MALARIA RISK AMONG FISHERMEN OF RUSINGA ISLAND, WESTERN KENYA: AN ASSESSMENT OF DIFFERENT MALARIA TRANSMISSION METRICS AND HUMAN BEHAVIOUR

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

I would like to dedicate this thesis to my beloved daughter Imani Hawi Okal, son Baraka Telo Okal and husband Michael Nyang’anga Okal for their love and support.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACTs	Artemisinin-based combination therapies
BCC	Behaviour change Communication
BGS	BG-Sentinel trap
BMU	Beach management Unit
BP	Boundary partner
C	Vectorial capacity
CDC	Centres for Disease Control
CI	Confidence interval
DOMC	Division of Malaria Control
ELISA	Enzyme-linked immunosorbent assay
EIR	Entomological inoculation rate
FOI	Force of infection
G	Gametocyte rate
HDSS	Health Demographics Surveillance System
HIV	Human Immunodeficiency Virus
HLC	Human landing Catch
HRP II	Histidine-rich protein II
IEC	Information, education and communication
ICIPE	International Centre of Insect Physiology and Ecology
IDRC	International Development Research Centre
IRS	Indoor Residual Spraying
ITT	Ifakara tent trap
K	Net infectiousness of humans
LLINs	Long Lasting Insecticidal Nets
Ma	Human biting rate
mFOI	Molecular force of infection
MOH	Ministry of Health
MOI	Multiplicity of infection
MM-X	Mosquito Magnet® - X
OBTs	Odour baited traps
OM	Outcome Mapping

OR	Odds Ratio
PCR	Polymerase chain reaction
PfPf	Proportion of fevers with *P. falciparum* parasitaemia
PIRE	Pacific Institute for Research and Evaluation
PR	Parasite Rate
PSC	Pyrethrum Spray Catches
QBC	Quantitative buffy coat microhaemoticrit centrifugation
RBCs	Red Blood Cells
RDTs	Rapid diagnostic tests
s. l.	*sensu lato*
s. s.	*sensu stricto*
SCR	Seroconversion rate
SPR	Slide positivity rate
SR	Sporozoite rate
USA	United States of America
WET	Window exit traps
WHO	World Health Organization

ABSTRACT

Studies were conducted to determine whether capture fishing and associated activities place individuals engaging in them at higher risk of contracting malaria. Consequently, a strategy was developed to create awareness of malaria risk among fisherfolk using a behaviour change monitoring and evaluation approach called Outcome Mapping. The first study sought to assess the burden of malaria and determine indoor and outdoor transmission on Rusinga Island. *Anopheles arabiensis* was found to be the most abundant malaria vector responsible for both indoor and outdoor transmission. No significant difference in the density of *An. arabiensis* found indoors and outdoors was observed ($p = 0.477$). Mosquitoes captured outdoors were found with sporozoites which suggest that individuals conducting fishing activities outdoors at night are at risk of receiving infectious bites. The prevalence of malaria was found to be 10.9% with *P. falciparum* as the most prevalent parasite. As expected malaria was significantly associated with individuals who did not use protective measures (Odds Ratio = 2.65, 95% Confidence Interval 1.76 – 3.91, $p<0.001$).

Based on these results, the spatial and temporal exposure to malaria mosquitoes in relation to fishing activities was conducted. Two peak mosquito biting times, revealing that fishermen found outdoors at beaches during specific times are at risk of receiving infectious bites, were identified. *Anopheles arabiensis* exhibited two biting peaks specifically between 10 - 11pm and 4 – 5am. Two peaks in capture fishing activity, which coincided with the peaks in mosquito biting patterns, were also observed. Of the total persons observed at the beach, 18.8% were counted between 9 – 10pm and 49.7% between 3 – 7am. No significant difference was found in the density of *An. arabiensis* at various distances from the shoreline. Compared to mosquitoes captured at close proximity to the shoreline, there was no significant difference in the numbers of *An. arabiensis* caught at 600 – 1200 metres ($p = 0.534$) and 1200 - 1800 metres ($p = 0.742$) away. Compared to individuals who live less than 600 metres from the shoreline, other individuals who lived 600 – 1200 metres ($p = 0.319$) and 1200 – 1800 metres ($p = 0.312$) away were not at a higher risk of malaria as measured by parasite prevalence.

Another investigation sought to characterize malaria prevalence among outpatients in health facilities and determine the association of the disease to individuals engaged in various livelihoods on the Island. A health facility-based survey revealed that fishermen and farmers were significantly

associated with malaria cases. Among patients engaged in different livelihoods, malaria was significantly associated with farmers (p = 0.003), and fishermen (p = 0.031) when compared to adults who were unemployed.

Based on these results, a study was carried out to assess risk factors for malaria infection among fishermen. The study provided knowledge regarding current behaviours exhibited by fishermen that aggravate malaria control. The risk of contracting malaria was three-fold among fishermen who conducted fishing activities in clothes that exposed their arms and legs (95% CI 1.11 – 14.23, p = 0.045) compared to those who dressed in long sleeved clothes. Bednets were not found to be an effective malaria control tool among fishermen since no difference in malaria infection was found among fishermen who used bednets as a personal malaria protection measure or not (OR = 0.38, 95% CI 0.05 – 1.45, p = 0.218).

The final study used an outcome mapping approach to measure malaria prevention behaviour change among pre-trained fisherfolk on Rusinga Island. A health intervention to create awareness, educate and foster behaviour change was implemented among fisherfolk. The most significant change was observed in attendance of project meetings by project partners. Most of the malaria prevention and control efforts focused on use of treated bed nets and wearing long-sleeved clothing outdoors.

In conclusion, the fishermen of Rusinga Island are at risk of contracting malaria because of a temporal convergence between their outdoor activities and peak mosquito biting times. In addition, they are at risk because they do not frequently sleep under treated bednets. Outcome mapping can be used as a tool to demonstrate malaria prevention behaviour change among fisherfolk.

CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Malaria is a mosquito-borne disease caused by the *Plasmodium* parasite (WHO, 2015). The disease is responsible for morbidity and mortality in many developing countries. According to the World Health Organization, malaria caused approximately 438,000 deaths and 214 million cases of which 88% were in Africa (WHO, 2015). Malaria impedes economic development not only by causing premature deaths but also through lost productivity, absenteeism, huge medical costs and negative impact on fertility, and countrys' savings and investments (Sachs and Malaney, 2002).

The disease is transmitted through the infectious bite of a female *Anopheles* mosquito. In Sub-Saharan Africa, malaria is mainly transmitted between humans by female mosquitoes belonging to the *Anopheles gambiae* and *Anopheles funestus* complexes (White, 1974; Gillies and Coetzee, 1987). Major malaria vectors are known to preferentially feed on humans at night and rest indoors (Gillies and De Meillon, 1968). Consequently, malaria vector control strategies are targeted at indoor biting and resting mosquitoes. The front-line tools which include Long lasting insecticidal nets (LLINs) and Indoor residual spraying (IRS) have been documented to reduce malaria transmission in several areas (Okumu and Moore, 2011; Bekele *et al.*, 2012; Fullman *et al.*, 2013). However, the effectiveness of the strategies has resulted in exerted selective pressure on malaria mosquitoes which requires them to adapt accordingly, thus forcing them to change their behavioural patterns in order to obtain a blood meal (Reddy *et al.*, 2011; Russell *et al.*, 2011). Insecticidal pressure has resulted in malaria mosquitoes either biting outdoors or early in the evening before sleeping time (Durnez and Coosemans, 2013). Furthermore, malaria transmission has been reported to be sustained by secondary malaria vectors in several areas (Antonio-Nkondjio *et al.*, 2006; Manh *et al.*, 2010). These secondary vectors are known to be zoophilic and feed on animals outdoors thus evading indoor-targeted vector control strategies.

Humans who are outdoors between dusk and dawn are at risk of receiving infectious mosquito bites. Individuals in fishing communities exhibit such behaviour due to the nature of their livelihood activities. The front-line vector control strategies do not target outdoor malaria vectors placing those individuals who are found outdoors from early evening till dawn at risk. The relationship between malaria and humans is complex and is affected by peoples' daily behavioural patterns (Butraporn *et al.*, 1986). The importance of human behaviour in malaria control has been documented (Williams *et al.*, 2002). It is posited that human behaviour linked to livelihoods can sustain outdoor malaria transmission. There is a dearth of information on the relationship between malaria and individuals who conduct livelihood activities outdoors in malaria endemic areas in Africa. Rusinga Island in western Kenya is a malaria endemic area where fishing is the main livelihood activity (Fillinger and Lindsay, 2006). Current malaria control measures on Rusinga Island include Long Lasting Insecticidal Nets (LLINs), Indoor Residual Spraying (IRS) and case management. This study investigated the burden and entomological risk of malaria transmission among fisherfolk and the effect of targeted behaviour change in relation to malaria to reduce occupational exposure of fishermen to mosquito vectors in a rural community in western Kenya.

1.2 LITERATURE REVIEW

1.2.1 Global burden of malaria

Malaria is a major public health problem in sub-Saharan Africa. It is often called a disease of the poor primarily because it exerts its greatest toll in poor countries (Worrall *et al.*, 2003; Fobil *et al.*, 2010; Ricci, 2012; Berthelemy *et al.*, 2013; Sledge and Mohler, 2013; Tusting *et al.*, 2013b). The burden of malaria in 1900 was distributed worldwide with the exception of few countries (Hay *et al.*, 2004). A century later, concerted efforts led to the elimination of the disease in 111 countries (Cotter *et al.*, 2013). However, more than 97 countries continue to report active malaria transmission in spite of mortality having reduced by 47% worldwide from the year 2000 (WHO, 2014). A total of 584,000 malaria-related deaths and 197 million cases of malaria were estimated to occur in 2013 (WHO, 2014). The disease is a major cause of poverty and a hindrance to socio-economic development. It has serious economic and social impacts in Africa affecting both Governments and individuals. On individuals it inflicts heavy treatment costs, lost days of work, absence from school, and expenses for burial in case of deaths. Governments in endemic countries incur huge expenditure which include purchase of drugs and medical supplies, staffing at health facilities, loss of labour, and purchase and distribution of malaria interventions (Sachs and Malaney, 2002).

1.2.1.1 Malaria in Kenya

Kenya has an estimated population of 40 million people, 77% of whom are at risk of contracting malaria (Noor *et al.*, 2009). In 2010, clinical malaria accounted for 34 per cent of outpatient visits and 15% of hospital admissions (DOMC, 2011). The country loses an estimated 17 million working hours to the disease each year (DOMC, 2009). The most vulnerable group to malaria infections are pregnant women and children under 5 years of age (Guyatt and Snow, 2001; DOMC, 2014). In 2005, the disease was estimated to cause 20% of all deaths in children under the age of five years (MOH, 2006).

A decline in the burden of malaria in Kenya has been observed in recent years resulting in low malaria transmission intensity in most parts of the country (Okiro *et al.*, 2007; O'Meara *et al.*, 2008; Okech *et al.*, 2008). This has been attributed to scaling up of insecticide treated bed net coverage, indoor residual spraying and advent and use of Artemisinin-based combination therapies (*ACTs*) (Bhattarai *et al.*, 2007; Ceesay *et al.*, 2008; O'Meara *et al.*, 2008). According to the 2010 malaria indicator survey, the highest prevalence of malaria of 38% was reported in Nyanza province (DOMC, 2011). Prevalence in other zones was recorded as less than 5%. It was also reported that malaria prevalence is nearly three times as high in rural areas (12%) as it is in urban areas (5%) (DOMC, 2011). At a national level *Plasmodium falciparum*, which causes the most severe form of the disease, is the predominant species (98.2%) while *P. malariae* accounts for 1.8% of cases (DOMC, 2009).

1.2.2 Malaria parasites

Malaria is a caused by a parasite belonging to the genus *Plasmodium*. The disease can be caused by single or multiple infections of five *Plasmodium* species. These include *P. falciparum*, *P. malariae*, *P. ovale, P. vivax* (Gilles, 1993) and *P. knowlesi* (Cox-Singh *et al.*, 2008; Lee *et al.*, 2009; Cox-Singh *et al.*, 2010). Among the five, *P. falciparum* and *P. vivax* have the highest prevalence. *Plasmodium falciparum* is the most widely distributed species in sub-Saharan Africa, has the highest morbidity and mortality rates, and causes the most severe complications (Gupta *et al.*, 1994). Outside Africa, *P. vivax* is responsible for over 50% of malaria infections and causes the highest malaria burden in South America and Central Asia (Mendis *et al.*, 2001). Malaria caused by *P. vivax* has the widest geographical distribution since it can survive at lower temperatures (Warrell and Gilles, 2002) compared to *P. falciparum*. Optimal temperatures within which *P. falciparum* can develop are 16°C and 19°C while *P. vivax* are 14°C to 15°C (Gage *et al.*, 2008). Two malaria parasites, namely *P. ovale*, and *P. malariae* are responsible for few malaria cases that occur in Africa. The fifth parasite, *P. knowlesi* is mainly found in South East Asia (Lee *et al.*, 2011; Singh and Daneshvar, 2013), but has been reported in Islands of India (Tyagi *et al.*, 2013). *Plasmodium knowlesi* infections are often uncomplicated but at times can be severe and fatal (Cox-Singh *et al.*, 2010). There is a rise in cases of hospital admissions caused by *Plasmodium knowlesi* infections (Cox-Singh *et al.*, 2010).

Several studies have reported the outcome of malaria surveys conducted among people engaging in different livelihood types. Malaria morbidity among people who work indoors has also been studied. A study conducted among hospital workers in Equatorial Guinea revealed that 12.7% of 102 workers tested positive for falciparum malaria (Neuberger *et al.*, 2010). In Columbia, South America, mosquitoes collected outdoors in canoes belonging to fishermen and mangrove swamps tested positive for *P. vivax* (Escovar *et al.*, 2013). Rubber tappers who work outdoors at night in Southern Thailand were confirmed to have outdoor-transmitted *P. falciparum* and *P. vivax* infections (Pattanasin *et al.*, 2012). Fishermen in Rameswaram island who engage in outdoor nocturnal fishing activities in India tested positive for *P. falciparum* malaria (Rajagopalan *et al.*, 1984). Tuck *et al.* (2003a) revealed that British troops deployed to high malaria risk areas in Sierra Leone suffered from an outbreak of *P. falciparum* malaria. The prevalence of malaria parasitemia was recorded as 32.1% among individuals in a farming community who usually stay overnight in farming huts in Cote d'ivoire (Matthys *et al.*, 2006). Majority of the infections were pure *P. falciparum* while one individual had a mixed infection of *P. falciparum* and *P. malariae*.

1.2.2.1 Life cycle of the malaria parasite

The life cycle of the malaria parasite is complex with several transformation stages in both the mosquito vector and human host (Wirth, 2002; Crompton *et al.*, 2010). An infected mosquito injects sporozoites and anticoagulant saliva (Stark and James, 1996) into a person's blood. The sporozoites travel through the blood stream to the liver and invade the hepatocytes (liver cells). Within the hepatocytes, the sporozoites can undergo initial growth followed by liver schizogony to form schizonts that contain up to 30,000 merozoites (Jones and Hoffman, 1994; Fujioka and Aikawa, 2002). The liver schizogony stage takes approximately 1 to 2 weeks and is asymptomatic. *Plasmodium vivax* and *P. ovale* sporozoites also differentiate into dormant forms called hypnozoites that may cause disease relapses after weeks, months or years (Wirth, 2002). These dormant stages do not appear in *P. falciparum*, *P. malariae* and *P. knowlesi*.

The merozoites exit the liver cells and re-enter the bloodstream where they invade Red Blood Cells (RBCs). The schizonts burst releasing thousands of merozoites. The merozoites cause the RBCs to burst in the blood (Figure 1) and undergo its asexual stage characterized by many rounds of invasion and replication inside the host's RBCs (Beier, 1998). Within the RBC, and between 24 – 72 hours (depending on the *Plasmodium* species) the merozoites differentiate into immature trophozoites (ring form), which develops into mature trophozoites that undergoes asexual reproduction and form a schizont that contains 8 to 36 merozoites each. The schizont eventually bursts and releases merozoites which invade the RBCs and the cycle continues (Beier, 1998). This results in an increase of RBCs getting infected by the parasite as well as an increase in parasite levels in the bloodstream. When the erythrocytes rupture they release parasite debris that induce pathogenesis, for instance fever. This is the stage at which the symptomatic phase of the infection begins.

Some of the merozoites that infect the RBCs develop into gametocytes (Alano, 2007) which is the sexual stage of the parasite. The gametocytes circulate in the blood waiting to be extracted by a blood questing mosquito. Once inside a female *Anopheles* mosquito, the male and female gametocytes merge to form a diploid zygote in the insects' midgut (Simonetti, 1996). The zygotes develop into a motile ookinete that actively burrows into the midgut wall and forms an oocyst which grows between the basal lamina and the epithelium of the mosquito's midgut. The oocyst undergoes sporogony through repeated nuclear division to form tens of thousands of active haploid sporozoite-stage parasites. The sporozoites burst open the oocyst and migrate to the haemocoel and then then into the salivary glands of the mosquito (Simonetti, 1996) ready to infect a susceptible individual through a mosquito bite (Beier, 1998).

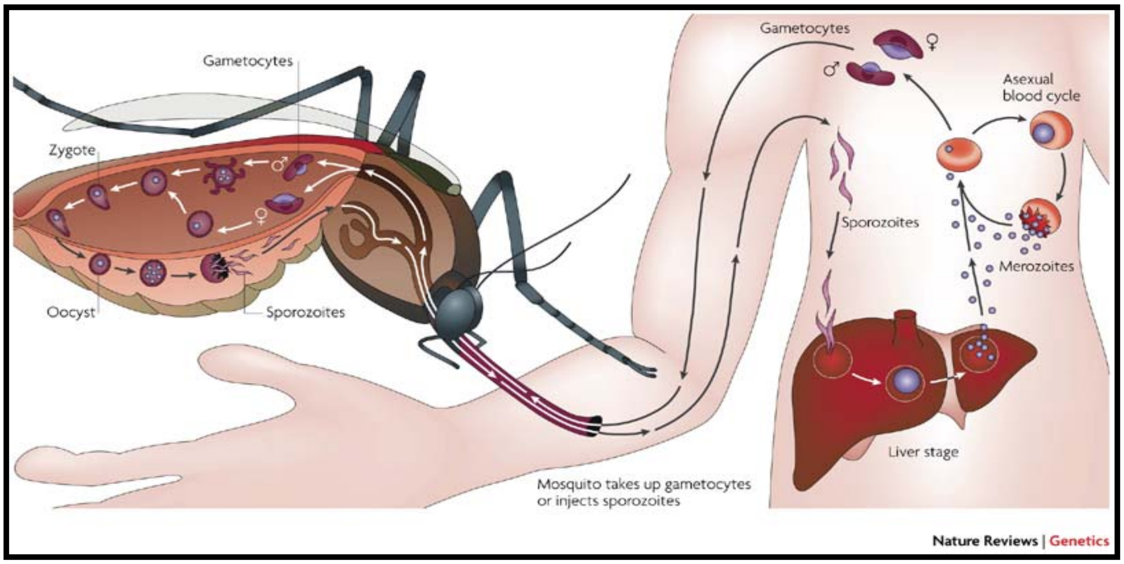

Figure 1: The *Plasmodium* spp. life cycle. After Su (2007).

1.2.3 Malaria vectors

Human malaria parasites are transmitted by female mosquitoes belonging to the genus *Anopheles* (Gillies and Coetzee, 1987). There are approximately 430 species of *Anopheles* mosquito species, about 70 of which are known malaria vectors (White, 1974; Service and Townson, 2002; Service, 2004). In many localities only one to two mosquito species are responsible for malaria transmission (White, 1974). In Africa malaria is mainly transmitted by four mosquito species namely *An. gambiae* Giles, *An. coluzzii* (formerly *An. gambiae s.s.* M form) Coetzee and Wilkerson, *Anopheles arabiensis* Patton and *Anopheles funestus sensu stricto* Giles (Gillies and De Meillon, 1968; White, 1974; Coetzee *et al.*, 2013). *Anopheles gambiae, An. coluzzii* and *An. arabiensis* belong to the *Anopheles gambiae* species complex (Coetzee *et al.*, 2000; Coetzee *et al.*, 2013).

Some Anopheline species are documented to be secondary vectors (also known as incidental vectors) majorly because of their minor importance in local malaria transmission (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987). These vectors include *An. coustani* Laveran, *An. squamosus* Theobald, *An. paludis* Theobald, *An. ziemanni* Grünberg, *An. brunnipes* Theobald, *An. flavicosta* Edwards, *An. gibbinsi* Evans, *An. hancocki* Edwards, *An. hargreavesi* Evans, *An. welcomei* Theobald, *An. pretoriensis* Theobald, *An. rufipes* Gough and *An. pharoensis* Theobald (Gillies and De Meillon, 1968). Secondary vectors are important as they are responsible for extending malaria transmission periods in various parts of Sub-Saharan Africa (Mukiama and Mwangi, 1989; Wanji *et al.*, 2003; Awono-Ambene *et al.*, 2004). Many of these vectors have been reported to be have exophagic and exophilic traits (Gillies and De Meillon, 1968). It has been postulated that these vectors sustain malaria transmission in areas where indoor-targeting tools have been used, specifically LLINs and IRS (Antonio-Nkondjio *et al.*, 2006).

The three vectors largely responsible for malaria transmission in Kenya include *Anopheles gambiae, An. arabiensis,* and *An. funestus* (Okara *et al.*, 2010; Kweka *et al.*, 2013). Reports indicate that *An. gambiae* is the abundant species found in high transmission areas (Okara *et al.*, 2010). In the recent past, siblings of the *An. funestus* complex have been reported in western Kenya (Kawada *et al.*, 2012; McCann *et al.*, 2014). Interestingly, a new malaria vector was recently discovered in the western Kenya highlands (Stevenson *et al.*, 2012). The three dominant malaria vectors (*An. gambiae, An. arabiensis* and *An. funestus*) have been reported to breed in the littoral zones of Lake Victoria in western Kenya (Minakawa *et al.*, 2012). A secondary vector, *Anopheles rivulorum* Leesoni was the dominant vector reported to inhabit floating mats of the water hyacinth. These malaria mosquitoes may be important in transmitting malaria among fishermen working outdoors. For the sake of this thesis, only malaria mosquitoes found on the African continent are described further.

1.2.3.1 The *Anopheles gambiae* complex

Mosquitoes belonging to the *An. gambiae* complex are the most efficient vectors of malaria in Africa (White, 1974; Coetzee *et al.*, 2000; Coetzee, 2004). The complex consists of eight morphologically indistinguishable sibling species namely *An. gambiae sensu stricto* Giles (hereafter referred to as *An. gambiae*), *An. arabiensis* Patton, *Anopheles bwambae* White, *Anopheles merus* Dönitz, *Anopheles melas* Theobald, *Anopheles quadriannulatus* species Theobald, *An. coluzzii* Coetzee and Wilkerson, and *An. amharicus* Hunt, Wilkerson and Coetzee (Coetzee *et al.*, 2013). Two species within the complex namely *An. gambiae* and *An. arabiensis* are responsible for most malaria transmission in Africa (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987; Coetzee *et al.*, 2000; Coetzee, 2004). In the savannah, *An. funestus* and *An. gambiae* display seasonal fluctuations in abundance during rainy and dry seasons. *Anopheles funestus* densities peak towards the end of the rainy season and at the onset of the dry season (Gillies and De Meillon, 1968) while densities of *An. gambiae* peak at the onset of the rainy season.

Several behavioural traits predispose *An. gambiae* as a major human malaria vector (Takken and Knols, 1999; Day, 2005). Females of this species have a focused preference for human over animal blood (anthropophagy) and exhibit distinct nocturnal host-seeking patterns (White, 1974), although variations to these behavioural trait have been reported in Eastern Africa (Duchemin *et al.*, 2001) and West Africa where they have been reported to feed on cattle and horses (Diatta *et al.*, 1998; Duchemin *et al.*, 2001; Lefevre *et al.*, 2009). In addition, these mosquitoes blood feed (endophagy) and rest (endophily) inside human dwellings (Githeko *et al.*, 1996; Clements, 1999; Costantini *et al.*, 1999). They also oviposit in water bodies created through anthropogenic activities (Day, 2005). The vectorial capacity of *An. arabiensis* is slightly lower than that of *An. gambiae* because of the opportunistic behavior that is seen in its ability to feed on other animals when humans are unavailable (Gillies and Coetzee, 1987; Githeko *et al.*, 1996).

Anopheles arabiensis is distributed all over Africa, but tends to be found more in drier areas than *An. gambiae* s.s. Two species in this complex namely *An. melas* and *An. merus* have been reported to be brackish-water breeding malaria vectors found along the coast of West and East Africa, respectively. *Anopheles melas* is anthropophillic while *An. merus* is zoophilic. *Anopheles quadriannulatus* is found in Ethiopia and south east Africa, and has not been documented as a malaria vector as it feeds on animals. *Anopheles bwambae* has been documented to be a malaria vector, is only found in the Semliki forest in Uganda and breeds in mineral springs (Gillies and Coetzee, 1987). *Anopheles coluzzii* is found in West Africa, although one study documented its existence in Zimbabwe (Coetzee *et al*., 2013). *Anopheles amharicus* is only found in Ethiopia (Coetzee *et al*., 2013). *Anopheles arabiensis* has been reported to be a major vector in areas along the Lake Victoria shore in western Kenya (Minakawa *et al*., 2002; Shililu *et al*., 2003; Futami *et al*., 2014). This vector is highly zoophilic and exophagic and may be important in outdoor malaria transmission among fishermen.

1.2.3.2 The *Anopheles funestus* group

Anopheles funestus consists of a group of nine species including *An. funestus* Giles (the nominal taxon, sometimes referred to as *An. funestus sensu stricto*), *Anopheles rivulorum* Leeson, *Anopheles parensis* Gillies, *Anopheles vaneedeni* Gillies and Coetzee, *Anopheles leesoni* Evans, *Anopheles fuscivenosus* Leeson, *Anopheles aruni* Sobti, *Anopheles brucei* Service and *Anopheles confusus* Evans and Lesson (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987; Cohuet *et al*., 2003; Spillings *et al*., 2009). Species in the *An. funestus* group are morphologically similar but can be distinguished by molecular methods (Koekemoer *et al*., 2002; Choi *et al*., 2012). *Anopheles funestus,* which is endophilic and anthropophilic, and *An. rivulorum* which is exophilic and zoophilic are the only two species within the *An. funestus* group that have been incriminated as malaria vectors (Gillies and De Meillon, 1968; Wilkes *et al*., 1996; Cohuet *et al*., 2004). The other species within the group are zoophilic (Gillies and De Meillon, 1968).

The two vectors in the group, *An. funestus s.s.* and *An. rivulorum* are widely distributed in Sub-Saharan Africa (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987). *Anopheles funestus s.s* as well as other sibling species like *An. leesoni*, *An. rivulorum* and *An. vaneedeni* have been recently found in western Kenya (Kweka *et al*., 2013). *Anopheles rivulorum* has been documented to be a malaria vector of minor importance (Kawada *et al*., 2012), and since it is an outdoor feeder, may be of importance in outdoor malaria transmission among fishermen. In Tanzania, *An. rivulorum* has also been reported to be a minor malaria vector (Wilkes *et al*., 1996). *Anopheles confusus* is found in eastern and southern Africa while *An. vaneedeni* is only found in southern Africa. *Anopheles parensis* is distributed in Swaziland, South and eastern Africa. Three species in the complex specifically *An. fuscivenosus*, *An. aruni*, and *An. brucei* are rare and found in Zimbabwe, Zanzibar and Nigeria, respectively.

1.2.5 Mosquito host-seeking behaviour

Female mosquitoes need to obtain a blood meal for egg development and maturation. Mosquitoes locate their preferred blood meal hosts using visual, physical and chemical cues (Sutcliffe, 1987; Takken, 1991; Takken and Knols, 1999; Zwiebel and Takken, 2004). Visual and physical cues comprising of heat and moisture play a crucial role in orienting mosquitoes at close vicinity to the host (Sutcliffe, 1987; Costantini *et al.*, 1999; Olanga *et al.*, 2010). Chemical cues are composed of chemicals emanating from breath and sweat (Braks and Takken, 1999; Healy and Copland, 2000; Smallegange *et al.*, 2005) and are used by mosquitoes as host markers at distances of 0 – 70 metres (Gillies and Wilkes, 1969; Olanga *et al.*, 2010).

Mosquitoes locate blood meal hosts through a process that comprises of responses to internal and external stimuli. A host seeking mosquito will initially engage in an appetitive search (Sutcliffe, 1987). In this behaviour the mosquito flies upwind in search of hosts. In a field setting, for instance a village, the mosquito would fly searching for chemical stimuli that signifies the presence of a host. Guided by host-specific stimuli, the mosquito switches to a behaviour called activation. Through a process called attraction, the mosquito comes in the immediate vicinity of the host. The above behaviours are guided by chemical, physical (heat and moisture) (Olanga *et al.*, 2010) and visual cues (Takken and Knols, 1999). The mosquito then lands on the host, probes the skin and initiates blood feeding. A nocturnal activity like fishing increases the chances of a mosquito encountering a potential blood meal host, and malaria transmission, outdoors. This livelihood activity influences the exposure of humans to malaria mosquitoes.

1.2.6 Sampling adult malaria vectors

Several techniques have been documented for sampling adult mosquitoes (Service, 1976). The choice of a particular sampling tool depends on the entomological investigation, for instance indoor or outdoor collections. Different sampling methods are used to estimate human exposure to mosquito bites and monitor impact of interventions. Mosquitoes are also captured to determine species distribution, abundance and behaviour. Information on indoor and outdoor mosquito biting patterns is useful for determining the host-vector contact and choice of malaria control strategy (Pates and Curtis, 2005).

The Human landing Catch (HLC) is a widely used sampling method that has been viewed as the gold standard for measuring malaria transmission. The method is used to capture host-seeking and host-biting mosquitoes with the aim of estimating human exposure to mosquito bites. It entails the use of a human volunteer as bait to lure host seeking mosquitoes. A volunteer sits either indoors or outdoors at night with legs exposed and waits for a mosquito to land on his body. The mosquito is then aspirated and placed in a holding container. The HLC method is labour-intensive, requires continuous supervision and skilled catchers. Variation in attractiveness of humans to mosquitoes (Lindsay *et al.*, 1993a; Mukabana *et al.*, 2002) is an important factor to be considered when using this method. The HLC method has a lot of ethical issues as collectors are exposed to infectious mosquito bites. However, a study conducted in the recent past reports that increased malaria risk due to HLC should not be a concern if proper administration of prophylaxis is adhered to (Gimnig *et al.*, 2013). It has previously been reported that Odour baited traps (OBTs) were more productive in collecting higher densities of mosquitoes than HLC (Kweka and Mahande, 2009).

Other methods for sampling adult mosquitoes include Centres for Disease Control (CDC) light traps (Sudia, 1962; Lines *et al.*, 1991), Pyrethrum Spray Catches (PSC) (Service, 1976), electric nets (Knols *et al.*, 1998), exposure-free bed net traps (Mathenge *et al.*, 2002), cloth resting boxes (Harbison *et al.*, 2006), clay pots (Odiere *et al.*, 2007), window exit traps (WET) (Mouatcho *et al.*, 2007), Ifakara tent trap (ITT) (Govella *et al.*, 2010a), and backpack aspirators (Maia *et al.*, 2011). Traps like BG-Sentinel (BGS) trap (Maciel-de-Freitas *et al.*, 2006), Mosquito Magnet-X traps (MM-X®) (Njiru *et al.*, 2006), and Suna trap (Hiscox *et al.*, 2014) have been baited with attractants to lure host-seeking mosquitoes in various studies. Here I only review the MM-X® and Suna traps as they were the trapping tools used in studies reported in this thesis.

1.2.6.1 The MM-X® trap

The MM-X® trap (American Biophysics Corporation, RI, USA) has been used for sampling (Mboera *et al.*, 2000) and mass trapping of mosquitoes (Kline, 1999; Kline *et al.*, 2006) in the field as well in semi-field set ups (Njiru *et al.*, 2006; Cilek *et al.*, 2011). The MM-X trap has been baited with octenol strips and dry ice (Xue *et al.*, 2008), synthetic attractants (Okumu *et al.*, 2010; Olanga *et al.*, 2010; Cilek *et al.*, 2012), and foot odours from worn socks (Njiru *et al.*, 2006). The traps use a counterflow mechanism whereby one smaller fan exhausts air while a bigger fan sucks in air via an inlet (Figure 2). Mosquitoes are sucked into the plastic jar where they remain trapped. The traps work best when hung 15 centimetres above the ground (Njiru *et al.*, 2006).

1.2.6.2 Suna trap

The Suna trap is a new trap that was developed for use in mass trapping malaria mosquitoes in Rusinga Island, western Kenya (Hiscox *et al.*, 2012; Hiscox *et al.*, 2014). The study in western Kenya is the only one documented to use the Suna trap. The trap is hung outdoors approximately 30 cm from the ground. The Suna trap has one fan at the top of the intake funnel that creates an air current that sucks in mosquitoes (Figure 2).

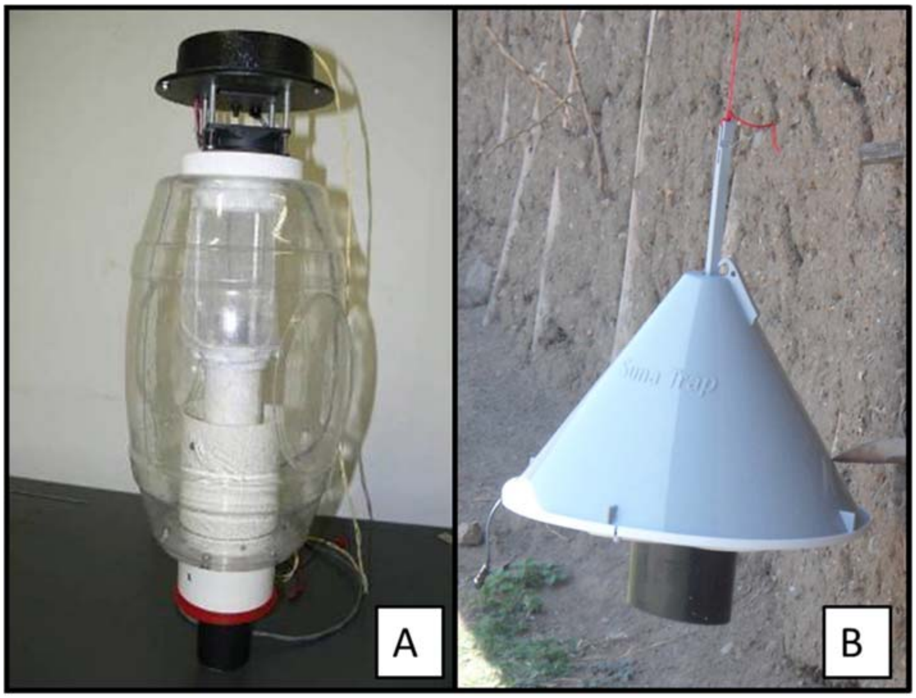

Figure 2: Pictures of MMX (panel A) and Suna (panel B) traps.

1.2.7 Malaria transmission outdoors

Outdoor malaria transmission has always occurred in Africa. It is important to note that outdoor transmission does not translate into failure of indoor vector control strategies. Indoor malaria transmission is more documented and studied because the most effective malaria vector, namely *An. gambiae* s.s. rests indoors (endophilic), and prefers to feed on humans (anthropophilic). Vector control strategies aimed at reducing indoor malaria transmission, specifically LLINs and IRS have been successful in reducing malaria (Bekele *et al.*, 2012;

Fullman *et al.*, 2013). However, IRS has minimal impact on outdoor resting or feeding vectors, while LLINs do not affect vectors that feed early in the evening or outdoors. This allows mosquitoes that prefer to bite outdoors (exophagic), feed on animal hosts (zoophilic) and rest outdoors (exophilic) to maintain transmission. Due to insecticidal pressure exerted by indoor vector control strategies, shifts have been observed in mosquitoes (Durnez and Coosemans, 2013). The two authors describe different types of shifts namely species shift, shifts to exophagy, shifts to zoophily, shifts to early morning or evening biting, and shifts to exophily.

Several studies have reported outdoor malaria transmission as well as early biting patterns of malaria vectors. A study in Tanzania revealed that 10% of exposure to infectious mosquito bites occurred outdoors (Killeen *et al.*, 2006). Shililu *et al.* (2004) reported that 56.5% of mosquito bites occurred outdoors in Eritrea. A study in Muheza, northern Tanzania showed that 12% of infective bites occurred before people went to bed (Maxwell *et al.*, 1998). A recent study in Cambodia showed that 29% of infected bites occurred before sleeping time (Durnez *et al.*, 2013). A study in Senegal revealed that a higher proportion of *An. funestus* mosquitoes engage in diurnal (early morning) feeding compared to nocturnal feeding (Sougoufara *et al.*, 2014).

Outdoor malaria transmission continues to occur even in areas of high LLIN and IRS coverage. Several studies indicate the reduction in densities of the major endophilic vectors of malaria, namely *An. gambiae* and *An. funestus* while the exophilic vector like *An. arabiensis* maintain the same densities (Durnez and Coosemans, 2013). In light of the types of shifts observed in malaria vectors due to indoor vector control strategies, there is a gap in malaria protection (Figure 3), particularly for individuals who work outdoors and around the time before people go to sleep. Controlling outdoor malaria transmission requires different strategies from those targeting indoor vectors. There is a need to develop novel tools targeting outdoor malaria transmission.

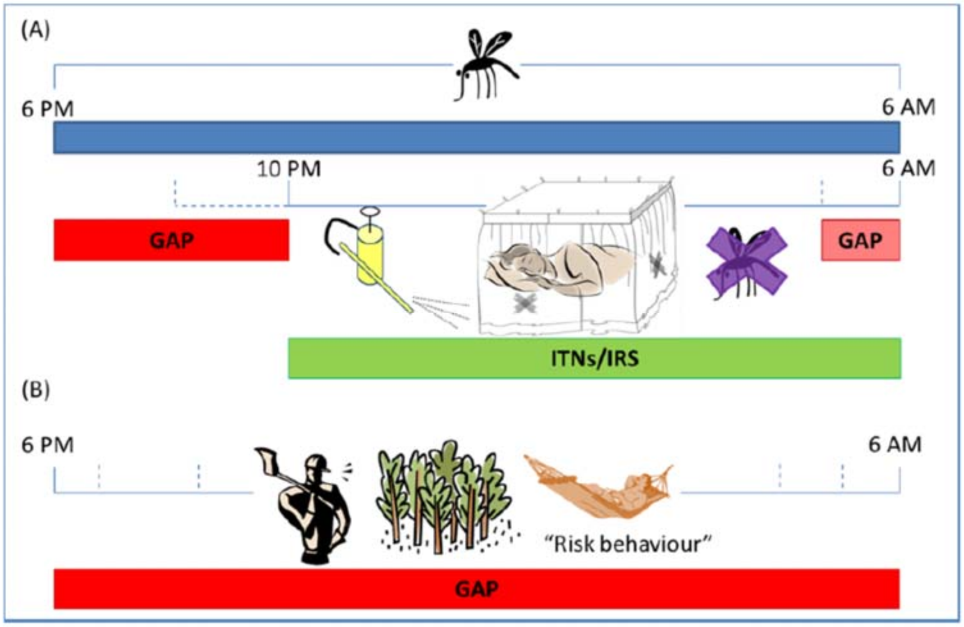

Figure 3: Malaria protection gaps associated with indoor insecticide use (After Durnez and Coosemans, 2013). The illustration shows the timeline of two distinct activities conducted by people between 6am and 6pm. Panel A describes indoor activities and malaria protection used indoors. Panel B shows examples of activities termed risk behaviours conducted outdoors during the time that mosquitoes are actively host-seeking.

1.2.8 Malaria diagnosis

Prompt and accurate diagnosis is an important component of malaria control programmes. Clinical diagnosis is the most practiced and least expensive method of malaria diagnosis. This type of diagnosis is based on signs, symptoms and physical examination. However, a clinical diagnosis is challenging because of the non-specificity of the symptoms (Tangpukdee *et al.*, 2009). The symptoms of uncomplicated malaria include fever, headache, joint pains, chills, abdominal pain, nausea, diarrhea, which are similar to those of common illnesses for instance, bacterial infections. Diagnosis specificity is impaired due to the overlapping of symptoms with those of other diseases which can lead to indiscriminate use of antimalarials (Reyburn *et al.*, 2004; Nankabirwa *et al.*, 2009). The World Health Organization recommends prompt diagnosis of malaria prior to treatment (WHO, 2010).

Laboratory malaria diagnosis involves identifying malaria parasites or antigens or products in the patients' blood (WHO, 2010). Different techniques are used to diagnose malaria infection and include microscopy, rapid diagnostic tests (RDTs), indirect fluorescent antibody test, Enzyme-linked immune assay, quantitative buffy coat microhaemoticrit centrifugation (QBC) method and polymerase chain reaction (PCR) (Tangpukdee *et al.*, 2009). For purposes of the studies described in the thesis, I will review two diagnostic methods namely light microscopy and RDTs.

1.2.8.1 Light microscopy

Malaria is conventionally diagnosed using microscopic examination of stained blood samples (WHO, 2009). Common fixatives used to stain the blood films include Giemsa, Wright's, or Field's stains (Warhurst and Williams, 1996). Malaria diagnosis using Giemsa stain is the gold standard for laboratory diagnosis (WHO, 2009). Parasite identification and microscopic detection is conducted on thick films of stained blood while *Plasmodium* species confirmation is done on thin blood films.

Briefly, the patients' finger is cleaned with 70% alcohol and pricked using a sterile lancet. A drop of blood is placed on a microscopic glass slide and a blood film prepared. For a thick blood smear, the blood is stirred in a circular motion with the corner of a slide. A lot of care is taken to ensure the blood film is not too thick. The blood is allowed to dry without a fixative. Once dry, the spot is stained with diluted Giemsa (10%) for approximately 20 minutes and placed in saline buffered water for about 3 minutes. The slide is allowed to air dry and is ready for visual examination under a light microscope. A thin blood film is prepared by using a spreader slide to smear the blood on the glass slide. The spreader is held at a 45^{o} angle and used to steadily smear the blood film across the slide. The blood film is allowed to air dry and is fixed with absolute methanol. Once dry the sample is fixed with Giemsa stain for approximately 20 minutes and dipped in and out of a container with buffered water. The slide is allowed to air dry and can be examined under a light microscope.

This technique has been widely accepted majorly because it is cheap, able to identify *Plasmodium* parasite to species and is simple to execute (Warhurst and Williams, 1996). However, the technique is labour intensive, time consuming and requires trained and skilled personnel to prepare slides (Guerin *et al.*, 2002; Moody, 2002) and identify species at low parasitemia (Kilian *et al.*, 2000).The greatest shortcoming of this technique is low sensitivity. The lowest number of parasites an average microscopist can count is between 50 – 100 parasites per µl (Kilian *et al.*, 2000). Blood films with lower numbers of parasites need expert microscopists (Milne *et al.*, 1994; Kilian *et al.*, 2000) who are rare to find in hard to reach malaria endemic areas.

1.2.8.2 Rapid diagnostic tests (RDTs)

Malaria RDTs detect *Plasmodium* parasites in the blood through an antibody-antigen reaction on a nitrocellulose strip in a cassette (WHO, 2003b). Reactions on the strip are expressed as bands that are red in colour. Rapid diagnostic tests have gained popularity because they are easy to use and do not require electricity, skilled personnel or specialized equipment (Bell *et al.*, 2006). They can easily diagnose malaria in health facilities that do not have equipment to diagnose malaria using light microscopy. The test kits provide results in approximately 15 to 20 minutes (Murray *et al.*, 2003; Talman *et al.*, 2007). Rapid diagnostic tests contain specific anti-malaria antibodies that detect malaria antigen in blood. Most test kits target *P. falciparum* –specific protein, for instance, histidine-rich protein II (HRP-II) (Talman *et al.*, 2007). Some tests distinguish mixed infections from non-*P. falciparum* infections. These test kits do not provide an estimation of parasite density.

1.2.9 Measurement of the malaria burden and transmission

Malaria transmission intensity in different areas within an endemic region can vary greatly due to the populations' immunity and vectoral capacity of local vectors (Smith *et al.*, 2010). According to Tusting *et al.* (2014), malaria transmission refers to the process by which a malaria parasite is spread from the mosquito into a person's blood, through the individual's liver, back into the blood, picked up by a mosquito and ends up completing its cycle within the mosquito. Transmission can be measured at three points, namely the parasite, mosquito and human (Carter and Mendis, 2006; Hay *et al.*, 2008). There exist 13 metrics of transmission, each one conveying an important step in the malaria transmission process. The metrics include sporozoite rate (SR), parasite rate (PR), human biting rate (Ma), entomological inoculation rate (EIR), force of infection (FOI), molecular force of infection (mFOI), gametocyte rate (G), seroconversion rate (SCR), vectorial capacity (C), net infectiousness of humans (k), multiplicity of infection (MOI), proportion of fevers with *P. falciparum* parasitaemia (PfPf), and slide positivity rate (SPR) (Tusting *et al.*, 2014). For purposes of this thesis, the Parasite Rate (PR) commonly known as prevalence rate, and Sporozoite rate (SR) are reviewed.

1.2.9.1. Parasite Rate

Parasite prevalence in humans is calculated as the proportion of persons infected with malaria parasites at a given point in time (Tusting *et al.*, 2014). In malariometric studies, PR is the most widely used metric to determine malaria endemicity (Hay and Snow, 2006). This metric has been traditionally used to determine malaria prevalence within a population. A sample of the human population is usually recruited into a cross-sectional survey and blood samples analyzed for malaria parasites. Diagnostic tools namely RDTs, microscopy, and PCR are used to determine the presence of malaria parasites. The accuracy of parasite prevalence within a population can be influenced by human factors of the recruited individual, the choice of diagnostic test and the distribution of malaria parasites within the population at that point in time (Tusting *et al.*, 2014).

1.2.9.2. Sporozoite Rate

Sporozoite rate (SR) is the proportion of mosquitoes infected with malaria parasites in their salivary glands. A sample of the malaria mosquitoes captured or even total number of malaria mosquitoes captured are tested for the presence of sporozoites. Mosquitoes can be captured through various methods, most commonly CDC light traps, PSC, and window exit traps. In a review by Hay *et al.* (2000), two methods namely enzyme-linked immunosorbent assay (ELISA) and dissection of mosquito salivary glands were used to determine SR. Another review by (Kelly-Hope and McKenzie, 2009) reported the use of PCR to identify sporozoites.

1.2.10 Definition of risk

Risk is described as the probability that an event will occur (Burt, 2001). According to the dictionary of epidemiology, risk is often used to express the probability that a particular outcome will occur following a particular exposure (Last, 2001). There is a dearth of information on malaria risk behaviours in relation to livelihood activities, particularly fishing activities in Africa. The study described in this thesis explores how fishing activities are associated with malaria risk in western Kenya. The study focuses on risks in relation to potential exposure to malaria vectors and malaria infection.

1.2.11 Malaria and human activities

Human activities have been strongly associated with increased intensity of malaria in various parts of the world. Several studies have shown the association between human activities and malaria (Mboera *et al.*, 2013). Malaria and human activities are related in at least two ways. Firstly, humans engage in livelihood activities that create mosquito breeding sites and foster mosquito productivity. The man-made sites are formed when individuals dig the ground to plant crops (Diuk-Wasser *et al.*, 2007; Yasuoka and Levins, 2007; Jarju *et al.*, 2009), extract minerals (Yapabandara and Curtis, 2004), create fishing ponds (Maheu-Giroux *et al.*, 2010), make bricks (Carlson *et al.*, 2004), and create dams (Keiser *et al.*, 2005). Secondly, there is evidence that individuals who conduct activities outdoors at night are at a higher risk of contracting malaria (Escovar *et al.*, 2013). These individuals are usually working at night conducting economic activities, for instance farmers who work in rubber plantations outdoors at night (Pattanasin *et al.*, 2012) and military personnel who conduct activities outdoors at night (Tuck *et al.*, 2003a). Fishermen who harvest fish at night are also at a higher risk of contracting malaria because they work during peak mosquito biting times (Escovar *et al.*, 2013). For purposes of this thesis, studies on malaria and human activities will be limited. However, literature related to malaria and fishing is reviewed more extensively.

Agricultural production systems including type of farming, farming practices, location of farms and farming technologies could lead to environmental changes that create suitable ecological and climatic conditions for the breeding and survival of *Anopheline* mosquitoes. Rice irrigation is notably the most documented agricultural system that is linked to spread of malaria. A study conducted in Mali revealed that rice irrigation increases the densities of *An. gambiae* (Diuk-Wasser *et al.*, 2007). *An. gambiae* and *An. arabiensis* mosquitoes have been reported to breed in rice paddies in western (Imbahale, 2011) and Central Kenya (Mwangangi *et al.*, 2006a; Mwangangi *et al.*, 2006b; Muturi *et al.*, 2007; Muturi *et al.*, 2008; Mwangangi *et al.*, 2008; Mwangangi *et al.*, 2010). Several studies have documented that there is less malaria in communities living in close proximity to irrigation schemes when compared with populations living further away, which is partially explained by enhanced incomes that facilitate better protective measures to be taken (Boudin *et al.*, 1992; Carnevale *et al.*, 1999; Ijumba *et al.*, 2002). This is the so-called paddies paradox, where agricultural development resulting in increased income for the community is likely to improve access to malaria treatment and may support an increased use of malaria preventive devices.

Overnight stays in farming huts have been reported to be risk factors for malaria (Somboon *et al.*, 1998; Matthys *et al.*, 2006; Pattanasin *et al.*, 2012). The risk associated with malaria and staying overnight in these temporary farming huts was shown to be 17-fold higher in individuals who slept in the huts rather than at home (Matthys *et al.*, 2006). The farming huts are temporary in structure made of wood and thatched grass and open eaves and therefore porous to mosquitoes. The huts are located next to rice farming fields that are breeding sites for malaria mosquitoes.

Mining in Africa has been documented to be associated with malaria. Clay is the main ingredient in the production of bricks in Africa. The soil is excavated from the ground in large-scale which leaves a large number of shallow pits. The pits readily accumulate water especially during the rainy season and can support high densities of mosquito larvae. Brick-making pits have been reported to contribute to malaria transmission in western Kenya (Carlson *et al.*, 2004). The pits were found to have the highest number of *Anopheles* larvae and houses near the pits recorded higher numbers of adult malaria mosquitoes compared to houses further away. Both functional and abandoned brick-making pits were found with malaria mosquito larvae (Carlson *et al.*, 2004). Brick or sand pits have also been reported in The Gambia as mosquito larval habitats (Majambere *et al.*, 2008). Excavation of soil to re-plaster houses and brick making has led to an increase in burrow pits in Tanzania (Mboera *et al.*, 2013). This activity is mainly conducted during the dry season and could sustain malaria transmission during this season.

Malaria has been reported as an occupational disease among military personnel posted in various countries around the world. This is notably reported to be among troops from developed countries who are stationed in malaria-endemic areas in Asia and Africa (Croft *et al.*, 2005). British troops deployed to Sierra Leone in the year 2000 suffered from a malaria outbreak during their operations (Tuck *et al.*, 2003a; Tuck *et al.*, 2003b). Military personnel from Brazil who were deployed to Angola also suffered from malaria in the year 2000 (Sanchez *et al.*, 2000). Other reported cases of malaria among the military populations include U.S.A marines in Somalia (Newton *et al.*, 1994), and Italian soldiers deployed to Somalia and Mozambique (Peragallo *et al.*, 1995).

Construction activities have been associated with malaria. Deforestation for infrastructural development has been linked to environmental changes that support the increase in malaria breeding and transmission. Manga *et al.* (1995) reported that the construction of the Nsimalen airport in Nsimalen village, southern Cameroon required deforestation which led to malaria epidemiological changes in a surrounding village, specifically a shift from *An. moucheti* to *An. gambiae* as the main malaria vector species in the area. Fillinger and others (2004) reported the importance and availability of different larval habitats in Mbita, western Kenya. Out of the 53 habitats recorded, 70% of them were man-made. Cement-lined pits constituted approximately 80% of habitats during the dry and rainy seasons (Fillinger *et al.*, 2004). *Anopheles* mosquitoes colonized these habitats all year through. Cement-lined pits were created whenever a brick or stone house was constructed in the area. The pits stored water for construction purposes and were never demolished after completion of the house (Fillinger *et al.*, 2004).

1.2.11.1 Malaria and fishing

Two types of fishing methods are practiced worldwide which include capture fisheries and fish farming. Capture fisheries involves hunting of wild fish while fish farming, which is a subset of aquaculture, is the rearing of fish in controlled conditions. Small scale fish farming has been practiced in western Kenya for almost 50 years. In 1969, Lockart *et al.* reported that 1,000 fish ponds in western Kenya were well maintained in 1959 but started deteriorating in 1961. Reasons cited for this abandonment were bad farming methods because fish farming entailed more work than farmers had anticipated (Lockhart *et al.*, 1969). Water bodies without fish have been documented to habour mosquito larvae (Fletcher *et al.*, 1992) while those with fish have also been reported to contain mosquito larvae (Vittor *et al.*, 2009; Imbahale *et al.*, 2013). A study in western Kenya revealed that *An. gambiae* s.l. was the most abundant mosquito species found in both active and abandoned fish ponds (Howard and Omlin, 2008). It has also been reported that mosquito larval densities correspond with habitat size (Minakawa *et al.*, 2005). Fish ponds in western Kenya measure approximately 20 metres by 10 metres and 3 metres deep thus hold a large amount of water. If such ponds are abandoned they create large breeding grounds for malaria vectors. Vittor (2003) reported that the presence of a fish pond close to a house was a risk factor for *Plasmodium falciparum* malaria. In 2006, it was reported that households located closer to a fish pond had a higher number of self-reported malaria episodes than households further away (Simpson, 2006).

Fingerponds are excavated ponds at the landward edge of swamps. The ponds extend like fingers out of the swamp (hence the term “fingerponds”). Soil from the pond is heaped between the ponds to form raised beds for crop cultivation. The ponds are stocked with fish through natural flooding in the rainy season. As the water recedes, the trapped fish are supposed to be cultured using manure, crop and household wastes to fertilize the ponds and feed the fish. The main aim of creating finger ponds was to increase food production especially during the dry seasons (Bailey *et al.*, 2005). They were introduced in the Lake Victoria basin in the early 1990s (Denny and Turyatunga, 1992) and proven to harbor fish especially Tilapia (Bailey *et al.*, 2005; Kipkemboi *et al.*, 2006; Van Dam *et al.*, 2006). Water in these excavated ponds is stagnant and provides good breeding grounds for malaria mosquitoes if not periodically flooded with fresh water. Fingerponds are at times dug by farmers who carry out farming activities by the lake shore (Kipkemboi *et al.*, 2007). Fish is reared in these ponds while malaria mosquitoes breed inadvertently.

The Lake Victoria fishing community comprises of fishermen, artisanal fish processors, small-scale fish traders and industrial processors. Thus fishery activities play a vital role in the lives of the communities in terms of employment and nutrition. Malaria is cited as one of the leading vector borne diseases that affect fishermen on Lake Victoria (Omwega *et al.*, 2006). The prevalence of malaria among the residents of the Lake Victoria basin remains high. The environment associated with the lake maintains high numbers of malaria vectors. *Anopheles rivulorum* a secondary malaria vector has been reported to be the dominant malaria vector that breeds on floating water hyacinth on Lake Victoria (Minakawa *et al.*, 2012). The three major vectors of malaria namely *Anopheles gambiae* and *Anopheles funestus, Anopheles arabiensis* were also found to inhabit areas along the lake shore (Minakawa *et al.*, 2012). It is plausible that these vectors sustain malaria transmission among fishermen while they conduct livelihood activities around the shore and in the lake.

Several studies have documented the association between fishing communities and malaria. Outdoor activities like nocturnal fishing were recorded to hamper malaria transmission in rural areas of India (Barai *et al.*, 1982). The lagoons and coastal areas in West Africa have been reported to have higher malaria transmission rates due to high densities of *An. melas* and *An. gambiae* (Akogbeto, 2000). In Uganda, high malaria prevalence rates were recorded among pregnant women in a fishing community (Woodburn *et al.*, 2009). A study by Sogoba *et al.* (2007) revealed that high adult mosquito densities and many larval habitats were recorded during the dry season in fishing hamlets in Mali. These mosquitoes were postulated to sustain malaria transmission in neighbouring villages prior to the rainy season.

Fishing was reported to facilitate outdoor exposure to malaria mosquitoes in Brazil (Sa *et al.*, 2005). Malaria was reported as an occupational disease among fishermen and seafarers in Poland (Jaremin *et al.*, 1993; Jaremin *et al.*, 1996). These individuals conduct activities in endemic areas, specifically West Africa and import malaria into Poland. Malaria was also reported as a work-related health risk among seamen involved in the fishing industry of Lithuania (Scerbaviciene and Pilipavicius, 1999, 2009). Fishermen travelled to South America, Africa, and Asia in search of fish. Sevilla-Casas (1993) reported the high risk of malaria associated with individuals who engage in economic activities that require movement in the malaria endemic area of Naya river basin in Colombia. In Colombia, studies have shown that fishermen may be at risk of malaria when conducting diurnal activities where the outdoor biting mosquito *An. neivai* is the major vector (Carvajal *et al.*, 1989; Solarte *et al.*, 1996). Recently, a study by Escovar *et al.* (2013) showed that the peak biting times of the mosquito *An. neivai* coincided with fishing activities conducted in mangroves and marshlands in Colombia. These results strongly indicated that fishermen are at risk of malaria while conducting their daily activities.

There have been several reports of alternative uses of LLINs in fishing villages (Minakawa *et al.*, 2008a; Lover *et al.*, 2011). In Kenya, LLINs were commonly used to capture fish in lake Victoria and to dry fish in fishing beaches because they dried fish faster (Minakawa *et al.*, 2008a). In The Democratic Republic of Timor-Leste in Southeast Asia, bed nets were re-purposed / re-made to several types of fishing nets (Lover *et al.*, 2011). Several individuals interviewed during that study reported that they prefer to use the nets because they immobilize small fish and shrimps. Whereas the reports on misuse of bednets are low, this activity impedes malaria control efforts.

Malaria has been cited as the most important health hazard among fishermen in Lake Victoria (Kitakule and Reynolds, 1991) in Uganda. Many fishing communities live in houses made of temporary material that are porous to mosquitoes. Fisherfolk living in these environments are at risk of exposure both while they are working outdoors or when they are indoors during peak mosquito biting times. The studies reported in this thesis are focused on the relationship between fishing activities and malaria in the western Kenya Island of Rusinga in Lake Victoria.

1.2.12 Malaria prevention and control

Malaria prevention and control efforts are targeted at either the *Anopheles* vector or the *Plasmodium* parasite. The World Health Organization Global Malaria Program (WHO/GMP) advocates for the combined use of three malaria interventions (WHO, 2013). These include ACTs, LLINs and IRS. Control of *Plasmodium* parasites entails the use of effective antimalarial medicines (Wistanley and Ward, 2006) that contain artemisinin-based compounds combined with other compounds (WHO, 2010; Banek *et al.*, 2014). Long Lasting Insecticidal nets offer personal protection by acting as a physical barrier and chemical between the sleeper and mosquitoes. Pyrethroid insecticides used to impregnate mosquito nets have insecticidal properties that cause mortality and excito-repellent properties that reduce house entry (Lines *et al.*, 1987; Miller *et al.*, 1991). Insecticide-related properties of LLINs have been shown to be effective in providing protection (Lengeler, 2004; Muller *et al.*, 2006) even at community level (Killeen and Smith, 2007; Le Menach *et al.*, 2007). The community-wide effects include a reduction in feeding frequency of malaria vectors (Charlwood *et al.*, 2001), reduced survival and reduction in mosquito densities (Magesa *et al.*, 1991; Robert and Carnevale, 1991).

The combined use of LLINs, IRS and ACTs have been reported to reduce malaria cases in several parts of Africa (Bhattarai *et al.*, 2007; Ceesay *et al.*, 2008; O'Meara *et al.*, 2008; Chizema-Kawesha *et al.*, 2010; Ngomane and de Jager, 2012). The current arsenal of vector control strategies specifically LLINs and IRS either alone or in combination are effective against indoor biting malaria mosquitoes that are active at night (Mabaso *et al.*, 2004; John *et al.*, 2009; Kleinschmidt *et al.*, 2009; Pluess B. *et al.*, 2010; Hamel *et al.*, 2011; Okumu and Moore, 2011; Bekele *et al.*, 2012; Fullman *et al.*, 2013). However, they are not effective in areas where malaria transmission mainly occurs outside human dwellings (Pates and Curtis, 2005; Tirados *et al.*, 2006; Geissbuhler *et al.*, 2007; Griffin *et al.*, 2010; Van Bortel *et al.*, 2010; Yohannes and Boelee, 2012).

Other vector control methods, for instance larviciding with microbial insecticides (Fillinger *et al.*, 2003; Fillinger and Lindsay, 2006; Tusting *et al.*, 2013a) and environmental management (Killeen *et al.*, 2002; Keiser *et al.*, 2005) have been documented to effectively reduce malaria transmission. Biological control using fish has been reported to reduce densities of immature mosquitoes in water bodies, but there is no evidence to support its reduction in malaria prevalence in communities (Walshe *et al.*, 2013). Repellents used for personal protection have been documented as other malaria prevention and control methods (Rozendaal, 1997). Insecticide-treated clothes (ITCs) have also been proven to be effective in reducing malaria transmission (Rowland *et al.*, 1999; Macintyre *et al.*, 2003; Kimani *et al.*, 2006). It has been hypothesized that spatial vapour repellents are most effective when used outdoors to protect spaces (Killeen and Moore, 2012). The authors argue that the protective properties offered by this method can extend to other individuals close to the point of application. Spatial repellents do not need to be applied on skin or clothing when people are outdoors, thus are friendly for outdoor use (Killeen and Moore, 2012).

Not all strategies in the arsenal of malaria prevention and control tools can be effectively used by fishermen. Due to the nature of their livelihood activities, fishermen are outdoors from early evening till dawn. Outdoor malaria transmission is less compared to indoor transmission, thus few strategies have been developed and thoroughly tested to reduce outdoor transmission (Killeen *et al.*, 2011). Strategies like LLINs and IRS that target indoor biting and resting mosquitoes will not effectively protect fishermen. Repellents that are applied on the skin may prove to be a more effective preventive measure as they can be used outdoors during peak mosquito biting times. Another malaria control strategy that is effective among this group is the use of ACTs to treat malaria. Insecticide-treated clothes are another strategy that can be effective in reducing malaria infection among fishermen.

1.2.12.1 Challenges in malaria elimination

The burden of malaria has been on a decline over the last few years. However, there are several major challenges that need to be addressed before elimination is achieved (Cotter *et al.*, 2013). One major challenge against malaria elimination is that the disease is widespread. Reported malaria deaths in 2010 were estimated at 655,000 and malaria cases at 219 million (WHO, 2012). Another challenge that has been widely reported is resistance of malaria vectors to insecticides (Ranson *et al.*, 2011). Resistance of malaria parasites to Artemisinin drugs has also been reported for *Plasmodium falciparum*, the most widespread and lethal malaria parasite. The reports are from South East Asia in countries like Cambodia, Thailand and Burma (WHO, 2012; Tulloch *et al.*, 2013). Mobility of people makes it difficult to contain malaria in areas of possible eradication. Additionally, this movement threatens the containment of Artemisinin-resistant malaria in endemic areas and can possibly result in the re-introduction of malaria in malaria-free areas (Liu *et al.*, 2013).

Plasmodium vivax is the second most important malaria parasite that has been under-researched. Malaria caused by *P. vivax* is harder to control as it is difficult to diagnose by microscopists. Lack of diagnosis leads to *P. vivax* parasites infecting the liver and remaining dormant until a time when they can cause a relapse of the disease (Sulistyaningsih *et al.*, 2010). Additionally, in South East Asia, the fifth malaria parasite, *Plasmodium knowlesi* is becoming widespread (Cox-Singh *et al.*, 2008; White, 2008; Figtree *et al.*, 2010; Khim *et al.*, 2011; William *et al.*, 2011; Goh *et al.*, 2013). The parasite was previously known only to infect old world monkeys called macaques (Garnham, 1966; Lee *et al.*, 2011). It is difficult to eradicate a disease that has zoonotic reservoirs (Liu *et al.*, 2013). Human conflict has also posed a challenge in malaria eradication as they disrupt malaria control efforts which may eventually lead to resurgence of the disease in a given area (Liu *et al.*, 2013). Governments that fail to provide financial support in areas that have few cases of malaria hamper malaria control efforts. It is imperative that more financial support be routed towards areas that are well on their way to eradicate malaria (Liu *et al.*, 2013).

1.2.13 Outcome Mapping

Outcome mapping (OM) is a planning, monitoring and evaluation tool (Earl *et al.*, 2001). The concept was first developed by Barry Kibel from the Pacific Institute for Research and Evaluation (PIRE) who called it Outcome Engineering or Journey Mapping. In the late 1990s, the International Development Research Centre (IDRC) in Canada adopted the Outcome engineering approach and thereafter developed the Outcome Mapping tool (Earl *et al.*, 2001).

The Outcome Mapping approach has hardly been used in malaria control studies or programs. Outcome mapping was used in a study reported in the last chapter of this thesis to foster and measure behaviour change among fishermen. The ultimate aim of the study reported herein was to identify behaviours that place fishermen at risk of malaria and eventually influence these individuals through a health-based intervention to adopt positive behaviours. The transformation process was planned, monitored and evaluated using Outcome Mapping.

Outcome mapping has been adopted in several studies to measure behaviour change in various disciplines (Jones, 2007), for instance health (Price *et al.*, 2014) and agriculture (Leksmono *et al.*, 2006; Nyangaga *et al.*, 2010a; Nyangaga *et al.*, 2010b; Nyangaga and Schaeffer, 2011). A recent review reported several studies in Eastern Africa that employed the Outcome mapping approach to foster behaviour change among various institutions in different disciplines (IIRR, 2012). Researchers in south western Uganda near Lake Mburo National Park adopted Outcome mapping to create awareness on transmission of zoonotic diseases influenced by cattle domestication by locals (IIRR, 2012). Daily movement of wild animals into the lands of the locals was perceived to be a risk factor for zoonotic diseases. Through Outcome mapping, the project was able to track key behaviour changes among the pastoralists that led to a reduction in transmission of zoonotic diseases in cattle. In Kenya, OM was used to measure behaviour change among farmers who are at risk of zoonotic *Cryptosporidium parvum* associated with dairy farming (Nyangaga *et al.*, 2012). The researchers in that study reported positive changes in the behaviour of fifty dairy farmers with regard to contracting the zoonotic disease.

Outcome mapping is a methodology for planning, monitoring and evaluating development initiatives to attain social change. The Outcome mapping methodology that can be adapted to a wide range of contexts and disciplines (Jones, 2007). At the planning stage, OM provides a framework that helps the project team identify individuals or groups it intends to work with so as to bring about positive behaviour change. For monitoring, Outcome mapping offers a set of tools whose function is to gather data on the change process. At the evaluation stage, OM provides a framework that enables data collection on specific changes that led to longer and more transformative change.

1.2.13.1 Elements of Outcome Mapping

Outcome mapping provides a set of tools that guide teams to identify desired change and determine ways to achieve the change (Jones and Hearn, 2009). The Outcome mapping approach focuses on one end result, namely outcomes also known as behaviour change (Earl *et al.*, 2001). This is the innovative element in the approach. The behaviour change is monitored in actors (called Boundary Partners), whom the program anticipates to influence (Smutylo, 2005). Planning, monitoring and evaluation efforts are conducted around Boundary partners. Behaviour change in OM terms refer to changes in behaviour, activities, relationships and actions of individuals, groups or organizations. The OM approach is based on the principle that social transformation is through the concerted effort of individuals or groups who work towards one common goal. Outcome mapping provides a method through which a programme can plan its activities together with boundary partners who are the people charged with the responsibility of bringing change and well-being to their communities (beneficiaries). Outcome mapping is a participatory learning process both for the programme teams and boundary partners.

Outcome mapping provides a set of tools that enable programs to plan, monitor and evaluate their work (Earl *et al.*, 2001). The set of tools are presented in 12 steps that are classified into three stages (Figure 4). It is not compulsory for users of the OM approach to use all 12 steps. Different elements of OM can be used to cater to the needs of a project. For purposes of this thesis, only those elements used in studies reported herein are discussed further. The first stage is called Intentional design that consists of seven elements namely vision, mission, boundary partners (BPs), outcome challenges, progress markers, strategy maps and organizational practices. These elements answer the questions: Why?; Who?; What?; and How?

Project staff, together with the actors with whom they want to effect change in a community discuss the elements that are of importance or will apply to their programme. The vision statement reflects on why the programme wants to conduct its activities. It is usually a broad general statement on what the project foresees to accomplish. The mission explains how the programme intents to achieve its objectives. Boundary partners are people, groups or organizations who have an influence in the society with whom a programme interacts with as well as share the same vision. The programme usually anticipates opportunities to influence

BPs. Outcome challenges describe the ideal behaviours of boundary partners and their contribution to the vision. Progress markers define the behaviour change of each boundary partner (Nyangaga and Schaeffer, 2011). These markers are graduated as it has been noted that behaviour change is a gradual process. Progress markers are graduated into levels namely expect to see, like to see and Love to see (Earl *et al.*, 2001). Behavioural changes that are easiest to achieve are placed under the level 'expect to see'. Like to see behaviours are changes that indicate more learning and engagement has been achieved. Behaviours that are advanced and closely related to the vision are under 'Love to see' category (Earl *et al.*, 2001).

The second stage is called Outcome and performance monitoring and describes elements that help the programme monitor its activities. The elements include Monitoring priorities, Outcome journals, Strategy journals, and Performance journals. An Outcome journal helps a programme to track the achievement of individual behavioural changes (progress markers) in Boundary partners. This document captures details on specific behavioural changes, sources of evidence of change, actors who contributed to the change, and the quality of change that is graded from Low to High (Earl *et al.*, 2001). Information collected in the Outcome journal is useful as it informs the programme team of advances in behaviour change and provides an opportunity for them to foster changes that would improve performance of the Boundary partner. Outcome journals are to be filled periodically as often as the project team and boundary partners decide. A lot of information in collected using the Outcome journal that will need to be assessed individually in the third stage called Evaluation. This step involves the assessment of change in one issue or behaviour in depth.

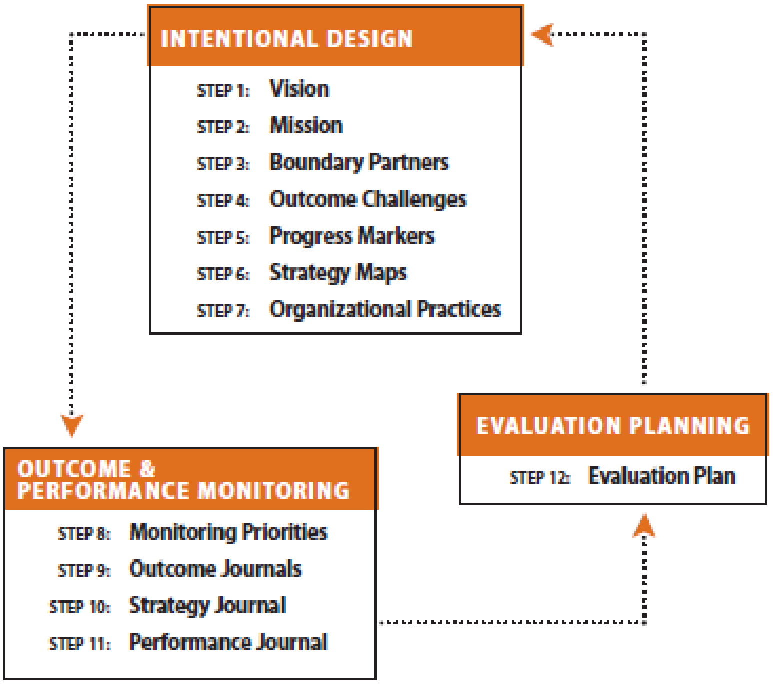

Figure 4: The 12 elements of Outcome mapping

1.2.13.2 Malaria and behavior change communication (BCC)

Behaviour change communication (BCC) is a tool that has been used over decades to transform groups of people with regard to improving their health. It involves the use of targeted tailored messages whose aim is to promote healthy behaviours. The ultimate aim is to enable individuals and affected communities to maximize the use of health interventions. This approach encompasses the use of Information, Communication and Information (IEC) materials as well as various strategies that range from campaigns to interpersonal communication (Wakefield *et al.*, 2010). Behaviour Change Communication has been successfully used in several programmes that aim to mitigate risk behaviours with regard to diseases including HIV prevention and health areas like family planning and hygiene promotion (Curtis *et al.*, 2001; Albarracin *et al.*, 2003; Bertrand *et al.*, 2006).

It has recently been reported that BCC is useful in malaria control if applied in the proper manner (Koenker *et al.*, 2014). Several initiatives have noted the importance of human behavior in malaria control (Williams *et al.*, 2002). The success or failure of control programmes majorly rely on the scale of behavioural responses of populations at risk of the disease (Setbon and Raude, 2009). It has been reported that the manner in which people respond to health risks depends on complex processes that are related to past experiences, attitudes, beliefs, information, and emotions which in turn may result in behaviour change (Setbon and Raude, 2009). Several studies have reported the success of quality BCC in malaria interventions (Minja *et al.*, 2001; Deribew *et al.*, 2012; Keating *et al.*, 2012; Shaw *et al.*, 2012; Bowen, 2013).

Behaviours that hamper effective malaria control are well known. Inconsistent or non-use of bed nets is one behaviour that has been well documented as a barrier to effective malaria control (Korenromp *et al.*, 2003; Eisele *et al.*, 2009; Widmar *et al.*, 2009; Githinji *et al.*, 2010; Littrell *et al.*, 2011; Ngondi *et al.*, 2011). Lack of adherence to dose completion of malaria drugs has also been documented to be a barrier to malaria control efforts (Littrell *et al.*, 2011). Addition of BCC elements to malaria control interventions can help reduce behavioural barriers that hinder proper uptake and use (Koenker *et al.*, 2014). The study in this thesis aimed to use BCC, together with OM, as a tool to change malaria risk behaviours among fishermen.

1.2 PROBLEM STATEMENT

In spite of immense progress in its control over the last decade, malaria still is a leading global public health problem with its worst impact in sub-Saharan Africa (WHO, 2014) . The disease was responsible for more than 584,000 deaths and 198 million disease cases annually in 2013 (WHO, 2014). Malaria is transmitted by female mosquitoes of the *An. gambiae* species complex - mosquitoes that are nocturnal and prefer to feed on humans indoors (Gillies and De Meillon, 1968). For this reason, two tools (LLINs and IRS) that target indoor feeding and resting mosquitoes are considered the gold standard for controlling the mosquitoes and preventing new malaria infections. While these tools are great and have demonstrated an unparalleled impact on malaria since 2000, their effectiveness could be limited on outdoor biting vector populations leaving people exposed to malaria infection outdoors (Killeen, 2014). These tools might fail to protect potential victims of malaria that engage in nocturnal and outdoor activities (Durnez and Coosemans, 2013).

Although malaria transmission was initially believed to occur only indoors, there in an increase of reports of outdoor transmission (Reddy *et al.*, 2011; Russell *et al.*, 2011). These reports suggest that people who engage in livelihood activities conducted outdoors during mosquito peak biting hours could be exposed to malaria vectors. Practical examples include rubber tappers in southern Thailand and fishermen near the Colombian pacific who have been demonstrated to be at significantly higher risks for infectious with malaria compared to residents whose livelihood activities are restricted to daytime (Pattanasin *et al.*, 2012; Escovar *et al.*, 2013).

Taking all this information into account, it is predictable that fishermen near Lake Victoria who are active during the peak biting period of the local malaria vectors, *An. gambiae,* may be unknowingly exposing themselves and their communities to malaria as they conduct their day to day economic activities. Moreover, such persons may act as reservoirs for the disease parasite hindering elimination efforts.

1.3 JUSTIFICATION AND SIGNIFICANCE OF THE STUDY

The consistent decline in Malaria transmission and cases in most parts of Africa (Ceesay *et al.*, 2008; Ngomane and de Jager, 2012), including Kenya (Okiro *et al.*, 2007; O'Meara *et al.*, 2008; Okech *et al.*, 2008), should restore confidence in vector control as an important part of malaria control. In areas where LLIN’s and IRS are implemented, the densities of indoor resting and indoor biting vectors and consequently the burden of malaria have declined remarkably (Bhattarai *et al.*, 2007; Chizema-Kawesha *et al.*, 2010; Ngomane and de Jager, 2012; Fullman *et al.*, 2013). However, the effectiveness of these interventions is limited by the dynamic behaviors of both the mosquito vector and human hosts: on one hand, mosquitoes in many areas have become resistant to insecticides (Coetzee *et al.*, 1999; Chandre *et al.*, 2000; Nauen, 2007; Mathias *et al.*, 2011; Ranson *et al.*, 2011) and are more inclined to outdoor biting (Suwonkerd *et al.*, 1990; Zhang and Yang, 1996; Van Bortel *et al.*, 2010) thus effectively avoiding both LLINs and IRS (Durnez and Coosemans, 2013). On the other hand, many people continue to stay outdoors in areas such as Rusinga, late at night beyond the protective cover of the two frontline interventions in pursuit of livelihood. The latter problem can only be managed by an attempt at human behavior change without impeding normal livelihood activities. There is therefore an urgent need for new approaches at influencing behavior change sustainably and guided by in depth knowledge of the risk factors for fishermen. This study is a pioneer attempt to protect fishermen on Rusinga Island through by targeted behavior change. Hypothetically, success with such an intervention would reduce the incidence and prevalence of malaria among Rusinga fisherfolk and improve productivity in the industry by alleviating loss of production caused by morbidity and mortality due to malaria.

1.4 OBJECTIVES OF THE STUDY

1.4.1 General objective

To assess the risk of malaria infection among fishermen of Rusinga Island and measure malaria prevention behaviour change among fisherfolk

1.4.2 Specific objectives

1. To estimate malaria burden and transmission in fishing villages on Rusinga Island
2. To correlate spatial and temporal exposure to malaria transmission and burden in fishing villages on Rusinga Island
3. To determine the proportional contribution of malaria to outpatient visits in health facilities on Rusinga Island
4. To determine risk factors for malaria infection among fishermen
5. To measure behaviour change in relation to malaria risks among fisher folk on Rusinga Island

CHAPTER 2: GENERAL METHODS

2.1 Study area

The study was conducted in Rusinga Island (0°35'–0°44' South; 34°11'–34°22' East), located in Homabay county in western Kenya (Figure 5). The island is one of many found in Lake Victoria and covers an area of 42km^2. The topography of the island is rocky and hilly with partial vegetation cover. The long rainy season extends from March to June while the short rainy season occurs in October and November. Total annual rainfall ranges between 700 – 800 mm. Malaria on Rusinga is mesoendemic with peak incidence cases reported shortly after the rainy seasons (Mutero *et al.*, 1998). Rusinga is home to a population of approximately 24,000 people (Kaneko *et al.*, 2007; Opiyo *et al.*, 2007). The residents of Rusinga are involved in two main livelihood activities namely artisanal capture fishing and small-scale trading (Opiyo *et al.*, 2007). Other livelihood activities for instance farming, boat making, and net weaving, occur on a smaller scale.

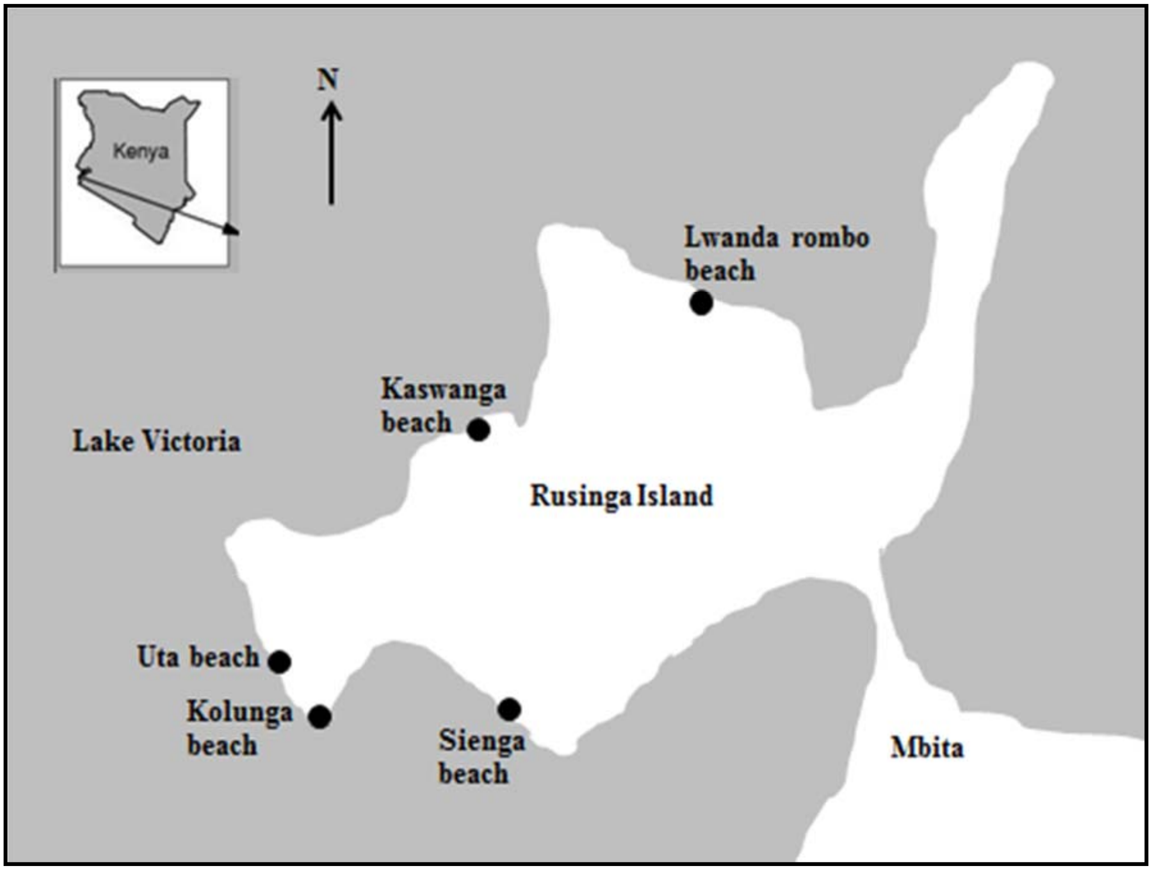

Figure 5: Fishing beaches in Rusinga Island, western Kenya. The dots indicate major fishing beaches where this study was carried out.

2.2. Mosquito Identification

2.2.1 Morphological identification of malaria mosquitoes

Malaria mosquitoes were first separated by sex. *Anopheles* mosquitoes were identified using morphological features described by Gillies and Coetzee (Gillies and Coetzee, 1987). *Anopheles gambiae* s.l. was distinguished from *An. funestus* mosquitoes. Specimens were placed under a light microscope for identification. Anophelines were identified by a dark spot found on the upper margins of the wings. *Anopheles funestus* was identified by focusing on the elongated palp that is segmented into three. Characteristic features of *An. funestus* include a pale spot on the second dark area, a light spot between the two dark spots found on vein 6, two dark spots on vein 6 and lack of fringes on vein 6. Distinctive features of *An. gambiae* s.l. include conspicuous bands on tersus 1 – 4, third pre-apical dark area on vein 1 with a pale interruption, and speckles on hind legs.

2.2.2. Morphological identification of malaria mosquitoes by the Polymerease Chain Reaction (PCR)

A sample of the female *Anopheles gambiae* s.l. mosquito catch was identified to sibling species and molecular form by PCR (Scott *et al.*, 1993). The molecular assay sought to separate *An. gambiae* s.s. from *An. arabiensis* mosquitoes. The samples previously stored in crystals of silica gel in eppendorf tubes were arranged on a plastic rack. The Eppendorf tubes were then labeled with code numbers (for instance, 1, 2, 3, etc) on top of each tube which served as a PCR identity number. One leg from each mosquito was placed in a separate micro-centrifuge PCR tubes. A 15 µl PCR master mix was then added to each PCR tube. The contents of the PCR master mix are indicated in Table 1. The specific contents of the master mix were then multiplied by the number of samples that were assayed.

Table 1: Contents of the PCR master mix and their specific volumes.

Reagent	**Concentration**	**Volume for 1 sample** (µl)
PCR Water	n/a	7.7
5X PCR buffer (**No $MgCl_2$**)	1X	3.0
2mM dNTP mix	160µM of each	1.2
25mM $MgCl_2$	1.5mM	1.0
Primer UN	0.4µM	0.6
Primer GA	0.4µM	0.6
Primer AR	0.4µM	0.6
Taq DNA Polymerase	1.5U/ul	0.3
Total		**15µl**

The tubes were shut tightly and placed in a thermo cycler machine to duplicate DNA material. Positive and negative controls were considered during the assay. For positive controls, 1 µl was added while nothing was added to the negative control. The standard reaction conditions were as follows: 94°C for 5 minutes, then 30 cyclic conditions of 94°C for 30sec, then 45°C for 30 sec followed by 72°C for 4 minutes and final extension at 72°C for 10 minutes. The samples were then stored in the fridge at 4°C until ready for loading into gel.

Agarose gel was prepared using 1.5 grams of agarose powder transferred to 100ml of 1xTAE buffer in a conical flask. The mixture was shaken then heated in a microwave for approximately two minutes till the agarose powder dissolved and the liquid appeared clear. Well moulds were prepared by setting gel combs as necessary. Once the agarose powder was dissolved, it was cooled using running tap water while gently whirling it on the palm of the hand. When the liquid was almost cool, 5 µl of Ethidium Bromide was added and the mixture whirled then poured into a gel tray to let it solidify. The combs were removed and carefully placed in the gel tank covered with 1xTAE buffer. Approximately 2 µl spot of loading dye on parafilm was mixed with 5 µl of PCR template or ladder before the mixture was loaded into each well. One 1kb/100bp ladder was used at the beginning of each row (specifically the first well of each row). The voltage of the power pack was set at 90˚C and left to run for 45 minutes until the two dyes were well apart. As the current was applied, DNA that is negatively charged travelled to the positively charged terminal inducing separation. The dyes enhanced the tracking of DNA separation while Ethidium Bromide enhanced visualization. The gel was then removed and placed in a UV transilluminator where DNA bands were visualized and photographs taken.

2.3 Molecular identification of sporozoites by ELISA

Mosquitoes received from the field were arranged according to their mosquito identification number. The head and thorax of each mosquito was tested for presence of *Plasmodium falciparum* circumsporozoite antibodies (CSA) by the Enzyme-linked immunosorbent assay (ELISA) method. The method described by Wirtz *et al*. (1989) was used in this study. The head and thorax of mosquitoes were separated from the abdomen and the two placed in a labeled Starstedt PVC microfuge tube. After cutting all mosquito parts were stored at -70ºC. Approximately 50 µl of blocking buffer NP40 was added to each mosquito that was tested. One hour later, the mosquito parts were ground with a pestle attached to a motor driven grinder. The pestle was rinsed with two 100 µl volumes of blocking buffer and together with the rinse in the tube made up a total volume of 250 µl. The pestle was rinsed two times in PBS-Tween and dried to prevent contamination between mosquitoes. The ground mosquitoes were stored overnight at -20ºC, or at -70ºC if stored for longer periods.

Precisely 50 µl of MAb solution containing the *P. falciparum* capture antibodies was placed in each well of the 96-well ELISA plate. The MAb solution was removed from the plates by banging on paper towels. Each well was filled with approximately 200 µl of blocking buffer and incubated at room temperature for one hour. The blocking buffer was aspirated out and 50 µl of the mosquito triturate added to the wells. Positive and negative controls were added and incubated for two hours. The mosquito titrate was transferred and each well washed twice with 200 µl of PBS-Tween. Precisely 50 µl of MAb-peroxidase conjugate solution diluted in 50ul blocking Buffer was added to each well. The plates were incubated in a dark place for one hour. The MAb-peroxidase conjugate was transferred and wells washed four times with PBS-Tween. A Peroxidase substrate was added to each well (100 µl) and left to incubate for 30 minutes. Absorbance was read at 414nm using an ELISA plate reader.

2.4 Mosquito traps

2.4.1 Mosquito Magnet-X trap

Mosquito Magnet-X (MM-X®) (American Biophysics Corporation, RI, USA) traps were mainly used to capture mosquitoes in this study. The trap was made of a clear oval-shaped plastic container called the collection container (Figure 6). Two tubes were enclosed within the container, specifically the attractant and collection tubes. Carbon dioxide was delivered though a small hole found at the black hood above the collection container. The MM-X trap had two fans, namely exhaust fan and attractant plume fan. The larger exhaust fan sucked in air while the smaller attractant plume fan blew air out of the trap. Details on use of MM-X traps are described in Chapters 3 and 4.

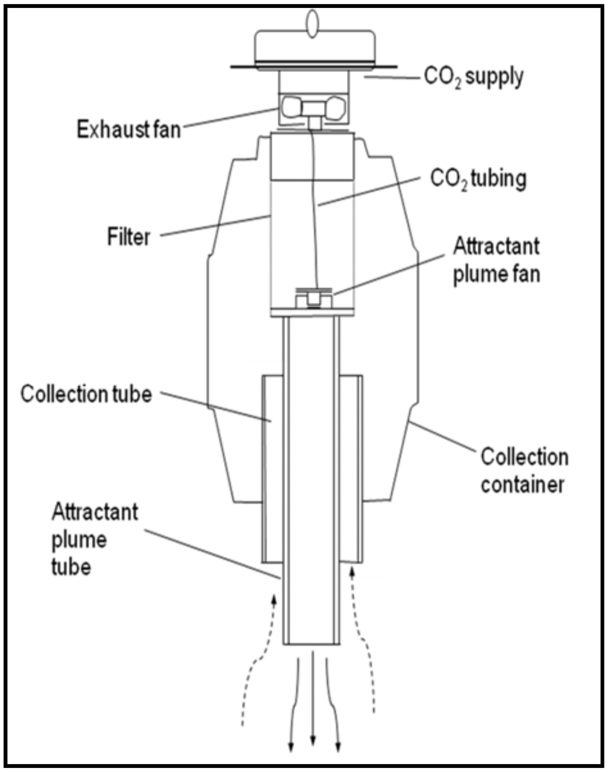

Figure 6: Illustration of MM-X trap indicating various parts of the trap

2.4.2 Suna trap

The Suna trap was designed to capture malaria mosquitoes (Biogents®). The trap contains one fan (ventilator) that sucks air into the trap (Figure 7). The air is pushed upwards and gets trapped in the conical-shaped plastic container. The catch bag made of thin netting material is hung at the centre of the trap beneath the plastic lid of the trap. Attractants can be added to the trap by hanging them between the plastic lid and the catch bag. When air is sucked into the trap from the bottom, it becomes saturated with the attractant. The saturated air then leaves through the small holes at the bottom of the trap. Mosquitoes are attracted to the trap, are sucked in and cannot escape because of the presence of the shutter. Carbon dioxide can be connected to the trap through a nozzle at the bottom of the Suna trap. The captured mosquitoes eventually die in the catch bag because of dehydration. Details on the use of the Suna trap are highlighted in Chapter 4.

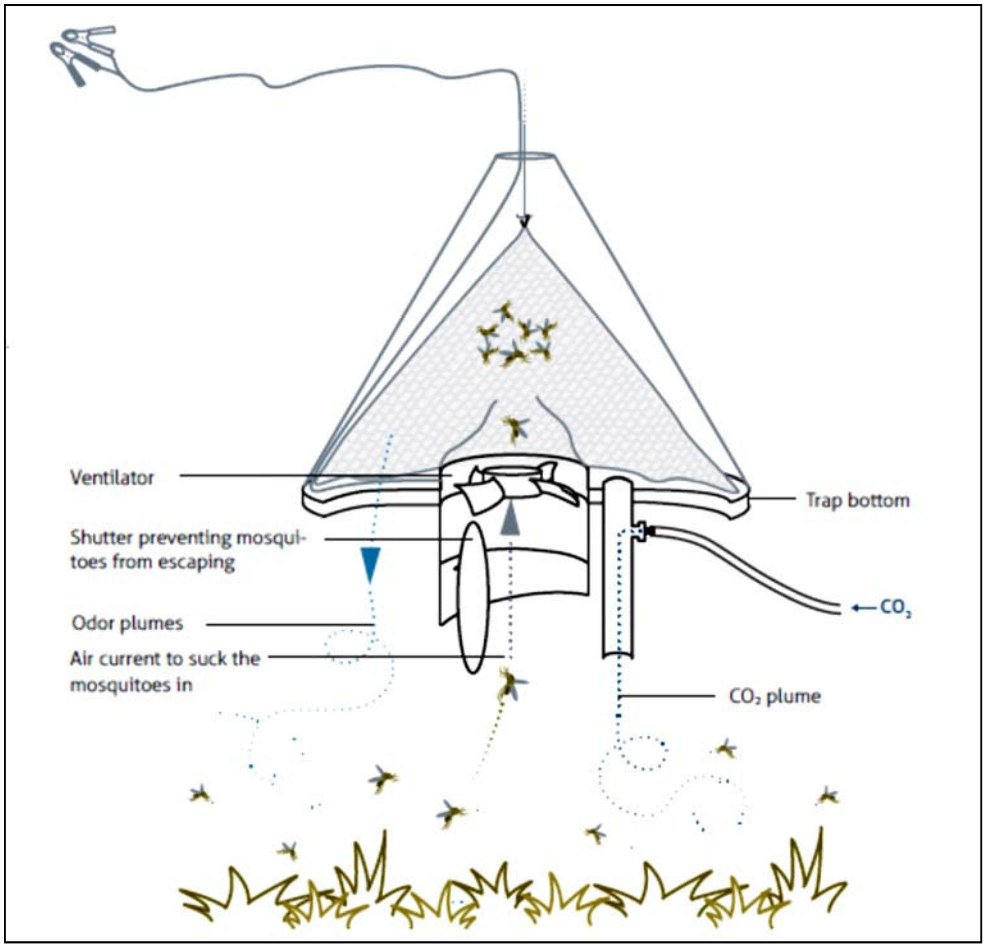

Figure 7: Illustration of Suna trap depicting its components. The blue arrow indicates odour plumes that are leaving the trap while the grey arrow demonstrates the movement of air into the trap. Adapted from Hiscox *et al*. (2014)

2.5 Synthetic attractant used as bait in MM-X and Suna trap

The MM-X traps were baited with a synthetic mosquito attractant known as Mbita blend. The attractant consisted of several chemical compounds that included 3-methyl- 1-butanol

(0.000001%), tetradecanoic acid (0.00025%), ammonia (2.5%), (S)-lactic acid (85%), 1-butylamine (0.000001%), and 2-butanone (99.5%). The six synthetic compounds were impregnated on nylon strips and used to dispense the attractants (Okumu *et al.*, 2010; Mukabana *et al.*, 2012). Nylon strips measuring 24 cm by1 cm were cut from brown nylon stockings made from 90% polyamide and 10% spandex (Bata Shoe Company, Kenya). Each nylon strip was dipped in a glass vial containing one chemical compound measuring one millilitre. The soaked strips were placed on a rack at room temperature for five hours. The six strips were then tied together using a safety pin and placed in the attractant tube of the MM-X trap. Impregnated nylon strips were replaced every three months. Carbon dioxide was derived from fermenting 17.5g of yeast (Angel Yeast Co. Ltd., China) with 2 litres of tap water, 250ml of molasses and in a 3-litre bottle (Mweresa *et al.*, 2014). Molasses is a dark and sweet by-product made during the extraction of sugar from sugarcane (Mumias Sugar Company Ltd, Kenya). Carbon dioxide was produced at a rate of 80ml per minute (Mweresa *et al.*, 2014) and delivered through thin PVC pipes that connected the fermentation bottle and the MM-X trap. Vaseline petroleum jelly was applied at the point where the PVC pipe was connected to the fermentation bottle to prevent ants from climbing up the trap and feeding on trapped mosquitoes. The same attractant described above with the exception of carbon dioxide was used as bait in the Suna trap. Instead 2-butanone, a compound that has recently been demonstrated to mimic carbon dioxide was used.

CHAPTER 3: EVALUATION OF THE MALARIA BURDEN AND TRANSMISSION IN THE FISHING VILLAGES OF RUSINGA ISLAND, WESTERN KENYA

3.1 BACKGROUND

Malaria is endemic in western Kenya (Mutero *et al.*, 1998; Noor *et al.*, 2009; Manoti, 2013) where transmission is sustained by three vectors namely *An. gambiae*, *An. arabiensis* and *An. funestus* (Minakawa *et al.*, 2002; Shililu *et al.*, 2003; Futami *et al.*, 2014). Fishing is a major source of food and livelihood among people living around the Lake Victoria shores of Mbita sub-county (Weckenbrock, 2005; Opiyo *et al.*, 2007). And the majority of the adult population living on Rusinga Island in the sub-county is involved with artisanal capture fishing activities. These individuals capture fish close to the shoreline and further into the lake.

Fishing is predominantly conducted at night. This suggests that the majority of the adult male population of Rusinga Island is outdoors at night. Mosquitoes prefer to feed at night (White, 1974; Mathenge *et al.*, 2001), a situation that places individuals engaging in nocturnal outdoor activities at a higher biting risk (Durnez and Coosemans, 2013). Fishing is deemed an occupational risk as it increases the chance of receiving infective mosquito bites (Escovar *et al.*, 2013). In general outdoor malaria transmission presents a major challenge to malaria elimination (Killeen, 2014). Thus, it is not surprising that malaria cases remain high despite the use of indoor vector control interventions (Durnez and Coosemans, 2013; Killeen, 2014) on Rusinga Island.

There is need to determine outdoor malaria transmission potential in relation to human livelihood activities (Durnez and Coosemans, 2013). Availability of this information is critical towards effective implementation of vector control interventions. To date few entomological studies have been conducted to determine indoor and residual transmission of malaria on Rusinga Island. Similarly, few malaria parasitological studies have been conducted on the Island. This study sought to determine relative abundance and density of vectors of malaria, and their contribution to indoor and outdoor malaria transmission potential on Rusinga Island, which is characterized by extensive outdoor capture fishing livelihood activities. A malaria parasitological survey was also conducted to determine the burden of malaria and its association with geographical location and use of malaria preventive measures among residents of Rusinga Island.

3.2 METHODOLOGY

3.2.1 Study site

The study was conducted on Rusinga Island, located in Mbita sub-county in western Kenya. The Island is predominantly rural and is connected to the Mbita mainland via a causeway (Figure 5). Rusinga has no main town and mainly relies on the neighbouring Mbita town for regional transportation, access to banking services and other urban economic services. The Island covers an area of 42km^2 and is mainly composed of rocky and hilly terrain. The centre of the Island is characterized by a large hill called Ligogo. The vegetation on Rusinga is mainly shrubland that is partially cleared for cultivation of subsistence crops like maize, vegetables, potatoes and millet. The island is administratively divided into Rusinga East and Rusinga West locations. The dominant ethnic group is Luo who mainly engage in artisanal capture fishing and small-scale trading (Opiyo *et al.*, 2007). Subsistence farming of drought-resistant crops is also conducted by Rusinga residents. Malaria in Mbita sub-county is perennial with peaks in malaria cases recorded in July, shortly after the long rainy season (Mutero *et al.*, 1998).

Fish is the main economic item on the island. Fishing on Rusinga Island is mainly conducted by the adult male population. Women are mostly involved in fish processing and fish trading. Other fishing-related activities that are carried out on the island include boat making and fish net repair. Fishing beaches are located all over the island with different beaches specialized in the capture of one or two species of fish. Fishing is conducted all year round, both day and night but predominantly at night. An annual fishing ban is enforced on Silver fish (*Rastrineobola argentea*) between April and August to enable the species to proliferate.

3.2.2 Experimental design

A longitudinal entomological survey was conducted in Gunda village. Mosquitoes were collected using the MM-X trap. Mosquitoes were collected for four nights in a week, with alternating nights for indoor and outdoor trapping. Experiments were replicated over 72 nights, thus 36 nights indoors and 36 nights outdoors.

A cross-sectional malaria survey was conducted in all villages in Rusinga Island. A population sample of Rusinga residents was calculated using the formula used in calculating sample size in prevalence studies proposed by Daniel (1999) and Naing *et al*. (2006). In this formula, n =

sample size, Z = Z statistic for a level of confidence, P = expected prevalence or proportion (usually stated as a proportion of one; if prevalence is 20%, then $P = 0.2$), and d = precision.

$$n = \frac{Z^2 P(1 - P)}{d^2}$$

The prevalence was reported as 24% in a malaria survey reported on the island in 1998 (Mutero *et al.*, 1998). The sample size for malaria parasite prevalence surveys was calculated assuming an expected prevalence of 24%, 5% level of significance ($d = 0.05$), and 95% confidence interval (CI) ($Z = 1.96$). A sample of 280 people was estimated as the minimum number per zone. Rusinga Island had previously been locally divided into eight zones that each consisted of approximately two to four villages. The sample size required for the malaria survey in the whole island was 2,240.

Participants were randomly selected from a Health Demographic Surveillance System (HDSS) database of Rusinga Island that was compiled in 2012 (Hiscox *et al.*, 2012). Prior to the malaria survey, participants were mobilized by project staff. The project members visited the homes of selected participants and requested them to turn up at a primary school within their zone to undergo a malaria test. Eight Primary schools all around Rusinga were used as centres for the malaria study.

3.2.3 Contribution of malaria vectors to indoor and outdoor transmission

A longitudinal entomological survey was conducted to determine the densities of mosquitoes and their species composition inside and outside houses. Mosquitoes were collected using MM-X traps powered using 12-volt batteries and baited with a synthetic mosquito attractant known as Mbita blend. The constituents of the attractant are described in chapter two of this thesis. Mosquitoes were collected from six randomly selected houses in Gunda village. Houses were spaced a minimum of 25 metres apart (Hill *et al.*, 2007). The houses were similar in structure with roofs made of iron sheets, walls made of mud and open eaves. Each house had two rooms. Household members were supplied with untreated mosquito nets that were used throughout the study. The MM-X traps were placed above a bed occupied by a human host and towards its foot end. Outdoor biting mosquitoes were collected by traps hung within the peri-domestic environment approximately 15 cm off the ground. The MM-X traps were operated from 6pm till 7am the following morning. Mosquitoes were collected from the same houses throughout the study.

3.2.3.1 Mosquito identification

Captured mosquitoes were sorted by sex, counted and identified morphologically using the keys of Gillies and DeMeillion (Gillies and Coetzee, 1987) as described in chapter two. Anopheline mosquitoes were preserved in silica gel waiting further processing. The legs of female malaria mosquitoes were used to identify the sibling species of mosquitoes using the PCR technique (Scott *et al.*, 1993) as described in the previous chapter.

3.2.3.2 Sporozoite infection

The head and thorax of female Anopheline mosquitoes were tested for the presence of *Plasmodium* sporozoite using immunological techniques. The presence of *Plasmodium falciparum* circumsporozoite antigens (CSA) was tested using the Enzyme-linked immunosorbent assay (ELISA) (Wirtz *et al.*, 1989).

3.2.4 Burden of malaria and its association with geographical location and use of malaria preventive measures

A cross-sectional malaria survey was conducted to determine malaria prevalence. Each participant's body temperature was measured using an electronic thermometer. Fever was defined as temperature above 37.5ºC. Participants were tested for malaria using blood slide microscopy. A consent form was signed prior to the conduct of a malaria test. Finger-prick blood samples were obtained for blood smears from participants. The blood smears were stained with 10% Giemsa for approximately 10 minutes and examined for malaria parasites microscopically using 100 high-powered microscopic fields under oil immersion. The blood smears were examined by two experienced microscopists. The first microscopist recorded malaria positivity and malaria species identity. The second microscopist conducted a quality assurance examination on the slides and his findings were recorded as the final microscopy result. A slide was categorized as negative if no malaria parasite was seen after scanning 100 microscope fields. Individuals found positive for malaria parasites received a treatment dose of artemether-lumefantrine (AL) according to the national policy (MOPHS and MMS, 2010). Malaria prevalence was calculated as the proportion of participants with malaria from the total number of individuals tested. A questionnaire was administered during the malaria survey to determine the association between malaria infection and geographical location and use of preventive measures.

3.2.5 Ethical considerations

Ethical clearance was obtained from Kenya Ethical Review Committee at the Kenya Medical Research Institute (NON-SSC No. 280). Consent was obtained from participants prior to the malaria survey. Written consent was sought from parents and care givers of children to allow minors to participate in the study. Consent was also obtained from heads of households that provided approval for mosquito collection in houses.

3.2.6 Statistical analysis

The data analysis for this study was done using R statistical software version 2.15.2. The response variable was derived from count data (mosquito numbers). The untransformed data was analyzed by fitting generalized linear models (O'Hara and Kotze, 2010) with a poisson regression. The packages mass, effects, epicalc, multcomp, lme4, gee, geepack and aod (Team, 2011) were loaded before running the analysis. The night mosquitoes were captured (day of experiment) was included in the model as a factor. Variables were considered to be significant at $p<0.05$. Data collected during the malaria survey was analyzed using multivariate logistic regression. The outcome of the malaria test (whether positive or negative for malaria parasites) was treated as the dependent variable. The effect of other variables, specifically sex, location and age group were also analyzed. Statistical significance was set at $p<0.05$.

3.3 RESULTS

3.3.1 Contribution of malaria vectors to indoor and outdoor transmission

A total of 1,684 mosquitoes were collected during the entomological survey (Table 2) carried out over a period of 72 nights. The species caught were: *An. gambiae* s.l., *An. funestus* s.l., *Culex* species, *Mansonia* species, *Aedes* species and other anophelines. Among the collected mosquitoes 74 (4.4%) were malaria vectors while 1,610 (95.6%) were non-malaria vectors. Of the total anophelines collected, *An. gambiae* s.l. was the most abundant malaria vector captured both indoors and outdoors (62%). *An. funestus* was the second most abundant malaria vector (38%). Overall, *Culex* spp. were the most abundant species collected both indoors and outdoors (1,493).

A total of 74 female anopheline mosquitoes were collected from both indoor and outdoor sites (Table 2). A higher number of *An. gambiae* s.l. mosquitoes were captured outdoors (27) compared to indoors (19). However, this finding was not statistically different (p = 0.477). As well, there was no statistical difference between the number of *An. funestus* s.l. females captured indoors and outdoors (p = 0.153).

Of the 46 *An. gambiae* s.l. mosquitoes subjected to PCR analysis, 36 (92.3%) were identified as *An. arabiensis*. Of the 19 females captured indoors, 12 (92.3%) were identified as *An. arabiensis* and one (7.7%) was *An. gambiae* s.s. However, 6 samples failed to amplify. Of the 27 *An. gambiae* s.l. mosquitoes captured outdoors, 24 (92.3%) were *An. arabiensis*, two (7.7%) were *An. gambiae s.s*. and one did not amplify. This indicates that *An. arabiensis* is the dominant malaria vector among siblings of the *An. gambiae* complex on Rusinga Island.

There was a significant difference in the number of *Culex* spp. captured indoors compared to outdoors (p<0.001). Among the 1,493 *Culex* spp. mosquitoes caught 665 (52.4%) were caught indoors. No statistical difference was found between *Mansonia* spp. (p = 0.681), *Aedes* spp. (p = 0.291) and other *Anopheline* spp. (p = 0.995) captured indoors and outdoors.

3.3.1.2 Sporozoites in malaria vectors

A total of 62 malaria vectors were tested for *Plasmodium falciparum* sporozoite infection of which 28 were captured indoors and 34 outdoors. Overall, 4 *An. Arabiensis* mosquitoes tested positive for sporozoites of which 2 were captured indoors and 2 outdoors.

Table 2: Number of mosquitoes captured inside and outside houses in Gunda village on Rusinga Island.

Species	**N**	**Mosquitoes collected**			**Total**
		Indoors (%)	**Outdoors (%)**	**p-value**	
An. gambiae s.l. females	72	19 (41.3)	27 (58.7)	0.477	46
An. gambiae s.l. males	72	3 (60)	2 (40)	0.499	5
An. funestus s.l. females	72	9 (32.1)	19 (67.9)	0.153	28
An. funestus s.l. males	72	0 (0)	0 (0)	1.000	0
Culex species females	72	665 (52.4)	584 (47.6)	<0.001	1249
Culex species males	72	132 (54.1)	112 (45.9)	0.038	244
Mansonia spp females	72	44 (45.4)	53 (54.6)	0.681	97
Mansonia species males	72	0 (0)	0 (0)	1.000	0
Aedes species females	72	7 (63.6)	4 (36.4)	0.291	11
Aedes species males	72	1 (100)	0 (0)	0.997	1
Other species	72	3 (100)	0 (0)	0.995	3

N refers to the number of trapping nights

3.3.2 Burden of malaria and its association with geographical location and use of malaria preventive measures

A total of 2,318 individuals were recruited in the malaria survey. They consisted of individuals of all ages. The age of study participants ranged from 1 year to 102 years. The median age was 14 years and 57% of participants were below 18 years of age. Of the individuals enrolled in the study, 1,263 (54.4%) were female while 1,055 (45.6%) were male.

3.3.2.1 Prevalence of malaria and distribution of *Plasmodium* species

Overall, blood samples from 252 (10.9%) participants were positive for malaria parasites. Of the 2,318 people who enrolled in the study, three malaria species were identified namely *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium ovale*. Among the 252 individuals with malaria parasites, 222 (88.1%) were infected with *P. falciparum*, 10 (3.96%) with *P. malariae*, 2 (0.79%) with *P. ovale*, 16 (6.34%) with a mixed infection of *P. falciparum* and *P. malariae*, and 1 (0.39%) with *P. falciparum* and *P. ovale* and 1 (0.39%) with a mixed infection of all three malaria species (Table 3).

Table 3: Malaria infection among individuals from Rusinga East and West

			Malaria parasite species					
Location	**N**	**n**	***P. f***	***P. m.***	***P. o.***	***P. f. +P. m.***	***P. f. +P. o.***	***P. f. + P. m. + P. o.***
Rusinga East	1248	120	105	6	0	9	0	0
Rusinga West	1070	132	117	4	2	7	1	1
Total	**2318**	**252**	**222**	**10**	**2**	**16**	**1**	**1**

N refers to the total number of participants; n refers to the number of individuals positive for *Plasmodium* parasites; *P.f. Plasmodium falciparum*; *P.m. Plasmodium malariae*; *P.o. Plasmodium ovale*

3.3.2.2 Association between malaria infection and location and malaria preventive measures

There was a significant association between malaria parasitemia and location (OR = 1.5, 95% CI 1.14 – 1.97, p = 0.003) (Table 4). Individuals who did not use malaria protective measures whilst sleeping were two times more likely to get malaria (95% CI 1.76 – 3.91, p<0.001) compared to those who did.

Table 4: Factors associated with malaria parasitemia among residents of Rusinga Island

Variable	**Odds Ratio (95% CI)**	**p-value**
Location		
Rusinga East	ref	ref
Rusinga West	1.5 (1.14 – 1.97)	0.003
Malaria preventive measures		
Yes	ref	ref
None	2.65 (1.76 – 3.91)	<0.001

3.4 DISCUSSION

The study demonstrated that malaria transmission occurs both indoors and outdoors in Rusinga Island. Both indoor and outdoor transmission are sustained by *An. arabiensis* which was found to be the most abundant malaria vector in the study site. Futami *et al.* (2014) recently reported *An. arabiensis* as the major malaria vector in the study area. This species is traditionally known to be an opportunistic vector that prefers to feed outdoors (White, 1974) on humans and animals depending on availability (Muriu *et al.*, 2008). Several studies have reported *An. arabiensis* as partially responsible for indoor malaria transmission in several areas (Tirados *et al.*, 2006; Muturi *et al.*, 2008; Kerah-Hinzoumbe *et al.*, 2009; Mwangangi *et al.*, 2013b). The finding that no significant difference was found in density of *An. arabiensis* found indoors and outdoors is most likely explained by the low density of malaria vectors. It is statistically difficult to show differences in indoor and outdoor mosquito densities when general mosquito densities are low.

A recent report by Futami *et al.* (2014) revealed that *Anopheles arabiensis* replaced *An. gambiae s.s.* as the main malaria vector in the study area. Similar cases of species shifts among populations of malaria vectors have been reported in Kenya (Bayoh *et al.*, 2010; Mutuku *et al.*, 2011; Mwangangi *et al.*, 2013a) and Tanzania (Russell *et al.*, 2010). The species shifts among *An. gambiae* and *An. arabiensis* populations was reported to occur after wide coverage, ownership, and use of Long lasting insecticidal nets (LLINs) (Bayoh *et al.*, 2010; Mutuku *et al.*, 2011). *Anopheles arabiensis* has been known to survive in areas with wide coverage of both LLINs and IRS (Russell *et al.*, 2011; Mwangangi *et al.*, 2013a; Bayoh *et al.*, 2014). Killeen and others (2014) indicated that *An. arabiensis* mosquitoes have adopted a behaviour that is instrumental in avoiding prolonged exposure to insecticides. These malaria vectors exit houses immediately after entry if a human host is sleeping under an LLIN (Killeen, 2014), an act that suggests that the mosquito either enters another house or searches for a blood meal host outdoors. This particular behaviour has been reported in several studies (Kitau *et al.*, 2012; Okumu *et al.*, 2013a; Okumu *et al.*, 2013b).

Malaria mosquitoes mainly bite at night, thus individuals who use indoor control tools are usually protected from malaria. The front-line malaria vector control interventions specifically Indoor residual spraying (IRS) and LLINs are effective indoors, which is a major space for insecticidal exposure (Huho *et al.*, 2013). These indoor interventions do not protect individuals who are found outdoors at night, for instance fishermen. Fishing is the main livelihood activity predominantly conducted at night by majority of the male adult population on Rusinga Island. Individuals who engage in fishing and its related activities by the shoreline at night appear to be at risk of malaria. Huho and others (2013) indicate that human behaviour is an important determinant in the place where malaria transmission occurs. Outdoor transmission persists in areas with intense nocturnal outdoor activities (Durnez and Coosemans, 2013; Killeen, 2014).

The overall malaria prevalence assessed through a population-based survey in Rusinga was 10.9%. This finding represents an estimate of *Plasmodium* prevalence across all age groups in western Kenya, unlike national surveys that tend to focus on sub-populations like children. A similar prevalence study conducted on the Island in November 1998 revealed that the prevalence was 24.4%. This suggests that there has been a reduction in the burden of malaria in the study area probably due to the increased use of LLINs in areas around the shores of Lake Victoria (Futami *et al.*, 2014).

The predominant malaria species in Rusinga Island was *P. falciparum*. This parasite is the major species found in Kenya. This finding is consistent with those of other studies conducted in Mbita sub-county (Mutero *et al.*, 1998; Gouagna *et al.*, 2003; Manoti, 2013). In our study, *Plasmodium ovale* single-species and mixed infections were detected in a few individuals. This parasite has never been documented in Mbita sub-county. Reported cases of malaria infections caused by *P. ovale* are rare and could be because of under-diagnosis or low transmission (Faye *et al.*, 1998; Faye *et al.*, 2002). The parasite is known for its low densities (Collins and Jeffery, 2005; Mueller *et al.*, 2007) which contribute to difficulties in diagnosis. Identification of malaria parasites by microscopy largely depends on the skills and experience of the microscopist (Bell *et al.*, 2006). Malaria in health facilities in Rusinga is diagnosed microscopically by staining blood smears with Giemsa. The greatest shortcoming of this diagnostic technique is low sensitivity. This may explain the lack of patients diagnosed with malaria caused by *P. ovale* in the study area. Cases of *P. ovale* infections have been reported in other areas of western Kenya (Munyekenye *et al.*, 2005; Bashir *et al.*, 2013).

Our study demonstrated that individuals who did not use malaria protective measures whilst sleeping were at a higher risk of malaria. The majority of the adult population on Rusinga Island engage in outdoor nocturnal fishing activities and are less likely to sleep under treated bednets consistently. Similar findings have been reported in other parts of Africa (Amuta *et al.*, 2014; Chirebvu *et al.*, 2014). A review conducted by Pulford *et al* (2011) documented several reasons why people do not sleep under LLINs. Spending time elsewhere, for instance, at the work place at night, was cited as a reason for not using an LLIN even when one was available

(Pulford *et al.*, 2011). Fishermen of Rusinga often do not sleep in their houses and are not protected from malaria vectors in their work place at night.

An increase in outdoor biting has been reported in several areas in sub-Saharan Africa (Govella *et al.*, 2010b; Reddy *et al.*, 2011; Russell *et al.*, 2011). Although approximately 80% of malaria transmission occurs indoors (Huho *et al.*, 2013), outdoor malaria transmission is still important. Indoor vector control interventions are effective and have been reported to reduce malaria transmission in several areas (Okumu and Moore, 2011; Bekele *et al.*, 2012; Fullman *et al.*, 2013), but are insufficient and extra effort is required to eliminate malaria. This should be especially applied in settings with intense regular nocturnal outdoor human activities. Since malaria transmission occurs where mosquitoes bite humans, vector control strategies need to be developed that also target outdoor biting mosquito populations (Lindblade, 2013) in order to achieve malaria elimination (Takken and Knols, 2009; Ferguson *et al.*, 2010; mal, 2011; Govella and Ferguson, 2012).

3.5 Conclusion

Malaria is mesoendemic on Rusinga Island with *P. falciparum* as the predominant parasite species. The rather significant and unexpected contribution of *P. malariae* and *P. ovale* to the overall malaria prevalence on Rusinga Island underscores the epidemiological importance of these species in the big push towards eliminating malaria. Lack of consistent use of malaria protective measures such as treated bed nets predisposed individuals to malaria infection. This study shows the existence of outdoor transmission in Rusinga Island that suggests that individuals involved in nocturnal outdoor activities in the study area are at risk of malaria. Vector control strategies that target outdoor biting mosquitoes should be sought to supplement indoor strategies like LLINs and IRS.

CHAPTER 4: SPATIAL AND TEMPORAL EXPOSURE TO MALARIA TRANSMISSION AND BURDEN IN FISHING VILLAGES IN RUSINGA ISLAND, WESTERN KENYA

4.1 BACKGROUND

Malaria vectors are traditionally known to bite at night indoors when their human hosts are asleep. This justifies the current front-line vector control strategies, specifically Long Lasting Insecticidal Nets (LLINs) and Indoor Residual Spraying (IRS), that target mosquitoes that bite and rest indoors (Killeen, 2014). The two interventions have been proven to be effective in reducing malaria transmission in several parts of sub-Saharan Africa (Mabaso *et al.*, 2004; Pluess B. *et al.*, 2010; Okumu and Moore, 2011; Bekele *et al.*, 2012; Fullman *et al.*, 2013). However, Individuals found outdoors at night beyond the protective range of indoor interventions are at risk of malaria (Durnez and Coosemans, 2013). An increase in outdoor biting has been reported in several areas (Govella *et al.*, 2010b; Reddy *et al.*, 2011; Russell *et al.*, 2011) and biting patterns observed (Escovar *et al.*, 2013; Kabbale *et al.*, 2013).

Outdoor capture fishing is the major livelihood activity on Rusinga, an island located within the Winam Gulf of Lake Victoria in western Kenya (Opiyo *et al.*, 2007). Fisherfolk practising capture fishing do so both at daytime and at night. Rusinga is a malaria endemic area with fluctuating densities of malaria transmitting mosquitoes. A lot fishing-related activities here are rudimentary in nature and confined to the shorelines and in the nearby shoreline waters. The three main malaria vectors in the area include *An. gambiae* s.s., *An. arabiensis* and *An. funestus* (Futami *et al.*, 2014). *Anopheles arabiensis* is currently the most abundant malaria vector on Rusinga Island and is generally characterized as an outdoor biter that feeds on both animals and humans depending on availability in Central parts of Kenya (Muriu *et al.*, 2008). The Lake Victoria shoreline of Mbita sub-county, which Rusinga Island is part, is associated with both permanent and semi-permanent water bodies that form breeding sites for mosquitoes (Weckenbrock, 2005; Minakawa *et al.*, 2012). These breeding habitats contain mosquito larvae during the rainy and dry seasons (Weckenbrock, 2005). It is reported that such water bodies are sentinel breeding sites for malaria vectors (Minakawa *et al.*, 2012). We sought to investigate possible consequent high adult vector densities around the shoreline.

We hypothesize that the risk of malaria amongst fishermen in Rusinga Island is greatest in areas close to the shoreline. Here, fishermen work and live in clustered houses found in fishing beaches around the shoreline. Moreover, other areas of the shoreline are used as farms whose owners take advantage of free lake water. Houses are not clustered in these farms but are relatively sparsely spaced further inland. Fishing-related activities are conducted along the shoreline and concentrated in fishing beaches

The main objective of this study was to determine the spatial and temporal exposure to malaria mosquitoes and infection in relation to fishing activities. This study sought to determine the (a) temporal association between mosquito and capture fishing activities and (b) spatial relationship between *Anopheles* mosquito density, malaria prevalence and distance from Lake Victoria shoreline.

4.2 METHODOLOGY

4.2.1 Study area

The study was carried out on Rusinga Island located in Mbita sub-county in western Kenya. The people of Rusinga engage in two main livelihood activities namely artisanal capture fishing and small scale trading (Opiyo *et al.*, 2007). Fishing is carried out during the day but predominantly at night. Three main fish species of commercial importance are exploited on Rusinga Island. These include the Nile perch (*Lates niloticus*), silver fish (*Rastrineobola argentea*), and tilapia (*Oreochromis niloticus*). Rusinga Island is a mesoendemic malarious area. Malaria is the main cause of outpatient hospital visits in Mbita sub-county, which Rusinga island is a part of (Mutero *et al.*, 1998).The prevalence of the disease was recorded as 24% on the Island (Mutero *et al.*, 1998).

Fishing beaches are densely populated with individuals involved in capture fishing and related activities. Houses in these beaches are usually occur as slum-like clusters. The groups of individuals involved in capture fishing activities include fishing crew, fish traders, fish processors, boat owners and auxiliary stakeholders e.g. boat makers/repairers, net menders/repairers, middlemen etc. Areas away from the shoreline are sparsely populated compared to the fishing beaches. Majority of the adult male population are fishermen. Women engage in fishing activities but at a smaller scale compared to men. Fishing-related activities that are conducted by adult females include fish processing (mainly sun-drying and deep frying) and small scale fish trading.

Fishing beaches of Rusinga are specialized in the capture of one or two species of fish. Two sites were chosen for the study, namely Nyangera and Sienga village. Kolunga fishing beach is located along the shoreline area of Nyagera village while Sienga beach is found along the shores of Sienga village. Both fishing beaches specialize in the harvest of silverfish which is called *Omena* by the local *Dholuo* natives. This species of fish is mainly harvested at night compared to the Nile perch and tilapia fish which are regularly harvested during the day. Structures found clustered in the fishing beaches include residential houses, local offices of the Beach Management Unit officials, local open kiosks, and make-shift structures locally called *Abila* that are used as storage units or offices for the fishing crew or sleeping places.

4.2.2 Temporal association between capture fishing and mosquito activity patterns

An entomological field study was conducted in Kolunga fishing beach. The behavioural biting pattern of mosquitoes at the fishing beach was recorded while fishing and its related activities observed. Mosquitoes at the fishing beach were trapped using odour-baited MM-X counter flow traps powered by 12-volt batteries. The synthetic attractants used as bait is described in chapter three of this thesis. Three traps spaced 25 metres apart were hung on metallic tripod stands close to housing structures at the beach. The stands were mounted approximately 30metres away from the shoreline and the traps hung 30cm above the ground.

The experiment started at 6pm and hourly collections of trapped mosquitoes were done till 7am the next morning. After every hour, the trap was stopped, unhung and set aside to facilitate removal of mosquitoes. The hood of the trap that holds two fans was unscrewed and the open hole covered with mosquito netting. Trapped mosquitoes were removed using an aspirator and transferred to a pre-labeled paper cup. The unhung trap was replaced by another trap that ran for the next one hour. During the experimental period, observations were done on the people at the fishing beach. The numbers of people conducting capture fishing and capture fishing related activities, for instance net mending and purchasing fish, at the beach were recorded at hourly intervals.

4.3.3 Variation of mosquito densities over distance from the shoreline

The study site for this experiment was Sienga village in Rusinga West location. The relative densities of mosquitoes found within a transect measuring 1km along the shoreline and 2km inland was determined. Variation in mosquito numbers from the shoreline was compared. The transect was divided into three sections from the shoreline, each measuring approximately 600 metres inland by 1 km along the shoreline. A total of 30 houses were randomly selected for inclusion in the study from a list of houses in Sienga village. The houses were limited to those that have solar-powered Suna traps (Hiscox *et al.*, 2014) positioned outdoors and operated from 6 pm to 6 am. The traps were previously placed outside houses in Sienga village as part of a larger project with the aim of mass trapping malaria vectors on Rusinga Island. The traps were baited with a synthetic blend impregnated on nylon strips as described in chapter three. Mosquito collections were made four times in a week. Captured mosquitoes were aspirated and transferred to pre-labelled paper cups. The paper cups were transported to the International Centre of Insect Physiology and Ecology (*icipe*) in Mbita Point, and placed in a -20ºC freezer for approximately 1 hour to kill the mosquitoes.

4.3.3.1 Identification of mosquitoes

Captured mosquitoes were sorted by sex and identified morphologically to separate vectors of human malaria from non-vectors (Gillies and Coetzee, 1987). *Anopheles gambiae* s.l. mosquitoes were subjected to PCR analysis and identified to species level (Scott *et al.*, 1993). Details on procedures used to identify mosquitoes are indicated in chapter two.

4.3.4 Local variation in malaria prevalence in a fishing village in Rusinga

A malaria survey was conducted in November 2013 in Sienga village. A total of 300 participants were randomly chosen among individuals who lived within the transect described earlier. Project staff visited participants in their homes and tested them for malaria. Rapid Diagnostic tests (SD Bioline Malaria Pf/Pan®, Korea) were used. These test kits determine whether an individual has a single infection of *Plasmodium falciparum* or mixed infections of *Plasmodium falciparum* and other *Plasmodium* species. Temperature was also measured to determine whether the participant had a fever which is defined as body temperature above 37.5ºC. Individuals who had taken malaria medication two weeks before administering the malaria test were not included in the study. The approximate distance of each house from the shoreline was determined using Google Earth. Other parameters recorded included age and occupation of the human subjects tested for malaria and their record of bed net use.

4.3.5 Ethical considerations and approval

Written informed consent was obtained from household heads to include their houses in the study. Similarly, individuals who willingly participated in the malaria survey signed written consent forms. Ethical approval for the study was sought and obtained from the Ethical Review Committee of the Kenya Medical Research Institute (NON-SCC No. 280). Approval to capture mosquitoes at Kolunga fishing beach was sought from Beach Management Unit Chairman of Kolunga.

4.3.6 Statistical analysis

Data was analyzed using the R statistics software (version 2.15.2). The association between mosquito numbers and distance from the shoreline was determined using Generalized Linear Models (GLM) fitted with a Poisson regression. The effect of day was tested in the model (refers to the night the experiment was conducted). The variation in malaria prevalence in Sienga village was determined using logistic regression. The test was performed to determine the contribution of age and use of bed net to malaria infection. The level of significance was set at $p<0.05$.

4.4 RESULTS

4.4.1 Temporal association between capture fishing and mosquito activity patterns

A total of 1,577 female mosquitoes were captured during the survey conducted for 73 nights. Caught mosquitoes comprised of 278 (17.63%) malaria vectors and 1,299 (82.37%) non-malaria vectors. Of the total mosquitoes captured 47 (3%) were *An. gambiae* s.l., 231 (14.6%) were *An. funestus* s.l., 1,270 (80.5%) were *Culex* spp, 25 (1.6%) were *Mansonia* spp, and 4 (0.2%) were *Aedes* spp. Of the 47 members of *An. gambiae s.l.* tested by PCR, 46 successfully amplified, from which 41 (89.1%) were identified as *An. arabiensis* and 5 (10.9%) as *An. gambiae* s.s.

Two peak periods of nocturnal biting activity were observed in the malaria vectors (Figure 8). *Anopheles arabiensis* exhibited two peaks specifically between 10 - 11pm and 4 – 5am. Of the total *An. arabiensis* captured, 13 (31.7%) were captured between 10 – 11pm and 11 (26.8%) between 4 - 5am. The time period between the two peaks was characterized by a decline in biting activities. *Anopheles funestus* also exhibited peak nocturnal biting activity between 10 – 11pm. The highest record of *An. funestus* mosquitoes was 69 (30%) and recorded between 10 – 11pm.The number of mosquitoes caught after 11pm decreased till 3am when there was a gradual increase in biting activity. The second peak of the *An. funestus* biting activity was observed between 5 – 6am.

A total of 4914 people were counted at the fishing beach. Two peaks in fishing activities at the beach were observed which coincided with the peak in mosquito biting patterns. The first peak in the number of people at the beach was noted between 9 - 11pm. The number of people observed to be at the beach between 9 – 10pm was 410 (8.3%) while 594 (10.5%) were counted between 10 – 11pm. The second peak in human activities was observed between 3 – 7am. Cumulatively, the number of people counted between 3 – 4am was 122 (2.5%), 4 – 5am was 281 (5.7%) and 5 – 6am was 724 (14.5%). The number of people counted between 6 – 7am was 1,340 (27%).

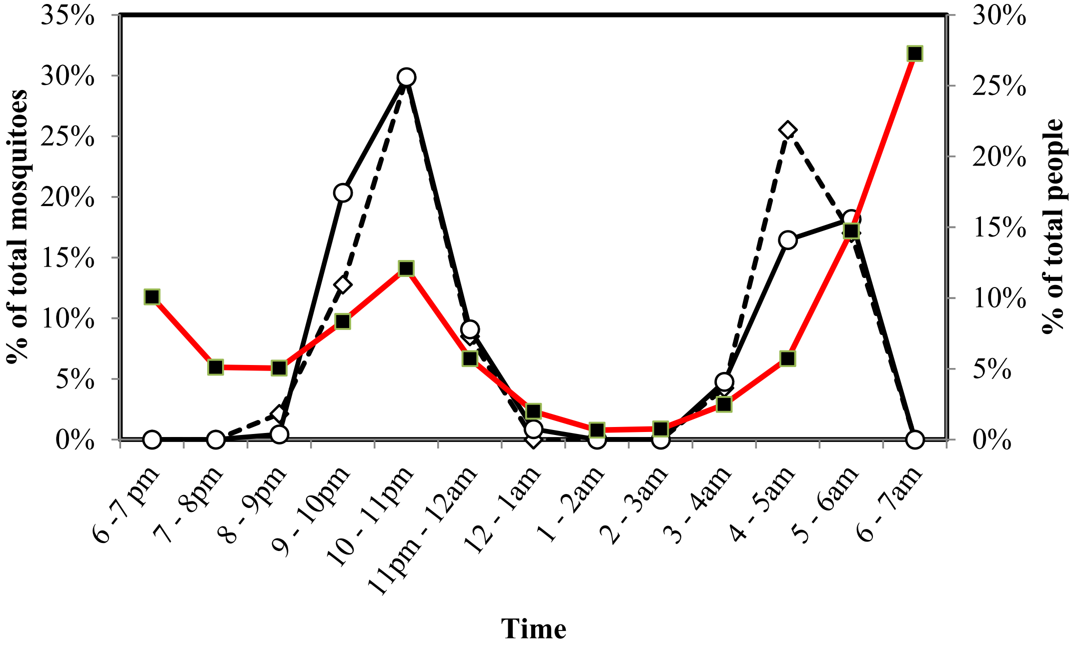

Figure 8: Mean hourly proportions of malaria mosquitoes and humans observed at night at Kolunga fishing beach on Rusinga Island. The red line indicates the number of people while the dashed and straight lines represent *An. gambiae s.l.* and *An. funestus* mosquitoes, respectively.

4.4.2 Variation of mosquito densities over distance from the shoreline

A total of 4,338 female mosquitoes were captured during the 78 nights that the experiment was conducted. Of these mosquitoes 25 (0.6%) were malaria vectors and 4,313 (99.4%) were non-vectors. From the 25 malaria vectors captured, 15 (60%) were identified as *An. gambiae* s.l. and 10 (40%) as *An. funestus* s.l. No significant difference was found in the density of *An. gambiae* s.l. and *An. funestus* s.l. at various distances from the shoreline.

Compared to mosquitoes captured at close proximity to the shoreline, there was no significant difference in the numbers of *An. gambiae* s.l. caught at 600 – 1200 metres (p = 0.534) and 1200 - 1800 metres (p = 0.742). Similarly, the density of *An. funestus* mosquitoes captured outside houses located <600m from the shoreline were not significantly different from those caught in houses 600 – 1200 metres (p = 0.426) and 1200 and 1800 metres (p = 0.424) from the shoreline. Majority of *Culex* mosquitoes were captured near the shoreline. Compared to *Culex* spp. captured <600 metres from the shoreline, there was a significant difference in the density of *Culex* mosquitoes collected 600 – 1200 (p<0.001) and 1200 – 1800 metres (p<0.001) away. Compared to *Mansonia* spp. captured in the area along the shoreline, a significant difference was observed in the density of these mosquitoes caught at 1200 – 1800 metres (p = 0.018), but not at 600 – 1200 metres (p = 0.705). The highest numbers of *Mansonia* mosquitoes were captured between 1200 – 1800 metres. Compared to *Aedes* mosquitoes caught <600 metres from the shoreline, there was no significant difference in their densities at 600 – 1200 metres (p = 0.997) and 1200 – 1800 metres (p = 0.382). Day was tested as a factor that may affect mosquito abundance and was only found to be significantly associated with the number of *Culex* spp. captured (p = 0.033) compared to the other species.

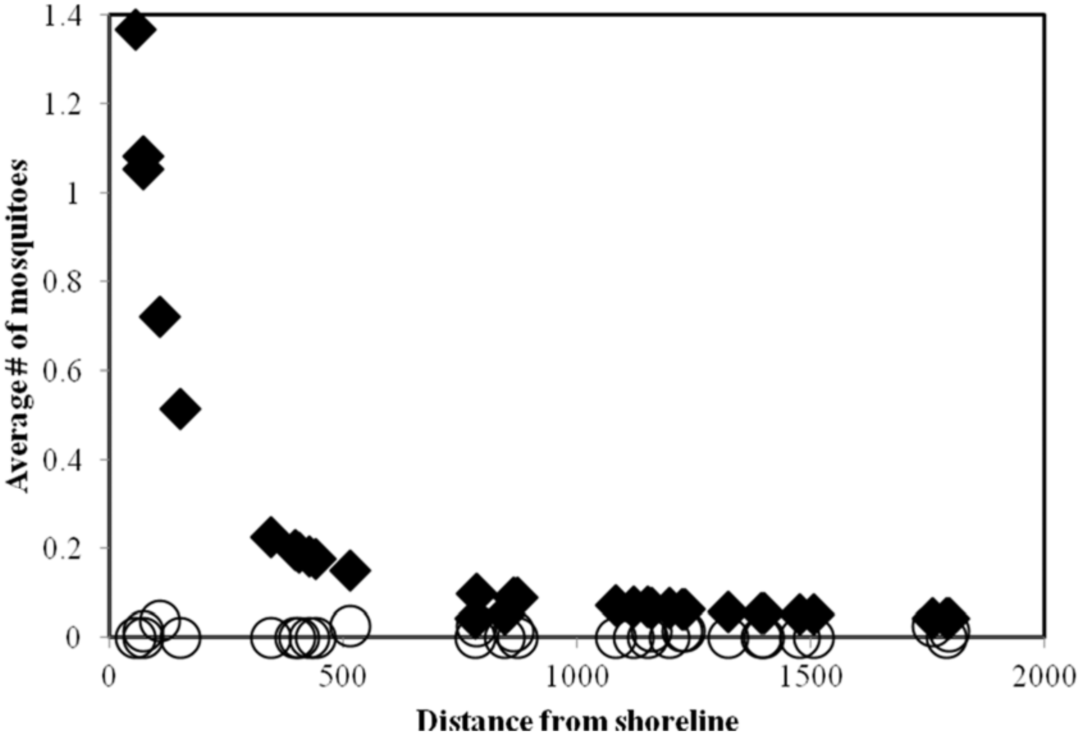

Figure 9: Mean counts of *An. gambiae* s.l. (full circles) and *An. funestus* (diamond) mosquitoes at various distances from the shoreline up to 2km inland.

4.4.3 Local variation in malaria prevalence in fishing village in Rusinga

A total of 301 individuals were screened for malaria parasites of which 44 (14.6%) tested positive. *Plasmodium falciparum* accounted for 32 (72.7%) and mixed infections of *P. falciparum* and other *Plasmodium* species accounted for 12 (27.3%) of the positive cases. Compared to individuals who live less than 600 metres from the shoreline, other individuals who lived 600 – 1200 metres (p = 0.319) and 1200 – 1800 metres (p = 0.312) away were not at a higher risk of malaria (Table 5). No statistical difference was found among individuals who slept under treated bed nets compared to those who did not. There was a significant difference in malaria prevalence among individuals aged 5 to 14 years (p = 0.015) compared to those above 30 years. However, there was no significant association between malaria prevalence and individuals below 5 years of age (p = 0.219), 15 – 29 years (p = 0.446) when compared to adults above the age of 30.

Table 5: Association between distance from the shoreline, use of treated bednets, age group and malaria prevalence

Variable	Case / n (%)	OR (95% CI)	p-value
Distance from shoreline			
0 – 600 m	16/100 (16%)	ref	ref
600 – 1200 m	9/100 (9%)	1.5 (0.6 – 3.8)	0.319
1200 – 1800 m	19/101 (18%)	0.6 (0.2 – 1.6)	0.312
Use of bednet			
Yes	35/263 (13%)	ref	ref
No	9/38 (23%)	2.0 (0.7 – 5.4)	0.161
Age group			
0 – 4 years	10/68 (6.5%)	2.1 (0.6 – 8.3)	0.219
5 – 14 years	22/97 (22.6%)	4.1 (1.4 – 14.6)	0.015
15 – 29 years	8/74 (10.8%)	1.6 (0.4 – 6.4)	0.446
Above 30 years	4/62 (14.7%)	ref	ref

OR refers to Odds Ratio, CI refers to confidence interval.

4.5 DISCUSSION

The finding of malaria vectors outdoors at the fishing beach suggests that outdoor malaria transmission occurs in Rusinga Island. *Anopheles arabiensis* and *An. funestus* were the main malaria vectors captured outdoors at Kolunga fishing beach. We observed that *An. arabiensis* exhibited low biting activities before 10pm. Both *An. arabiensis* and *An. funestus* exhibited similarities in mosquito activity patterns, with the first peak observed between 10 - 11pm, while the second between 4 – 6am. According to our observations, the peaks noted within these times coincided with those of people found at the fishing beach. This places these individuals at risk of receiving infectious bites. Biting activity in *An. gambiae* s.l. mosquitoes have been noted to be low before 10pm (Githeko *et al.*, 1996; Maxwell *et al.*, 1998).

We observed several activities conducted by fishermen between 6 and 10pm namely net mending, cleaning and lighting lamps used during fishing, arranging and loading fishing gear onto boats. During this time period, fishermen also spend time at the beach in make-shift structures called *Abilas*. The structures are porous to mosquito entry as they have multiple mosquito entry points including open eaves. Time spent in the *Abila* is used in planning fishing routes and strategies and eating supper.

The findings of this study imply that fish traders are at risk of malaria based on the time they are found at the lake shore. These individuals are mostly women who wait at the fishing beach before dawn (Weckenbrock, 2005). The women wait for fishermen to come back to shore with their daily catch and buy silverfish, a transaction conducted in the presence of boat owners. This activity conducted between 3 and 6am places fishermen, female traders and boat owners at risk of malaria. A study recently conducted by Escovar *et al*. (2013) showed that fishermen were at risk of receiving mosquito bites while conducting fishing activities in Colombia. Malaria mosquitoes were found in canoes used by fishermen at dawn while conducting fishing activities (Escovar *et al.*, 2013) at a distance of 200m offshore.

A study conducted in Rusinga Island in 2004 revealed that many breeding sites were located around the Lake Victoria shoreline (Weckenbrock, 2005). It was presumed that a higher number of malaria mosquitoes were likely to be found in areas in close proximity to the shoreline compared to distances further inland. Our findings indicate that areas around the shoreline do not have higher numbers of adult malaria mosquitoes than those further away. It is important to interpret this finding with caution as the density of adult malaria mosquito densities during the study was low. The finding of no difference in the densities of malaria mosquitoes at several distances from the shoreline differs from that of other studies conducted in western Kenya. Distance from breeding sites has been shown to influence distribution of malaria vectors. In a study conducted by Zhou *et al*. (2004) in areas around the Yala river in western Kenya, malaria mosquitoes were abundant in houses located in <200m from the river compared to houses found 600 – 800m, and 1200 – 1400m. This was attributed to the high number of breeding sites found along river Yala. A study conducted by Minakawa *et al*. (2002) revealed that distance of a house from breeding sites was significantly associated with abundance of adult *An. gambiae* mosquitoes in houses in Mbita district. In The Gambia, higher densities of *An. gambiae* mosquitoes were found in houses close to the flood plain of river Gambia (Lindsay *et al.*, 1993b).

The study reported herein demonstrates that the risk of malaria on Rusinga is not associated with the distance of an individuals' house from the shoreline. However, the sample of malaria mosquitoes collected in this study do not provide enough evidence to certify this assertion. The fact that many breeding sites are located along the shores of Lake Victoria does not imply an increase in mosquito biting activity. It is possible that malaria mosquitoes on Rusinga fly away from the shoreline up to distances of 2km in search of humans. Malaria mosquitoes are known to fly up to distances of below 3km in search of blood meal hosts (Kaufmann and Briegel, 2004). It is important to note that breeding sites were not identified in the study reported herein. The finding that no significant difference in the density of malaria vectors captured at various distances from the shoreline could also be explained by the few numbers of mosquitoes captured in the study area. A recent report by Futami *et al*. (2014) revealed a stunning reduction in densities of malaria vectors in the study area. However, studies have shown that high malaria transmission can be sustained by small vector populations (Trung *et al.*, 2004).

Though it is often assumed that malaria burden is associated with large water bodies, this was not the case in our study. No significant association in malaria infection and individuals living at various distances from the lake's shoreline was observed in this study. These water bodies are often presumed to support high numbers of malaria vectors which eventually results in an increase in malaria cases. It has been shown that the distance of houses from breeding sites is significantly associated with the risk of malaria (Gunawardena *et al.*, 1998; Staedke *et al.*, 2003). These results are similar to those obtained by Yewhalaw *et al*. (2013) in Ethiopia where malaria incidence was not significantly associated with distance from a dam. In contrast, residing in close proximity to river Yala was found to be significantly associated with *Plasmodium* infection among individuals in western Kenya (Munyekenye *et al.*, 2005). Individuals living within 100m from fish ponds in Cote d'Ivoire were at a higher risk of malaria compared to their counterparts who lived more than 500m from the ponds (Matthys *et al.*, 2006). Another study reported that malaria incidence was significantly correlated to distance of a large water body (Lautze *et al.*, 2007) with the highest infection rates observed among people living in close proximity to a dam. In the present study area, fishermen were not at risk of malaria infection because of the distance of their house from the Lake Victoria shoreline.

Malaria prevalence was significantly associated with school-going children aged 5 to 14 years when compared to adults. This finding is supported by results from a study conducted by Manoti (2013) who reported that malaria prevalence in the study area was highest among children between 5 – 14 years. Malaria has been shown to be highest among school children (Smith *et al.*, 2007; Brooker *et al.*, 2009) because of a decline in the *Plasmodium falciparum* parasite rate in children as they develop immunity while they mature into adulthood (Baird *et al.*, 1993; Baird, 1995).

The risk of malaria on Rusinga Island is not associated with the distance of a house from the lake, but rather on the time that individuals are found outdoors at night. Residents of Rusinga live in an area characterized by stable malaria transmission coupled with low malaria mosquito densities. Indoor vector control tools are the only interventions currently used to reduce malaria transmission in the area (Futami *et al.*, 2014). However, malaria transmission may be sustained in the area because of outdoor capture fishing activities.

4.6 Conclusion

The overlap between mosquito biting activity and human activity at the shoreline may increase the risk of malaria transmission outdoors around peak biting times. The study did not show a significant difference in mosquito densities at various distances from the shoreline. Similarly, no association was found between malaria infection and distance from the shoreline.

CHAPTER 5: CHARACTERIZING MALARIA PREVALENCE IN OUTPATIENTS OF HEALTH FACILITIES IN RUSINGA ISLAND, WESTERN KENYA

5.1 BACKGROUND

Despite intense efforts by the Government of Kenya through its National malaria control program to control malaria, the disease is still a public health problem in Rusinga Island and many parts of Kenya (DOMC, 2014). Government and non-governmental programmes, consistent with global malaria eradication campaigns, have intensified the distribution of and promoted civic education for the use of LLINs for malaria prevention. It is therefore expected that malaria cases should have in general reduced consistent with the global decline in malaria cases and mortality (Gething *et al.*, 2011). However, malaria transmission is a complex of many factors including but not limited to the competence and ecology of vectors in a given area, the uptake of effective interventions, and the demographic characteristics of a population (Kelly-Hope and McKenzie, 2009). It is also possible therefore that in different eco-epidemiological settings within the country, the incidence and prevalence of malaria is secondarily affected by local socio-economic factors that determine the risk for malaria transmission within different groups of people.

Rusinga is an island in the Homabay County of Western Kenya where rudimentary fishing and small scale farming constitute the major sources of livelihood (Opiyo *et al.*, 2007). Both activities are done close to the lake shore and are often associated with long periods out of door in the night during the peak biting period of local malaria vectors. For this reason, it is possible that even though bednet use is extensive in the island, the majority of farmers and fishermen are still exposed to malaria and might act as reservoirs for the disease parasites. After all, high malaria prevalence is not uncommon in individuals that engage in outdoor and nocturnal livelihood activities. Rice farmers in Cote d'Ivoire (Assi *et al.*, 2013) and fishing communities in Uganda (Woodburn *et al.*, 2009) have been demonstrated to be at a higher risk of malaria than people from the same community whose livelihood engagements are more indoor and diurnal. Rubber farmers in Thailand (Pattanasin *et al.*, 2012), military personnel (Tuck *et al.*, 2003a), and fishermen in Colombia pacific (Escovar *et al.*, 2013) all who work at night during peak mosquito biting times and have a higher prevalence for malaria than the general community.

In the least developed countries, fever is the main reason why people seek health care (Petit and van Ginneken, 1995; Feikin *et al.*, 2011). For decades clinical symptom have been used to diagnose malaria (Font *et al.*, 2001; Othnigue *et al.*, 2006). This has led to over-diagnosis of malaria, over-prescription of malaria drugs, and misdiagnosis and inappropriate treatment of non-malarial fevers (Reyburn *et al.*, 2004; Nankabirwa *et al.*, 2009) especially since a reduction in the proportion of fever cases caused by malaria has been observed in the recent past (D'Acremont *et al.*, 2010). Now it is known that the etiology of fever cannot be determined by clinical diagnosis alone (Crump *et al.*, 2011a; Crump *et al.*, 2011b) and the WHO has consequently recommended parasitological evidence as mandatory diagnostic test for malaria without which malaria treatment should not be administered regardless of malaria endemicity. Parasitological evidence is now a prerequisite to malaria treatment in Kenya: patients who present with fever are diagnosed for malaria using Rapid Diagnostic Tests (RDTs) or microscopy before treatment (MOPHS and MMS, 2010).

Data collected in health facilities can be useful in determining the burden of malaria (Cibulskis *et al.*, 2007), detecting pockets of high malaria transmission (Bousema *et al.*, 2010) and monitoring the coverage of malaria control interventions (Abdulla *et al.*, 2002; Skarbinski *et al.*, 2008). Data on malaria cases collected in this manner has the advantage of instant availability for individuals who are interested in malaria control (Shiff *et al.*, 2013). This study therefore sought to characterize malaria endemicity in Rusinga Island using health facility-based malaria survey to test the hypotheses that persons involved in outdoor and nocturnal livelihood activities in Rusinga island constitute the majority of malaria cases and could be asymptomatic harbors' (reservoirs) of the disease. Specifically, the study aimed to i) categorize patients who visited Government and private health facilities with different demographic indices, ii) determine the prevalence of different malaria parasites species in patients visiting health facilities, iii) quantify non-malaria fevers, and iv) determine the association between malaria and occupation.

5.2 METHODOLOGY

5.2.1 Study area

The study was conducted in Rusinga Island, located in Mbita sub-county in western Kenya. The main malaria control methods used on the Island include Long Lasting Insecticidal Nets (LLINs) and prompt treatment of malaria. The Island has five health facilities. Three are owned and run by the Government of Kenya and two are sponsored privately by the Slovakian

Republic and individuals from the U.S.A. The Government health facilities include Tom Mboya Health Centre, Kamasengre Dispensary and Waware Dispensary. The two privately owned health facilities are Humanist Healthcare Centre and Rusinga Island Trust (Kageno) Dispensary. Tom Mboya Health Centre is the largest health facility on Rusinga Island. The health facilities located in Rusinga West sub-location include Kamasengre Dispensary, Tom Mboya Health Centre, and Rusinga Island Trust (Kageno) Dispensary. Waware Dispensary and Humanist Healthcare Centre are found in Rusinga East sub-location. The health facilities are staffed by clinical officers, nurses, laboratory technologists and support staff.

5.2.2 Health facility selection and recruitment of participants

A health facility-based malaria survey was conducted in four health facilities on Rusinga. The four reference health facilities included Tom Mboya Health Centre, Waware Dispensary, Rusinga Island Trust (Kageno) Dispensary and Humanist Healthcare Centre. Health facilities with a history of high attendance were chosen for this study of which two were Government-owned (Tom Mboya Health Centre and Waware Dispensary) and two private (Rusinga Island Trust Kageno dispensary and Humanist Healthcare Centre). Kamasengre Dispensary was not selected because it had very low attendance rates. Individuals seeking outpatient services between February and August 2013 were recruited into the study. All outpatient visitors were illegible to participate in the study except individuals who accompanied patients to the health facilities or guardians of children. Two research assistants collected data during workdays. Each assistant was assigned two health facilities which they visited daily on a rotational basis.

5.2.3 Malaria diagnosis

Prior to collection of blood samples, the axillary body temperature was measured using ear thermometers. Fever was defined as a body temperature of >37.5°C. Symptoms of ill health as narrated by the patient were recorded. A finger prick was used to obtain blood samples from participants. Blood smears were made on microscopic slides, air dried, and stained with 10% Giemsa's for 10 minutes. Blood smears were examined under oil immersion for presence of malaria parasites. The stained slides were initially examined by an experienced microscopist who recorded malaria positivity, malaria species identity, malaria parasite density and presence of gametocytes of malaria parasites. The slides were later subjected to a quality assurance examination by a second independent and experienced microscopist whose examination was recorded as the final result. A slide was categorized as negative if no parasites were observed after examining 100 fields.

5.2.3.1 Definition of malaria infection

Patients were diagnosed with malaria infection if they had a history of fever within the last 24 hours, had a body temperature above 37.5ºC during the health facility visit, presented with headache, joint pains, vomiting among other common malaria symptoms, and their blood smears showed positive results for either *P. falciparum* or *P. malariae*. Individuals found with malaria were treated according to the National guidelines (MOPHS and MMS, 2010).

5.2.4 Demographic characteristics of study participants

The number of patients seeking outpatient services from Government and private health facilities was recorded. A questionnaire was administered in the local language (Luo) to individuals who participated in the study. Demographic characteristics the participants including sex, age, occupation and area of residence was recorded. Data on the use of a treated bed net was also collected. Due to the wide range of livelihood activities conducted by participants, the demographic characteristic occupation was categorized by type of job. This included blue, pink and white collar jobs. Blue collar jobs are those that involve manual labour. For purposes of this thesis, we excluded fishermen and farmers from the list of manual labourers. Pink collar jobs are those that are traditionally performed by women and are related to customer interaction, and any other service-oriented work. White collar jobs are those performed in an office or administrative setting.

5.2.5 Prevalence of malaria and parasite species among patients in health facilities

The prevalence of malaria, calculated as the proportion of individuals who tested positive for malaria out of the total number of people tested, was recorded in each health facility. The number of people infected with single *Plasmodium* species infection and those with mixed species infections was determined. Malaria positivity rates between patients visiting Government and private health facilities were compared.

5.2.6 Proportion of patients presenting non-malaria fevers

Clinical symptoms as narrated by patients were recorded. The proportion of individuals presenting with fever but who did not have patent malaria parasitaemia was determined.

5.2.7 Association between malaria and occupation

The administered questionnaire included questions on occupation. The participants' occupation was only recorded if the individual was an adult. The relationship between malaria infection and a participants' occupation were determined.

5.2.10 Ethical considerations

Consent forms were signed by prospective study subjects before participation in the study. Informed consent was obtained from parents or guardians who accompanied children to a health facility. The study was approved by the Ethical Review Committee of the Kenya Medical Research Institute (KEMRI). Malaria tests and interviews were conducted after a consent form was signed by a participant. Free malaria medicine (artemether-lumefantrine) was provided to individuals found positive for malaria.

5.2.11 Statistical analysis

Statistical analysis was performed using R software (version 2.15.3). Descriptive statistics were used to analyze demographic characteristics of patients. The relationship between malaria infection and demographic characteristics and bed net use was determined by logistic regression. The association between symptoms among malaria positive and negative individuals was also determined using logistic regression.

5.3 RESULTS

5.3.1 Demographic characteristics of study participants

A total of 1644 patients 1023 (62.2%) of whom visited Government health facilities and 621 (37.8%) accessed the private ones were recruited in the study (Table 6). The patients consisted of 940 (57.2%) females and 704 (42.9%) males. Majority of the patients were children aged 0 to 4 years old (26.1%) while the least number were from the age group above 45 years old (7.2%). The mean age of individuals who attended Government health facilities were 15.4 years and 18.1 years among patients visiting private facilities. Of the total patients recruited into the study, 1215 (66%) were from Rusinga West while 429 (34%) were from Rusinga East sub locations. Among the study subjects, 42.8% (n = 704) were students, 23% (n = 378 were non-school going children, 9% (n = 148) were fishermen, 8.6% (n = 141) were traders, and 3.1% (n = 51) were farmers.

Of the 562 individuals listed with an occupation, fishermen and traders had the highest health facility attendance. Out of 562 adults, 73 (13%) fishermen visited Government health facilities while 74 (13.2%) accessed private facilities. Of the 562 individuals who had an occupation, 94 (16.7%) involved in trading activities attended Government health facilities while 47 (8.4%) visited private facilities. Of the 562 adults that enrolled in the study, 87 (15.5%) unemployed individuals visited Government health facilities while 28 (5%) attended Private facilities. Of the total number of patients who visited the health facilities, 1402 (85.2%) slept under a treated bednet of which 871 (62.1%) visited Government health facilities while 531 (37.8%) visited private facilities.

Table 6: Characteristics of participants with respect to health provider

Variable	Government health facilities	Private health facilities
Number of patients (n = 1644)	1023 (62.2%)	621 (37.8%)
Sex (n = 1644)		
Female	590 (35.9%)	350 (21.3%)
Male	433 (26.3%)	271 (16.5%)
Age group (n = 1644)		
0 – 4 years	297 (18.1%)	132 (8%)
5 – 14 years	333 (20%)	221 (13.4%)
15 – 29 years	236 (14.3%)	145 (8.8%)
30 – 44 years	87 (5.2%)	73 (4.4%)
Above 45 years	70 (4.2%)	50 (3%)
Mean age (years)	15.41	18.11
Occupation (n = 562)		
Blue collar job (Manual labour jobs e.g., construction worker)	20 (3.6%)	19 (3.4%)
Farmer	21 (3.7%)	31 (5.5%)
Fisherman	73 (13%)	74 (13.2%)
Unemployed	87 (15.5%)	28 (5%)
Pink collar job (Service industry jobs e.g., Hairdresser)	7 (1.2%)	12 (2.1%)
Trader	94 (16.7%)	47 (8.4%)
White collar job (Office jobs e.g., Administrator,	31 (5.5%)	18 (3.2%)
Area of residence (n = 1644)		
Rusinga East	397 (24.1)	42 (2.6%)
Rusinga West	626 (30.8)	579 (35.2%)
Slept under treated bednet (n=1402)	871 (62.1%)	531 (37.8%)

Percentages are in brackets.

5.3.2 Prevalence of malaria and parasite species among patients in health facilities

The prevalence of malaria among individuals attending outpatient services in Humanist Healthcare Centre was 42%, Waware Dispensary was 42%, Tom Mboya Health Centre was

36%, and Rusinga Island Trust (Kageno) was 31%. Majority of the patients sought treatment from Government health facilities. The prevalence among patients who visited Government health facilities was 38.3% while those who accessed private ones was 41.2% (Table 7). There was no significant difference in malaria positivity rates between the two types of health facilities (p = 0.243). Among patients with malaria, 631 (97.4%) were caused by *P. falciparum*, 9 (1.3%) by *P. malariae*, and 8 (1.2%) were mixed infections of *P. falciparum* and *P. malariae*.

Table 7: Distribution of malaria cases and species with respect to type of health facility

Health facility type	**Number examined**	**Malaria cases**	**Species distribution**			**Prevalence**	**p value**
			P.f.	*P.m*	*P.f. + P.m.*		
Government	1023	392	380	6	5	38.3%	ref
Private	621	256	251	3	3	41.2%	0.243
Total	1644	648	631	9	8		

P.f. refers to *Plasmodium falciparum* and *P.m.* to *Plasmodium malariae*

5.3.3 Proportion of patients presenting non-malaria fevers

The proportion of febrile and afebrile patients with or without confirmed malaria is presented in Table 8. Approximately 34.5% of all fever episodes were not associated with malaria parasitemia. Fever (p<0.001) were significantly associated with malaria infection. Of the total number of patients recruited during the study period, 424 (25.8%) had malaria but did not present fever (Table 8). The number of afebrile malaria positive individuals (25.8%) was higher compared to their malaria positive febrile counterparts (13.6%), (p < 0.001).

Table 8: Calculation of the proportion of malaria and non-malaria fevers among patients attending health facilities on Rusinga

Symptom	Malaria -ve	Malaria +ve	p-value
Afebrile	878 (53.4%)	424 (25.8%)	ref
Febrile	118 (7.2%)	224 (13.6%)	<0.001

5.3.4 Association between malaria and occupation

Compared to individuals who are jobless, malaria was significantly associated with those involved in farming (p = 0.003) and fishing (p = 0.031) activities. No association between malaria and persons engaged in blue collar (p = 0.063), pink collar (p = 0.626), white collar (p = 0.588) and trading (0.319) was observed.

Table 9: Relationship between malaria and occupation among patients visiting health facilities on Rusinga Island

Variable	OR (95% CI)	p value
Occupation		
Unemployed	ref	ref
Blue collar jobs	2.16 (0.94 - 4.89)	0.063
Farming	2.93 (1.41 - 6.13)	0.003
Fishing	1.91 (1.07 - 3.50)	0.031
Pink collar jobs	0.72 (0.15 - 2.38)	0.626
Trading	1.36 (0.74 - 2.54)	0.319
White collar jobs	1.25 (0.53 - 2.81)	0.588

5.4 DISCUSSION

Despite intense control activities conducted by the National malaria control programme and considerable high bednet use (up to 62.1%), our study confirmed that malaria was a highly prevalent disease among individuals seeking outpatient services at health facilities in Rusinga Island with an upward of 38.3% of outpatient visits because of the disease. The majority of the Rusinga population was found to prefer government health facilities compared to privately owned health facility (62.2%). The adult patient population consisted majorly of unemployed (87%) the vast majority who went to government-facilities. Of all occupations, fishermen and farmers had the highest odds of malaria infection (1.91 and 2.93 respectively).

This study consistent with past studies, and government malaria reports shows malaria to be one of the major reasons for the largest proportion of health facility visits (DOMC, 2011). Gouagna and others (2003), more than a decade ago in a health facility-based study done in Mbita sub-county which Rusinga Island is part, showed similar results. The National malaria control programme recently reported that malaria accounted for 34% of outpatient health facility visits in the whole of Kenya (DOMC, 2011). According to the World Health Organization (2003a), malaria accounts for 25 to 40% of outpatient health facility visits in malaria-endemic countries in Africa. This suggests that malaria is still the major public health problem in Rusinga with significant economic burden on the health system. Additional strategies of its control are thus necessitated in the area.

Our study showed greater utilization of outpatient services in Government health facilities than private ones. This is probably influenced by the lower cost of medicare in government compared to private facilities. Individuals who visit government health facilities pay approximately Ksh. 120 to access outpatient services while those who visit private health facilities averagely pay Ksh. 1,000. Service charges have been shown to affect the choice of health care provider by individuals in poor households (Mbugua *et al.*, 1995). Individuals who visited private health facilities were probably those who could afford them as suggested in many studies (Uzochukwu and Onwujekwe, 2004; Chaturvedi *et al.*, 2009; Mebratie *et al.*, 2014). Notably, the vast majority of patients in the private clinics in Rusinga were fishermen. The average weekly income of a fisherman who harvests fish from Lake Victoria is Ksh. 7,750 (Omwega *et al.*, 2006) which much higher than the minimum monthly wage of Ksh. 5,436 earned by an unskilled labourer in the agricultural industry (KNBS, 2015). Fishermen could therefore comfortably afford to pay for services offered in private health facilities.

The prevalence of malaria among patients seeking outpatient services in the current study was recorded as 42% in Humanist Healthcare Centre, 42% in Waware Dispensary, 36% in Tom Mboya Health Centre and 31% in Rusinga Island Trust (Kageno). The prevalence reported in this study is much higher than the population-based cross-sectional malaria prevalence reported in the same study site (Olanga *et al.*, 2015). These results are slightly lower compared to that of Mutero *et al*. (1998) who conducted a similar study in a nearby health facility within Mbita sub-county and found that in 1995 and 1996 in Mbita health centre (currently called Mbita sub-county Hospital) and Sindo hospital ranged from 42% to 48%. This represents progress for malaria control in the area. However, the results must be interpreted with caution since data collected from the four health facilities in Rusinga Island are approximately 15 kilometres from Mbita sub-county Hospital where Mutero and others (1998) collected their data. In addition, their finding was based on hospital records of Mbita and Sindo hospitals and was biased to symptomatic malaria rather than parasitological confirmation. It is interesting to note that over a period of 18 years, malaria decline in health facilities in Mbita sub-county has not been as dramatic as implied for the global prevalence of the disease. One must wonder whether the behavior of residents of the area especially since the bednet use in the study area (37.8 - 62.1%) is well within the global average of 44%.

In this study, fishing and farming were associated with high likelihood of outpatients having malarial parasites. These two activities are conducted outdoors usually at a time when mosquitoes actively engage in host seeking. This might explain the high infection rate. Fishing is the main livelihood activity conducted in the study area (Opiyo *et al.*, 2007). Fishermen also constitute the highest number of outpatient visits in health facilities. In previous studies in Colombia, Escovar *et al.* (2013) showed that fishermen who harvest fish by the river at night are at risk of malaria because their activities coincide with peak malaria mosquito biting times. A study conducted in Botswana similarly showed an association between malaria and individuals who conduct late outdoor activities (Chirebvu *et al.*, 2014). Farming on Rusinga Island is mainly for subsistence purposes and is usually conducted very early in the morning to avoid the hot sun. The primary malaria vector on Rusinga Island is *An. arabiensis* (Futami *et al.*, 2014), a vector that preferentially bites hosts outdoors and bites humans and animals depending on availability of hosts (Muriu *et al.*, 2008). Individuals who conduct livelihood activities outdoor and at night or in the early morning hours on Rusinga Island are thus be exposed to outdoor malaria transmission. Alternative methods for protecting such people are a necessary step towards malaria elimination.

This study recorded a large number of febrile patients who did not test positive for malaria parasites. This adds evidence that fever is not necessarily an accurate diagnosis for malaria parasites and against presumptive treatment of the diseases. A proportional increase in non-malaria fevers has been reported in areas where malaria control has been effective (D'Acremont *et al.*, 2010; D’Acremont and Bosman, 2013). The emphasis by the government and WHO on confirmatory parasitological testing is thus commendable as it not only reduces the risk resistance to antimalarials but also antibiotics (D’Acremont and Bosman, 2013).

This study was limited by potential biases in parasite diagnosis stemming from microscopy being used as the only diagnosis tool for malaria. The accuracy of parasite prevalence studies in a population is known to be influenced by the choice of a diagnostic tests (Tusting *et al.*, 2014). Moreover, the results could have been biased by the experience of individual microscopists. Microscopy is also limited to infections of between 50 – 100 parasites per µl (Kilian *et al.*, 2000) unlike molecular methods e.g., PCR that are relatively more sensitive to lower infection levels detecting extremely low numbers of parasites.

5.5 Conclusion

Majority of individuals on Rusinga Island sought outpatient services from Government health facilities. The prevalence of malaria among individuals attending Government facilities was not significantly different from those in private facilities. Approximately a third of all fever episodes were not associated with malaria parasites. This study demonstrated an association between malaria and individuals involved in fishing and farming activities. There is a need to develop vector control tools that protect individuals who work outdoors at night from outdoor malaria transmission.

CHAPTER 6: FACTORS DETERMINING MALARIA RISK AMONG FISHERMEN OF RUSINGA ISLAND, WESTERN KENYA

6.1 BACKGROUND

Malaria is a public health problem in western Kenya (DOMC, 2011). The disease is endemic in areas around the shores of Lake Victoria (Mutero *et al.*, 1998; Noor *et al.*, 2009; Manoti, 2013). The predominant malaria vectors in the region include *Anopheles gambiae*, *An. arabiensis* and *An. funestus* (Minakawa *et al.*, 2002; Shililu *et al.*, 2003; Fillinger *et al.*, 2004; Futami *et al.*, 2014). The current malaria vector control tools used in western Kenya include Long Lasting Insecticidal Nets (LLINs) and Indoor Residual Spraying (IRS). These strategies are used to protect individuals against malaria by targeting indoor resting and feeding mosquitoes and not outdoor biting mosquitoes (Durnez and Coosemans, 2013). *Anopheles arabiensis* is the main vector reported in Mbita District (Minakawa *et al.*, 1999; Minakawa *et al.*, 2012; Futami *et al.*, 2014) and is described as opportunistic, as it feeds on man or animals depending on availability (White, 1974; Muriu *et al.*, 2008).

Individuals who reside in areas around Lake Victoria largely depend on the environment for their livelihoods. Artisanal fishing is a major livelihood activity carried out among rural communities that live in these areas. The activity is conducted both inshore (around the shoreline) and offshore (further into the lake) depending on availability of fish species. Inshore fishing is usually conducted by fishermen harvesting fish using hooks and nets. Fishing nets are used in beach seining which involves pulling nets placed in a U-pattern ashore. Fish found within the netted area are captured and drawn ashore. Female fishermen in western Kenya mostly engage in beach seining compared to other forms of fishing that require sailing into the lake. Seine fishing using canoes is also practiced in rural fishing communities. Offshore fishing is mainly conducted by men in canoes equipped with nets and hooks.

Several fishery activities have been posited to aggravate malaria on Rusinga Island in western Kenya. Fishermen require bait to effectively attract and capture fish. Earthworms are commonly used as bait in capture fisheries which motivates individuals to dig into soil leaving holes in search of earthworms in swampy areas close to the lake shore. These holes eventually fill up with water and form breeding sites for malaria mosquitoes (Mukabana, personal communication). Construction of fingerponds to retain water and trap fish (Bailey *et al.*, 2005; Kipkemboi *et al.*, 2006; Van Dam *et al.*, 2006) also promotes mosquito breeding. These ponds are constructed by digging depressions inland near the shore to achieve slow and continuous entry and exit of water to trap fish (Bailey *et al.*, 2005).

In theory people who engage in outdoor activities at night are at higher risk of contracting malaria partly because (i) local vectors in Africa mostly bite at night (White, 1974), (ii) this enhances contact with vectors that prefer to feed outdoors (Durnez and Coosemans, 2013), and (iii) they are not protected by indoor interventions, specifically LLINs (Killeen, 2014). Fishing activities are carried out outdoors partly during the day and predominantly at night. The latter presents an occupational risk among fishermen as it predisposes them to potentially infectious mosquito bites. In addition, a study conducted by Weckenbrock (2005) in a fishing village in Rusinga Island revealed that many breeding sites containing *Anopheles* larvae were found in areas around the lake Victoria shoreline even during the dry season. The present study sought to determine whether demographic characteristics, housing characteristics, environmental factors, occupational factors, population movement and use of malaria personal protection measures are potential drivers of malaria morbidity among the fishermen on Rusinga Island.

6.2 METHODOLOGY

6.2.1 Study site

The study was conducted on Rusinga Island, Homabay County in western Kenya. The residents of Rusinga are involved in two main livelihood activities namely artisanal capture fishing and small-scale trading (Opiyo *et al.*, 2007). Other livelihood activities for instance farming, boat making, and net weaving, occur on a smaller scale.

Fishing is an activity conducted all year round for both subsistence and commercial purposes. This livelihood activity is conducted throughout the day and night. Different fish species are harvested in Lake Victoria but three are of commercial importance. These are tilapia (*Oreochromis niloticus*), nile perch (*Lates niloticus*) and silver fish (*Rastrineobola argentea*). Fishing is conducted both inshore and offshore depending on availability of fish species. Artisanal fishing is mainly conducted by men while fishing-related activities like fish processing is conducted by women. The predominant language spoken in the area is dholuo. Rusinga residents mainly live in traditional houses constructed with mud walls and iron sheet roofs and have open eaves.

6.2.2 Recruitment of participants

The fishermen were recruited from three fishing beaches randomly chosen from a list of beaches. The three fishing beaches included Kaswanga, Luanda Rombo and Uta. Lists of fishermen registered at these beaches were obtained from the Beach Management Units (BMUs). In September and October, 2013, fishermen randomly selected (using random computer generated numbers) from the list were enrolled in the study. Participants were requested to show up on specific days at the fishing beaches where they were tested for malaria and interviewed.

6.2.3 Sample size

The Sample size was estimated using the formula for prevalence surveys (Naing *et al.*, 2006). The prevalence was reported as 10.9% in a population-based malaria survey previously conducted in the same area (Chapter 3). Values assumed in the formula include 95% confidence interval (CI) level ($Z = 1.96$), and 5% level of significance ($d = 0.05$). The estimated sample size required for the malaria survey was 149 participants.

6.2.4 Malaria diagnosis

A cross-sectional malaria survey was conducted among fishermen. Malaria cases were diagnosed using active case detection. A blood sample was collected from each consenting individual by a finger prick in order to test for presence of malaria parasites. Presence of infection was determined using a rapid diagnostic test (RDT) kit (SD Bioline Malaria Pf/Pan®, Korea). The RDT kit contains antibodies specific to *Plasmodium falciparum* and other *Plasmodium* species (*P. vivax*, *P. ovale* and *P. malariae*). Each individual's body temperature was measured and fever was defined as temperature above 37.5ºC. A participant was classified as positive for malaria based on the RDT results regardless of whether they were febrile or not. Individuals tested positive for malaria were provided with one full doze of artemether-lumefantrine (AL) consistent with the National malaria treatment guidelines (MOPHS and MMS, 2010).

6.2.5 Characteristics of respondents and distribution of malaria parasites

Demographic data specifically age, sex, area of residence and occupation were recorded. Malaria prevalence was calculated as the proportion of participants found with malaria infection out of the total number tested for malaria.

6.2.6 Risk factors for malaria infection

A structured questionnaire was verbally administered in Luo, the predominant language in the area. The questionnaire included questions on demographic variables, housing characteristics, environmental risk factors, occupational variables, population movement and malaria personal protection measures. Demographic questions included age, sex and type of fisherman. One housing characteristic namely type of house was investigated in relation to malaria risk. A house was classified as make-shift if its walls and roof were made of iron sheet. A house with walls made of mud was categorized as semi-permanent while one with walls made of bricks or stone was categorized as permanent. Data was collected on environmental factors, specifically distance from participants' house to lakeshore and location of fishing beach. Occupational variables included time of work, use of light while fishing, distance of work area from the shoreline, and frequency of fishing. The distance of work area from the shoreline was determined by the type of artisanal fishery conducted on Rusinga Island characterized as inshore or offshore. Inshore fishing (also known as fishing in shallow waters) describes fishing that is conducted close to the shores, a distance of approximately 0 – 300 metres. Offshore fishing (fishing in deep waters) refers to fishing conducted further from the shore, in this study we considered it to be beyond 300 metres from the shoreline. Two categories were created under frequency of fishing variable, specifically regular and irregular. Regular fishing was used to describe fishing that was conducted at regular intervals, between five to seven times a week while irregular fishing applied to fishing carried out intermittently. One variable under population movement was investigated; specifically travel to other fishing beaches. Parameters recorded under personal mosquito protection measures included use of bed nets, frequency of their usage and mode of dress while at work.

6.2.7 Ethical considerations

Participants enrolled in the study at their own will and were well informed about the study. A consent form was signed prior to data collection. The study was approved by the Ethical Review Committee of the Kenya Medical Research Institution (NON-SSC No. 280).

6.2.8 Data analysis

Data were analyzed using R statistical software version 2.15.2. Univariate and multivariate logistic regression was used to identify factors associated with malaria. The outcome of the malaria test (positive or negative) was identified as the dependent variable. Potential risk factors (demographic factors, housing and occupational characteristics, population movement and malaria prevention measures) were classified as independent variables. Univariate analysis using logistic regression was performed on each independent variable. Odds Ratios (OR), 95% Confidence intervals (95% CI) and p values (P) were obtained. Statistical significance was set at $p<0.2$. Variables with probability values less than 0.2 were advanced to the multivariate analysis. The individual effect of variables on malaria was analyzed while controlling for other variables in the multivariate analysis. Combinations of two-way interaction terms were assessed. Probability values less than 0.05 were considered as statistically significant.

6.3 RESULTS

6.3.1 Demographic characteristics of recruited participants

A total of 152 people were recruited into the study, 105 (69%) were male and 47 (31%) were female (Table 10). Ninety two (60.5%) of the respondents were aged 15 to 34 years while 60 (39.5%) were above 35 years old. Majority of the participants were married (73.7%). The remaining were either single (19%), widowed (6.6%) or divorced (0.7%). Ninety four percent of fishermen interviewed were from the Luo tribe. Majority of the fishermen (60.5%) recruited into the study had attained a secondary school level of education. With respect to type of fisherman, the livelihoods of 42 (27.6%), 51 (33.7%), and 59 (38.8%) individuals were dependent on capturing tilapia, nile perch, and silver fish, respectively.

6.3.2. Malaria diagnosis

Of the total number of people tested, only 16 (10.5%) were found with malaria. *Plasmodium falciparum* was responsible for 87.5% (14) of infections while 12.5% (2) were mixed infections. There were no reports of severe malaria among the study participants. Among the 16 fishermen found with malaria parasites, 15 (93.8%) were afebrile.

Table 10: Demographic characteristics of the respondents

Variable	**n (%)**
Age group	
15 – 34	92 (60.5%)
Above 35	60 (39.5%)
Sex	
Female	47 (31%)
Male	105 (69%)
Marital status	
Single	29 (19%)
Married	112 (73.7%)
Divorced	1 (0.7%)
Widowed	10 (6.6%)
Tribe	
Borana	1 (0.7%)
Kikuyu	2 (1.3%)
Luhya	3 (1.9%)
Luo	144 (94.7%)
Maasai	1 (0.7%)
Nandi	1 (0.7%)
Education	
Never attended school	8 (5.3%)
Primary	92 (60.5%)
Secondary	46 (30.2%)
College	6 (4%)
Type of fisherman	
Tilapia fisherman	59 (38.8%)
Silver fish fisherman	42 (27.6%)
Nile perch fisherman	51 (33.7%)

6.3.3 Risk factors for malaria infection among fishermen

Potential malaria risk factors were classified into six groups of variables namely demographics, housing characteristics, environmental factors, occupational characteristics, population movement and malaria protection measures.

Based on the univariate analysis, there was no association between malaria and age. There was no significant difference observed between fishermen aged 15 – 34 years and those above 35 years of age (OR = 0.991, 95% CI 0.29 – 2.06, p = 0.864) in relation to malaria infection. Fishermen who harvest Silverfish (OR = 1.236, 95% CI 0.27 – 5.54, p = 0.774) and nile perch (OR = 1.834, 95% CI 0.54 – 7.27, p = 0.343) were not significantly associated with malaria compared to those who harvest tilapia (Table 11). No significant difference was observed between women and men (OR = 0.719, 95% CI 0.24 – 2.34, p = 0.549) in relation to *Plasmodium* infection. In this study, malaria infection was not significantly related to fishermen who live in semi-permanent houses (OR = 2.10, 95% CI 0.28 – 7.81, p = 0.857) and make-shift structures (OR = 1.15, 95% CI 0.31 – 17.47, p = 0.445) when compared to those who live in houses constructed with permanent materials.

Two environmental risk factors were tested for association with malaria infection. Location of the fishing beach was not significantly associated with malaria parasitemia. Compared to fishermen who live in Kaswanga beach, their counterparts who lived in Luanda Rombo (OR = 1.72, 95% CI 0.36 – 0.17, p = 0.49) and Uta beaches (OR = 2.82, 95% CI 0.78 – 13.29, p = 0.136) were not at a higher risk of malaria. Malaria mosquito breeding habitats have been shown to be located near the Lake Victoria shoreline in Mbita district (Weckenbrock, 2005; Minakawa *et al.*, 2008b; Minakawa *et al.*, 2012). Fishermen mainly live in houses found in clusters located at the fishing beaches within a distance of 500m from the shoreline. In this study, the distance of the respondents' house from the shoreline was not statistically significant for malaria infection (OR = 1, 95% CI 0.34 – 3.09, p = 1) as an equal proportion of individuals living at 500 metres and more than 500 metres from the shoreline were found infected with malaria.

Under occupational characteristics, no significant difference was observed between fishermen who work during the day or night (OR = 0.873, 95% CI 0.30 – 2.57, p = 0.779) in relation to *Plasmodium* infection. Usage of light during fishing was also not significantly associated with malaria (OR = 0.661, 95% CI 0.16 – 1.86, p = 0.415) when compared to non-usage. Distance of work area from the shoreline and frequency of fishing activities showed no statistically significant association with the presence of malaria parasitemia (Table 11).

Travel was identified as a significant risk factor for malaria. At the 0.2 level, there was a statistical difference of malaria infection among fishermen who did not migrate to other fishing beaches than those who migrated (OR = 3.96, 95% CI 1.11 – 12.71, p = 0.122). In the multivariate analysis, fishermen who travelled out of their fishing beaches to others were three times more likely to get malaria compared to their counterparts who did not travel (95% CI 0.96 – 12.03, p = 0.047) (Table 12).

Based on the univariate analysis, there was no statistically significant difference in malaria infection among fishermen who used bednets as a personal malaria protection measure than those who did not (OR = 0.38, 95% CI 0.05 – 1.45, p = 0.218). Similarly, frequency of bednet usage was not significantly associated with malaria (Table 11). At the $p<0.2$ level, a significant association was observed between malaria infection and fishermen who exposed their arms and legs while working (OR = 2.74, 95% CI 0.90 – 10.21, p = 0.093) compared to those who covered their arms and legs. In the multivariate analysis the risk of contracting malaria was three-fold among fishermen who conducted fishing activities in clothes that exposed their arms and legs (CI 95% 1.11 – 14.23, p = 0.045) compared to those who dressed in long sleeved clothes after controlling for travel to other fishing beaches.

Table 11: Univariate analyses of potential risk factors for malaria infection among fishermen

Variable	Case / n (%)	OR (95% CI)	p-value
Demographic characteristics			
Age group			
15 – 34	10 / 92 (10.9%)	ref	ref
Above 35	6 / 60 (10%)	0.91 (0.29 – 2.06)	0.864
Sex			
Female	6 / 47 (12.8%)	ref	ref
Male	10 / 105 (9.5%)	0.71 (0.24 – 2.34)	0.549
Type of fisherman			
Tilapia fishermen	8 / 59 (13.6%)	ref	ref
Silver fish fishermen	4 / 42 (9.5%)	1.23 (0.27 – 5.54)	0.774
Nile perch fishermen	4 / 51 (7.8%)	1.84 (0.54 – 7.27)	0.343
Housing characteristics			
Type of house			
Permanent	2 / 23 (8.7%)	ref	ref
Semi-permanent	11 / 111 (9.9%)	2.10 (0.28 – 7.81)	0.857
Makeshift	3 / 18 (16.7%)	1.15 (0.31 – 17.47)	0.445
Environmental risk factors			
Distance of house from shoreline			
Above 500 m	6 / 57 (10.5%)	ref	ref
Below 500 m	10 / 95 (10.5%)	1 (0.34 – 3.09)	1
Location of fishing beach			
Kaswanga	3/51 (5.9%)	ref	ref
Lwanda Rombo	4/41 (9.8%)	1.72 (0.36 – 0.17)	0.490
Uta	9/60 (15%)	2.82 (0.78 - 13.29)	0.136
Occupational characteristics			
Time of work			
Day	7/62 (11.3%)	ref	ref
Night	9/90 (10%)	0.87 (0.3 – 2.57)	0.799
Light usage while fishing			
No	12/100 (12%)	ref	ref
Yes	4/52 (7.7%)	0.61 (0.16 – 1.86)	0.415

Distance of work area from the shoreline			
Offshore	9/98 (9.2%)	ref	ref
Inshore	7/54 (13%)	1.47 (0.49 – 4.20)	0.469
Frequency of fishing			
Irregularly	8/69 (11.6%)	ref	ref
Regularly	8/83 (9.6%)	0.81 (0.28 – 2.33)	0.696
Population movement			
Travel to other fishing beaches			
No	11/126 (8.7%)	ref	ref
Yes	5/26 (19.2%)	3.96 (1.11 – 12.71)	0.122
Malaria protection measures			
Mode of dress			
Arms and legs covered	4/69 (5.7%)	ref	ref
Arms and legs exposed	12/83 (14.4%)	2.74 (0.9 – 10.21)	0.093
Malaria measures used			
Bednets	14/113 (12.3%)	ref	ref
None	2/39 (5.1%)	0.38 (0.05 – 1.45)	0.218
Frequency of bednet usage			
Every night	11/82 (13.4%)	ref	ref
Sometimes	3/30 (10%)	0.71 (0.15 – 2.50)	0.630
Do not use at all	2/40 (5%)	0.33 (0.05 – 1.34)	0.174

CI = Confidence interval; OR = Odds Ratio

Table 12: Multivariate analysis of risk factors for malaria infection among fishermen

Variable	**OR (95% CI)**	**p-value**
Mode of dress		
Arms and legs covered	ref	ref
Arms and legs exposed	3.56 (1.11 – 14.23)	0.045
Travel to other fishing beaches		
No	ref	ref
Yes	3.50 (0.96 – 12.03)	0.047

CI = Confidence interval; OR = Odds Ratio

6.4 DISCUSSION

This study identified predictors of malaria risk among fishermen. Malaria was associated to fishermen who wore clothing that exposed their arms and legs, and travelled out of Rusinga Island. Findings in this study show that wearing clothing that exposes arms and legs is a risk factor for malaria. Fishermen spend a lot of time outdoors from dusk till dawn, which already exposes them to malaria mosquitoes. Thus individuals who dress in clothing that expose their arms and legs during peak mosquito biting times are at an even greater risk of malaria. Dressing was also proven to be a risk factor among individuals in Malaysia in Asia. Kaur and others (2009) showed that individuals who did not cover their upper and lower limbs had the highest prevalence of malaria compared to their counterparts who did not cover their limbs. Similarly, a study on individuals in northern Thailand showed that wearing short-sleeved clothes outdoors was a risk factor for the disease (Pichainarong and Chaveepojnkamjorn, 2004). However, another study conducted by Pattanasin and others (2012) in Thailand on rubber tappers did not support this finding. The rubber tappers conduct their livelihood activities outdoors and begin working at midnight which coincides with the time when local malaria vectors actively engage in host seeking. It is possible that the difference in the results of the study described herein and the one by Pattanasin *et al.* (2012) had to do with their description of dressing. Pattanasin and colleagues (2012) focused on wearing long-sleeved shirts thus concentrating on the upper body, rather than the upper and lower limbs like in our study. Similarly, dressing in short-sleeved clothing had no association with malaria infection as reported in other studies (Chaveepojnkamjorn and Pichainarong, 2005; Neuberger *et al.*, 2010). Dressing in long sleeved clothing that covers arms and legs is known to offer a certain degree of protection against biting insects (Rozendaal, 1997). It would seem proper for the fishermen of Rusinga Island to dress in clothing that offers protection against malaria mosquitoes.

Fishermen who dress in clothes that do not cover exposed parts of their bodies increase the exposure of receiving infected mosquito bites. This lifestyle requires adoption of malaria preventive measures, such as wearing long sleeved and long legged clothes to reduce contact with infected malaria vectors. A study by Schoepke *et al*. (1998) showed that wearing long-sleeved clothes was an effective personal malaria protection measure used by European tourists visiting East Africa. However, it is important to note that our study had some limitations. We did not categorize the mode of dress by investigating specific parts of the body that were covered or not, specifically either upper body, lower body or both.

In this study, fishermen who travelled out of the study area to conduct fishing activities elsewhere were at a higher risk of malaria. Travel among fishermen involves movement between islands in Lake Victoria in search of areas with high densities of fish. Fishermen usually travel to Mageta, Mfangano, Ngodhe, Remba and Ringiti Islands. Although the number of fishermen who tested positive for malaria and travelled to other fishing beaches was low (5), this variable was shown to be a risk factor for malaria. This finding should be interpreted with caution as the low number of malaria positive individuals reduces the power to safely draw conclusions about the finding. Population movement has been reported to be significantly associated with malaria in several studies (Rajagopalan *et al*., 1986; Sinhanetra-Renard, 1986; Martens and Hall, 2000). A study conducted among workers in tea estates of Kericho in western Kenya showed a significant association between malaria parasitemia and travel to malaria endemic areas (Shanks *et al*., 2005). It is important to note a difference in movement in the study conducted by Shanks *et al*. (2005) and that reported herein. Tea estate workers travelled from a highland epidemic area to malaria endemic areas while fishermen travelled from malaria endemic areas to areas of unknown endemicity. Malaria prevalence in islands in Lake Victoria has been recorded as approximately 40% (Kabatereine *et al*., 2011). The aforementioned study was conducted in Ugandan Islands. There is a dearth of information about malaria endemicity in many Kenyan Islands of Lake Victoria. The densities and species composition of malaria vectors in the islands of Lake Victoria are well documented (Futami *et al*., 2014). The vectors include *Anopheles gambiae*, *An. arabiensis* and *An. funestus*. We are therefore unsure whether the prevalence of malaria is higher in these islands that fishermen travel to in search of fish compared to Rusinga Island that is described as mesoendemic. Factors related to population movement, for instance the frequency of movement were not investigated in this study.

The results of the present study indicate that there was no difference in malaria infection among fishermen who slept under bednets compared to their counterparts who did not. Our findings suggest that bednets are not an effective tool for malaria control among fishermen. Fishermen who work at night are not likely to sleep under bednets. They are not adequately unprotected by choice but because they depend on outdoor activities for their livelihood. Bednets have been documented to be an effective malaria prevention and control tool (Lengeler, 2004; Muller *et al.*, 2006). This tool is most effective when used indoors to protect sleepers. Fishermen work outdoors either inshore or offshore thus do not benefit from the protective effect offered by bednets. These individuals spend time at the shore to either unload their catch or take long breaks in make-shift structures called *Abila*. These temporary structures are usually located at the shoreline and rarely equipped with bednets. A review conducted by Pulford *et al* (2011) documented several reasons why people do not sleep under bed nets. Spending time elsewhere, for instance, at the work place at night, was cited as a reason for not using a bednet even though one was available (Pulford *et al.*, 2011).

In our study, there was no difference in malaria infection among fishermen who live in make-shift structures compared to those who reside in semi-permanent and permanent houses. This finding may be explained by the small sample size of residential *Abilas* observed in the study. One fishing beach that was randomly chosen, specifically Uta beach did not have any A*bila*. These make-shift structures usually have both walls and roofs made from corrugated iron sheets and are unsuitable for IRS. The *abilas* allow for free mosquito entry through eaves. Houses have been proven to be the main site for human-vector contact (Snow, 1987; Gamage-Mendis *et al.*, 1991) and those constructed with semi-permanent material create favourable microenvironments for malaria mosquitoes (Schofield and White, 1984) to survive and feed. Several studies have associated higher densities of malaria mosquitoes with houses with open eaves (Lindsay *et al.*, 2002; Lindsay *et al.*, 2003; Kirby *et al.*, 2008; Lwetoijera *et al.*, 2013). Poorly constructed houses have also been associated with malaria infection (Gamage-Mendis *et al.*, 1991; Konradsen *et al.*, 2003). *Abilas* are usually owned by boat owners who use the structures i) to store fishing gear, ii) as an office for planning fishing routes, iii) to light pressure lamps used in capturing silverfish, and iv) to take short breaks to eat dinner. The houses are normally shared by large numbers of fishermen who use them in shifts and have no steadfast rental or owner-occupier status, thus do not contain beds or beddings.

Although indoor interventions have been proven to be effective, they only affect malaria vectors that feed and rest indoors. This creates a gap in malaria protection, particularly for individuals who work outdoors around the time that people go to sleep (Durnez and

Coosemans, 2013). Controlling outdoor malaria transmission requires different strategies from those targeting indoor vectors. There is a need to develop novel tools targeting outdoor malaria transmission.

6.5 Conclusion

Behavioural factors are related to increased malaria risk among fishermen. Wearing clothing that exposes arms and legs is a risk factor for malaria among fishermen of Rusinga Island. Travel to other fishing beaches was significantly associated with malaria among fishermen. Bed nets are not an effective malaria control tool among fishermen. There is a need for a health-based educational intervention in the area to create awareness among the fisherfolk. Use of long sleeved shirts and trousers should be encouraged so as to cover up exposed skin when working outdoors. Vector control tools that protect individuals outdoors during peak malaria mosquito biting times need to be developed.

CHAPTER 7: USING OUTCOME MAPPING TO MEASURE MALARIA-PREVENTION BEHAVIOUR CHANGE AMONG FISHERFOLK

7.1 BACKGROUND

Fishing is a major livelihood activity along the shoreline of Lake Victoria in western Kenya. The area around Lake Victoria is characterized as malaria endemic (Mutero *et al.*, 1998; Gouagna *et al.*, 2003; Noor *et al.*, 2009; DOMC, 2011), with fluctuating but significant densities of malaria mosquitoes (Futami *et al.*, 2014). Malaria is transmitted through the infected bite of a female *Anopheles* mosquito. The disease was shown to be responsible for 48% of hospital outpatient visits in areas around the shoreline in 1998 (Mutero *et al.*, 1998).

Previous studies conducted in areas around the Lake Victoria shoreline have demonstrated that human behaviour is associated with malaria risk (chapter 6). People living in areas along the shoreline engage in fishing activities that are related to malaria in at least two ways. Firstly, individuals engage in livelihood activities that create mosquito breeding sites and foster mosquito productivity (Mukabana et al, unpublished data). The man-made sites are formed when individuals dig into the ground in swampy areas in search of earthworms used as fish bait. The holes formed hold water that supports mosquito breeding. Trenches dug inland at the shoreline to achieve slow entry and exit of lake water create a perfect area to retain water, trap fish and support immature malaria mosquitoes. Boats abandoned by fishermen because they are either old or not in use because of the annual fishing ban are purposely filled with water to prevent the wood from cracking. The water serves as a suitable breeding site for malaria mosquitoes. Fish farmers who conduct fish rearing activities promote mosquito breeding when they abandon their fish ponds. The large hole left in the ground fills up with water during the rainy season creating mosquito breeding grounds. Secondly, fisherfolk prefer to wear clothing that exposes their arms and legs while working, an act that exposes them to infectious mosquito bites (Chapter 6).

The fisherfolk of Lake Victoria are responsible for the creation of health risks related to malaria. There is a need for fisherfolk to be aware of the predisposing activities and respond accordingly to alleviate malaria risk. A behaviour change intervention was developed to create awareness and educate members of the capture fishing community about livelihood activities related to malaria. An outcome mapping approach was used as a tool to plan, monitor and evaluate behavioural change among fisherfolk.

Outcome Mapping (OM) is an approach designed for projects that focus on changes in behaviour (Earl *et al.*, 2001). It is a tool that supports projects throughout their life span as it consists of several components that assist in the planning, monitoring and evaluation processes (Earl *et al.*, 2001). The primary focus of Outcome mapping is behavioural changes (defined as Outcomes in OM terms) among the individuals and/or groups that a project interacts with (Earl *et al.*, 2001). The individuals or groups are known as Boundary Partners and can be influenced to foster the expected behavioural change among communities. The community that ultimately benefits from the changes in behaviour of Boundary partners is called beneficiaries. All activities conducted in OM revolve around Boundary partners. The OM approach is based on the principal that social change is achieved through concerted effort from several people who have a mutual goal.

Outcome mapping has been adopted in several studies to measure behaviour change in various disciplines (Jones, 2007), for instance health (Price *et al.*, 2014) and agriculture (Leksmono *et al.*, 2006; Nyangaga *et al.*, 2010a; Nyangaga *et al.*, 2010b; Nyangaga and Schaeffer, 2011). This study was designed to measure behaviour change in relation to malaria among fisherfolk involved in capture fishing activities on Rusinga Island. The present study sought to engage Boundary Partners on discussions on issues related to malaria risk and fishing. In addition, investigations were conducted to determine how malaria discussions translated to behaviour change among members of the fishing community. Specifically, the study also sought to i) develop vision and missions statements, ii) identify Boundary Partners, iii) develop Outcome challenges and Progress markers, iv) design a behaviour change intervention, and v) monitor behaviour change in Boundary Partners using Outcome Journals.

7.2 METHODOLOGY

7.2.1 Study area

The study was carried out in Rusinga Island, Mbita district in western Kenya. Capture fishing is a major source of food and income for residents of Rusinga Island (Opiyo *et al.*, 2007). Majority of the male adult population on the island engages in artisanal fishing activities. Females are also involved in fishing activities on a smaller scale and fishing-related activities on a larger scale. Other activities conducted on Rusinga include fish trading, net mending, traditional fish processing, boat making, boat repair, subsistence farming and small-scale trading. Two fishing beaches were randomly chosen as study sites namely Luanda rombo and

Uta. Each fishing beach is administratively managed by a Beach Management Unit that consists of approximately 10 elected members.

7.2.2 Development of the vision and mission statements

The formulation of the vision and mission are part of step 1 and 2 respectively in the OM model (Figure 4). Vision statements are developed to provide inspirational focus thus describe an ideal world that describes all positive changes the project will bring about (Earl *et al.*, 2001). Mission statements contain information about activities that will be undertaken that will contribute to the vision (Earl *et al.*, 2001).

7.2.3 Identification of Boundary Partners

Groups and institutions that are stakeholders in the fishing industry were identified. Stakeholders were identified as groups of people who i) are affected by the actions of the behaviour change intervention, ii) who have influence among the beneficiaries of the project (members of the fishing community), and iii) who have an interest in the success of the behaviour change intervention. The search for stakeholders was limited to those in Rusinga Island. Of the stakeholders identified, those that had the most influence and power over individuals of the fishing community were identified as Boundary partners.

7.2.4 Development of Outcome challenges and progress markers

A stakeholder workshop was conducted in October 2013 during which an Outcome mapping model was developed and an outcome challenge statement and progress markers defined. The behavioural results that this research aimed to attain were clearly stated in the outcome challenge and progress markers. Outcome challenge statements were formulated that described the manner in which behaviors among boundary partners change. Progress markers defined the behavioural change of each boundary partner (Nyangaga and Schaeffer, 2011). These markers were graduated based on the understanding that behavior change is a gradual process. The progress markers were graduated into three levels namely expect to see, like to see and love to see issues (Earl *et al.*, 2001). Behavioural changes that were easiest to achieve were placed under the level ‘expect to see’ while those that were advanced and required more learning and engagement were indicated under ‘Like to see’ levels. Behaviours that were deeply transformative and were conducted without the help of the research team were classified under ‘Love to see’.

7.2.5 Behaviour change intervention

The behaviour change intervention involved creating awareness and educating Boundary partners. Several meetings were conducted by project staff and Boundary partners whereby fishing activities related to malaria were discussed. Boundary partners were educated on malaria transmission and causes, ecology of mosquitoes, and diagnosis, treatment, prevention and control of malaria. Strategies for malaria prevention and control tailored for fisherfolk were identified and discussed among boundary partners and research team members. The partners were also provided with Information, Education and Communication (IEC) materials that were used and distributed during their health programmes. These materials included wall calendars, training guides and brochures. The IEC materials were provided to beneficiaries and helped in creating awareness of fishing activities related to malaria.

7.2.6 Monitoring behaviour change in Boundary Partners using Outcome journals

Outcome journals were used as data collection tools to track behavioural changes in boundary partners over time. Progress markers were listed in Outcome Journals that were used to document progress marker achievement among Boundary Partners. Each boundary partner had a specific outcome journal. The journals were filled in during joint monthly feedback meetings conducted by research team members and Boundary Partners.

7.3 RESULTS

7.3.1 Development of the vision and mission statements

The first step in Outcome mapping involves defining the vision of a project. The vision was stated as ‘The project envisions the achievement of a malaria-free Rusinga whereby fishing communities holistically use available malaria control tools to prevent and control malaria’. The project envisioned a period when fisherfolk would be aware about the relationship between fishing activities and malaria. Members of the fisherfolk community would take appropriate preventive and control measures to alleviate malaria risk.

The mission was to undertake research to identify potential risk factors for malaria and determine malaria burden and transmission among fishermen and support the implementation of strategies to prevent and control malaria. The project was to develop and promote a plan of action for awareness creation among fisherfolk. The project aimed to provide and support a platform for sharing malaria prevention strategies to control malaria in Rusinga Island.

7.3.2 Identification of Boundary Partners

Two Boundary partners were identified namely Uta and Luanda Rombo Beach Management Units (BMUs) as shown in the OM framework (Figure 11). The BMUs were identified as Boundary partners because of their influence in the fishing community. Fishing beaches are administratively organized by BMUs, each of which is led by a Chairman. The BMU Chairmen are powerful and usually set rules that govern activities conducted at their designated fishing beaches. The two Boundary partners run health programmes within their groups and this presented an opportunity for influence. These Boundary partners were charged with the responsibility of bringing change and well-being to their fishing communities (beneficiaries). It was anticipated that the BMUs would promote healthy malaria-related behaviour–in their fishing beaches. Two strategic partners were identified namely Mbita District Public Health Office under the Ministry of Health and Mbita Department of Fisheries under the Ministry of Agriculture, Livestock and Fisheries. The two strategic partners were stakeholders in the health and fishing industries. The partners were chosen as strategic because the project staff recognized an opportunity to interact but not influence them.

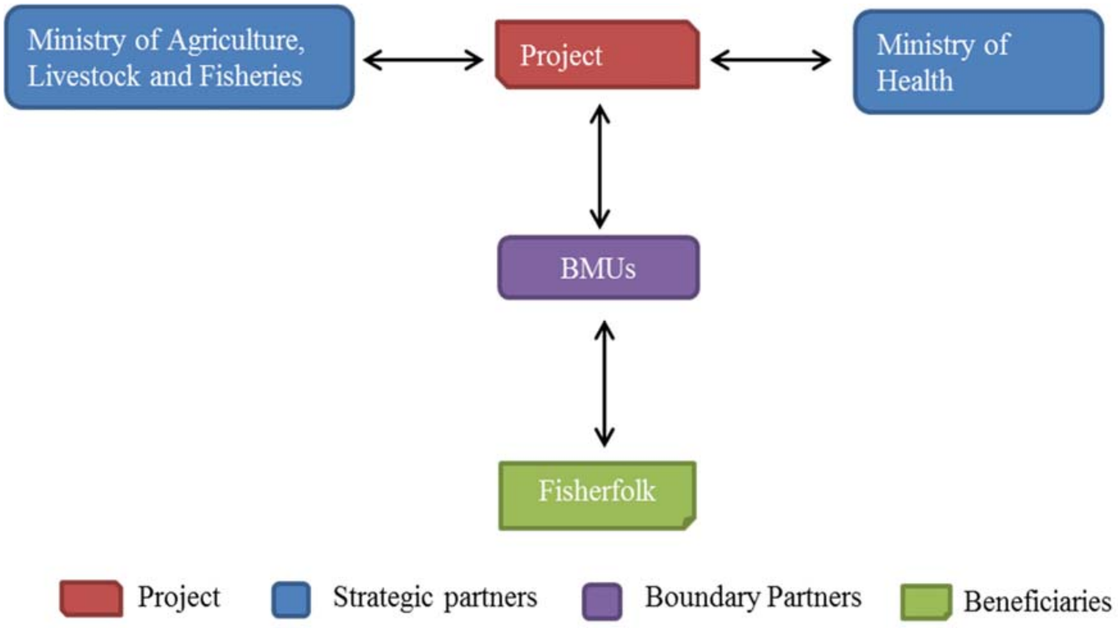

Figure 10: Outcome Mapping framework

7.3.2 Development of Outcome challenges and progress markers

Outcome challenge statements were formulated that described the manner in which behaviour among members of BMUs would change. The program intended to see members of BMUs understanding the link between malaria, the environment and fishing activities and participate in activities that disseminate this information to fisherfolk. The BMU members were to adopt malaria preventive strategies and showcase positive behaviour in the fishing community.

Progress markers developed for BMUs were graduated into three levels namely ‘expect to see’, ‘like to see’ and ‘love to see’ (Table 13). Three progress markers were formulated under the expect to see category. The easiest behaviours that BMUs were expected to exhibit included, i) attendance and active participation in meetings conducted by the project, ii) take up an active role in discussions related to malaria and fishing within their community, and iii) increase knowledge about causes and transmission of malaria. Three progress markers were developed under the like to see category. The BMUs were to display the following behaviours, i) share knowledge about malaria with colleagues from other BMUs who operate in other fishing beaches, ii) attempting to influence the decisions of fishermen with regards to malaria prevention and control, and iii) adopting malaria prevention and control methods. Behaviours that were hardest to achieve were listed under the love to see category. The BMUs were to sustain malaria-related activities after the projects’ lifespan. Lastly, the BMUs were to introduce by-laws that ban fishing-related activities that promote mosquito breeding at their fishing beaches.

7.3.3 Monitoring behaviour change in Boundary Partners using Outcome journals

Several outcomes were achieved by the Luanda Rombo and Uta Beach Management Units. Members of BMUs attended all meetings, actively participated in meetings conducted by the project and eventually became aware of the causes and transmission of malaria (Table 13). These individuals actively engaged in discussions with members of the fishing community and BMU members from other fishing beaches about fishing activities related to malaria. During weekly meetings with community members, IEC materials, namely calendars and brochures were distributed to fishermen, fish traders, boat owners, boat repairers, net menders and ladies who process fish.

The BMU members adopted the recommended malaria control and prevention practices that were discussed during meetings with project staff. These included periodic drainage of water from abandoned fishing boats, sleeping under a treated mosquito net, wearing long-sleeved clothing that offers some degree of protection against biting mosquitoes, prompt health seeking behaviour to diagnose suspected malaria and treatment. However, a few strategies for malaria prevention and control that were previously discussed were not implemented by many BMU members. A strategy to locally rear fish baits, specifically earthworms in containers instead of harvesting them was discussed but was never initiated. The practice of digging into the ground in swampy areas to harvest earthworms did not cease. In addition, trenches dug inland at the shoreline to support growth of immature fish were not periodically drained. These trenches continued to support immature mosquitoes.

Another outcome that was not achieved was that the BMUs did not develop by-laws at their fishing beaches that prohibit the conduct of fishing activities that aggravate mosquito breeding. Nevertheless, BMU members used their weekly meetings as a platform to discuss malaria issues with members of their fishing beaches during and after the project duration.

Table 13: Level of progress marker achievement among members of Beach Management Units (BMUs)

Progress marker	Level of marker achievement among BMUs
Expect to see	
Attending and actively participating in meetings conducted by the project	H
Taking an active role in discussions related to fishing and malaria within their fishing community	H
Understand better / increase knowledge on the causes and transmission of malaria	H
Like to see	
Sharing malaria knowledge with members of other BMUs from other fishing beaches	M
BMUs attempting to influence the decisions of fishermen regarding malaria prevention and control	H
BMU members adopting malaria control and prevention strategies	H
Love to see	
BMUs sustaining the project even after its lifespan	H
Introduce by-laws at fishing beaches that ban fishing-related activities that promote mosquito breeding	L

The table above show the level of progress marker achievement in boundary Partners. Low (L) means that the boundary partner did not change their actions or behaviour. Medium (M) means that a change in behaviour was observed, and High (H) shows that a positive action was observed without the help of project staff.

7.4 DISCUSSION

Outcome mapping was influential in identifying partners with whom the programme worked and the expected behavioural changes (Smutylo, 2005). The programme identified leaders of Beach Management Units as individuals who can influence the behavior of individuals involved in fishing activities. The programme brought together leaders of the fisherfolk community and researchers to create awareness among fisherfolk on fishing activities that promote malaria and identify strategies to mitigate malaria risk. A major change in behaviour was observed in attendance and participation of BMUs in these meetings. However, this change in behaviour could have been influenced by the provision of a sitting allowance provided to BMU members. Each participant received approximately USD 5 for attending each meeting. Allowances are usually given as financial motivation to encourage participation in meetings (Vian *et al.*, 2013). The meetings were also recognized as fora where co-learning was enabled, information and ideas generated and shared among the groups.

In this study, Boundary partners were educated by project staff on causes and transmission of malaria. The OM approach has been reported to be suitable for programmes that entail capacity building (Jones and Hearn, 2009). An increase in knowledge on causes and transmission of malaria was an outcome that was observed among all boundary partners. This knowledge was publicized to other members of the fishing community through weekly meetings. Members of the BMUs also increased awareness of fishing activities related to malaria among fisherfolk.

Malaria knowledge was translated well into action by members of the BMUs. Prior to the intervention, boat owners abandoned fishing boats during the fishing ban that targets Silverfish (*Rastrineobola argentea*) between April and August every year. A change was observed among these individuals whereby boats were drained off of water once a week. African malaria mosquitoes prefer to breed in temporary, open, clear, sunlit pools of water (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987) like those found in abandoned boats. It appears that members of the BMUs understood the risks associated with abandoned boats and mosquito breeding.

In the recent past, members of BMUs used to dress in short-sleeved clothing that would place them at risk of receiving mosquito bites while conducting late outdoor activities. Members of BMUs initiated the practice of dressing in long-sleeved clothing while conducting fishing related activities outdoors. Long-sleeved clothing has been shown to offer some degree of protection against biting insects (Rozendaal, 1997). Members of BMUs became aware that outdoor nocturnal activities place them at risk of malaria. Spending time outdoors at night exposes them to mosquito bites.

Members of the BMUs demonstrated positive behavior change in relation to bednet usage. This implies that they learned the benefits of sleeping under bednets. Treated mosquito nets have been documented to be an effective malaria prevention and control tool (Lengeler, 2004; Muller *et al.*, 2006). Human behaviour, specifically use of treated bed nets have been shown to reduce malaria cases in African communities (Fullman *et al.*, 2013). Another major outcome that was realized was improved health seeking behaviour. The BMU members were able to recognize malaria symptoms and seek proper treatment at health facilities.

Two malaria prevention and control measures were discussed during project meetings, but not implemented by many members of the BMUs. The members did not locally rear earthworms in containers, but instead continued searching for earthworms in swampy areas near the lake shore. In addition, trenches were not periodically drained of water to disrupt mosquito breeding in these sites. The lack of adoption of the malaria prevention measures may be explained by the diffusion of innovations theory. According to the theory, individuals adopt innovations at different rates. Some individuals in the society adopt innovations at a slower rate compared to others. This is probably due to the individuals' interest and evaluation (Rogers, 2003).

Members of BMUs were less successful in demonstrating change with regards to local policy. By-laws at fishing beaches that prohibit the conduct of fishing activities that aggravate mosquito breeding were not developed. This change in behaviour was not observed probably because of the short lifespan of the programme. Behaviour change reported in this study was only reported within a 6-month period. This suggests that behaviour change interventions should be employed for a longer period of time to facilitate progress.

Several initiatives have noted the importance of human behavior in malaria control (Williams *et al.*, 2002). Human behaviour whether deliberate or not can promote the spread of infectious diseases (Inhorn and Brown, 1990), such as malaria. The success of malaria interventions heavily rely on the behavioural responses of affected populations (Heggenhougen *et al.*, 2003; Setbon and Raude, 2009). Appropriate malaria prevention and control tools should be used in ecological settings influenced by local human behavioural patterns.

7.5 Conclusion

Outcome mapping can be used as a tool to document behaviour change among members of Beach Management Units. The OM framework enabled sharing of information among research team members and Boundary partners. The behaviour change intervention was well perceived among Boundary Partners. The most significant change was observed in attendance of project meetings by Boundary Partners. Most of the malaria prevention and control efforts focused on use of treated bed nets and wearing long-sleeved clothing outdoors. Members of Beach Management Units exhibited different behaviour because of the contribution of several actors, including the project members and strategic partners.

CHAPTER 8: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1 General Discussion

Fishing is a major livelihood activity conducted outdoors at night in malaria endemic areas around the shores of Lake Victoria in western Kenya. Outdoor malaria transmission was found to occur on Rusinga Island as infected malaria mosquitoes were captured outdoors. This study demonstrated that individuals conducting fishing activities outdoors at night were at risk of receiving infectious bites. Human behaviour is a determinant of the place malaria transmission will occur (Huho *et al.*, 2013), thus transmission will persist if nocturnal activities are conducted outdoors. Outdoor transmission presents a challenge to malaria control efforts that should eventually culminate to elimination of the disease (Durnez and Coosemans, 2013; Killeen, 2014). Several studies have reported an increase in outdoor transmission in the evenings and early mornings (Govella *et al.*, 2010b; Russell *et al.*, 2011).

Malaria mosquitoes on Rusinga Island were found to be low in density. Evidence collected in this thesis implies that a small population of malaria vectors is responsible for malaria cases on Rusinga Island. Small populations of malaria vectors can sustain high malaria transmission in endemic areas (Trung *et al.*, 2004). Low densities of malaria vectors also imply that malaria vectors on Rusinga Island are highly efficient and maintain stable malaria transmission throughout the year. Elimination of malaria from Rusinga Island will require an increase in the use of interventions that target indoor transmission as well as novel interventions to control outdoor transmission.

Fishermen and individuals involved in fishing-related activities were found to be at risk of malaria because of their presence at the shoreline that coincides with peak mosquito biting activity of malaria vectors. *Anopheles arabiensis* was found to be a crepuscular vector on Rusinga Island. It showed a peak in biting activity after dusk and just before dawn around the same time when fishermen are active. Individuals who harvest fish a few metres from the shoreline by boat are similarly at risk of receiving infectious bites. Fish traders who wait for fishermen to sail back to shore between 4am and 6am to purchase fish were also found to be at risk of malaria. *Anopheles arabiensis* is traditionally known to be an outdoor biter (Muriu *et al.*, 2008), a characteristic trait that places fishermen found outdoors at night at high risk. A study conducted by Escovar *et al*. (2013) in Colombia found a similar association between fishermen and local malaria vectors.

Outdoor transmission presents a challenge to malaria control as most interventions are targeted for indoor use (Durnez and Coosemans, 2013; Killeen, 2014). Malaria transmitting mosquitoes traditionally bite people indoors at night when they are asleep (Huho *et al.*, 2013). One frontline tool for vector control, specifically LLINs target indoor biting mosquitoes and protect humans by either repel/kill strategies. Another tool, namely IRS targets indoor resting mosquitoes that rest on walls after a blood meal with the ultimate aim of killing the vectors. Although bed nets have been found to be an effective malaria prevention and control tool (Lengeler, 2004; Muller *et al.*, 2006) they were not found to be an effective tool among fishermen in this study. Fishermen of Rusinga who harvest fish at night are less likely to sleep under treated bed nets consistently. Not all fishermen who work at night engage in fishing activities daily. They probably sleep under treated bed nets during the nights that they are off duty. This inconsistency in bed net usage limits the protection offered by bednets. On the other hand, IRS has only been conducted once in the study area in the year 2012 thus cannot be truly included as a frontline tool for malaria vector control on Rusinga Island.

The health facility-based study conducted among individuals engaged in various livelihood activities revealed that fishermen and farmers were associated with malaria cases. Fishermen and farmers were also the two main livelihood categories who sought outpatient services. Individuals who conduct the two activities are usually found outdoors during peak malaria biting times. A study conducted in Botswana similarly showed an association between malaria and individuals who conduct late outdoor activities (Chirebvu *et al.*, 2014). Fishing on Rusinga Island is conducted all year round; an activity that places fishermen at risk throughout the year, unlike farming that is mostly conducted at specific times of the year.

One factor under personal protection measures, specifically mode of dress while at work was identified as a risk factor for malaria among fishermen. Fishermen who wear clothing that do not cover their arms and legs were found to be at a higher risk of malaria. This finding was also found to be a risk factor among individuals in Malaysia and Thailand (Pichainarong and Chaveepojnkamjorn, 2004; Kaur, 2009). Long-sleeved clothing offers some protection against biting insects (Rozendaal, 1997) and offers fishermen a simple cost-effective malaria prevention method.

It was important for fisherfolk to become aware of behaviours that place them at risk of malaria in their community and consequently change. A behaviour change intervention was conducted among fisherfolk of Rusinga Island and an Outcome mapping approach used for tracking and measuring changes in behaviour. Several malaria-related changes in behaviour were observed which indicated that Outcome mapping can be used to plan and monitor behaviour change among fisherfolk. The biggest change in behaviour was observed in attendance of project meetings by Beach Management Units (BMUs). This led to capacity building that entailed mutual learning and the exchange of ideas (Jones and Hearn, 2009). The BMU members promoted positive behaviour change through action. A key outcome that was achieved was the use of malaria prevention and control tools, especially use of treated bed nets and wearing long-sleeved clothing outdoors among members of the two BMUs. This change to healthy behaviour is a major step towards the reduction of behaviour that hampers malaria control. Changes in the content of weekly BMU meetings were also observed. Fishing activities related to malaria was frequently discussed during the meetings. The BMU members shared information regarding malaria and fishing activities with other BMU members from other fishing beaches. The BMU members increased the awareness of malaria and encouraged behaviour change among their colleagues.

The findings of this study helped to understand the risk of malaria on Rusinga Island. Fishermen unknowingly expose themselves to malaria. It is plausible that fishing as a means of livelihood outweighs the health risks associated with the activity. A few novel interventions to control outdoor transmission have been reported, ranging from outdoor traps (Okumu, 2010, 2013) to toxic sugar baits (Müller, 2010), but none have generated enough evidence to warrant employment at a national programmatic level (Killeen, 2014). Larval source management is a useful strategy that has been used to control malaria vectors outdoors. The application of larvicides to aquatic habitats has been proven to be successful in reducing immature malaria vectors and adult females by >90% in Mbita sub-county (Fillinger and Lindsay, 2006). Other strategies that can be used to control outdoor malaria vectors include insecticide-treated clothing (Macintyre *et al.*, 2003; Kimani *et al.*, 2006) and spatial repellents (Achee, 2012). However, these strategies may be adopted in the near future and will eventually protect individuals like fishermen. However, prior to that, the fisherfolk community can engage in discussions to encourage use of the existing vector control tools.

8.2 Limitations of the study

The study had a few limitations. Firstly, the Suna trap used to capture mosquitoes outdoors shredded adult mosquitoes. Although some individual mosquitoes were trapped unharmed, it was hard to quantify the total number of mosquitoes that were captured. Identification of mosquitoes was difficult using body parts thus only the heads were identified and used to obtain an accurate number.

Secondly, the fact that fisherman who harvest silverfish were not active during the fishing ban (April - July) was a limitation. The long rainy season occurs between April and July but data specific to malaria infection could not be collected. Thus malaria surveys could not be conducted during the period of high malaria transmission. Malaria surveys could only be carried out when all fishermen were active.

Lastly, the dearth of information on the use of Outcome mapping in malaria studies was a limitation. The OM approach has not been widely used in the field of human health either. The study reported in this thesis is the first study that applies and reports the use of Outcome mapping in malaria research and development activities.

8.3 Future work and recommendations

- Further investigations need to be undertaken to determine whether fishermen receive more infected mosquito bites compared to individuals who sleep indoors at night. Serological tests need to be conducted on blood samples to determine antibody responses against *Plasmodium falciparum*. Individuals exposed to infected mosquito bites can remain seropositive for *P. falciparum* antibody responses for long periods of time. These antibodies persist markedly longer than individual malaria infections thus seroprevalence rates could provide a reliable tool for assessing malaria endemicity.
- Follow-up research on the risk of malaria among individuals who conduct other outdoor activities at the fishing beach, for instance small-scale trading, should be conducted. These individuals spend a lot of time outdoors from early in the morning till late in the evening.
- The relationship between fishing and travel to other areas should be investigated further.
- Malaria studies among fishermen in different ecological settings should be conducted. This would enable one to determine whether the current malaria risk associated with fishing activities is unique to Rusinga Island.

8.4 Conclusion

This study demonstrated that fishing in Rusinga Island is an occupational risk that increases the probability of receiving infected mosquito bites. Majority of the adult male population on the island is at risk of malaria. The most common front line malaria control intervention, specifically treated bed nets is used irregularly by fishermen. Based on these findings, outdoor vector control strategies are urgently needed to supplement indoor strategies because the proportion of outdoor malaria vectors has increased on Rusinga Island in the recent past. In addition, fishermen need to be encouraged to utilize existing interventions like treated bed nets whenever they are indoors at night. Behaviour change communication programmes are needed to encourage fishermen all over Rusinga to protect them from malaria.

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Appendix 1: Survey questionnaire for chapter three

Malaria survey in Rusinga Island

Questionnaire ID ________________________ Date ______________________

Interviewer's Name ______________________ House & HH ID _____________

***Instructions for the interviewer**: Each question should have only one answer. Tick only one answer.*

Demographic characteristics

1. What is your **name**?

   ____

2. Are you **male** or **female**?

   Male ☐ Female ☐

3. What is your **age**?

   ____

4. What is your **marital status**?

   Single ☐ Married ☐ Divorced ☐ Separated ☐

   Widow/widower ☐

5. What is your **tribe** (ethnicity)?

   ____

6. What is your **highest level of education**?

   Never attended school ☐ Primary school ☐ Secondary school ☐

   College/university ☐ Other (*specify*)

   ____

7. What is **your main occupation**?

   Fisherman ☐ Other (*specify*) ________________

8. Which **sub-location** in Rusinga do you live?

   Rusinga East ☐Rusinga West ☐

Use of malaria preventive measures

9. Which **malaria preventive measure** do you currently use?

   Bednets ☐ Insecticide sprays ☐ Repellents ☐ None ☐

   Other (*specify*) ________________

10. Do you **own** a mosquito net?

    Yes ☐ No ☐

11. How often do you **sleep** under the net?

Every night ☐ Sometimes ☐ Only during the rainy season ☐ Not used ☐ Do not own a bednet ☐

Malaria status

12. What is the participants' temperature? __________

13. Presence of fever?

Yes ☐ No ☐

14. Presence of other malaria symptoms (joint pain, headache, vomiting, diarrhea, shivering, chills, sweating)?

Yes ☐ No ☐

15. Presence of malaria parasites?

Yes ☐ No ☐

16. Presence of *P. falciparum* or mixed infection?

P. falciparum ☐ Mixed infection ☐ None ☐

17. Taken malaria medicine in the past two weeks?

Yes ☐ No ☐

Appendix 2: Survey questionnaire for chapter four

Malaria survey in Sienga village

Questionnaire ID ________________________ Date ______________________

Interviewer's Name ______________________ Distant interval ____________

House & HH ID __________________________

***Instructions for the interviewer**: Each question should have only one answer. Tick only one answer.*

Demographic characteristics

18. What is your **name**?

____

19. Are you **male** or **female**?

Male ☐ Female ☐

20. What is your **age**?

____

21. What is your **marital status**?

Single ☐ Married ☐ Divorced ☐ Separated ☐

Widow/widower ☐

22. What is your **tribe** (ethnicity)?

____

23. What is your **highest level of education**?

Never attended school ☐ Primary school ☐ Secondary school ☐

College/university ☐ Other (*specify*)

____

24. What is **your main occupation**?

Fisherman ☐ Other (*specify*) _________________

25. What is the **occupation** of the **household head**?

Fisherman ☐ Other (*specify*) _________________

Use of malaria preventive measures

26. Which **malaria preventive measure** do you currently use?

Bednets ☐ Insecticide sprays ☐ Repellents ☐ None ☐

Clothing that covers arms and legs ☐ Other (*specify*) ________________

27. Do you **own** a mosquito net?

Yes ☐ No ☐

28. How often do you **sleep** under the net?

Every night ☐ Sometimes ☐ Only during the rainy season ☐ Not used ☐ Do not own a bednet ☐

Malaria status

29. What is the participants' temperature? __________

30. Presence of fever?

Yes ☐ No ☐

31. Presence of other malaria symptoms (joint pain, headache, vomiting, diarrhea, shivering, chills, sweating)?

Yes ☐ No ☐

32. Presence of malaria parasites?

Yes ☐ No ☐

33. Presence of *P. falciparum* or mixed infection?

P. falciparum ☐ Mixed infection ☐ None ☐

34. Taken malaria medicine in the past two weeks?

Yes ☐ No ☐

Appendix 3: Survey questionnaire for chapter five

CONTRIBUTION OF MALARIA TO OUTPATIENT VISITS IN HEALTH FACILITIES IN RUSINGA ISLAND

Health facility ______________________ **Filled in by** ________________________

DATE	PATIENTS' NAME	Age	Sex	Zone	Occupation	Occup. Of HH	Fever (Y/N)	Temp.	Other symptoms	Plasmodium spp.	Gametocytes (Y/N)	Parasitemia level	Treat ment	Net use	Outdoor night activity 1 - 2 wks ago	History of malaria	Use malaria drugs 2 weeks ago (Y/N)

Appendix 4: Survey questionnaire for chapter six

Risk factors for malaria among fishermen of Rusinga Island

Questionnaire ID ________________________ Date ____________________

Interviewer's Name ______________________ Fishing beach _____________

Instructions for the interviewer*: Each question should have only one answer. Tick only one answer.*

Demographic characteristics

1. What is your name?

   ____

2. Are you male or female?

   Male ☐ Female ☐

3. What is your age?

   ____

4. What is your marital status?

   Single ☐ Married ☐ Divorced ☐ Separated ☐

   Widow/widower ☐

5. What is your tribe (ethnicity)?

   ____

6. What is your **highest level of education**?

   Never attended school ☐ Primary school ☐ Secondary school ☐

   College/university ☐ Other (*specify*) ______________________________

7. What is your **main occupation**?

   Fisherman ☐ Other (*specify*) ________________

8. Which species of fish do you **mostly harvest**?

   Omena ☐ Tilapia ☐ Nile perch ☐ Other (*specify*) _________________

Malaria knowledge

9. Is malaria a serious problem to you?

Yes ☐ No ☐

10. What causes malaria?

Plasmodium parasites ☐ Don’t know ☐

Other (*specify / wrong answer*) ________________________________

11. What are the symptoms of malaria?

Headache, fever, joint pain, vomiting, etc (correct answer) ☐ Don’t know ☐

Other (specify / *wrong answer*) ________________

12. What transmits malaria?

Mosquitoes ☐ Don’t know ☐

Other (*specify / wrong answer*) ________________

13. What is the breeding place for mosquitoes?

Anything that has stagnant water ☐ Don’t know ☐

Other (*specify / wrong answer*) ____________

14. What is the best method to prevent or control malaria?

Sleeping under bed nets, draining stagnant water, etc (correct answer) ☐ Don’t know ☐ Other (*specify / wrong answer*) ____________

15. What time malaria mosquitoes bite?

Day (6am to 6pm) ☐ Night (6pm to 6am) ☐

Housing characteristics

16. What material is the **wall** of the house you sleep in made of?

Mud or Iron sheets (semi-permanent) ☐ Bricks/Stone/Cemented (permanent) ☐

17. Does the house you sleep in have a **ceiling**?

Yes ☐ No ☐

18. Does the house you sleep in have open or closed **eaves**?

Open ☐ Closed ☐

19. How many **doors** does the house you sleep in have?

One ☐ Two ☐ More than two ☐

20. How any **windows** does the house you sleep in have?

One ☐ Two ☐ More than two ☐ None ☐

21. How many **rooms** does the house you sleep in have?

One ☐ Two ☐ More than two ☐

22. How **far** do you live from the shoreline?

<100 m ☐ Between 100 m – 500 m ☐ >500 m ☐

Sleeping arrangements

23. Where do you **mostly sleep**?

Abila owned by boat owner ☐ A friends' house who is also a fisherman ☐

My own house ☐ Rental house ☐

Other (*specify*) ____________

24. How many **people sleep** in that house?

One ☐ Two ☐ More than two ☐

25. What do you **sleep on**?

Bed ☐ Mat on the floor ☐ Other (*specify*) ____________

Livelihood activities

26. What **time** do you fish?

Day (6am to 6pm) ☐ Night (6pm to 6am) ☐

27. How many **hours** do you spend fishing?

0 – 5 hours ☐ 5 – 10 hours ☐ 10 – 15 hours ☐ 15 – 20 hours ☐

28. How do you **dress** while fishing?

Arms and legs covered ☐ Arms and legs exposed ☐

29. When are you **mostly bitten** by mosquitoes?

At home ☐ At work on the lake ☐ At work on the beach/land ☐

30. How **frequently** do you fish?

Everyday ☐ Weekdays ☐ Weekends ☐ Irregularly ☐

31. Which **fishing-related activity** apart from actual fishing do you conduct?

Net repair ☐ Boat repair ☐ Harvesting fish baits ☐ None ☐

Other (*specify*) ____________________

32. What **time** do you conduct this fishing-related activity?

Day (6am to 6pm) ☐ Night (6pm to 6am) ☐

33. **Where** do you conduct the fishing-related activity?

On the lake ☐ On land ☐

34. Do you **fish with light**?

Yes ☐ No ☐

35. What is the **source of light**?

Osram lamp ☐ Torch ☐ Pressure lamp ☐ Moon ☐

Sun ☐ Other (*specify*) ____________

Population movement

36. Are you originally **from Rusinga**?

Yes ☐ No ☐

37. How long have you **been** in Rusinga?

All my life ☐ Less than 6 months (temporary) ☐

Between 6 months and 1 year (long-term) ☐

38. How **far** into the lake do you work?

Shoreline ☐ Shallow waters ☐ Deep waters ☐

Estimate distance (meters/km) from the shoreline if not sure ______

39. Do you usually **move** from one fishing beach to another?

Yes ☐ No ☐

40. If yes, how many times in the **last 1 year** did you move from beach to beach?

____

41. Do you move between the **same fishing beaches**?

Yes ☐ No ☐

42. How **long** do you stay in one fishing beach before moving to another?

0 – 3 months ☐ 3 – 6 months ☐ More than 6 months ☐

Don’t move from beach to beach ☐

Social/Evening/Recreational activities

43. Have you engaged in any **overnight entertainment activity** over the last 2 weeks?

Yes ☐ No ☐

44. Which activity was this?

____

45. What time do you **bath**?

Day (6am to 6pm) ☐ Night (6pm to 6am) ☐

46. How **long** do you take to bath?

Less than 15 minutes ☐ More than 15 minutes ☐

47. **Where** do you bath?

At the shores ☐ In a house ☐

Use of malaria preventive measures

48. Which malaria preventive measure do you currently use?

Bednets ☐ Insecticide sprays ☐ Repellents ☐ None ☐

Clothing that covers arms and legs ☐ Other (*specify*) ________________

If the answer to Qn 41 is ***NOT*** *bednets, skip to the next section on Malaria status*

49. Who does the bed net you sleep under belong to?

Myself ☐ Owner of *abila* ☐ Do not know ☐ Do not have a bednet ☐

Other (*specify*) __________________

50. Who sleeps under the bednet in the house where you mostly sleep?

Myself ☐ Other fishermen ☐ No bednet available ☐

Other (*specify*) __________________

51. How old is that bednet?

Less than 1 year ☐ 1 to 3 years ☐ More than 3 years ☐ I do not know ☐

Do not have a bednet ☐

52. What is the condition of the bednet?

Good ☐ Poor (has holes or is torn) ☐ Do not have a bednet ☐

53. Where did you get the bednet?

Supermarket ☐ Local shop ☐ NGO ☐ Hospital ☐ Don’t remember ☐

Do not have a bednet ☐

54. What type of bednet is it?

Conical ☐ Rectangular ☐ Do not have a bednet ☐

Other (*specify*) ___________________

55. What colour is the bednet?

Green ☐ White ☐ Blue ☐ Do not have a bednet ☐

Other (*specify*) _______________________

56. How often do you sleep under the net?

Every night ☐ Sometimes ☐ Only during the rainy season ☐ Not used ☐

Do not own a bednet ☐

57. What time do you sleep or enter the bednet?

Between 6 pm and 10 pm ☐ Between 10pm and 3am ☐ Between 3am and 6am ☐

During the day ☐

Malaria status

58. What is the participants' temperature? __________

59. Presence of fever?

Yes ☐ No ☐

60. Presence of other malaria symptoms (joint pain, headache, vomiting, diarrhea, shivering, chills, sweating)?

Yes ☐ No ☐

61. Presence of malaria parasites?

Yes ☐ No ☐

62. Presence of *P. falciparum* or mixed infection?

P. falciparum ☐ Mixed infection ☐ None ☐

Use of temporary shelter called *abila*

63. Do you spend time in an *abila*?

Yes ☐ No ☐

64. What specific time do you spend in the *abila* (e.g. 5pm to 8pm) (specify)?

____

65. What activity do you conduct in the *abila* within the time you've stated?

____

66. What material is the wall of the *abila* made of (specify)?

____

67. What material is the roof of the *abila* made of (specify)?

____

68. Does the *abila* have open eaves?

Yes ☐ No ☐

69. How many people are usually in the *abila* at the same time with you?

____

70. Where are you between 6pm to 9pm during work days?

____

71. Where are you between 4am to 6am during work days?

____

72. Was the *abila* sprayed during IRS activities?

Yes ☐ No ☐

Appendix 5: Outcome journal

Outcome Journal

Boundary Partner: BMUs
Work Dating From/To:
Contributors to monitoring update:

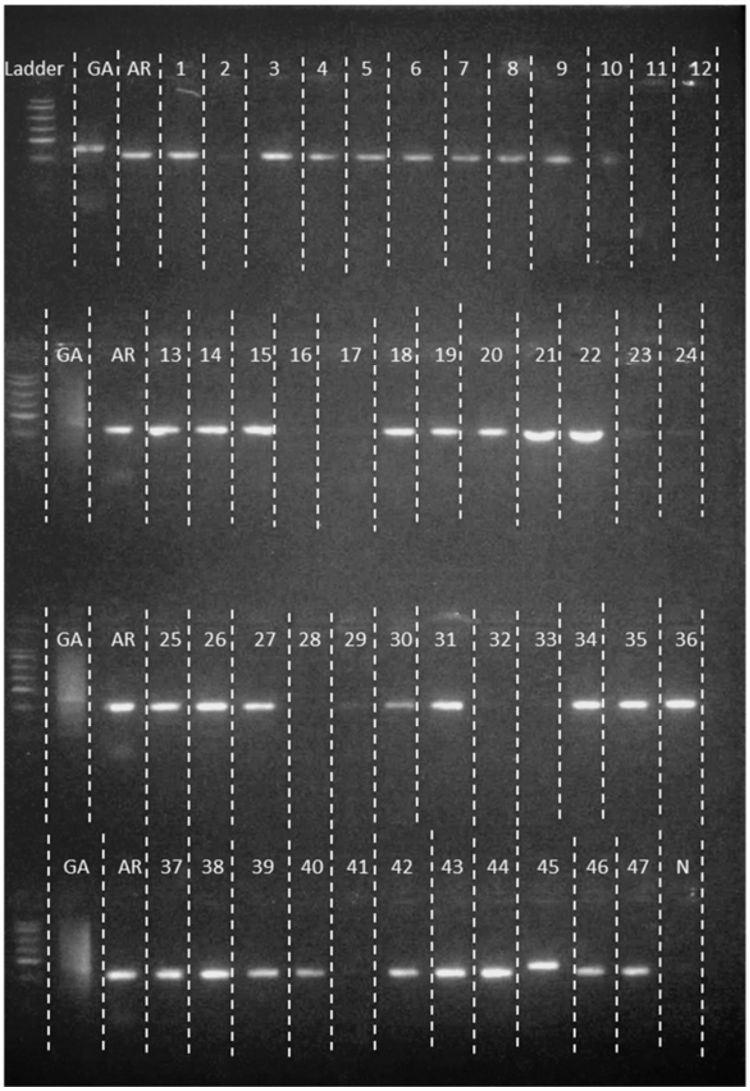
OUTCOME CHALLENGE: Members of Beach Management Unit are publicizing the problem of malaria at community level. They incorporate malaria talks into their forums where malaria and fishing-related activities are discussed and solutions debated.		
LMH **LOW = ACTIONS ARE AGAINST THE BEHAVIOUR OR THE GOAL HAS BEEN IGNORED** **MEDIUM = THE PARTNER IS PASSIVE / THERE IS NOTHING NEGATIVE TO REPORT** **HIGH = THE ACTION HAS BEEN TAKEN IN FAVOUR OF THE OUTCOME**		
EXPECT TO SEE		**Who?**
LMH		
OOO	**1** BMUs attending and actively participating in meetings conducted by the IPMA project	
OOO	**2** BMUs taking an active role in discussions related to fishing and malaria within their fishing community	
OOO	**3** Understand better/increase knowledge on the causes and transmission of malaria	
LIKE TO SEE		
OOO	**4** BMUs sharing malaria knowledge with members of BMUs from other fishing beaches	
OOO	**5** BMUs trying to influence the decisions of fishermen regarding malaria prevention and control (through meetings and individual contact)	
OOO	**6** BMU members adopting malaria control and prevention strategies	
LOVE TO SEE		
OOO	**7** BMUs willing to sustain the project even after its life span	
OOO	**8** Introduce by-laws at fishing beaches that ban fishing-related activities that promote mosquito breeding	
OOO	**9** BMUs adopting malaria control strategies in their routine fishing activities thus making it part of their culture	
Description of change:		

Contributing factors and actors:
Source of evidence:
Unanticipated change:

Lessons and required program changes/reactions:

NOTE: An outcome journal is designed for each boundary partner. Information detailing the reason for change, people and circumstances that contributed to the change, evidence of the change, a record of unanticipated change, and lessons for the program are also recorded.

Appendix 6: Photograph of an agarose gel taken in a uv transilluminator to identify sibling mosquito species



Results of PCR assay to identify sibling species of *An. gambiae* complex. Ladder refers to Hyper ladder, N refers to negative control, GA refers to positive control for *Anopheles gambiae*, AR refers to positive control for *Anopheles arabiensis*, 36 were identified as *An. arabiensis*, 3 were *An. gambiae* s.s.

Appendix 7: Published Paper 1

Olanga *et al. Parasites & Vectors* (2015) 8:250
DOI 10.1186/s13071-015-0860-z

Parasites & Vectors

RESEARCH **Open Access**

Parasites and vectors of malaria on Rusinga Island, Western Kenya

Evelyn A Olanga[1,2], Lawrence Okombo[1], Lucy W Irungu[2] and Wolfgang R Mukabana[1,2*]

Abstract

Background: There is a dearth of information on malaria endemicity in the islands of Lake Victoria in western Kenya. In this study malaria prevalence and *Plasmodium* sporozoite rates on Rusinga Island were investigated. The contribution of different *Anopheles* species to indoor and outdoor transmission of malaria was also determined.

Methods: Active case detection through microscopy was used to diagnose malaria in a 10% random sample of the human population on Rusinga Island and a longitudinal entomological survey conducted in Gunda village in 2012. Nocturnally active host-seeking mosquitoes were captured indoors and outdoors using odour-baited traps. *Anopheles* species were tested for the presence of *Plasmodium* parasites using an enzyme linked immunosorbent assay. All data were analyzed using generalized linear models.

Results: Single infections of *Plasmodium falciparum* (88.1%), *P. malariae* (3.96%) and *P. ovale* (0.79%) as well as multiple infections (7.14%) of these parasites were found on Rusinga Island. The overall malaria prevalence was 10.9%. The risk of contracting malaria was higher among dwellers of Rusinga West than Rusinga East locations (Odds Ratio [OR] = 1.5, 95% Confidence Interval [CI] 1.14 – 1.97, P = 0.003). Parasite positivity was significantly associated with individuals who did not use malaria protective measures (OR = 2.65, 95% CI 1.76 – 3.91, p < 0.001). A total of 1,684 mosquitoes, including 74 anophelines, were captured. Unlike *Culex* species, more of which were collected indoors than outdoors (P < 0.001), the females of *An. gambiae* s.l. (P = 0.477), *An. funestus* s.l. (P = 0.153) and *Mansonia* species captured indoors versus outdoors were not different. The 46 *An. gambiae* s.l. collected were mainly *An. arabiensis* (92.3%). Of the 62 malaria mosquitoes tested, 4, including 2 indoor and 2 outdoor-collected individuals had *Plasmodium*.

Conclusion: The rather significant and unexpected contribution of *P. malariae* and *P. ovale* to the overall malaria prevalence on Rusinga Island underscores the epidemiological importance of these species in the big push towards eliminating malaria. Although current entomological interventions mainly target indoor environments, additional strategies should be considered to prevent outdoor transmission of malaria.

Keywords: Malaria, Malaria prevalence, *Plasmodium falciparum*, *P. malariae*, *P. ovale*, Malaria, *Anopheles*, *Culex*, *Mansonia*, Indoor transmission, Outdoor transmission, Capture fishing, Rusinga Island, Kenya

Background

Malaria is a public health problem in Kenya despite intense deployment of vector control tools. Malaria prevalence is highest in areas around the shores of Lake Victoria in western Kenya [1,2]. The main tools used for vector control in this region include long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) [3]. *Anopheles gambiae* s.l. has been reported to be the main malaria vector in western Kenya [3-6]. The abundance and density of this vector has been clearly documented in the islands of Lake Victoria [4]. However, there is a dearth of information on the role of anophelines in indoor and outdoor transmission in the aforementioned islands. In addition, little information on malaria parasites in these islands is available. Compared to other areas in western Kenya where epidemiological studies have been conducted [5], very little is known on malaria transmission in the islands of Lake Victoria. This study

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sought to determine malaria prevalence, *Plasmodium* sporozoite rates and the contribution of different *Anopheles* species to indoor and outdoor malaria transmission on Rusinga Island, western Kenya. The association between morbidity and use of malaria preventive measures was also evaluated.

Methods

Study site

This study was conducted on Rusinga Island, which is located in Mbita sub-county in western Kenya. Rusinga Island is predominantly rural and is connected to the mainland via a causeway at Mbita Point Township. Residents of Rusinga Island rely on the neighbouring Mbita town for regional transportation, access to banking services and other urban economic services. The island covers an area of 42 km^2 and the terrain is mainly rocky and hilly. The centre of the Island is characterized by a large hill called Ligogo. Shrubs constitute the main vegetation on the island but the land has been extensively deforested. The island is divided into two administrative sections namely Rusinga East and Rusinga West sub-locations. The dominant ethnic group is *Luo* who mainly engage in artisanal capture fishing and small-scale trading [7]. Subsistence farming of drought-resistant crops is also practised. The subsistence crops cultivated on Rusinga Island include maize, vegetables, potatoes and millet. Malaria in Mbita sub-county is perennial with peaks occurring in July, shortly after the long rainy season [8]. Fishing is the main economic activity on the island and is mainly conducted by adult males. Women are mostly involved in fish processing and trading. Other fishing-related activities that are carried out on the island include boat making and fish net repair.

Malaria burden and parasite diversity on Rusinga Island

A cross-sectional malaria survey was conducted in all villages on Rusinga Island in October and November 2012 to determine malaria parasite diversity and prevalence. A sample size of 2,240 study participants was determined using the formula proposed by Daniel [9] and Naing *et al.* [10]. The selected study participants were mobilized by project staff performing home visits and requesting human research subjects to turn up at the nearest one of eight primary schools that served as sentinel malaria testing sites.

Each participant's body temperature was measured using an electronic thermometer (Hangzhou Hua'an Medical & health instruments Co. Ltd., China). Fever was defined as temperature above 37.5°C. Malaria infection status was tested microscopically using participants' finger pricked blood smears stained with 10% Giemsa. The blood smears were examined by two experienced microscopists. The first one recorded malaria positivity and identified malaria parasites to species level. The second microscopist performed quality assurance before a final result was determined. A slide was categorized as negative if no malaria parasite was seen after scanning 100 microscope fields. Individuals found positive for malaria were treated with a dose of artemether-lumefantrine (AL) according to the Kenya national guidelines for the diagnosis, treatment and prevention of malaria [11]. Malaria prevalence was calculated as the proportion of participants with malaria from the total number of individuals tested.

Gametocyte prevalence was measured to determine the level of human to mosquito malaria transmission potential. The study participants were screened for gametocytes of *P. falciparum* only. Blood smears were considered negative if no gametocyte was detected after examining 100 fields. Gametocyte prevalence was calculated as the proportion of human subjects harbouring gametocytes from the overall number tested.

A questionnaire was administered during the malaria survey to determine the association between malaria infection and use of preventive measures. The respondent's demographic characteristics and use of malaria prevention measures were correlated to their malaria infection status.

Malaria vector species and transmission potential on Rusinga Island

A longitudinal entomological survey was conducted in Gunda village in Rusinga West sub-location from June to December 2012. Nocturnally active host-seeking mosquitoes were captured and their indoor and outdoor densities and species composition determined. The mosquitoes were collected using odour-baited MM-X traps (American Biophysics, Corp., North Kingston, RI) containing a synthetic mosquito attractant known as Mbita blend [12].

The mosquitoes were collected from six randomly selected village houses spaced at least 25 metres apart [13]. The houses were similar in structure with roofs made of iron sheets, walls made of mud and open eaves. Each house had two rooms. Household members were supplied with untreated mosquito nets that were used throughout the study. Each house was fitted with one MM-X trap per night. The MM-X traps were placed above the foot end of a bed occupied by a human subject. Outdoor biting mosquitoes were collected by hanging traps within the peri-domestic environment approximately 15 cm off the ground. The MM-X traps were operated from 6 pm till 7 am the following morning. Mosquitoes were collected for four nights in a week, with alternating nights for indoor and outdoor trapping. The same houses were used throughout the study. Experiments were replicated over 72 nights, 34 nights with six traps per night indoors (i.e. 204 trap nights) and 38 nights with 6 traps per night outdoors (i.e. 228 trap nights).

Captured mosquitoes were counted, sorted by sex and identified morphologically using the keys of Gillies and DeMeillion [14]. Anopheline mosquitoes were preserved using silica gel awaiting further processing. Legs of female mosquitoes were used to identify the sibling species of *An. gambiae* s.l. using the PCR technique [15]. The head and thorax of female anopheline mosquitoes were tested for the presence of *Plasmodium* sporozoites using an enzyme linked immusorbent assay (ELISA) [16]. The infection rate of mosquitoes captured during the entomological survey was measured as the proportion of mosquitoes found to contain sporozoites.

Ethical considerations

Ethical clearance was obtained from the Kenya Ethical Review Committee located at the Kenya Medical Research Institute (NON-SSC No. 280). Consent was obtained from participants prior to the malaria survey. Written consent was sought from parents and care givers of children to allow minors to participate in the study. Consent was also obtained from heads of households that provided approval for mosquito collection in houses.

Statistical analysis

Data were analysed using R statistical software version 2.15.2. Data collected during the malaria survey were analyzed using multivariate logistic regression. The outcome of the malaria test (whether positive or negative for malaria parasites) was treated as the dependent variable. The effect of other variables, specifically sex, location and age group were also analyzed. The response variable in the entomology survey was derived from count data (mosquito numbers). The untransformed data was analyzed by fitting generalized linear models [17] with a poisson regression. The packages mass, effects, epicalc, multcomp, lme4, gee, geepack and aod [18] were loaded before running the analysis. The night mosquitoes were captured (day of experiment) was included in the model as a factor. Statistical significance was set at $P < 0.05$.

Results

Malaria burden and parasite diversity on Rusinga Island

A total of 2,318 individuals from all age groups participated in the malaria survey. The age of study participants ranged from 1 year to 102 years. The majority of the participants were between the age 0 – 14 (n = 1197, 51.7%), while the least were above 60 (n = 131, 5.7%). The median age was 14 years. Of the individuals enrolled in the study 1,263 (54.4%) were female while 1,055 (45.6%) were male (Table 1). Participants from Rusinga East were 1,248 (53.8%) while those from Rusinga West were 1,070 (46.1%).

Table 1 Characteristics of study participants of the survey carried out to determine malaria burden and parasite diversity on Rusinga Island

Variable	n (%)
Sex	
Female	1263 (54.4%)
Male	1055 (45.6%)
Age group	
0 – 14	1197 (51.7%)
15 – 30	520 (22.4%)
30 – 45	330 (14.2%)
46 – 60	140 (6%)
Above 60	131 (5.7%)
Median age	14
Location	
Rusinga East	1248 (53.8%)
Rusinga West	1070 (46.1%)
Use of malaria preventive measures	
Yes	2133 (92%)
No	185 (8%)

Overall, blood samples from 252 (10.9%) participants had malaria parasites. Three malaria species were identified namely *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium ovale*. Among the 252 individuals harboring malaria parasites 222 (88.1%) were infected with *P. falciparum*, 10 (3.96%) with *P. malariae* and 2 (0.79%) with *P. ovale*. Of the remaining 18 individuals with malaria parasites, 16 (6.34%) had mixed infections of *P. falciparum* and *P. malariae*, 1 (0.39%) had a mixed infection of *P. falciparum* and *P ovale* and 1 (0.39%) had a mixed infection of all 3 malaria species (Table 2). The overall prevalence of mixed *Plasmodium* infections was 7.14%.

Out of the total number of blood smears screened for gametocytes 16 (0.7%) participants tested positive. School-going children had the highest burden of gametocytes. Of the 16 individuals with gametocytes 11 (69%) were 0 – 14 years old, 1 (6.25%) was in the 15 – 30 year age bracket, another 1 (6.25%) in the 30 – 45 year age bracket and 3 (18.7%) were in the 45 – 60 year old age bracket (Table 3). Among the gametocyte carriers 6 (37.5%) were from Rusinga East and 10 (62.5%) from Rusinga West. There was a significant association between malaria parasitemia and location (OR = 1.5, 95% CI 1.14 – 1.97, p = 0.003) (Table 4). Individuals who did not use malaria protective measures whilst sleeping were two times more likely to get malaria (95% CI 1.76 – 3.91, $p < 0.001$) compared to those who did (Table 4).

Table 2 Malaria infection among individuals from Rusinga East and West locations

Location	N	n	Malaria parasite species					
			Pf	*Pm*	*Po*	*Pf + Pm*	*Pf + Po*	*Pf + Pm + Po*
Rusinga East	1248	120	105	6	0	9	0	0
Rusinga West	1070	132	117	4	2	7	1	1
Total	2318	252	222	10	2	16	1	1

N refers to the total number of participants; n refers to the number of individuals positive for *Plasmodium* parasites. *Pf* refers to *Plasmodium falciparum*, *Pm* to *Plasmodium malariae* and *Po* to *Plasmodium ovale*.

Malaria vector species and transmission potential on Rusinga Island

A total of 1,681 mosquitoes, including both males and females, were collected (Table 5). The species caught were *An. gambiae* s.l., *An. funestus* s.l., *Culex* species, *Mansonia* species, and *Aedes* species. Among the collected mosquitoes 79 (4.7%; constituting 74 females and 5 males) belonged to the genus *Anopheles* while 1,602 (95.3%; constituting 1,357 females and 245 males) were culicine species. Of the total female anophelines collected, *An. gambiae* s.l. was the most abundant malaria vector (n = 46; 62%). Female *An. funestus* mosquitoes accounted for 38% (n = 28) of the total female anophelines collected. Overall, *Culex* species were the most abundant non-malaria mosquitoes collected (n = 1,493).

Although a higher number of female *An. gambiae* s.l. mosquitoes were captured outdoors (n = 27) than indoors (n = 19) these catches did not differ significantly (P = 0.477). Similarly, there was no statistical difference between the number of female *An. funestus* s.l. mosquitoes captured indoors versus outdoors (P = 0.153). Of the 46 *An. gambiae* s.l. mosquitoes subjected to polymerase chain reaction (PCR) analysis, 39 were successfully identified. The rest (7 samples) failed to amplify. Of the 19 *An. gambiae* s.l. females captured indoors, 12 (92.3%) were identified as *An. arabiensis* and 1 (7.7%) as *An. gambiae* s.s. Of the 19 *An. gambiae* s.l. females captured indoors, 6 failed to amplify. Of the 27 *An. gambiae* s.l. mosquitoes captured outdoors, 24 (92.3%) were identified as *An. arabiensis* and 2 (7.7%) as *An. gambiae s.s.* Only 1 specimen of the 27 *An. gambiae* s.l. mosquitoes captured outdoors did not amplify. These data indicate that *An. arabiensis* is the dominant malaria vector among siblings of the *An. gambiae* complex on Rusinga Island.

Table 3 Proportion of participants with gametocytes in the survey carried out to determine malaria burden and parasite diversity on Rusinga Island

Variable	N	n
Age group		
0 -14	1197	11 (0.47%)
15 – 30	520	1 (0.04%)
30 – 45	330	1 (0.04%)
45 -60	140	3 (0.14%)
Above 61	131	0 (0%)
Sub-location		
Rusinga East	1248	6 (0.26%)
Rusinga West	1070	10 (0.43%)

N refers to the total number of participants; n refers to the number of individuals positive for gametocytes parasites.

Table 4 Factors associated with malaria parasitemia among residents of Rusinga Island

Variable	Odds ratio (95% CI)	p-value
Location		
Rusinga East	ref	ref
Rusinga West	1.5 (1.14 – 1.97)	0.003
Malaria preventive measures		
Yes	ref	ref
None	2.65 (1.76 – 3.91)	<0.001

Of the total 62 malaria vectors tested for the presence of *Plasmodium falciparum* sporozoites 28 and 34 were captured indoors and outdoors, respectively. Overall 4 mosquitoes were sporozoite positive of which 2 (7.14%) were captured indoors (1 *An. arabiensis* and 1 *An. funestus*) and the other 2 (5.88%) outdoors (1 *An. arabiensis* and 1 *An. funestus*). The overall sporozoite infectivity rate was 6.45% (4/62). Site-specific sporozoite rates were 7.14% for indoors and 5.88% for outdoors.

There was a significant difference in the number of *Culex* spp. captured indoors compared to those captured outdoors (P < 0.001). Among the 1,249 female *Culex* species of mosquitoes caught 665 (52.2%) were caught indoors and 585 (46.8%) were collected outdoors. No statistical difference was found between the females of *Mansonia* (P = 0.681) and *Aedes* mosquito species (P = 0.291) captured indoors and outdoors. *Culex* species yielded the largest collection of male mosquitoes (n = 244), with significantly higher numbers of the mosquitoes being collected indoors (n = 132; 54.1%) than outdoors (n = 112; 45.9%) (P = 0.038).

Discussion

In this study, single infections of *Plasmodium falciparum*, *P. malariae* and *P. ovale* as well as multiple infections of these species were observed. The overall malaria prevalence on Rusinga Island was 10.9%. The risk of contracting malaria was higher among dwellers of Rusinga West than Rusinga East locations. Parasite positivity was significantly associated with individuals who did not use malaria protective measures. The numbers of females of the main malaria vectors namely *Anopheles gambiae s.l.* (consisting largely of *An. arabiensis*) and *An. funestus* collected indoors and outdoors did not differ significantly. This was also the case with females of species of *Mansonia*. On the

Table 5 Number of mosquitoes captured inside (204 trap nights) and outside (228 trap nights) houses in Gunda village on Rusinga Island

Species	Sum of mosquitoes collected		p-value	Total
	Indoors (%)	Outdoors (%)		
An. gambiae s.l. females	19 (41.3)	27 (58.7)	0.477	46
An. gambiae s.l. males	3 (60)	2 (40)	0.499	5
An. funestus s.l. females	9 (32.1)	19 (67.9)	0.153	28
An. funestus s.l. males	0 (0)	0 (0)	1.000	0
Culex species females	665 (52.4)	584 (47.6)	<0.001	1249
Culex species males	132 (54.1)	112 (45.9)	0.038	244
Mansonia spp females	44 (45.4)	53 (54.6)	0.681	97
Mansonia species males	0 (0)	0 (0)	1.000	0
Aedes species females	7 (63.6)	4 (36.4)	0.291	11
Aedes species males	1 (100)	0 (0)	0.997	1
Total	880	801	–	1,681

contrary significantly more male and female *Culex* species were collected indoors than outdoors. Interestingly the ratios of *An. gambiae* versus *An. arabiensis* in samples collected indoors versus outdoors were similar, *albeit* with overall higher numbers of mosquitoes being collected outdoors. Similarly, the numbers, and more or less the ratios, of malaria infected mosquitoes collected indoors and outdoors were equal.

Malaria prevalence among residents of Rusinga Island was 10.9%. However, a prevalence study conducted on the Island in November 1998 revealed that the prevalence was 24.4% [8]. This suggests that there has been a reduction in the burden of malaria in the study area probably due to the increased use of LLINs [4]. However, the prevalence of malaria reported in this study is generally lower than the 40% reported by Noor et al. around the shores of Lake Victoria in 2009 [1]. Identification of malaria parasites by microscopy largely depends on the skills and experience of the microscopist [19], thus it is likely that the prevalence rate of malaria reported in this study is an underestimate.

Single infections of *P. falciparum, P. malariae* and *P. ovale* as well as multiple infections of these parasites were found in the study area. The predominant malaria species on Rusinga Island was *P. falciparum*. This finding is consistent with those of other studies conducted in Mbita sub-county [8,20,21]. *Plasmodium malariae* was reported as a minor species responsible for malaria infections on Rusinga Island. Previous studies conducted in the area also documented *P. malariae* as being responsible for single and co-infections of malaria in Mbita sub-county [20,21]. In this study, single as well as mixed infections of *Plasmodium ovale* were detected in a few individuals. Reported cases of malaria infections caused by *P. ovale* are rare and could be because of under-diagnosis or low transmission rates [22,23]. The parasite is known for its low densities [24,25] which contribute to difficulties in diagnosis. Furthermore, the greatest shortcoming of the diagnostic technique used in this study i.e. microscopic examination of patients' peripheral blood smears stained with Giemsa is low sensitivity. This may explain the small number of individuals diagnosed with *P. ovale* malaria in the study area. Cases of *P. ovale* infections have been reported in other areas of western Kenya [26,27].

Malaria burden was found to be unevenly distributed with individuals from Rusinga West location recording more malaria cases. This can be attributed to the higher number of malaria mosquito breeding sites found in Rusinga West compared to Rusinga East (Mukabana, unpublished data). Most of the breeding sites recorded in Rusinga West were man-made [28] and created to sustain livelihood activities. With regards to outdoor fishing activities, more fishing activities are conducted in Rusinga West compared to East. A higher number of fishing beaches engaged in nocturnal fishing activities are found in Rusinga West (five) compared to East (three).

It is not surprising that the gametocyte prevalence detected by microscopy was as low as 0.7%. A study conducted in Mbita hospital, less than two kilometres from Rusinga Island reported a 0.9% prevalence of gametocytes among patients seeking outpatient services [21]. Gametocytes have been known to be low in densities thus can easily go undetected when screened microscopically [29]. Of the total parasite load in an infected person, the proportion of gametocytes has been reported to be 0.2% and 5.7% in children and adults, respectively [30]. Gametocytes are the sexual stages of the malaria parasite responsible for transmission from human to mosquito. This study shows that a small subpopulation of humans is infective and may be responsible for maintaining malaria transmission on Rusinga Island. It is important to note that this infective subpopulation may be higher if the presence of gametocytes is detected using molecular techniques.

This study demonstrated that individuals who did not use malaria protective measures whilst sleeping indoors at night were at a higher risk of malaria. Similar findings have been reported in other parts of Africa [31,32]. The majority of the adult population on Rusinga Island are fishermen who engage in nocturnal outdoor activities and therefore hardly use LLINs consistently. A review conducted by Pulford *et al.* [33] documented several reasons why people do not sleep under LLINs. Spending time elsewhere, for instance, at the work place at night, was cited as a reason for not using an LLIN even when one was available [33]. An increase in outdoor biting has been reported in several areas in sub-Saharan Africa [34-36]. Although approximately 80% of malaria transmission occurs indoors [37], outdoor malaria transmission is still important. Indoor vector control interventions are effective and have been reported to reduce malaria transmission in several areas [38-40], but are insufficient and extra effort is required to eliminate malaria. This should be especially applied in settings with intense regular nocturnal outdoor human activities. Since malaria transmission occurs where mosquitoes bite humans, vector control strategies need to be developed that also target outdoor biting mosquito populations [41] in order to achieve malaria elimination [42-45].

The lack of a statistical difference between the numbers of female malaria vectors collected indoors and outdoors, with higher numbers of the mosquitoes being collected outdoors than indoors, underscores the epidemiological importance of understanding and investing in outdoor transmission control in the current big push towards eliminating malaria [46]. It is likely that *An. arabiensis* and *An. funestus* are responsible for transmitting malaria indoors and outdoors in the study area. *Anopheles arabiensis* is known to be opportunistic, preferring to feed outdoors [47] on humans and animals depending on availability [48]. Several studies have also reported *An. arabiensis* as being partially responsible for indoor malaria transmission in several areas [49-52]. *Anopheles funestus* s.s. Giles has been identified as the main vector among siblings in the *An. funestus* group found in Mbita sub-county [4]. This vector prefers to rest indoors and feed on humans [53,54].

The findings of this study corroborate those of Futami *et al.* [4] who reported that *An. arabiensis* had replaced *An. gambiae* as the main malaria vector in the study area. Similar cases of species shifts among populations of malaria vectors have been reported elsewhere in Kenya [3,55,56] and Tanzania [57]. The species shifts among *An. gambiae* and *An. arabiensis* populations were reported to occur after wide coverage, ownership, and use of LLINs [3,55]. *Anopheles arabiensis* has been known to survive in areas with wide coverage of both LLINs and Indoor Residual Spraying (IRS) [5,34,56]. Killeen [46] indicated that *An. arabiensis* mosquitoes have adopted a behaviour that is instrumental in avoiding prolonged exposure to insecticides. These malaria vectors exit houses immediately after entry if a human host is sleeping under an LLIN [46], suggesting that the mosquito either enters another house or searches for a blood meal host outdoors. This particular behaviour has been reported in several studies [58-60].

Interestingly the ratios of *An. arabiensis* versus *An. funestus* in samples collected indoors versus outdoors were similar. This touches on the lack of bias of the sampling tool for indoor and outdoor collections. A possible explanation is that since outdoor collections were done on the outer walls of houses, we were dealing with the same mosquito subpopulations. The finding that no significant difference was found in density of *An. arabiensis* and *An. funestus* found indoors and outdoors is most likely explained by the few number of mosquitoes captured. It is statistically difficult to show differences in indoor and outdoor mosquito densities when general mosquito densities are low. It is unlikely that the low density of anopheline mosquitoes captured on Rusinga Island reflects a lack of efficacy of the MM-X trap as a sampling tool. The trap has been successfully used in sampling wild mosquitoes in sub-Saharan Africa [12,61-63]. More specifically, MM-X traps baited with the Mbita blend (as used in this study) have been shown to trap high numbers of *Anopheles* mosquitoes in other areas of western Kenya [12]. Also worth noting is that although there were few anophelines the numbers of culicine mosquitoes trapped in this study were high, indicating good sampling efficacy of the MM-X traps.

High densities of *Culex* mosquito species were recorded in this study. Other non-malaria vectors captured included *Mansonia* and *Aedes* spp. The presence of these mosquitoes indicates the potential transmission of arboviruses in Mbita sub-county. However, arboviral diseases have not been diagnosed in health facilities in Mbita sub-county, probably due to lack of diagnostic tools. We posit that the aforementioned non-malaria vectors are involved in nuisance biting in the area. One point is that targeting *Anopheles* species and leaving out culicine species may reduce acceptance of malaria control interventions among target communities because of the continuing biting menace from the unaffected *Culex* population [64-66].

It was interesting to note that the numbers and more or less the ratios, of malaria infected mosquitoes collected indoors and outdoors were equal. Malaria mosquitoes mainly bite at night when humans are asleep

[47]. Thus, individuals who use indoor mosquito control tools are presumably protected from malaria infective bites. The front-line malaria vector control interventions specifically IRS and LLINs are effective indoors, which is a major space for insecticidal exposure [37]. These indoor interventions do not protect individuals who spend a significant part of their time outdoors and at night. This is the case of the capture fishing community of Rusinga Island where, in addition, the local malaria vectors prefer to blood-feed at night [4,47,67-69]. Huho and others [37] indicate that human behaviour is an important determinant in the place where malaria transmission occurs and it is strongly argued that outdoor transmission persists in areas with intense nocturnal outdoor activities [46,70]. The convergence between outdoor nocturnal fishing activities, preference for night-biting by local malaria vectors and the similarity in sporozoite rates between malaria mosquitoes collected indoors and outdoors exemplifies the vicious cycling of the disease in rural Africa.

Malaria mosquitoes on Rusinga Island were found to be low in density. This may be explained by climatic conditions, particularly fluctuating rainfall intensity experienced in recent years in the study area. The low adult mosquito density has previously been reported by Futami *et al.* [4] who compared densities of *An. gambiae* s.l. females in selected years from 1999 to 2010. Futami and others [4] reported a 95% decline in densities of *An. gambiae* s.l. which was attributed to an increase in bednet coverage. Evidence collected in the study reported herein implies that a small population of malaria vectors is responsible for malaria cases on Rusinga Island. Small populations of malaria vectors can sustain high malaria transmission in endemic areas [71]. Low densities of malaria vectors also imply that malaria vectors on Rusinga Island are highly efficient and maintain stable malaria transmission throughout the year. Elimination of malaria from Rusinga Island will require an increase in the use of interventions that target indoor transmission as well as novel interventions to control outdoor transmission.

Conclusion

The rather significant and unexpected contribution of *P. malariae* and *P. ovale* to the overall malaria prevalence on Rusinga Island underscores the epidemiological importance of these species in the big push towards eliminating malaria. Although current entomological interventions mainly target indoor environments, additional strategies should be invented to prevent outdoor transmission of malaria.

Abbreviations

CI: Confidence interval; CSA: circumsporozoite antibodies; ELISA: Enzyme-linked immunosorbent assay; HDSS: Health Demographic Surveillance System; IRS: Indoor residual spraying; MM-X: Mosquito magnet-x trap; PCR: Polymerase chain reaction; OR: Odds ratio.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

EAO, LWI and WRM conceived and designed the experiments. LO was instrumental in the acquisition of data. EAO and WRM analysed the data and wrote the manuscript. All authors read and approved the final manuscript.

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