide susceptibility status of *An. arabiensis* in the Ghibe River basin, southwestern Ethiopia.

5.2 Material and Methods

5.2.1 Description of the study area

This study was conducted in Ghibe and Darge study sites in Ghibe River basin, southwestern Ethiopia. Detail description of the study area is presented in chapter 3 (section 3.2.1) and chapter 4 (section 4.2.1) above.

5.2.2 Malaria prevalence data collection

Malaria prevalence results from November 2014 to October 2016 for Darge and Ghibe study sites were recorded from Abeshge woreda health management and information system (HMIS) data base. Malaria cases were sorted by study sites, sex and age of patients confirmed by Rapid Diagnostic Tests (RDTs). Monthly malaria prevalence (cases per 1,000 populations per month) was calculated to determine the level of malaria transmission in each study site across months of the year.

5.2.3 Mosquito collection and identification

Adult *Anopheles* mosquitoes were collected from indoors and outdoors using CDC light trap, PSC, pit shelter and mouth aspirators from January 2015 to October 2016 (section 4.2.2). *Anopheles* mosquitoes were identified into species using keys of Gillies and Coetzee (1987) and were kept in individual tubes containing silica gel for further laboratory testing.

5.2.4 Dissection and longevity determination

The abdomens of blood unfed *Anopheles* mosquitoes were placed on a drop of physiological saline on a clean microscopic slide. The thorax was gently held with forceps and the ventral side was placed upward with the abdomen in the solution. Using a fine tip forceps, the seventh and eighth abdominal segments were removed by pulling them away slowly. Accessory tissues were removed and ovaries were isolated and transferred to a new slide and allowed to dry. After they were air dried, they were observed under compound microscope at 20x magnification. Females in which their ovaries have tightly coiled tracheoles (skein) were determined as nulliparous whereas those ovaries with stretched tracheoles were parous mosquitoes (WHO, 2011). Parity rate was determined as proportion of parous mosquitoes to the total mosquitoes dissected. The survival rate of *An. arabiensis* was determined using parity rate (PR) and the gonotrophic cycle and expressed as the cube root of the proportion of parous mosquitoes considering three days for its gonotrophic period as described by Davidson (1954) and WHO (2011).

5.2.5 Malaria sporozoite ELISA

Heads and thoraces of female *Anopheles* mosquitoes were processed for detection of circumsporozoite proteins (CSP) of *P. falciparum* and *P. vivax* sporozoites using an ELISA following the procedure described in Beier *et al*. (1987). Briefly, the head and thorax of a mosquito was placed in a 1.5 ml Eppendorf tube and ground by adding 50 µl grinding buffer (Igepal CA-630 in blocking buffer or BB). The pestle was rinsed with two 100 µl volumes of BB resulting in the final volume of 250 µl mosquito triturate.

Monoclonal antibody (mAb) solution of *P. falciparum*, *P. vivax* 210 (Pv-210) and *P. vivax* 247 (Pv-247) of 50 µl was added in each well of separate plates assigned for each species. The plates

were covered and incubated over night at room temperature in a dark cabinet. Plate contents were well aspirated and washed five times. Wells were then filled with 200 µl BB and incubated for 1hr at room temperature. The well contents were aspirated and washed five times. A positive control (*P. falciparum*, Pv-210 and Pv-247) in each respective plate, six negative controls (unfed *An. arabiensis* from ALIPB laboratory) and mosquito triturates at an amount of 50 µl per well were loaded, covered and incubated for 2 hours. Well contents were aspirated and washed 5 times using 200 µl PBS-Tween-20.

Fifty microliter of *P. falciparum*, Pv-210 and Pv-247 peroxidase conjugated solutions were added to each well to the respective plates and incubated for 1 hour. Well contents were aspirated and plates were washed thrice with 200 µl PBS-Tween-20. One hundred microliter ABTS substrate solutions were added per well. The plates were covered and incubated for 30 minutes and were visually read after 30 minutes and 1 hour. Positive reactions, which were appeared green, were determined by reading plates at 414 nm using an ELISA plate reader; and mosquitoes were considered infected if ELISA absorbance values (at 30 min) exceeded the mean plus 3 times standard deviation of six control mosquitoes on the same plate (Beier *et al*., 1987). All positive samples were re-tested for confirmation and CSP rates and EIRs were determined.

5.2.6 Insecticide susceptibility study

This portion has relation with malaria transmission in the study area because those *Anopheles* mosquitoes developed resistance might have the potential to transmit the disease though insecticide based vector control interventions were implemented.

5.2.6.1 Mosquito collection

Mosquito larvae and pupae were collected from their breeding sites from February to March 2016 in Darge study site using plastic scoop and transferred into small white plastic larval trays. *Anopheles* larvae and pupae were sorted and transported with water in plastic bottles to the laboratory of the ALIPB. The larvae fed with larval food (dogs' biscuit) to develop into adults. Adult *An. gambiae* s.l. (= *An. arabiensis*) mosquitoes were morphologically identified and non-blood fed 3-5 days old females were used for the insecticide bioassays.

5.2.6.2 Insecticide susceptibility test procedure

Mosquitoes were tested against nine insecticides using papers impregnated with the WHO discriminating concentrations. The insecticides belonged to four different insecticide classes including deltamethrin (0.05%), permethrin (0.75%), lambdacyhalothrin (0.05%), cyfluthrin (0.15%), DDT (4%), malathion (5%), fenitrothion (1.0%), propoxur (0.1%) and bendiocarb (0.1%). Paper impregnated with risella oil, olive oil and silicone oil were used for the control groups of organochlorine, organophosphate/carbamate and pyrethroids insecticides, respectively. WHO insecticide impregnated papers were obtained from Universiti Sains Malaysia.

Six clean white papers (12cm x 15 cm) were rolled into cylinder shape and each were inserted into the holding tubes and fastened with steel spring-wire clip to the side of the tube. Twenty five 3-5 days old blood unfed female mosquitoes were aspirated from the cage into each holding tubes in four replicates per test. In addition, twenty five mosquitoes in two replicates were used as a control for each insecticide. The slide tubes were closed and the tubes were set into the upright position for an hour. Insecticide impregnated papers were inserted into each of the four replicates of exposure tubes and respective oil impregnated papers were inserted into two control

exposure tubes. The mosquitoes were gently blown from each holding tubes to the exposure tubes and set into vertical position for 60 minutes (WHO, 2013). For DDT and pyrethroids insecticides knockdown mosquitoes were recorded at 10′, 15′, 20′, 30′, 40′, 50′ and 60′. The test was performed at ALIPB laboratory at 27 ± 2 °C and 60 ± 10 relative humidities.

After an hour (except for fenitrothion which was exposed for two hours) of exposure the mosquitoes were transferred from exposure tubes back into the holding tubes and a cotton wool soaked in sugar water were placed on the mesh screen from the side of the holding tubes and allowed to stay for 24 hours. At the end of 24hrs, dead mosquitoes were counted and recorded. Any knocked down mosquitoes were recorded as dead.

5.2.7 Data analysis

Monthly malaria prevalence was calculated as the number of laboratory-confirmed malaria cases in a given month among 1,000 populations. It was expressed as the proportion of number of confirmed malaria cases to the total population of the area (WHO, 2012). Descriptive statistics was used to see the variation in malaria prevalence with age and sex. Pearson correlation analysis was used to determine the association between malaria cases and monthly rainfall. Parity rate was determined as the proportion of parous females to the total females dissected. Sporozoite rate (SRs) is the number of mosquitoes found positive to CSP antigens divided by the total number of mosquitoes examined, expressed as a percentage (WHO, 2011). The daily EIR was estimated based on CDC light trap catches (see Chapter 4, section 4.3.1) and using the factor determined in Adami Tullu district in Ethiopia for *An. arabiensis* as a CDC light trap caught 0.35 times that of indoor human landing catch (HLC) (Kenea *et al*., 2017). As a result, the daily EIR was calculated as 0.35 x (number of sporozoite positive ELISAs/ number of mosquitoes tested) x

(number of mosquitoes collected by CDC light trap/ number of CDC light trap catches) (Drakeley *et al*., 2003; Kenea *et al*., 2017). EIR was not estimated for mosquitoes collected with other collection methods because no CSP positive mosquitoes were identified. Monthly EIR was obtained by multiplying the daily EIR with the number of respective days of that month (Beier, 2002). The monthly EIRs in each study site were summed up to calculate the annual EIR (Kent *et al*., 2007). For insecticide susceptibility study, mean mortality of the four replicates was determined. Mortality less than 90% indicated development of resistance in the tested population (WHO, 2013). Data were analyzed with IBM SPSS statistical for Windows (IBM corp., Armonk, NY), version 20.0 and all statistical tests were considered significantly different at $p < 0.05$.

5.3 Results

5.3.1 Malaria prevalence in the study area

Retrospective and current malaria prevalence data analysis using one-sample T test indicated that, malaria cases were higher in Ghibe (3.6 per 1,000 population; 95%CI = 2.2-4.9; $t = 5.4$; $p < 0.001$) than Darge (0.3 per 1,000 population; 95%CI = 0.1-0.4; $t = 3.7$; $p = 0.001$) (Figure 5.1). In Ghibe, malaria cases were recorded throughout the study period except in January and February 2016 and with a peak records in May and June 2015. Though no substantial malaria cases were recorded in Darge, high cases were recorded in November 2014, February 2015 and July 2016 and no malaria cases were recorded in many of the months during the study period. There was fluctuation in monthly malaria cases during the study period. There was statistically significant variation in monthly malaria cases in Darge ($p = 0.001$) and Ghibe ($p < 0.001$). Pearson correlation analysis showed positive correlation between malaria cases and monthly

rainfall with none significant association in both study sites (Darge r = 0.019, p = 0.93; Ghibe r = 0.360, p = 0.081).

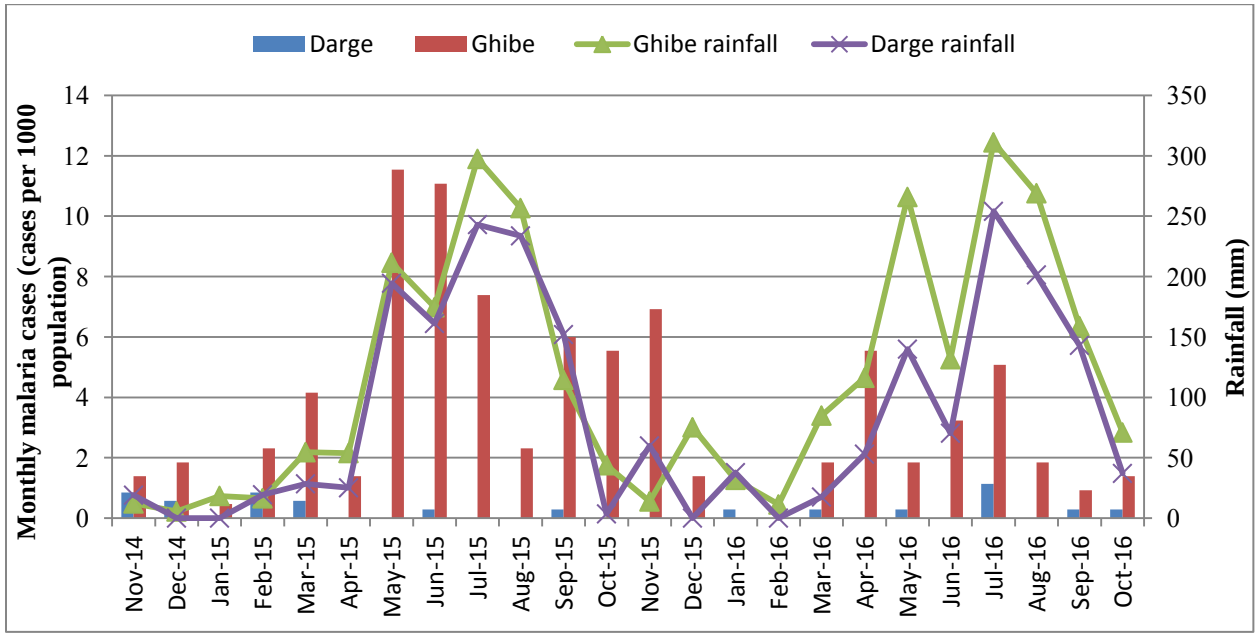
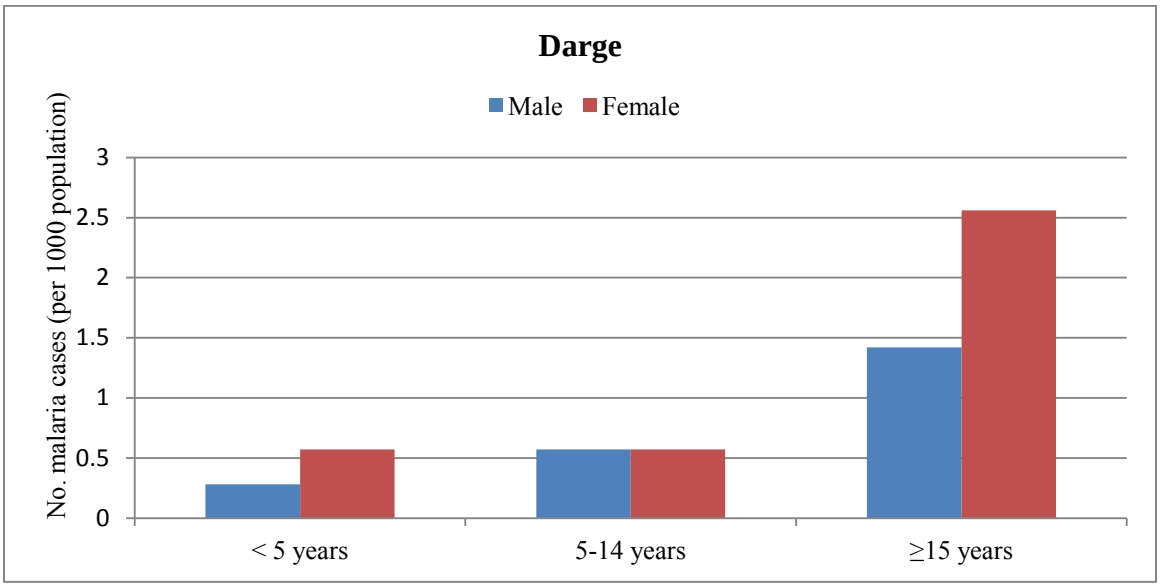


Figure 5.1 Monthly total confirmed malaria cases (cases per 1,000 populations) in the Ghibe River basin, southwestern Ethiopia (November 2014 to October 2016)

Confirmed malaria cases were significantly higher in a population aged above 14 years in both study sites (4.0 episodes in Darge and 45.2 in Ghibe per 1,000 people) and no significant variations were observed between ages less than five years and 5-14 years throughout the study period. In Darge, females were affected more than males while in Ghibe males were affected more than females for age greater than 14 years ($p < 0.05$) (Figure 5.2).

a)



b)

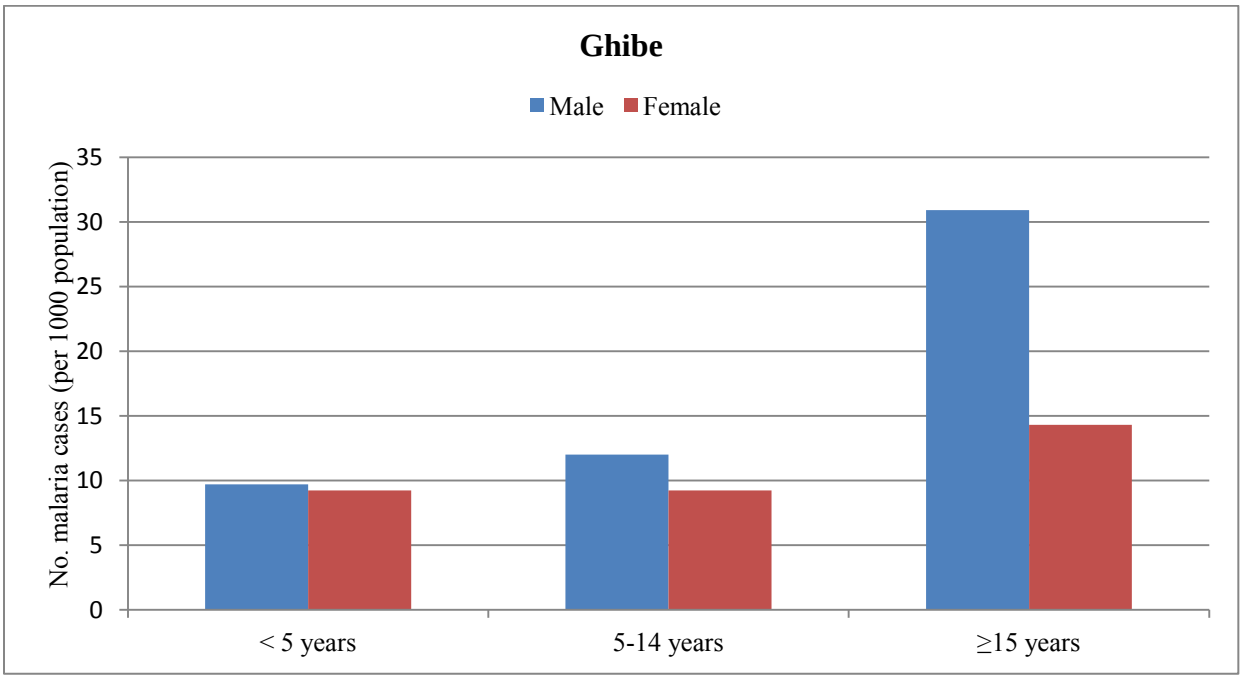


Figure 5.2 Confirmed malaria cases distribution in age and sex (a, Darge; b, Ghibe) in Ghibe River basin, southwestern Ethiopia (November 2014-October 2016)

5.3.2 Parity rate and EIR

In total, 126 *An. arabiensis* were dissected for determination of parity rate. Survival rate of *An. arabiensis* was between 5 and 9 days in Darge and Ghibe, respectively which has an average of 7 days (Table 5.1).

Table 5.1 Parity rate and longevity of *An. arabiensis* in Ghibe River basin, southwestern Ethiopia

Study site	No. dissected	Parous	PR	*p*	Age
Darge	19	11	0.58	0.83	5
Ghibe	107	76	0.71	0.9	9

PR = parasite rate, *p* = probability of surviving one day after blood-meal

In total, 1,801 *Anopheles* mosquitoes (1,668 from Ghibe and 133 from Darge) belonged to twelve species were tested for *Plasmodium* CSP using ELISA, of these 1,620 (90%) were *An. arabiensis* (Table 5.2). Out of the total tested, sprozoites were detected only in two of the *An. arabiensis* (one *P. vivax* i.e. Pv-210 and one *P. falciparum*) collected using CDC light traps and no other species of *Anopheles* was detected positive. Sporozoite rates were 0.07% for *P. vivax* and 0.07% for *P. falciparum* in *An. arabiensis* tested in Ghibe and zero in Darge. *Anopheles arabiensis* tested had overall *P. vivax* and *P. falciparum* sporozoite rates of 0.06% each.

In Ghibe, the overall EIRs for *P. falciparum* and *P. vivax* were zero and 1.86, respectively for the year 2015 while zero and 1.22 infective bites/person/year for *P. vivax* and *P. falciparum*, respectively for the year 2016 (Appendix 5.1). No *Plasmodium* positive *Anopheles* mosquitoes were identified from Darge so that EIR was not analyzed.

Table 5.2 Sporozoite infection rates of *Anopheles* mosquitoes in two study sites in Ghibe River basin, southwestern Ethiopia (January 2015 - October 2016)

	An. arabiensis	*An. coustani*	*An. pharoensis*	*An. demeilloni*	*An. pretoriensis*	*An. christyi*	*An. rupicolus*	*An. nili*	*An. tenebrosus*	*An. rivulorum*	*An. ardensis*	*An. zeimanni*
Ghibe												
No. tested	1521	74	9	13	14	6	14	2	8	1	5	1
No. PvS+ (%)	1(0.07)	0	0	0	0	0	0	0	0	0	0	0
No. PfS+ (%)	1(0.07)	0	0	0	0	0	0	0	0	0	0	0
Darge												
No. tested	99	17	0	8	0	1	4	3	0	1	0	0
No. PvS+ (%)	0	0	0	0	0	0	0	0	0	0	0	0
No. PfS+ (%)	0	0	0	0	0	0	0	0	0	0	0	0
Total												
No. tested	1620	91	9	21	14	7	18	5	8	2	5	1
No. PvS+ (%)	1(0.06)	0	0	0	0	0	0	0	0	0	0	0
No. PfS+ (%)	1(0.06)	0	0	0	0	0	0	0	0	0	0	0

PvS+ = number *P. vivax* sporozoite positive; PfS+ = number *P. falciparum* sporozoite positive; values in parentheses indicate sporozoite rates.

5.3.3 Susceptibility of *An. arabiensis* to insecticides

In total 1,350 blood unfed female *An. arabiensis* mosquitoes were used for the insecticide susceptibility test. Knockdown analysis showed that, until the 15th minute mosquitoes were not knockdown to all the insecticides tested. As compared to other insecticides, permethrin and lambdacyhalothrin had low knockdown effect to the tested mosquitoes (Figure 5.3). Due to lack of knockdown of 50% of the population within tested time, Probit analysis was not run to determine KDT_{50} and KDT_{95} for DDT and pyrethroids insecticides.

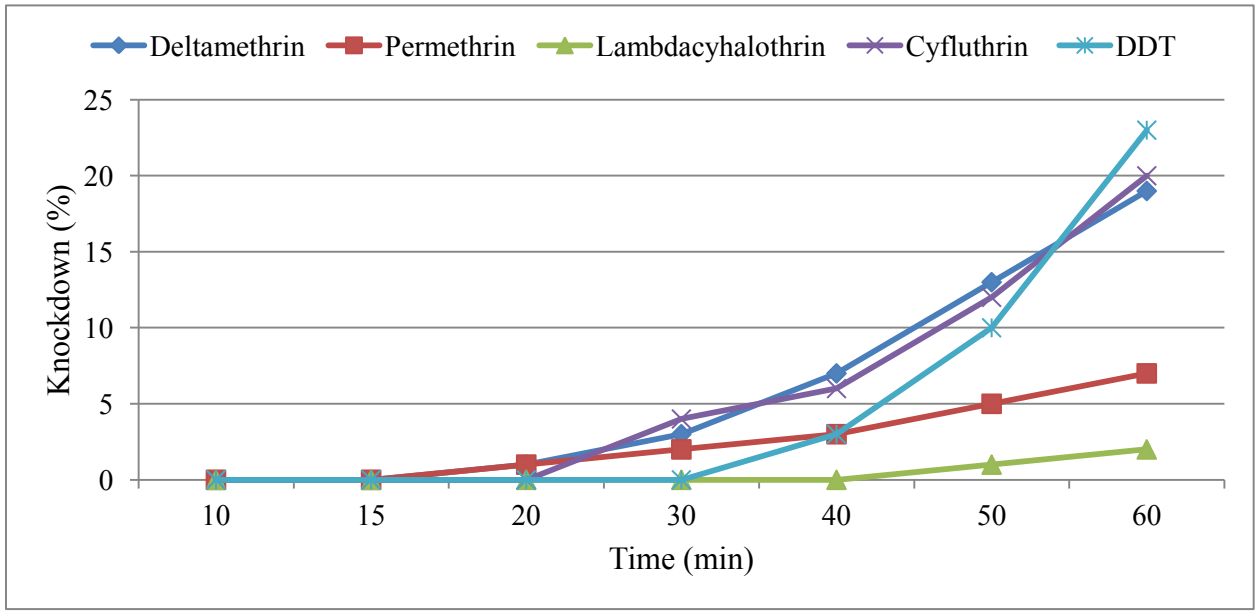


Figure 5.3 Knockdown rates of *An. arabiensis* over 60 minutes after exposure to DDT and pyrethroids in Darge study site in Ghibe River basin, southwestern Ethiopia (February to March, 2016)

Anopheles arabiensis developed insecticide resistance to all the tested insecticides except fenitrotion which killed 100% of the tested mosquitoes (Figure 5.4). They showed mortality of 88.0% (95%CI = 80.0-97.0%) to bendiocarb and 75.0% (95%CI = 68.9-81.1%) to propoxur.

Anopheles arabiensis showed least mortality to permethrin (9.0%, 95% CI = 2.9-15.1%) and lambdacyhalothrin (10.0%, 95%CI = 3.6-16.4%) as compared to other insecticides.

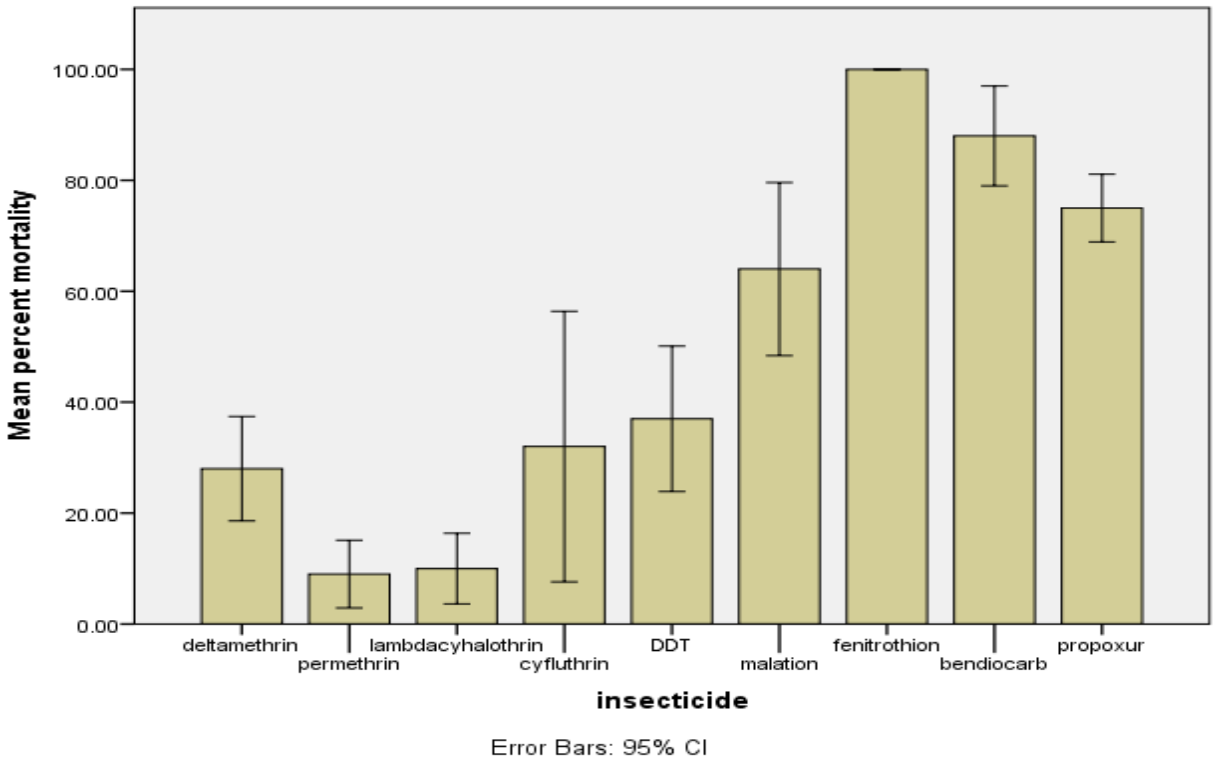


Figure 5.4 Susceptibility of *An. arabiensis* 24 hrs after exposure to different insecticides in Darge in Ghibe River basin, southwestern Ethiopia (February to March, 2016)

5.4 Discussion and conclusions

Malaria cases were very low in the study area during the study period. This observed low malaria prevalence might be associated with scale-up of malaria control interventions as operated throughout malaria endemic areas of the country (Jima *et al*., 2010). Malaria prevalence rates were lower in Darge as compared to Ghibe. It was suggested that, though malaria prevalence reduced in many malaria endemic areas of the country, its transmission become more and more focal so that some areas might have substantial risk of malaria than others (Jima *et al*., 2010).

Hence, the variation between our study sites was presumably due to variation in the type of house construction materials (Degefa *et al*., 2015), distance to the town (Drakeley *et al*., 2003) and altitudinal variation (Animut *et al*., 2013; Woyessa *et al*., 2013; Kibret *et al*., 2017). Except in few months, malaria cases were recorded throughout the year in Ghibe and it was in line with the study conducted in Kola Diba, northwest Ethiopia which showed occurrence of malaria in almost every month (Alemu *et al*., 2012). Unlike our study, high malaria prevalence was recorded in suburbs of Jimma town, southwestern Ethiopia with overall prevalence of 6.3% (Degefa *et al*., 2015).

Though highest peak of malaria cases were observed as a trend after the main rainy seasons from June to September (Alemu *et al*., 2012), our study did not show such a trend. This might be due to low amount of rainfall for an extended period as a result of El Niño effect in the country occurred in 2015/16 (personal observation). This and other meteorological factors which were not considered in our study might have effect in low record of malaria cases in the study area. Monthly total rainfall determine malaria transmission in creating breeding habitats for the immature stages of malaria vectors and in increasing the relative humidity which is very important for longevity of adult mosquitoes (Alemu *et al*., 2011). Malaria episodes might be reduced due to malaria control interventions, unfavorable weather conditions due to climate change and increased awareness of the community on use of LLINs (Alemu *et al*., 2012). Study showed that, large scale distribution of LLINs and ACT resulted in tremendous reduction in malaria cases and deaths particularly in children under-five years of age (Otten *et al*., 2009; Aregawi *et al*., 2014).

In Ghibe, males were affected more than females. This might be because of agricultural purpose, irrigational activities and to protect the crop from hippopotamus males stay outside at night.

However, in Darge females were affected more than males and this might be males go to bed early at night than females and could be protected with LLINs. In our study sites, malaria cases were higher in people aged greater than 14 years. This was in line with the study of Alemu *et al*. (2012) in that age groups between 15-44 years were highly affected with malaria. In contrary to our study, malaria cases were higher in children aged less than five years and between 5 to 14 years of age than the other age groups in study conducted in south-central Ethiopia (Woyessa *et al*., 2013; Gari *et al*., 2016).

The expected longevity of *An. arabiensis* sampled was shorter (7 days on average) in our study than that of *An. arabiensis* collected from other area which was 14 days as indicated by Gari *et al*. (2016). The sporozoite rate for *An. arabiensis* was very low (0.07% for *P. vivax* and *P. falciparum* in Ghibe and zero in Darge) as compared to 1.5% and 0.3% for *P. falciparum* and *P. vivax*, respectively in suburbs of Jimma town (Degefa *et al*., 2015), 1.18% for *P. falciparum* Zway area, central Ethiopia (Kibret *et al*., 2010) and 4.1% in lowland areas around dams in Ethiopia (Kibret *et al*., 2017). These differences might be due to variation in sampling season of *Anopheles* mosquitoes in which mosquito collection was during peak malaria transmission season or due to availability of small-scale irrigation or dam construction in the previous studies. The population dynamics and adult age structure of *Anopheles* mosquitoes are important when determining a given population's ability to transmit malaria (Beck-Johnson *et al*., 2013). In line with the study conducted in south-central Ethiopia (Animut *et al*., 2013), our study revealed that, *Plasmodium* infections were detected in *An. arabiensis* collected at the end of short rainy season (May) and long rainy season (October). However, *An. arabiensis* and *An. pharoensis* were also observed infected with *P. falciparum* sporozoites both in the dry and short rainy seasons in areas

with irrigation scheme of Zway (Kibret *et al*., 2010). *Plasmodium* infected *An. pharoensis* were not identified in our study. This might be due to the low number of mosquitoes tested.

Our study revealed that, annual EIR was very low (1.86 infective bites/person/year for *P. vivax* and 1.22 infective bites/person/year for *P. falciparum*) in Ghibe and was zero in Darge during the study period. This might be associated with an extended low rainfall during the study period (Kent *et al*., 2007). Recent study showed that, EIR by *An. arabiensis* collected from near dam constructed at lowland area was very high with a value of 129.8 infective bites/person/year (Kibret *et al*., 2017). Studies also showed that, variation in EIR could also be observed between the periphery and the center within the same town (Drakeley *et al*., 2003; Degefa *et al*., 2015).

Though malaria prevalence was low in the study area, the annual EIR was not < 1 infective bites/person/year in Ghibe but zero in Darge. This indicated that, a substantial reduction in malaria prevalence was not achieved in Ghibe. This is because, EIR is a more direct measure of malaria transmission intensity (Beier *et al*., 1999). Study in south-central Ethiopia showed that, all the tested *Anopheles* mosquitoes were negative for *P. falciparum* and *P. vivax* CSP (Gari *et al*., 2016; Kenea *et al*., 2016). There is uneven distribution in mosquito biting among humans. In some locations, few people might be bitten largely by mosquitoes and these people might have a higher probability of infection with malaria and more likely remain infected though overall prevalence fall in a population. Most of the mosquitoes become infected when they bite these group of people and likely not if bite other people. So that, the probability of the mosquitoes to be infected by malaria parasite gets decline due to the chance of biting more likely uninfected people (Churcher *et al*., 2015).

In our study, few *An. arabiensis* were knocked down to DDT and pyrethroids tested for within 80 minutes of tested time. Unlike our study, though they showed resistance to knockdown and mortality, *An. arabiensis* had KDT_{50} less than 40 minutes to the pyrethroid insecticides tested in Chano, south-west Ethiopia (Massebo *et al*., 2013). Development of resistance to all insecticides had been observed including the carbamates which were considered as insecticides of choice for IRS as malaria vector control intervention in Ethiopia (Yewhalaw *et al*., 2011). In line with our study, *An. gambiae* s.l. (*An. arabiensis*) developed resistant to DDT, permethrin and deltamethrin in different parts of the country (Yewhalaw *et al*., 2010; Balkew *et al*., 2010). However, the mortality of *An. gambiae* s.l. using deltamethrin recorded by Yewhalaw *et al*. (2010) was 82.2% which is about 3 fold (28%) of mortality recorded in our study. The mortality of *An. arabiensis* to the tested insecticides was lower than that of the mortality observed in *An. arabiensis* from Chano, south-west Ethiopia except for DDT (Massebo *et al*., 2013).

In line with our study, resistance development by *An. arabiensis* was reported to deltamethrin, lambdacyhalothrin, permethrin and alphacypermethrin (Balkew et al., 2010; Gari *et al*., 2016) but was susceptible to bendiocarb (Gari *et al*., 2016) and propoxur (Yewhalaw *et al*., 2011; Gari *et al.*, 2016). Intensive application of insecticide to agricultural pests and for the control of malaria vectors (IRS and LLINs) was highly practiced in the study area which might result in the development of insecticide resistance by malaria vectors and might be cross resistance developed among insecticides with similar mode of action (Yewhalaw *et al*., 2011).

Mortality of *An. arabiensis* after exposure to propoxur (75%) and to bendiocarb (88%) which showed development of resistance was in line with other studies in member of *An. gambiae* s.l. mosquitoes to carbamates conducted in Guinea Conakry (Vezenegho *et al*., 2009), Lagos, south-western Nigeria (Oduola *et al*., 2012), Ghana (Hunt *et al*., 2012), Benin (Aïkpon *et al*., 2013) and

northwest Tanzania (Matowo *et al*., 2015). Development of resistance to carbamates might be due to the use of insecticides in households as IRS or due to cross resistance with organophosphates applied on agricultural fields to control pests (Aïkpon *et al.*, 2013). However, it needs further investigation to evaluate the biochemical mechanism whether cross resistance was developed between carbamates and organophosphates in *An. arabiensis*. The development of resistance to the insecticides tested, which are used as impregnation of bed nets and for IRS may compromise malaria vector control using these interventions. These control interventions become efficient when the vectors are susceptible to the available insecticides used for impregnation of nets and spraying of houses (Massebo *et al*., 2013).

Our study has limitations in lack of records in HMIS data for the Plasmodia to know the predominant species in the study area. Human landing catch which is considered as the gold standard method for the study of malaria transmission intensity was not included in the study due to ethical concerns. Small number of *An. arabiensis* was dissected to determine parity rate and longevity, so that it might result in bias of the result.

In conclusion, this study revealed that malaria cases were high in people aged greater than fourteen years of age. This might be because people within this age group stayed outdoors at night due to different agricultural activities. *Anopheles arabiensis* is the main malaria vector in the study area and showed high rate of resistance development to all the four classes of insecticides tested for. This may compromise malaria control and subsequent elimination using IRS and ITNs. These imply the need of additional vector control interventions like LSM and screening houses in addition to LLINs and IRS. It also needs further investigation whether carbamate resistance development was due to cross resistance with organophosphates or not in major malaria vector.

Chapter 6. Knowledge, attitude and practice of the community towards malaria prevention and control options in Ghibe River basin, southwestern Ethiopia

6.1 Introduction

Malaria is one of the major public health problems in Ethiopia. In the country, 68% of the population lives in malarious areas, of these 27% were in high malaria transmission settings. It was estimated that, 2.8 million cases and 4,900 deaths were attributed to malaria in 2015 (WHO, 2016).

ITNs/LLINs were distributed free of charge for all age groups and treatment with ACT is also free of charge for all age groups in public health sectors starting from the year 2004 (WHO, 2016). After scale up of vector control interventions and prompt treatments, malaria morbidity and mortality is declining in Ethiopia as most parts of the globe (WHO, 2016). However, factors like population movement to malarious regions, deforestation, dam construction, expansion of irrigation, climate change, and drug and insecticide resistance more likely sustain malaria transmission (Sachs and Malaney, 2002; Kibret *et al*., 2010; 2017). Due to the efforts made for malaria elimination, it might declines further through time and its transmission become unstable and due to lack of functional immunity in the population it makes the risk of infection more and more sever (Snow, 2015).

Malaria and socio-economic status of the people living in malaria endemic areas affect each other. People with low income could be infected more with malaria or malaria could result in poverty by reducing the economic growth of the society due to different factors associated with

disease burden (de Castro and Fisher, 2012). In reducing poverty, vector abundance and species composition could be altered through urbanization and increase access to diagnosis and prompt treatment of infected individuals resulting from improved health system (Snow, 2015).

Housing condition and its structure; house construction materials (Ghebreyesus *et al*., 2000; Yé *et al*., 2006), ceiling modifications (Atieli *et al*., 2009), and open eaves, presence of domestic animals in a house and number of people sleeping in a room (Ghebreyesus *et al*., 2000; Pålsson *et al*., 2004) affect malaria infections and vector abundance in the houses. Mosquitoes prefer to rest in thatched roof and mud houses due to the suitability of houses for their entry and create conducive environment for mosquitoes to rest indoors (Dery *et al*., 2015).

Knowledge and awareness of malaria transmission route and proper utilization of control interventions are very important in malaria elimination programs (Ferreira *et al*., 2012; Yadav *et al*., 2014). Distance to the health facility from the household, individual education level, and household income influence malaria treatment seeking behaviour (Lowassa *et al*., 2012; Yadav *et al*., 2014). In addition, the presence of electricity (Yamamoto *et al*., 2010) and the time people are engaged in daily activities or stayed outside at night might determine whether they could be exposed to mosquito bites or not (Wotodjo *et al*., 2015).

Population distribution and settlements are important for understanding a variety of social and economic determinants used for planning interventions that encompass the community living in malaria endemic areas (Linard *et al*., 2012). Understanding the demographic factors and the communities' awareness about malaria transmission and the way they protect themselves from malaria vectors bite are very important to know and evaluate effectiveness of malaria control interventions (Mora-Ruiz *et al*., 2014). Hence, the aim of this study was to investigate the socio-

economic factors, local communities' understanding of malaria transmission, recognition of signs and symptoms, perceptions of cause, treatment-seeking patterns, preventive measures and practices of the communities in two localities in Ghibe River basin, southwestern Ethiopia.

6.2 Material and Methods

6.2.1 Study area

The study was conducted in Ghibe and Darge study sites, southwestern Ethiopia. Brief description of the study area is presented in Chapter 3, section 3.2.1 and Chapter 4, section 4.2.1

6.2.2 Study design and data collection

The study was designed as a descriptive cross-sectional survey using a semi-structured questionnaire and by visual inspection. The first part of a questionnaire comprised demographic information of the household heads while in the second part the number of occupants in the house, housing condition and assets, adult residents' attitudes and understanding of malaria transmission, recognition of signs and symptoms, and preventive measures and practices were recorded. Information on property ownership (radio, television, mobile phone, domestic animals, and electricity) was also recorded.

The questionnaire was prepared in English (Appendix 6.1) and translated to Amharic. In Darge, a person who knows Kembata language was additionally used for those who did not understand Amharic. The questionnaire was administered to 175 (81 in Ghibe and 94 in Darge) randomly selected household heads in January 2015 from the total 1,151 household heads. Only one person per household; the head of household or a responsible adult aged greater than 18 years in the absence of household head was interviewed. The aim of the study was explained to the household heads, and only those who gave verbal consents were participated in the study. Data

were analyzed with IBM SPSS statistical for Windows (IBM corp., Armonk, NY), version 20.0 and Microsoft Office Excel 2007.

6.2.3 Ethical considerations

Approval to conduct the study was obtained from the ethical committees of College of Natural Sciences Institutional Ethics Review Board, Addis Ababa University (Reference No. CNSDO/213/07/15). Consent was also obtained from Abeshge district (woreda) administration and the manager of each study site after the purpose of the investigation was explained to them. Verbal consent was obtained from those individuals who were involved in the study.

6.3 Results

6.3.1 Socio-demographic characteristics of respondents

In total, 175 household heads were interviewed including 81 (46.3%) from Ghibe and 94 (53.7%) from Darge (Table 6.1). One hundred thirty four (76.6%) respondents were male. Forty five (25.7%) of the respondents were aged between 20 and 30 years and the remaining were more than 30 years of age. The large proportion (55.4%) of the respondents had 4 to 6 family size and 66 (37.7%) of them did not get access to education. The largest proportions (72.0%) of respondents were farmers.

Table 6.1 Demographic characteristic of respondents in Ghibe and Darge study sites in Ghibe River basin, southwestern Ethiopia (January 2015)

Characteristics	n	%
Study site		
Ghibe	81	46.3
Darge	94	53.7
Sex of respondents		
Male	134	76.6
Female	41	23.4
Age of respondents in years		
20-30	45	25.7
31-40	55	31.4
41-50	40	22.9
>50	35	20.0
Number of family member in a house		
1-3	55	31.4
4-6	97	55.4
7-9	23	13.2
Education level of household head		
No education	66	37.7
Primary level	82	46.9
Secondary level	23	13.1
Above secondary	4	2.3
Household head occupation		
Farmer	126	72.0
Employee	19	10.9
Retired	6	3.4
Daily laborer	10	5.7
Merchant	14	8.0

House characteristics of the respondents are depicted in Table 6.2. One hundred and eight (61.7%) of the participants houses were made of thatched roof, 133 (76%) without ceiling, and all with stick and mud walls. The majority of respondents' houses (95.4%) had closed eaves. There were different floor types in the respondents' houses; 76 (43.4%) were soil, 69 (39.4%) were plastic laid on the ground, 27 (15.5%) plastered with dung. The large proportion of respondents houses were with single room (n = 117, 66.9%), single door (n = 108, 61.7%) and without door screen (n = 174, 99.4%). Sixty six (37.7%) of respondents houses were without windows and 84 (77.1%) of those respondents houses with window did not have window screen.

Table 6.2 Conditions of respondents houses in Ghibe and Darge study sites in Ghibe River basin, southwestern Ethiopia (January 2015)

House structure	n	%
Roofing type		
Corrugated	67	38.3
Thatched	108	61.7
Presence of ceiling		
Yes	42	24.0
No	133	76.0
Wall material		
Stick and mud	175	100
Eave condition		
Closed	167	95.4
Open	8	4.6
Floor type		
Cement	3	1.7
Dung	27	15.5
Plastic	69	39.4
Soil	76	43.4
Number of rooms		
One	117	66.9
Two	45	25.7
Three or above	13	7.4
Number of doors		
One	108	61.7
Two	65	37.2
Three or above	2	1.1
Door screen		
Yes	1	0.6
No	174	99.4
Number windows		
No window	66	37.7
One	73	41.7
Two	32	18.3
Three or above	4	2.3
Window screen		
Yes	25	22.9
No	84	77.1

6.3.2 Awareness of the community towards malaria transmission and its prevention

Awarness of the respondents on malaria transmission and its prevention is shown in Table 6.3. One hundred nine (62.3%) respondents knew that malaria is transmitted by mosquitoes bite and 65 (37.1%) did not know how to be infected with malaria. One hundred and eight (61.7%) of the respondents replied that, malaria could be protected with ITN/LLINs, 43 (24.6%) with spraying insecticides in houses, 39 (22.3%) with draining stagnant water and 149 (85.1%) said visiting health centers to get appropriate treatment but 51 (29.1%) of respondents did not know how to be protected from malaria infections.

Table 6.3 Knowledge of respondents on malaria transmission and it prevention in Ghibe and Darge study sites in Ghibe River basin, southwestern Ethiopia (January 2015)

Knowledge of malaria transmission	**n**	**%**
How to be infected with malaria?		
Do not know	65	37.1
Mosquitoes bite	109	62.3
Stored water	1	0.6
What are the ways of malaria prevention?		
Do not know	51	29.1
Cleaning around homestead	8	4.6
ITN/ LLINs	108	61.7
Spray houses with insecticides	43	24.6
Draining stagnant water	39	22.3
Wearing cloth at night	2	1.1
Drinking clean water	1	0.6
Smoking houses	1	0.6
Proper feeding	1	0.6
How to cure from malaria?		
Do not know	18	10.3
Medication	149	85.1
Resting	3	1.7
Using onion	5	2.9

Of the total respondents, 146 (83.4%), 140 (80%) and 109 (62.3%) knew fever, head ache and lack of appetite, respectively are signs and symptoms of malaria infections among others. Twenty three (13.1%) did not know the signs and symptoms of malaria disease (Figure 6.1).

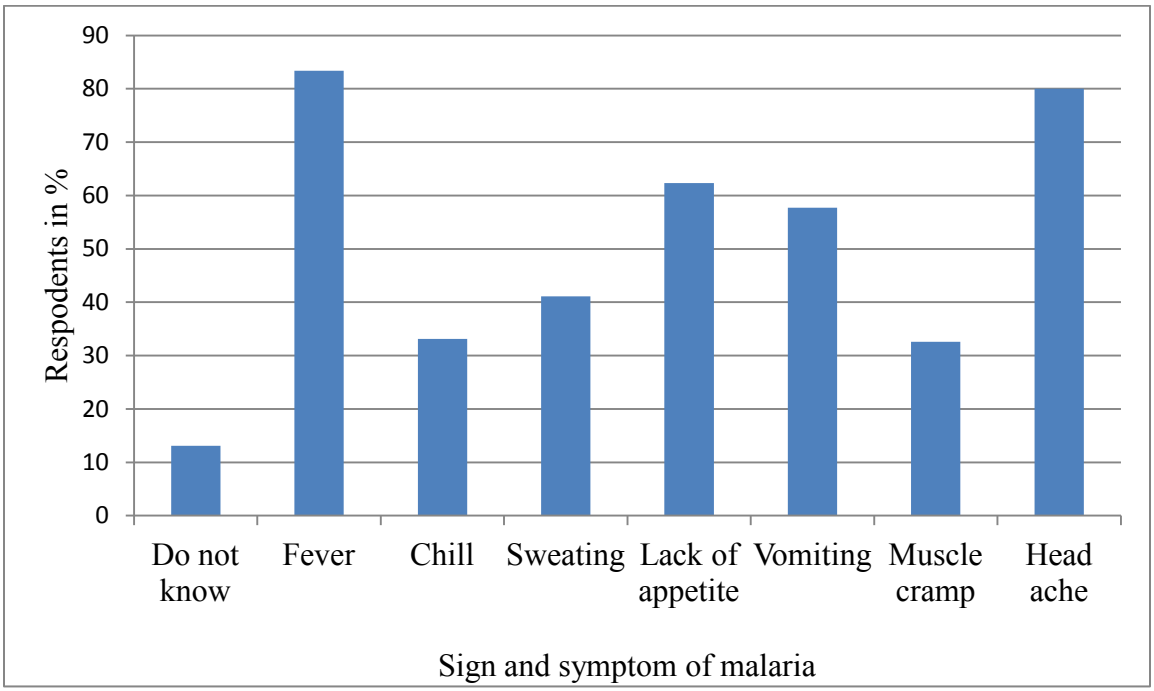


Figure 6.1 Response on knowledge of sign and symptom of malaria in Ghibe and Darge study sites in Ghibe River basin, southwestern Ethiopia (January 2015)

One hundred sixty five (94.3%) respondents possessed at least one LLIN in their house (Table 6.4). Among those who owned nets, 74 (42.3%) were used nets sometimes. Thirty one (17.7%) of the respondents stayed outdoors at night for different activities. Ninety six (54.9%) of respondents' houses were sprayed with residual insecticide within the last six months but 20 (11.4%) of respondents' houses were never sprayed at all.

Table 6.4 Responses on LLINs and IRS related information in Ghibe and Darge in Ghibe River basin, southwestern Ethiopia (January 2015)

LLINs ownership and usage	**n**	**%**
Number of LLINs in the house		
No net	10	5.7
1	63	36.0
2	87	49.7
≥3	15	8.6
Proper utilization of LLINs		
Not used	10	5.7
Always	91	52.0
Sometimes	74	42.3
Reason for not utilizing nets properly		
No net	10	11.9
Low mosquito abundance	25	29.8
Not comfortable	49	58.3
Do you stay outdoors at night?		
No	144	82.3
Guard	17	9.7
Cattle herding	8	4.6
Ventilation	6	3.4
IRS		
Never	20	11.4
Last 3 months	0	0
Last 6 months	96	54.9
Last 12 months	42	24.0
Before a year	17	9.7

6.3.3 Respondents property ownership

Among the respondents, 4 (2.3%), 51 (29.1%), 119 (68.0%), 51 (29.1%) and 105 (60.0%) owned sheep, goats, cattle, donkeys and chicken, respectively (Table 6.5). Domestic animals of most of the respondents were tethered outdoor or in separate houses during the night time. One hundred thirty seven (78.3%) and 133 (76.0%) respondents owned mobile phone and houses with electricity, respectively.

Table 6.5 Response on domestic animals and household assets ownership in Ghibe and Darge in Ghibe River basin, southwestern Ethiopia (January 2015)

Description		**n**	**%**
Domestic animals tethered indoor/outdoor at night			
Sheep	Indoor	1	25.0
	Outdoor	3	75.0
Goat	Indoor	3	5.9
	Outdoor	48	94.1
Cattle	Indoor	15	12.6
	Outdoor	104	87.4
Donkey	Indoor	5	9.8
	Outdoor	46	90.2
Chicken	Indoor	7	6.7
	Outdoor	98	93.3
Household assets			
Television		34	19.4
Radio		85	48.6
Mobile phone		137	78.3
Electricity		133	76.0
Fridge		3	1.7
Donkey driven cart		11	6.3

6.4 Discussion and conclusions

Results obtained from the respondents showed that, most of the respondents were males, aged greater than twenty years, between four and six family members per household, 62.3% of them had primary or more education level but 37.7% without access to education and most were farmers. Individuals with higher education might have more health conscious and more likely to adhere to public health education than illiterate people (Legesse *et a*l., 2007; Lowassa *et al*., 2012). However, if the societies have overall knowledge on malaria transmission, level of formal education might not be correlated with malaria risk (Forero *et al*., 2014).

Our study revealed that, 61.7% and 76.0% of the participants houses were made of thatched roof and without ceiling, respectively and all with stick and mud walls. However, the majority of respondents' houses were with closed eaves. Though they could not build houses with corrugated roofs, they have to be encouraged to construct ceiling made of low cost and easily accessible materials. In mud walls and thatched roofed houses, high densities of malaria vectors were recorded (Lwetoijera *et al.,* 2013). However, the presence of simple ceilings in the houses constructed with traditional design substantially reduced exposure to the bites of malaria vectors (Lindsay *et al*., 2003). The presence of open eaves enables host odours to be released from the room and attracting mosquitoes into the roof space and can get easy access to enter houses. Though closing eaves is easy and inexpensive, people might not prefer due to its influence in reducing air flow in hot and humid environments where malaria transmission is mostly endemic. This can be compensated by netting the open eaves and using insect-screen ceilings to reduce the biting rate of malaria vectors (Lindsay *et al*., 2003). In poorly constructed houses, mosquito densities are higher and this increases the malaria risk to residents (Konradsen *et al*., 2003).

Construction of houses with single room, single door and one or without window was common which might reduce cost incurred to construct large houses with many rooms, doors and windows. The majority of houses (99.4%) were also without door screens and window screens (77.1%). High densities of malaria vectors were sampled in houses with low number of windows, doors and rooms and smaller houses are more likely to concentrate human odour (Lwetoijera *et al*., 2013). In a room many people sleep at night might have higher risk of attracting those mosquitoes prefer to enter houses (Konradsen *et al*., 2003).

The respondents result showed that, 62.3% of them knew malaria is transmitted by mosquito bite. This result was far lower than those studies conducted in different areas in Ethiopia; 95.6%, (Abate *et al*., 2013), 78.8% (Yimer *et al*., 2015) and 95.5% (Gutasa *et al*., 2015) but higher than that of the study conducted in rural northwest Tanzania, 56% (Mazigo *et al*., 2010) and on pregnant women in southern Ethiopia, 15.6% (Fuge *et al*., 2015). The presence of large number of people who did not know how malaria is transmitted from infected people to others might have impact on malaria control. Lack of knowledge the way malaria is transmitted, the time malaria vectors are more active, the areas where vectors concentrate and where they reproduce is necessary to implement protective and preventive measures (Ferreira *et al*., 2012).

Most of the respondents knew at least one, but 13.1% did not know the common signs and symptoms of malaria. Likewise, at least 80% of the respondents knew fever and headache are the signs and symptoms of malaria as of other studies in rural northwest Tanzania (Mazigo *et al*., 2010) and northeastern Ethiopia (Abate *et al*., 2013).

The majority of respondents involved in the study knew at least one of the measures that used to control malaria transmission. Among these respondents, 61.7%, 24.6% and 22.3% described

using ITNs/LLINs, house spraying with insecticides and draining stagnant water that used as a potential breeding for mosquitoes, respectively useful for the control and prevention of malaria. However, there were very few people who considered malaria could be controlled with wearing clothes at night, drinking clean water, smoking houses and proper feeding. The knowledge of the respondent on malaria control methods was low as compared to other studies in the country (Abate *et al*., 2013; Yimer *et al*., 2015). This reduced response to the main control interventions in our study might be due to the surveying time which coincides with dry season where people might not give attention in malaria transmission.

Among the total respondents, 5.7% of them did not possess LLINs in their house. This was because some of them came recently from other areas after the nets were distributed while others constructed their own home recently after marriage. From those who had nets, 42.3% did not utilize all the night due to low mosquitoes abundance during low rainy seasons or lack of comfort by nets due to reduced ventilation, torn, dirt and harbor bedbugs. In line with our study, people did not use LLINs regularly during the dry season in other study elsewhere (Animut *et al*., 2014) and considered nets were not comfortable due to increased heat, dirt, being old, presence of holes, bad smell and lack of knowledge how to install it (Abate *et al*., 2013; Fuge *et al*., 2015). Study on pregnant women in eastern India showed, in low malaria transmission settings people knew little about malaria as compared to those live in high malaria transmission settings (Sabin *et al*., 2010). Though IRS was implemented yearly before the onset of the main rain, there were respondents (11.4%) whose houses were not sprayed. These were due to newly construction of houses and lack of information when the spray was undertaken. Missing such houses might serve as a potential reservoir for malaria vectors. Though control interventions

scaled up, if the community did not utilize them properly malaria control might not be achieved (Animut *et al*., 2014).

Most household heads had mobile phone, radio and electricity at their home. Most respondents tethered their domestic animals outdoors or in separately constructed houses but there were also peoples who share their home with domestic animals at night and was common in Darge. Study in northern Ethiopia showed that, malaria incidence was significantly higher in children of families who kept animals in their houses compared to those who had a separate shelter for animals (Ghebreyesus *et al*., 2000). There were respondents (7.7%) who stayed outdoors at night for different activities. This people might not be protected with LLINs and IRS which are effective to control vectors entering houses. Staying outdoors at night for few hours, increase the probability of a person to be infected with malaria (Wotodjo *et al*., 2015). The limitation of this study was lack of design to see the association of socio-demographic characteristics and awareness of the communities and also with malaria prevalence survey and the entomological study.

In conclusion, among the participants of this study, most of them knew the most recognized signs and symptoms of malaria illness, use of LLINs and prompt treatment with drugs administered by health professionals in health facilities as the major protective methods of malaria. However, there was paucity of knowledge the way malaria is transmitted from infected to healthy individual and other malaria control interventions including IRS and larval source management. Extra effort will be needed to educate the community to engage in proper utilization of malaria control interventions in houses and those which require community participation especially in LSM.

Chapter 7. General conclusions and recommendations

7.1 Conclusions

This study implicated investigation of the *Anopheles* mosquitoes species composition, characterization of *Anopheles* larval habitat, blood meal sources, sporozoite infection rates, malaria prevalence and insecticide susceptibility of the main malaria vector *An. arabiensis* in Ghibe and Darge study sites in Ghibe River basin, southwestern Ethiopia. Based on the results obtained it was concluded as:

Anopheles gambiae s.l. (= *An. arabiensis*) is the predominant species in the study area. This species of mosquito larvae breed in different types of larval habitats in large proportion in all types of habitats both during the dry and rainy seasons except in swamps.

Anopheles mosquito larvae were observed breeding in different types of larval habitats. Among the identified habitats, larval density was proportionately higher in pools at river edges, borrow pits and pools in drying river beds as compared to other types of larval habitats. Most of the temporary larval habitats were formed immediately after rains while pools at river edges and pools in drying river beds were formed during reduced amount of rainfall when the rivers retreat. Hence, the rivers found along this study area serve as refugia during the dry seasons and enable the malaria vectors to sustain throughout the year. *Anopheles arabiensis* larval densities were significantly higher in habitats without surface debris, sunlit, near human habitations, without vegetation near larval habitats and lack of emergent plants within the habitats.

Anopheles arabiensis was the most abundant species in the adult stages in collections made using different methods. Its density was high in houses near to the river (<500m from the river) than houses far from the river (>500m) and in CDC light traps set outdoors than indoors.

Unlike *Anopheles* mosquitoes collected from resting places, most of the mosquitoes collected with CDC light traps were blood unfed. Blood meal analysis using ELISA indicated that, like that of the primary malaria vectors, *An. arabiensis*, other species of *Anopheles* mosquitoes (*An. coustani*, *An. demeilloni* and *An. nili*) which are less important as malaria vectors were also identified fed on human blood. In the long run, these may serve as potential transmitters of the disease in the future if attention will not be given.

Anopheles arabiensis showed opportunistic behavior in their feeding. Those collected from human houses were mostly fed on human blood while those collected from cattle sheds (outdoors) mostly fed on bovine blood. Thus, the ability to adapt feeding on non-human hosts and resting outdoors might compromise malaria control and subsequent elimination with the existing vector control interventions which mostly rely on LLINs and IRS.

Retrospective data showed that, malaria prevalence was high in Ghibe than in Darge study site. In Ghibe, malaria infections were recorded throughout the study period except in January and February 2016. Adult *Anopheles* mosquitoes densities were also higher in Ghibe as compared to Darge. Sporozoite infected *An. arabiensis* mosquitoes were detected from collections made in Ghibe study site. These implied that, malaria infections were higher in Ghibe than in Darge study sites.

Anopheles arabiensis showed high rate of resistance development to all the four classes of insecticides tested for. High rate of insecticide resistance was detected in pyrethroids insecticides

which are the only class of insecticide classes used for impregnation of nets. Insecticide resistance was also pronounced in carbamate insecticides which are used for IRS in malaria endemic areas of the country.

Among the participants of awareness study on malaria transmission and its prevention, most of them knew the most recognized signs and symptoms of malaria illness, use of LLINs and prompt treatment with drugs administered by health professionals in health facilities as the major protective methods of malaria. However, there were people who did not know the way malaria is transmitted.

7.2 Recommendations

While planning for malaria control program that incorporate larval control interventions, both dry and rainy seasons should be considered. However, larval habitats might be reduced during the dry seasons, so that application of control interventions during this season might be more effective and should targer habitats formed at the river fringes after the rivers receded following reduction of rainfalls.

For the reduction of malaria risks in the community, vector control strategies should target houses closer to the river than those houses far from the rivers.

It might be difficult to control malaria with the current control interventions due to the presence of the principal malaria vector indoors and outdoors which may lead to increase the community to be exposed to their bites. Thus, it needs looking for additional vector control interventions in addition to LLINs and IRS. This might include LSM, oviposition traps, zooprophylaxis and ouder baited traps.

Some species were identified during adult surveys but not identified when collected at larval stages indicated that, in malaria vectors surveillance considering both the adult and larval stages is very important not to miss the species available in specific area.

Insecticide resistance development by the principal malaria vector to all the four insecticide classes will confront malaria control and subsequent elimination using IRS and LLINs. It needs further investigation on carbamate resistance which has been started to be observed in recent years in the country. The development of insecticide resistance to different insecticides needs to look for alternative vector control methods.

There was knowledge gap the way malaria is transmitted from infected to healthy individual and other malaria control interventions including IRS and LSM. Awareness creation to the community should be practiced in malaria endemic areas in proper utilization of malaria control interventions in houses and those which require community participation especially in LSM.

The present study has potential limitations. Larval samplings were conducted on monthly basis so that mosquito larvae might be missed during the study period to show the whole picture of the species abundance and larval habitats productivity. Water temperature of the larval habitat was recorded only during larval sampling time and was not measured at different times of the day. On the other hand, environmental variables including detailed analysis of water chemistry, mosquito predators and competitors to know the most predictor of larval habitat characteristics were not included in the study. The study could not incorporate window exit trap to study the indoor feeding and resting behavior of *Anopheles* mosquitoes as they leave the houses after feeding and/or resting. The study has limitations in lack of records in HMIS data for the Plasmodia to know the predominant species in the study area. Human landing catch which is

considered as the gold standard method for the study of malaria transmission intensity was not included in the study due to ethical concerns. Small number of *An. arabiensis* was dissected to determine parity rate and longevity. In knowledge, attitude and practice study, sample size was not calculated following design of data collection procedure, lack of design to see the association of socio-demographic characteristics and awareness of the communities with malaria prevalence survey and the entomological study.

References

Abate, A., Degarege, A., Erko, B. (2013). Community knowledge, attitude and practice about malaria in a low endemic setting of Shewa Robit Town, northeastern Ethiopia. *BMC Public Health*, **13**:312.

Abeku, T. A., van Oortmarssen, G. J., Borsboom, G., de Vlas, S. J., Habbema, J. D. F. (2003). Spatial and temporal variations of malaria epidemic risk in Ethiopia: factors involved and implications. *Acta Trop.*, **87**: 331-340.

Abeku, T. A. (2007). Response to malaria epidemics in Africa. *Emerg. Infect. Dis*., **13**(5): 681-686.

Afrane, Y. A., Lawson, B. W., Brenya, R., Kruppa, T., Yan, G. (2012). The ecology of mosquitoes in an irrigated vegetable farm in Kumasi, Ghana: abundance, productivity and survivorship. *Parasit. Vectors*, **5**:233.

Afrane, Y. A., Lawson, B. W., Githeko, A. K.. Yan, G. (2005). Effects of microclimatic changes caused by land use and land cover on duration of gonotrophic cycles of *Anopheles gambiae* (Diptera: Culicidae) in western Kenya highlands. *J. Med. Entomol*. **42**(6): 974-980.

Ahmed, M. A., Cox-Singh, J. (2015). *Plasmodium knowlesi* - an emerging pathogen. *ISBT Sci. Series*, **10**(1): 134-140.

Aïkpon, R., Agossa, F., Ossè, R., Oussou, O., Aïzoun, N., Oké-Agbo, F., Akogbéto, M. (2013). Bendiocarb resistance in *Anopheles gambiae* s.l. populations from Atacora department in Benin, West Africa: a threat for malaria vector control. *Parasit. Vectors*, **6**:192.

Alemu, A., Abebe, G., Tsegaye, W., Golassa, L. (2011). Climatic variables and malaria transmission dynamics in Jimma town, South West Ethiopia. *Parasit. Vectors*, **4**:30.

Alemu, A., Muluye, D., Mihret, M., Adugna, M., Gebeyaw, M. (2012). Ten year trend analysis of malaria prevalence in Kola Diba, North Gondar, Northwest Ethiopia. *Parasit. Vectors*, **5**:173.

Animut, A., Balkew, M., Gebre-Michael, T., Lindtjørn, B. (2013). Blood meal sources and entomological inoculation rates of anophelines along a highland altitudinal transect in south-central Ethiopia. *Malar. J*., **12**:76.

Animut, A., Gebre-Michael, T., Balkew, M., Lindtjørn, B. (2012). Abundance and dynamics of anopheline larvae in a highland malarious area of south-central Ethiopia. *Parasit. Vectors,* **5**:117.

Animut, A., Negash, Y., Kebede, N. (2014). Distribution and utilization of vector control strategies in a malarious village of Jabi Tehnan District, north-western Ethiopia. *Malar. J*., **13**:356.

Aregawi, M., Lynch, M., Bekele, W., Kebede, H., Jima, D., Taffese, H. S., Yenehun, M. A., Lilay, A., Williams, R., Thomson, M., Nafo-Traore, F., Admasu, K., Gebreyesus, T. A., Marc Coosemans, M. (2014). Time series analysis of trends in malaria cases and deaths at hospitals and the effect of antimalarial interventions, 2001–2011, Ethiopia. *PLoS ONE*, **9**(11): e106359. doi:10.1371/journal.pone.0106359.

Armstrong, J. C. (1969). *Plasmodium ovale* endemic in Ethiopia. *Trans. R. Soc. Trop. Med. Hyg*., **63**(2): 287-288.

Atieli, H., Menya, D., Githeko, A.. Scott, T. (2009). House design modifications reduce indoor resting malaria vector densities in rice irrigation scheme area in western Kenya. *Malar. J.,* **8**:108.

Atieli, H. E., Zhou, G., Afrane, Y., Lee, M-C., Mwanzo, I., Githeko, A. K., Yan, G. (2011). Insecticide-treated net (ITN) ownership, usage, and malaria transmission in the highlands of western Kenya. *Parasit. Vectors*, **4**:113.

Autino, B., Noris, A., Russo, R., Castelli, F. (2012). Epidemiology of malaria in endemic areas. *Mediterr. J. Hematol. Infect. Dis.*, **4**(1): e2012060, DOI 10.4084/MJHID.2012.060.

Baird, J. K. (2007). Neglect of *Plasmodium vivax* malaria. *Trends Parasitol.*, **23**(11): 533-539.

Bayoh, M. N., Mathias,D. K., Odiere, M. R., Mutuku, F. M., Kamau, L., Gimnig, J. E., Vulule, J. M., Hawley, W. A., Hamel, M. J., Walker, E. D. (2010). *Anopheles gambiae*: historical population decline associated with regional distribution of insecticide-treated bed nets in western Nyanza Province, Kenya. *Malar. J*., **9**:62.

Balkew, M., Ibrahim, M., Koekemoer, L. L., Brooke, B. D., Engers, H., Aseffa, A., Gebre-Michael, T.. Elhassen, I. (2010). Insecticide resistance in *Anopheles arabiensis* (Diptera: Culicidae) from villages in central, northern and south west Ethiopia and detection of *kdr* mutation. *Parasit. Vectors*, **3**:40.

Bashar, K., Tuno, N., Ahmed, T. U., Howlader, A. J. (2012). Blood-feeding patterns of *Anopheles* mosquitoes in a malaria-endemic area of Bangladesh. *Parasit. Vectors*, **5**:39.

Bayoh, M. N., Lindsay, S. W. (2003). Effect of temperature on the development of the aquatic stages of *Anopheles gambiae* sensu stricto (Diptera: Culicidae). *Bull. Entomol. Res.*, **93**: 375-381.

Bayoh, M. N., Walker, E. D., Kosgei, J., Ombok, M., Olang, G. B., Githeko, A. K., Killeen, G. F., Otieno, P., Desai, M., Lobo, N. F., Vulule, J. M., Hamel, M. J., Kariuki, S., Gimnig, J. E. (2014). Persistently high estimates of late night, indoor exposure to malaria vectors despite high coverage of insecticide treated nets. *Parasit. Vectors*, **7**:380.

Beck-Johnson, L. M., Nelson, W. A., Paaijmans, K. P., Read, A. F., Thomas, M. B., Bjørnstad, O. N. (2013). The effect of temperature on *Anopheles* mosquito population dynamics and the potential for malaria transmission. *PLoS ONE* **8**(11): e79276. doi:10.1371/journal.pone.0079276.

Beier, J. C., Perkins, P. V., Wirtz, R. A., Koros, J., Diggs, D., Gargan, T. P., Koech, D. K. (1988). Blood meal identification by direct enzyme-linked immunosorbent assay (ELISA) tested on *Anopheles* (Diptera: Culicidae) in Kenya. *J. Med. Entomol*., **25**(1): 9-16.

Beier, J. (2002). Vector incrimination and entomological inoculation rates. *Methods Mol. Med.*, **72**: 3-10.

Beier, J. C., Killeen, G. F., Githure, J. I. (1999). Short report: entomologic inoculation rates and *Plasmodium falciparum* malaria prevalence in Africa. *Am. J. Trop. Med. Hyg.*, **61**(1): 109-113.

Beier, J. C., Perkins, P. V., Wirtz, R. A., Whitmire, R. E., Mugambi, M., Hockmeyer, W. T. (1987). Field evaluation of an enzyme-linked immunosorbent assay (ELISA) for *Plasmodium falciparum* sporozoite detection in Anopheline mosquitoes from Kenya. *Am. J. Trop. Med. Hyg*, **36**(3): 459-468.

Bloland, P. B. (2001). *Drug Resistance in Malaria*. World Health Organization, WHO/CDS/CSR/DRS/2001.4.

Bøgh, C., Clarke, S. E., Pinder, M., Sanyang, F., Lindsay, S. W. (2001). Effect of passive zooprophylaxis on malaria transmission in the Gambia. *J. Med. Entomol*., **38**(6): 822-828.

Breman, J. G., Brandling-Bennett, A. D. (2011). The challenge of malaria eradication in the twenty-first century: Research linked to operations is the key. *Vaccine*, **29S**: D97- D103.

Brooker, S., Clarke, S., Njagi, J. K., Polack, S., Mugo, B., Estambale, B., Muchiri, E., Magnussen, P., Cox, J. (2004). Spatial clustering of malaria and associated risk factors during an epidemic in a highland area of western Kenya. *Trop. Med. Int. Health*, **9**(7): 757-766.

Bugoro, H., Cooper, R. D., Butafa, C., Iro'ofa, C., Mackenzie, D. O., Chen, C-C., Russell, T. L. (2011). Bionomics of the malaria vector *Anopheles farauti* in Temotu Province, Solomon Islands: issues for malaria elimination. *Malar. J*., **10**:133.

Carneiro, I., Roca-Feltrer, A., Griffin, J. T., Smith, L., Tanner, M., Schellenberg, J. A., Greenwood, B., Schellenberg, D. (2010). Age-patterns of malaria vary with severity, transmission intensity and seasonality in sub-Saharan Africa: A systematic review and pooled analysis. *PLoS ONE,* **5**(2): e8988. doi:10.1371/journal.pone.0008988.

Churcher, T. S. , Trape, J-F., Cohuet, A. (2015). Human to mosquito transmission efficiency increases as malaria is controlled. *Nat. Commun*., **6**:6054 doi: 10.1038/ncomms7054.

Coetzee, M. (2004). Distribution of the African malaria vectors of the *Anopheles gambiae* complex. *Am. J. Trop. Med. Hyg.,* **70**(2): 103-104.

Coetzee, M., Craig, M., le Sueur, D. (2000). Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitol. Today*, **16**(2): 74-77.

Coetzee, M., Fontenille, D. (2004). Advances in the study of *Anopheles funestus*, a major vector of malaria in Africa. *Insect Biochem. Mol. Biol*., **34**: 599-605.

Coetzee, M., Hunt, R. H., Wilkerson, R., Torre, A. D., Coulibaly, M. B., Besansky, N. J. (2013). *Anopheles coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex. *Zootaxa,* **3619**(3): 246-274.

Collins, W. E., Jeffery, G. M. (2005). *Plasmodium ovale*: parasite and disease. *Clin. Microbiol. Rev*., **18**(3): 570-581.

Collins, W. E., Jeffery, G. M. (2007). *Plasmodium malariae*: parasite and disease. *Clin. Microbiol. Rev*., **20**(4): 579-592.

Coluzzi, M. (1984). Heterogeneities of the malaria vectorial system in tropical Africa and their significance in malaria epidemiology and control. *Bull. World Health Organ*., **62**: 107-13.

Coluzzi, M., Sabatini, A., Petrarca, V., Di Deco, M. A. (1979). Chromosomal differentiation and adaptation to human environments in the Anopheles gambiae complex. *Trans. R. Soc. Trop. Med. Hyg*., **73**(5): 483-497.

Corbel, V., N'Guessan, R., Brengues, C., Chandre, F., Djogbenou, L., Martin, T., Akogbéto, M., Hougard, J. M., Rowland, M. (2007). Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa. *Acta Trop*., **101**: 207-216.

Costantini, C., Badolo, A., Ilboudo-Sanogo, E. (2004). Field evaluation of the efficacy and persistence of insect repellents DEET, IR3535, and KBR 3023 against *Anopheles gambiae* complex and other Afrotropical vector mosquitoes. *Trans. R. Soc. Trop. Med. Hyg*., **98**: 644-652.

Culleton, R., Ndounga, M., Zeyrek, F. Y., Coban, C., Casimiro, P. N., Takeo, S., Tsuboi, T., Yadava, A., Carter, R., Tanabe, K. (2009). Evidence for the transmission of

Plasmodium vivax in the Republic of the Congo, West Central Africa. *J. Infect. Dis*., **200**:1465-1469.

Dabiré, K. R., Diabaté, A., Djogbenou, L., Ouari, A., N'Guessan, R., Ouédraogo, J-B., Hougard, J-M., Chandre, F., Baldet, T. (2008). Dynamics of multiple insecticide resistance in the malaria vector *Anopheles gambiae* in a rice growing area in South-Western Burkina Faso. *Malar. J*., **7**:188.

Davidson G. (1954). Estimation of the survival rate of anopheline mosquitoes in nature. *Nature*. **174**: 792-793.

de Castro, M. C., Fisher, M. G. (2012). Is malaria illness among young children a cause or a consequence of low socioeconomic status? Evidence from the united Republic of Tanzania. *Malar. J*., **11**:161.

Degefa, T., Zeynudin, A., Godesso, A., Haile Michael, Y., Eba, K., Zemene, E., Emana, D., Birlie, B., Tushune, K., Yewhalaw, D. (2015). Malaria incidence and assessment of entomological indices among resettled communities in Ethiopia: a longitudinal study. *Malar. J*., **14**:24.

Demissew, A., Balkew, M. and Girma, M. (2016). Larvicidal activities of chinaberry, neem and Bacillus thuringiensis israelensis (Bti) to an insecticide resistant population of Anopheles arabiensis from Tolay, Southwest Ethiopia. *Asian Pac. J. Trop. Biomed*., **6**(7): 554-561.

Deressa, W., Ali, A., Berhane, Y. (2006). Review of the interplay between population dynamics and malaria transmission in Ethiopia. *Ethiop. J. Health Dev.,* **20**(3):137-144.

Dery, D. B., Asante, K. P., Zandoh, C., Febir, L. G., Brown, C., Adjei, G., Antwi-Dadzie, Y., Mahama, E., Tchum, K., Dosoo, D., Amenga-Etego, S., Adda, R., Mensah, C., Owusu-Sekyere, K. B., Anderson, C., Krieger, G., Owusu-Agyei, S. (2015). Baseline malaria

vector transmission dynamics in communities in Ahafo mining area in Ghana. *Malar. J*., **14**:142.

Devi, N. P., Jauhari, R. K. (2006). Climatic variables and malaria incidence in Dehradun, Uttaranchal, India. *J. Vect. Borne Dis.,* **43**, 21-28.

Dida, G. O., Gelder, F. B., Anyona, D. N., Abuom, P. O., Onyuka, J. O., Matano, A-S., Adoka, S. O., Kanangire, C. K., Owuor, P. O., Ouma, C., Ofulla, A. V. O. (2015). Presence and distribution of mosquito larvae predators and factors influencing their abundance along the Mara River, Kenya and Tanzania. *Springer Plus*, **4**:136.

Drakeley, C., Schellenberg, D., Kihonda, J., Sousa, C. A., Arez, A. P., Lopes, D., Lines, J., Mshinda, H., Lengeler, C., Schellenberg, J. A., Tanner, M., Alonso, P. (2003). An estimation of the entomological inoculation rate for Ifakara: a semi-urban area in a region of intense malaria transmission in Tanzania. *Trop. Med. Int. Health*, **8**(9): 767-774.

Edi, C. V. A., Koudou, B. G., Jones, C. M., Weetman, D., Ranson, H. (2012). Multiple-insecticide resistance in *Anopheles gambiae* mosquitoes, Southern Côte d'Ivoire. *Emerg. Infect. Dis*., **18**(9): 1508-1511.

Edillo, F. E., Toure´, Y. T., Lanzaro, G. C., Dolo, G., Taylor, C. E. (2004). Survivorship and distribution of immature *Anopheles gambiae* s.l. (Diptera: Culicidae) in Banambani village, Mali. *J. Med. Entomol.,* **41**(3): 333-339.

Faye, O., Konate, L., Mouchet, J., Fontenille, D., Sy, N., Hebrard, G., Herve, J. P. (1997). Indoor resting by outdoor biting females of *Anopheles gumbiue* complex (Diptera: Culicidae) in the Sahel of Northern Senegal. *J. Med. Entomol*., **34**(3): 285-289.

Ferreira, I. M., Yokoo, E. M., Souza-Santos, R., Galvão, N. D., Atanaka-Santos, M. (2012). Factors associated with the incidence of malaria in settlement areas in the district of Juruena, Mato Grosso state, Brazil. *Ciência & Saúde Coletiva*, **17**(9): 2415-2424.

Fillinger, U., Kannady, K., William,G., Vanek, M. J., Dongus, S., Nyika, D., Geissbühler, Y., Chaki, P. P., Govella, N. J., Mathenge, E. M., Singer, B. H., Mshinda, H., Lindsay, S. W., Tanner, M., Mtasiwa, D., de Castro, M. C., Killeen, G. F. (2008). A tool box for operational mosquito larval control: preliminary results and early lessons from the Urban Malaria Control Programme in Dar es Salaam, Tanzania. *Malar. J*., **7**:20.

Fillinger, U., Lindsay, S. W. (2011). Larval source management for malaria control in Africa: myths and reality. *Malar. J*., **10**:353.

Fillinger, U., Sonye, G., Killeen, G. F., Knols, B. G. J., Becker, N. (2004). The practical importance of permanent and semipermanent habitats for controlling aquatic stages of *Anopheles gambiae* sensu lato mosquitoes: operational observations from a rural town in western Kenya. *Trop. Med. Int. Health,* **9**(12): 1274-1289.

Floore, T. G. (2006). Mosquito larval control practices: past and present. *J. Am. Mosq. Control Assoc*., **22**(3): 527-533.

FMoH (2007). *Entomological Profile of Malaria in Ethiopia*. Federal Ministry of Health Ethiopia. Addis Ababa, Ethiopia.

FMoH (2016). *Ethiopia National Malaria Indicator Survey 2015*. Addis Ababa: Federal Democratic Republic of Ethiopia, Ministry of Health.

FMoH (2014). *Health and Health Related Indicators 2005 E.C (2012/2013)*. Policy Planning Directorate, Federal Ministry of Ethiopia.

FMoH and WHO (2007). *Entomological profile of malaria in Ethiopia*. Federal ministry of health Ethiopia and world health organization.

Forero, D. A., Chaparro, P. E., Vallejo, A. F., Benavides, Y., Gutiérrez, J. B., Arévalo-Herrera, M., Herrera, S. (2014). Knowledge, attitudes and practices of malaria in Colombia. *Malar. J*., **13**:165.

Frances, S. P., Marlow, R. M., Jansen, C. C., Huggins, R. L., Cooper, R. D. (2005). Laboratory and field evaluation of commercial repellent formulations against mosquitoes (Diptera: Culicidae) in Queensland, Australia. *Australian J. Entomol.,* **44**: 431-436.

Franco, A. O., Gomes, M. G. M., Rowland, M., Coleman, P. G., Davies, C. R. (2014). Controlling malaria using livestock-based interventions: A one health approach. *PLoS ONE*, **9**(7): e101699. doi:10.1371/journal.pone.0101699.

Fuge, T. G., Ayanto, S. Y., Gurmamo, F. L. (2015). Assessment of knowledge, attitude and practice about malaria and ITNs utilization among pregnant women in Shashogo District, Southern Ethiopia. *Malar. J*., **14**:235.

Gallup, J. L., Sachs, J. D. (2001). The economic burden of malaria. *Am. J. Trop. Med. Hyg.,* **64**(1, 2)S: 85-96.

Gari, T., Kenea, O., Loha, E., Deressa, W., Hailu, A., Balkew, M., Gebre-Michael, T., Robberstad, B., Overgaard, H. J., Lindtjørn, B. (2016). Malaria incidence and entomological findings in an area targeted for a cluster-randomized controlled trial to prevent malaria in Ethiopia: results from a pilot study. *Malar. J*., **15**:145.

Gatton, M. L., Chitnis, N., Churcher, T., Donnelly, M. J., Ghani, A. C., Godfray, H. C. J., Gould, F., Hastings, I., Marshall, J., Ranson, H., Rowland, M., Shaman, J., Lindsay, S. W.

(2013). The importance of mosquito behavioral adaptations to malaria control in Africa. *Evolution*, **7**(4): 1218-1230.

Gething, P. W., Patil, A. P., Smith, D. L., Guerra, C. A., Elyazar, I. R. F., Johnston, G. L., Tatem, A. J., Hay, S. I. (2011). A new world malaria map: *Plasmodium falciparum* endemicity in 2010. *Malar. J.*, **10**:378.

Ghebreyesus, T. A., Haile, M., Witten, K. H., Getachew, A., Yohannes, M., Lindsay, S. W., Byass, P. (2000). Household risk factors for malaria among children in the Ethiopian highlands. *Trans. R. Soc. Trop. Med. Hyg*., **94**: 17-21.

Gillies, M. T., Coetzee, M. (1987): *A Supplement to the Anophelinae of Africa South of the Sahara (Afrotropical Region)*. Johannesburg. Publications of the South African Institute for Medical Research no. 55: South African Institute for Medical Research.

Gimnig, J. E., Ombok, M., Kamau, L., Hawley, W. A. (2001). Characteristics of larval anopheline (Diptera: Culicidae) habitats in western Kenya. *J. Med. Entomol*., **38**(2): 282-288.

Gimnig, J. E., Otieno, P., Were, V., Marwanga, D., Abong'o, D., Wiegand, R., Williamson, J., Wolkon, A., Zhou, Y., Bayoh, M. N., Lobo, N. F., Laserson, K., Kariuki, S., Hamel, M. J. (2016). The effect of indoor residual spraying on the prevalence of malaria parasite infection, clinical malaria and anemia in an area of perennial transmission and moderate coverage of insecticide treated nets in western Kenya. *PLoS ONE,* **11**(1): e0145282. doi:10.1371/journal.pone.0145282.

Githeko, A. K., Lindsay, S. W., Confalonieri, U. E., Patz, J. A. (2000). Climate change and vector-borne diseases: a regional analysis. *Bull. World Health Organ.*, **78**(9): 1136-1147.

Githeko, A. K., Service, M. W., Mbogo, C. M., Atieli, F. A., Juma, F. O. (1994). Sampling *Anopheles arabiensis, An. gambiae* sensu lato and *An. funestus* (Diptera: Culicidae) with CDC light-traps near a rice irrigation area and a sugarcane belt in western Kenya. *Bull. Entomol. Res.,* **84**: 319-324.

Gone, T., Balkew, M., Gebre-Michael, T. (2014). Comparative entomological study on ecology and behaviour of *Anopheles* mosquitoes in highland and lowland localities of Derashe District, southern Ethiopia. *Parasit. Vectors,* **7**:483.

Govella, N. J., Ferguson, H. (2012). Why use of interventions targeting outdoor biting mosquitoes will be necessary to achieve malaria elimination. *Front. Physiol*., **13**:199.

Griffin, J. T., Hollingsworth, T. D., Okell, L. C., Churcher, T. S., White, M., Hinsley, W., Bousema, T., Drakeley, C. J., Ferguson, N. M., Basáñez, M-G., Ghani, A. C. (2010). Reducing *Plasmodium falciparum* malaria transmission in Africa: a model-based evaluation of intervention strategies. *PLoS Med*, **7**(8): e1000324. doi:10.1371/journal.pmed.1000324.

Grover-Kopec, E., Kawano, M., Klaver, R. W., Blumenthal, B., Ceccato, P. and Connor, S. J. (2005). An online operational rainfall-monitoring resource for epidemic malaria early warning systems in Africa. *Malar. J.*, **4**:6.

Gu, W., Novak, R. J. (2005). Habitat-based modeling of impacts of mosquito larval interventions on entomological inoculation rates, incidence, and prevalence of malaria. *Am. J. Trop. Med. Hyg.,* **73**(3): 546-552.

Gu, W., Utzinger, J., Novak, R. J. (2008). Habitat-based larval interventions: a new perspective for malaria control. *Am. J. Trop. Med. Hyg.,* **78**(1): 2-6.

Guerra, C. A., Howes, R. E., Patil, A. P., Gething, P. W., Van Boeckel, T. P., Temperley, W. H., Kabaria, C. W., Tatem, A. J., Manh, B. H., Elyazar, I. R. F., Baird, J. K., Snow, R. W., Hay, S. I. (2010). The international limits and population at risk of *Plasmodium vivax* transmission in 2009. *PLoS Negl. Trop. Dis.,* **4**(8): e774. doi:10.1371/journal.pntd.0000774.

Gutasa, A., Assefa, D., Kumela, K. (2015). Assessment of knowledge and practice of the community towards malaria and its treatment in Jiren kebele, Jimma town Oromia, Ethiopia. *Advances Pharmacol. Pharmacy*, **3**(2): 23-29.

Habtewold, T., Prior, A., Torr, S. J., Gibson, G. (2004). Could insecticide-treated cattle reduce Afrotropical malaria transmission? Effects of deltamethrin-treated Zebu on *Anopheles arabiensis* behaviour and survival in Ethiopia. *Med. Vet. Entomol*., **18**: 408-417.

Hadis, M., Lulu, M., Makonnen, Y., Asfaw, T. (1997). Host choice by indoor-resting *Anopheles arabiensis* in Ethiopia. *Trans. R. Soc. Trop. Med. Hyg.,* **91**: 376-378.

Hay, S. I., Sinka, M. E., Okara, R. M., Kabaria, C. W., Mbithi, P. M., Tago, C. C., Benz, D., Gething, P. W., Howes, R. E., Patil, A. P., Temperley, W. H., Bangs, M. J., Chareonviriyaphap, T., Elyazar, I. R. F., Harbach, R. E., Hemingway, J., Manguin, S., Mbogo, C. M., Rubio-Palis, Y., Godfray, H. C. J. (2010). Developing global maps of the dominant anopheles vectors of human malaria. *PLoS Med.,* 7(2): e1000209. doi:10.1371/journal.pmed.1000209.

Hemingway, J., Ranson, H., Magill, A., Kolaczinski, J., Fornadel, C., Gimnig, J., Coetzee, M., Simard, F., Roch, D. K., Hinzoumbe, C. K., Pickett, J., Schellenberg, D., Gething, P.,

Hoppé, M., Hamon, N. (2016). Averting a malaria disaster: will insecticide resistance derail malaria control? *Lancet*, **387**(10092): 1785-1788.

Huang, F., Zhou, S., Zhang, S., Wang, H., Tang, L. (2011). Temporal correlation analysis between malaria and meteorological factors in Motuo County, Tibet. *Malar. J.*, **10**:54.

Hunt, R. H., Coetzee, M., Fettene, M. (1998). The *Anopheles gambiae* complex: a new species from Ethiopia. *Trans. R. Soc. Trop. Med. Hyg*., **92**: 231-235.

Hunt, R. H., Fuseini, G., Knowles, S., Stiles-Ocran, J., Verster, R., Kaiser, M. L., Choi, K. S., Koekemoer, L. L., Coetzee, M. (2012). Insecticide resistance in malaria vector mosquitoes at four localities in Ghana, West Africa. *Parasit. Vectors*, **4**:107.

Imbahale, S. S., Paaijmans, K. P., Mukabana, W. R., van Lammeren, R., Andrew K Githeko, A. K., Takken, W. (2011). A longitudinal study on *Anopheles* mosquito larval abundance in distinct geographical and environmental settings in western Kenya. *Malar. J.*, **10**:81.

Jawara, M., Pinder, M., Drakeley, C. J., Nwakanma, D. C., Jallow, E., Bogh, C., Lindsay, S. W., Conway, D. J. (2008). Dry season ecology of *Anopheles gambiae* complex mosquitoes in The Gambia. *Malar. J.*, **7**:156.

Jima, D., Getachew, A., Bilak, H., Steketee, R. W., Emerson, P. M., Graves, P. M., Gebre, T., Reithinger, R., Hwang, J., the Ethiopia Malaria Indicator Survey Working Group (2010). Malaria indicator survey 2007, Ethiopia: coverage and use of major malaria prevention and control interventions. *Malar. J.,* **9**:58.

Jongwutiwes, S., Putaporntip, C., Iwasaki, T., Sata, T., Kanbara, H. (2004). Naturally acquired *Plasmodium knowlesi* malaria in human, Thailand. *Emerg. Infect. Dis*., **10**(12): 2211-2213.

Joveen-Neoh, W. F., Chong, K. L., Wong, C. M. V. L., Tiek Ying Lau, T. K. (2011). Incidence of malaria in the interior division of Sabah, Malaysian Borneo, based on nested PCR. *J. Parasitol. Res*., **2011**:6.

Kibret, S., Alemu, Y., Boelee, E., Tekie, H., Alemu, D., Petros, B. (2010). The impact of a small-scale irrigation scheme on malaria transmission in Ziway area, Central Ethiopia. *Trop. Med. Int. Health*, **15**(1): 41-50.

Kibret, S., Wilson, G. G., Tekie, H., Petros, B. (2014). Increased malaria transmission around irrigation schemes in Ethiopia and the potential of canal water management for malaria vector control. *Malar. J*., **13**:360.

Kibret, S., Wilson, G. G., Ryder, D., Tekie, H., Petros, B. (2017). Malaria impact of large dams at different eco-epidemiological settings in Ethiopia. *Trop. Med. Health*, **45**:4.

Kirby, M. J., Green, C., Milligan, P. M., Sismanidis, C., Jasseh, M., Conway, D. J., Lindsay, S. W. (2008). Risk factors for house-entry by malaria vectors in a rural town and satellite villages in The Gambia. *Malar. J.*, **7**:2.

Kelly-Hope, L., Ranson, H., Hemingway, J. (2008). Lessons from the past: managing insecticide resistance in malaria control and eradication programmes. *Lancet Infect. Dis.,* **8**: 387-89.

Kenea, O., Balkew, M., Gebre-Michael, T. (2011). Environmental factors associated with larval habitats of anopheline mosquitoes (Diptera: Culicidae) in irrigation and major drainage areas in the middle course of the Rift Valley, central Ethiopia. *J. Vect. Borne Dis.,* **48**(85): 85-92.

Kenea, O., Balkew, M., Tekie, H., Gebre-Michael, T., Deressa, W., Loha, E., Lindtjørn, B,. Overgaard, H. J. (2016). Human-biting activities of *Anopheles* species in south-central Ethiopia. *Parasit. Vectors*, **9**:527.

Kenea, O., Balkew, M., Tekie, H., Gebre‐Michael, T., Deressa, W., Loha, E., Lindtjørn, B., Overgaard, H. J. (2017). Comparison of two adult mosquito sampling methods with human landing catches in south‐central Ethiopia. *Malar. J.*,**16**:30.

Kent, R. J., Thuma, P. E., Mharakurwa, S., Norris, D. E. (2007). Seasonality, blood feeding behavior and transmission of *Plasmodium falciparum* by *Anopheles arabiensis* after an extended drought in southern Zambia. *Am. J. Trop. Med. Hyg.,* **76**(2): 267-274.

Ketema, T., Getahun, K., Bacha, K. (2011). Therapeutic efficacy of chloroquine for treatment of *Plasmodium vivax* malaria cases in Halaba district, South Ethiopia. *Parasit. Vectors*, **4**:46.

Killeen, G. F. (2014). Characterizing, controlling and eliminating residual malaria transmission. *Malar. J.*, **13**:330.

Killeen, G. F., Seyoum, A., Sikaala, C., Zomboko, A. S., Gimnig, J., Govella, N. J., White, M. T. (2013). Eliminating malaria vectors. *Parasit, Vectors*, **6**:172.

Kitron, U., Spielman, A. (1989). Suppression of transmission of malaria through source reduction: antianopheline measures applied in Israel, the United States, and Italy. *Rev. Infect. Dis*., **2**(3): 391-406.

Koennraadt, C. J. M., Takken, W. (2003). Cannibalism and predation among larvae of the *Anopheles gambiae* complex. *Med. Vet. Entomol.,* **17**: 61-66.

Konradsen, F., Amerasinghe, P., van der Hoek, W., Amerasinghe, F., Perera, D., Piyaratne,

M. (2003). Strong association between house characteristics and malaria vectors in Sri Lanka. *Am. J. Trop. Med. Hyg.,* **68**(2): 177-181.

Konradsen, F., Matsuno, Y., Amerasinghe, F. P., Amerasinghe, P. H., van der Hoek, W. (1998). *Anopheles culicifacies* breeding in Sri Lanka and options for control through water management. *Acta Trop.,* **71**: 131-138.

Kweka, E. J., Zhou, G., Lee, M-C., Gilbreath, T. M., Mosha, F., Munga, S., Githeko, A. K., Yan, G. (2011). Evaluation of two methods of estimating larval habitat productivity in western Kenya highlands. *Parasit. Vectors*, **4**:110.

Lardeux, F. J., Tejerina, R. H., Quispe, V., Chavez, T. K. (2008). A physiological time analysis of the duration of the gonotrophic cycle of *Anopheles pseudopunctipennis* and its implications for malaria transmission in Bolivia. *Malar. J.*, **7**:141.

Legesse, Y., Tegegn, A., Belachew, T., Tushune, K. (2007). Knowledge, attitude and practice about malaria transmission and its preventive measures among households in urban areas of Assosa Zone, western Ethiopia. *Ethiop. J. Health Dev.,* **21**(2): 157-165.

Li, L., Bian, L., Yan, G. (2008). A study of the distribution and abundance of the adult malaria vector in western Kenya highlands. *Inter. J. Health Geog.,* **7**:50.

Livadas, G. A., Georgopoulos, G. (1953). Development of resistance to DDT by *Anopheles sacharovi* in Greece. *Bull. World Health Organ.*, **8**: 497-511.

Linard, C., Gilbert, M., Snow, R. W., Noor, A. M., Tatem, A. J. (2012). Population distribution, settlement patterns and accessibility across Africa in 2010. *PLoS ONE*, **7**(2): e31743. doi:10.1371/journal.pone.0031743.

Lindblade, K. A. (2013). Commentary: Does a mosquito bite when no one is around to hear it? *Int. J. Epidemiol*., **42**: 247-249.

Lindsay, S. W., Martens, W. J. M. (1998). Malaria in the African highlands: past, present and future. *Bull. World Health Organ.*, **76** (1): 33-45.

Lindsay, S. W., Jawara, M., Paine, K., Pinder, M., Walraven, G. E. L., Emerson, P. M. (2003). Changes in house design reduce exposure to malaria mosquitoes. *Trop. Med. Intern. Health*, **8**(6): 512-517.

Liu, X. B., Liu, Q. Y., Guo, Y. H., Jiang, J. Y., Ren, D. S., Zhou, G. C., Zheng, C. J., Liu, J. L., Chen, Y., Li, H-S., Li, H-Z., Li, Q. (2012). Random repeated cross sectional study on breeding site characterization of *Anopheles sinensis* larvae in distinct villages of Yongcheng City, People's Republic of China. *Parasit. Vectors*, **5**:58.

Lowassa, A., Mazigo, H. D., Mahande, A. M., Mwang'onde, B. J., Msangi, S., Mahande, M. J., Kimaro, E. E., Elisante, E., Kweka, E. J. (2012). Social economic factors and malaria transmission in Lower Moshi, Northern Tanzania. *Parasit. Vectors*, **5**:129.

Luchavez, J., Espino, F., Curameng, P., Espina, R., Bell, D., Chiodini, P., Nolder, D., Sutherland, C., Lee, K-S., Singh, B. (2008). Human Infections with *Plasmodium knowlesi*, the Philippines. *Emerg. Infect. Dis*., **14**(5):811-813.

Lwetoijera, D. W., Kiware, S. S., Mageni, Z. D., Dongus, S., Harris, C., Devine, G. J., Majambere, S. (2013). A need for better housing to further reduce indoor malaria transmission in areas with high bed net coverage. *Parasit. Vectors*, **6**:57.

Mala, A. O., Irungu, L. W. (2011). Factors influencing differential larval habitat productivity of *Anopheles gambiae* complex mosquitoes in a western Kenyan village. *J. Vect. Borne Dis.*, **48**:52-57.

Martens, W. J. M., Jetten, T. H., Focks, D. A. (1997). Sensitivity of malaria, schistosomiasis and dengue to global warming. *Climatic Change,* **35:** 145-156.

Massebo, F., Balkew, M., Gebre-Michael, T., Lindtjørn, B. (2013). Blood meal origins and insecticide susceptibility of Anopheles arabiensis from Chano in South-West Ethiopia. *Parasit. Vectors*, **6**: 44.

Massebo, F., Balkew, M., Gebre-Michael, T., Lindtjørn, B. (2015). Zoophagic behaviour of Anopheline mosquitoes in southwest Ethiopia: opportunity for malaria vector control. *Parasit*. *Vectors*, **8**: 645.

Matowo, J., Kitau, J., Kaaya, R., Kavishe, R., Wright, A., Kisinza, W., Kleinschmidt, I., Mosha, F., Rowland, M., Protopopoff, N. (2015). Trends in the selection of insecticide resistance in *Anopheles gambiae* s.l. mosquitoes in northwest Tanzania during a community randomized trial of longlasting insecticidal nets and indoor residual spraying. *Med. Vet. Entomol.,* **29**: 51-59.

Mayagaya, V. S., Nkwengulila, G., Lyimo, I. N., Kihonda, J., Mtambala, H., Ngonyani, H., Russell, T. L., Ferguson, H. M. (2015). The impact of livestock on the abundance, resting behaviour and sporozoite rate of malaria vectors in southern Tanzania. *Malar. J*., **14**:17.

Mazigo, H. D., Obasy, E., Mauka, W., Manyiri, P., Zinga, M., Kweka, E. J., Mnyone, L. L., Heukelbach, J. (2010). Knowledge, attitudes, and practices about malaria and its control in rural northwest Tanzania. *Malar. Res. Treat*., **2010**.

Mboera, L. E. G., Kihonda, J., Braks, M. A. H., Knols, B. G. J. (1998). Short report: influence of centers for disease control light trap position, relative to a human-baited bed net, on catches of *Anopheles gambiae* and *Culex quinquefasciatus* in Tanzania. *Am. J. Trop. Med. Hyg.,* **59**(4): 595-596.

Mbogo, C. M., Mwangangi, J. M., Nzovu, J., Gu, W., Yan, G., Gunter, J. T., Swalm, C., Keating, J., Regens, J. L., Shililu, J. I., Githure, J. I., ad Beier, J. C. (2003). Spatial and temporal heterogeneity of *Anopheles* mosquitoes and *Plasmodium falciparum* transmission along the Kenyan coast. *Am. J. Trop. Med. Hyg.,* **68**(6): 734-742.

Mekonnen, S. K., Aseffa, A., Medhin, G., Berhe, N., P Velavan, T. P. (2014). Re-evaluation of microscopy confirmed *Plasmodium falciparum* and *Plasmodium vivax* malaria by nested PCR detection in southern Ethiopia. *Malar. J*., **13**:48.

Mereta, S. T., Yewhalaw, D., Boets, P., Ahmed, A., Duchateau, L., Speybroeck, N., Vanwambeke, S. O., Legesse, W., Meester, L. D., Goethals, P. L. M. (2013). Physico-chemical and biological characterization of anopheline mosquito larval habitats (Diptera: Culicidae): implications for malaria control. *Parasit. Vectors*, **6**:320.

Meyrowitsch, D. W., Pedersen, E. M., Alifrangis, M., Scheike, T. H., Malecela, M. N., Magesa, S. M., Derua, Y. A., Rwegoshora, R. T., Michael, E., Simonsen, P. E. (2011). Is the current decline in malaria burden in sub-Saharan Africa due to a decrease in vector population? *Malar. J*., **10**:188.

Minakawa, N., Mutero, C. M., Githure, J. I., Beier, J. C., Yan, G. (1999). Spatial distribution and habitat characterization of Anopheline mosquito larvae in western Kenya. *Am. J. Trop. Med. Hyg.,* **61**(6): 1010-1016.

Moiroux, N., Gomez, M. B., Pennetier, C., Elanga, E., Djènontin, A., Chandre, F., Djègbé, I., Guis, H., Corbel, V. (2012). Changes in *Anopheles funestus* biting behavior following universal coverage of long-lasting Insecticidal nets in Benin. *J. Infect. Dis*., **206**: 1622-1629.

Mora-Ruiz, M., Penilla, R. P., Ordóñez, J. G., López, A. D., Solis, F., Torres-Estrada, J. L., Rodríguez, A. D. (2014). Socioeconomic factors, attitudes and practices associated with malaria prevention in the coastal plain of Chiapas, Mexico. *Malar. J.*, **13**:157.

Mordecai, E. A., Paaijmans, K. P., Johnson, L. R., Balzer, C., Ben-Horin, T., Moor, E., McNally, A., Pawar, S., Ryan, S. J., Smith, T. C., Lafferty, K. D. (2013). Optimal temperature for malaria transmission is dramatically lower than previously predicted. *Ecol. Letters*, **16**(1): 22-30.

Moreno, M., Saavedra, M. P., Bickersmith, S. A., Lainhart, W., Tong, C., Alava, F., Vinetz, J. M., Conn, J. E. (2015). Implications for changes in *Anopheles darlingi* biting behaviour in three communities in the peri-Iquitos region of Amazonian Peru. *Malar J.,* **14**:290.

Mouchet, J., Manguin, S., Sircoulon, J., Laventure, S., Faye, O., Onapa, A. W., Carnevale, P., Julvez, J., Fontenille, D. (1998). Evolution of malaria in Africa for the past 40 years: impact of climatic and human factors. *J. Am. Mosq. Control Assoc.,* **14**(2): 121-130.

Moyes, C. L., Henry, A. J., Golding, N., Huang, Z., Singh, B., Baird, J. K., Newton, P. N., Huffman, M., Duda, K. A., Drakeley, C. J., Elyazar, I. R. F., Anstey, N. M., Chen, Q., Zommers, Z., Bhatt, S., Gething, P. W., Hay, S. I. (2014). Defining the geographical range of the *Plasmodium knowlesi* reservoir. *PLoS Negl. Trop. Dis*., **8**(3): e2780. doi:10.1371/journal.pntd.0002780.

Munga, S., Minakawa, N., Zhou, G., Mushinzimana, E., Barrack, O. J., Githeko, A. K., Yan, G. (2006). Association between land cover and habitat productivity of malaria vectors in Western Kenyan highlands. *Am. J. Trop. Med. Hyg.,* **74**(1): 69-75.

Muriu, S. M., Muturi, E. J., Shililu, J. I., Mbogo, C. M., Mwangangi, J. M., Jacob, B. G., Irungu, L. W., Mukabana, R. W., Githure, J. I., Novak, R. J. (2008). Host choice and multiple blood feeding behaviour of malaria vectors and other anophelines in Mwea rice scheme, Kenya. *Malar. J.*, **7**:43.

Murray, C. J. L., Rosenfeld, L. C., Lim, S. S., Andrews, K. G., Foreman, K. J., Haring, D., Fullman, N., Naghavi, M., Lozano, R., Lopez, A. D. (2012). Global malaria mortality between 1980 and 2010: a systematic analysis. *Lancet,* **379**: 413-431.

Mutero, C. M., Blank, H., Konradsen, F., van der Hoek, W. (2000). Water management for controlling the breeding of *Anopheles* mosquitoes in rice irrigation schemes in Kenya. *Acta Trop*., **76**: 253-263.

Muturi, E. J., Mwangangi, J., Shililu, J., Jacob, B. G., Mbogo, C., Githure, J., Novak, R. J. (2008). Environmental factors associated with the distribution of *Anopheles arabiensis* and *Culex quinquefasciatus* in a rice agro-ecosystem in Mwea, Kenya. *J. Vector Ecol.,* **33** (1): 56-63.

Mwangangi, J. M., Mbogo, C. M., Muturi, E. J., Nzovu, J. G., Githure, J. I., Yan, G., Minakawa, N., Novak, R., Beier, J. C.(2007). Spatial distribution and habitat characterisation of *Anopheles* larvae along the Kenyan coast. *J. Vect. Borne Dis.,* **44**: 44-51.

Mwangangi, J. M., Mbogo, C. M., Orindi, B. O., Muturi, E. J., Midega, J. T., Nzovu, J., Gatakaa, H., Githure, J., Borgemeister, C., Keating, J., Beier, J. C. (2013). Shifts in malaria vector species composition and transmission dynamics along the Kenyan coast over the past 20 years. *Malar. J*., **12**:13.

Mwangangi, J. M., Muturi, E. J., Shililu, J., Muriu, S. M., Jacob, B., Kabiru, E. W., Mbogo, C. M., Githure, J., Novak, R. (2008). Contribution of different aquatic habitats to adult

Anopheles arabiensis and *Culex quinquefasciatus* (Diptera: Culicidae) production in a rice agroecosystem in Mwea, Kenya. *J. Vector Ecol.,* **33**(1): 129-138.

Mwangangi, J. M., Muturi, E. J., Shililu, J., Muriu, S. M., Jacob, B., Kabiru, E. W., Mbogo, C. M., Githure, J., Novak, R. (2006). Survival of immature *Anopheles arabiensis* (Diptera: Culicidae) in aquatic habitats in Mwea rice irrigation scheme, central Kenya. *Malar. J.,* **5**:114.

Mwangangi, J., Shililu, J., Muturi, E., Gu, W., Mbogo, C., Kabiru, E., Jacob, B., Githure, J., Novak, R. (2006). Dynamics of immature stages of *Anopheles arabiensis* and other mosquito species (Diptera: Culicidae) in relation to rice cropping in a rice agro-ecosystem in Kenya. *J. Vector Ecol.,* **31**(2): 245-251.

Mwangangi, J. M., Shililu, J., Muturi, E. J., Muriu, S., Jacob, B., Kabiru, E. W., Mbogo, C. M., Githure, J., Novak, R. J. (2010). *Anopheles* larval abundance and diversity in three rice agro-village complexes Mwea irrigation scheme, central Kenya. *Malar. J.,* **9**:228.

N'Guessan, R., Corbel, V., Akogbéto, M., Rowland, M. (2007). Reduced efficacy of insecticide treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. *Emerg. Infect. Dis.*, **13**(2): 199-206.

Nkya, T. E., Akhouayri, I., Poupardin, R., Batengana, B., Mosha, F., Magesa, S., Kisinza, W., David, J-P. (2014). Insecticide resistance mechanisms associated with different environments in the malaria vector Anopheles gambiae: a case study in Tanzania. *Malar. J.*, **13**: 28.

Noor, A. M., Kinyoki, D. K., Mundia, C. W., Kabaria, C. W., Mutua, J. W., Alegana, V. A., Fall, I. S., Snow, R. W. (2014). The changing risk of *Plasmodium falciparum* malaria

infection in Africa: 2000-10: a spatial and temporal analysis of transmission intensity. *Lancet*, **383**: 1739-1747.

Nwane, P., Etang, J., Chouaïbou, M., Toto, J. C., Koffi, A., Mimpfoundi, R., Simard, F. (2013). Multiple insecticide resistance mechanisms in *Anopheles gambiae* s.l. populations from Cameroon, Central Africa. *Parasit. Vectors*, **6**:41.

Nyanjom, S. R. G., Chen, H., Gebre-Michael, T., Bekele, E., Shililu, J., Githure, J., Beier, J. C., Yan, G. (2003). Population genetic structure of *Anopheles arabiensis* mosquitoes in Ethiopia and Eritrea. *J. Hered*., **94**(6): 457-463.

Oduola, A. O., Idowu, E. T., Oyebola, M. K., Adeogun, A. O., Olojede, J. B., Otubanjo, O. A., Awolola, T. S. (2012). Evidence of carbamate resistance in urban populations of *Anopheles gambiae* s.s. mosquitoes resistant to DDT and deltamethrin insecticides in Lagos, South-Western Nigeria. *Parasit. Vectors*, **5**:116.

Oguike, M. C., Betson, M., Burke, M., Nolder, D., Stothard, J. R., Kleinschmidt, I., Proietti, C., Bousema, T., Ndounga, M., Tanabe, K., Ntege, E., Culleton, R., Sutherland, C. J. (2011). *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri* circulate simultaneously in African communities. *Int. J. Parasitol*., **41**: 677-683.

Opondo, K. O., Weetman, D., Jawara, M., Diatta, M., Fofana, A., Crombe, F., Mwesigwa, J., D'Alessandro, U., Donnelly, M. J. (2016). Does insecticide resistance contribute to heterogeneities in malaria transmission in The Gambia? *Malar. J*., **15**:166.

Otten, M., Aregawi, M., Were, W., Karema, C., Medin, A., Bekele, W., Jima, D., Gausi, K., Komatsu, R., Korenromp, E., Low-Beer, D., Grabowsky, M. (2009). Initial evidence of reduction of malaria cases and deaths in Rwanda and Ethiopia due to rapid scale-up of malaria prevention and treatment. *Malar. J*., **8**:14.

Paaijmans, K. P., Wandago, M. O., Githeko, A. K., Takken, W. (2007). Unexpected high losses of *Anopheles gambiae* larvae due to rainfall. *PLoS One*, **2**(11):e1146.

Padonou, G. G., Sezonlin, M., Ossé, R., Aizoun, N., Oké-Agbo, F., Oussou, O., Gbédjissi, G., Akogbéto, M. (2012). Impact of three years of large scale Indoor Residual Spraying (IRS) and Insecticide Treated Nets (ITNs) interventions on insecticide resistance in *Anopheles gambiae* s.l. in Benin. *Parasit. Vectors*, **5**:72.

Pålsson, K., Jaenson, T. G. T., Dias, F., Laugen, A. T., BjöRkman, A. (2004). Endophilic *Anopheles* mosquitoes in Guinea Bissau, West Africa, in relation to human housing conditions. *J. Med. Entomol*., **41**(4): 746-752.

Pappa, V., Reddy, M., Overgaard, H. J., Abaga, S., Caccone, A. (2011). Short report: estimation of the human blood index in malaria mosquito vectors in Equatorial Guinea after indoor antivector interventions. *Am. J. Trop. Med. Hyg.,* **84**(2): 298-301.

Peterson, I., Borrell, L. N., El-Sadr, W., Teklehaimanot, A. (2009). Individual and household level factors associated with malaria incidence in a highland region of Ethiopia: a multilevel analysis. *Am. J. Trop. Med. Hyg.,* **80**(1): 103-111.

Ranson, H., Abdallah, H., Badolo, A., Guelbeogo, W. M., Kerah-Hinzoumbé, C., Yangalbé-Kalnoné, E., Sagnon, N., Simard, F., Coetzee, M. (2009). Insecticide resistance in *Anopheles gambiae:* data from the first year of a multi-country study highlight the extent of the problem. *Malar. J*., **8**:299.

Ranson, H., N'Guessan, R., Lines, J., Moiroux, N., Nkuni, Z., Corbel, V. (2011). Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? *Trends Parasitol*., **27**(2): 91-98.

Ribeiro, J. M. C., Seulu, F., Abose, T., Kidane, G., Teklehaimanot, A. (1996). Temporal and spatial distribution of anopheline mosquitos in an Ethiopian village: implications for malaria control strategies. *Bull. World Health Organ*., **74**(3): 299-305.

Roberts, L., Enserink, M. (2007). Malaria. Did they really say …eradication? *Science*, **318**: 1544-1545.

Rowland, M., Downey, G., Rab, A., Freeman, T., Mohammad, N., Rehman, H., Durrani, N., Reyburn, H., Curtis, C., Lines, J., Fayaz, M. (2004). DEET mosquito repellent provides personal protection against malaria: a household randomized trial in an Afghan refugee camp in Pakistan. *Trop. Med. Int. Health*, **9**(3): 335-342.

Russell, T. L., Beebe, N. W., Bugoro, H., Apairamo, A., Chow, W. K., Cooper, R. D., Collins, F. H., Lobo, N. F., Burkot, T. R. (2016). Frequent blood feeding enables insecticide‐treated nets to reduce transmission by mosquitoes that bite predominately outdoors. *Malar. J*., **15**:156.

Russell, T. L., Beebe, N. W., Cooper, R. D., Lobo, N. F., Burkot, T. R. (2013). Successful malaria elimination strategies require interventions that target changing vector behaviours. *Malar. J*., **12**:56.

Russell, T. L., Govella, N. J., Azizi, S., Drakeley, C. J., Kachur, S. P., Killeen, G. F. (2011). Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. *Malar. J*., **10**:80.

Ryan, J. R., Stoute, J. A., Amon, J., Dunton, R. F., Mtalib, R. M., Koros, J., Owour, B., Luckhart, S., Wirtz, R. A., Barnwell, J. W., Rosenberg, R. (2006). Evidence for transmission of *Plasmodium vivax* among a Duffy antigen negative population in western Kenya. *Am. J. Trop. Med. Hyg.,* **75**(4): 575-581.

Sabin, L. L., Rizal, A., Brooks, M. I., Singh, M. P., Tuchman, J., Wylie, B. J., Joyce, K. M., Yeboah-Antwi, K., Singh, N., Hamer, D. H. (2010). Attitudes, knowledge, and practices regarding malaria prevention and treatment among pregnant women in eastern India. *Am. J. Trop. Med. Hyg.,* **82**(6): 1010-1016.

Sachs, J., Malaney, P. (2002). The economic and social burden of malaria. *Nature*, **415**: 680-685.

Santana-Morales, M. A., Afonso-Lehmann, R. N., Quispe, M. A., Reyes, F., Berzosa, P., Benito, A., Valladares, B., Martinez-Carretero, E., (2012). Microscopy and molecular biology for the diagnosis and evaluation of malaria in a hospital in a rural area of Ethiopia. *Malar. J.,* **11**:199.

Sattler, M. A., Mtasiwa, D., Kiama, M., Premji, Z., Tanner, M., Killeen, G. F., Lengeler, C. (2005). Habitat characterization and spatial distribution of *Anopheles* sp. mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. *Malar. J.,* **4**:4.

Schunk, M., Kumma, W. P., Miranda, I. B., Osman, M. E., Roewer, S., Alano, A., Löscher, T., Bienzle, U., Mockenhaupt, F. P. (2006). High prevalence of drug-resistance mutations in *Plasmodium falciparum* and *Plasmodium vivax* in southern Ethiopia. *Malar. J.*, **5**:54.

Sena, L. D., Deressa, W. A., Ali, A. A. (2014). Analysis of trend of malaria prevalence in south-west Ethiopia: a retrospective comparative study. *Malar. J.*, **13**:188.

Seyoum, A., Sikaala, C. H., Chanda, J., Chinula, D., Ntamatungiro, A. J., Hawela, M., Miller, J. M., Russell, T. L., Briët, O. J. T., Killeen, G. F. (2012). Human exposure to anopheline mosquitoes occurs primarily indoors, even for users of insecticide treated nets in Luangwa Valley, South-east Zambia. *Parasit. Vectors*, **5**:101.

Shaukat, A. M., Breman, J. G., McKenzie, F. E. (2010). Using the entomological inoculation rate to assess the impact of vector control on malaria parasite transmission and elimination *Malar. J.*, **9**:122.

Shililu, J., Ghebremeskel, T., Seulu, F., Mengistu, S., Fekadu,H., Zerom, M., Ghebregziabiher, A., Sintasath, D., Bretas, G., Mbogo, C., Githure, J., Brantly, E., Novak, R., Beier, J. C. (2003). Larval habitat diversity and ecology of anopheline larvae in Eritrea. *J. Med. Entomol*., **40**(6): 921-929.

Shililu, J., Mbogo, C., Ghebremeskel, T., Githure, J., Novak, R. (2007). Mosquito larval habitats in a semiarid ecosystem in Eritrea: impact of larval habitat management on *Anopheles arabiensis* population. *Am. J. Trop. Med. Hyg.*, **76**(1): 103-110.

Sikaala, C. H., Killeen, G. F., Chanda, J., Chinula, D., Miller, J. M., Russell, T. L., Seyoum, A. (2013). Evaluation of alternative mosquito sampling methods for malaria vectors in lowland South-East Zambia. *Parasit. Vectors*, **6**: 91.

Silver, J. B. (2008). *Mosquito Ecology: Field Sampling Methods*, 3rd edn. The Netherlands: Springer.

Singh, B., Sung, L. K., Matusop, A., Radhakrishnan, A., Shamsul, S. S. G., Cox-Singh, J., Thomas, A., Conway, D. J. (2004). A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. *Lancet,* **363**: 1017-1024.

Sinka, M. E., Bangs, M. J., Manguin, S., Rubio-Palis, Y., Chareonviriyaphap, T., Coetzee, M., Mbogo, C. M., Hemingway, J., Patil, A. P., Temperley, W. H., Gething, P. W., Kabaria, C. W., Burkot, T. R., Harbach, R. E., Hay, S. I. (2012). A global map of dominant malaria vectors. *Parasit. Vectors*, **5**:69.

Smith, D. L., McKenzie, F. E., Snow, R. W., Hay, S. I. (2007). Revisiting the basic reproductive number for malaria and its implications for malaria control. *PLoS Biol,* **5**(3): e42. doi:10.1371/journal.pbio.0050042.

Smith, T., Charlwood, J. D., Takken, W., Tanner, M., Spiegelhalter, D. J. (1995). Mapping the densities of malaria vectors within a single village. *Acta Trop*., **59**: 1-18.

Snow, R. W. (2015). Global malaria eradication and the importance of *Plasmodium falciparum* epidemiology in Africa. *BMC Medicine*, **13**:23.

Snow, R. W., Amratia, P., Kabaria, C. W., Noor, A. M., Marsh, K. (2012). The changing limits and incidence of malaria in Africa: 1939-2009. *Advances Parasitol*., **78**: 169-262.

Sogoba, N., Doumbia, S., Vounatsou, P., Baber, I., Keita, M., Maiga, M., Traoré, S. F., Touré, A., Dolo, G., Smith, T., Ribeiro, J. M. C. (2007). Monitoring of larval habitats and mosquito densities in the Sudan savanna of Mali: implications for malaria vector control. *Am. J. Trop. Med. Hyg.*, **77**(1): 82-88.

Sougoufara, S., Diédhiou, S. M., Doucouré, S., Diagne, N., Sembène, P. M., Harry, M., Trape, J-P., Sokhna, C., Ndiath, M. O. (2014). Biting by *Anopheles funestus* in broad daylight after use of long-lasting insecticidal nets: a new challenge to malaria elimination. *Malar. J*., **13**:125.

Sriwichai, P., Karl, S., Samung, Y., Sumruayphol, S., Kiattibutr, K., Payakkapol, A., Mueller, I., Yan, G., Cui, L., Sattabongkot, J. (2015). Evaluation of CDC light traps for mosquito surveillance in a malaria endemic area on the Thai-Myanmar border. *Parasit. Vectors*, **8**: 636.

Staedke, S. G., Nottingham, E. W., Cox, J., Kamya, M. R., Rosenthal, P. J., Dorsey, G. (2003). Short report: proximity to mosquito breeding sites as a risk factor for clinical malaria

episodes in an urban cohort of Ugandan children. *Am. J. Trop. Med. Hyg.*, **69**(3): 244-246.

Tabbabi, A., Bousse`s, P., Rhim, A., Brengues, C., Daaboub, J., Nissaf Ben-Alaya-Bouafif, A., Fontenille, D., Bouratbine, A., Simard, F., Aoun, K. (2015). Larval habitats characterization and species composition of anopheles mosquitoes in Tunisia, with particular attention to *Anopheles maculipennis* complex. *Am. J. Trop. Med. Hyg*., **92**(3): 653–659.

Tadesse, F. G., Pett, H., Baidjoe, A., Lanke, K., Grignard, L., Sutherland, C., Hall, T., Drakeley, C., Bousema, T., Mamo, H. (2015). Submicroscopic carriage of *Plasmodium falciparum* and *Plasmodium vivax* in a low endemic area in Ethiopia where no parasitaemia was detected by microscopy or rapid diagnostic test. *Malar. J*., **14**:303.

Tekie, H. (1989). *The Ecology of Anthropophilic Anopheles Mosquitoes in the Ghibe Horticulture Development Farm*. MSc Thesis, Addis Ababa University, Addis Ababa. pp. 85.

Tesfaye, S., Belyhun, Y., Teklu, T., Mengesha, T., Petros, B. (2011). Malaria prevalence pattern observed in the highland fringe of Butajira, Southern Ethiopia: A longitudinal study from parasitological and entomological survey. *Malar. J*., **10**:153.

The Carter Center (2012). *Summary proceedings 3rd annual malaria control program review Ethiopia and Nigeria*: February 24, 2012, The Carter Center, Atlanta, Georgia.

The malERA Consultative Group on Vector Control (2011). A research agenda for malaria eradication: vector control. *PLoS Med*, **8**(1): e1000401. doi:10.1371/journal.pmed.1000401.

Tirados, I., Costantini, C., Gibson, G., Torr, S. J. (2006). Blood-feeding behaviour of the malarial mosquito *Anopheles arabiensis*: implications for vector control. *Med. Vet. Entomol.,* **20**: 425-437.

Tirados, I., Gibson, G., Young, S., Torr, S. J. (2011). Are herders protected by their herds? An experimental analysis of zooprophylaxis against the malaria vector *Anopheles arabiensis*. *Malar. J*., **10**:68.

Tuno, N., Okeka, W., Minakawa, N., Takagi, M., Yan, G. (2005). Survivorship of *Anopheles gambiae* sensu stricto (Diptera: Culicidae) larvae in western Kenya highland forest. *J. Med. Entomol.,* **42**(3): 270-277.

Vezenegho, S. B., Brooke, B. D., Hunt, R. H., Coetzee, M., Koekemoer, L. L. (2009). Malaria vector composition and insecticide susceptibility status in Guinea Conakry, West Africa. *Med. Vet. Entomol.,* **23**: 326-334.

Walker, K., Lynch, M. (2007). Contributions of *Anopheles* larval control to malaria suppression in tropical Africa: review of achievements and potential. *Med. Vet. Entomol.,* **21**: 2-21.

Weeto, M. M., Koekemoer, L. L., Kamau, L., Hunt, R. H. and Coetzee, M. (2004). Evaluation of a species-specific PCR assay for the *Anopheles funestus* group from eleven African countries and Madagascar. *Trans. R. Soc. Trop. Med. Hyg*., **98**: 142-147.

White, G. B. (1974). *Anopheles gambiae* complex and disease transmission in Africa. *Trans. R. Soc. Trop. Med. Hyg*., **68**(4): 278-298.

White, N. J. (2008). *Plasmodium knowlesi:* The fifth human malaria parasite. *Clin. Infect. Dis*., **46**:172-173.

WHO (2006a). *The Africa malaria report 2006*. World Health Organization, Regional Offices for Africa and Eastern Mediterranean.

WHO (2006b). *Indoor residual spraying: Use of indoor residual spraying for scaling up global malaria control and elimination, WHO Position Statement*. World Health Organization, Global Malaria Program. Geneva, Switzerland.

WHO (2011). *Malaria entomology and vector control. Guide for participants: Training module on malaria control*. World Health Organization, Geneva, Switzerland.

WHO (2012a). *Management of severe malaria: A practical handbook*. 3rd ed. World Health Organization. Global Malaria Program, Geneva, Switzerland.

WHO (2012b). *Disease Surveillance for Malaria Control: An Operational Manual*. Geneva, Switzerland, World Health Organization.

WHO (2013a). *Epidemiological Approach for Malaria Control: Guide for Participants*. 2nd ed. World Health Organization. Geneva, Switzerland.

WHO (2013b). World Malaria Report 2013. World Health Organization. Geneva, Switzerland.

WHO (2013c). *Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes.* WHO Document Production Services, Geneva, Switzerland. World Health Organization.

WHO (2015a). *Guidelines for the Treatment of Malaria*. 3rd ed. World Health Organization. Geneva, Switzerland.

WHO (2015b). World Malaria Report 2015. World Health Organization. Geneva, Switzerland.

WHO (2016). World Malaria Report 2016. World Health Organization. Geneva, Switzerland.

Worrall, E., Fillinger, U. (2011). Large-scale use of mosquito larval source management for malaria control in Africa: a cost analysis. *Malar. J.*, **10**:338.

Wotodjo, A. N., Diagne, N., Gaudart, J., Richard, V., Raoult, D., Cheikh Sokhna, C. (2015). Malaria risk factors in Dielmo, a Senegalese malaria-endemic village, between October and November of 2013: a case-control study. *Am. J. Trop. Med. Hyg*., **92**(3): 565-568.

Woyessa, A., Deressa, W., Ali, A., Lindtjørn, B. (2012). Prevalence of malaria infection in Butajira area, south-central Ethiopia. *Malar. J*., **11**:84.

Woyessa, A., Deressa,W., Ali, A., Lindtjørn, B. (2013). Malaria risk factors in Butajira area, south-central Ethiopia: a multilevel analysis. *Malar. J*., **12**:273.

Woyessa, A., Gebre-Micheal, T., Ali, A. (2004). An indigenous malaria transmission in the outskirts of Addis Ababa, Akaki Town and its environs. *Ethiop. J. Health Dev.,* **18**(1): 2-7.

Yadav, K., Dhiman, S., Rabha, B., Saikia, P. K., Veer, V. (2014). Socio-economic determinants for malaria transmission risk in an endemic primary health centre in Assam, India. *Infect. Dis. Poverty*, **3**:19.

Yamamoto, S., Louis, V. R. Sié, A., Sauerborn, R. (2010). Household risk factors for clinical malaria in a semi-urban area of Burkina Faso: a case-control study. *Trans. R. Soc. Trop. Med. Hyg*., **104**: 61-65.

Yé, Y., Hoshen, M., Louis, V., Séraphin, S., Traoré, I., Sauerborn, R. (2006). Housing conditions and *Plasmodium falciparum* infection: protective effect of iron-sheet roofed houses. *Malar. J.,* **5**:8.

Yé, Y., Louis, V. R., Simboro, S., Sauerborn, R. (2007). Effect of meteorological factors on clinical malaria risk among children: an assessment using village-based meteorological stations and community-based parasitological survey. *BMC Public Health*, **7**:101.

Yeshiwondim, A. K., Gopal, S., Hailemariam, A. T., Dengela, D. O., Patel, H. P. (2009). Spatial analysis of malaria incidence at the village level in areas with unstable transmission in Ethiopia. *Int. J. Health Geog.*, **8**:5.

Yimer, F., Animut, A., Erko, B., Mamo, H. (2015). Past five-year trend, current prevalence and household knowledge, attitude and practice of malaria in Abeshge, south-central Ethiopia. *Malar. J.*, **14**:230.

Yohannes, M., Boelee, E. (2012). Early biting rhythm in the afro-tropical vector of malaria, *Anopheles arabiensis*, and challenges for its control in Ethiopia. *Med. Vet. Entomol.,* **26:** 103-105.

Yewhalaw, D., Bortel, W. V., Denis, L., Coosemans, M., Duchateau, L., Speybroeck, N. (2010). First evidence of high knockdown resistance frequency in *Anopheles arabiensis* (Diptera: Culicidae) from Ethiopia. *Am. J. Trop. Med. Hyg.,* **83**(1):122-125.

Yewhalaw, D., Wassie, F., Steurbaut, W., Spanoghe, P., Bortel, W. V., Denis, L., Tessema, D. A., Getachew, Y., Coosemans, M., Duchateau, L., Speybroeck, N. (2011). Multiple insecticide resistance: an impediment to insecticide-based malaria vector control program. *PLoS ONE* **6**(1): e16066. doi:10.1371/journal.pone.0016066.

Zhou, G., Minakawa, N., Githeko, A. K., Yan, G. (2004). Association between climate variability and malaria epidemics in the East African highlands. *PNAS*, **101**(8): 2375-2380.

Zhou, G., Munga, S., Minakawa, N., Githeko, A. K., Yan, G. (2007). Spatial relationship between adult malaria vector abundance and environmental factors in western Kenya highlands. *Am. J. Trop. Med. Hyg.,* **77**(1): 29-35.

Appendices

Appendix 3.1. Environmental variables and distribution of *Anopheles* larvae the study sites at Ghibe River basin, southwestern Ethioia (November 2014-October 2016).

Environmental	*An. gambiae* s. l.			*An. christyi*			*An. pharoensis*		
Factors	Mean rank	U	*p*	Mean rank	U	*p*	Mean rank	U	*p*
Habitat permanence									
Temporary	81.69	3137	0.455	79.9	2960.5	0.019*	81.46	3115	0.047*
Permanent	87.37			89.96			87.69		
Presence of algae									
Present	90.08	2808	0.235	91.56	2722.5	0.09	84.85	3111.5	0.686
Absent	80.77			79.98			83.55		
Surface debris									
Absent	90.21	2117	0.009*	79.05	2267	0.00*	83.23	2746	0.429
Present	68.6			96.27			85.92		
Intensity of light									
Shade	41.5	228	0.028*	74	423	0.36	93.42	426.5	0.237
Lit	85.58			84.37			83.65		

	An. gambiae s.l.				*An. christyi*				*An. pharoensis*			
	Mean rank	χ^2	df	*p*	Mean rank	χ^2	df	*p*	Mean rank	χ^2	df	*p*
Water perimeter (m)												
<10m	85.96	5.17	2	0.075	83.45	2.13	2	0.343	81.3	68.74	2	0.00*

10-100m	83.3				90.88				83.23			
>100m	40.25				74				150			
Turbidity												
Low	87.31	0.58	2	0.748	90.33	5.34	2	0.06	85.71	1.95	2	0.376
Medium	80.54				79.9				85			
High	83.78				80.67				80.69			
Distance to nearest house (m)												
≤100	94.62	10.57	3	0.014*	76.04	24.91	3	0.00*	81.14	4.57	3	0.206
101-200	82.36				83.41				88.31			
201-300	63.26				99.83				84.69			
301-400	60.9				116.33				79			
Canopy cover												
Open	89.75	7.955	2	0.019*	84.42	0.454	2	0.797	81.54	9.469	2	0.009*
Tree	64.32				83.36				92.86			
Shrub	83.33				74				79			
Emergent plant												
Absent	93.22	8.65	3	0.034*	82.33	7.54	3	0.057	81.29	11.84	3	0.008*
Grass	71.81				91.57				80.28			
Weeds	89				82.3				86.75			
Grass + weeds	68.9				73.5				95.6			
Substrate type												
Gravel	71.69	2.066	2	0.356	94.44	21.06	2	0.00*	84.03	0.838	2	0.658
Mud	87.37				77.66				84.81			
Stone	78.42				100.24				81.3			

Appendix 5.1 Sporozoite rate and EIR of *An. arabiensis* in Ghibe study site of Ghibe River basin, southwestern Ethiopia (January 2015 - October 2016)

Months	Daily PvSR	Monthly PvEIR	Daily PfSR	Monthly PfEIR
Jan-15	0	0	0	0
Feb-15	0	0	0	0
Mar-15	0	0	0	0
Apr-15	0	0	0	0
May-15	0	0	0	0
Jun-15	0	0	0	0
Jul-15	0	0	0	0
Aug-15	0	0	0	0
Sep-15	0	0	0	0
Oct-15	0.09	1.86	0	0
Nov-15	0	0	0	0
Dec-15	0	0	0	0
2015 Total	**0.09**	**1.86**	**0**	**0**
Jan-16	0	0	0	0
Feb-16	0	0	0	0
Apr-16	0	0	0	0
May-16	0	0	0.1	1.22
Jun-16	0	0	0	0
Jul-16	0	0	0	0
Aug-16	0	0	0	0
Sep-16	0	0	0	0
Oct-16	0	0	0	0
2016 Total	**0**	**0**	**0.1**	**1.22**

PvSR = *P. vivax* sporozoite rate, PvEIR = *P. vivax* entomologic inoculation rate, PfSR = *P. falciparum* sporozoite rate, PfEIR = *P. falciparum* entomologic inoculation rate, results in bold showed annual EIR.

Appendix 6.1 Questionnaire prepared for the knowledge, attitude and practice study

Dear Respondent

Malaria represents one of the major causes of high rate of death and disability in most parts of Ethiopia and other region of the world especially the sub Saharan Africa. We are working a research on malaria and its vector mosquitoes. Kindly assist by filling the questionnaire yourse lf/ we will assist if you could not do so. The questions are designed to guide and help evaluate your understanding on malaria and its control methods that may affect the malaria prevalence and malaria vectors distributions.

In anticipation of your kind cooperation.

Thank you.

I. Socio-demography of the participant

a. Age of household head: __

b. Sex of household head: Male ☐ Female ☐

c. Educational level of household head:

No education ☐ primary ☐ Secondary ☐ more than secondary ☐

d. Location: Rural ☐ Urban ☐

e. Occupation of household head:

Farmer ☐ Employee ☐ Merchant ☐ if any_____

II. Risk factors associated with malaria infections

a. Number living in the house holds: age <5 _____ 6-12____ 13-17_____ 18-64____ >65____
b. Do you know how malaria is transmitted? Yes ☐ No ☐
c. If you know how it is transmitted? _____.
d. Source of water: Tap water ☐ Fetching from protected ☐ spring ☐ River ☐ Well ☐ Pond ☐ Open stream ☐
e. Latrine type: private toilet ☐ public toilet ☐ open field ☐
f. Housing condition:
   i) Roof material: iron sheet ☐ concrete ☐ tiles/asbestos ☐ thatched ☐ if any____
   ii) Presence of ceiling: yes ☐ no ☐
   iii) Wall material: mud ☐ brick ☐ wood/timber ☐ cement ☐ stones ☐ iron/metal ☐
   iv) Eves: present ☐ absent ☐
   v) Flooring material: Earth/sand ☐ dung ☐ wood plank ☐ palm/bamboo ☐

ceramic tiles ☐ cement ☐ carpet ☐

vi)Number of rooms per household member: 1 ☐ 2☐ >3 ☐

vii)Number of windows: 1 ☐ 2 ☐ 3 ☐ >3 ☐

viii) Presence of window screening: yes ☐ no ☐

iv) Number of doors: 1 ☐2 ☐ 3 ☐ >3 ☐

x) Presence of door screening: yes ☐ no ☐

g) Available mammal hosts kept in the compound: sheep ☐ goat ☐ cattle ☐

donkey ☐ hourse ☐ Chicken ☐ if any _____

h) Activities conducted during outdoors: farming ☐ water fetching ☐ keeping livestock ☐

i) The house received indoor residual spraying (IRS) in the previous years: Yes ☐ No ☐

j) The last time the house was sprayed with insecticides for malaria prevention: <6 months ☐
6-12 months ☐ > a year ☐

k) The last time the net was obtained and treated: <6 months ☐ 6-12 months ☐ > a year ☐

l) Do you use LLINs properly? Not used ☐ Always ☐ Sometimes ☐

m) How many LLINs are available in the room?_____

n) Physical assets owned by the household: motorbike ☐ bicycle ☐ fridge ☐
television ☐ radio ☐ mobile phone ☐ access to electricity ☐