UNIVERSITY OF NAIROBI

MALARIA TRANSMISSION DYNAMICS IN SENTINEL SITES AND MIXED CROP IRRIGATION SCHEME IN WESTERN KENYA

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A thesis submitted in fulfilment of the requirements for the award of the degree of Doctor of Philosophy in Applied Parasitology of the University of Nairobi

Department of Biology

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DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination or presented for a degree award in any other university.

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ABSTRACT

Knowledge of the effects of environmental changes on malaria vector bionomics and surveillance of malaria transmission is important in understanding malaria epidemiology. This study aimed to provide information on the effects of a recently established irrigation scheme in Kenya on the bionomics and transmission of malaria vectors. In addition, establish long-term changes in malaria transmission profiles and map hotspot transmission regions in western Kenya. Entomological and parasitological surveys were undertaken in Kombewa, Iguhu, and Marani, whereas only entomological surveys were conducted in irrigated and non-irrigated agroecosystems in Homa bay in western Kenya. Malaria vector speciation, blood meal sources, and sporozoite infections were analyzed using polymerase chain reaction (PCR). Malaria parasite species and gametocytes were identified microscopically. Maps of hotspots for malaria transmission were generated using ArcGIS software. The predominant malaria vector species was *Anopheles arabiensis* in Homa Bay County in western Kenya. The mean indoor and outdoor densities of *An. arabiensis* were higher in the irrigated study zone compared to the non-irrigated zone. The *An. funestus* mean indoor resting density was higher in the irrigated zone, while only a few *An. funestus* were collected in the non-irrigated zone. In the irrigated zone, the outdoor human blood index (HBI) of *An. arabiensis* was almost twofold higher (outdoor, 5.20%; indoor, 3.90%) than indoor, whereas in the non-irrigated zone, the blood meals were of bovine origin. The indoor HBI of *An. funestus* (49.43%) was 14-fold higher than that of *An. arabiensis* (3.44%) in the irrigated zone, whereas in the non-irrigated zone, none of the *An. arabiensis* tested positive for human blood. The annual entomological inoculation rates (EIRs) for *An. arabiensis* in the irrigated zone were 0.71 infective bites per person per year (ib/p/year) and were higher indoors (0.41 ib/p/year) than outdoors (0.30 ib/p/year). Malaria transmission was not detected in the non-irrigated zone. Asymptomatic malaria

parasite prevalence decreased in Kombewa, Iguhu, and Marani from 2005 to 2008. However, since 2011, parasite prevalence has resurged by >40% in Kombewa and Marani. Malaria vector densities have similarly shown reductions from 2005 to 2008 in Kombewa, Iguhu, and Marani; thereafter, they rose steadily until 2014 before decreasing. A greater infection risk was observed in Kombewa compared to Iguhu and Marani. The median time and probability of non-infection during malaria episodes were lowest in Kombewa compared to Iguhu and Marani. A gender bias towards males in infection was observed. During 2018 to 2019, the annual EIRs were 5.12, 3.65, and 0.50 (ib/p/year) at Kombewa, Iguhu, and Marani, respectively. Cold spots and hotspots of spatially clustered elevated vector abundance and *Anopheles* larval densities were detected in Marani. The results show that irrigated agriculture may cause an upsurge in malaria transmission. Malaria prevalence remains high or has resurged in some sites in western Kenya despite continuous intervention efforts. Targeted control of those at risk of asymptomatic infection who serve as human reservoirs might effectively decrease malaria transmission and prevent resurgences. Incorporation of additional vector control tools such as larval source management to complement the long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) in the irrigation scheme is recommended. Further studies on socio-behavioural factors and biological sex differences in malaria infection is advised. Geospatial technology is recommended for effective vector-borne disease prevention, control, and eventually elimination.

DEDICATION

I dedicate this thesis to my dear parents, Dr. Andrew M'mene and Mrs. Joyce Ondeto for their constant support and encouragement during the time of my study; my siblings Rebecca, Bradford, and Steverink for their moral support and push for tenacity; my amazing nieces Leticia, Mitchell, Laurelle, Gwendolyn, Aryiela, Olivia, and doting nephew C'morn for the joy they bring in my life.

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3. **Ondeto B.M.**, Zhong D., Atieli H., Zhou G., Lee M., Muriu S.M., Odongo D., Ochanda H., Githeko A., & Yan G. (2020, November). Effects of irrigation system on *Anopheles arabiensis* vectorial capacity in western Kenya. Poster presented at the 69th American Society of Tropical Medicine and Hygiene (ASTMH) Conference held at the Metro Toronto Convention Centre, Toronto, Ontario, Canada. https://www.astmh.org/getmedia/71a0e5e6-7c06-48a4-ab47-d50c4ec870a2/ASTMH-2020-Abstract-Book.pdf
4. **Ondeto B.M.**, Zhou G., Lee M., Atieli H., Muriu S.M., Odongo D., Ochanda H., Githeko A., & Yan G. **(**2019, November). Effect of mixed crop irrigation system on malaria vector population in western Kenya. Poster presented at the 68th ASTMH Conference held at the Gaylord National Resort and Convention Center, National Harbor, Maryland, United States of America (USA). https://www.astmh.org/ASTMH/media/2019-Annual-Meeting/ASTMH-2019-Abstract-Book.pdf
5. **Ondeto B.M.**, Zhou G., Lee M., Atieli H., Muriu S.M., Odongo D., Ochanda H., Githeko A., & Yan G. (2019, September). Effect of irrigation on malaria vector population in western Kenya. Poster presented at the 6th PAMCA Annual Conference and Exhibition held at the Hilton Hotel, Yaoundé, Cameroon.

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LIST OF ABBREVIATIONS

KEMRI- Kenya Medical Research Institute

CDC- Centers for Disease Control and Prevention

HLCs- Human landing catches

PSC- Pyrethrum spray catches

EIR- Entomological inoculation rate

ICEMR- International Center of Excellence for Malaria Research

HBI- Human blood index

IRS- Indoor residual spraying

ITNs- Insecticide-treated nets

LLINs- Long-lasting insecticidal nets

ACT- Artemisinin-based combination therapy

IPTi- Intermittent preventive treatment in infants

WHO- World Health Organization

CO_2- Carbon dioxide

DDT- Dichlorodiphenyltrichloroethane

DNA- Deoxyribonucleic acid

PBS- Phosphate-buffered saline

PCR- Polymerase chain reaction

GPS- Global positioning system

ddH_2O- double-distilled water

NBMM- Negative binomial mixed model

AIC- Akaike information criterion

ANOVA- Analysis of variance

MICE- Multiple imputation by chained equations

MUERC- Maseno University Ethics Review Committee

UCI IRB- University of California, Irvine Institutional Review Board

RNA- Ribonucleic acid

qPCR- Quantitative polymerase chain reaction

S.E.- Standard error

ZINB- Zero-inflated negative binomial

IQR- Interquartile range

CHAPTER 1

1.0 INTRODUCTION

1.1 Background

Globally, malaria continues to be a serious health concern; in 2021, approximately 247 million cases were reported, leading to over 619,000 deaths, with 96% of these deaths occurring in Africa (WHO, 2022b). According to WHO (WHO, 2022b), 12,000 malaria-related fatalities and an estimated 3.4 million malaria cases were recorded in Kenya in 2021.

Malaria is caused by a protozoan of the genus *Plasmodium* (Sato, 2021). The human malarial parasites *Plasmodium falciparum, P. vivax, P. ovale, P. malariae,* and *P. knowlesi* are exclusively transmitted by *Anopheles* mosquitoes (Nicoletti, 2020). In Africa, *Anopheles gambiae* complex and *An. funestus* group are the dominant malaria vectors, with minor roles being played by *An. coustani* and *An. pharoensis* (Afrane, Bonizzoni, & Yan, 2016; Wiebe *et al*., 2017). *Anopheles gambiae, An. coluzzi,* and *An. arabiensis* predominate in the *An. gambiae* complex, whereas *An. funestus* s.s. predominates in the *An. funestus* group (Massey *et al*., 2016; Wiebe *et al*., 2017). The vectorial capacity of these vectors is attributed to their vector-host contact, a crucial aspect in malaria transmission that is highly influenced by their densities, longevity, and anthropophilic behavior, upon which vector control strategies have been formulated (Brady *et al*., 2016).

In Africa, chronic hunger, food insecurity, and famine continue to affect millions of people (Wudil *et al*., 2022), largely as a result of the region's heavy dependence on rain-fed farming and natural or anthropogenic changes to the physical environment (Sasson, 2012; Wudil *et al*., 2022). In an effort to curb food insecurity, the government of Kenya, in partnership with development partners, has supported irrigated agriculture (NIA, 2019). Environmental changes, for instance, the building of dams and agricultural irrigation systems, have been linked to an increase in vector-

borne diseases that are endemic, such as malaria, schistosomiasis, and onchocerciasis (Myers, 2012). This could be attributed to an increase in breeding habitats and enhanced habitat stability and productivity. In studies carried out, the construction of dams and irrigation schemes has caused an upsurge in malaria, while in other studies there has been no impact or, in some cases, a decrease in malaria transmission (Hawaria & Kibret, 2023; Mary *et al.*, 2023; Muriuki *et al*., 2016). Consequently, the effect of environmental changes on malaria transmission differs and relies upon a number of factors, such as mosquito ecology, epidemiological setting, socioeconomic conditions, and existing malaria control strategies (Keiser *et al*., 2005).

Long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and administration of artemisinin-based combination therapy (ACT) are the primary malaria intervention strategies in Kenya (National Malaria Control Programme (NMCP), Kenya National Bureau of Statistics (KNBS), 2016). The World Health Organization (WHO) has reported a reduction in malaria-related deaths and attributes this to interventions (WHO, 2020). Despite the milestones that have been achieved in malaria control, malaria programs face numerous challenges. The extensive coverage of LLINs and application of IRS may have caused: a shift in vector composition; an increased proportion of *An. arabiensis* replacing *An. gambiae* (Carnevale & Manguin, 2021); a shift to early biting (Ojuka *et al*., 2015); a shift to outdoor biting (Ototo *et al*., 2015); and the emergence of insecticide resistance. Moreover, the development of drug resistance threatens the effectiveness of malaria treatment (Kozlov, 2021). In studies carried out in western Kenya, these interventions have led to different outcomes in malaria control, with some areas indicating a decline in malaria transmission, while in others transmission has remained unchanged or has resurged (Zhou *et al*., 2016).

Malaria transmission hotspots are locations within a larger transmission area where the transmission intensity is noticeably greater compared with the usual level in that context (Carter & Mendis, 2002). Numerous markers, including parasitological, serological, and entomological, can be used to identify hotspots of malaria transmission (Bousema *et al*., 2012). Identifying malaria hotspots is critical for malaria elimination efforts. Hotspots signify areas where targeted control actions and interventions can be carried out, which are likely to be more effective than untargeted interventions (Bousema *et al*., 2012).

The purpose of the research was to evaluate the effect of a recently established irrigation scheme in Homa Bay, in western Kenya, on vector bionomics and malaria transmission. Secondly, the study aimed to evaluate long-term changes in the malaria transmission profile in Kombewa, Iguhu, and Marani in western Kenya. Lastly, the study provided essential information on malaria transmission hotspots in a Kenyan highland in western Kenya. These findings will be used to evaluate new interventions, provide crucial data on the effectiveness of core vector interventions, and help policymakers plan and guide future malaria interventions.

1.2 Research Objectives

1.2.1 General Objectives

To assess the malaria transmission dynamics in sentinel sites and mixed crop irrigation scheme in malaria lake endemic and highland epidemic prone areas in western Kenya

1.2.2 Specific Objectives

1. To evaluate the effect of mixed crop irrigation scheme on malaria transmission in Homa bay in western Kenya.

2. To assess the malaria transmission dynamics in sentinel sites in malaria lake endemic areas in western Kenya.
3. To determine the malaria transmission hotspots in a highland epidemic prone area in western Kenya.

1.3 Research Hypothesis

1. Environmental modification (irrigation schemes) has an impact on malaria vector bionomics and transmission.
2. High coverage with malaria control interventions has led to long-term changes in the malaria transmission profile.
3. Multi-layer data (i.e., entomological and parasitological) can be used to identify malaria transmission hot spots precisely.

1.4 Research Questions

1. What is the impact of environmental modifications (irrigation schemes) on malaria vector bionomics and transmission?
2. What are the long-term changes in the malaria transmission profile in sites with heterogeneous malaria transmission and high coverage of malaria control interventions in western Kenya?
3. Can the use of multi-layer data (i.e., entomological and parasitological) be used to identify malaria transmission hot spots precisely?

1.5 Problem Statement

The government of Kenya, together with international aid agencies, has undertaken agricultural irrigation farms to curb food insecurity and alleviate poverty. However, the impact of these environmental changes on the exposure and susceptibility of humans to vector-borne infectious diseases is not fully understood. Current malaria interventions targeting indoor biting mosquitoes have been reported to lead to a shift in vector behavior and species composition. An update on long- term evaluation of changes in vector behaviour, species composition, and malaria transmission profiles following intensified malaria control interventions in western Kenya is lacking.

1.6 Justification

The research seeks to evaluate the impact of environmental modification activities (irrigation schemes) on the adult malaria vector species composition, abundance, and transmission intensity in western Kenya. The impact of environmental modifications on malaria transmission will provide critical information for improving malaria control and elimination efforts in dynamic situations that are influenced by water resource projects. The study will also evaluate baseline information related to malaria parasite prevalence and vector densities as well as long-term changes in malaria transmission profiles and patterns of asymptomatic malaria infection in three sites in western Kenya. Long-term surveillance of malaria transmission dynamics is an essential intervention as it evaluates the impact of the current interventions, accurately measures the changing malaria epidemiology, and directs the planning of future control and elimination efforts. Moreover, the study endeavors to evaluate the occurrence of malaria hotspots in a low malaria transmission site in western Kenya that is prone to epidemics. Targeted control of pockets of

transmission might effectively reduce infection, potentially decrease malaria transmission, and prevent resurgences.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1. Malaria Parasite and Vector

Protozoa belonging to the genus *Plasmodium* are the causative agents of malaria (Sato, 2021). The human malaria parasites *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi* (Sato, 2021) are transmitted by mosquitoes in the genus *Anopheles* (Nicoletti, 2020). In humid Africa, the vectorial system is dominated by *Anopheles gambiae* and *Anopheles coluzzi* (Coetzee *et al*., 2013); in arid regions, *Anopheles arabiensis* predominates, whereas *Anopheles funestus* is ubiquitous (Coetzee, 2004). *Anopheles arabiensis* is anthropophilic or zoophilic, rests indoors or outdoors, and is distributed in dry savannah environments and woodlands; however, it can occur in forested areas where land disturbances or clearance have occurred (Coetzee *et al*., 2000b). *Anopheles gambiae*, *An. coluzzi*, and *An. funestus* are mainly anthropophilic, endophagic, and endophilic (Sinka *et al*., 2010).

2.2. Life Cycle of *Plasmodium* and Mosquito

2.2.1 Life Cycle of *Plasmodium*

Fig. 2.1 depicts the *Plasmodium* developmental phases. Female *Anopheles* mosquitoes are the definitive hosts, while humans are the intermediate hosts. The parasitized female *Anopheles* mosquito inoculates the human host with sporozoites while it ingests blood. Sporozoites invade liver cells and reproduce asexually over 7 to 10 days, maturing into schizonts before bursting and releasing merozoites. Merozoites enter the erythrocytes, reproduce asexually, and progress through the ring, trophozoite, and schizont stages. Merozoites bud from the mature schizonts, which rapture, releasing merozoites that enter more erythrocytes and repeating the cycle. Some of the

merozoites differentiate into gametocytes. The female *Anopheles* mosquito suck blood from a human, having both male (microgametocytes) and female (macrogametocytes) that develop into gametes in the mosquito's gut. Fertilization of the microgametes and macrogametes leads to a zygote, which matures into a motile ookinete that penetrates the mosquito's midgut wall and develops into an oocyst. The oocyst undergoes multiple rounds of asexual multiplication, grows, ruptures, and releases sporozoites in the mosquito's body cavity, which subsequently travel and infect the mosquito's salivary glands, completing the life cycle (Fujioka & Aikawa, 1999).

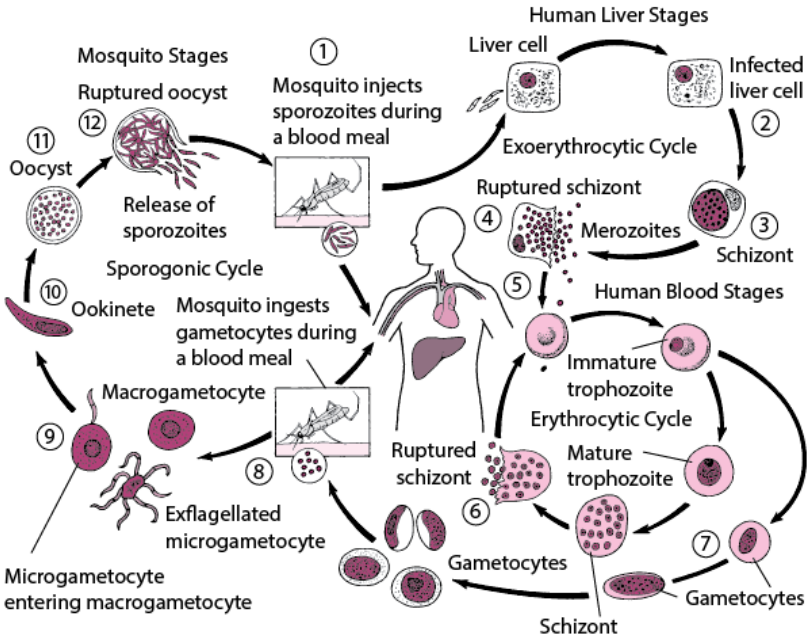


Fig. 2. 1 Life cycle of malaria parasite.

Source: https://www.msdmanuals.com/professional/multimedia/table/plasmodium-life-cycle accessed on 23/05/2022.

2.2.2 Life Cycle of Mosquito

As illustrated in Fig. 2.2, a mosquito's development phases comprise the egg, larva, pupa, and adult. Depending on the species, female mosquitoes lay their eggs either on water surfaces or moist

soil. Eggs hatch into larvae after 24 to 48 hours, depending on the temperature. The larva molts four times and grows larger after each molt. During the larval stage, the larva is active and breathes using a siphon or spiracles, depending on the species. Depending on the availability of food and environmental conditions, they can take 5 days, weeks, or even months in the larval stage before pupating into a pupa. The pupa is in the resting stage and does not feed; however, the pupa are mobile and breathe using 'trumpets'. The pupa takes 1-3 days before emerging into an adult. The adult feeds on plant sugars such as nectar for their source of energy. Blood feeding and mating occur a couple of days after the adults emerge. The female seeks vertebrate hosts for blood for the development of her eggs. The entire life cycle of mosquitoes can take 10 days or 1 year, depending on temperature and species characteristics.

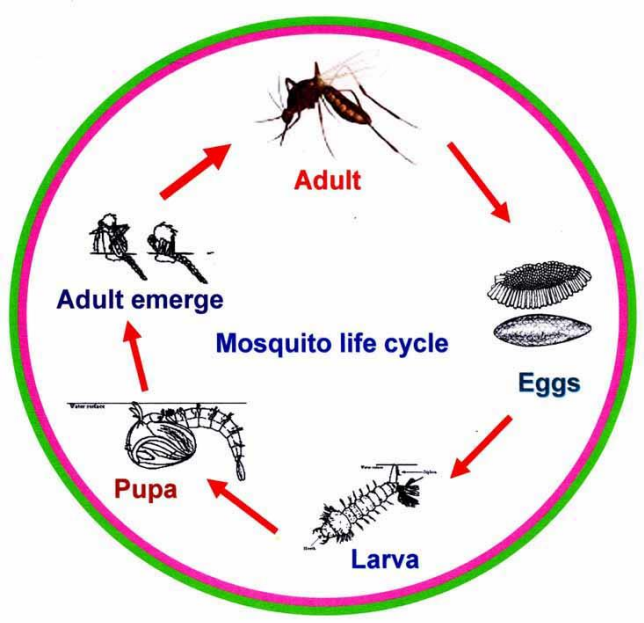


Fig. 2. 2 Life cycle of mosquito

Source: http://healthylifedigest.blogspot.com/2009/02/studying-mosquito-life-cycle-to-best.html accessed on 23/05/2022.

2.3. Malaria Mode of Transmission

The primary mode of malaria transmission is by the bite of an infected female *Anopheles* mosquito (WHO, 2012). Other modes of transmission include congenital transmission, which involves the transfer of parasitized red cells from an infected mother to a child either transplacentally or during parturition (WHO, 2012). Congenital malaria has been reported due to the four common human *Plasmodium* species in both malaria-endemic and non-endemic areas (Harrington & Duffy, 2008). First reported in 1911, malaria can also be transmitted by the transfusion of blood from infected people (Fong, 2020). In endemic areas, the risk of acquiring transfusion malaria is higher compared to non-endemic areas (Fong, 2020). The use of contaminated needles and syringes among drug addicts or accidental needle-stick injuries among health care professionals have also been reported to transmit malaria (WHO, 2016b).

2.4. Epidemiology of Malaria

Malaria still remains a public health concern that continues to cause disease and death, as indicated in World Malaria Reports (WHO, 2019, 2020, 2021). According to a recent report, the malaria burden remains highest in sub-Saharan Africa, with approximately 95% of malaria cases and deaths occurring there (WHO, 2021). In sub-Saharan Africa, most malaria deaths are caused by *Plasmodium falciparum* and mainly occur in children under the age of 5 (WHO, 2021). *Plasmodium vivax* is an important cause of malaria illness in Southeast Asia and South America (Menkin-smith & Winders, 2022). Malaria epidemiology varies geographically depending on malaria transmission intensity in an area and is classified as holo-, hyper-, meso-, and hypo-endemic. Hence, based on the intensity of malaria transmission, populations or regions can be classified as stable malaria areas: Transmission occurs all year round; older children and adults

have acquired partial immunity; young children are susceptible to severe malaria; or unstable malaria areas: Intermittent malaria may be annual, biannual, or vary; malaria epidemics are common; malaria immunity is usually low or absent (WHO, 2012). Malaria endemicity classification is based on parasite prevalence and spleen rates in children aged 2-9 years, and clinical classification is based on two major forms, uncomplicated and severe malaria (WHO, 2012).

The pattern and intensity of malaria transmission are greatly influenced by climatic factors, mainly temperature, rainfall, and relative humidity (Hay *et al*., 2000). The variations in minimum and maximum temperatures affect the development of the malaria parasite and mosquito vector. The optimum temperature needed for the parasite to complete its development in mosquitoes is 27°C, and 25-27°C is needed for the development of mosquito larvae (Christiansen-Jucht *et al*., 2015; Shapiro *et al*., 2017). Mosquitoes breed in water, so the right amount of rainfall is crucial for them to breed. Malaria vectors mainly prefer breeding in stagnant freshwater collections created after rainfall (Sinka *et al*., 2011; Sinka *et al*., 2010). The relative humidity affects the activity and survival of mosquitoes. Relative humidity above 60% is favorable for the mosquito to live at least 8-10 days to transmit the malarial parasite (Bayoh, 2001).

Non-climatic factors also affect malaria transmission, and these include: (1) the type of vector in an area. It is only *Anopheles* mosquitoes that exclusively transmit human malaria parasite and this species differ in their capacity to transmit the parasite (Nicoletti, 2020); (2) the type of malaria parasite influence the intensity of malaria transmission and *Plasmodium falciparum* is known to be virulent (Otto *et al*., 2018); (3) agricultural development, irrigation channels, dams, and ponds, create breeding sites for mosquitoes which may increase malaria transmission (Hawaria & Kibret, 2023; Mary *et al*., 2023); (4) malaria incidence is generally lower in urban settings compared to

rural settings due to limited breeding sites, polluted water that is unfavorable for mosquito breeding, and more access to health care and malaria prevention interventions in urban settings (Doumbe-Belisse *et al*., 2021). *Anopheles stephensi*, a novel invasive species that colonizes plastic and man-made containers, may increase malaria transmission in urban areas of Africa (Takken & Lindsay, 2019). Migration or recurrent visits by persons from malaria-endemic areas to urban areas in search of opportunities for work and education may further aggravate the situation (Sinka *et al*., 2020; Takken & Lindsay, 2019); (5) migration and movement of populations from areas free of malaria or low transmission to high transmission areas. This poses a problem as the migrants lack immunity against the disease and, in addition, may bring back the parasite to malaria-free areas, thus leading to epidemics (Martens & Hall, 2000); (6) the level of immunity to malaria in the human host affects the pattern of malaria transmission and severity of disease (Doolan *et al*., 2009). Those with weak immunity, such as pregnant women and children under the age of 5, are at high risk (Doolan *et al*., 2009). While malaria control strategies have focused on pregnant women and children under the age of 5, there is an emerging risk among school-age children aged 5 years to teen age, whose parasite prevalence in asymptomatic infections has risen and serves as human reservoirs (Laishram *et al*., 2012; Mensah *et al*., 2021; Walldorf *et al*., 2015). If this group is not targeted in malaria control, they may increase transmission and frustrate malaria elimination efforts (Mensah *et al*., 2021); (7) mosquitoes may develop insecticide resistance, which implies that they are no longer killed by the insecticides used in vector strategies such as LLINs and IRS (Riveron *et al*., 2018); and (8) the development of drug resistance by the parasite due to repeated use or improper use of the antimalarial drugs may lead to patients not being cured and the parasite being transmitted to susceptible individuals (Hanboonkunupakarn & White, 2016).

2.5. Malaria Vector Behaviour

Malaria vector behaviours include host-seeking, blood-feeding, and resting behaviours that are crucial factors in malaria transmission. Understanding malaria vector behavior is important for planning effective control measures and accelerating progress towards malaria elimination.

2.5.1 Host-seeking Behaviour

Host-seeking behavior is defined as the in-flight orientation of an avid female toward a potential blood meal host (Bowen, 1991). Host-seeking normally begins shortly after sunset (Laarman, 1958; Reisen, Lothrop, & Meyer, 1997). Cues that mosquitoes use to locate a host are CO_2 from exhaled breath, body odours, the host's visual silhouette, and elevated levels of heat and humidity (Takken & Knols, 2010). One of the key factors in malaria transmission is the level of human-vector contact, which is estimated by calculating the entomological inoculation rate (EIR) (Amoah *et al*., 2021). Estimating the EIR is critical for determining the intensity of malaria transmission and assessing the effectiveness of vector control interventions (Ferguson *et al*., 2010). To estimate EIR, host-seeking *Anopheles* mosquitoes must be sampled to determine the human biting rate and sporozoite infection rate, both of which are important in calculating EIR (Hay *et al*., 2000).

Human landing catches (HLC) have been considered the gold standard method for estimating mosquito-human contact. The limitation of this method is that it is a labour-intensive procedure requiring highly trained collectors, extensive supervision, variation in the skill of the collectors or their individual attractiveness to mosquitoes, and ethical concerns associated with potential exposure to infectious mosquito bites (Gimnig *et al*., 2013; Kilama *et al*., 2014; WHO, 2013). Hence, other methods have been employed to study mosquito host-seeking behaviour indoors and/or outdoors, including Centers for Disease Control and Prevention (CDC) light traps (indoor and outdoor), human-baited double net traps (outdoor), human-odor-baited CDC light

traps (outdoor), animal-baited traps (indoor and outdoor), odor traps (outdoor), Ifakara tent traps (outdoor), and Mbita traps (indoor and outdoor) (Degefa *et al*., 2020; Govella *et al*., 2009; Mathenge *et al*., 2004; Tangena, Thammavong, Hiscox, Lindsay, & Brey, 2015; WHO, 2013). Nevertheless, the limitation of these methods is that they don't allow hourly anopheline collections to determine the biting cycle which is mainly from HLC.

2.5.2 Blood Feeding Behaviour

Mosquito blood feeding behaviour is classified into two categories: (i) an anthropophilic mosquito, defined as a mosquito that prefers feeding on human hosts (WHO, 2016c); and (ii) a zoophilic mosquito, which prefers feeding on animal hosts (Durnez & Coosemans, 2013). *Anopheles* mosquitoes with a tendency to feed on human blood are considered important vectors of malaria (Killeen, 2014). For a mosquito to transmit the malaria parasite to humans, it must ingest at least two blood meals to facilitate parasite uptake and transmission (Shaw *et al*., 2020). Host feeding preferences in mosquitoes are partly genetically determined, but other factors also play an important role, including host availability, nutritional requirements, intrinsic host preferences of the species, and vector density (Martínez-de la Puente, Dunn, & Gangoso, 2021). The choice and preference of *Anopheles* mosquito hosts depend on the local ecological conditions and are site-specific (Garrett-Jones, Boreham, & Pant, 1980).

Traditional techniques for blood meal identification, precipitin tests, and enzyme-linked immunosorbent assays (ELISA) have limitations in their ability to identify vertebrate hosts to only those whose anti-sera have been generated, low species-specificity, and restricting identification to order, family, or genus (Borland & Kading, 2021; Burkot *et al*., 1981; Washino & Tempelis, 1983). Advancement in technology has led to the development of molecular techniques such as multiplexed polymerase chain reaction (PCR), real-time PCR, high-resolution melting analysis,

digital PCR, next-generation sequencing, microsphere assays, mass spectrometry, and stable isotope analysis that have high species-specificity and enable genus- and species-level identification (Borland & Kading, 2021; Kent & Norris, 2005).

2.5.3 Resting Behaviour

Mosquito resting behavior is classified into two groups: (i) an endophilic mosquito, defined as a mosquito that prefers resting indoors, inside a human dwelling (Pates & Curtis, 2005); and (ii) an exophilic mosquito, which prefers resting outdoors, outside the human dwelling. In sub-Saharan Africa, *An. gambiae* and *An. funestus* are largely endophilic (Githeko, Service, Mbogo, & Atieli, 1996), although exophily has been reported in the presence of Insecticide-treated nets (ITNs) and IRS (Durnez & Coosemans, 2013). In contrast, *An. arabiensis* is more exophilic (Mahande *et al*., 2007; Mnzava et., 1995), although endophily has been reported (Fornadel & Norris, 2008). Mosquito resting behavior is relatively plastic, with potential for variation between and within species (Githeko *et al*., 1996; Lines *et al*., 1986). Malaria vectors' resting behavioral plasticity maintains residual malaria transmission and reduces the effectiveness of the core malaria vector interventions (Killeen & Chitnis, 2014).

Various collection methods are used to sample mosquitoes resting indoors or outdoors at natural or artificial resting sites (van de Straat *et al*., 2021). These collection methods include pyrethrum spray catch/collection (PSC) (indoor), pit trap/shelter (outdoor), clay pot (outdoor), sticky pot (outdoor), resting box (outdoor), sticky resting box (indoor, outdoor), barrier traps (outdoor), urine-trap (outdoor), malaise trap (outdoor), manual and prokopack aspirator (indoor, outdoor) (Degefa *et al*., 2019; Odiere *et al*., 2007; Pombi *et al*., 2014; van de Straat *et al*., 2021; Vazquez-Prokopec *et al*., 2009; WHO, 1975).

2.6. Impact of Environmental Modification on Malaria Transmission

Environmental modifications such as the construction of dams and agricultural irrigation schemes have been linked to an increase in vector-borne diseases that are endemic, such as malaria, schistosomiasis, and onchocerciasis (Gbakima, 1994). This could be attributed to an increase in suitable breeding habitats and enhanced habitat stability and productivity (Briet *et al*., 2003; Diuk-Wasser *et al*., 2005; Ijumba *et al*., 2002; Muturi *et al*., 2006).

Several examples of water development projects have led to variations in the impact of malaria transmission in Latin America, Asia, and the Pacific. The transmission pattern in India's Punjab region switched from malaria epidemic-prone to endemic due to irrigation (Sharma & Mehrotra, 1982). Malaria rates quadrupled in regions irrigated by India's Bargi dam (Singh & Mishra, 2000). Dams and irrigation schemes have been widely built throughout Southeast Asia, resulting in extensive deforestation and a dramatic decline in the highly effective forest malaria vector *An. dirus* (Sanchez-Ribas *et al*., 2012). The majority of manmade lakes in Latin America have been connected to worsening health conditions in high-risk locations, owing mostly to the comeback of numerous vector-borne diseases (Sanchez-Ribas *et al*., 2012).

Irrigated agro-ecosystems in Africa have been reported to either increase or reduce malaria transmission; however, in some cases, irrigation schemes have no impact on malaria transmission (Faye *et al*., 1995; Sissoko *et al*., 2004; Yohannes *et al*., 2005). In the Gezira-Managil scheme in Sudan and the irrigation scheme in Cameroon, there has been an increase in malaria transmission (el Gaddal *et al*., 1985; Robert *et al*., 1992). Irrigated agro-ecosystems have also been reported to change malaria transmission patterns from seasonal to perennial or increase the degree of endemicity (el Gaddal *et al*., 1985). In contrast, a reduction in malaria transmission was reported in irrigated rice cultivations in Mali (Sissoko *et al*., 2004) and Lower Moshi, Tanzania (Ijumba *et*

al., 2002) as compared to the adjacent non-irrigated areas, and this could be attributed to increased wealth that was implicated in the increased acquisition and use of insecticide-treated nets and anti-malarial drugs in irrigation projects, leading to reduced malaria incidence (Diuk-Wasser *et al*., 2005; Henry *et al*., 2003; Ijumba *et al*., 2002). The introduction of an irrigation scheme in the Senegal River Delta and central Côte d'Ivoire had no impact on malaria transmission (Diakité et al., 2015; Faye et al., 1995).

Several water resource development initiatives in Kenya have produced or exacerbated malaria. The Kano irrigation scheme is reported to have generated optimal breeding environments for *Anopheles gambiae*, resulting in increasing malaria intensity in the area (Muriuki *et al*., 2016; Mutero *et al*., 2000). Malaria transmission has shifted from seasonal to perennial in the Hola-Bura irrigation schemes (Mutero *et al*., 2000). Muturi et al. reported in the Mwea irrigation scheme that there was no difference in malaria transmission by *An. arabiensis* between irrigated and non-irrigated areas but reduced malaria transmission by *An. funestus* in irrigated areas (Muturi *et al*., 2008). Malaria has become endemic in the Pekerra irrigation scheme as a result of the formation of permanent breeding grounds for *Anopheles gambiae* as a result of the irrigation of meadows using water diverted from irrigation canals (Amadi, 2018; GOK, 1987). The hydro-electric generating reservoirs of Masinga, Kindaruma, Gitaru Kamburu, and Kiambere, which run along the Tana River, have altered the ecology of the area, altering topographic and hydrological aspects and increasing the prevalence of malaria (GOK, 1988; Okuku *et al*., 2015).

The variation in the impact of water development projects on malaria transmission is likely dependent on the ecology of local mosquito vectors, underlying ecological factors, epidemiologic setting, socioeconomic conditions, and existing malaria control measures (Keiser *et al*., 2005), whose complexity can only be understood through site-specific evaluation of these parameters.

2.7. Malaria Hot Spots

Malaria transmission hotspots are defined as geographical areas within a wider area of transmission in which the transmission intensity is significantly higher than the average level in the surrounding area of that setting and are widely observed in malaria-endemic regions (Carter & Mendis, 2002). Markers that are used to identify malaria hot spots include asymptomatic parasite carriage, reported fever, serological findings, and mosquito densities (Bousema *et al*., 2010). The identification of malaria transmission hot spots is significant for malaria control and elimination efforts. Hotspots signify areas where targeted control interventions can be carried out and are expected to be more efficient than untargeted interventions (Bousema *et al*., 2012). Spatially targeted interventions supplement LLINs and intermittent preventive treatment (IPT) to reduce malaria transmission (Bousema *et al*., 2012). Targeted interventions in hot spots of malaria transmission can be achieved through targeted vector control activities, targeted interventions to reduce the human infectious reservoir by reactive screening and treatment with antimalarial drugs, and targeted vaccination of high-risk groups (Bousema *et al*., 2012).

2.8. Malaria Control Interventions

2.8.1 Malaria Vector Control

Various methods are used in the control of malaria vectors, which include chemical control, biological control, and environmental control.

2.8.1.1 Chemical Control

Currently, the main methods of adult malarial vector control are IRS and ITNs, both of which use chemicals to kill the malaria vectors (Greenwood, 2009) that bite and rest indoors (WHO, 2013).

Insecticides recommended by WHO for the control of malaria vectors are synthetic compounds that are divided into four classes: organochlorines (e.g., DDT, dieldrin), organophosphates (e.g., malathion, fenitrothion), carbamates (e.g., propoxur), and pyrethroids (e.g., permethrin, deltamethrin, and lambdacyhalothrin). Insecticide-treated nets have been regarded to reduce morbidity and mortality in adults and children under the age of five in highly malaria-endemic countries (Aregawi *et al*., 2011; Lengeler, 2004; O'Meara.,2010; WHO, 2020). Indoor residual spraying programs have also been successful in several parts of sub-Saharan Africa (Fullman *et al*., 2013; Mabaso *et al*., 2004; Oguttu *et al*., 2017).

The challenge facing this control strategy is the development of insecticide resistance. Insecticide resistance is defined by the WHO (WHO, 1957) as 'the development of an ability or strain of some organisms to tolerate doses of a toxicant that would prove lethal to a majority of individuals in a normal population of the same species'. The mechanisms of insecticide resistance in malaria vectors that have been described are altered target site of action for the insecticide, metabolic resistance, reduced penetration of the insecticides, and behavioural resistance (Hemingway, 2000; Hemingway & Karunaratne, 1998; Plapp, 1976; WHO, 1957).

2.8.1.2 Biological Control

The use of the bacterial pathogens *Bacillus thuringiensis israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) in the control of immature stages of malaria vectors by disrupting the midgut lining of mosquito larvae has been reported (Das & Amalraj, 1997; Fillinger *et al*., 2003). The use of these products is environmentally safe for humans and non-target organisms, easy to handle, cost-effective, capable of being produced locally, and doesn't require expensive equipment for application (Becker *et al*., 1992; Fillinger *et al*., 2003).

The use of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* is being tested against adult malaria vectors (Mnyone *et al*., 2009; Scholte *et al*., 2008; Scholte *et al*., 2004). The method involves spraying walls with suspension fungal spores, and upon exposure, the fungus invades and multiplies inside the mosquito, killing it within 15 days and thus reducing parasite transmission (Blanford *et al*., 2005; Scholte *et al*., 2005). The challenges facing these methods of control are fungal spore viability, fungal specificity, and the development of resistance in mosquitoes (Blanford *et al*., 2005; Kanzok & Jacobs-Lorena, 2006; Scholte *et al*., 2005; Scholte *et al*., 2004).

According to WHO, genetic control is 'the use of any condition or treatment that can reduce the reproductive potential of noxious forms [of the insect] by altering or replacing the hereditary material' (WHO, 1964).The sterile insect technique is a genetic control method that is still under study. The technique entails the mass rearing and release of sterile males, which, upon mating with the wild females, are unable to produce viable offspring, thus leading to the decline or eradication of the population (Knipling, 1955). The male mosquitoes are sterilized by irradiation or chemosterilization (Knipling, 1955). This method has been successful in controlling agricultural pests and has the potential to be integrated into the malaria vector control strategy (Townson, 2009). The challenges facing this control strategy are the loss of male fitness after sterilization and the need to produce sufficient numbers of sterile males (Alphey, 2002; Dyck *et al*., 2005; Phuc *et al*., 2007; Sharma *et al*., 1978; Thomas *et al*., 2000).

The other genetic control strategy is gene drives defined as 'selfish genetic elements that are transmitted to progeny at super-Mendelian (>50%) frequencies' (Bier, 2022). Gene-drive elements can be used to suppress mosquito populations or modify them, rendering them incapable of transmitting pathogens (Nash *et al*., 2019). Gene drives have the potential to completely change

vector management and disease control. They provide a potentially effective strategy for battling infectious diseases and lessening their impact. The challenges with this control strategy are that it is still being studied to determine its efficacy and safety; it can take many generations for drives to spread throughout populations; in some countries, the public has a negative perception of genetically modified organisms; there are ethical concerns; there may be unintended consequences; and there are regulatory obstacles (Collins, 2018; Kormos *et al*., 2022).

2.8.1.3 Environmental Control

Environmental management for malaria vector control is the planning, organization, carrying out, and monitoring of activities for the modification and/or manipulation of environmental factors or their interaction with man with a view to preventing or minimizing vector propagation and reducing man-vector pathogen contact (Esslinger, 1981). The malaria vector control strategies involve a well-constructed drainage system to prevent the formation of small water bodies suitable for mosquito larvae, the installation of house screens, the use of larvivorous fish, settlements away from vector sources, and personal protection (Shiff, 2002; Walker & Lynch, 2007). This approach has had some success in controlling mosquitoes in some countries, including Rome, Israel, Brazil, and Egypt, and it is still used as an alternative approach in some areas (Killeen *et al*., 2002; Utzinger *et al*., 2001). The challenge facing this control strategy is draining temporary water habitats formed during the rainy season; despite this challenge, there has been renewed interest in integrating this approach into malaria vector control (Ault, 1994). The efficacy of environmental control strategies has been found to depend on adapting such approaches to match the habitat requirements of the local vector species and local environmental conditions, conducting entomological monitoring, integrating these strategies into agricultural practices, and coordinating activities at the local and regional level (Lacey & Lacey, 1990; Walker & Lynch, 2007).

Larval source management (LSM) is the management of aquatic habitats (water bodies) that are potential larval habitats for mosquitoes, in order to prevent the completion of development of the immature stages (WHO, 1964). Larval source management (LSM), as a supplementary tool for malaria control in Africa, has recently attracted fresh interest (Fillinger & Lindsay, 2011). Reduction in malaria burden has been reported where LSM has been integrated with core vector control interventions (Fillinger, Ndenga, Githeko, & Lindsay, 2009). In many African countries, LSM is still less frequently used to control malaria due to a lack of understanding of the ecology of the local larval mosquito vectors, the expense of wide scale implementation, dedicated and laborious implementation (Dambach *et al*., 2016; Gowelo *et al*., 2020; Worrall & Fillinger, 2011). Habitat modification and larviciding are the most common methods of LSM. Habitat modification comprises manipulating larval habitats by draining water, filling, and leveling land, which permanently disrupts habitats and makes them unsuitable for mosquito breeding (Karunamoorthi, 2011). Larviciding is often achieved with the application of a bacterial larvicide, such as *Bti* or *Bs* (Walker & Lynch, 2007).

2.8.1.4 Integrated Vector Management

Adoption of a variety of control techniques through Integrated Vector Management (IVM) has gained favor in an effort to curb the spread and impact of malaria. An IVM approach is ‘a rational decision-making process for the optimal use of resources for vector control’ (Beier *et al*., 2008). It is essentially a management strategy for increasing the efficiency, effectiveness, and ecological soundness of vector control treatments given the tools and resources available (Chanda *et al*., 2008). Geographical information system (GIS) advancements have helped to more exact mapping of mosquito species distribution, breeding regions, and disease transmission. These can be utilized

in IVM to direct targeted control efforts, increase cost-effectiveness, and reduce unnecessary ecological disruption or damage.

2.8.2 Malaria Case Management and Prevention

2.8.2.1 Malaria Diagnosis and Treatment

Malaria case management includes prompt parasitological diagnosis and effective treatment (WHO, 2022a). The methods used in the parasitological diagnosis of uncomplicated malaria are light microscopy and rapid diagnostic tests (WHO, 2012). In addition, molecular diagnosis (PCR), which is highly sensitive, may be applied in research settings for the identification of morphologically similar species (*P. malariae* and *P. knowlesi*), to distinguish new infections from relapse and recrudescence, and for surveillance in areas where malaria elimination is ongoing (Tedla, 2019). The WHO recommends the use of ACTs for the effective treatment of *falciparum* malaria. These combinations include artemether plus lumefantrine, artesunate plus amodiaquine, artesunate plus mefloquine, artesunate plus sulfadoxine-pyrimethamine, and dihydroartemisinin plus piperaquine, which are recommended as first-line antimalarial treatments (WHO, 2022a).

2.8.2.2 Malaria Prevention

The WHO recommends Intermittent Preventive Treatment with Sulfadoxine Pyrimethamine (IPTp-SP) for all pregnant women living in areas of moderate-to-high malaria transmission in Africa (WHO, 2014, 2022a). The WHO also recommends the administration of chemopreventive measures such as seasonal malaria chemoprevention and intermittent preventive treatment in infants (IPTi) in areas of moderate-to-high transmission in Africa to reduce the risk of infection and disease (WHO, 2014, 2022a). Another preventive malaria measure recommended by WHO is the widespread use of the RTS,S/AS01 (RTS,S) malaria vaccine among children in regions with moderate to high *P. falciparum* malaria transmission (WHO, 2022a).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Study Sites

3.1.1 Homa Bay

The study was conducted in Kimira-Oluch Irrigation Scheme (0°26'44" S; 34°31'28.0" E) and its vicinity, which lies in an area of 110 km^2 and is located approximately 10 km north of the town of Homa Bay, in Homa Bay County, western Kenya (Fig. 3.1). Homa bay County is a semi-arid, malaria endemic area (Omondi *et al*., 2022) that experiences a bimodal rainfall pattern, with a mean annual rainfall of 1,226 mm. The long rainy season occurs between April and June, while the short rainy season occurs from October to November. The hot and dry season is from January to March. The mean annual temperature is 25.7 °C, with a minimum of 18.3 °C and a maximum of 29 ^{o}C. Relative humidity varies from 52% to 67%. The study site was stratified into irrigated and non-irrigated zones depending on proximity to the irrigation scheme. Each zone consisted of 10 clusters (cluster radii varied from 0.25-1 km) with populations ranging from 50-250 residents in each cluster. The irrigated zone is within a concrete canal and flood irrigation systems (Fig. 3.2). The crops grown under this irrigation scheme mainly include maize, beans, kales, tomatoes, pawpaw, bananas, watermelons, and rice grown in paddies. The non-irrigated zone is located about 5-10 km from the irrigated zone.

In an effort to reduce malaria burden in the lake endemic zone, vector control interventions were instigated between 2006 and 2008 through the use of LLINs and IRS. The first mass LLIN distribution occurred in 2006, followed by successive rounds of distribution in 2011, 2014, 2017, and 2021 (MOH, 2016b; 'National Malaria Control Programme- Kenya', 2021; Ng'ang'a, Aduogo, & Mutero, 2021). Insecticide residual spraying was first implemented in Rachuonyo

district in 2008, followed by successive rounds in 2009 to 2012 and 2017 to 2021 in targeted areas (Gimnig *et al*., 2016; PMI, 2013; PMI, 2021). According to a recent study conducted by Orondo *et al*. (Orondo *et al*., 2021) at the study site, the use of LLINs and IRS in the irrigated and non-irrigated zones is similar.

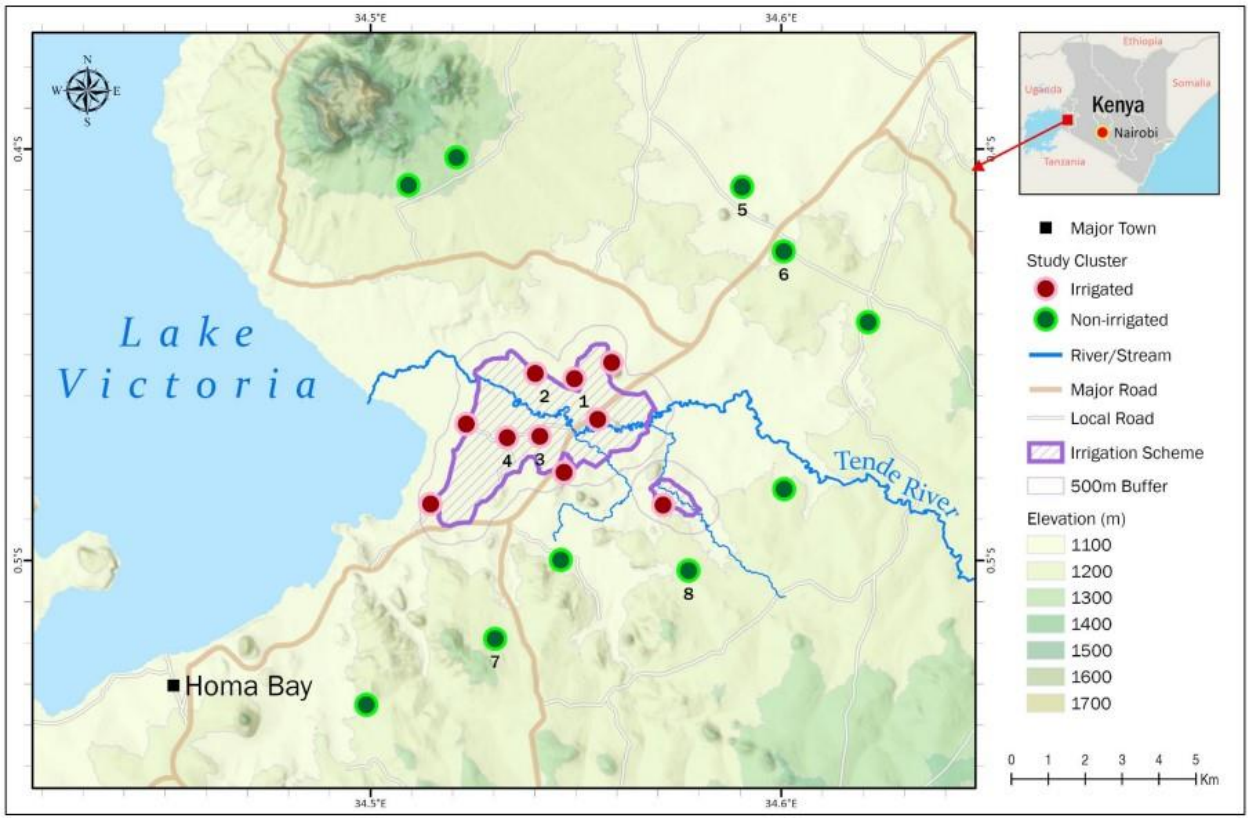


Fig. 3. 1 The site map indicates the study clusters in Homa Bay, Kenya.

The red dots represent the clusters within the irrigated zone and the green dots represent the clusters within the non-irrigated zone. Clusters labelled with number 1 ~ 8 also have been surveyed monthly for malaria vectors population dynamics research.

Fig. 3. 2 The images indicate the (a) flood, (b) and (c) concrete canal irrigation systems in the irrigated zone in Homa Bay in western Kenya.

3.1.2 Iguhu, Kombewa, and Marani

The study was also conducted at three sites with different altitudes, topographies, and malaria transmission intensities in western Kenya (Fig. 3.3). Parasitological surveys were conducted in selected government primary schools, while entomological surveys were conducted in households located within the study sites. The first study site was Kombewa (0°07'10" S; 34°29'04" E), located in Kisumu County, and a lowland setting with an altitude of 1150-1300 m above sea level. Kombewa has approximately 23,000 inhabitants, occupying a rolling terrain bisected by small streams with vast mosquito breeding habitats. This site has a holoendemic transmission intensity. Parasitological surveys were conducted in four selected primary schools (Akonya, Diemo, Kamonye, and Okode) located within the study site (Kapesa *et al*., 2018). The second study site was Iguhu (0°08'53" N; 34°47'16" E), located in Kakamega County, and a highland site with an altitude of 1430-1580 m above sea level. Iguhu has approximately 24,000 residents and is

characterized by large, flat-bottomed valleys with slow-flowing streams, thus leaving stagnant water in some areas, which favours *Anopheles* breeding. This site has mesoendemic transmission intensity. Parasitological surveys were conducted in two selected primary schools, Ivonda and Iguhu, located within the study site (Kapesa *et al*., 2018). The third study site was Marani (0°35'13" S; 34°48'11" E), located in Kisii County, and a highland site with an altitude of 1540-1740 m above sea level. Marani has approximately 19,000 residents and is characterized by very narrow valleys, which permit water to flow very fast, leading to few mosquito breeding habitats. This site has a hypoendemic transmission intensity and is prone to malaria epidemics. Parasitological surveys were conducted in three selected primary schools (Gesangora, Nyasaga, and Kiraeni) (Kapesa *et al*., 2018) and selected households located within the study site.

The climate in western Kenya consists mainly of a bimodal pattern of rainfall: a long rainy season between April and June and a short rainy season between October and November (Zhou *et al*., 2011). The hot and dry season is from January to February, while the cool and dry season is from July to September (Zhou *et al*., 2011). All sites have shown variations in monthly cumulative precipitation and monthly mean maximum and minimum temperatures, ranging from 29.1°C and 14.5°C, respectively (Kapesa *et al*., 2017; B. Ndenga *et al*., 2006; Zhou *et al*., 2011).

Plasmodium falciparum is the primary malaria parasite species in the three sites (Zhou *et al*., 2011). The first mass distribution of LLINS in 2006 in western Kenya led to a decline in both asymptomatic malaria and clinical cases (Kapesa *et al*., 2017). The second mass distribution in 2011 was characterized by a positive response at Iguhu, but Kombewa and Marani experienced sustained high *P. falciparum* transmission and infection resurgences, respectively, despite a third round of LLINs distributions in 2015 (Kapesa *et al*., 2017).

The predominant malaria vector species in the study sites are *Anopheles gambiae* s.s., *An. arabiensis,* and *An. funestus* (Degefa *et al*., 2017; Zhou *et al*., 2011). In the lowland site, *An. funestus* is the most abundant and infectious malaria vector, while in the highland sites, *An. gambiae* s.s. is the main vector responsible for Plasmodial transmission. Recent studies in this region have observed an increase in the proportion of *An. arabiensis* in the highlands as a result of vector interventions using LLINs and IRS; these measures may be suppressing the more anthropophilic and endophilic *An. gambiae* s.s. and killing fewer of the more zoophilic *An. arabiensis* (Ototo *et al*., 2011). Hence, high bednet coverage in western Kenya may explain decreases in vector densities of *An. gambiae* s.s. in the three sites, reductions of *An. funestus* in Iguhu and Kombewa, and temporal alterations in feeding behavior of *An. gambiae* to earlier host seeking (Ototo *et al*., 2015).

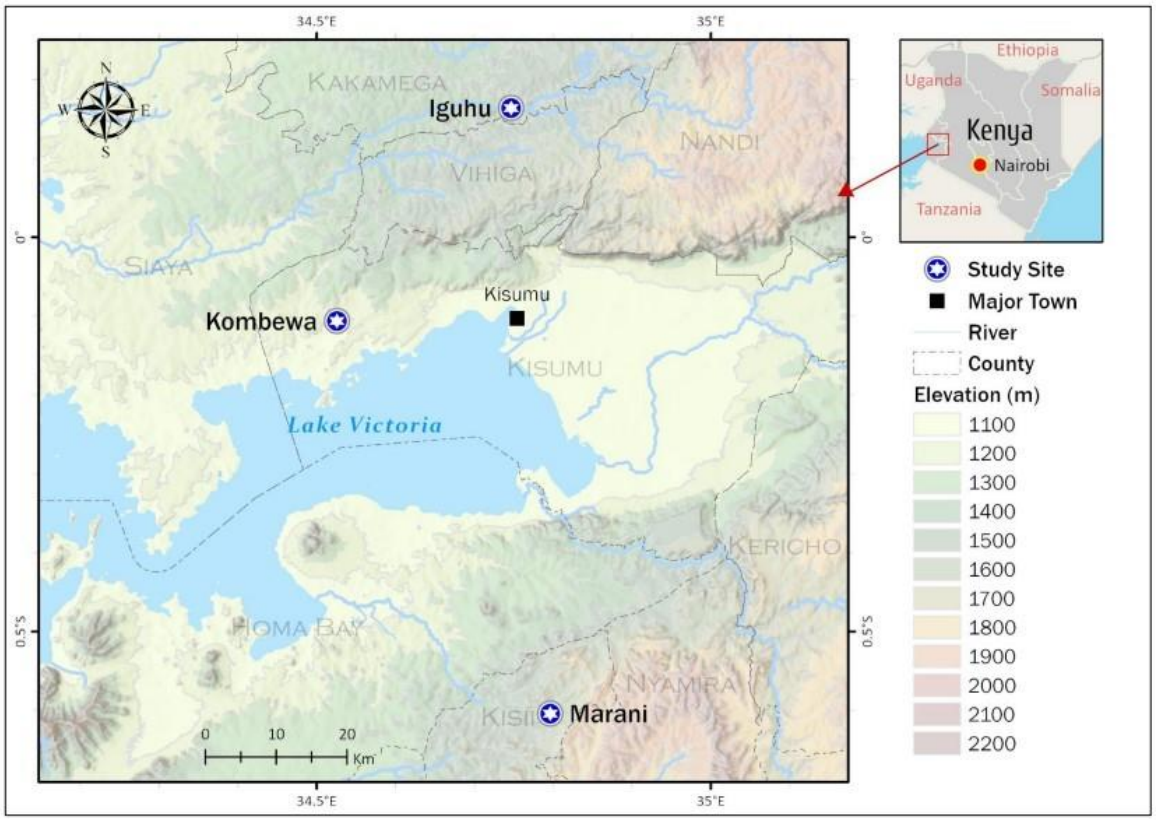


Fig. 3. 3 Map of the study sites in western Kenya.

3.2 Study Design

3.2.1 Study Design for Objective 1

Seasonal surveys were conducted in the dry (Jan-Mar) and wet (Apr-Jun) seasons in 2019 using five different trapping methods (Fig. 3.4). Indoor and outdoor host-seeking vector collection using CDC light traps and HLCs was undertaken in two randomly selected clusters in each zone. There were 160 trap-nights for each trap. Indoor and outdoor resting vector collection using PSCs (indoor), clay pots (outdoor), and pit shelters (outdoor) was undertaken in four randomly selected clusters in each zone. There were a total of 320 trap-nights for each trap and 144 for pit shelters. Longitudinal adult vector surveillance was conducted using PSCs in four clusters in each zone for malaria vector population dynamics research between 2018 and 2019 (Fig. 3.4).

3.2.2 Study Design for Objective 2

This study was based on longitudinal parasitological and adult vector surveillance that commenced in 2002 (Iguhu) and 2003 (Kombewa and Marani). Snapshots of this data were taken in a three-year interval from 2005 to 2014, which forms the baseline for the current study conducted between 2018 and 2019 (Fig. 3.4). Longitudinal adult vector surveillance was conducted monthly using PSC in Kombewa, Iguhu, and Marani between January 1st, 2018 and October 31st, 2019 (Fig. 3.4). A cohort of volunteer school-aged children aged 5-15 years were followed for monthly *Plasmodium falciparum* surveys between January 1st, 2018 and October 31st, 2019 in Kombewa, Iguhu, and Marani (Fig. 3.4). The parasitology survey was undertaken to determine malaria parasite prevalence, gametocyte prevalence, parasite density, and *P. falciparum* infection patterns that included duration and probability of non-infections.

The sample size calculation was based on the local parasite prevalence, ranged from 6.2 to 47.1% (Zhou *et al*., 2011). The sample size was calculated using a prevalence of 50%, which gives the best sample size. The sample size was calculated with a 95% confidence interval and a precision level of 5% (Munyekenye *et al*., 2005):

$$n = \frac{z^2 (pq)}{d^2}$$

Where:

n = sample size

z = the critical value of the standard normal distribution at the 5% level (1.96)

p = the malaria prevalence estimate

$q = 1 - p$

d = the precision level

At least 150 volunteer school children were sampled at each site and each month for parasite prevalence determination.

3.2.3 Study Design for Objective 3

A cross-sectional survey was conducted in the community to describe the asymptomatic parasite carriage, mosquito abundance (adult and larval mosquitoes), and the location of aquatic larval sites (Fig. 3.4). The survey was conducted in July and August 2018, after the long rainy season.

3.3 Laboratory Processes

3.3.1 Entomological Processes

All adult mosquitoes collected were transferred to the ICEMR laboratory in Homa Bay for further analysis. Mosquitoes were sorted and anophelines identified to species as previously described (Gillies & Coetzee, 1987). Female *Anopheles* mosquitoes were physiologically classified according to their gonotrophic stages, which were: unfed, blood-fed, half-gravid, and gravid. The samples were stored in individually labeled eppendorf tubes (1.5 ml) and preserved by refrigeration at -20 °C.

3.3.1.1 DNA Extraction

DNA was extracted from the legs and wings of each mosquito (for species identification), the abdomen of a freshly fed mosquito (for blood meal analysis), and the head and thorax of each mosquito (for sporozoite analysis) using the Chelex protocol by Musapa et al. (Musapa *et al*., 2013) with modifications. Briefly, the mosquito specimen was placed into eppendorf tubes (1.5 ml) and homogenized with 200 µl of 1X PBS/10% saponin solution. The submerged mosquito was ground into a uniform suspension and incubated at room temperature for 20 minutes. The samples were centrifuged at 14,000 x g for 2 minutes, and the supernatant was discarded. The pellet was

re-suspended in 200 µl of 1X PBS, centrifuged again at 14,000 x g for 2 minutes, and pipetted the supernatant. Samples were air dried for 10 minutes, and the pellet was re-suspended in 250 µl of 20% w/v Chelex-100 resin suspension in deionized water. The sample suspension was boiled in a water bath on a floating rack for 10 minutes. The sample was then centrifuged again at 14,000 x g for 1 minute, and the ensuing DNA solution was transferred into a pre-labeled storage vial for use as a template in PCR applications.

3.3.1.2 Sibling Mosquito Species Identification

Sibling species in the *An. gambiae* complex and the *An. funestus* group were speciated by conventional PCR as described by Scott *et al.* (Scott *et al.*, 1993) and Koekemoer *et al.* (Koekemoer *et al.*, 2002), respectively. For the *Anopheles gambiae* complex, amplification reactions were performed in volumes of 13 µl containing 6.5 µl of PCR green master mix (2x), 0.5 µl of each primer (10 µM) (UN, GA, AR) (Table 3.1), 4 µl of ddH_2O, and 1 µl of DNA extract. The amplification reaction consisted of an initial denaturation step of 95 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, with a final extension step of 72 °C for 6 min. The products of the PCR reaction were electrophoresed and visualized on a SmartGlow-stained 1.5% agarose gel along with a 100 bp DNA ladder. Positive results were interpreted by the presence of a band at the expected amplicon size (*An. gambiae* s.s.- 390 bp; *An. arabiensis*- 315 bp) (Scott *et al.,* 1993). For the *Anopheles funestus* complex, amplification reactions were performed in volumes of 13 µl containing 6.5 µl of PCR green master mix (2x), 0.5 µl of each primer (10 µM) (FUN, ITS2A) (Table 3.1), 4.5 µl of ddH_2O and 1 µl of DNA extract. The amplification reaction consisted of an initial denaturation step of 95 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 45 s, with a final extension step of 72 °C for 6 min. The products of the PCR reaction were electrophoresed and visualized on a

SmartGlow-stained 1.5% agarose gel along with a 100 bp DNA ladder. Positive results was interpreted by the presence of a band at the expected amplicon size (*An. funestus* s.s.- 505 bp) (Koekemoer *et al*., 2002).

3.3.1.3 Molecular Detection of Blood Meal Sources

The blood meal sources of freshly fed *Anopheles* mosquitoes were analyzed by multiplexed PCR. The PCR followed a protocol by Kent *et al*. (Kent & Norris, 2005) with slight modifications. Briefly, the reaction was performed in a final volume of 13 µl that contained 6.5 µl of PCR green master mix (2x), 0.5 µl of each primer (10 µM) (Pig573F, Human741F, Goat894F, Dog368F, Cow121F, and UNREV1025) (Table 3.1), 2.5 µl of ddH_2O, and 1 µl of DNA extract. The amplification reaction consisted of an initial denaturation step of 95 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, with a final extension step of 72 °C for 6 min. The products of the PCR reaction were electrophoresed and visualized on a SmartGlow-stained 1.5% agarose gel. Positive results were interpreted by the presence of a band at the expected amplicon size (pig - 453 bp; human - 334 bp; goat - 132 bp; dog - 680 bp; cow - 561 bp) (Kent & Norris, 2005).

3.3.1.4 Molecular Detection of Sporozoite Infections

The sporozoite infections in *Plasmodium* species of *Anopheles* mosquitoes were analyzed using the multiplexed real-time quantitative PCR (qPCR) assay. The assay was performed using the published species-specific 18 s ribosomal RNA probes and primers for *Plasmodium falciparum*, *P. malariae*, and *P. ovale* (Table 3.1) (Shokoples *et al*., 2009; Veron *et al*., 2009). Briefly, PCR was conducted in a final volume of 12 µl that contained 6 µl of PerfeCTa qPCR ToughMix, Low ROX Master mix (2X), 0.5 µl of each probe (2 µM), 0.4 µl of each forward primer (10 µM), 0.4 µl of each reverse primer (10 µM), 0.1 µl of double-distilled water, and 2 µl of sample DNA. The

amplification reaction consisted of a hold stage at 50 °C for 2 min and 95 °C for 2 min, followed by 45 cycles of 95 °C for 3 s and 60 °C for 30 s. The standard curve of a positive control containing plasmid DNA was included with 3 negative controls.

3.3.2 Parasitological Processes

A finger-prick sample was collected from each school-aged child, and thick and thin blood smears were prepared on labeled slides for examination at the ICEMR laboratory in Homa Bay. In the laboratory, the smears were stained with 4% Giemsa for 30 minutes. The stained smears were examined using magnification at 100x oil immersion to identify malaria parasite species and gametocytes. Malaria parasite counts were scored against 200 leukocytes when the slide was positive; however, the whole slide was carefully examined before declaring the slide negative. For quality control of the blood smear reading, a second microscopist carried out random checks on the slide counts (Munyekenye *et al*., 2005). Parasite density was expressed as parasites per µl, assuming a count of 8,000 white blood cells per µl of blood (Slutsker *et al*., 1994). Parasite density was calculated using the following formula (WHO, 2016a):

$$\text{Parasites / µl blood} = \frac{\text{No. of parasites counted x 8000 white cells/µl}}{\text{No. of white cells counted}}$$

3.4 Data Management and Analysis

Data were entered in Microsoft Excel 2010 datasheets, and analyses were done using R statistical software (version 4.0.3; R Foundation for Statistical Computing, Vienna, Austria) and JMP Pro 16 (SAS Institute, Inc.). Means (95% confidence interval, CI) and proportions were calculated for vector and parasite populations. The density of adult anopheline mosquitoes in each study site/zone was calculated as the average number of females per house per night (f/h/n). The human blood index (HBI) for each mosquito species was calculated as the proportion of mosquito samples that had fed on humans out of the total number tested (Garrett-Jones, 1964). Sporozoite rates for each

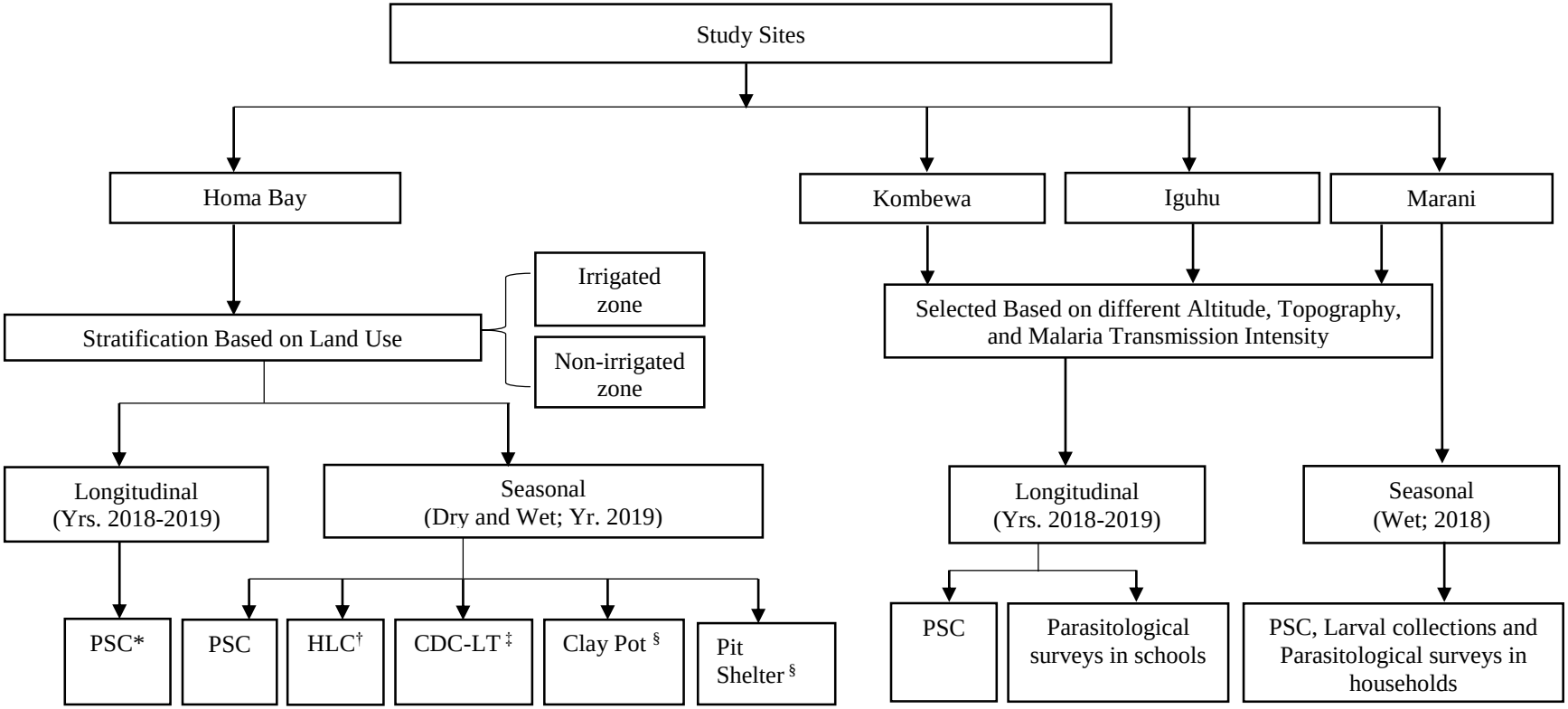
site/zone and vector species were calculated as the proportion of *Anopheles* mosquito samples positive for *Plasmodium* spp. out of the total number tested. The human biting rate was calculated as the product of blood-fed females per person per night and the HBI. Annual EIRs for each site/zone and vector species were calculated as the product of the sporozoite rate and the human biting rates (Macdonald, 1957).

In addition, the following analyses were carried out to evaluate the impact of mixed crop irrigation schemes on malaria transmission: Several models were evaluated for the analysis of vector density, and the model with the lowest akaike information criterion (AIC) and variables of interest was selected as the best model. In the analysis of seasonal data, a negative binomial mixed model (NBMM) was fitted to analyze *Anopheles* densities outdoors, indoors, and by trapping methods (HLC and CDC light trap). Zone and trapping methods were fitted as fixed variables in the outdoor and indoor models, while zone, trapping methods, and location (indoor and outdoor) were considered fixed variables in the trapping method model. In the indoor and trapping method models, house number and date were used as covariates, whereas house number and cluster were used as covariates in the outdoor model. In the analysis of longitudinal data, a NBMM with repeated measures was fitted to compare *Anopheles* densities and seasonality in the two zones by adjusting for months. Zone and season were fitted as the fixed variables, and year: (date: cluster), date: cluster, and cluster were considered as covariates. The Chi-square test was used to compare differences in vector species gonotrophic stage proportions between indoor and outdoor collections and also between zones.

To assess the malaria transmission in sentinel sites, the following analysis was also performed: The variations in parasite prevalence between different time periods at Kombewa, Iguhu, and Marani were compared using the Tukey-Kramer HSD test of analysis of variance

(ANOVA) with repeated measures. In addition, the differences in vector densities between different time periods at each site were compared using non-parametric Wilcoxon rank-sum tests. Means (95% confidence interval, CI) and proportions were calculated for vector and parasite populations. For the primary malaria species, *Plasmodium falciparum*, parasite/gametocyte prevalence for each site, each month, was expressed as the percentage of microscopically positive samples over the total number of samples tested. The Chi-square test was used to determine statistical differences in the parasite/gametocyte prevalence among the study sites and parasite prevalence by age and gender categories in each study site. Geometric mean parasite density and variations in proportion by month infected in the age and gender at each site were compared using Wilcoxon/Kruskal-Wallis tests. The variations in the distribution of the proportion of surveys being infected among the study sites were determined using the Tukey-Kramer HSD test of ANOVA. A Multiple Imputation by Chained Equations (MICE) simulation was done to impute the missing data in the time-to-event analysis. A Kaplan-Meier curve was built to analyze the probability of non-infection during malaria episodes at each study site. The log-rank test was applied to compare the probability of non-infection during malaria episodes in the three study sites, adjusted for multiple comparisons with Bonferroni corrections. Wald approximations were used for hazard ratio 95% confidence interval limit effects. Hazard ratios for the asymptomatic malaria infections were compared with proportional hazards fit by study sites, gender, and age groups. Vector density variation among study sites was compared using Wilcoxon/Kruskal-Wallis tests. Differences in the mean annual rainfall and mean annual maximum and minimum temperatures between the study sites were computed using the Tukey-Kramer HSD test of ANOVA with repeated measures.

Lastly, the following analyses were also carried out to determine the malaria transmission hotspots: Hotspots were defined spatially using the Getis-Ord Gi* statistic (Getis & Ord, 1992) and were used to detect three types of hotspots: hotspots of positive PCR tests; hotspots of adult *Anopheles* mosquito abundance; and hotspots of *Anopheles* larval densities. This statistic detects different spatial clustering patterns, such as hotspots and cold spots, and its statistical significance is determined. The Gi* statistic returned for each feature in the dataset is a z-score. For statistically significant positive z-scores, the larger the z-score, the more intense the clustering of high values (hot spot). For statistically significant negative z-scores, the smaller the z-score, the more intense the clustering of low values (cold spot). The Getis-Ord Gi* statistic was also used to assess the spatial co-location of *Plasmodium* infections and adult *Anopheles* mosquito abundance, and *Anopheles* larval densities. Mapping of hotspots and cold spots over the entire study area was done using ArcGIS software (version 10.3; ESRI, USA).



* PSC: Pyrethrum Spray Catches; Indoor only

† HLC: human landing catches; Indoor and outdoor

‡ CDC-LT: CDC light trap; Indoor and outdoor

§ Clay Pot and Pit Shelter; Outdoor only

Fig. 3. 4 Study design flow chart.

Table 3. 1 Primers and probes sequences for *An. gambiae* complex, *An. funestus* group, mammalian blood host, and *Plasmodium* spp. identification

Primer or probe	5' - 3' sequence	Product size (bp)
UN primer[a]	GTGTGCCCCTTCCTCGATGT	–
GA primer[a]	CTGGTTTGGTCGGCACGTTT	390
AR primer[a]	AAGTGTCCTTCTCCATCCTA	315
ITS2A primer[b]	TGTGAACTGCAGGACACAT	–
FUN primer[b]	GCATCGATGGGTTAATCATG	505
Pig573F primer[c]	CCTCGCAGCCGTACATCTC	453
Human741F primer[c]	GGCTTACTTCTCTTCATTCTCTCCT	334
Goat894F primer[c]	CCTAATCTTAGTACTTGTACCCTTCCTC	132
Dog368F primer[c]	GGAATTGTACTATTATTCGCAACCAT	680
Cow121F primer[c]	CATCGGCACAAATTTAGTCG	561
UNREV1025 primer[c]	GGTTGTCCTCCAATTCATGTTA	–
Fal-F primer[d]	CCG ACT AGG TGT TGG ATG AAA GTG TTA A	–
Falcprobe[d]	Quasar 670-AGC AAT CTA AAA GTC ACC TCG AAA GAT GAC T-BHQ-2	–
Viv-F primer[d]	CCG ACT AGG CTT TGG ATG AAA GAT TTT A	–
Vivprobe[d]	TAMRA-AGC AAT CTA AGA ATA AAC TCC GAA GAG AAA ATT CT-BHQ-2	–
Ova-F primer[d]	CCG ACT AGG TTT TGG ATG AAA GAT TTT T	–
Ovaprobe[d]	VIC-CGA AAG GAA TTT TCT TAT T-MGBNFQ	–
Mal-F primer[d]	CCG ACT AGG TGT TGG ATG ATA GAG TAA A	–
Malaprobe[d]	FAM-CTA TCT AAA AGA AAC ACT CAT-MGBNFQ	–

[a] *Anopheles gambiae* complex species-specific primer sequences (Scott *et al*., 1993).

[b] *Anopheles funestus* group species-specific primer sequences (Koekemoer *et al*., 2002).

[c] Species-specific cytochrome b primer sequence in mammalian blood hosts (Kent & Norris, 2005).

[d] *Plasmodium* species-specific primer and probe sequences (Shokoples *et al*., 2009).

CHAPTER 4

4.0 MALARIA VECTOR BIONOMICS AND TRANSMISSION IN IRRIGATED AND NON-IRRIGATED SITES IN WESTERN KENYA

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4.1 Abstract

Irrigation not only helps to improve food security but also creates numerous water bodies for mosquito production. This study assessed the effect of irrigation on malaria vector bionomics and transmission in a semi-arid site with ongoing malaria vector control program. The effectiveness of CDC light traps in the surveillance of malaria vectors was also evaluated relative to the HLC method. Adult mosquitoes were sampled in two study sites representing irrigated and non-irrigated agroecosystems in western Kenya using a variety of trapping methods. The mosquito samples were identified to species and assayed for host blood meal source and *Plasmodium* spp. sporozoite infection using PCR. *Anopheles arabiensis* was the dominant malaria vector in the two study sites and occurred in significantly higher densities in irrigated study site compared to the non-irrigated study site. The difference in indoor resting density of *An. arabiensis* during the dry and wet seasons was not significant. Other species, including *An. funestus*, *An. coustani* and *An. pharoensis* were collected. The *An. funestus* indoor resting density was 0.23 in irrigated study site while almost none of this species was collected in the non-irrigated study site. The HBI for *An. arabiensis* in the irrigated study site was 3.44% and significantly higher than 0.00% for the non-irrigated study site. In the irrigated study site, the HBI of *An. arabiensis* was 3.90% and 5.20% indoor and outdoor, respectively. The HBI of *An. funestus* was 49.43% and significantly higher compared to 3.44% for *An. arabiensis* in the irrigated study site. The annual entomologic inoculation rate for *An. arabiensis* in the irrigated study site was 0.41 and 0.30 infective bites/person/year indoor and

outdoor, respectively, whereas no transmission was observed in the non-irrigated study site. The CDC light trap performed consistently with HLC in terms of vector density. These findings demonstrate that irrigated agriculture may increase the risk of malaria transmission in irrigated areas compared to the non-irrigated areas and highlight the need to complement the existing malaria vector interventions with novel tools targeting the larvae and both indoor and outdoor biting vector populations.

Keywords: Irrigation, Vector density, Vector bionomics, Malaria transmission, *Anopheles*

4.2 Introduction

In Africa, food insecurity and famine continue to affect millions of people (Baro & Deubel, 2006). Given that nearly half of potential arable land in Africa occur in areas with irregular rainfall pattern, many countries have adopted irrigated agriculture as a key strategy to meet the rising demand for food (Blank, Mutero, & Murray-Rust, 2002). This effort has improved crop production by enabling the reclamation of arid and semi-arid lands, enhancing crop yield, extending the crop-growing season, and reducing the risk of crop failure (Keiser *et al*., 2005; Oomen, Wolf, & Jobin, 1988; Yohannes *et al*., 2005). In addition, irrigation projects have led to improved nutrition and socioeconomic conditions for the vulnerable population (Bryan *et al*., 2019). Despite these socioeconomic benefits, irrigated agriculture creates numerous water bodies that may support large populations of mosquitoes including malaria vectors although this may not necessarily lead to increased risk of malaria transmission (Muriu *et al*., 2008; Patz *et al*., 2004).

In Sudan, introduction of the Gezira-Managil scheme in the Nile river Valley led to an increased densities of *An. arabiensis* exacerbating malaria outbreaks (Oomen *et al*., 1988). Similarly, irrigation schemes increased vector densities and malaria incidences in Kenya (Muriuki

et al., 2016), Ethiopia (Kibret *et al*., 2014), and Malawi (Mangani *et al*., 2022). In contrast, reduction in malaria transmission was reported in irrigated rice cultivations of Mali (Sissoko *et al*., 2004) and Lower Moshi Tanzania (Ijumba *et al*., 2002) as compared to the adjacent non-irrigated areas. Reduced transmission could be attributed to, increased wealth that was implicated in the increased acquisition and use of insecticide treated nets (ITNs) and anti-malarial drugs in irrigation projects leading to reduced malaria incidence (Diuk-Wasser *et al*., 2005; Henry *et al*., 2003; Ijumba *et al*., 2002). However, in some cases introduction of irrigation schemes had no impact on malaria transmission (Diakité *et al*., 2015; Faye *et al*., 1995). Worthwhile noting is that in areas of stable malaria transmission, the introduction of irrigated agriculture has little or no impact on malaria transmission (Ijumba & Lindsay, 2001; Ijumba *et al*., 2002) nevertheless in semi-arid savannah zone of Africa irrigated rice cultivation can alter malaria transmission pattern from seasonal to perennial (Dolo *et al*., 2004; Sissoko *et al*., 2004). Hence, the impact of water development projects on malaria transmission is variable and likely depends on the ecology of local mosquito vectors, underlying ecological factors, epidemiologic setting, socioeconomic conditions, and existing malaria control measures (Keiser *et al*., 2005). Thus, its complexity can only be understood through site-specific evaluation of these parameters.

Insecticide-based vector control interventions mainly LLINs and IRS have been implemented to reduce malaria transmission with significant impacts. These tools have resulted in dramatic reduction in the proportion of endophagic and anthropophilic malaria vector species such as *Anopheles gambiae, An. coluzzii,* and *An. funestus* and a proportionate increase in *An. arabiensis*, which tend to be exophagic and less anthropophilic. However, previous studies indicate that vectors can develop resistance to insecticides or adapt to the presence of insecticides by becoming partially zoophilic and exophilic. Hence with the scale-up of LLINs and widespread use

of IRS, there is likely to be a shift in vector dominance from the highly endophilic *An. gambiae*/*An. coluzzi* and *An. funestus* to the more zoophilic and exophilic *An. arabiensis* (Abong'o *et al.*, 2020; Bayoh *et al.*, 2010; Futami *et al.*, 2014).

There is a pressing need to enhance our understanding on the effect of irrigation in a site where there is malaria vector control. The study aims to assess the effect of a recently established irrigation scheme in Homa Bay, Kenya on malaria vector bionomics and transmission. Vector control intervention using LLINs and IRS with organophosphate, pirimiphos-methyl (Actellic® 300CS) was being undertaken during the study period. Long-term success of the current malaria control efforts, ITNs and IRS, is dependent on continuous operational surveillance of the mosquito vectors, thus an effective mosquito sampling tool is required. Hence the secondary goal was to compare the trap effectiveness of CDC light traps against the gold standard, HLC. Results of this study will serve as the baseline vector bionomics and malaria transmission pattern for the evaluation of the success of core vector interventions and inform policy makers in planning and guiding future interventions especially in irrigated areas where there is scale up of LLINs distribution and application of IRS.

4.3 Materials and Methods

4.3.1 Seasonal Survey

4.3.1.1 CDC Light Traps

The CDC light traps were set both indoor and outdoor to assess vector host-seeking behavior (Fig. 4.1 a, b) (WHO, 1975). The indoor CDC light trap was set 1 m beside an occupied bed at a height of 1.5 m off-ground and the outdoor trap was set within 5 m away from the front door at a height

of 1.5 m off-ground. Vector collections were undertaken in five randomly selected houses in each cluster from 6 pm to 6 am for four consecutive nights once per season.

4.3.1.2 Human Landing Catches

Human landing catches were conducted both indoor and outdoor to assess vector host-seeking behavior (Gimnig *et al*., 2013; WHO, 2013). In each compound, vectors were collected indoors (at the house entrance) and outdoors 5 m away from the sentinel indoor collection house (Fig. 4.1 c, d). Collection was undertaken by four volunteers, two in each of the indoor and outdoor stations, who alternated after 6 hours. Hourly collections were done from 6 pm to 6 am each night, with 45 minutes of collection and a 15 minute break per hour. Each hourly collection was placed in individually labeled paper cups and maintained with a 10% sugar solution pad and then placed in a cool box. The same collectors conducted HLCs every night and were rotated between positions (indoor vs. outdoor). All collections were supervised by a team leader. Vector collections were undertaken in five randomly selected houses in each cluster for four consecutive nights once per season. All collectors were provided with anti-malarial chemo-prophylaxis during the study period.

4.3.1.3 Pyrethrum Spray Catches

Indoor resting vector collections using PSCs (Fig. 4.1 e) were undertaken in 20 randomly selected houses in each cluster once per season from 6 am to 9 am following WHO protocol (WHO, 1975).

4.3.1.4 Clay Pots

Outdoor resting vectors were assessed using clay pots outdoors (≤5 m away from the house) placed behind the house close to the bedroom where there is minimal human activities to minimize disturbance (Fig. 4.1 f). The 20 l capacity clay pots were ~0.5 m in height, 45 cm in diameter on wide base and a 20 cm diameter opening as described by Odiere *et al*. with modifications (Odiere

et al., 2007). During setting, the pots were filled with 2 l of rainwater to increase humidity (Ng'Habi *et al*., 2010) and tilted at 45° to the ground. Vectors collections were undertaken in 20 randomly selected houses in each cluster once per season. One pot was set at 6 pm in each of the 20 houses and mosquito collected the following morning between 6 am and 9 am using a hand-held Prokopack aspirator. The rainwater was then poured out to avoid creating vector breeding habitats.

4.3.1.5 Pit Shelters

Pit shelters were dug (1.5 m in depth, 1.5 m in length and 1 m in width) within 20 m of each selected house according to the method of Muirhead-Thomas (Fig. 4.1 g) (Muirhead-Thomson, 1958). In each of the four vertical sides, approximately 0.6 m from the bottom of the pit, cavities were dug to a depth of about 0.3 m. The mouth of the main pit was shaded from above using an artificial shelter. Vector collections were undertaken in one randomly selected house in each cluster for five consecutive nights monthly per season. Vector collection was undertaken between 6 am and 9 am inside the cavities by using a hand-held Prokopack aspirator according to WHO protocol (WHO, 1975). To prevent the development of vector breeding habitats, water that filled the pit shelters during the rains was drained out to avoid creating vector breeding habitats.

Fig. 4. 1 Vector sampling tools used for outdoor and/or indoor host-seeking/ resting malaria vector.

(**a**) Indoor CDC light trap, (**b**) outdoor CDC light trap, (**c**) indoor human landing catches, (**d**) outdoor human landing catches, (**e**) pyrethrum spray catches, (**f**) clay pot, (**g**) pit shelter). Pictures were captured in the field.

4.3.2 Longitudinal Surveillance

Temporal indoor resting vector population abundance was determined by conducting monthly surveys by PSCs in five randomly selected houses in each cluster. Application of IRS was undertaken in the study area by the National Malaria Control Program (Kenya) during the dry seasons in February of 2018 and 2019.

4.3.3 Vector Species Identification

All adult mosquitoes collected were transferred to the ICEMR laboratory in Homa Bay, sorted and anophelines identified morphologically to species as previously described (Gillies & Coetzee, 1987). Female *Anopheles* mosquitoes were physiologically classified according to their gonotrophic stages: unfed, blood-fed, half-gravid, and gravid. For species identification, DNA was extracted from the legs and wings of each specimen using the Chelex protocol by Musapa *et al*. (Musapa *et al*., 2013). Sibling species in *An. gambiae* s.l. and *An. funestus* were speciated by conventional PCR as described by Scott et al. (Scott *et al*., 1993) and Koekemoer et al. respectively (Koekemoer *et al*., 2002).

4.3.4 Molecular Detection of Blood Meal Sources and Sporozoite Infections

The abdomen of *Anopheles* mosquito specimens were carefully separated from the head and thorax and DNA extracted (Musapa *et al*., 2013). The blood meal sources of each freshly fed *Anopheles* mosquitoes were analyzed by multiplexed PCR as described by Kent et al. (Kent & Norris, 2005).

The DNA extracted (Musapa *et al*., 2013) from the head and thorax of each mosquito specimen was used to determine sporozoite infections of *Plasmodium* spp. by using the multiplexed real-time qPCR assay. The assay was performed using the published species-specific 18 s ribosomal RNA probes and primers for *Plasmodium falciparum*, *P. malariae and P. ovale* (Shokoples *et al*., 2009; Veron *et al*., 2009).

4.3.5 Ethics approval and consent to participate

Prior to beginning of the study, ethical approval was obtained from the Maseno University Ethics Review Committee (MUERC Protocol No. 00456) (**Appendix 1 and 2**) and the University of California, Irvine Institutional Review Board (UCI IRB). Permission was obtained from household heads and chiefs of the study site. Written informed consent was obtained from household heads

and all collectors (**Appendix 3**). The collection methods used in this study were undertaken in accordance with the principles of the Declaration of Helsinki.

4.4 Results

4.4.1 Seasonal Survey

4.4.1.1 Vector Species Composition

A total of 3,556 female *Anopheles* mosquitoes belonging to four species were collected using the five trapping methods during the study period. *Anopheles gambiae* s.l. was the predominant anopheline species accounting for 79.2%, followed by *An. coustani* (15.6%), *An. pharoensis* (4.6%), and *An. funestus* group (0.6%). In addition, 1,140 male *Anopheles* mosquitoes and 17,387 *Culex* species were collected (males, n=3,776; females, n=13,611). A total of 958 specimens (941 *An. gambiae* s.l. and 17 *An. funestus*) were analyzed for sibling species identification. Of these, 765 (81.3%) *An. gambiae* s.l. and 7 (41.2%) *An. funestus* were successfully amplified and all were confirmed as *An. arabiensis* and *An. funestus* s.s., respectively.

4.4.1.2 Indoor and Outdoor Vector Density

The mean density of the female *An. arabiensis* mosquitoes varied by zone and collection method (Fig. 4.2, Table 4.1, Table 4.2, Table 4.3, Table 4.4). Only few An. *funestus* were collected in the trapping methods, the mean density was not analyzed.

In the irrigated zone, the outdoor mean density of *An. arabiensis* was significantly higher compared to the non-irrigated zone ($Z = -8.276$, $df = 776$, $P < 0.001$) (Fig. 4.2, Table 4.1, Table 4.2). Similarly, in the irrigated zone, the indoor mean density of *An. arabiensis* was significantly higher compared to the non-irrigated zone ($Z = -9.403$, $df = 628$, $P < 0.001$) (Fig. 4.2, Table 4.1, Table 4.3).

Pit shelters (Z = 6.433, df = 776, $P < 0.001$) and clay pots (Z = 3.117, df = 776, $P < 0.01$) yielded a significantly higher outdoor density of *An. arabiensis* than CDC light traps whereas, the difference in outdoor mean density of *An. arabiensis* between HLC and CDC light trap was not significant (Z = 0.966, df = 776, $P = 0.334$) (Table 4.2). There was no significant difference in the indoor mean density of *An. arabiensis* from PSC and HLC compared to CDC light traps (All, $P > 0.001$) (Table 4.3).

Overall, the mean density of *An. arabiensis* was higher in the irrigated zone than in the non-irrigated zone. There was no significance difference in the mean density of *An. arabiensis* during the dry and wet seasons (All, $P > 0.001$) (Table 4.2, Table 4.3, and Table 4.4).

4.4.1.3 HLC and CDC Light Trap Comparison

The HLC and CDC light trap yielded a significantly higher host-seeking density of *An. arabiensis* in the irrigated zone than non-irrigated zone (Z = -9.841, df = 631, $P < 0.001$) (Fig. 4.2, Table 4.4). There was no significant difference between HLC and CDC light traps in terms of the *An. arabiensis* mean host-seeking density (Z = 0.351, df = 631, $P = 0.725$) (Fig. 4.2, Table 4.4). The results indicated that CDC light trap performed consistently with HLC in terms of vector density. The mean indoor and outdoor host-seeking density of *An. arabiensis* from HLC and CDC light traps collections varied significantly (Z = -3.175, df = 631, $P < 0.01$) with the highest mean host-seeking density collected indoors (Fig. 4.2, Table 4.4).

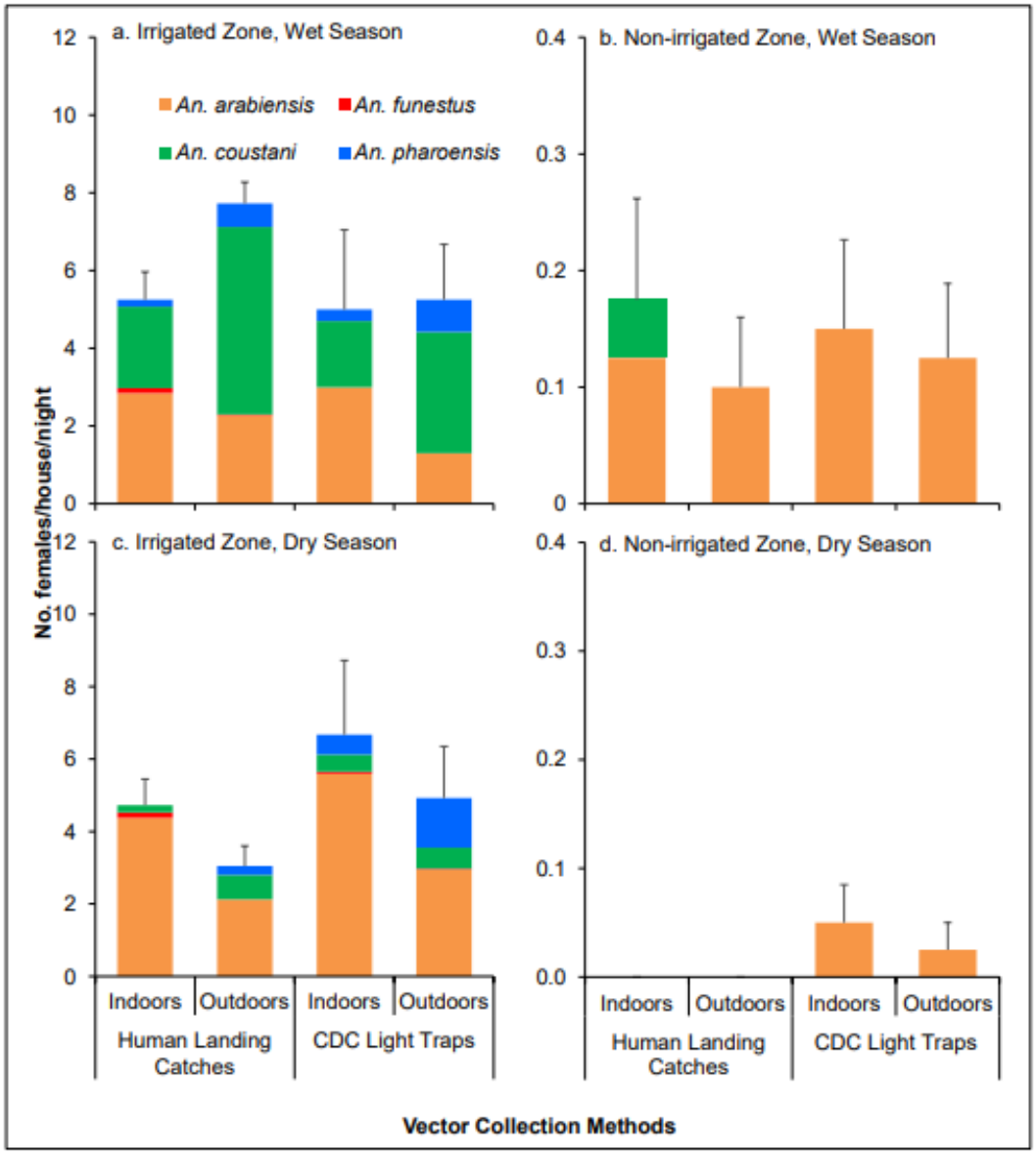

Fig. 4. 2 Host-seeking female *Anopheles* mosquito densities collected in 2019.

Error bars were for the standard error for the total *Anopheles* mosquitoes collected. n=160 trap-nights for each trap.

Table 4. 1 Resting densities of female *Anopheles* mosquito

Study site and Species	Dry Season			Wet Season		
	Indoor	Outdoor		Indoor	Outdoor	
	PSC[a]	Clay pot	Pit shelter	PSC	Clay pot	Pit shelter
Irrigated zone						
An. arabiensis	4.36 (2.89, 5.84)	3.34 (2.29, 4.39)	9.75 (5.26, 14.24)	2.08 (0.88, 3.27)	1.88 (1.27, 2.48)	11.42 (8.50, 14.34)
An. funestus	0.01 (0, 0.04)	0.03 (0, 0.06)	0	0	0	0.04 (0, 0.09)
An. coustani	0	0	0.15 (0, 0.32)	0.01 (0, 0.04)	0.01 (0, 0.04)	0.04 (0, 0.09)
Non-irrigated zone						
An. arabiensis	0.06 (0.01, 0.12)	0.11 (0.02, 0.21)	0.30 (0, 0.73)	0.35 (0.15, 0.55)	0.21 (0.09, 0.33)	0.77 (0.48, 1.05)
An. funestus	0	0	0	0	0	0.04 (0, 0.09)

[a] PSC, pyrethrum spray catches

Seasonal sampling conducted in the dry (Jan-Mar, 2019) and wet (Apr-Jun, 2019) seasons

n = 320 trap-nights for each trap; n = 144 trap-nights for pit shelter

Mean (95% CI)

Table 4. 2 Negative binomial mixed model and linear model analysis of differences of outdoor density of female *An. arabiensis* by different zones and collection methods

Model Number	**Model Type**	**Random Variables**	**Fixed Variables/ Coefficients**	**Estimate**	**S.E.[a]**	**z**	***p***	**AIC[b]**
1[d]	NBMM[e]	house number, cluster	intercept	-0.007683	0.306343	-0.025	0.97999	2150.2
			zone	-2.852747	0.344714	-8.276	<0.001	
			method clay pot	0.663984	0.213009	3.117	0.00183	
			method hlc	0.257399	0.266529	0.966	0.33417	
			method pit shelter	2.052270	0.319039	6.433	<0.001	
2	NBMM	cluster	intercept	1.3267	0.3059	4.338	<0.001	2280.4
			zone	-3.0253	0.4356	-6.944	<0.001	
3	NBMM	date	intercept	1.0777	0.1623	6.642	<0.001	2228.4
			zone	-2.9237	0.1659	-17.625	<0.001	
4	NBMM	house number	intercept	0.6267	0.1336	4.692	<0.001	2191.4
			zone	-2.8812	0.2314	-12.454	<0.001	

[a] S.E., standard error

[b] AIC, akaike information criterion

[c] hlc, human landing catches

[d] Best model selected with the lowest AIC

[e] NBMM, negative binomial mixed model

Table 4. 3 Negative binomial mixed model and linear model analysis of differences of indoor density of female *An. arabiensis* by different zones and collection methods

Model Number	**Model Type**	**Random Variables**	**Fixed Variables/ Coefficients**	**Estimate**	**S.E.[a]**	**z**	***p***	**AIC[b]**
1	NBMM[e]	house number, cluster	intercept	1.02809	0.45856	2.242	0.0250	1718.5
			zone	-3.10285	0.46602	-6.658	<0.001	
			method hlc[c]	-0.39704	0.32712	-1.214	0.2248	
			method psc[d]	-0.20615	0.26353	-0.782	0.4340	
			bed nets	0.20595	0.14552	1.415	0.1570	
			occupants	-0.14435	0.05652	-2.554	0.0107	
2[f]	NBMM	house number, date	intercept	0.71118	0.36250	1.962	0.0498	1695.2
			zone	-3.12624	0.33248	-9.403	<0.001	
			method hlc	-0.32106	0.31501	-1.019	0.3081	
			method psc	-0.05661	0.32143	-0.176	0.8602	
			bednets	0.21325	0.13769	1.549	0.1214	
			occupants	-0.12140	0.05393	-2.251	0.0244	

[a] S.E., standard error

[b] AIC, akaike information criterion

[c] hlc, human landing catches

[d] psc, pyrethrum spray catches

[e] NBMM, negative binomial mixed model

[f] Best model selected with the lowest AIC

Table 4. 4 Negative binomial mixed model and linear model analysis of differences of HLC and CDC light trap host-seeking density of female *An. arabiensis*

Model Number	**Model Type**	**Random Variables**	**Fixed Variables/ Coefficients**	**Estimate**	**S.E.[a]**	**z**	***p***	**AIC[b]**
1	NBMM[d]	house number, cluster	intercept	0.54096	0.37791	1.431	0.15230	1512.2
			zone	-3.54422	0.38894	-9.112	<0.001	
			method hlc[c]	0.09948	0.28923	0.344	0.73087	
			bed nets	0.41517	0.16019	2.592	0.00955	
			occupants	-0.12036	0.07180	-1.676	0.09367	
			location	-0.40592	0.14549	-2.790	0.00527	
2[e]	NBMM	house number, date	intercept	0.32559	0.34050	0.956	0.3390	1478.3
			zone	-3.56800	0.36257	-9.841	<0.001	
			method hlc	0.09417	0.26810	0.351	0.7254	
			bed nets	0.28635	0.14940	1.917	0.0553	
			occupants	-0.02062	0.07123	-0.290	0.7722	
			location	-0.41860	0.13186	-3.175	0.0015	

[a] S.E., standard error

[b] AIC, akaike information criterion

[c] hlc, human landing catches

[d] NBMM, negative binomial mixed model

[e] Best model selected with the lowest AIC

4.4.1.4 Gonotrophic Status of Female *Anopheles* Mosquitoes

The gonotrophic status of *An. arabiensis* variation was significantly higher indoor compared to outdoor collections using CDC light trap during the dry season ($\chi^2 = 11.94$, df = 3, $P = 0.03$). In contrast, there was no significant difference during the wet season ($\chi^2 = 3.08$, df = 3, $P = 0.38$) (Fig. 4.3). There was also no significance difference in the gonotrophic status of *An. arabiensis* between indoor and outdoor collections using HLC during the dry ($\chi^2 = 5.68$, df = 3, $P = 0.13$) and wet seasons ($\chi^2 = 1.11$, df = 3, $P = 0.78$) (Fig. 4.3). Most of the *An. arabiensis* collected by HLC and CDC light trap were unfed (Fig. 4.3). Due to the small number of mosquito collections in HLC and CDC light traps in non-irrigated zone, the gonotrophic status was not analyzed.

The gonotrophic status of *An. arabiensis* variation was significantly higher in the irrigated zone than in the non-irrigated zone in PSC ($\chi^2 = 10.51$, df = 3, $P = 0.02$) and clay pots ($\chi^2 = 14.64$, df = 3, $P = 0.01$) collections whereas there was no significance difference in pit shelter ($\chi^2 = 6.87$, df = 3, $P = 0.08$) collections (Fig. 4.4). Pit shelters and PSC yielded a higher proportion of blood-fed *An. arabiensis* compared to clay pots that captured mostly half-gravid *An. arabiensis* (Fig. 4.4).

4.4.1.5 Blood Meal Indices

The majority of the blood meals were of bovine origin (71.6%). Only, 0.6% of mosquitoes samples had human blood meals and less than 1% of the samples had blood meals of goat, pig or dog origin (Table 4.5). In the irrigated zone, outdoor HBI of *An. arabiensis* was almost 2-fold higher (outdoor, 5.20%; indoor, 3.90%) than indoor whereas in the non-irrigated zone, the blood meals were of bovine origin (Table 4.5).

4.4.1.6 Sporozoite Rate and Entomological Inoculation Rate

Sporozoite-positive *An. arabiensis* samples were detected in the irrigation zone only and the sporozoite rate was 2-fold higher indoors than outdoors (Table 4.6). None of the *An. funestus* samples tested positive for sporozoites (Table 4.6). The annual EIR for *An. arabiensis* in irrigated zone was 0.71 infective bites/person/year (ib/p/year) and was higher indoors than outdoors (Table 4.7). Malaria transmission was not detected in the non-irrigated zone (Table 4.7).

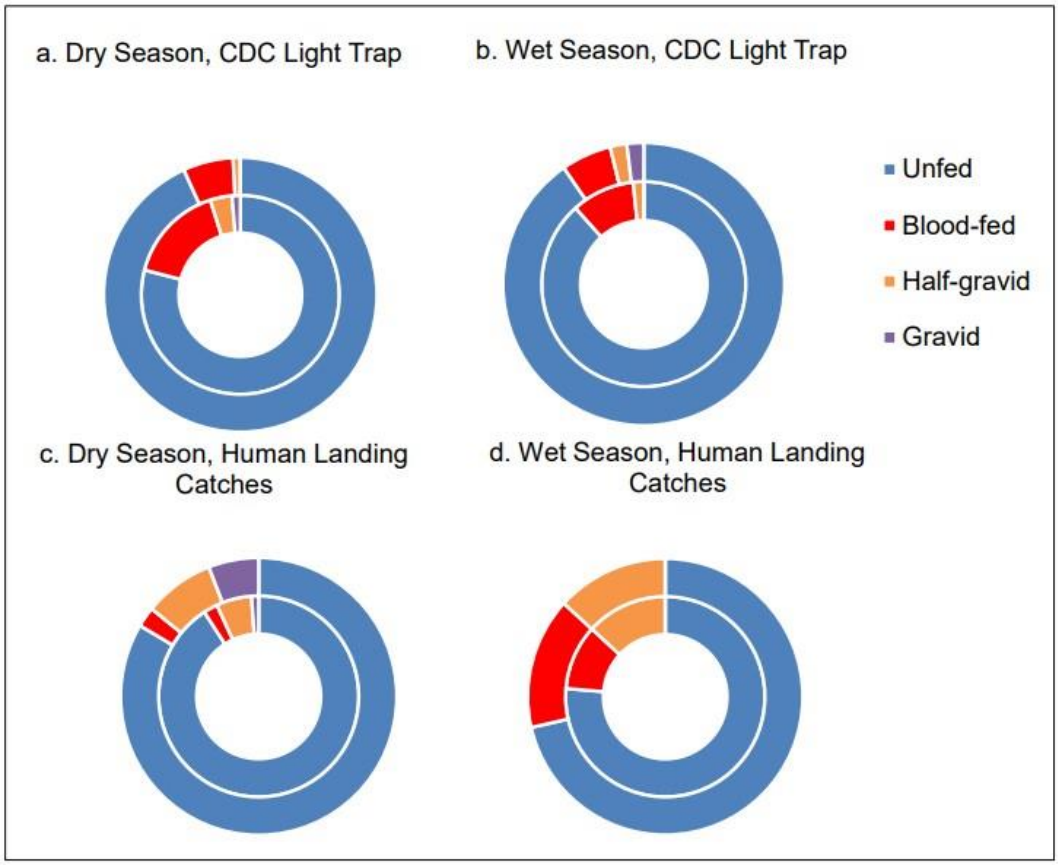

Fig. 4. 3 Gonotrophic status of female *An. arabiensis* mosquitoes sampled in irrigated zones in 2019.

Outer rings referred to outdoor collection; inner rings referred to indoor collection

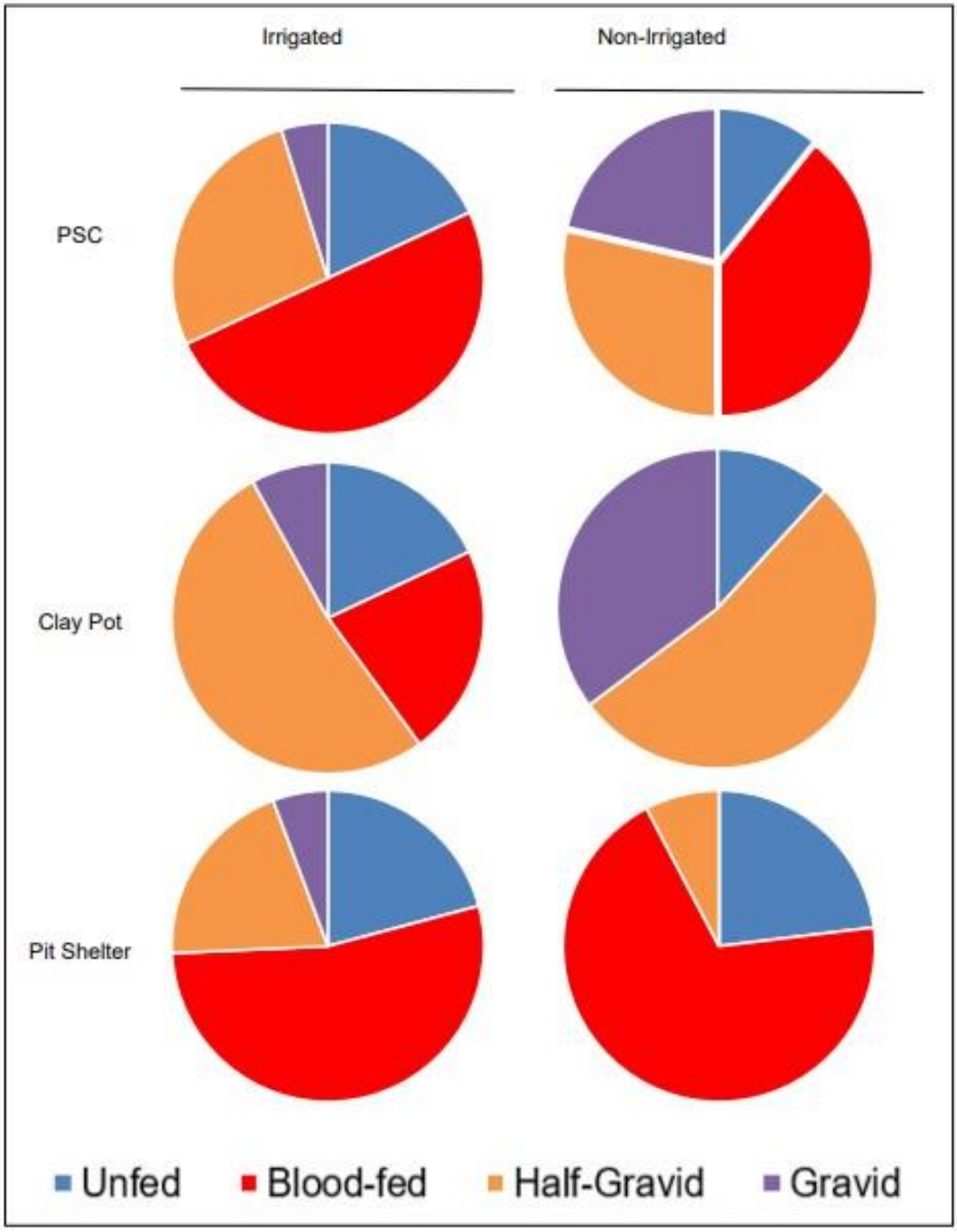

Fig. 4. 4 Gonotrophic status of resting female *An. arabiensis* mosquitoes sampled in 2019.

Table 4. 5 The host feeding preferences of *An. arabiensis* mosquitoes sampled in 2019

Blood-meal origins	Indoor		Outdoor			
	PSC[a] (%)		Clay pot (%)		Pit shelter (%)	
	Irrigated Zone	Non-irrigated Zone	Irrigated Zone	Non-irrigated Zone	Irrigated Zone	Non-irrigated Zone
No. tested	205	13	114	1	136	6
Human	2 (1.0)	0 (0)	1 (0.9)	0 (0)	0 (0)	0 (0)
Bovine	157 (76.6)	10(76.9)	67 (58.8)	1(100.0)	101 (74.3)	4(66.7)
Human + bovine	4 (2.0)	0 (0)	0 (0)	0 (0)	8 (5.9)	0 (0)
Human + dog	2 (1.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Bovine + dog	1 (0.5)	0 (0)	1 (0.9)	0 (0)	0 (0)	0 (0)
Pig	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Human + goat	0 (0)	0 (0)	1 (0.9)	0 (0)	0 (0)	0 (0)
Human + bovine + dog	0 (0)	0 (0)	2 (1.8)	0 (0)	0 (0)	0 (0)
Human + dog + goat	0 (0)	0 (0)	1 (0.9)	0 (0)	0 (0)	0 (0)
Goat	0 (0)	0 (0)	2 (1.8)	0 (0)	0 (0)	0 (0)
Dog	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.7)	0 (0)
Bovine + goat	0 (0)	0 (0)	0 (0)	0 (0)	3 (2.2)	0 (0)
Bovine + pig	0 (0)	0 (0)	1 (0.9)	0 (0)	0 (0)	0 (0)
Unknown	38 (18.5)	3(23.1)	38 (33.3)	0 (0)	23 (16.9)	2(33.3)
HBI[b]	**3.90%**	**0.00%**	**4.39%**	**0.00%**	**5.88%**	**0.00%**

[a] PSC, pyrethrum spray catches

[b] Human blood index (HBI) was calculated as the number of mosquito positive for human blood meal (including mixed blood meal) divided by the total number tested

Table 4. 6 The sporozoite rate of *An. arabiensis* and *An. funestus* mosquitoes sampled in 2019

Zone	Location	Method	*An. arabiensis*			*An. funestus*		
			Mosquito tested	No. Positive	Sporozoite rate (%)	Mosquito tested	No. Positive	Sporozoite rate (%)
Irrigated Zone	Indoors	PSC[a]	170	5	2.9	1	0	0
		CDC LT[b]	228	1	0.4	2	0	0
		HLC[c]	246	4	1.6	11	0	0
		Sub-total	644	10	1.6	14	0	0
	Outdoors	Clay pot	238	3	1.3	1	0	0
		Pit shelter	198	0	0	1	0	0
		CDC LT	101	1	1	1	0	0
		HLC	150	2	1.3	1	0	0
		Sub-total	687	6	0.8	4	0	0
Non-irrigated Zone	Indoors	PSC	14	0	0	0	0	-
		CDC LT	8	0	0	0	0	-
		HLC	3	0	0	0	0	-
		Sub-total	25	0	0	0	0	-
	Outdoors	Clay pot	18	0	0	0	0	-
		Pit shelter	24	0	0	2	0	0
		CDC LT	5	0	0	0	0	-
		HLC	5	0	0	0	0	-
		Sub-total	52	0	0	2	0	0

[a] PSC, pyrethrum spray catches

[b] CDC LT, CDC light trap

[c] HLC, human landing catches

Table 4. 7 The annual EIR of *An. arabiensis* and *An. funestus* mosquitoes sampled in 2019

Study site and Species	Indoors				Outdoors			
	HBI[a] (%)	Sporozoite Rate (%)	Mosquito Density	Annual EIR[b]	HBI (%)	Sporozoite Rate (%)	Mosquito Density	Annual EIR
Irrigated zone								
An. arabiensis	3.90	1.60	3.58	0.41	5.20	0.80	3.96	0.30
An. funestus	-	0.00	0.04	-	-	0.00	0.02	-
Non-irrigated zone								
An. arabiensis	0.00	0.00	0.14	0.00	0.00	0.00	0.21	0.00
An. funestus	-	-	0.00	-	-	0.00	0.01	-

[a] HBI, human biting index

[b] EIR, entomological inoculation rate

- Not tested

4.4.2 Longitudinal Surveillance

A total of 2,474 female anophelines were collected between January 1st, 2018 and December 31st, 2019, consisting of 2,248 (90.9%) *An. gambiae* s.l., 225 (9.1%) *An. funestus*, and 1 (0.04%) *An. coustani*. 760 specimens (621 *An. gambiae* s.l. and 139 *An. funestus*) were analyzed for sibling species identification. For the *An. gambiae* s.l. specimens, PCR results indicated that 99.7% were *An. arabiensis* and 0.3% *An. gambiae* s.s. All the *An. funestus* subjected to species identification were confirmed as *An. funestus* s.s. Overall, *An. arabiensis* was the dominant vector of malaria in the study sites. After adjusting for month, *Anopheles arabiensis* indoor resting density was 2.19 in irrigated zone and significantly higher than 0.21 in the non-irrigated zone ($Z = -4.690$, $df = 1540$, $P < 0.001$) (Fig. 4.5, Table 4.8). The difference in indoor resting density of *An. arabiensis* during the dry and wet seasons was not significant ($Z = -1.055$, $df = 1540$, $P = 0.292$) (Table 4.8). The *An. funestus* indoor resting density was 0.23 in irrigated zone while only few *An. funestus* were collected in the non-irrigated zone (Fig. 4.5). The study clearly indicated that the malaria vector species were more abundant in the irrigated zone than in the non-irrigated zone. In the irrigated zone, the HBI of *An. funestus* (49.43%) was 14-fold higher than *An. arabiensis* (3.44%) whereas in the non-irrigated zone none of the *An. arabiensis* tested positive for human blood (Table 4.9).

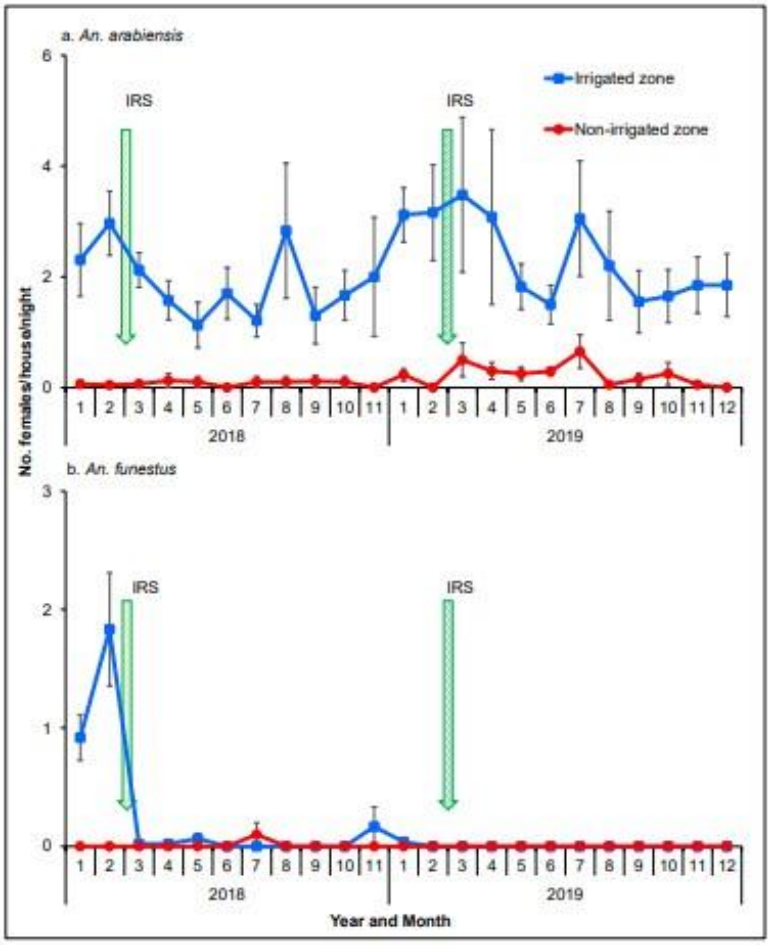

Fig. 4. 5 Indoor resting density of female *Anopheles* mosquitoes.

Error bars were for the standard error for the *Anopheles* mosquitoes collected. Abbreviation: IRS, indoor residual spraying.

Table 4. 8 Negative binomial and linear models analysis of differences of indoor resting density of female *An. arabiensis* in irrigated and non-irrigated zones

Model Number	**Model Type**	**Random Variables**	**Fixed Variables/ Coefficients**	**Estimate**	**S.E.[a]**	**z**	***p***	**AIC[b]**
1	NBMM[c]	cluster	intercept	0.1749	0.3729	0.469	0.639	4023.3
			zone	-2.5058	0.5744	-4.362	<0.001	
2	NBMM	date	intercept	0.4320	0.1300	3.322	0.000894	4108.7
			zone	-2.3646	0.2151	-10.991	<0.001	
3	NBMM	date:cluster, cluster	intercept	-0.1579	0.3356	-0.470	0.638	3935.8
			zone	-2.4618	0.5262	-4.679	<0.001	
4[d]	NBMM	year:(date:cluster), date:cluster, cluster	intercept	-0.08174	0.34388	-0.238	0.812	3938.7
			zone	-2.47435	0.52763	-4.690	<0.001	
			season	-0.16377	0.15528	-1.055	0.292	

[a] S.E., standard error
[b] AIC, akaike information criterion
[c] NBMM, negative binomial mixed model
[d] Best model selected with the 2nd lowest AIC and variables of interest

Table 4. 9 Host feeding preferences of *An. arabiensis* and *An. funestus* mosquitoes sampled in 2018 and 2019

Blood-meal origins	*An. arabiensis* (%)		*An. funestus* (%)	
	Irrigated Zone	Non-irrigated Zone	Irrigated Zone	Non-irrigated Zone
No. tested	494	27	87	0
Human	7 (1.6)	0 (0)	39 (44.8)	0 (0)
Bovine	390 (78.8)	22 (81.5)	30 (34.5)	0 (0)
Human + bovine	5 (1.0)	0 (0)	3 (3.4)	0 (0)
Human + dog	4 (0.8)	0 (0)	0 (0)	0 (0)
Bovine + dog	4 (0.8)	0 (0)	0 (0)	0 (0)
Pig + dog	3 (0.6)	0 (0)	0 (0)	0 (0)
Goat	2 (0.4)	0 (0)	0 (0)	0 (0)
Dog	2 (0.4)	0 (0)	0 (0)	0 (0)
Bovine + goat	2 (0.4)	0 (0)	0 (0)	0 (0)
Pig	1 (0.2)	1 (3.7)	1 (1.1)	0 (0)
Human + bovine + pig	1 (0.2)	0 (0)	0 (0)	0 (0)
Bovine + dog + pig	1 (0.2)	0 (0)	0 (0)	0 (0)
Human + pig	0 (0)	0 (0)	1 (1.1)	0 (0)
Unknown	72 (14.5)	4 (14.8)	13 (14.9)	0 (0)
HBI[a]	**3.44%**	**0.00%**	**49.43%**	**0.00%**

[a] Human blood index (HBI) was calculated as the number of mosquito positive for human blood meal (including mixed blood meal) divided by the total number tested

4.5 Discussion

The distribution of *An. arabiensis* is generally concentrated in the drier savannah environments where rainfall is < 1000 mm (Coetzee *et al*., 2000a). *Anopheles funestus* was scarcely collected in the study site. Other anopheline species that were reported, and occurred only in the irrigated zone, were *An. coustani* and *An. pharoensis*. These two mosquitoes species have previously been considered as secondary vectors of malaria in Africa (Afrane *et al*., 2016) but recent studies have shown that they could play an important role in malaria transmission (Kerah-Hinzoumbé *et al*., 2009; Kibret *et al*., 2012; Mwangangi *et al*., 2013). Thus, it is prudent to integrate them in malaria vector surveillance and control strategies particularly where they are abundant.

A significant variation in vector density was observed in the irrigated and non-irrigated zones which is consistent with previous studies that the introduction of irrigation schemes leads to an increase in vector density and abundance (Briet *et al*., 2003; Diuk-Wasser *et al*., 2005; Ijumba *et al*., 2002; Muturi *et al*., 2008). In the irrigated zone, the irrigated canals, seepage areas, and flooded irrigated fields serve as the main larval habitats and provide stable mosquito breeding habitats during the dry season when other larval habitats dry up. In contrast, the low *An. arabiensis* density in the non-irrigated zone may be due to the temporary and parched nature of aquatic habitats (rain pools, rice fields, and edges of seasonal swamps) during the dry season. These observations have also been reported in similar studies in the Mwea Irrigation Scheme and the neighboring non-irrigated agroecosystems (Muturi *et al*., 2006). The indoor resting density of *An. funestus* was generally low however, their indoor abundance was relatively high during the dry season of 2018 in the irrigated zone prior to the application of the IRS in the study site. Thereafter, *An. funestus* was rarely collected from our study. This can be attributed to the application of Actellic® 300CS IRS which has been shown to significantly reduce the indoor resting density of

An. funestus in the same area (Abong'o *et al*., 2020). Indoor residual spraying is known to be highly effective on endophilic and anthropophilic mosquito species such as *An. funestus* due to high exposure to the wall sprayed insecticides.

The indoor and outdoor host-seeking density of *An. arabiensis* varied significantly with the highest biting densities collected indoors. Studies conducted over three decades ago by Githeko *et al*. showed that *An. arabiensis* was more likely to bite indoor than outdoor before the scale up of vector control intervention in western Kenya (Githeko *et al*., 1994a; Githeko *et al*., 1996). In the present study, the endophagic tendency of *An. arabiensis* was still observed despite the high LLINs coverage and application of IRS in the study sites. This could be attributed to behaviour of this species whereby it may enter a house protected with malaria vector control interventions in search of unprotected host, but exit without fatal exposure to insecticide-treated surfaces (Asale *et al*., 2014; Kitau *et al*., 2012; Okumu *et al*., 2013). Furthermore, in recent studies conducted in the study area, *An. arabiensis* has been reported to have developed resistance to pyrethroids, and which could also be a factor contributing to endophagy in the species (Orondo *et al*., 2021). The scale-up of LLINs and IRS since 2006 by the Kenya National Malaria Control Programme may be implicated in the development of insecticide resistance in malaria vectors in the study area. Nonetheless, outdoor biting behaviour was observed for this mosquito species which is consistent with other studies within its distribution range (Gatton *et al*., 2013).

It is worth mentioning that when comparing the traps deployed in the study, the mean vector density varied significantly between traps. The outdoor density of *An. arabiensis* was significantly higher in pit shelters and clay pots than for CDC light traps, in contrast, the indoor density of *An. arabiensis* was not significantly different between traps. Such variations was likely driven by differences in vector behaviour, vector species composition, and history of malaria

interventions rather than differences in the efficiency between the traps. Wide variation in the vector density of each trapping method has also been observed by Gedefa *et al* in a study conducted in western and attributed this variations to vector behaviour (Degefa *et al*., 2019).

Human landing catches has been considered the gold standard method for estimating mosquito-human contact. However, it is a labor-intensive procedure requiring highly trained collectors, extensive supervision, variation in the skill of the collectors or their individual attractiveness to mosquitoes, and ethical concerns associated with potential exposure to infectious mosquito bites (Knols, de Jong, & Takken, 1995; WHO, 2013). Our results indicate that CDC light trap is an effective trapping alternative to HLC for continuous operational surveillance of mosquito vectors within the study sites. The limitation of the CDC light trap is that it doesn't allow hourly anopheline collections to determine the biting cycle; however, the CDC light trap can be equipped with a collection bottle rotator that collects mosquitoes at 2-hour intervals (Kawada *et al*., 2014; Ototo *et al*., 2015). In a recent study conducted in western Kenya and southwestern Ethiopia, human-odour-baited CDC light traps (HBLT) collected twice the number of outdoor host-seeking *An. arabiensis* and *An. funestus* compared to non-baited CDC light traps (Degefa *et al*., 2020). Thus, it will be important to evaluate the effectiveness of this tool in the study sites that could be a better outdoor surveillance tool than the non-baited CDC light trap.

Anthropophily was highest in *An. funestus* compared to *An. arabiensis* in the irrigated zone. These findings are consistent with previous studies that have reported *An. funestus* s.s. to exhibit anthropophagic behavior in Kenya (Githeko *et al*., 1994b; Mwangangi *et al*., 2003) and in other parts of Africa (Dadzie *et al*., 2013; Mzilahowa *et al*., 2012; Tanga *et al*., 2011). However, in recent reports they have been shown to also feed on bovine (Degefa *et al*., 2017; Ogola *et al*., 2018) in the presence of LLINs. This plasticity of the feeding behavior of the vector may influence

malaria transmission, leading to residual transmission after the densities of endophilic and endophagic vectors have been reduced by the interventions (Afrane *et al*., 2016; Durnez & Coosemans, 2013). The life histories of *An. arabiensis* population of southern Tanzania were simulated in a model by Killeen *et al*. and estimated that two thirds of the vector feeds outdoor in an area where bednet usage is high (Killeen *et al*., 2016). Studies have indicated that *An. arabiensi*s exhibits behavior that mediates residual transmission such as feeding outdoors on humans or cattle and rapidly exiting houses without fatal exposure to insecticide-treated surfaces (Killeen *et al*., 2016). Findings of the present study demonstrated that *An. arabiensis* fed on humans both indoors and outdoors with a higher HBI outdoors and predominantly fed on bovine. However, it remains capable of transmitting malaria whenever it can feed on humans.

There was a significant difference in the risk of malaria transmission by *An. arabiensis* in the two zones, with higher transmission risk in the irrigated zone. These results show that irrigation has an effect on malaria transmission and *An. arabiensis* played a significant role in transmission. In addition, this species contributed almost equally to both indoor and outdoor transmission. In many studies, irrigated areas have been associated with increased malaria transmission than neighboring non-irrigated areas (Kibret *et al*., 2014; Mangani *et al*., 2022; Muriuki *et al*., 2016; Yohannes *et al*., 2005) however, in some cases, introduction of irrigation schemes reduces (Ijumba *et al*., 2002; Sissoko *et al*., 2004) or has no impact on malaria transmission (Diakité *et al*., 2015). Hence, the impact of water development projects on malaria transmission is variable and the transmission dynamic likely depends on the local epidemiological setting. Our data also suggest that the zoophagic behaviour of *An. arabiensis* could be accounting for the low transmission in the irrigated zone whereas the low vectors densities limited transmission in the non-irrigated zone.

The zoophagic tendency of *An. arabiensis* indicates zooprophylaxis may be a potential strategy for malaria control.

The limitation of our study is the lack of information on the movement of endophagic mosquitoes as they exit the house after feeding and/or resting. This information would have improved the understanding of the effect of insecticide-based vector control interventions in the houses on the normal movement, density, and reticence feeding of endophilic species (Müller *et al*., 2017; WHO, 1975).

CHAPTER 5

5.0 A PROSPECTIVE COHORT STUDY OF *PLASMODIUM FALCIPARUM* MALARIA IN THREE SITES OF WESTERN KENYA

(This chapter has been published in Parasites and Vectors, 2022 https://rdcu.be/cZmdq)

5.1 Abstract

Malaria in western Kenya is currently characterized by sustained high Plasmodial transmission and infection resurgence, despite positive responses in some areas following intensified malaria control interventions since 2006. This study aimed to evaluate long-term changes in malaria transmission profiles and to assess patterns of asymptomatic malaria infections in school children aged 5-15 years at three sites in western Kenya with heterogeneous malaria transmission and simultaneous malaria control interventions. The study was conducted from 2018 to 2019 and is based on data taken every third year from 2005 to 2014 during a longitudinal parasitological and mosquito adult surveillance and malaria control programme that was initiated in 2002 in the villages of Kombewa, Iguhu, and Marani. *Plasmodium* spp. infections were determined using microscopy. Mosquito samples were identified to species and host blood meal source and sporozoite infection were assayed using PCR. *Plasmodium falciparum* was the only malaria parasite evaluated during this study (2018-2019). Asymptomatic malaria parasite prevalence in school children decreased in all sites from 2005 to 2008. However, since 2011, parasite prevalence has resurged by >40% in Kombewa and Marani. Malaria vector densities showed similar reductions from 2005 to 2008 in all sites, rose steadily until 2014, and decreased again. Overall, Kombewa had a higher risk of infection compared to Iguhu (χ^2=552.52, *df*=1, *P*<0.0001) and Marani (χ^2=1127.99, *df*=1, *P*<0.0001). There was a significant difference in probability of non-

infection during malaria episodes (Log-Rank test, χ^2=617.59, *df*=2, *P*<0.0001) in the study sites, with Kombewa having the least median time of non-infection during malaria episodes. Gender bias towards males in infection was observed (χ^2=27.17, *df*=1, *P*<0.0001). The annual EIRs were 5.12, 3.65, and 0.50 infective bites/person/year at Kombewa, Iguhu, and Marani, respectively, during 2018 to 2019. Malaria prevalence in western Kenya remains high and has resurged in some sites despite continuous intervention efforts. Targeting malaria interventions to those with asymptomatic infections who serve as human reservoirs might decrease malaria transmission and prevent resurgences. Longitudinal monitoring enables detection of changes in parasitological and entomological profiles and provides core baseline data for the evaluation of vector interventions and guidance for future planning of malaria control.

Keywords: Malaria, Transmission, Resurgence, *Plasmodium falciparum*

5.2 Introduction

Globally, an estimated 241 million cases of malaria were reported in 2020 resulting in approximately 627,000 deaths; 96% of these deaths occurred in Africa and children aged less than five years accounted for 77% of these deaths (WHO, 2021). In Kenya, an estimated 2.7 million malaria cases and 12,600 deaths attributed to malaria were reported in 2020 (WHO, 2021). Since 2000, malaria mortality and morbidity have declined significantly in African countries, including Kenya, and has been attributed chiefly to the scale-up of ITN distributions, IRS, and ACTs (WHO, 2020). Nonetheless, malaria remains a major public health concern in Africa.

Kenya's Ministry of Health began the country's first free mass long-lasting insecticidal net (LLIN) distribution in 2006 to children under five years and pregnant women, followed by a second distribution in 2011 aiming for universal coverage in targeted areas (MOH, 2016b).

Thereafter, there have been three successive rounds of distribution in 2014, 2017, and 2021 to boost LLIN coverage and replace worn nets (MOH, 2016b; NMCP, 2021; Zhou *et al*., 2016). Indoor residual spraying applications began in 2005 to prevent epidemics in malaria epidemic-prone areas in the highlands (DOMC & KNBS, 2011). To reduce the malaria burden in the Lake Victoria endemic zone, IRS was implemented in targeted districts from 2008 to 2012 DOMC & KNBS, 2011; Gimnig *et al*., 2016; PMI, 2013). However, IRS was not applied from 2012 to 2016 due to the detection of widespread pyrethroid resistance in malaria vector populations and lack of a registered, non-pyrethroid insecticide in the country (AIRS, 2018; NMCP & KNBS, 2016). After five years of no treatments, IRS was restarted in 2017 with the micro-encapsulated organophosphate insecticide pirimiphos-methyl (Actellic® 300CS) and applied during successive rounds from 2018 to 2021 in two targeted counties (Migori and Homa Bay) located in the Lake Victoria endemic zone, where intense malaria transmission occurs throughout the year (PMI, 2021). Artemisinin-based combination therapies began in 2004 after several years of sulfadoxine-pyrimethamine treatments (1998-2003) and earlier recognition of widespread antimalarial drug failures (e.g. chloroquine) (Okech *et al*., 2008; Okiro *et al*., 2007). Malaria control programmes face numerous challenges, among them development of pyrethroid resistance in malaria vectors (Ondeto *et al*., 2017), changes in vector dominance and behavior (Bayoh *et al*., 2010; Ojuka *et al*., 2015; Takken, 2002), and the emergence of antimalarial drug resistance (Kozlov, 2021). In an effort to mitigate insecticide resistance, the WHO has recommended conducting IRS with organophosphate and neonicotinoid insecticides and using pyrethroid-piperonyl butoxide (PBO) nets synergized treated nets (WHO, 2021), which have been distributed in targeted counties in Kenya from 2020 to 2021.

Despite these malaria control efforts, areas in western Kenya are experiencing heterogeneity in malaria transmission after interventions, with some areas indicating a decline in transmission, while in others, transmission has remained unchanged or has resurged (Kapesa *et al*., 2017; Ototo *et al*., 2015; Zhou *et al*., 2011, 2016). A study in western Kenya linked these contrasting outcomes to malaria vector species composition shifts, insecticide resistance, and climatic warming (Kapesa *et al*., 2017). Similar observations of varying outcomes in malaria control have been observed elsewhere in Africa (Nkumama *et al*., 2017).

This study aimed to evaluate long-term changes in malaria transmission profiles and patterns of asymptomatic malaria infection in three sites with different transmission intensities in western Kenya after distributions of new pyrethroid-PBO treated LLINs and applications of new IRS formulations. Hopefully, the results presented here will help in assessing vector interventions, serve as a baseline for the evaluation of new interventions, and guide future control planning by the Kenya National Malaria Control Programme.

5.3 Material and methods

5.3.1 Historic *Plasmodium falciparum* Parasite Prevalence and Vector Densities

This study was based on longitudinal parasitological and adult vector surveillance that commenced in 2002 (Iguhu) and 2003 (Kombewa and Marani) (Zhou *et al*., 2011) to date. Snapshots of these data were taken every three years from 2005 to 2014 (Kapesa *et al*., 2017; Ototo *et al*., 2015; Zhou *et al*., 2016). Data (years 2005, 2008, 2011, and 2014) from this period form the basis for the current study conducted between 2018 and 2019.

5.3.2 Parasitological Surveys

A cohort of 514 volunteer school-aged children aged 5-15 years were enrolled (January-March, 2018) for monthly *Plasmodium* spp. surveys between 1 Jan. 2018 and 31 Oct. 2019 in Kombewa, Iguhu, and Marani. The sample size was calculated based on the size of the study population and parasite prevalence from a previous study (Zhou *et al*., 2016). Consent was obtained from parents or guardians before children could participate in the study. Children with no reported chronic or acute illness, except malaria, were allowed to participate in the study. At the sampling time, children who were found to have fever were referred to the nearest government health facility for diagnosis and treatment according to Kenyan government malaria treatment guidelines (MOH, 2016a).

Blood samples were collected using the finger-prick method, and thick and thin smears prepared on labeled slides for malaria parasite species identification and parasite counts using microscopy. Malaria parasite counts were scored against 200 leukocytes. A second microscopist carried out random checks on the slide counts to ensure microscopy quality. Parasite density was expressed as parasites per µl, assuming a count of 8,000 white blood cells per µl of blood (Slutsker *et al*., 1994). *Plasmodium* spp. infection data collected from all participants were subjected to prevalence analyses, however, only participants with at least six months of follow-up were included in the *Plasmodium* spp. infection pattern analyses, including duration and probability of non-infections.

5.3.3 Entomological Surveys

Collections of indoor resting vector populations were conducted monthly by the PSC method (WHO, 1975) in 30 randomly selected houses in each study site between 1 Jan. 2018 to 31 Oct. 2019. Mosquitoes were identified morphologically as either *Anopheles gambiae* s.l. or *An*.

funestus (Gillies & Coetzee, 1987). DNA was extracted (Musapa *et al*., 2013) from the legs and wings of each mosquito specimen to speciate sibling species in *An*. *gambiae* s.l. and *An*. *funestus* using conventional PCR, as described by Scott et al. (Scott *et al*., 1993) and Koekemoer et al. (Koekemoer *et al*., 2002) , respectively. The DNA extracted from the abdomen of each freshly fed female mosquito was used to identify host blood meal sources using a multiplexed PCR assay (Kent & Norris, 2005). The DNA extracted from the head and thorax of each mosquito specimen was used to determine sporozoite infections of *Plasmodium* spp. by using a multiplexed real-time qPCR assay (Shokoples *et al*., 2009; Veron *et al*., 2009).

5.3.4 Climatic Data

Mean monthly rainfall, maximum and minimum temperature from 2018 to 2019 were obtained from the Kenya Meteorological Department for meteorological stations in Kakamega (for Iguhu), Kisii (for Marani), and Kisumu (for Kombewa).

5.3.5 Ethics approval and consent to participate

The study obtained ethical approval from the Maseno University Ethics Review Committee (MUERC Protocol No. 00660) (**Appendix 4**) and the University of California, Irvine Institutional Review Board (UCI IRB). Written informed consent was obtained from children's parents/guardians before they were enrolled in the study and household heads (**Appendix 5**).

5.4 Results

5.4.1 Historic *Plasmodium falciparum* Parasite Prevalence and Vector Densities

Changes in parasite prevalence and vector densities in Kombewa, Iguhu, and Marani are shown in Table 5.1 from 2005 to 2014. Similar trends in parasite prevalence was observed in the three sites, i.e., declining parasite prevalence from 2005 to 2008 in all sites, and a rebounding trend in

prevalence from 2008 in Iguhu and 2011 in Kombewa and Marani (Table 5.1). In Kombewa, parasite prevalence decreased slightly from 2005 (51.16%, 95% CI 46.79-55.54) to 2008 (48.06%, 95% CI 41.61-54.51) (Tukey-Kramer HSD test, $P>0.05$), then declined sharply from 2008 to 2011 (29.80%, 95% CI 19.50-40.10) (Tukey-Kramer HSD test, $P=0.006$). After that, it rose significantly to 45.86% (95% CI 39.34-52.38) in 2014 (Tukey-Kramer HSD test, $P=0.02$). In Iguhu, a sharp decline in parasite prevalence was observed from 2005 (26.61%, 95% CI 21.88-31.34) to 2008 (6.45%, 95% CI 4.58-8.32) (Tukey-Kramer HSD test, $P<0.0001$), and rose steadily to 16.82% (95% CI 13.52-20.12) in 2014 (Tukey-Kramer HSD test, $P=0.002$). In Marani, a steady decline of parasite prevalence was observed from 2005 (1.95%, 95% CI 0.82-3.09) to 2011 (0.35%, 95% CI 0.05-0.66) (Tukey-Kramer HSD test, $P=0.04$), after which there was a sharp rise in 2014 (4.44%, 95% CI 3.37-5.51) (Tukey-Kramer HSD test, $P<0.0001$).

The indoor resting densities of *An. gambiae* s.l. and *An. funestus* varied significantly in all sites. The vector densities showed reductions from 2005 to 2008 in all sites, thereafter rose steadily until 2014 (Table 5.1). Studies from 2005 and 2008 indicate that the indoor resting densities of malaria vectors decreased sharply in Kombewa from 1.04 (95% CI 0.14-1.93) to 0.31 (95% CI 0.15-0.47) f/h/n for *An. gambiae* s.l. (Wilcoxon test, $Z=1.24$, $P=0.21$) and from 2.14 (95% CI 1.16-3.12) to 0.52 (95% CI 0.21-0.83) f/h/n for *An. funestus* (Wilcoxon test, $Z=3.38$, $P=0.0007$). Similarly, a decline was observed in Iguhu with a reduction from 2.56 (95% CI 0.21-4.91) to 0.36 (95% CI 0.24-0.48) f/h/n for *An. gambiae* s.l. (Wilcoxon test, $Z=1.62$, $P=0.11$) and that of *An. funestus* changed significantly from 0.29 (95% CI 0.09-0.49) to 0.02 (95% CI 0.01-0.04) f/h/n (Wilcoxon test, $Z=4.03$, $P<0.0001$). In Marani, *An. gambiae* s.l. densities decreased from 0.03 (95% CI 0.00-0.05) to 0.01 (95% CI 0.00-0.02) f/h/n (Wilcoxon test, $Z=1.07$, $P=0.29$) between 2005 and 2008, while no *An. funestus* were found during the two years. Between 2008 and 2014,

the population of indoor resting vectors rose steadily in Kombewa (*An. gambiae* s.l., Wilcoxon test, Z=3.23, *P*=0.001; *An. funestus*, Wilcoxon test, Z=2.51, *P*=0.01), Iguhu (*An. gambiae* s.l., Wilcoxon test, Z=1.47, *P*=0.14; *An. funestus*, Wilcoxon test, Z=4.17, *P*<0.0001) and Marani (*An. gambiae* s.l., Wilcoxon test, Z=3.00, *P*=0.003; *An. funestus*, Wilcoxon test, Z=4.41, *P*<0.0001).

5.4.2 *Plasmodium falciparum* Parasite Prevalence, Gametocyte Prevalence, and Parasite Density

In the 2018-2019 survey, only *P. falciparum* was found and evaluated. The *P. falciparum* prevalence in Kombewa was significantly higher compared to Iguhu (χ^2=552.52, *df*=1, *P*<0.0001) and Marani (χ^2=1127.99, *df*=1, *P*<0.0001) (Fig. 5.1). Compared to 2011, parasite prevalence in 2018-2019 has resurged by >40% in Kombewa and Marani, whereas in Iguhu, it has decreased by 7.3%. There were no significant differences in *P. falciparum* prevalence between males and females in all sites except Kombewa, and no significant differences in *P. falciparum* prevalence between age groups at all sites (Table 5.2).

The *P. falciparum* gametocyte prevalence was significantly higher in Kombewa compared to Iguhu and Marani (χ^2=7.69, *df*=2, *P*=0.02) (Fig. 5.1).

In Kombewa, there was a significant difference in the geometric means of *P. falciparum* density between the two age groups, with higher parasite density in the 5-10 years old group. Similarly, males had higher parasite density compared to females (Table 5.2).

Table 5. 1 Historic *Plasmodium falciparum* parasite prevalence and vector densities in Kombewa, Iguhu, and Marani in western Kenya

Study Sites	Kombewa			Iguhu			Marani		
Year	Parasite prevalence (%)[a]	*An. gambiae* s.l density[b]	*An. funestus* density[b]	Parasite prevalence (%)[a]	*An. gambiae* s.l. density[b]	*An. funestus* density[b]	Parasite prevalence (%)[a]	*An. gambiae* s.l. density[b]	*An. funestus* density[b]
2005	51.16 (46.79, 55.54)	1.04 (0.14, 1.93)	2.14 (1.16, 3.12)	26.61 (21.88, 31.34)	2.56 (0.21, 4.91)	0.29 (0.09, 0.49)	1.95 (0.82, 3.09)	0.03 (0.00, 0.05)	0.00 (0.00, 0.00)
2008	48.06 (41.61, 54.51)	0.31 (0.15, 0.47)	0.52 (0.21, 0.83)	6.45 (4.58, 8.32)	0.36 (0.24, 0.48)	0.02 (0.01, 0.04)	0.43 (0.11, 0.74)	0.01 (0.00, 0.02)	0.00 (0.00, 0.00)
2011	29.80 (19.50, 40.10)	0.54 (0.37, 0.70)	0.95 (0.58, 1.32)	13.59 (9.55, 17.64)	0.37 (0.28, 0.47)	0.12 (0.04, 0.21)	0.35 (0.05, 0.66)	0.05 (0.01, 0.09)	0.29 (0.11, 0.47)
2014	45.86 (39.34, 52.38)	0.78 (0.49, 1.07)	1.38 (1.05, 1.70)	16.82 (13.52, 20.12)	0.55 (0.35, 0.75)	0.31 (0.22, 0.40)	4.44 (3.37, 5.51)	0.11 (0.03, 0.19)	0.59 (0.46, 0.71)

[a] Variations in parasite prevalence between different time periods at each study site were compared using Tukey-Kramer HSD test of analysis of variance (ANOVA) with repeated measures.

[b] Variations in vector densities between different time periods at each study site were compared using non-parametric Wilcoxon rank-sum tests.

Mean (95%CI)

Table 5. 2 Malaria infection in Kombewa, Iguhu, and Marani in western Kenya

Sites			No. Samples	Mean Parasite Prevalence (%)	*P*	Mean Parasite Density (/µl)	*P*	Proportion of Months Infected (%)[a]	*P*
Kombewa	**Age**	5-10 yrs	734	45.23 (41.63, 48.83)	χ^2=2.88, *d.f.*=1, *P*>0.05	1836.27 (1359.46, 2313.07)	Z=4.49, *P*<0.0001	35.61 (29.41, 41.80)	Z=0.16, *P*>0.05
		11-15 yrs	1,408	41.41 (38.83, 43.98)		1218.25 (894.33, 1542.17)		36.00 (32.35, 39.65)	
	Gender	Female	1,036	36.97 (34.03, 39.91)	χ^2=27.17, *d.f.*=1, *P*<0.0001	1330.03 (929.91, 1730.14)	Z=3.32, *P*<0.001	32.83 (28.44, 37.22)	Z=1.65, *P*>0.05
		Male	1,106	48.10 (45.16, 51.05)		1523.46 (1159.46, 1887.45)		38.49 (34.14, 42.85)	
Iguhu	**Age**	5-10 yrs	620	14.52 (11.74, 17.29)	χ^2=2.47, *d.f.*=1, *P*>0.05	2290.11 (698.57, 1657.88)	Z=1.65, *P*>0.05	15.80 (9.24, 22.36)	Z=0.09, *P*>0.05
		11-15 yrs	2,011	12.08 (10.66, 13.51)		2948.64 (963.01, 4934.27)		14.71 (12.16, 17.25)	
	Gender	Female	1,669	12.28 (10.71, 13.86)	χ^2=0.57, *d.f.*=1, *P*>0.05	2826.15 (703.63, 4948.67)	Z=0.41, *P*>0.05	14.06 (11.37, 16.74)	Z=0.58 *P*>0.05
		Male	962	13.31 (11.16, 15.45)		1900.00 (207.32, 3592.68)		16.19 (11.76, 20.62)	
Marani	**Age**	5-10 yrs	592	2.53 (1.27, 3.80)	χ^2=0.15, *d.f.*=1, *P*>0.05	288.00 (33.06, 542.94)	Z=0.69, *P*>0.05	3.93 (1.93, 5.92)	Z=1.93 *P*>0.05
		11-15 yrs	1,980	2.83 (2.10, 3.56)		349.29 (24.27, 674.30)		6.10 (5.18, 7.01)	
	Gender	Female	1,541	2.73 (1.91, 3.54)	χ^2=0.02, *d.f.*=1, *P*>0.05	235.24 (122.29, 348.19)	Z=1.26, *P*>0.05	5.55 (4.50, 6.60)	Z=0.46 *P*>0.05
		Male	1,031	2.81 (1.80, 3.82)		482.76 (0, 1115.95)		6.09 (4.70, 7.47)	

[a] Individuals enrolled between January to March 2018, with at least 6 tests done in the cohort were used.

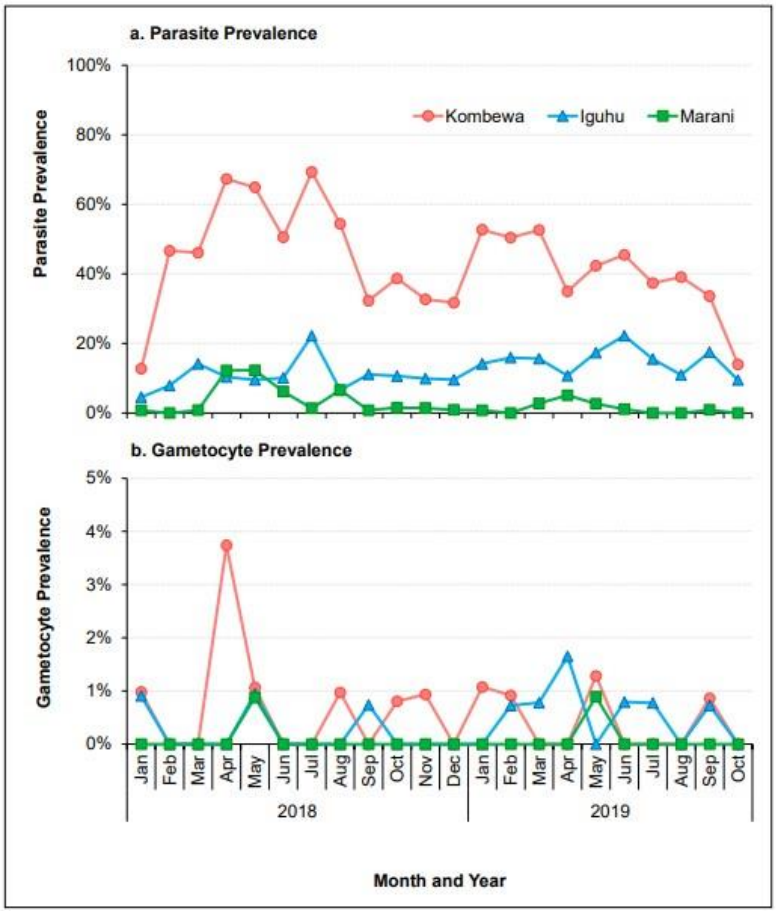

Fig. 5. 1 *Plasmodium* parasite prevalence and gametocyte prevalence in Kombewa, Iguhu, and Marani in western Kenya.

Differences in the parasite/gametocyte prevalence among study sites were determined using Chi-square test

5.4.3 *Plasmodium falciparum* Infection Patterns

The proportion of months infected varied greatly in Kombewa (35.9%), Iguhu (14.9%), and Marani (5.8%) (Tukey-Kramer HSD test, $P<0.0001$) (Fig. 5.2). No significant age and gender variations were found in the proportion of months infected in the study sites (Table 5.2). Figure

5.3 indicates the distribution of malaria infection patterns in the age and gender groups in the study sites.

As shown in Figure 5.4, the median time of non-infection during malaria first episode was 1.90 (interquartile range (IQR): 1.61-2.19) months, 5.46 (IQR: 4.30-6.62) months, and 10.86 (IQR: 9.03-12.69) months in Kombewa, Iguhu, and Marani, respectively. Median time from first to second malaria episodes was 1.95 (IQR: 1.64-2.32) months, 10.37 (IQR: 8.18-12.57) months, and 65.96 (IQR: 35.38-122.98) months in Kombewa, Iguhu, and Marani, respectively. When exploring time intervals from second to third malaria episodes, the median time was 3.24 (IQR: 2.56-4.10) months, 29.10 (IQR: 16.20-52.25) months, and 491.07 (IQR: 60.23-4003.94) months in Kombewa, Iguhu, and Marani, respectively. The median time of non-infection for all malaria episodes was 17.30 (IQR: 16.77-17.75) months, 23.26 (IQR: 22.40-24.13) months, and 33.40 (IQR: 30.14-34.65) months in Kombewa, Iguhu, and Marani, respectively. There was a significant difference in probability of non-infection during malaria 1st episode (Log-Rank test, χ^2=171.78, df=2, P<0.0001), 1st to 2nd episodes (Log-Rank test, χ^2=179.33, df=2, P<0.0001), 2nd to 3rd episodes (Log-Rank test, χ^2=245.77, df=2, P<0.0001) and all episodes (Log-Rank test, χ^2=617.59, df=2, P<0.0001) in the study sites.

For male gender, Kombewa and Iguhu sites were statistically significant risk factors associated with asymptomatic malaria infection (Table 5.3). Unadjusted hazard ratios for the infection were significantly higher in Kombewa and Iguhu compared to Marani, with similar results after adjustment for gender and age. Females had a significantly lower unadjusted hazard ratio for the infection than males, but was insignificant after adjusted for sites and ages.

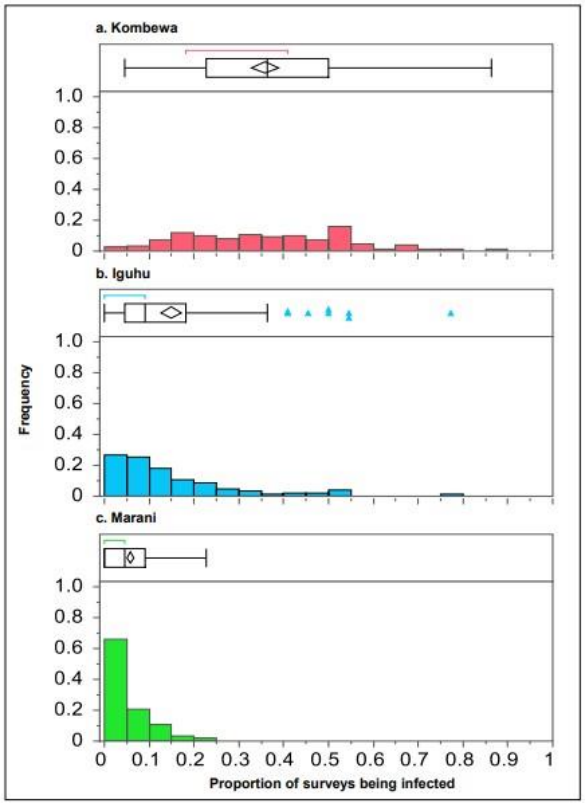

Fig. 5. 2 Distribution of the proportion of surveys being infected in Kombewa, Iguhu, and Marani in western Kenya.

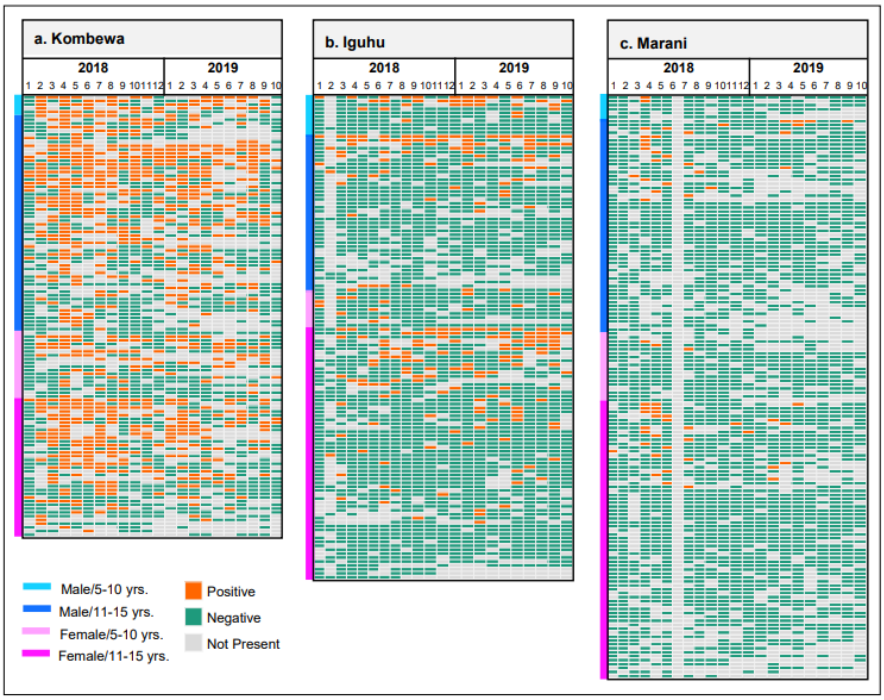

Fig. 5. 3 Heat map showing the *Plasmodium falciparum* infection patterns in Kombewa, Iguhu, and Marani in western Kenya.

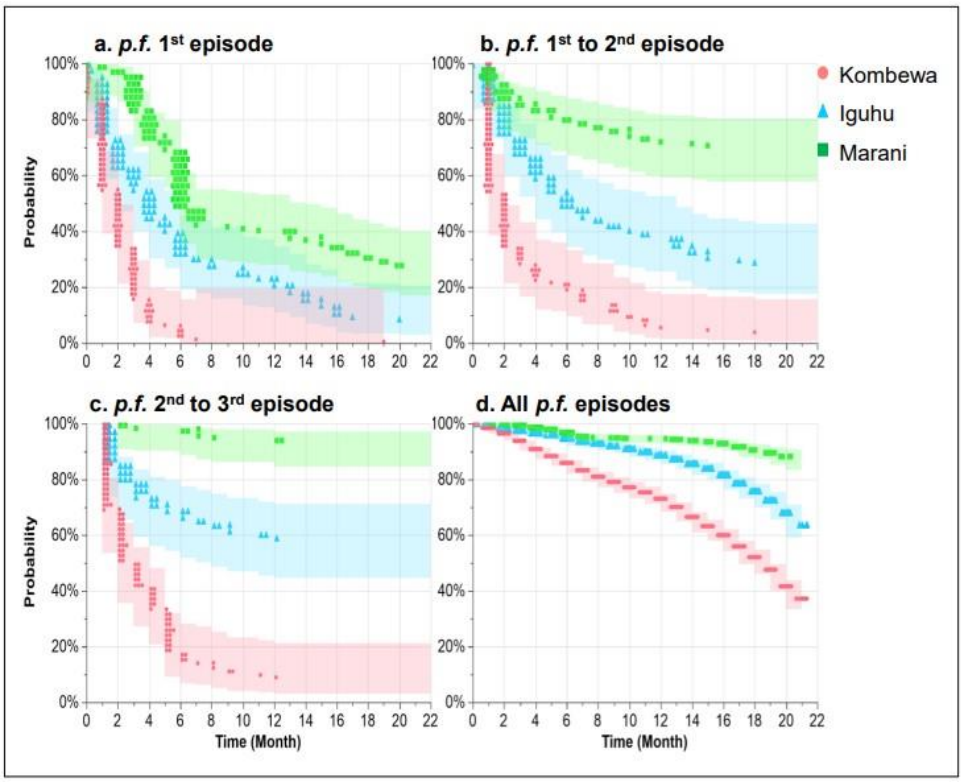

Fig. 5. 4 Kaplan-Meier probability of non-infection in Kombewa, Iguhu, and Marani in western Kenya.

Abbreviation: *p.f.*, *Plasmodium falciparum*. The probability of non-infection during malaria episodes in the study sites were compared using Log-Rank test.

Table 5. 3 Hazard ratios for the infection in Kombewa, Iguhu, and Marani in western Kenya

Predictors		Hazard ratio (95% CI[a])			
		Unadjusted	*P*[b]	Adjusted	*P*[b]
Sites	**Marani**	ref		ref	
	Kombewa	6.22 (5.32, 7.27)	<0.0001	6.12 (5.22, 7.16)	<0.0001
	Iguhu	2.58 (2.17, 3.07)	<0.0001	2.59 (2.18, 3.08)	<0.0001
Gender	**Male**	ref		ref	
	Female	0.74 (0.67, 0.82)	<0.0001	0.97 (0.89, 1.02)	0.005
Age	**5-10 yrs**	ref		ref	
	11-15 yrs	0.84 (0.75, 0.95)	0.007	1.01 (0.90, 1.15)	0.84

[a] Wald approximations were used for ratio confidence limits effects.

[b] *P* values were calculated with proportional hazards fit.

5.4.4 Vector Species Composition and Densities

A total of 583 female anophelines were collected between 1 Jan. 2018 to 31 Oct. 2019, comprising 458 (78.6%) *An. gambiae* s.l. and 125 (21.4%) *An. funestus*. Of these, 479 specimens (391 *An. gambiae* s.l. and 88 *An. funestus*) were analyzed for sibling species. For the *An. gambiae* s.l. specimens, PCR results indicated that 77.8% were *An. gambiae* s.s. and 22.2% *An. arabiensis* in Kombewa, 85.7% *An. gambiae* s.s. and 14.3% *An. arabiensis* in Iguhu, and 33.3% *An. gambiae* s.s. and 66.7% *An. arabiensis* in Marani. All the *An. funestus* subjected to species identification from the study sites were confirmed as *An. funestus* s.s.

The mean indoor resting densities of *An. gambia*e s.l. were significantly different among the study sites (Wilcoxon test, χ^2=253.44, *df*=2, *P*<0.0001), with Iguhu having the highest densities and Marani lowest densities (Fig. 5.5; Table 5.4). Also, the mean densities of *An. funestus* were significantly different among study sites (Wilcoxon test, χ^2=26.03, *df*=2, *P*<0.0001), with Kombewa having the highest densities and Marani lowest densities (Fig. 5.5; Table 5.4). Compared to 2014, vector density has decreased by >60% in all sites except in Iguhu, where *An. gambiae* s.l. density decreased slightly by 9%.

5.4.5 Blood Meal Indices and Annual Entomological Inoculation Rate

The blood meals of *An. gambiae* s.l. and *An. funestus* were mostly of bovine (55.3%) and human (90.4%) origin, respectively, in both Kombewa and Iguhu (Table 5.5). Due to the small number of mosquito collections in Marani, the HBI was not analyzed. Overall, the HBI of *An. gambiae* s.l. and *An. funestus* was 41.10% and 88.00%, respectively.

The annual EIR of *An. funestus* was threefold higher in Kombewa compared to Iguhu (Table 5.6). In Iguhu, the annual EIR of *An. gambiae* s.l. was threefold higher than the corresponding value of *An. funestus* (Table 3). Due to the small number of mosquito collections in

Marani, the annual EIR was not analyzed. The overall total annual EIRs were 5.12, 3.65 and 0.50 infective bites/person/year (ib/p/yr) at Kombewa, Iguhu, and Marani, respectively.

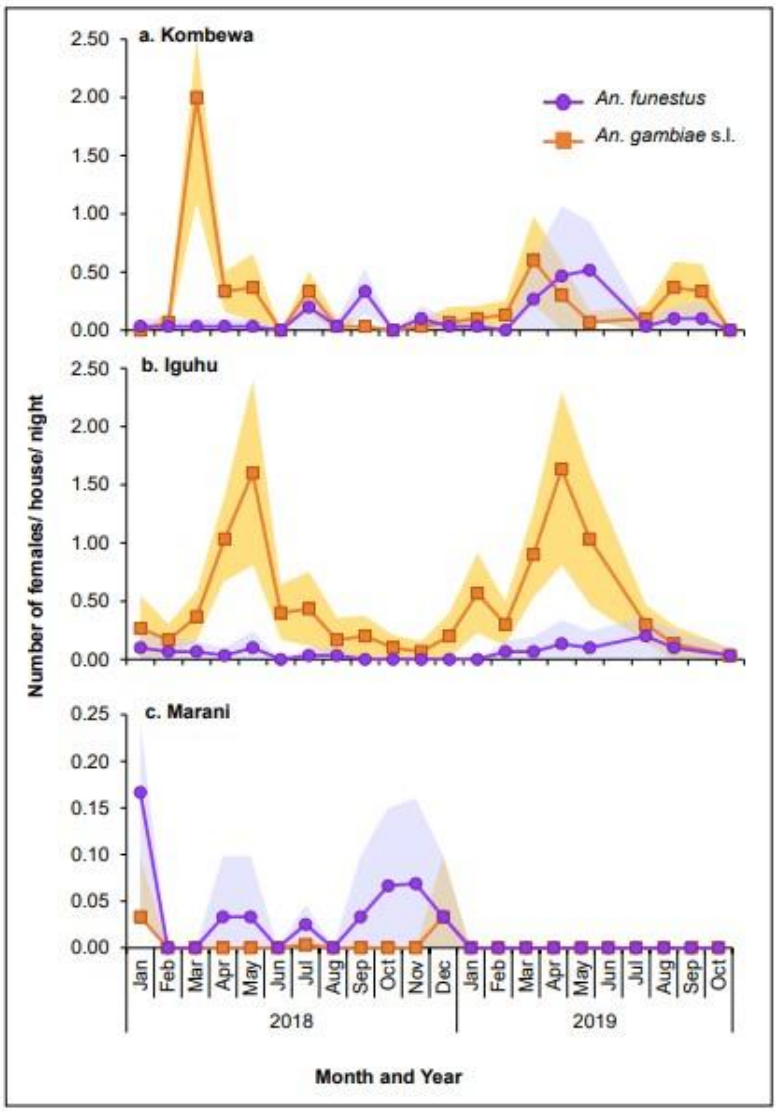

Fig. 5. 5 Indoor resting densities of *Anopheles* mosquitoes in Kombewa, Iguhu, and Marani in western Kenya.

Differences in vector density among study sites was compared using Wilcoxon/Kruskal-Wallis tests.

Table 5. 4 Indoor resting densities of *An. gambiae* s.l. and *An. funestus* in Kombewa, Iguhu, and Marani in western Kenya

Sites	Density (Female/house/night) 2018		Density (Female/house/night) 2019	
	***An. gambiae* s.l.**[a]	***An. funestus***[a]	***An. gambiae* s.l.**[a]	***An. funestus***[a]
Kombewa	0.27 (0.05, 0.49)	0.07 (0.01, 0.13)	0.24 (0.08, 0.40)	0.18 (0.00, 0.38)
Iguhu	0.42 (0.20, 0.64)	0.04 (0.00, 0.08)	0.61 (0.28, 0.94)	0.09 (0.00, 0.19)
Marani	0.00 (0.00, 0.02)	0.03 (0.01, 0.05)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)

[a] Differences in vector density among study sites was compared using Wilcoxon/Kruskal-Wallis tests

Mean (95% CI)

Table 5. 5 Host feeding preferences of *Anopheles* mosquitoes in Kombewa, Iguhu, and Marani in western Kenya

Study sites and blood-meal origins	**Kombewa**		**Iguhu**		**Marani**	
	***An. gambiae* s.l. (%)**	***An. funestus* (%)**	***An. gambiae* s.l. (%)**	***An. funestus* (%)**	***An. gambiae s.l.* (%)**	***An. funestus* (%)**
No. tested	63	59	87	35	1	6
Human	18 (28.57)	44 (74.58)	29 (33.33)	23 (65.71)	1 (100.00)	2 (33.33)
Bovine	29 (46.03)	4 (6.78)	45 (51.72	1 (2.86)	0 (0.00)	3 (50.00)
Goat	0 (0.00)	1 (1.69)	1 (1.15)	0 (0.00)	0 (0.00)	0 (0.00)
Pig	1 (1.59)	2 (3.39)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Dog	1 (1.59)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Human + bovine	5 (7.93)	0 (0.00)	2 (2.30)	8 (22.86)	0 (0.00)	0 (0.00)
Human + dog	0 (0.00)	4 (6.78)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Human + pig	0 (0.00)	1 (1.69)	0 (0.00)	2 (5.71)	0 (0.00)	1 (16.67)
Human + goat	4 (6.35)	2 (3.39)	2 (2.30)	1 (2.86)	0 (0.00)	0 (0.00)
Human + bovine + pig + goat	0 (0.00)	0 (0.00)	1 (1.15)	0 (0.00)	0 (0.00)	0 (0.00)
Bovine + goat	1 (1.59)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Pig + goat	1 (1.59)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Unknown	3 (4.76)	1 (1.69)	7 (8.05)	0 (0.00)	0 (0.00)	0 (0.00)
HBI[a]	**42.86%**	**86.44%**	**39.08%**	**97.14%**	**100.00%**	**50.00%**

[a] Human blood index (HBI) was calculated as the number of mosquitoes positive for human blood-meal (including mixed blood-meal) divided by the total number tested

Table 5. 6 The EIR of *Anopheles* mosquitoes in Kombewa, Iguhu, and Marani in western Kenya

Study site and Species	Mean no. of sleepers/ house	Mosquito density	Sporozoite rate	HBI[a]	Annual EIR[b]
Kombewa					
***An. gambiae* s.l.**	2.96	0.26	0.15	0.43	2.07
An. funestus		0.12	0.24	0.86	3.05
Iguhu					
***An. gambiae* s.l.**	3.35	0.50	0.13	0.39	2.76
An. funestus		0.06	0.14	0.97	0.89
Marani					
***An. gambiae* s.l.**	3.11	0.00	0.00	1.00	0.00
An. funestus		0.02	0.44	0.50	0.50

[a] HBI, human biting index

[b] EIR, entomological inoculation rate

5.4.6 Climatic Data

Rainfall among the three study sites were not statistically different (ANOVA, $F_{(2, 69)}=1.24$, $P>0.05$). The mean annual maximum (ANOVA, $F_{(2, 69)}=29.72$, $P<0.0001$) and minimum (ANOVA, $F_{(2, 69)}=77.19$, $P<0.0001$) temperatures were significantly different among the sites (Fig. 5.6). The mean annual temperature between Iguhu and Marani was not significantly different (Tukey-Kramer HSD test, $P=0.0004$), whereas it was significantly different between Iguhu and Kombewa and between Marani and Kombewa (Tukey-Kramer HSD test, all $P<0.0001$) (Fig. 5.6).

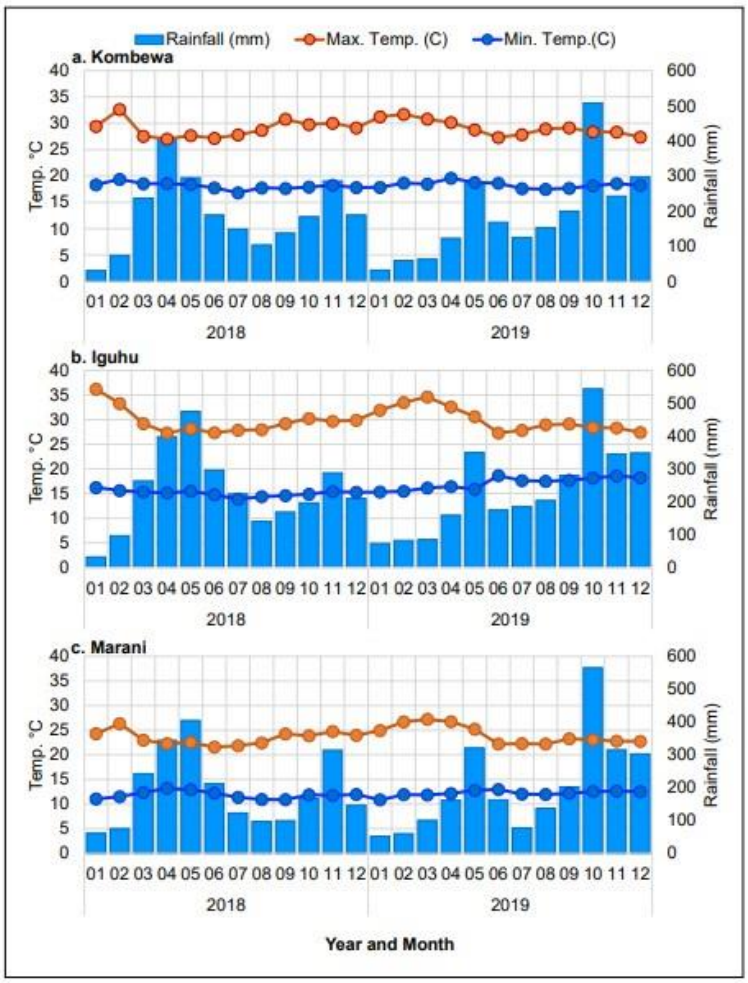

Fig. 5. 6 Variations in monthly maximum temperature, minimum temperature, mean temperature and monthly rainfalls in Kombewa, Iguhu, and Marani in western Kenya.

5.5 Discussion

Parasite prevalence has been decreasing since 2005 in the three sites and is likely associated with a reduction in vector abundance after free mass LLIN distributions after 2006, application of IRS, and increased use of ACT treatment (Gimnig *et al*., 2016; Zhou *et al*., 2011). However, there has been an observed resurgence of parasite prevalence since 2008 (Iguhu) and 2011 (Kombewa and Marani) and malaria vector densities since 2008 in all sites. These changes may be attributed to worn-out bednets and irregular use of nets; reduced optimum efficacy of LLINs over time; development of pyrethroid resistance in malaria vectors, and less coverage of IRS in epidemic-prone areas (DOMC & KNBS, 2011; Zhou *et al*., 2011). Additionally, in 2014 the resurgence in malaria transmission observed in Marani may also be explained by the increase in ambient temperatures between 2012 and 2015 and high rainfall in 2014 (Kapesa *et al*., 2017). The sharp decrease in indoor resting vector densities since 2014 is likely due to continuous scaling up of LLINs in the study area. Nevertheless, despite the decrease in vector densities, persistent malaria transmission in the context of extensive malaria vector control has been observed, and this could be attributed to outdoor vector biting and resting behavior to avoid physical contact with insecticide treated materials, changes in vector behavior to early-evening biting and early exiting from houses, as reported in western Kenya and other parts of Africa (Bamou *et al*., 2021; Ototo *et al*., 2015; Wanjala *et al*., 2015).

The 2018-2019 study observed a higher prevalence of gametocytes in Kombewa and Iguhu than in Marani and shows that the populations living in Kombewa and Iguhu maintain a large reservoir of infectious gametocytes, thus leading to stable and continuous malaria transmission. In contrast, the population living in highland village of Marani consists of a high proportion of susceptible individuals, and consequently, under suitable climatic conditions, may experience

malaria resurgences (Wanjala *et al*., 2011). Hence, monitoring air temperature and precipitation data is crucial in predicting vector and parasite dynamics, particularly in the highlands where slight changes in these parameters could lead to malaria epidemics (Kapesa *et al*., 2017).

Many factors have been associated with heterogeneity in malaria risk, and include biotic, abiotic, and socio-economic factors (Essendi *et al*., 2019). Kombewa had the highest risk and hazard ratio of asymptomatic malaria infections in the study. Furthermore, the median time interval and probability of non-infection during malaria episodes was least in Kombewa compared to other study sites, indicating increased malaria exposure. The study further reported a gender bias towards males in asymptomatic malaria infection. Briggs *et al*. (2020) (Briggs *et al*., 2020) observed that the sex-based difference might be elucidated by a slower clearance of infection in males than females due to differences in immune responses (Bernin & Lotter, 2014; Briggs *et al*., 2020; Fish, 2008). In other studies, this sex-based difference has been postulated to socio-behavioral factors that place men at a higher risk (Camargo *et al*., 1996; Pathak *et al*., 2012). Higher risk of malaria in male children and adolescents is likely linked to an array of physiological and behavioral changes that could contribute to the observed gender bias in this study. The possible explanations put forward for the gender difference in malaria infection include roles of sex hormones in the functioning of the immune system, immunological factors, cultural factors, and vector exposure, such as not sleeping under a net (Bernin & Lotter, 2014; Finda *et al*., 2019; Fish, 2008; Pathak *et al*., 2012). Therefore, research studies on sex-based differences in infectious diseases such as malaria are essential in providing optimum disease management for both genders (Bernin & Lotter, 2014). In Kombewa, young children had a higher parasite density than older individuals. The declining risk of parasitaemia as age increases has been documented in other parts of Africa with

stable malaria transmission, since individuals develop semi-immunity after continued exposure to infectious mosquito bites (Rogier *et al*., 1999; Sarpong *et al*., 2015).

Studies conducted over two decades ago showed that the HBI of indoor resting *An. gambiae* s.s. in western Kenya was 96-97%, indicating that they had fed exclusively on humans (Githeko *et al*., 1994b; Shililu *et al*., 1998). However, in this investigation, the overall HBI of *An. gambiae* s.l. in all study sites was only 41.1%. This behavioral plasticity in host seeking suggests that there has been a shift in blood meal sources, which could be attributed to extensive bednet coverage in the region (Ndenga *et al*., 2016). Conversely, *An. funestus* was highly anthropophilic, an observation that has been previously made in Kenya and other parts of Africa (Githeko *et al*., 1994b; Mzilahowa *et al*., 2012; Tanga *et al*., 2011). Furthermore, in studies conducted in Kombewa, the highly anthropophilic *An. funestus* has been reported to have high resistance to pyrethroids, and changes in their biting behavior could be a major factor sustaining high transmission in the area amidst extensive malaria vector control (Kapesa *et al*., 2017; Ototo *et al*., 2015).

The EIRs obtained in previous studies by Githeko et al. (Githeko *et al*., 1993) and Beier et al. (Beier *et al*., 1990) were exceedingly high (91-416 ib/p/yr) in western Kenya. Since then, there has been a decline in the annual *P. falciparum* inoculation rates, as observed by Ndenga *et al*. (2016), who reported the total annual EIRs as 31.1, 16.6, and 0.4 ib/p/yr at Kombewa, Iguhu, and Marani, respectively (Ndenga *et al*., 2006). In the current study, the lower inoculation rates recorded could be attributed to reduced vector densities and to some extent, a shift to non-human feeding by the malaria vectors due to high bednet coverage in the study areas (Ndenga *et al*., 2016). Nevertheless, *An. funestus* and *An. gambiae* s.l. played major roles in malaria transmission in

Kombewa and Iguhu, respectively, despite the comparatively low vector densities, indicating high vectorial efficiency of these anophelines in transmitting malaria in the region.

One limitation of our study was that parasitological surveys were based on microscopy only, which may not detect light Plasmodial infections compared to highly sensitive PCR-based techniques. Hence, the *P. falciparum* prevalence and infection pattern may have been underestimated. A second limitation was the lack of long-term information on outdoor malaria transmission dynamics, which may have provided insight to the resurgence in *P. falciparum* transmission despite continuous intervention efforts.

CHAPTER 6

6.0 IDENTIFICATION OF MALARIA HOTSPOTS DEFINED BY ASYMPTOMATIC PARASITE CARRIAGE, MALARIA VECTOR ABUNDANCE, AND *ANOPHELES* LARVAL DENSITIES IN MARANI IN WESTERN KENYA

6.1 Abstract

The transmission of malaria often shows spatial heterogeneity. This heterogeneity reduces the effectiveness of control strategies; however, targeting control strategies at hotspots of transmission may represent a highly effective strategy for reducing transmission and could be essential for malaria elimination. This study aims to identify and map hot and cold spots of malaria transmission in a low-endemic area that is prone to epidemics in the western Kenyan highlands. A cross-sectional survey was conducted in the community with 575 individuals (children and adults) and an entomological survey in 300 households. The Getis-Ord Gi* statistic was used to detect hotspots of malaria transmission based on asymptomatic malaria prevalence detected by qPCR, *Anopheles* larval densities, and densities of *Anopheles* mosquitoes in PSC. Hot and cold spots of elevated vector abundance and *Anopheles* larval densities were detected. We observed partial co-location between asymptomatic malaria infections and hotspots of *Anopheles* larval densities and vector abundance. Geospatial technology is essential for the analysis of spatial and temporal data, and the tool is particularly important in targeted control interventions that may effectively reduce the malaria burden and eventually eliminate it. Further studies are required to understand the environmental, socio-demographic, and behavioural factors determining malaria risk.

Keywords: Hotspots, Malaria, Asymptomatic parasitemia, Mosquito

6.2 Introduction

It is well known that the transmission of many infectious diseases, including malaria, shows marked heterogeneity in space and time (Woolhouse *et al*., 1997). Variation in the risk of malaria occurs at a global or micro-epidemiological scale (Bejon *et al*., 2010; Bousema *et al*., 2010; Ernst *et al*., 2006; Gething *et al*., 2011; Kleinschmidt *et al*., 2001; Woolhouse *et al*., 1997) and results in apparent clustering of transmission intensity that exceeds the average level, forming hotspots of transmission. Malaria hotspots are defined as 'a geographical part of a focus of malaria transmission where transmission intensity exceeds the average level' (Bousema *et al*., 2012). Malaria transmission in these hotspots is driven by numerous factors including environmental factors (distance to the nearest larval habitats, wind direction and velocity, vegetation cover, land use, altitude, topography, rainfall, temperature, hydrology, and soils), genetic factors (red blood cell genetic polymorphisms), level of host immunity, and socio-economic factors (urbanization, housing structure and condition, household occupancy, access to treatment and vector control interventions, agricultural practices, wealth, education, and human mobility) (Amoako *et al*., 2014; Bousema *et al*., 2010; Clark *et al*., 2008; Ernst *et al*., 2009; Gamage-Mendis *et al*., 1991; Ghebreyesus *et al*., 1999; Hawkes *et al*., 2019; Hay *et al*., 2005; Lacroix *et al*., 2005; Mackinnon *et al*., 2005; Midega *et al*., 2012; Reyburn *et al*., 2005; Rodríguez-Rodríguez *et al*., 2021; Tusting *et al*., 2015; Williams, 2006; Yadouléton *et al*., 2010).

Hotspots are important because they can serve as reservoirs of malaria parasites, thereby fueling transmission or resurgences of malaria, reduce the effectiveness of malaria control, and hence threaten the gains made in the reduction in malaria burden (Bousema *et al*., 2012, 2016; Nourein *et al*., 2011; Woolhouse *et al*., 1997). Nonetheless, to counter this effect, it is prudent to reliably identify and target malaria interventions in hotspots, as this approach will be highly

efficient, cost-effective, and benefit the whole community if the assumption that hotspots seed transmission in surrounding areas is correct (Bejon *et al*., 2014; Bousema *et al*., 2012; Smith *et al*., 2007; Woolhouse *et al*., 1997). Challenges in the identification of spatial heterogeneity in malaria transmission include the choice of the transmission indicator to measure, the choice of the geospatial method of detection, the spatial scale chosen, when to detect them, and their stability over time (Bousema *et al*., 2012; Mosha *et al*., 2014).

To target hotspots of transmission, tools are required to reliably identify these hotspots. Several approaches to identifying malaria transmission hotspots have been described using combinations of malaria incidence, asymptomatic parasite carriage, reported fever, drug use, serological responses to selected malaria antigens, vector abundance, and vector biting intensities (Bejon *et al*., 2010; Bousema *et al*., 2010; Ernst *et al*., 2006; Teuscher, 1992). Geographical clustering of asexual parasite carriage may be a more stable indicator of hotspots of transmission (Bejon *et al*., 2010) and serological markers of malaria exposure have been especially suggested in areas of moderate or low endemicity (Bousema *et al*., 2010).

Statistically, hotspots have been delineated using various methods that include cluster detection algorithms and geostatistical methods (Giorgi *et al*., 2018; Kabaghe *et al*., 2017). Cluster detection algorithms involve comparing the density of points (malaria infections) within a defined area with the distribution of all sampled points in the entire study area, and the most commonly applied tools are Kulldorf's spatial scanning statistic (SatScan) and Getis Ord-Gi* (Pullan *et al*., 2012; Sturrock *et al*., 2014). Spatial prediction algorithms use model-based geostatistics, kriging, or regression trees to estimate the malaria burden at unsampled locations informed by the available malaria data and relevant covariable information that includes intervention coverage, elevation, or land use to help predictions (Stresman *et al*., 2019). The analysis of each of these methods varies,

with the suitability of the tool depending on the specific research question, availability of data, and any causal spatial dynamics present (Stresman *et al*., 2019).

In this study, we will identify and map hotspots and cold spots of malaria transmission in a low-endemic area that is prone to epidemics in the western Kenyan highlands.

6.3 Material and Methods

6.3.1 Study Population

Parasitological and entomological surveys were conducted in selected households located within the study site. During the surveys, the geographical coordinates of households were determined using Global Positioning System (GPS) recording devices. Individual-level data was collected from all participants and assigned a unique personal identifier; furthermore, information on household characteristics such as type of construction materials used, presence or absence of window screens and eaves, and number of LLINs was recorded.

6.3.2 Malaria Parasite Prevalence

Asymptomatic malaria parasitemia surveys were conducted in the community, involving 575 individuals (children and adults) in 300 households. The survey was conducted in July and August 2018, after the long rainy season. Informed consent (assent for minors < 18 years) forms were obtained from the participants. At the time of sampling, participants who were found to have a fever, i.e., an axillary temperature $\geq 37.5°C$, were referred to the nearest government health facility for treatment according to Kenyan government malaria treatment guidelines. Blood samples were collected using the standard finger-prick method, and thin and thick blood smears were prepared on labeled slides for malaria parasite species identification using microscopy. Malaria parasite counts were scored against 200 white blood cells. All slides were examined by an experienced

laboratory technician, and a second technician carried out random checks on the slide counts for quality control purposes. Asymptomatic parasitemia was also measured using molecular detection of *Plasmodium* infections. Genomic DNA was extracted from dried blood spots on filter paper using the Chelex protocol by Musapa *et al*. (Musapa *et al*., 2013). Confirmation of the presence of parasite DNA was done by a multiplexed real-time qPCR assay using the published species-specific 18 s ribosomal RNA probes and primers for *Plasmodium falciparum*, *P. malariae*, *and P. ovale* (Shokoples *et al*., 2009; Veron *et al*., 2009).

6.3.3 Adult Mosquito and Larval Sampling

Indoor resting adult mosquitoes were sampled using PSC in 300 houses from 6 a.m. to 9 a.m., following WHO protocol (WHO, 1975). Mosquitoes collected were sorted and anophelines identified to species using morphological keys as previously described (Gillies & Coetzee, 1987).

The field team conducted intensive searches of aquatic habitats and characterized the habitats based on type, size, vegetation cover, and appearance. Mosquito larvae were collected from habitats in each village using the standard dipping method (350 ml dipper). Larval habitat types that were identified were mainly drainage ditches, fish ponds, and swamps/marshes. The larvae were sorted, counted, identified morphologically as *Anopheles* or culicine larvae, and classified as early instar stage (I and II) or late instar stage (III and IV). The geographical coordinates of each larval habitat were recorded using GPS recording devices.

6.3.4 Ethics approval and consent to participate

The study obtained ethical approval from the Maseno University Ethics Review Committee (MUERC Protocol No. 00660) (**Appendix 4**) and the University of California, Irvine Institutional Review Board (UCI IRB). Written informed consent was obtained from children's

parents/guardians and adults before they were enrolled in the study and household heads (**Appendix 5 and 6**).

6.4 Results

6.4.1 Study Population

The study area covered 30 square kilometers. 575 individuals living in 300 households within the study area were included in the survey (Table 6.1.). The LLINs ownership was 89.1%. The distribution of *Plasmodium* infection and adult vector density are indicated in Figure 6.1.

6.4.2 Spatial Clustering of Asymptomatic Parasitaemia

Blood samples were taken from a population with a varied age distribution. The majority of blood samples were taken from teenagers and adults of ages 16 and above (57.9%), followed by children aged between 5 and 15 years (25.7%), children aged between 1 and 4 years (15.1%), and the least from infants less than one year old (1.2%). The overall malaria parasite prevalence by qPCR tests was 8.1%. Spatial clustering of asymptomatic parasitemia could not be analysed due to only a few positive data points (Fig. 6.2).

Table 6. 1 Summary of parasitological and entomological surveys in the study site

Survey	**Characteristics**	
Parasitological surveys	Households/population	300/575
	Households with positive PCR tests	33 (11%)
	Prevalence asymptomatic infection (PCR)	8.1%
Entomological surveys	Households	300
	Households with *Anopheles*	7 (2.3%)
	Range of *Anopheles* captured/house	0-3
	Total *Anopheles* captured	8
	Total *An. gambiae* captured	1
	Total *An. funestus* captured	7
	Total larval habitats sampled	163
	Total *Anopheles* larvae sampled	1,182
	Total *An. gambiae* larvae sampled	380 (32.1%)
	Total *An. funestus* larvae sampled	802 (67.9%)

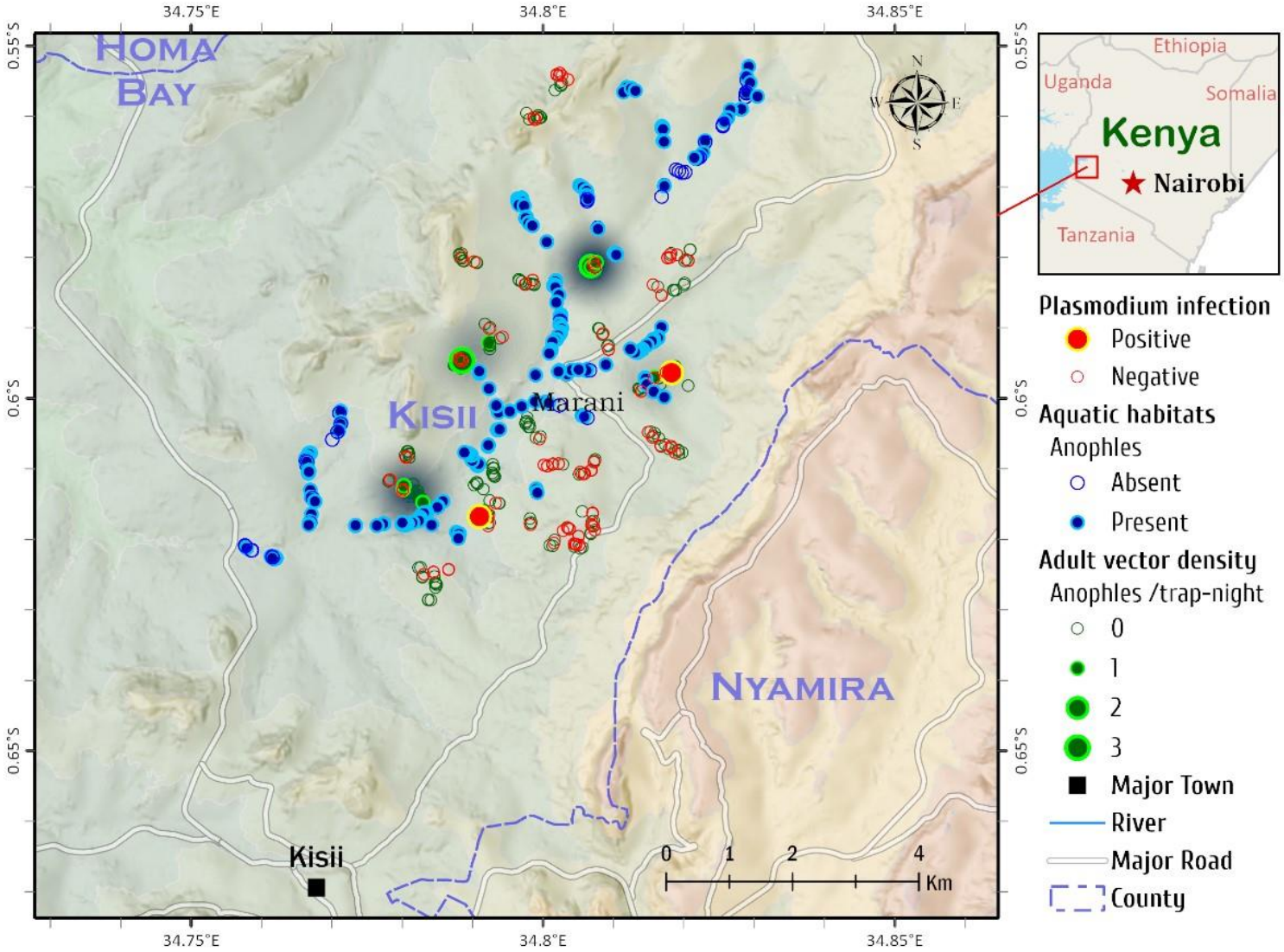

Figure 6. 1 Distribution of *Plasmodium* infection, aquatic habitats, and adult vector density in Marani, western Kenya

6.4.3 Spatial Clustering of Malaria Adult Vector Abundance

Anopheles mosquitoes were captured in only 2.5% of the surveyed houses, and a total of 8 mosquitoes were caught indoors. Spatial clustering analyses of adult vector abundance showed the presence of hotspots in the study site and that the *Anopheles* species aggregated in a few houses (Fig. 6.3).

6.4.4 Spatial Clustering of *Anopheles* larval densities

A total of 163 aquatic larval habitats were identified and sampled. The habitats that were identified consisted of drainage ditches (27.8%), swamps/ marshes (22.7%), fish ponds (19.5%), natural ponds/ rain pools (9.3%), river edges/ reservoir shorelines (6.6%), man-made ponds (4.9%), animal footprints (3.2%), and springs or brick pits (1.1%). A total of 1,182 *Anopheles* mosquito larvae were collected and were present in 51.5% (N=84) of the habitats. The most abundant were the early instar stage (59.7%, N=706) compared to the late instar stage (40.3%, N=476). A total of 99 pupae were collected. A total of 2,156 *Culex* larvae were collected. Potential predators (fish, frogs, *Gambusia*, backswimmers, dragon flies, and water bugs) were identified in all the habitats. Spatial clustering analyses of *Anopheles* larval densities showed the presence of hot and cold spots in the study site and that the *Anopheles* larval species aggregated in several habitats (Fig. 6.4).

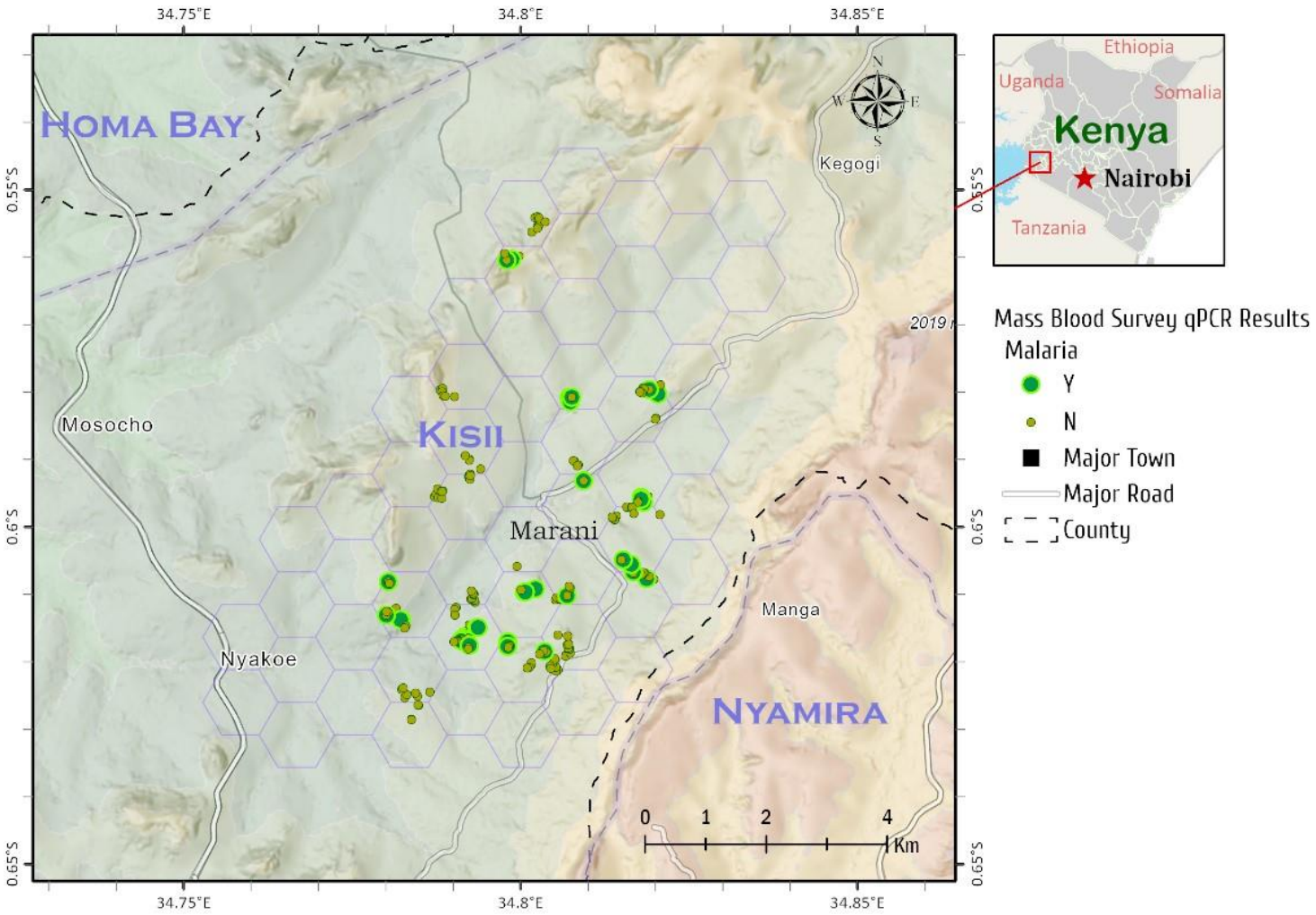

Figure 6. 2 A map of asymptomatic malaria infections detected by qPCR.

Abbreviation: qPCR, quantitative polymerase chain reaction

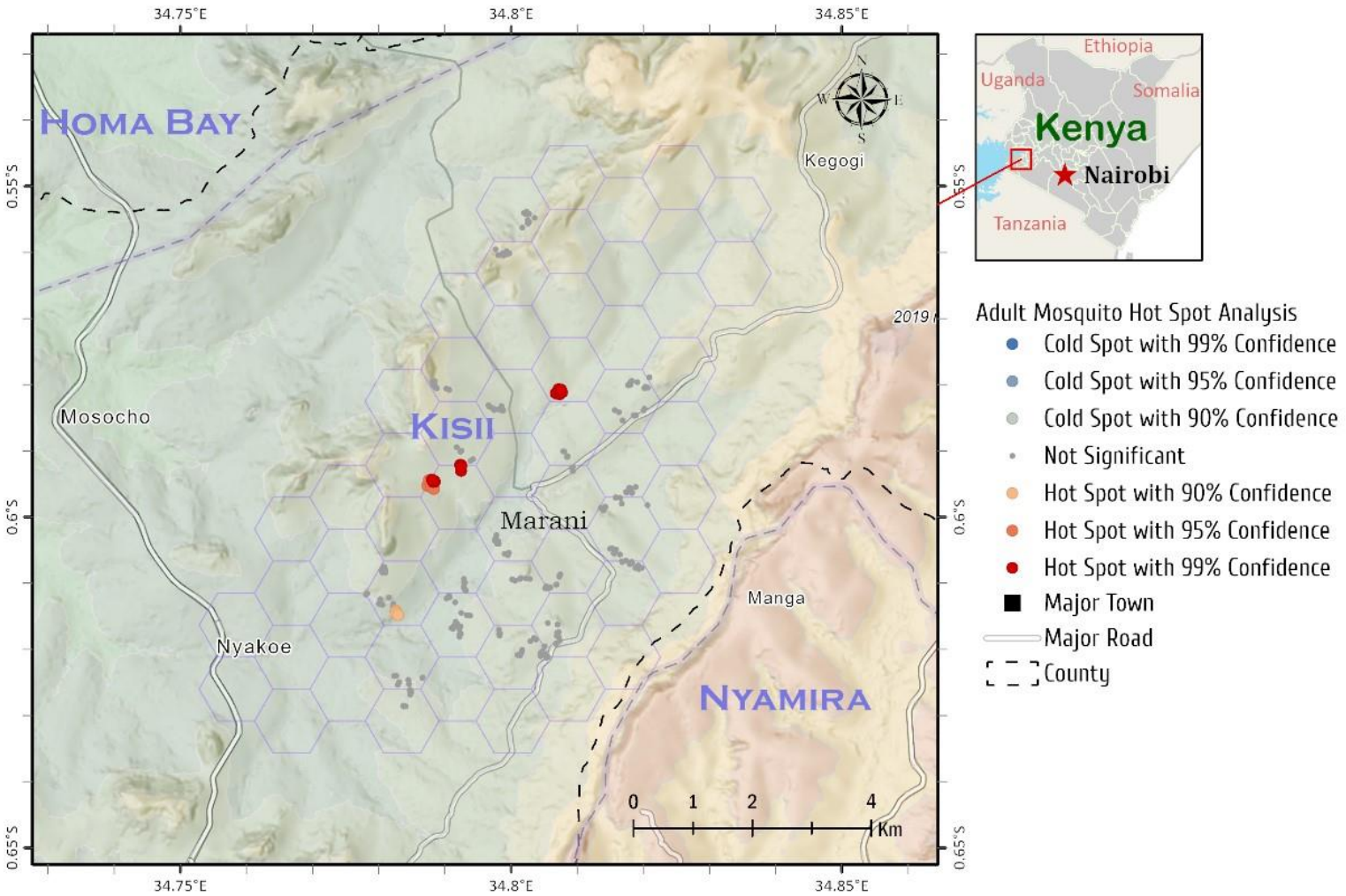

Figure 6. 3 Hot and cold spots of malaria adult vector abundance.

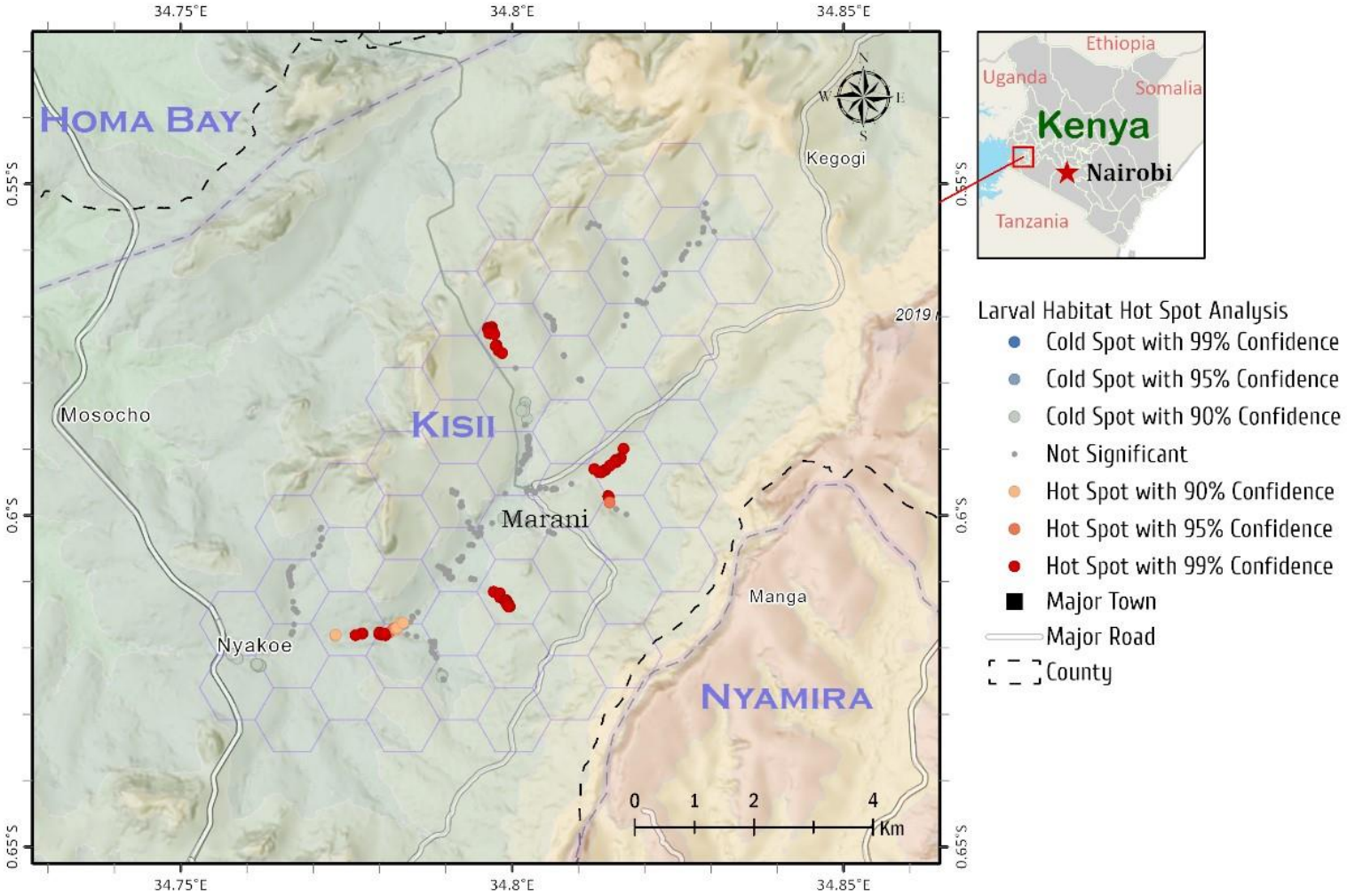

Figure 6. 4 Hot and cold spots of *Anopheles* larval densities.

6.4.5 Co-Location between Asymptomatic Parasitaemia and Adult Vector Abundance and Aquatic Larval Habitats

There was partial co-location between asymptomatic malaria infections and hotspots of vector abundance and *Anopheles* larval densities (Fig. 6.5, 6.6).

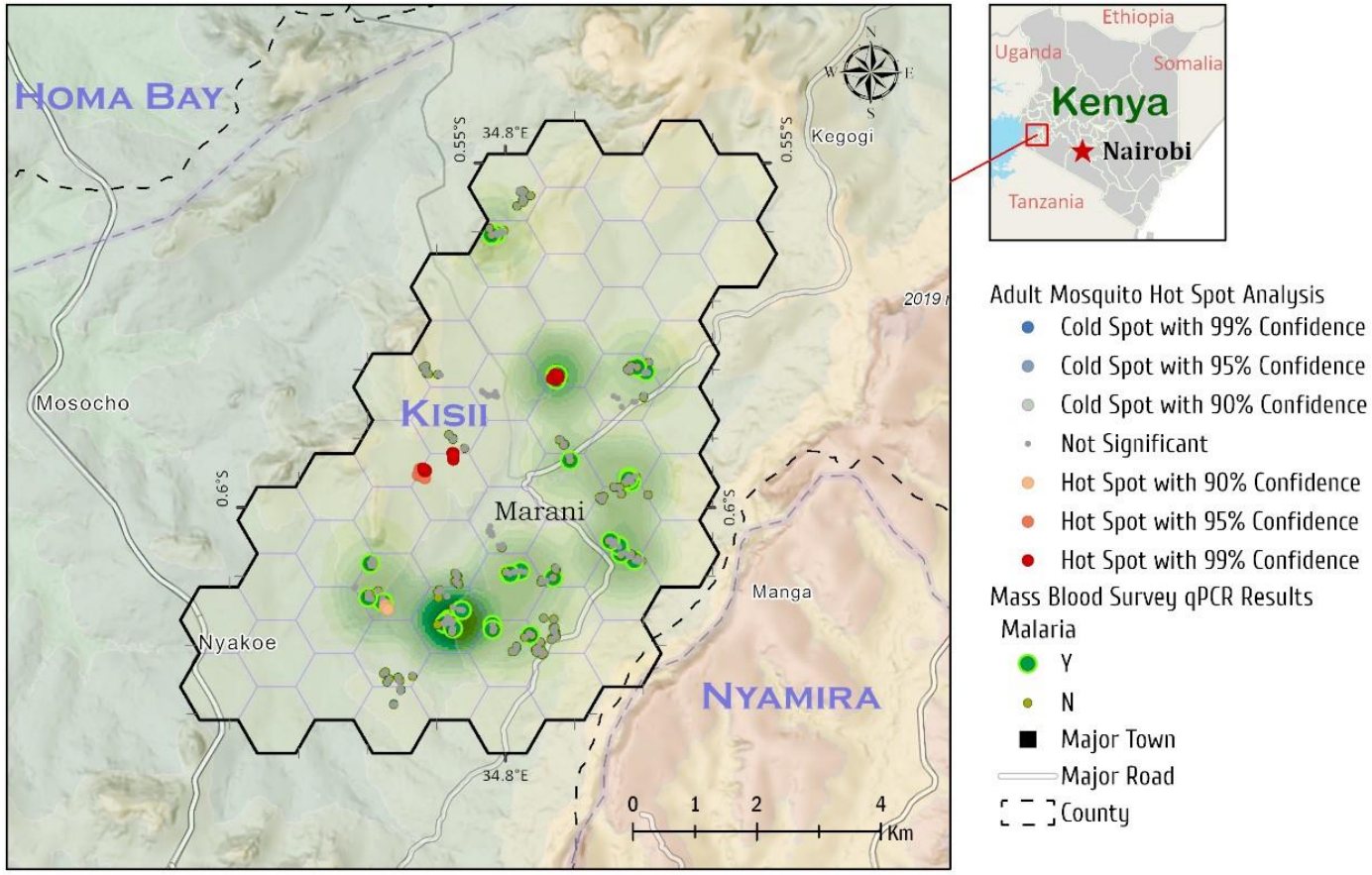

Figure 6. 5 Co-location between asymptomatic parasitaemia and adult vector abundance.

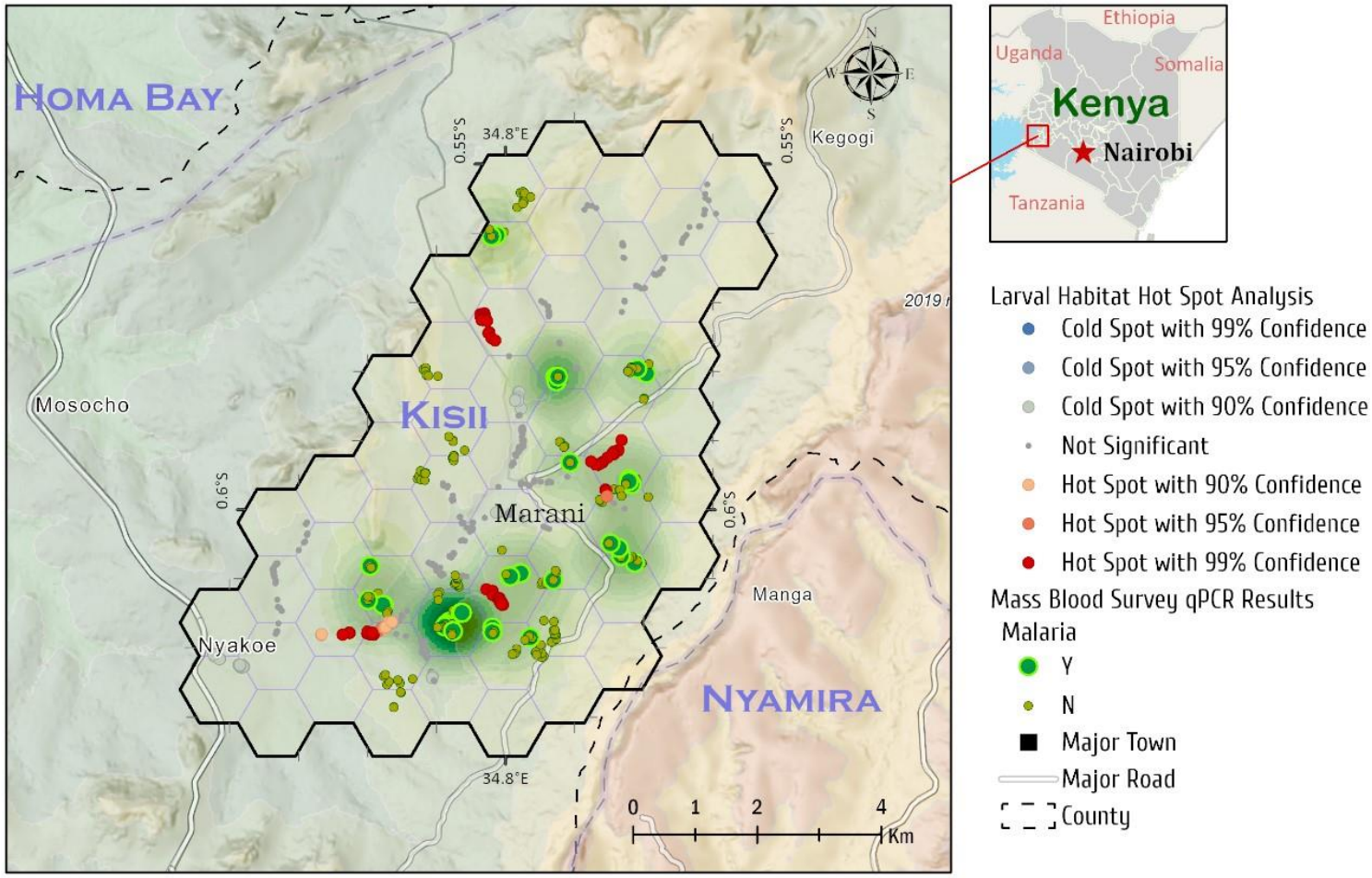

Figure 6. 6 Co-location between asymptomatic parasitaemia and *Anopheles* larval densities.

6.5 Discussion

We observed clusters of elevated vector abundance and *Anopheles* larval densities that we interpreted as potential hotspots of malaria transmission in a highland site in western Kenya. The identification of spatial clustering of elevated vector abundance and *Anopheles* larval densities was made possible by the utility of geospatial technology.

The hotspots analysis did not identify spatial clustering of asymptomatic parasitemia due to only a few positive data points. The study was cross-sectional and was conducted once at the end of the wet season, which could have led to the few positive data points. In addition, the study could have produced different results if it had also focused on the clinical characteristics or severity of malaria. Hotspots of malaria transmission are associated with factors such as distance to the breeding site (Staedke *et al*., 2003), environmental conditions (Lindsay & Snow, 1988), altitude (Reyburn *et al*., 2005), farming activities (Lindsay & Snow, 1988), house construction (Lindsay & Snow, 1988), bed net coverage (Clark *et al*., 2008), and socio-economic status (Baragatti *et al*., 2009). Identifying and targeting hotspots could be a highly cost-effective way of reducing and eventually eliminating the malaria burden (Bousema *et al*., 2012). Elimination of malaria will therefore require routine longitudinal parasitological indicators with high spatial and temporal resolution to detect hotspots of transmission so interventions can be conveyed in a targeted and timely manner (Mwakalinga *et al*., 2016).

We were able to identify cold and hotspots of vector abundance. Spatial clustering analyses of adult vector abundance showed the presence of hotspots in the study site and that the *Anopheles* species aggregated in a few houses. According to studies, households show spatial variation in mosquito densities (Lindsay et al., 1995). We observed that in the study area, asymptomatic parasitemia only partially overlapped with clusters of vector abundance. The lack of an overall

relationship between the spatial distribution of malaria infection and vector abundance may be explained by differences in heterogeneity in exposure to malaria-infected mosquitoes, with some households having a higher exposure to *Anopheles* mosquitoes but not infected mosquitoes (Bousema *et al*., 2010).

Spatial clustering analyses of *Anopheles* larval densities showed the presence of hot and cold spots in the study site and that the *Anopheles* larval species aggregated in several habitats. There was partial co-location between asymptomatic malaria infections and hotspots of *Anopheles* larval densities. Closer proximity to the nearest breeding site has been known as a risk factor for malaria (Oesterholt *et al*., 2006; Staedke *et al*., 2003). This observation is expected because the dispersion distance of *Anopheles* mosquitoes is limited and they probably seek hosts in the nearest household. The larval habitats produced a lower number of mature larvae and pupae compared to the early *Anopheles* larval instars. This has been described previously, and it is attributed to the early larval instars being highly subjected to predation (Mutuku *et al*., 2006), which reduces the association between larval numbers and adult mosquito densities (Mutuku *et al*., 2006).

One limitation of the study was the limited period of observation of the parasitological and entomological indicators that was assessed at a single time-point, which does not allow a definitive assessment of the spatial heterogeneity.

CHAPTER 7

7.0 GENERAL DISCUSSIONS, CONCLUSIONS, AND RECOMMENDATIONS

7.1 GENERAL DISCUSSIONS

The current study was conducted with the aim of assessing the effect of recently established irrigation scheme in Homa Bay County in western Kenya, on malaria transmission and vector bionomics. It further aimed at evaluating the long-term changes in malaria transmission profiles and patterns of asymptomatic malaria infection in three sites with different transmission intensities in western Kenya with ongoing malaria interventions. The malaria intervention measures at the three sites are similar with standardized monitoring and evaluation procedures (National Malaria Control Programme (NMCP), 2019). The results are crucial in providing an assessment of vector interventions, a baseline for the evaluation of new interventions, and guide future control planning by the Malaria Control Programmes. The study aimed at identifying and mapping malaria transmission hot and cold spots in a low-endemic area prone to epidemics in western Kenyan highlands.

In Chapter 4 the study elucidated the effect of concrete canal and flood irrigation systems on anopheline species composition, abundance, seasonality, behaviuor, and malaria transmission in irrigated and non-irrigated zones during implementation of LLINs and IRS vector control strategies. Overall, *An. arabiensis* was shown to be the predominant anopheline species (79.2%) and being more abundant in the irrigated zone compared to the non-irrigated zone. The high density of *An. arabiensis*, coupled with its behavioural plasticity poses a significant risk of malaria transmission especially those living within the irrigation zone.

The study also compared the effectiveness of CDC light traps against the gold standard, HLC. Human landing catches is labour-intensive, cannot be deployed widely, and has ethical

concerns associated with potential exposure to infectious mosquito bites (Knols *et al*., 1995; WHO, 2013). Hence, the study evaluated CDC light traps as an alternative sampling tool that would be inexpensive, risk-free, and widely deployable. The reliability of CDC light traps as a tool for continuous operational surveillance of malaria vectors was affirmed by the current study, which showed them to be equally effective in malaria vector sampling as the HLC.

In evaluating the long-term changes in malaria transmission profiles in the three study sites in western Kenya (Chapter 5), characterized by heterogeneous malaria transmission and intense malaria interventions (DNMP & ICF, 2021; Dulacha *et al*., 2022; NMCP & KNBS, 2016), a decrease in parasite prevalence and vector densities from 2005 to 2008 was observed. However, a resurgence of parasite prevalence since 2008 (Iguhu) and 2011 (Kombewa and Marani) and malaria vector densities since 2008 in all sites were observed. Since 2011 to 2019, parasite prevalence has resurged by >40% in Kombewa and Marani, whereas in Iguhu, it has decreased by 7.3%. The sharp decrease in indoor resting vector densities since 2014 to 2019 is likely due to the continuous scaling up of LLINs in the study area. The heterogeneity in malaria transmission observed, with some areas indicating a decline in transmission while in others transmission has remained high or has resurged despite continuous intervention efforts, is linked to malaria vector species composition shifts, insecticide resistance, and climatic warming, as observed by Kapesa and others (Kapesa *et al*., 2017).

The study demonstrated a disparity in asymptomatic malaria infections, with Kombewa showing a higher risk than Iguhu and Marani and an overall gender bias where a disproportionately higher number of males are asymptomatic malaria parasite carriers. The observed heterogeneity in malaria risk in the study sites might be associated with differences in socioeconomic, biotic, and abiotic factors (Essendi *et al*., 2019). The sex-based difference in malaria infections, particularly

observed in male children and adolescents, might be elucidated by physiological and behavioural changes that include the roles of sex hormones in the functioning of the immune system, immunological factors, cultural factors, and vector exposure, such as not sleeping under a net (Bernin & Lotter, 2014; Finda *et al*., 2019; Fish, 2008; Pathak *et al*., 2012).

The hotspots of *Anopheles* larval densities and vector abundance were identified in an epidemic-prone highland setting in western Kenya, as detailed in Chapter 6. The study showed a partial co-location between asymptomatic malaria infections and hotspots of *Anopheles* larval densities and vector abundance. Such findings suggest a potential breakthrough in the implementation of targeted interventions in high-risk areas for an improved impact on transmission, especially if the malaria transmission hotspots can be identified as recommended by Bousema and others (Bousema *et al*., 2012).

7.2 CONCLUSIONS

1. *Anopheles arabiensis* was the predominant malaria vector in both irrigated and non-irrigated agroecosystems in Homa Bay County in western Kenya. However, its role in malaria transmission varies between the two agroecosystems, with the irrigated zone recording a higher transmission potential, which could be reattributed to the high densities of *An. arabiensis* in the irrigated zone.
2. A small but significant population of a highly anthropophilic *An. funestus* group remains a key threat and contribution to malaria transmission in the irrigated and non-irrigated zones of Homa Bay County.
3. Indoor settings remain the most risky locations, though not statistically significant, for malaria transmission compared to outdoor settings, particularly in the irrigated zone, where the

propensity of receiving an infectious mosquito bite remained high at 0.4 ib/p/year from *An. arabiensis*.

4. Malaria prevalence, especially in asymptomatic cases, remains disproportionately high in the study area, indicating a potential resurgence of malaria infections. Such findings either demonstrate the ineffectiveness of the intervention strategies being implemented or adaptive behavioural changes in the vector and human populations that complicate the intervention efforts.
5. The majority of the asymptomatic malaria infection cases were associated with males residing in malaria-endemic study sites. This could potentially indicate that the male population remains a great contributor and source of malaria infections among the vulnerable population within the household and general community at large.
6. There was partial co-location in the occurrence of asymptomatic malaria infections with spatial clusters of *Anopheles* larval densities and adult population abundance. Such findings point to a potential time bomb in the fight against malaria, as an explosion in malaria cases may be witnessed in the near future with grave consequences if urgent intervention measures are not undertaken.

7.3 RECOMMENDATIONS

1. There is a need to re-evaluate the vector intervention strategies in the irrigation scheme and also consider the incorporation of additional vector control tools to complement the LLINs and IRS. Changes in vector behaviuor and high vector densities underscore the necessity of integrating larval source management, the use of mosaic and synergized insecticide-treated

nets, as well as cutting-edge technologies such as gene drive, drones, and artificial intelligence (AI)-enabled mosquito surveillance tools.

2. There's a need for further evaluation of the behaviour of *An. arabiensis* and its role in residual transmission, given its behavioural plasticity. A scenario where malaria transmission is occurring at outdoor locations due to the intensification of indoor intervention tools, especially LLINs and IRS, in western Kenya coupled with the high prevalence of asymptomatic malaria cases should be evaluated further.
3. More research on the movement of endophagic mosquitoes when they exit the house after feeding and/or resting is needed in the irrigation scheme. This finding will help researchers better understand the impact of insecticide-based vector control measures in houses on the normal movement, density, and feeding of endophilic species.
4. A strong coordination and collaboration between the Ministries of Health, Agriculture, Livestock, Fisheries, and Irrigation, and Environment and Forestry in devising strategies that boost crop production in irrigated agroecosystems while limiting the proliferation of vector populations is recommended.
5. Further research on gender disparities in malaria infection in the study area is advised. Studies on socio-behavioural factors and biological sex differences will aid malaria control implementers in determining the best disease management strategy for both genders.
6. Geospatial technology is essential for the analysis of spatial and temporal data and is particularly important in effective vector-borne disease prevention, control, and eventually elimination. Consequently, targeted control might effectively reduce those with asymptomatic infections, potentially decrease malaria transmission, and prevent resurgences.

The study findings and recommendations will be cascaded to county or sub-county levels for the benefit of the local communities in the following ways:

1. Collaboration with administrative and religious leadership will promote change in the community.
2. Organizing community outreach and disseminating the study findings and recommendations through walks, songs, and dance, school presentations (i.e., poems, choral verses, drama), and passing the message through lesos, t-shirts, and caps.
3. Advising the Ministry of Health to implement additional vector control measures, such as larval source management, to supplement core malaria vector interventions and prevent the spread of mosquito vector populations.
4. Collaborating with policymakers and communicating research findings to ensure that the results are turned into practice and policy.

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APPENDICES

APPENDIX 1: ETHICAL APPROVAL FROM MASENO UNIVERSITY ETHICS REVIEW COMMITEE

MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

Tel: +254 057 351 622 Ext: 3050
Fax: +254 057 351 221

Private Bag – 40105, Maseno, Kenya
Email: muerc-secretariate@maseno.ac.ke

FROM: Secretary - MUERC

DATE: 11th September, 2018

TO: Dr. Harrysone Atieli
Department of Public Health
School of Public Health and Community Development
Maseno University
P. O. Box, Private Bag, Maseno, Kenya

REF:MSU/DRPI/MUERC/00456/17

RE: Environmental Modification in Sub-Saharan Africa: Changing Epidemiology, Transmission and Pathogenesis of *Plasmodium falciparum* and *Plasmodium vivax* Malaria. Proposal Reference Number: MSU/DRPI/MUERC/ 00456/17

This is to inform you that the Maseno University Ethics Review Committee (MUERC) considered your valued application for extension of ethics approval of your study. The Committee commended the progress made and granted an **approval for continuation** of the study effective this 11th day of September, 2018 for a period of one (1) year

Please note that authorization to conduct this study will automatically expire on 10th September, 2019. If you plan to continue with the study beyond this date, please submit an application for continuation approval to the MUERC Secretariat by 15th August, 2019.

Approval for continuation of the study will be subject to successful submission of an annual progress report that is to reach the MUERC Secretariat by 15th August, 2019.

Please note that any unanticipated problems resulting from the conduct of this study must be reported to MUERC. You are required to submit any proposed changes to this study to the MUERC for review and approval prior to initiation. Please advice MUERC when the study is completed or discontinued.

Thank you.

MASENO UNIVERSITY SECRETARY 11 SEP 2018 ETHICS REVIEW COMMITTEE

Dr. Bonuke Anyona,
Secretary,
Maseno University Ethics Review Committee.

Cc: Chairman,
Maseno University Ethics Review Committee.

MASENO UNIVERSITY IS ISO 9001:2008 CERTIFIED

APPENDIX 2: ETHICAL APPROVAL FROM MASENO UNIVERSITY ETHICS REVIEW COMMITEE

MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

Tel: +254 057 351 622 Ext: 3050
Fax: +254 057 351 221

Private Bag – 40105, Maseno, Kenya
Email: muerc-secretariate@maseno.ac.ke

FROM: Secretary - MUERC

DATE: 11th September, 2019

TO: Dr. Harrysone Atieli
Department of Public Health
School of Public Health and Community Development
Maseno University
P. O. Box, Private Bag, Maseno, Kenya

REF: MSU/DRPI/MUERC/00456/17

RE: Environmental Modification in Sub-Saharan Africa: Changing Epidemiology, Transmission and Pathogenesis of *Plasmodium falciparum* and *Plasmodium vivax* Malaria. Proposal Reference Number: MSU/DRPI/MUERC/ 00456/17

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Approval for continuation of the study will be subject to successful submission of an annual progress report that is to reach the MUERC Secretariat by 15th August, 2020.

Please note that any unanticipated problems resulting from the conduct of this study must be reported to MUERC. You are required to submit any proposed changes to this study to the MUERC for review and approval prior to initiation. Please advice MUERC when the study is completed or discontinued.

Thank you.

MASENO UNIVERSITY SECRETARY 11 SEP 2019 ETHICS REVIEW COMMITTEE

Dr. Bernard Guyah
Ag. Secretary,
Maseno University Ethics Review Committee.

Cc: Chairman,
Maseno University Ethics Review Committee.

MASENO UNIVERSITY IS ISO 9001:2008 CERTIFIED

APPENDIX 3: CONSENT FORM FOR PARTICIPANTS

Appendix V: Consent Forms

Informed Consent

Form 1: CONSENT FORM FOR PARTICIPANTS

Title of Study: Environmental Modification in sub- Saharan Africa: Changing Epidemiology, Transmission and Pathogenesis of *Plasmodium falciparum* and *P. vivax* Malaria

This consent form will be explained and signed by each study participant

Name of Volunteer: _____,

Age of Volunteer: _____

1. **INVESTIGATORS CONDUCTING THE STUDY**:

   Dr. Harrysone Atieli, Maseno University, Kenya;

   Prof. Guiyun Yan, Program in Public Health, University of California, Irvine, USA;

2. **PURPOSE OF STUDY:**

   The International Centers of Excellence for Malaria Research (ICEMR) program studies the malaria vector (*Anopheles* mosquito) that transmits the malaria parasite. This study will look at the species composition, densities, and ecological behavior of the malaria vectors in Homa bay / Kendu bay.

3. **PROCEDURES TO BE FOLLOWED:**

   (1) Adult volunteers will be recruited to carry out human landing catches (HLC) mosquito collections indoors and outdoors. The indoor station will be set up in the living area of the house, while the outdoor station will be set up just outside the same house within 5 m of the front door.

   (2) The collections will begin at 1800 hrs. and end at 0600 hrs.

(3) The collector will sit on a stool and expose the lower parts of their legs.

(4) Hourly sampling of mosquitoes will be carried out with a mouth aspirator as they land on the legs and placed in time/ date-labeled paper cups.

(5) At the end of each hour, the old cup will be secured by placing a piece of cotton wool lightly soaked in a 10% sugar solution on top to keep the mosquitoes alive, and then placed in a cool box. The mosquitoes will be transported to the ICEMR laboratories in Homa bay for further analysis by qualified technicians.

(6) The same collectors will conduct HLC every night and will be rotated between positions (indoors vs outdoors).

(7) The collectors conducting HLC will be supervised through random spot checks throughout the study by a mobile team led by the investigators, who will also provide support and transport.

4. DISCOMFORTS AND RISKS:

During the collection of mosquitoes, you may be bitten, experience discomfort, and be exposed to malaria. To minimize these risks, you will be asked to take antimalarial tablets one week before the study and one week after the study is complete. Antihistamines will be administered in cases of skin irritation caused by mosquito bites.

5. BENEFIT TO PARTICIPANTS:

Although you will not benefit directly from participating in this study, you will make a major contribution to the knowledge known about malaria. In the future, others may benefit because scientists will learn how to improve malaria vector control strategies.

6. COST AND COMPENSATION:

You will be paid Ksh. 800 per shift for the time and travel required to collect the mosquitoes.

7. CONFIDENTIALITY:

We will protect your privacy. Your name will not appear in any reports or journals.

8. FREEDOM TO WITHDRAW:

Your participation in this study is voluntary and you may discontinue your participation at any time without prejudice.

9. CONSENT FORM:

The consent form will be explained to each study participant and signed by the investigators or the leading scientists conducting the study.

10. NEW FINDINGS:

You will be informed of any significant new findings developed during the course of this study.

11. <u>Do you have any questions?</u>

You can ask questions at any time. You can ask now. You can ask later. You can talk to me or someone else at any time during the study.

Harrysone Atieli	Maseno	0721347437	
Guiyun Yan	UC Irvine	1-949 824 0175	OR

For any questions pertaining to rights as a research participant, contact person is: **The Secretary, Maseno University Ethics Review Committee, Private Bag, Maseno; Telephone numbers: 057-51622, 0722203411, 0721543976, 0733230878; Email address:** muerc-secretariate@maseno.ac.ke; muerc-secretariate@gmail.com.

I HAVE READ AND UNDERSTAND THIS CONSENT FORM,

AND I AM WILLING TO PARTICIPATE IN THE STUDY.

Subject's Name (type or print)	Subject's Signature (consent)	Date
Witness' Name (type or print)	Witness' Signature (consent)	Date
Investigator's Name (type or print)	Investigator's Signature	Date

APPENDIX 4: ETHICAL APPROVAL FROM MASENO UNIVERSITY ETHICS REVIEW COMMITEE

MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

Tel: +254 057 351 622 Ext: 3050
Fax: +254 057 351 221
Private Bag – 40105, Maseno, Kenya
Email: muerc-secretariate@maseno.ac.ke

FROM: Secretary - MUERC

DATE: 15th February, 2019

TO: Dr. Harrysone Atieli
Department of Public Health
School of Public Health and Community Development
Maseno University
P. O. Box, Private Bag, Maseno, Kenya

REF:MSU/DRPI/MUERC/00660/19

RE: Ecology and Population Genetics of African Highland Malaria. Proposal Reference Number MSU/DRPI/MUERC/00660/19

This is to inform you that the Maseno University Ethics Review Committee (MUERC) determined that the ethics issues raised at the initial review were adequately addressed in the revised proposal. Consequently, the study is granted approval for implementation effective this 15th day of February, 2019 for a period of one (1) year. This is subject to getting approvals from NACOSTI and other relevant authorities.

Please note that authorization to conduct this study will automatically expire on 14th February, 2020. If you plan to continue with the study beyond this date, please submit an application for continuation approval to the MUERC Secretariat by 15th January, 2020.

Approval for continuation of the study will be subject to successful submission of an annual progress report that is to reach the MUERC Secretariat by 15th January, 2020.

Please note that any unanticipated problems resulting from the conduct of this study must be reported to MUERC. You are required to submit any proposed changes to this study to MUERC for review and approval prior to initiation. Please advice MUERC when the study is completed or discontinued.

Thank you.

MASENO UNIVERSITY SECRETARY 15 FEB 2019 ETHICS REVIEW COMMITTEE

Dr. Bernard Guyah
Ag. Secretary,
Maseno University Ethics Review Committee.

Cc: Chairman, Maseno University Ethics Review Committee.

APPENDIX 5: ASSENT FORM FOR MINORS

Form 1b: ASSENT FORM FOR MINORS *Version 1.0, July 25, 2017*

Title of Study: Malaria Transmission Dynamics in Sentinel Sites in Western Kenya

This assent form will be explained and signed by each study participant

Name of Volunteer: _____,

Age of Volunteer: _____

Who are we?

Our names are

Dr. Harrysone Atieli, and Dr. Guiyun Yan. Dr. Atieli is a Lecturer and research scientist in Maseno University. Dr. Yan is an Associate Professor in the Program in Public Health at the University of California at Irvine.

Why are we meeting with you?

We want to tell you about a study that involves children like yourself. We want to see if you would like to be in this study.

Why are we doing this study?

We are trying to determine malaria vector, transmission and parasite dynamics in East Africa.

What will happen to you if you are in the study?

A tiny amount of blood will be taken from your finger by pricking it to find out whether you are infected with malaria parasites.

Exclusion criteria:

Participants who are unwilling to participate in the study will be excluded. All minors will be excluded from questionnaire survey.

Discomforts and risks:

The finger-prick blood collection method causes slight discomfort. Sterile blood lancets (followed with sterile ethanol) will be used for every single person, the procedures will pose very minimal risk of being infected by other pathogens.

Benefit to participants:

You will not receive financial benefit from your participation, however, if you have a fever or are ill, you will be referred to the local clinic for care.

Reimbursement for medical treatment (This statement is required on all assent forms):

The present project will be responsible for diagnosis of malaria and referral to local clinics for evaluation. Competent staff member of the Kenyan Ministry of Health will perform the evaluation and provide appropriate treatment. The project will only cover the costs of the normal standard treatment of uncomplicated malaria approved by Kenyan Ministry of Health. The study will not attend to other disease unrelated to malaria. If other illness or diseases are identified during the malaria screening, we will provide referral to the appropriate local health authorities. In cases of emergencies, transport, whenever possible, will be provided to the nearest government medical facility.

Confidentiality:

Information related to you will be treated in strict confidence to the extent provided by law. Your identity will be coded and will not be associated with any published results. Your code number and identity will be kept in a locked file of the Principal Investigator and Kenyan Investigator.

Freedom to withdraw:

Your participation in this study is voluntary and you may discontinue your participation at any time without prejudice and without affecting future health care.

Assent form:

The consent form will be explained to each study participant and signed by the Investigators or the leading scientists conducting the study.

New findings:

You will be told of any significant new findings developed during the course of this study

Do you have any questions?

You can ask questions at any time. You can ask now. You can ask later. You can talk to me or you can talk to someone else at any time during the study.

Harrysone Atieli	Maseno	0721347437
Guiyun Yan	UC Irvine	1-949 824 0175

For any questions pertaining to rights as a research participant, contact person is: **The Secretary, Maseno University Ethics Review Committee, Private Bag, Maseno; Telephone numbers: 057-51622, 0722203411, 0721543976, 0733230878; Email address:** muerc-secretariate@maseno.ac.ke; muerc-secretariate@gmail.com.

IF YOU WANT TO BE IN THE STUDY, SIGN YOUR NAME ON THE LINE BELOW.

Signature of the Child: _____

Printed Name: _____Date: _____

Signature of the Parent/Guardian: _____

Printed Name: _____Date: _____

Signature of the Investigator: _____

Printed Name: _____Date: _____

(Each participant will be given a copy of this assent form)

APPENDIX 6: CONSENT FORM FOR ADULTS

Appendix V: Consent Forms Version 1.0, July 25, 2017

Form 1a. Informed Consent

Form 1: CONSENT FORM FOR ADULTS

Title of Study: Malaria Transmission Hot Spots in a Highland Site in Western Kenya

This consent form will be explained and signed by each study participant

Name of Volunteer: _____,

Age of Volunteer: _____

1. INVESTIGATORS CONDUCTING THE STUDY:

Dr. Harrysone Atieli, Maseno University, Kenya;

Prof. Guiyun Yan, Program in Public Health, University of California, Irvine, USA;

2. PURPOSE OF STUDY:

The purpose of this project is to determine hot spots of malaria transmission in a highland site in western Kenya.

3. PROCEDURES TO BE FOLLOWED:

(1) Thin and thick smears will be made and drops of blood will be preserved on filter paper. All participants will have a unique identifier number that links the participant with her/his laboratory test results, demography, location etc. Samples will be analyzed by PCR in the International Centers of Excellence for Malaria Research (ICEMR).

(2) We will ask you some questions about whether you had malaria in the past two weeks, whether you have traveled to malaria endemic areas, whether you have been using bednets and whether

you have taken antimalarial drugs. This information is important for us to determine how active malaria transmission occurs in your village.

4. **Inclusion criteria**: We will include all residents located in the study site who are willing to participate in the study, regardless of their age, sex, and economic status.
5. **Exclusion criteria**: Residents who are unwilling to participate in the study or change their willingness to participate at any point in time.
6. **DISCOMFORTS AND RISKS:**

   The finger-prick blood collection method causes slight discomfort. Sterile blood lancets (followed with sterile ethanol) will be used for every single person, the procedures will pose very minimal risk of being infected by other pathogens.

7. **BENEFIT TO PARTICIPANTS:**

   You will not receive financial benefit from your participation, however, if you have a fever or are ill, you will be referred to the local clinic for care.

8. **REIMBURSEMENT FOR MEDICAL TREATMENT (This statement is required on all consent forms):**

   The present project will be responsible for diagnosis of malaria and referral to local clinics for evaluation. Competent staff member of the Kenyan Ministry of Health will perform the evaluation and provide appropriate treatment. The project will only cover the costs of the normal standard treatment of uncomplicated malaria approved by Kenyan Ministry of Health. The study will not attend to other disease unrelated to malaria. If other illness or diseases are identified during the malaria screening, we will provide referral to the appropriate local health authorities. In cases of emergencies, transport, whenever possible, will be provided to the nearest government medical facility.

9. **CONFIDENTIALITY:**

Information related to you will be treated in strict confidence to the extent provided by law. Your identity will be coded and will not be associated with any published results. Your code number and identity will be kept in a locked file of the Principal Investigator and Kenyan Investigator.

10. FREEDOM TO WITHDRAW:

Your participation in this study is voluntary and you may discontinue your participation at any time without prejudice and without affecting future health care.

11. CONSENT FORM:

The consent form will be explained to each study participant and signed by the Investigators or the leading scientists conducting the study.

12. NEW FINDINGS:

You will be told of any significant new findings developed during the course of this study.

13. <u>Do you have any questions?</u>

You can ask questions at any time. You can ask now. You can ask later. You can talk to me or you can talk to someone else at any time during the study.

Harrysone Atieli	Maseno	0721347437	
Guiyun Yan	UC Irvine	1-949 824 0175	OR

For any questions pertaining to rights as a research participant, contact person is: **The Secretary, Maseno University Ethics Review Committee, Private Bag, Maseno; Telephone numbers: 057-51622, 0722203411, 0721543976, 0733230878; Email address:** muerc-secretariate@maseno.ac.ke; muerc-secretariate@gmail.com.

I HAVE READ AND UNDERSTAND THIS CONSENT FORM,

AND I AM WILLING TO PARTICIPATE IN THE STUDY.

_____	_____	_____
Subject's Name (type or print)	Subject's Signature (consent)	Date
_____	_____	_____
Witness' Name (type or print)	Witness' Signature (consent)	Date
_____	_____	_____
Investigator's Name (type or print)	Investigator's Signature	Date