# Indoor and outdoor biting behaviour of malaria vectors and the potential risk factors that enhance malaria in southern Malawi

Monicah M. Mburu

**Propositions**

1. A house with fully closed eaves reduces house entry by malaria vectors more than one with open eaves.
   (this thesis)

2. The biting activity of malaria vectors during the evening hours, when people are not yet protected by bed nets, enhances the risk of malaria transmission.
   (this thesis)

3. Multidisciplinary research teams are complex but of central importance for the elimination of diseases.

4. Agricultural subsidy programmes widen the gap between the rich and the poor.

5. Transactional relationships impede the fight against HIV/AIDs.

6. Structural adjustment economic policies for developing countries, although essential for broader economic development, are drivers of poverty.

7. The use of mobile phones promotes literacy in communities where such levels are otherwise low.

Propositions belonging to the thesis, entitled:
'Indoor and outdoor biting behaviour of malaria vectors and the potential risk factors that enhance malaria in southern Malawi'.

Monicah Mirai Mburu

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**Monicah M. Mburu**

**Thesis committee**

**Promotor**

Prof. Dr W. Takken

Personal chair at the Laboratory of Entomology

Wageningen University & Research

**Co-promotor**

Dr R.S. McCann

Researcher, Laboratory of Entomology

Wageningen University & Research

**Other members**

Prof. Dr J.E. Kammenga, Wageningen University & Research

Prof. Dr R.W. Sauerwein, Radboud University, Nijmegen

Dr M.M. van den Berg, Wageningen University & Research

Dr F.T. Muijres, Wageningen University & Research

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# Indoor and outdoor biting behaviour of malaria vectors and the potential risk factors that enhance malaria in southern Malawi

**Monicah M. Mburu**

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Monicah M. Mburu

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# Chapter 1

# General introduction

Chapter 1

## Introduction

Malaria continues to place a huge burden in communities living in malaria-endemic areas. In 2017, of the estimated 219 million malaria cases that occurred globally, 92% of them were in Africa (WHO 2018). Of the estimated 435,000 malaria deaths, the majority occurred in Africa. The primary malaria control measures target either the parasites or the vectors. Whereas treatment of malaria with drugs targets the parasites, vector control involves the use of long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS). These measures have significantly reduced malaria prevalence (Bhatt et al. 2015). Despite this recent gain in malaria control, the use of some of these measures such as IRS has declined in some regions (WHO 2018), most likely due to the resistance of mosquitoes to the insecticides used when spraying. Furthermore, a similar challenge applies to LLINs such that resistance of mosquitoes to these insecticides (Ranson et al. 2011, Strode et al. 2014, Hemingway et al. 2016) raises concern on the effectiveness of these tools for malaria control. As a result, residual transmission of malaria can be maintained by mosquitoes that avoid contact with insecticides by either exiting early from the houses or feeding and resting outdoors. Increasing rates of outdoor biting by malaria vectors have been observed in some regions (Reddy et al. 2011, Russell et al. 2011, Meyers et al. 2016). Residual transmission, outdoor biting and insecticide resistance may have an implication on malaria transmission. Therefore, there is a need for assessing the biting behaviour of malaria vectors to understand the dynamics of malaria transmission in regions. My Ph.D. study aimed at investigating the biting behaviour of malaria vectors in and around houses, and the underlying factors that promote malaria transmission for the purpose of understanding malaria epidemiology, residual transmission and improving malaria control strategies in Malawi. The combined knowledge is expected to help in designing interventions which protect against infectious bites outdoors and

impede vector access to houses. As a result, individuals will be protected from infective mosquito bites, leading to reduced malaria incidence and prevalence.

**Malaria parasites**

Human malaria, transmitted by *Anopheles* mosquitoes when they are biting, is caused by single or multiple infections of *Plasmodium* species namely *Plasmodium falciparum, P. ovale, P. vivax, P. malariae and P. knowlesi* (Cox-Singh et al. 2008, Lee et al. 2009, Krief et al. 2010). *Plasmodium falciparum* is the most widespread species, especially in sub-Saharan Africa, causing severe clinical complications (WHO 2018). *Plasmodium vivax* and *P. knowlesi* are prevalent in Southeast Asia (Cox-Singh et al. 2008, Lee et al. 2009) and *P. ovale* is most common in Africa but also prevalent in the western Pacific and the Asian mainland. *Plasmodium malariae*, which shares geographical coverage with *P. falciparum*, is irregular in prevalence (Collins and Jeffery 2005).

**Malaria vectors and transmission**

Human malaria parasites are transmitted by female mosquitoes of the genus *Anopheles*. Out of about 460 species of *Anopheles* mosquitoes, 70 are known to transmit malaria (White 1974). In Africa, the *Anopheles gambiae* sensu lato (s.l.) complex and *Anopheles funestus* group contain the most significant malaria vectors. The eight sibling species belonging to the *An. gambiae* s.l. complex include *An. gambiae* sensu stricto*, An. coluzzii, An. arabiensis, An. melas, An. merus, An. bwambae, An. quadriannulatus* (White 1974, Coetzee et al. 2000, Coetzee 2004, Coetzee et al. 2013) and *An. amharicus* (Hunt et al. 1998, Coetzee et al. 2013).

Malaria parasites require two hosts namely the vertebrate and invertebrate host to complete their lifecycle. The invertebrate host, a female mosquito from the genus *Anopheles*, uses visual,

chemical and physical cues to locate its (vertebrate) host for a blood meal (Sutcliffe 1987, Takken 1991, Takken and Knols 1999). Blood-feeding by female malaria mosquitoes is essential for egg maturation (Clements 1999) and transmission of malaria parasites. It's during the blood-feeding period that female malaria mosquitoes ingest sexual-stage *Plasmodium* parasites (gametocytes) from an infected host. The ingested gametocytes undergo fertilization in the mosquito's midgut, transform to ookinetes, and then into oocysts. The latter produce the infective stages, the sporozoites, which migrate to the salivary glands of the mosquito. Mosquito species are considered competent malaria vectors if they frequently contain sporozoites, feed on humans and are more abundant than others (Kiszewski et al. 2004). Upon the bite by a *Plasmodium*-infective mosquito, the injected sporozoites get into the liver. Here, the sporozoites produce merozoites, which in turn infect the red blood cells. The parasites reproduce asexually causing the bursting of cells, which culminates into symptoms of malaria such as general malaise and fever (Warrell and Gilles 2002). Some of these parasites develop into gametocytes which may be taken up by other female mosquitoes during blood-feeding thereby sustaining the cycle of malaria transmission.

**Feeding and resting behaviour of malaria vectors**

The most effective and efficient malaria vectors in Africa are *An. gambiae* s.s. and *An. funestus* (Sinka et al. 2010, Sinka 2013), largely due to a high degree of anthropophagy (the tendency to feed on human blood) (Gillies and De Meillon 1968, Highton et al. 1979, Githeko et al. 1994, Githeko et al. 1996b, Antonio-Nkondjio et al. 2002, Awolola et al. 2003, Mwangangi et al. 2003, Wanji et al. 2003, Temu et al. 2007, Dabire et al. 2008, Seyoum et al. 2012, Dadzie et al. 2013). *Anopheles arabiensis* is often seen as a less efficient vector than *An. gambiae* s.s. and *An. funestus* because of the higher plasticity in blood meal hosts utilized by this species (Takken and Verhulst

2013), but it is still a primary malaria vector across many regions of the continent, particularly since the introduction of LLINs (Sinka 2013).

*Anopheles gambiae* s.s. is highly endophilic (Highton et al. 1979, Mnzava et al. 1995, Githeko et al. 1996a, Faye et al. 1997) although some exophily has also been reported (Bockarie et al. 1994, Mahande et al. 2007a). Likewise, *An. funestus* is generally endophilic (Gillies 1954, Mnzava et al. 1995, Githeko et al. 1996a) although exophily has been reported (Fontenille et al. 1990). In contrast, *An. arabiensis* is mainly exophilic (Highton et al. 1979, Fontenille et al. 1990, Mnzava et al. 1995, Tirados et al. 2006, Mahande et al. 2007a) but some endophily has also been reported (Ameneshewa 1996, Faye et al. 1997). Similar to the association between anthropophily and vectorial capacity, the habit of *An. gambiae* and *An. funestus* to rest inside human dwellings enhances their efficiency in transmitting the malaria parasites (Beier 1996, Costantini et al. 1999, Takken and Knols 1999, Antonio-Nkondjio et al. 2002, Wanji et al. 2003, Cano et al. 2004, Sinka et al. 2010).

**Vector control and its challenges**

In 2007, the Bill and Melinda Gates Foundation announced a roadmap for the eradication of malaria (Gates 2007), a move that was supported by WHO and the Roll Back Malaria Partnership (RBM). The Global Malaria action plan was formulated in 2008 by RBM, which set out a strategy for reducing the malaria burden by (a) controlling malaria through "scaling up for impact" (SUFI) existing tools such as anti-malarial drugs to treat malaria cases, LLINs and IRS to reduce vector abundance; (b) eliminating malaria; and (c) research into new tools and approaches geared towards reducing disease transmission (RBM 2008). The use of LLINs and IRS has reduced malaria prevalence (Bhatt et al. 2015). However, the use of LLINs and IRS

targets malaria vectors that feed and rest indoors, but some portion of vectors may feed and rest outdoors, limiting the effectiveness of LLINs or IRS on these vectors. In addition, the indoor biting vectors may develop behavioural changes by exiting early from the houses to avoid contact with the insecticides. Resistance to insecticides has also been reported (Chandre et al. 1999, Ranson et al. 2011, Strode et al. 2014, Hemingway et al. 2016), which is likely to affect the effectiveness of LLINs and IRS leading to an upsurge in malaria cases (Maxmen 2012).

Other vector control strategies exist. These include larval source management, a combination of techniques with the ultimate purpose of preventing the development of immature mosquitoes into adults (Imbahale et al. 2012, Tusting et al. 2013). For example, biological control using predatory fish such as *Gambusia affinis* and *Tilapia nilotica* (Baird and Girard 1853, Howard et al. 2007) to prey on mosquito larvae has been employed to reduce malaria mosquito abundance, but this may not be effective due to lack of trained personnel on rearing of the fishes. Larviciding can also be employed to reduce the immature stages of malaria mosquitoes (Fillinger and Lindsay 2006, Bukhari and Knols 2009, Mbare et al. 2014), though questions remain on best practices for implementation. Therefore, my study focused on understanding the biting patterns of malaria vectors in terms of peaks and sites, and whether the presence of cattle near human domiciles would provide a zoophylactic effect or not against bites by malaria vectors. This knowledge will help in developing effective interventions for malaria control in southern Malawi.

**Biting patterns of malaria vectors**

The blood-feeding patterns, resting behaviour of malaria mosquitoes and human activities/behaviour are important for understanding host-vector relationship, dynamics of

disease transmission and development of malaria control strategies (Singh et al. 1998, Chaves et al. 2010). The risk of getting infected with malaria parasites can be estimated using various methods. For instance, human exposure to infective mosquitoes (Beier 1998). The human landing catch (HLC) is the most direct method of estimating human exposure to mosquito bites (Lines et al. 1991, Service 1993a, Davis et al. 1995, Kline 2006, Govella et al. 2010) because mosquitoes are caught as they land on humans. The biting times and preference for biting indoors or outdoors varies among mosquito species and across regions. These behaviours may also change over time in response to vector control measures such as LLINs (Reddy et al. 2011, Russell et al. 2011, Moiroux et al. 2012b). Therefore, assessing the biting patterns of malaria vectors and sites at which most biting occur is important because data on these parameters can provide the sites and times at which different interventions would be effective for vector control.

Furthermore, entomological monitoring is important for public health. Available tools for entomological monitoring, although effective, may be biased towards or specifically target certain portions of a mosquito population (e.g. host-seeking females or resting mosquitoes). Therefore, it is also important to understand the strengths and weaknesses of any sampling method to determine whether it is appropriate for addressing a specific question about the behaviour of malaria vectors (Mboera et al. 1998, Mboera 2005).

**Factors affecting the abundance of malaria vectors both indoors and outdoors**

Malaria transmission varies across regions (Beier 1998). The variation may be attributed to the distribution of major malaria vectors. The different vector species are mostly characterized by seasonal and geographical patterns and land use (Coluzzi et al. 1979, Coluzzi et al. 1985, Touré et al. 1994, Toure et al. 1996, Lindsay et al. 1998, Bayoh et al. 2001, Minakawa et al. 2002,

Herrel et al. 2004, Afrane et al. 2012, Moiroux et al. 2012a, Bashar and Tuno 2014). For instance, at relatively broad scales, *An. gambiae* and *An. arabiensis* can be characterized by levels of aridity (Lindsay et al. 1998, Coetzee et al. 2000) and by the ability to survive in certain altitudes, temperatures and humidity, though the two species are also sympatric in many regions. Warm temperatures and moist climate favour the abundance of *An. gambiae* and *An. funestus* (Lindsay et al. 1998, Minakawa et al. 2002, Siraj et al. 2014). At relatively finer scales (e.g. within a village), high incidence of malaria has been associated with housing conditions such as open eaves, grass-thatched roofs, nearby irrigated land and tethering livestock inside houses, all of which likely increase the abundance of malaria vectors (Lindsay et al. 1995, Ghebreyesus et al. 2000, Lindsay et al. 2002, Yé et al. 2006, Mutuku et al. 2011, Temu et al. 2012, Animut et al. 2013).

The association of livestock with malaria transmission remains debatable, but differences among vector species in host preference have been well documented (Takken and Verhulst 2013). Whereas *An. gambiae* and *An. funestus* are highly anthropophagic, *An. arabiensis* is quite plastic in its feeding behaviour, readily feeding on cattle in addition to humans (Ralisoa and Coluzzi 1987, Fontenille et al. 1992, Habtewold et al. 2004, Mahande et al. 2007a). WHO (1982) proposed that the presence of cattle around human dwellings would provide a prophylactic effect against biting by malaria vectors. This has been supported by various reports (Mahande et al. 2007b, Yamamoto et al. 2009, Franco et al. 2014, Donnelly et al. 2015, Massebo et al. 2015) but refuted by many authors from studies conducted in Pakistan (Bouma and Rowland 1995), The Gambia (Bøgh et al. 2001, Bøgh et al. 2002) and Ethiopia (Tirados et al. 2011). Because of these different results, more studies are highly recommended to evaluate the host preferences of

dominant malaria vectors in a region and the distances at which livestock should be kept to promote zooprophylaxis or prevent zoopotentiation (Donnelly et al. 2015).

**Improved housing to reduce malaria transmission**

One of the major sites of contact between humans and night-biting mosquito vectors is a house (Snow 1987, Gamage-Mendis et al. 1991). Most people infected with malaria acquire the infection indoors from mosquito vectors that entered the house through open eaves, which are the most preferred entry points for malaria mosquitoes (Snow 1987, Lindsay and Snow 1988, Njie et al. 2009). Poor house designs have been associated with increased numbers of mosquitoes into houses and higher levels of malaria (Lindsay et al. 2002, Lindsay et al. 2003, Mutuku et al. 2011, Wanzirah et al. 2015). Thatch roofs and mud walls provide better resting surfaces and more entry points to malaria mosquitoes (Ghebreyesus et al. 2000, Kirby et al. 2008). Various studies have tested the effect of improved housing using different materials to block mosquito entry such as nettings, ceilings (Atieli et al. 2009, Ogoma et al. 2009, Kampango et al. 2013) and sand, rubble and cement (Kirby et al. 2008). The results of house improvement showed significant reductions in malaria risk, which were attributed to fewer mosquitoes entering indoors, and hence, fewer mosquito bites. Therefore, structural house improvement (e.g. closed eaves and screened windows) is an established method of reducing mosquito entry. It could be complementary to other interventions such as LLINs for malaria control because the nets cover and protect all individuals in a house equally. However, when implemented at a large scale, house improvement may not be employed optimally. It is therefore critical to assess whether partial house improvement will have any effect on house entry by malaria vectors.

## This thesis

This thesis describes the biting behaviour of malaria mosquitoes in and around houses. Data on where most human-vector contact takes place, biting times of malaria mosquitoes and feeding behaviour of malaria vectors will help in understanding whether malaria is maintained by the vectors that either bite indoors, outdoors or on both locations. In addition, biological factors need to be explored to better understand the dynamics of malaria transmission and potential challenges for current malaria control interventions. Biological factors such as the presence of livestock in human domiciles may enhance biting by mosquitoes due to the availability of blood meal hosts which may have a negative effect on malaria control. In general, the study investigated the natural behaviour of malaria vectors such as host preference, indoor and outdoor biting and the house entry behaviour in southern Malawi. The work described in this thesis was based on the following specific primary objectives:

1. To assess whether indoor and outdoor biting malaria mosquitoes differ in behaviour relevant for malaria transmission.
2. To assess the impact of cattle on the resting behaviour of malaria vectors.
3. To evaluate the impact of fully and partially closed eaves on house entry by malaria vectors.

**Chapter 2** provides an overview of the methods of assessing the biting behaviour of malaria vectors, with emphasis on African malaria vectors, both indoors and outdoors, historical and contemporary evidence for species-level preference between indoor and outdoor biting, factors associated with variations in biting behaviour, and the current evidence for population-level changes in biting behaviour due to vector control interventions.

**Chapter 3** assesses the biting patterns of malaria vectors in southern Malawi. The preference for biting either indoors or outdoors is evaluated as well as the peaks in the biting times during the wet and dry seasons. The entomological inoculation rates are also calculated. Implications for malaria control are provided following the findings whereby, most biting occurred at times when people may be still awake and physically active, unprotected by bed nets. A considerable amount of biting also occurred outdoors. Therefore, recommendations for targeting these biting activities are provided.

**Chapter 4** evaluates the use of the Suna trap, an odour-baited trap, in sampling mosquitoes both indoors and outdoors. The efficiency of this trap in sampling mosquitoes is compared to that of the human landing catch method (HLC) and the Centers for Disease and Prevention Light Trap (CDC-LT). The simultaneous use of the Suna trap indoors and outdoors to sample mosquitoes is also assessed.

**Chapter 5** evaluates the impact of cattle on the resting behaviour of malaria vectors. The effect of cattle presence/absence (around houses) on malaria vectors resting indoors and outdoors is assessed. The distance at which cattle are placed around houses is considered to further assess whether this may have an effect on malaria vectors resting indoors and outdoors. The blood-fed malaria vectors are analysed to determine the hosts that the vectors fed on.

**Chapter 6** assesses the impact of fully and partially closed eaves on house entry by mosquitoes. House entry by malaria vectors is compared in houses with fully closed eaves, open eaves and three levels of partially closed eaves by use of a CDC-LT.

**Chapter 7** discusses the outcomes of this thesis which should guide the future vector-control strategies in southern Malawi.

## Chapter 2

# Indoor and outdoor biting behaviour of the human malaria vectors *An. gambiae* s.s., *An. coluzzii*, *An. arabiensis* and *An. funestus* s.s. in Africa – a Review

Monicah M. Mburu, Themba Mzilahowa, Alexandra Hiscox, Willem Takken, Robert S. McCann

To be submitted in a modified form

Chapter 2

**Abstract**

Knowledge of the biting behaviour of malaria vectors is crucial in understanding the role of the vectors in disease transmission. This helps in the development and implementation of effective tools for vector control. In recent years, it is increasingly reported that the introduction of area-wide vector control by long-lasting insecticide-treated bed nets (LLINs) and indoor residual spraying (IRS) may have caused shifts in vector behaviour. Here, we review available knowledge on anophelines biting behaviour with respect to its temporal and spatial aspects. We discuss methods of assessing the biting behaviour of malaria vectors, with emphasis on African malaria vectors, both indoors and outdoors, historical and contemporary evidence for species-level preference between indoor and outdoor biting, factors associated with variations in biting behaviour, and the current evidence for population-level changes in biting behaviour due to vector control interventions. We searched two electronic databases (Web of Science and PubMed) to retrieve studies (published from 1900 to 2018) that assessed the biting behaviour of the malaria mosquitoes *Anopheles gambiae* s.s., *An. coluzzii*, *An. arabiensis* and *An. funestus*, both indoors and outdoors and the methods that were applied to collect the mosquitoes. Additionally, studies with potential factors that may have influenced the biting patterns of the vectors were also retrieved. It is evident that the malaria vectors were biting both indoors and outdoors before the implementation of vector control. Additionally, variation in biting behaviour is common, with respect to both geographic locations and times. Whereas it is difficult to interpret whether the prolonged use of the LLINs and/or IRS have had an effect on the biting times of mosquitoes as collections were not performed before the implementation of vector control in many studies, an increasing volume of work has documented variations in biting behaviour, from predominantly indoors to indoors and outdoors, especially for *An. gambiae* s.l., and from entirely nocturnal, to

early evening and/or morning biting. Additionally, the biting behaviour of malaria vectors may be influenced by the availability of potential hosts in different locations. African malaria vectors, with a high degree of anthropophily, feed primarily indoors due to the availability of (human) hosts. However, variations in this behaviour are observed, where biting can occur both indoors and outdoors, and during a wider range of times. The area-wide introduction of LLINs and IRS may have affected this behaviour, as variations in behaviour have been found across Africa. Such behaviours would have a negative effect on malaria transmission in a region. Therefore, entomological monitoring to assess vector biting behaviour is essential for planning vector control interventions. Furthermore, the development of tools that can protect individuals from the early biting both indoors and outdoors is highly recommended.

**Keywords:** Malaria mosquitoes**,** Biting times, *Anopheles gambiae, Anopheles coluzzii, Anopheles arabiensis, Anopheles funestus,* LLINs, IRS, Hosts, Indoors, Outdoors.

## Introduction

Malaria continues to place a heavy burden on communities living in malaria-endemic areas, claiming thousands of individual lives per year (WHO 2018) and holding back economies (Drake and Lubell 2017). Trials conducted in the 1980-1990s demonstrated that the use of insecticide-treated nets or curtains (ITNs) can reduce malaria-related mortalities in Africa (Snow et al. 1988, Alonso et al. 1991, Lyimo et al. 1991, D'Alessandro et al. 1995, Binka et al. 1996, Nevill et al. 1996, Habluetzel et al. 1997, Diallo et al. 1999). Later on, there was a switch to the use of long-lasting insecticidal nets (LLINs), which led to the development of policies that steered the move for universal coverage of households with LLINs (Gates 2007, RBM 2008, 2015a, WHO 2015).

The use of ITNs and LLINs, together with indoor residual spraying (IRS) and treatment with artemisinin-based combination therapy, have significantly reduced *Plasmodium falciparum* infection prevalence and the incidence of clinical malaria in Africa since 2000 (Bhatt et al. 2015). The success of ITNs and LLINs is underpinned by the protection from infectious mosquito bites provided to net users and the reduction in mosquito population size caused by sufficient contact of mosquitoes with the insecticide in the nets. The high degree of endophily and endophagy exhibited by the dominant African malaria vectors has been, therefore, a key component of that success.

However, in recent years an increasing volume of work has documented biting behaviour of the African malaria vectors both indoors and outdoors, and during a wider range of times than previously recognized (Reddy et al. 2011, Russell et al. 2011, Sougoufara et al. 2014, Meyers et al. 2016). Furthermore, increasing levels of resistance of malaria vectors to the insecticides used on bed nets and for IRS have been reported (Chandre et al. 1999, Ranson et al. 2011, Hemingway et al. 2016, Ranson and Lissenden 2016) , which are associated with the widespread use of LLINs and IRS. With these developments, the long-term effectiveness of LLINs and IRS may be limited.

Here, we review the indoor and outdoor biting behaviour of malaria vectors in Africa, with a focus on the four most common species: *Anopheles gambiae* s.s. Giles, *An. coluzzii* Coetzee & Wilkerson, *An. arabiensis* Patton and *An. funestus* Giles. We discuss the feeding and resting behaviours of malaria vectors, historical and contemporary evidence for species-level preference between indoor and outdoor biting, methods of assessing the biting behaviour of malaria vectors, both indoors and outdoors, factors associated with variations in biting behaviour, and the current evidence for population-level changes in biting behaviour due to vector control interventions.

Chapter 2

**Search methodology**

We searched two electronic databases (Web of Science and PubMed) to retrieve studies (published from 1900 to 2018) that assessed the biting behaviour of malaria mosquitoes both indoors and outdoors and the methods that were applied to collect the mosquitoes. As search terms we used: ((mosquito OR mosquitoes AND malaria AND indoors OR indoor AND outdoors OR outdoor AND biting OR bite AND ITNS OR ITN AND LLIN OR LLINs AND shift OR shifts AND change OR changes AND Africa AND Sahara AND treated AND untreated)). Additionally, studies with potential factors that may have influenced the biting patterns of the vectors were also retrieved.

**Blood-feeding behaviour of African malaria vectors**

Feeding behaviour of malaria vectors comprises of sugar and blood-feeding. Whereas sugar feeding provides energy reserves for both male and female mosquitoes' survival, flight activity and reproduction, female mosquitoes require blood, which provides nutrients that are necessary for the maturation of their eggs (Clements 1999). After a complete blood-meal, the host-seeking behaviour ceases and the mosquitoes rest for the eggs to mature. After egg maturation, the mosquitoes search for a suitable site to lay their eggs and the cycle continues again.

Behaviour related to finding a blood meal host (host-seeking) is usually driven by olfactory cues given off by an individual host (Lehane 1991, Smallegange and Takken 2010). Additionally, at close range, the host-seeking behaviour of mosquitoes is also driven by visual cues, heat and moisture (Takken 1996). The most effective and efficient malaria vectors in Africa are *An. gambiae* s.s. and *An. funestus* (Sinka et al. 2010, Sinka 2013), largely due to high anthropophily (Gillies and De Meillon 1968, Highton et al. 1979, Githeko et al. 1994, Githeko et al. 1996b, Antonio-Nkondjio et al. 2002, Awolola et al. 2003, Mwangangi et al. 2003, Wanji et al. 2003,

Temu et al. 2007, Dabire et al. 2008, Seyoum et al. 2012, Dadzie et al. 2013). *Anopheles arabiensis* is often seen as a less efficient vector than *An. gambiae, An. coluzzii* and *An. funestus* because of the higher plasticity in blood meal hosts utilized by this species (Takken and Verhulst 2013), leading to an opportunistic feeding behaviour. In most cases, the fitness of a vector is enhanced when the vector feeds frequently on human blood (Scott et al. 1997, Scott and Takken 2012). For instance, it has been suggested that *An. funestus* may have evolved with humans, thus its adaptability and efficient use of man as a source of blood meal (Charlwood et al. 1995).

The resting behaviour of a mosquito constitutes of endophily and exophily. A mosquito exhibiting endophily prefers to rest indoors in human dwellings in the period between the end of blood-feeding and the onset of the search for a suitable site to lay eggs (Pates and Curtis 2005) while exophilic mosquitoes rest and spend this time outdoors (Paaijmans and Thomas 2011). *Anopheles gambiae* s.s. is highly endophilic (Highton et al. 1979, Mnzava et al. 1995, Githeko et al. 1996a, Faye et al. 1997) although some exophily has also been reported (Bockarie et al. 1994, Mahande et al. 2007a). Likewise, *An. funestus* is generally endophilic (Gillies 1954, Mnzava et al. 1995, Githeko et al. 1996a) although exophily has been reported (Fontenille et al. 1990). In contrast, *An. arabiensis* is mainly exophilic (Highton et al. 1979, Fontenille et al. 1990, Mnzava et al. 1995, Tirados et al. 2006, Mahande et al. 2007a) but some endophily has also been reported (Ameneshewa 1996, Faye et al. 1997). Similar to the association between anthropophily and vectorial capacity, the habit of *An. gambiae* and *An. funestus* to rest inside human dwellings enhances their efficiency in transmitting the malaria parasites (Beier 1996, Costantini et al. 1999, Takken and Knols 1999, Antonio-Nkondjio et al. 2002, Wanji et al. 2003, Cano et al. 2004, Sinka et al. 2010).

Chapter 2

**Methods of assessing the biting behaviour of malaria vectors both indoors and outdoors**

The main methods that have been used to assess the biting behaviour of malaria vectors are the human landing catch (HLC) and light traps. Light traps include the Centers for Disease Control and Prevention Light Trap (CDC-LT) and the rotator traps (Figure 1).

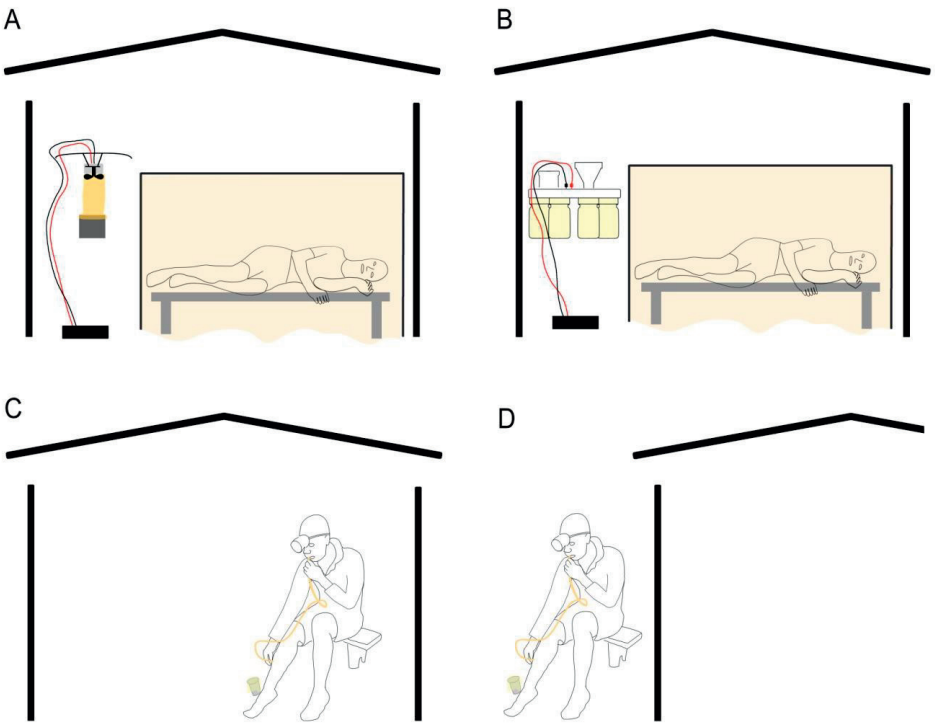


Figure 1: Schematic drawing of A. CDC-LT, B. Rotator trap, C. HLC indoors and D. HLC outdoors.

The HLC is the most frequently used method. Mosquitoes are collected from a human volunteer as they land on the skin to bite. The method estimates the peak biting times for vectors, the vectors'

indoor/outdoor biting preferences and the number of infectious bites that a single individual can receive per unit time. Many studies have used the HLC method to assess the biting behaviour of malaria vectors both indoors and outdoors (Beier et al. 1990, Aniedu 1993, Githeko et al. 1996b, Fontenille et al. 1997, Quinones et al. 1997, Mendis et al. 2000, Killeen et al. 2006, Geissbühler et al. 2007, Reddy et al. 2011, Russell et al. 2011, Moiroux et al. 2012b, Seyoum et al. 2012, Kabbale et al. 2013, Bayoh et al. 2014, Meyers et al. 2016). One limitation of the HLC is that it is labour intensive, requiring collectors to be alert and active throughout each night of the sampling period. Additionally, standardization of HLC across sampling points is restricted by differences among people in their attractiveness to mosquitoes and ability to collect mosquitoes. Furthermore, the HLC has raised ethical concerns because it exposes the human collectors to increased malaria risk but providing the collectors with a malaria prophylaxis reduces the malaria risk (Gimnig et al. 2013).

The CDC-LT requires less labour than the HLC and operates via a mechanical suction device that captures mosquitoes attracted to the trap. The trap usually has a bulb that provides incandescent light. For collecting malaria vectors in Africa, the trap is usually placed next to a person sleeping under a bed net (Lines et al. 1991), whereby the person acts as an attractive stimulant for mosquitoes (Garret-Jones and Magayuka 1975), but the mosquitoes are not able to reach the person because of the bed net. In Zimbabwe, the CDC-LT has been used to assess the biting patterns both indoors and outdoors (Sande et al. 2016). In other regions, the CDC-LT has been compared with the HLC method and variable results have been reported (Lines et al. 1991, Costantini et al. 1998b, Magbity et al. 2002, Govella et al. 2011, Overgaard et al. 2012b). The use of CDC-LT outdoors, however, is limited given the requirement of setting it next to an occupied bed net. One major drawback of the CDC-LT is that when used outdoors, mosquito

captures are low (Costantini et al. 1998b, Sande et al. 2016). In addition, variation among humans in their attractiveness to mosquitoes (Verhulst et al. 2011) still inhibits standardization of the CDC-LT.

Few studies have used the rotator trap to assess the biting behaviour of mosquitoes (Kawada et al. 2014, Ototo et al. 2015). The trap uses a similar mechanism as that of the CDC-LT with the addition that this trap has a programmable timer that allows segregation of catches at flexible schedules/times, which can then be compared with timed catches of the HLC.

Studies have compared the trapping efficiencies of the HLCs and the CDC-LT mostly indoors. These studies demonstrate that the two methods collect similar numbers of anophelines (Lines et al. 1991, Magbity et al. 2002, Mathenge et al. 2005, Ndiath et al. 2011, Sikaala et al. 2013). However, other studies report that the efficiency of the HLC in sampling host-seeking anophelines is higher than that of the CDC-LT (Mbogo et al. 1993, Govella et al. 2011, Overgaard et al. 2012b) or that the efficiency of the CDC-LT in collecting mosquitoes is higher than that of the HLC (Govella et al. 2009, Fornadel et al. 2010b, Wong et al. 2013) (Mburu et al. Chapter 3). A review by Kelly-Hope and McKenzie (2009) highlights the lack of comparability and consistency between various entomological sampling tools whereas that of Briët et al. (2015) showed that the CDC-LT catches were either similar to those of the HLC, higher or lower than those of the HLC.

Additionally, these methods have been used to assess the biting times of malaria vectors. The catches are mostly conducted on an hourly basis with few after every thirty minutes (Beier et al. 1990) or on a two-hourly basis (Aniedu 1993). In other cases, the hours of mosquito collections

vary with most studies leaving out the collections in the later morning hours (i.e. after 7 am) until recently, when these hours were also considered (Moiroux et al. 2012b, Sougoufara et al. 2014).

**Factors that may influence the biting behaviour of malaria vectors**

Biting behaviour of malaria vectors is affected by numerous factors, including host preference, physiological state, diurnal rhythm, but also environmental factors such as ambient temperature, relative humidity, house design, host density and distribution.

The high degree of anthropophagy exhibited by *Anopheles gambiae* s.s., *An. coluzzii* and *An. funestus* makes them the most effective and efficient malaria vectors in Africa (Gillies and De Meillon 1968, Highton et al. 1979, Githeko et al. 1994, Githeko et al. 1996b, Duchemin et al. 2001, Antonio-Nkondjio et al. 2002, Awolola et al. 2003, Mwangangi et al. 2003, Wanji et al. 2003, Temu et al. 2007, Dabire et al. 2008, Seyoum et al. 2012, Dadzie et al. 2013). Costantini et al. (1999) highlighted that anthropophagy may have evolved in two ways. Firstly, that the vectors sought a protective environment near human dwellings to avoid unfavourable climatic conditions. Secondly, that the vectors were exploiting humans as guides for larval habitats because some human activities would create water bodies suitable as larval habitats. Because of this strong association with humans, the location of humans relative to their housing influences the biting behaviour of anthropophagic mosquitoes.

The availability of hosts in specific locations can influence the biting behaviour of malaria vectors. For instance, in Senegal, Faye et al. (1997) found that *An. arabiensis* and *An. gambiae* s.s. were strongly exophagic. In this study area, most of the inhabitants slept outdoors, which likely explains the outdoor biting by both species. In Tanzania and Ethiopia, Mnzava et al. (1995) and Animut et al. (2013) documented that *An. arabiensis* was endophagic in settings where cattle were kept indoors. The availability of different hosts (human and cattle) in these studies

influenced the indoor biting behaviour of *An. arabiensis*, likely because this species bites humans or cattle indiscriminately, and the relative abundance of hosts or certain animal species may also affect the biting behaviour of malaria vectors (Day 2005, Takken and Verhulst 2013).

Housing has been regarded as a risk factor for malaria (Tusting et al. 2015, Tusting et al. 2017). This is because African malaria vectors are often highly endophilic, but the structural design of a house can affect the entry and exit of the mosquitoes. For instance, studies by Animut et al. (2013) found that open windows and open eaves were likely to be associated with the indoor entry of *An. arabiensis.* Whereas structural house improvements by closing eaves and windows have been associated with reduced indoor densities of malaria vectors (Atieli et al. 2009, Kirby et al. 2009, Ogoma et al. 2009, Kampango et al. 2013), it is unclear whether malaria vectors diverted from improved houses would switch to increased outdoor biting. To answer this question, more studies incorporating outdoor biting with regard to house improvement are needed.

In Mali, laboratory studies suggested that mosquitoes may utilize associative learning that enables them to know when and where (indoors or outdoors) hosts are located (Chilaka et al. 2012). Similarly, in Tanzania, McCall et al. (2001) found that *An. arabiensis* mosquitoes demonstrate site fidelity (ability to return to the locations where they had previously fed). A combination of such traits may be a driver for earlier outdoor feeding given that mosquitoes would recall that they were successful in biting the previous day outdoors even if their innate preference as species was for indoor biting.

Additionally, in recent studies, a genetic component has been linked with *An. arabiensis* feeding behaviour. Two genes, namely the odorant binding protein (*Obp5*) and odorant receptor (*Or65*), have been associated with host selection (Main et al. 2016). As a result, the mosquitoes may avoid hosts protected by LLINs by changing their biting times such as to the early evening and

late morning hours. The other assumption would be that such mosquitoes may feed on lesser preferred hosts to avoid contact with LLINs.

Furthermore, genetic variations have been associated with the resting behaviour of malaria vectors. In the Garki project in Nigeria, the numbers of *An. gambiae* s.l. in indoor and outdoor catches were almost the same (Molineaux and Gramiccia 1980) but differences were noted in the chromosomal inversion frequencies in *An. gambiae* s.l. that rested indoors and outdoors (Coluzzi et al. 1979); spraying of insecticides (propoxur) may have contributed to the decline of *An. gambiae* s.s. indoors, while the exophilic *An. arabiensis* mosquitoes were not vulnerable to the insecticides and therefore persisted to levels that would sustain the malaria transmission. It was concluded that the failure of interrupting malaria transmission in this area of Nigeria was caused by these differences in behaviour between *An. gambiae* s.s. and *An. arabiensis* (Molineaux and Gramiccia 1980).

On the other hand, scaling up of LLINs has been taking place rapidly in Africa since this strategy was adopted by the roll back malaria programme (WHO 1999). The use of LLINs reduces the feeding success of mosquitoes only if the vector's biting pattern coincides with the period that people are asleep and protected by a LLIN (Killeen and Moore 2012). Additionally, models have predicted that the indoor insecticide-based measures are likely to reduce the relative abundance of major vector species such as *An. gambiae* s.s., *An. coluzzii* and *An. funestus* (Sinka et al. 2016). However, LLIN use is limited against vectors that bite outdoors and those that evade contact with the insecticides impregnated on the bed nets, and therefore the outdoor fraction of the mosquito population is selected in favour of those biting indoors. Such strong selection can lead to the emergence of behavioural traits that were previously under-represented. A review by Takken

(2002) highlighted that such behavioural traits can be associated with changes in host preferences, biting times and locations.

**The biting behaviour of mosquitoes before the implementation of LLINs**

A study conducted in two neighbouring regions in Kenya, namely Saradidi and Kisian, in the 1980s using human landing catch method demonstrated that the biting activities of *An. gambiae* s.l. and *An. funestus* were higher indoors than outdoors in the latter region (*An. gambiae* s.l.: indoors mean number of bites per house/site 9.7, outdoors mean 3.1; *An. funestus:* indoors mean 3.4, outdoors mean 0.4). Interestingly, both indoor and outdoor biting activities of these two species were similar in the former region *An. gambiae* s.l.; indoor mean 3.3, outdoor mean 2.7; *An. funestus:* indoor mean 0.6; outdoor mean 0.5) (Beier et al. 1990). Additionally, the indoor entomological inoculation rates (EIRS) were 237 and 299 infectious bites/person/year in Saradidi and Kisian, respectively. Although Saradidi had fewer mosquitoes than Kisian, the sporozoite rates were higher outdoors in Saradidi (190 infective bites/person/year) than in Kisian (33 infective bites/person/year)

Furthermore, another study conducted in 1987 with the human landing catch (HLC) method both indoors and outdoors, demonstrated that whereas the biting activities of *An. gambiae* s.l. and *An. funestus* were higher indoors than outdoors, there was a close similarity in the biting times of each of the species in both locations. The maximum biting peaks of *An. gambiae* s.l. both indoors (n = 1038) and outdoors (n = 348) were from 21:00 h to 01:00 h and those for *An. funestus* were from 21:00 h to 03:00 h both indoors (n = 1007) and outdoors (n = 283) (Aniedu 1993) (Table 1). The authors suggest that, most likely, the populations biting in both locations were homogeneous or that the biting was intrinsic and not affected by environmental conditions. It is worth noting that

the implementation of LLINs had not begun in this region as the authors highlight that such plans were underway. Additionally, in Senegal, Fontenille et al. (1997) found that the most abundant species were *An. gambiae* s.s. and *An. arabiensis*. The highest biting peaks for both species occurred from 02:00 h to 06: 00 h both indoors and outdoors, whereby the indoor collections comprised of 58% and 56% of *An. gambiae* and *An. arabiensis*, respectively.

In other regions, studies have focused on the collection of mosquitoes at a single location. For instance, a study in South Africa focused on the outdoor biting using the HLC method (La Grange et al. 1997). Though no *An. gambiae* s.l. was collected, *An. funestus* mosquitoes were collected 2 hrs after sunset. The findings from these studies provide evidence on the presence of both indoor and outdoor biting in different regions before the implementation of LLINs.

Table 1: Biting times of malaria vectors in Africa

| Species | Country | Dates of collection | ITN use | Biting peaks indoors | Biting peaks outdoors | Number or % collected indoors | Number or % collected outdoors | Author |
|---|---|---|---|---|---|---|---|---|
| *An. gambiae* | Kenya | 1987 | No | 21: 00 h to 01:00 h | 21: 00 h to 01:00 h | 1038 | 348 | Aniedu et al. 1993 |
| *An. gambiae* | Senegal | 1993-1996 | Not reported | 02:00 h to 06:00 h | 02:00 h to 06:00 h | 58.20% | 41.80% | Fontenille et al. 1997 |
| *An. gambiae* | Tanzania | 2006 | Yes | 24:00 h to 02:00 h | 24:00 h to 02:00 h | Combined locations 75.6% | | Geissbuhler et al. 2007 |
| *An. gambiae* | Equatorial Guinea | 2007-2008 | Yes | 20:00 h to 22:00 h; 22:00 h to 24:00 h | 20:00 h to 22:00 h; 22:00 h to 24:00 h | 292 | 295 | Reddy et al. 2011 |
| *An. gambiae* | Equatorial Guinea | 7/1/1905 | Yes | 19:00 h to 02:00 h | 19:00 h to 24:00 h; 03:00 h to 05:00 h | 608 | 598 | Reddy et al. 2012 |
| *An. gambiae* | Kenya | 2011 | Yes | 21:00 h to 22:00 h; 23:00 h to 24:00 h; 02:00 h to 06:00 h | 21:00 h to 02:00 h; 04:00h to 05:00 h | Combined locations= 235 | | Bayoh et al. 2014 |
| *An. gambiae* | Benin | 2016 | Yes | Not reported | Not reported | 751 | 551 | Akogbeto et al. 2018 |
| *An. coluzii* | Benin | 2016 | Yes | Not reported | Not reported | 575 | 539 | Akogbeto et al. 2018 |
| *An. arabiensis* | Kenya | 1995 | Not reported | 02:00 h to 06:00 h | 03:00 h to 06:00 h | 1142 | 610 | Githeko et al. 1996 |
| *An. arabiensis* | Senegal | 1993-1996 | Not reported | 02:00 h to 06:00 h | 02:00 h to 06:00 h | 56% | 44% | Fontenille et al. 1997 |
| *An. arabiensis* | Tanzania | 2006 | Yes | 19:00 h to 22:00 h | 19:00 h to 20:00 h;22:00 h to 23:00 h | | | Geissbuhler et al. 2007 |
| *An. arabiensis* | Zambia | 2007-2008 | Not reported | 20:00 h to 24:00 h; 02:00 h to 03:00 h | 2000-0200;0300-0400hrs | | | Fornadel et al. 2008 |
| *An. arabiensis* | Zambia | 2007-2008 | Not reported | 19:00 h to 23:00 h; 24:00 h to 02:00 h | 19:00 h to 21:00 h; 23:00 h to 01:00 h | | | Fornadel et al. 2008 |
| *An. arabiensis* | Ethiopia | 2008 | Yes | 19:00 h to 20:00 h | None | | | Yohannes et al. 2012 |
| *An. arabiensis* | Kenya | 2011 | Yes | 18:00 h to 21:00 h; 22:00 h to 23:00 h; 24:00 h to 01:00 h | 18:00 h to 03:00 h | 63.30% | 36.70% | Bayoh et al. 2014 |

Table 1 Continued: Biting times of malaria vectors in Africa

| Species | Country | Dates of collection | ITN use | Biting peaks indoors | Biting peaks outdoors | Number or % collected indoors | Number or % collected outdoors | Author |
|---|---|---|---|---|---|---|---|---|
| *An. arabiensis* | Ethiopia | 2014 | Yes | 20:00 h to 21:00 h; 24:00 h to 01:00 h | 21:00 h to 22:00 h | 370 | 463 | Kenea et al. 2016 |
| *An. funestus* | Kenya | 1987 | No | 21:00 h to 03:00 h | 21:00 h to 03:00 h | 1007 | 283 | Aniedu et al. 1993 |
| *An. funestus* | Kenya | 1995 | No | 23:00 h to 06:00 h | 03:00 h to 06:00 h | 2353 | 357 | Githeko et al. 1996 |
| *An. funestus* | Kenya | 1995 | Yes | None | 19:00 h to 23:00 h | | Permethrin bed nets=697 | Mathenge et al. 2001 |
| *An. funestus* | Kenya | 1995 | Yes | None | 19.00 h to 20:00 h; 21:00 h to 22:00 h; 23:00 h to 24:00 h | | Control=221 | Mathenge et al. 2001 |
| *An. funestus* | Tanzania | 1997 | No_Yes | 22:00 h to 01:00 h | 22:00 h to 06:00 h | 100% | | Killeen et al. 2006 |
| *An. funestus* | Tanzania | 2004 | No_Yes | 03:00 h to 06:00 h | 18:00 h to 20:00 h; 22:00 h to 24:00 h | 76.10% | | Killeen et al. 2006 |
| *An. funestus* | Tanzania | 2009 | Yes | 19:00 h to 21:00 h | 19:00 h to 23:00 h | 50.50% | | Russell et al. 2011 |
| *An. funestus* | Kenya | not indicated | Yes | 01:00 h to 02:00 h | 20:00 h to 21:00 h | 69% | | Huho et al. 2012 |
| *An. funestus* | Zambia | not indicated | Yes | 04:00 h to 06:00 h | 24:00 h to 02:00 h | 52% | | Huho et al. 2012 |
| *An. funestus* | Burkina Faso | not indicated | Yes | 22:00 h to 23:00 h | 20:00 h to 21:00 h | 36% | | Huho et al. 2012 |
| *An. funestus* | Burkina Faso | not indicated | Yes | ….. | 20:00 h to 21:00 h | 22% | | Huho et al. 2012 |
| *An. funestus* | Benin | 2007-2008 | During LLIN distribution | 23:00 h to 02:00 h | 24:00 h to 02:00 h | 111 | 93 | Moiroux et al. 2012 |
| *An. funestus* | Benin | 2008-2009 | 1 year after distribution | 01:00 h to 03:00 h | 01:00 h to 06:00 h | 72 | 154 | Moiroux et al. 2012 |
| *An. funestus* | Benin | 2011 | 3 years after distribution | 03:00 h to 06:00 h | 03:00 h to 05:00 h | 59 | 92 | Moiroux et al. 2012 |
| *An. funestus* | Benin | 2007-2008 | During LLIN distribution | 01:00 h to 02:00 h | 02:00 h to 04:00 h | 93 | 75 | Moiroux et al. 2012 |
| *An. funestus* | Benin | 2008-2009 | 1 year after distribution | 05:00 h to 06:00 h | 04:00 h to 06:00 h | 121 | 96 | Moiroux et al. 2012 |
| *An. funestus* | Zambia | 2009-2011 | Yes | 24:00 h to 06:00 h | 24:00 h to 06:00 h | 58.60% | | Seyoum et al. 2013 |

Table 1 Continued: Biting times of malaria vectors in Africa

| Species | Country | Dates of collection | ITN use | Biting peaks indoors | Biting peaks outdoors | Number or % collected indoors | Number or % collected outdoors | Author |
|---|---|---|---|---|---|---|---|---|
| *An. funestus* | Benin | 2011 | 3 years after distribution | 03:00 h to 06:00 h | 05:00 h to 07:00 h | 479 | 419 | Moiroux et al. 2012 |
| *An. funestus* | Uganda | 2010 | Bugabula-LLINS | 19:00 h to 02:00 h | 19:00 h to 05:00 h | 39 | 39 | Kabbale et al. 2013 |
| *An. funestus* | Uganda | 2010 | Budiope -no LLINs | 19:00 h to 04:00 h | 19:00 h to 24:00 h | 453 | 411 | Kabbale et al. 2013 |
| *An. funestus* | Kenya | 2011 | Yes | 21:00 h to 05:00 h | 21:00 h to 04:00 h | 67.30% | 32.70% | Bayoh et al. 2014 |
| *An. funestus* | Kenya | 2012 | Yes | 18:00 h to 21:00 h | None | 11 | | Wamae et al. 2015 |
| *An. funestus* | Kenya | 2012 | Yes | 21:00 h to 06:00 h | None | 16 | | Wamae et al. 2015 |
| *An. funestus* | Ethiopia | 2014 | Yes | 20:00 h to 21:00 h; 23:00 h to 24:00 h | 21:00 h to 22:00 h; 05:00 h to 06:00 h; 01:00 h to 02:00 h | 22 | 38 | Kenea et al. 2016 |
| *An. funestus* | Zimbabwe | 2014 | Yes | 20:00 h to 23:00 h; 01:00 to 03:00 h | 20:00 h to 24:00 h; 01:00 h to 02:00 h | 68.50% | 31.50% | Sande et al. 2016 |
| *An. funestus* | Zimbabwe | 2014 | Yes | 20:00 h to 23:00 h; 01:00 h to 04:00 h | 20:00 h to 01:00 h; 01:00 h to 03:00 h | 69.10% | 30.90% | Sande et al. 2016 |
| *An. funestus* | Burkina Faso | 2012 | Yes | 18:00 h to 22:00 h | 18:00 h to 19:00 h; 21:00 h to 02:00 h | | | Dambach et al. 2018 |

Chapter 2

**Biting behaviour of mosquitoes during comparisons between treated and untreated bed nets**

Various studies have been conducted to assess the effect of the use of untreated and treated bed nets on the biting behaviour of mosquitoes. Whereas some studies have observed variations in the biting behaviour of mosquitoes, other studies have not. For instance, in western Kenya, Mathenge et al. (2001) found that the outdoor biting times of malaria vectors were similar in compounds with treated and untreated bed nets. The outdoor biting activities, of *An. gambiae s*.l. and *An. funestus* were highest from 23:00 h to 01:00 h and from 22:00 h to 24:00 h, respectively. Another study in The Gambia showed that the indoor and outdoor biting ratios were similar in regions with and without treated bed nets. Human landing catches were conducted simultaneously indoors and outdoors and 96% of these consisted of *An. gambiae* s.s. and 4% *An. arabiensis* (Quinones et al. 1997). Additionally, in the same country, other collections were conducted outdoors with the HLC method. The results showed that outdoor biting activities were similar in regions with and without treated bed nets and the biting occurred in the early evening hours (Lindsay et al. 1993). Furthermore, studies using the HLC method in Tanzania found that *An. gambiae* was predominantly endophagic and nocturnal before the use of bed nets in 1997 and after distribution of untreated nets in 2004 (Killeen et al. 2006). In Uganda, the outdoor biting densities of *An. gambiae* s.l. exceeded the indoor biting densities of this species in regions with (outdoors: n=346; indoors: n=299) and without LLINs (outdoors: n=1079; indoors: n=853). The indoor densities of *An. funestus* were similar to the outdoor biting densities in regions with (outdoors: n=39; indoors: n=39) and without LLINs (outdoors: n=411; indoors: n=453). For both species, the peak biting times occurred from 23:00 h to 05:00 h in regions with and without LLINs use (Kabbale et al. 2013).

Conversely, in other regions where the same comparisons were made on the effect of treated and untreated bed nets, it was reported that the vectors were biting early in the evening hours in both zones. This biting was apparent both indoors and outdoors, but the biting activity was higher outdoors (Mbogo et al. 1996). In addition, Githeko et al. (1996b) found that the biting activities of *An. gambiae* s.l. and *An. funestus* occurred mostly from 24:00 h to 06:00 h both indoors and outdoors in regions with and without treated curtains on the eaves. Interestingly, though *An. gambiae* s.s. was exophilic, it maintained its anthropophagy while *An. funestus* was more zoophagic than anthropophagic. Furthermore, in Burkina Faso, where comparisons were made with two types of LLINs and untreated nets, the results demonstrated that the anthropophagic *An. gambiae* s.s. fed on cattle in houses where the two types of LLINs were used (Dabiré et al. 2006).

**Biting behaviour of mosquitoes after the implementation of LLINs**

The biting activities of malaria vectors have been reported from midnight to the late night hours (Huho et al. 2013, Bayoh et al. 2014). These biting activities are hereafter referred to as the historic biting times. In Tanzania, the biting activity of *An. gambiae* s.s. followed the historic pattern both indoors and outdoors. However, that of *An. arabiensis* was highest between 20:00 h and at 22:00 h, indoors and outdoors, respectively; periods at which many individuals would still be active and unprotected by ITNs. Additionally, for both species, the outdoor biting activities were higher than the indoor biting (Geissbühler et al. 2007). Furthermore, in Ethiopia the biting behaviour of *An. arabiensis* in the early evening hours was consistent both before (Yohannes et al. 2005) and after the implementation of LLINs (Yohannes and Boelee 2012). In Mozambique where the use of LLINs had just been initiated, a study conducted in 1994-1996, found that *An. funestus* was twotimes more likely to bite indoors than outdoors and *An. arabiensis* was 1.5 times more likely to bite outdoors than indoors (Mendis et al. 2000). Conversely, in Ethiopia *An. funestus* and *An.*

*arabiensis* were more likely to bite outdoors than indoors with the highest peaks from 20:00 h to 22:00 h for both species (Kenea et al. 2016). In Zimbabwe, with the use of CDC-LT, 69% and 31% of the mosquitoes were collected indoors and outdoors, respectively. The biting activity of *An. funestus* was nocturnal with the highest peaks occurring from 22:00 h to 23:00 h and 02:00 h to 04:00 h (Sande et al. 2016). Furthermore in western Kenya, with the use of rotator traps, the biting activities of *An. gambiae* and *An. funestus* were highest from18:00 h to 22:00 h and from 04:00 h to 06:00 h, both indoors and outdoors (Ototo et al. 2015).

However, in other regions differences in the biting behaviour following the introduction of bed nets have been reported. For instance, Russell et al. (2011) reported that the nocturnal activity of *An. gambiae* s.l. (Killeen et al. 2006) declined after the distribution of LLINs and that outdoor biting was apparent in this region in the early evening hours. Additionally, in Equatorial Guinea, the biting activity of *An. gambiae* s.s. occurred mostly outdoors in the early evening hours (Reddy et al. 2011, Overgaard et al. 2012a). Furthermore, in Benin, the biting peak of *An. funestus* shifted from 02:00 h to the early morning hours (05:00 h) (Moiroux et al. 2012b) and in Senegal, the peak of this species occurred in the early morning hours (from 07: 00 h to 11:00 h) (Sougoufara et al. 2014).

**Discussion**

From the numerous studies conducted on biting behaviour of African anophelines, it is evident that variations in biting behaviour are common, with respect to both locations (indoors/outdoors) and times, even before the implementation of LLINs. In some areas, biting behaviour was not affected by the introduction of LLINs, while in other areas mosquitoes shifted their biting times to early evening or late morning. However, only a few studies assessed the biting behaviour of the vectors

before and after the implementation of LLINs, with conflicting results. The biting behaviour of malaria vectors may further be influenced by the availability of potential hosts in different locations (Takken and Verhulst 2013).

Earlier studies conducted in the 1980-1990s have demonstrated that *An. gambiae*, *An. arabiensis* and *An. funestus* mosquitoes were actively biting indoors and outdoors before the implementation of LLINs (Beier et al. 1990, Aniedu 1993, Fontenille et al. 1997). In Kenya and Senegal the biting peaks of both *An. gambiae* and *An. arabiensis* were from 02:00 h to 06:00 h (Githeko et al. 1996b, Fontenille et al. 1997) whereas those of *An. funestus* were either from 23:00 h to 06:00 h (Githeko et al. 1996b) or 21:00 h to 03:00 h (Aniedu 1993) in Kenya. We conclude that in the 1980-1990s, before the area-wide introduction of LLINs and IRS, the biting activities of the malaria vectors both indoors and outdoors in different regions exhibited a similar biting pattern at times when most people would be asleep but the biting peaks varied.

Following the implementation and area-wide use of LLINs, from studies conducted in 2001-2008, the biting activities of malaria vectors have been observed from the early evening hours. For instance, in Tanzania, the biting activity of *An. gambiae* s.l. occurred between 19:00 h to 06:00 h both indoors and outdoors, with peaks at 20:00 h (indoors) and 22:00 h (outdoors) for *An. arabiensis* and from 24:00 h to 02:00 h for *An. gambiae* s.s., both indoors and outdoors (Geissbühler et al. 2007). In Zambia, the indoor peaks of *An. arabiensis* were from 19:00 h to 03:00 h and the outdoor peaks of this species were from19:00 h to 04:00 h (Fornadel et al. 2010a). For *An. funestus*, studies demonstrated that the indoor biting times of this species were from 22: 00 h to 06: 00 h (Killeen et al. 2006) and the outdoor peaks were either from 19:00 h to 24:00 h or 22:00 h to 06:00 h (Mathenge et al. 2001, Killeen et al. 2006). Furthermore, studies conducted from 2008 to date in regions where the use of LLINs has been optimized, have observed similar

biting patterns with peaks of these species occurring at the early evening hours to either midnight or 06:00 h both indoors and outdoors as depicted in table one and as discussed in earlier sections (Reddy et al. 2011, Russell et al. 2011, Huho et al. 2013, Kabbale et al. 2013, Bayoh et al. 2014, Wamae et al. 2015, Kenea et al. 2016, Sande et al. 2016, Dambach et al. 2018).

Whereas it is difficult to interpret whether the area-wide use of the LLINs have had an effect on the biting times of mosquitoes as collections were not performed before the implementation of LLINs in many studies, a study by Lefèvre et al. (2009) in a region where LLIN use was high found that phenotypic plasticity exists in the feeding/biting behaviour of *An. gambiae* s.s. This was evident as human hosts were not accessible for a bite indoors (due to bed net coverage) and therefore the vectors fed on a lesser preferred host, cattle, outdoors. Furthermore, laboratory studies suggest that mosquitoes may utilize associative learning that enables them to know when and where (indoors or outdoors) hosts are exposed (Chilaka et al., 2012) and this may enhance indoor and/or outdoor biting. Further studies are recommended under field/natural conditions to support this finding (associative learning) (Killeen and Chitnis 2014).

The relative abundance of hosts will also affect the biting behaviour of malaria vectors (Day 2005, Takken and Verhulst 2013). As shown in this review, selection and location of hosts by the malaria vectors is highly dependent on the availability of hosts (Mnzava et al. 1995, Faye et al. 1997, Animut et al. 2013). Many African malaria vectors prefer to feed on humans, but a certain degree of plasticity allows them to switch to other hosts. This is well known for subpopulations of *An. arabiensis* which can survive entirely on non-human hosts (Braack et al. 1994). The strong reduction of *An. gambiae* s.s. since the introduction of LLINs (Bayoh et al. 2010, Meyrowitsch et al. 2011, Mutuku et al. 2011, Meyers et al. 2016) suggests that this species has

less plasticity in host choice compared to *An. arabiensis*. This has also been predicted by simulation models (Killeen et al. 2007, Sinka et al. 2016).

In other cases, the choice of a sampling tool for assessing the biting behaviour of malaria vectors may underestimate the densities of mosquitoes biting indoors or outdoors. For instance, in Zimbabwe, a study which utilized the use of the CDC-LT method demonstrated that the malaria vectors not only showed the historic biting times (from midnight to the late hours of the night) but also that some mosquitoes were biting in the early evening. Additionally, the results further showed that the biting activity was more prevalent indoors than outdoors (Sande et al. 2016). Whether the reduced outdoor biting was as a result of the inefficiency of the CDC-LT method is unclear but Costantini et al. (1998b) found that this method is less efficient outdoors.

Mostly, the observed differences in the biting behaviour of malaria vectors in some regions like Benin and Tanzania have been attributed to the use of LLINs (Russell et al. 2011, Moiroux et al. 2012b). Over-reliance on a single intervention such as the use of LLINs is likely to contribute to behavioural resistance (Mattingly 1962, Elliot 1972) which in turn alters the biting behaviour of the major malaria vectors. Shifts in the biting times of malaria vectors, for instance to the early evenings or late mornings when most people are unprotected, both indoors and outdoors, have an impact on malaria risk. This is because such behaviour enhances the feeding success by malaria vectors (Killeen and Chitnis 2014). Therefore, the probability of the vectors sustaining or enhancing residual malaria transmission in such cases remains high (Durnez and Coosemans 2013, Durnez et al. 2013, Killeen 2014, Killeen et al. 2017a). Measures that can be implemented to overcome the negative impact of these changes include the development of alternative interventions that target the vectors' changing behaviours. Recently, in Burkina Faso, it has been demonstrated that LLINs treated with permethrin plus pyriproxyfen provide better protection

against clinical malaria than the standard LLINs (Tiono et al. 2018). This development is important because the resistance of mosquitoes to permethrin-treated bed nets has been reported across many regions (Strode et al. 2014, Hemingway et al. 2016). Besides this development, further complementary measures are needed to tackle the vectors changing behaviours. For instance, house improvement protects all individuals in a house equally. This is being assessed in a number of regions (Killeen et al. 2017b, McCann et al. 2017b) as well as the use of insecticide-impregnated tubes along the eaves, which are the preferred entry points for mosquitoes (Knols et al. 2016, Sternberg et al. 2016, Oumbouke et al. 2018). The development of such protective measures that divert malaria vectors from human beings to alternative hosts like cattle is important, especially for species with an opportunistic host-feeding behaviour such as *An. arabiensis*. However, this would still sustain the densities of malaria vectors and therefore as suggested by Killeen et al. (2017a) the use of insecticide-treated cattle would be more effective in reducing the density of malaria vectors. Other complementary measures include the use of insecticide-treated clothes (Kimani et al. 2006, Banks et al. 2014) and larval source management (Fillinger and Lindsay 2006, Imbahale et al. 2011, Imbahale et al. 2012, McCann et al. 2017b). By targeting the larval habitats, the densities of emerging malaria vectors can be reduced significantly. The 'push-pull' approach can also be implemented either by the of use of attractive toxic sugar baits (Müller et al. 2010, Beier et al. 2012) or by use of attractants and repellents in traps (Menger et al. 2014a, Menger et al. 2016). Both methods would also reduce the densities of malaria vectors significantly. Recently, it was shown that mass trapping of malaria vectors with odour-baited traps caused a significant reduction in malaria risk as shown by reduced mosquito densities and malaria prevalence (Homan et al 2016).

## Conclusion

African malaria vectors with a high degree of anthropophily feed primarily indoors due to the availability of (human) hosts. However, variations in this behaviour are widely present, where biting can occur both indoors and outdoors, and during a wider range of times. The area-wide introduction of LLINs and IRS may have affected this behaviour, as variations in behaviour have been found across Africa, and are reported to be associated with the introduction of insecticide-based vector control. Such behaviours would have a negative effect on malaria transmission in a region. Therefore, entomological monitoring to assess vector biting behaviour is essential for planning vector control interventions. Furthermore, the development of tools that can protect individuals from the early evening and late morning biting, both indoors and outdoors, is highly recommended.

## Acknowledgement

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## Chapter 3

# Biting patterns of malaria vectors of the lower Shire valley, southern Malawi

Monicah M. Mburu, Themba Mzilahowa, Benjamin Amoah, Duster Chifundo, Kamija S. Phiri, Henk van den Berg, Willem Takken and Robert S. McCann

Submitted

Chapter 3

**Abstract**

Assessing the biting behaviour of malaria vectors plays an integral role in understanding the dynamics of malaria transmission in a region. Biting times and preference for biting indoors or outdoors varies among mosquito species and across regions. These behaviours may also change over time in response to vector control measures such as long-lasting insecticidal nets (LLINs). Data on these parameters can provide the sites and times at which different interventions would be effective for vector control. This study assessed the biting patterns of malaria vectors in Chikwawa district, southern Malawi. The study was conducted during the dry and wet seasons in 2016 and 2017, respectively. In each season, mosquitoes were collected indoors and outdoors for 24 nights in six houses per night using the human landing catch. Volunteers were organized into six teams of two individuals, whereby three teams collected mosquitoes indoors and the other three collected mosquitoes outdoors each night, and the teams were rotated among twelve houses. All data were analyzed using Poisson log-linear models. The most abundant species were *Anopheles gambiae* sensu lato (primarily *An. arabiensis*) and *An. funestus* s.l. (exclusively *An. funestus* s.s.). During the dry season, the biting activity of *An. gambiae* s.l. was constant outdoors across the categorized hours (18:00 h to 08:45 h), but highest in the late evening hours (21:00 h to 23:45 h) during the wet season. The biting activity of *An. funestus* s.l. was highest in the late evening hours (21:00 h to 23:45 h) during the dry season and in the late night hours (03:00 h to 05:45 h) during the wet season. Whereas the number of *An. funestus* s.l. biting was constant (P = 0.662) in both seasons, that of *An. gambiae* s.l. was higher during the wet season than in the dry season (P = 0.001). *Anopheles gambiae* s.l. was more likely to bite outdoors than indoors in both seasons. During the wet season, *An. funestus* s.l. was more likely to bite indoors than outdoors but during the dry season, the bites were similar both indoors and outdoors. The biting activity that occurred in the

early and late evening hours, both indoors and outdoors coincides with the times at which individuals may still be awake and physically active, and therefore unprotected by LLINs. Additionally, a substantial number of anopheline bites occurred outdoors. These findings imply that LLINs would only provide partial protection from malaria vectors, which would affect malaria transmission in this area. Therefore, protection against bites by malaria mosquitoes in the early and late evening hours is essential and can be achieved by designing interventions that reduce vector-host contacts during this period.

**Keywords**: Anophelines, Culicines, HLC, Biting, Indoors, Outdoors, Malawi

## Introduction

Vector control remains the most effective measure to prevent malaria transmission (WHO 2006, 2017, 2018). The most common methods of malaria vector control in the last 20 years have been the use of indoor residual spraying (IRS), conventional insecticide-treated nets and long-lasting insecticide treated nets (LLINs). These methods provide protection against mosquitoes that bite and rest indoors. The effectiveness of LLINs and IRS in reducing malaria vectors relies on the ability of the vectors coming into contact with the insecticides applied either on the nets or on the inner walls of houses (Killeen and Moore 2012). However, some malaria vector species bite outdoors at least as often as indoors (White et al. 1974, Joshi et al. 1975, Highton et al. 1979, Fornadel et al. 2010a, Kenea et al. 2016, Kenea et al. 2017). Additionally, prolonged use of LLINs may lead to changes in the biting preferences of malaria vectors from indoors to outdoors (Reddy et al. 2011, Russell et al. 2011, Padonou et al. 2012, Meyers et al. 2016). In both cases, the vectors biting outdoors are less vulnerable to the insecticides applied indoors (LLINs and IRS), and

outdoor biting can sustain or enhance the risk of malaria transmission (Gillies 1964, Antonio-Nkondjio et al. 2006, Killeen et al. 2013, Mwangangi et al. 2013, Killeen 2014). Besides biting location in relation to indoors or outdoors, knowledge about the peak biting times of malaria vectors is also critical for understanding the impact of LLIN use in a given region. It is evident that the biting behaviour of malaria vectors varies across regions (Pates and Curtis 2005). Thus, there is a need for assessing the biting behaviour of malaria vectors to assess the risk of malaria transmission in a given region. Historically, the highest biting activity of primary malaria vectors in Africa was reported to occur indoors from midnight to late night hours (Fontenille et al. 1990, Githeko et al. 1996b, Fontenille et al. 1997), and therefore, the use of bed nets gained interest because people sleeping under LLINs would be protected from most potentially infectious bites. Furthermore, these late-night biting mosquitoes would experience high mortality from the insecticide on the net, reducing vector populations. More recently, shifts in the peak biting times of malaria vectors have been reported following large-scale use of LLINs. For example, in Benin, the peak biting time of *An. funestus* populations shifted from 02:00 h to the early morning hours (05:00 h) (Moiroux et al. 2012b), and in Senegal the peak biting time of *An. funestus* was observed in the later morning hours (07:00 h to 11:00 h) (Sougoufara et al. 2014). In Tanzania, the biting activity of *An. arabiensis* and *An. funestus* s.s. was in the early night hours (20:00 h to 23:00 h) (Russell et al. 2011). These regions had high LLIN coverage, suggesting that the malaria vectors sought hosts at times when people were not protected by LLINs.

The most direct and favoured method of estimating malaria transmission entomologically is the human landing catch (HLC) (Lines et al. 1991, Service 1993a, Davis et al. 1995, Beier 1998, Kline 2006, Govella et al. 2010, Lima et al. 2014). The HLC estimates the peak biting times for vectors, the vectors' indoor/outdoor biting preferences and the number of infectious bites that a

single individual can receive per unit time (Charlwood and Graves 1987, Bockarie et al. 1996, Mboera 2005, Pates and Curtis 2005, Oyewole et al. 2007, Bayoh et al. 2014, Sougoufara et al. 2014). Data on these parameters can provide the times at which different interventions would be effective for vector control. In Malawi, the main malaria vectors are *An. gambiae* sensu stricto (s.s.), *An. arabiensis* and *An. funestus* (Spiers et al. 2002, Mzilahowa et al. 2012), but little is known about the biting behaviour of these vectors in the country. This study assessed the vectors' indoor/outdoor biting preferences and the peaks in their biting activities.

## Methods

### Study site

The study was conducted in two neighbouring villages, Mwalija (-15.96, 34.78) and Njereza (-15.96, 34.77), in Chikwawa District, southern Malawi. The villages are along the low-lying regions that are categorized as hot, wet and humid with high rates of malaria transmission (Kazembe et al. 2006, Kabaghe et al. 2018). Most houses are made of sun-dried or fire-baked bricks with grass-thatched or corrugated iron-sheet roofs. Residents of this region engage mostly in subsistence farming with maize and millet as main crops. The area has been poorly served by malaria vector control, and the mass distribution campaign of LLINs was only conducted for the first time in April 2016.

### Selection of households

The two villages in this study were part of a cluster-randomised control trial assessing the effects of larval source management and house improvement on malaria transmission (McCann et al.

2017b). The villages fell under the control arms of the trial (i.e. no larval source management or house improvement were implemented in these two villages).

Inclusion criteria were applied to ensure a degree of uniformity across the houses and these were: houses with grass thatched roofs and open eaves that were ≥ 25m apart and ≥ 100m away from any mosquito breeding habitat. Houses that were participating in other mosquito sampling efforts at the time of the current study as part of the cluster-randomised trial referenced above were excluded from the current study. A complete list of households in the two villages was used to randomly select twelve households for the study.

**Mosquito sampling**

Mosquito sampling was done during the early months of the dry season (May-June 2016) and following the peak of the rainy season (March-April 2017) using the HLC method (Figure 1). In each season, the sampling was conducted for 24 nights in 6 of the 12 houses each night. The same houses were used in both seasons. Human volunteers from the study houses were organized into six teams of two individuals. A pair of individuals collected mosquitoes in six houses each night, whereby three teams of HLC volunteers collected mosquitoes indoors, and the other three teams collected mosquitoes outdoors. The collections were from 17:00 h to 09:45 h and were divided into two shifts. The first volunteer in each team sampled mosquitoes from 17:00 h to 01:45 h and the second volunteer sampled from 02:00 h to 09:45 h. Each volunteer was provided with a headlight, wristwatch, pencil, mouth aspirator and mosquito holding containers. Prior to the study, all volunteers were trained in the HLC technique. The volunteers sat on stools exposing the lower part of their legs and collected mosquitoes that landed on their legs. The mosquitoes were placed in holding cups that had been pre-labeled with the house number, hour of collection and location

(indoors or outdoors). The volunteers collected mosquitoes for 45 min. and had a 15 min. break within every hour. A research nurse screened the volunteers for malaria on a weekly basis using a malaria rapid diagnostic test (mRDT; SD Bioline malaria Ag Pf HRP-2; Standard Diagnostics Inc, Korea). Additionally, all volunteers were provided with doxycycline daily as malaria prophylaxis from one week before the start of the study to one week after the end of the study.

Spot checks were conducted on random days and at random times by the research team and members from a local community watch group. Likewise, sporadic phone calls were made to volunteers' team leaders to check whether there were any challenges.


Figure 1: Typical house in the present study region (a) and HLC method (b).

**Identification of mosquitoes and detection of *Plasmodium falciparum* DNA**

In the laboratory, all mosquitoes were identified morphologically using the protocol by Gillies and Coetzee (1987). All anophelines were classified as *An. gambiae* s.l., *An. funestus* s.l. or *An.*

*tenebrous*. There was no further classification of the culicines beyond the subfamily level. Females from the *An. gambiae* species complex and the *An. funestus* species group were further identified to species level using polymerase chain reaction (PCR) (Scott et al. 1993, Koekemoer et al. 2002, Cohuet et al. 2003), respectively. For the *An. gambiae* species complex, the PCR included species-specific primers for *An. gambiae* s.s., *An. arabiensis*, and *An. quadriannulatus*. For the *An. funestus* species group, the PCR included species-specific primers for *An. funestus* s.s., *An. vandeeni*, *An. rivulorum*, *An. rivulorum-like*, *An. parensis*, and *An. leesoni*. The heads and thoraces of all female *An. gambiae* s.l. and *An. funestus* s.l. were tested for the presence of *P. falciparum* DNA using real-time polymerase chain reaction (RT-PCR) (Perandin et al. 2004) with a Ct value ≤ 37.0 as the cut-off for *P. falciparum* positive.

**Data analysis**

Assuming the Poisson distribution for the count of mosquitoes and applying the log link function to the Poisson rate parameter, generalized linear models were fitted to assess differences: a) in the biting times of mosquitoes, b) in vectors' indoor/outdoor biting preference and c) in the abundance of mosquitoes between seasons. Generalized estimating equations were used to account for repeated measures by house. Each of the differences was assessed in a separate model for each taxonomic group and, subsequently, for the pooled counts of all malaria vectors. The cooking locations, number of people that slept in the house during the night of data collection, use of bed-net and kind of livestock that stayed within 20m of the house during the night of data collection were included as covariates in each of the models. Door and roof types were not included in the analysis because all the doors were made of wood and all roofs were grass-thatched. Cooking

locations included: inside the house, on the veranda, outside the house but within 2m, and outdoors at more than 2m from the house. Livestock categories were comprised of cattle, goats, and chickens. As the human volunteers worked for 45 min within every hour, the average bites by mosquitoes were divided by 0.75 to obtain the hourly catch rate. The hourly bites were further categorized as early evening (18:00 h to 20:45 h), late evening (21:00 h to 23:45 h), early night (24:00 h to 02:45 h), late night (03:00 h to 05:45 h) and early morning (06:00 h to 08:45 h). Hourly collections at 17:00 h to 17:45 h and at 09:00 h to 09:45 h were low and were not considered in the analysis with the categorical hours. All data were analysed using SPSS Version 20.0. Entomological inoculations rates (EIRs) were estimated by pooling all the catches in all the locations (indoors and outdoors) and calculating the average bites. The averages were divided by 0.75 as earlier explained. This was then multiplied by the sporozoite rate that was estimated using RT-PCR.

## Results

### Abundance of mosquitoes during the dry season

Combined across all locations, a total of 1,032 mosquitoes was collected during the dry season. Of these, 25 were males (2 anophelines indoors and 4 outdoors; 11 culicines indoors and 8 outdoors) and 1007 were females. Of the 1007 females, 917 (91%) were culicines (400 indoors, 517 outdoors), 43 (4.3%) were *An. tenebrosus (*25 indoors, 18 outdoors) and 47 (4.7%) were malaria vector species. Of the 47 malaria vectors, 22 (46.8%) were *An. gambiae* s.l. (5 indoors and 17 outdoors) and 25 (53.2%) were *An. funestus* s.l. (16 indoors and 9 outdoors; Table 1). Of the 21 malaria vectors caught indoors, 14 were identified by PCR as *An. arabiensis* (n=4) and *An.*

*funestus* s.s. (n=10). DNA of seven of the twenty-one malaria vectors caught indoors failed to amplify (6 *An. funestus* s.l. and 1 *An. gambiae* s.l.). Of the 26 caught outdoors, 23 were identified by PCR as *An. arabiensis* (n=13), *An. gambiae* s.s. (n=1) and *An. funestus* s.s. (n=9). DNA of three of the twenty-six vectors caught outdoors failed to amplify (3 *An. gambiae* s.l.).

Of the 47 malaria vectors tested for the presence of *P. falciparum* DNA, only one was positive for *P. falciparum* (*An. funestus* s.s.). The sporozoite rate was 2.1% and the EIR was 3.4 infectious bites/person/year.

**Abundance of mosquitoes during the wet seasons**

Combined across all locations, a total of 1,408 mosquitoes was collected during the wet season. Of these, 18 were males (1 male anopheline outdoors, 10 culicines indoors and 7 outdoors) and 1390 were females. Of the 1,390 females, 1289 (92.7%) were culicines (568 indoors, 721 outdoors), 10 (1%) were *An. tenebrosus (*1 indoors, 9 outdoors) and 91 (6.5%) were malaria vector species. Of the 91 malaria vectors, 69 (75.8%) were *An. gambiae* s.l. (25 indoors and 44 outdoors) and 22 (24.2%) were *An. funestus* s.l. (17 indoors and 5 outdoors; Table 1). Of the 42 caught indoors, 40 were identified by PCR as *An. arabiensis* (n=18), *An. gambiae* s.s. (n=6) and *An. funestus* s.s. (n=16). DNA of two of the forty-two malaria vectors caught indoors failed to amplify (1 *An. funestus* s.l. and 1 *An. gambiae* s.l.). Of the 49 outdoor malaria vectors, 46 were identified by PCR as *An. arabiensis* (n=36), *An. gambiae* s.s. (n=4), *An. funestus* s.s. (n=5) and a hybrid of *An. arabiensis* and *An. gambiae* s.s. (n=1). DNA of three of the forty-nine vectors caught outdoors failed to amplify (3 *An. gambiae* s.l.).

Of the 91 malaria vectors tested for the presence of *P. falciparum* DNA, 4 were positive for *P. falciparum* (3 *An. funestus* s.s. and 1 *An. gambiae* s.s.). The sporozoite rate was 4.4% and the EIR was 13.5 infectious bites/person/year.

The abundance of female *An. gambiae* s.l. was lower in the dry season than in the wet season (Risk ratio (RR) = 0.32, 95% confidence intervals (CI) = [0.20-0.52], P = 0.001) but that of female *An. funestus* s.l. did not differ between the two seasons (RR = 1.06, CI = [0.56-2.06], P = 0.854).

Table 1: Mosquito collection during the dry and wet seasons

| **Mosquito collection** | **Indoors** | | **Outdoors** | | **Totals** | |
|---|---|---|---|---|---|---|
| | Dry season | Wet season | Dry season | Wet season | Dry season | Wet season |
| **No. of nights** | 72 | 72 | 72 | 72 | 144 | 144 |
| *An. gambiae* s.l. | 5 | 25 | 17 | 44 | 22 | 69 |
| *An. funestus* s.l. | 16 | 17 | 9 | 5 | 25 | 22 |
| *An. tenebrosus* | 25 | 1 | 18 | 9 | 43 | 10 |
| Female culicines | 400 | 568 | 517 | 721 | 917 | 1289 |
| Male anophelines | 2 | 0 | 4 | 1 | 6 | 1 |
| Male culicines | 11 | 10 | 8 | 7 | 19 | 17 |

**Biting times of mosquitoes**

During the dry season, the indoor and outdoor biting by malaria vectors (combined across all species) exhibited bi-modal and uni-modal peaks, respectively. For the indoor biting, the first peak was observed between 21:00 h to 21:45 h and the second peak was at 23:00 h to 23:45 h. For the outdoor biting, the peak was observed between 20:00 h to 20:45 h (Figure. 2). Considering each species complex/group separately, the biting activity of *An. gambiae* s.l. was lower indoors than

outdoors (RR = 0.29, CI = [0.11-0.80], P= 0.016). The biting activity of *An. gambiae* s.l., outdoors, was constant across all the categorized hours (18:00 h to 08:45 h) ($P \geq 0.05$). Whereas there was no biting activity observed in the early morning hours, indoors, for *An. gambiae* s.l., the biting rates of this species were constant from the late evening hours to the late night hours (21:00 h to 05:45 h) ($P \geq 0.05$) (Figure. 3A). *Anopheles funestus* s.l. biting rates did not differ between indoors and outdoors in the dry season (RR = 1.78, CI = [0.79-4.02], P = 0.167). The biting rate of *An. funestus* s.l. indoors was highest during the late evening hours (21:00 h to 23:45 h) but absent in the early morning hours. The outdoor biting rates of this species were constant from 18:00 h to 05:45 h ($P \geq 0.05$) (Figure. 3B).

During the wet season, the indoor and outdoor biting by malaria vectors (combined across all species) exhibited uni-modal peaks. The highest activity of indoor biting was from 02:00 h to 04:00 h and that of outdoor biting was at 21:00 h (Figure. 2). Similar to the dry season, the biting activity of *An. gambiae* s.l. in the wet season was lower indoors than outdoors (RR = 0.57, CI = [0.35-0.93], P = 0.024). Outdoors, the peak biting time of *An. gambiae* s.l. occurred in the late evening hours (21:00 h to 23:45 h) and this biting activity was higher than that observed in the early evening hours (P = 0.001), early night hours (P = 0.037) and late night hours (P = 0.001). The indoor biting rates of *An. gambiae* s.l. in the wet season were constant from 18:00 h to 05:45 h ($P \geq 0.05$) (Figure. 3A). *Anopheles funestus* s.l. was more likely to bite indoors than outdoors in the wet season (RR= 3.4, CI = [1.25-9.22], P = 0.016). The peak biting time of *An. funestus* indoors in the wet season was in the late night hours (03:00 h to 05:45 h) and was similar to the biting activity that was observed in the early night hours (P = 0.317) but different from the biting activities in the early evening hours (P = 0.021) and in the late evening hours (P = 0.021). The outdoor biting rates of *An. funestus* s.l. were constant from 21:00 h to 05:45 h ($P \geq 0.05$) (Figure. 3B).

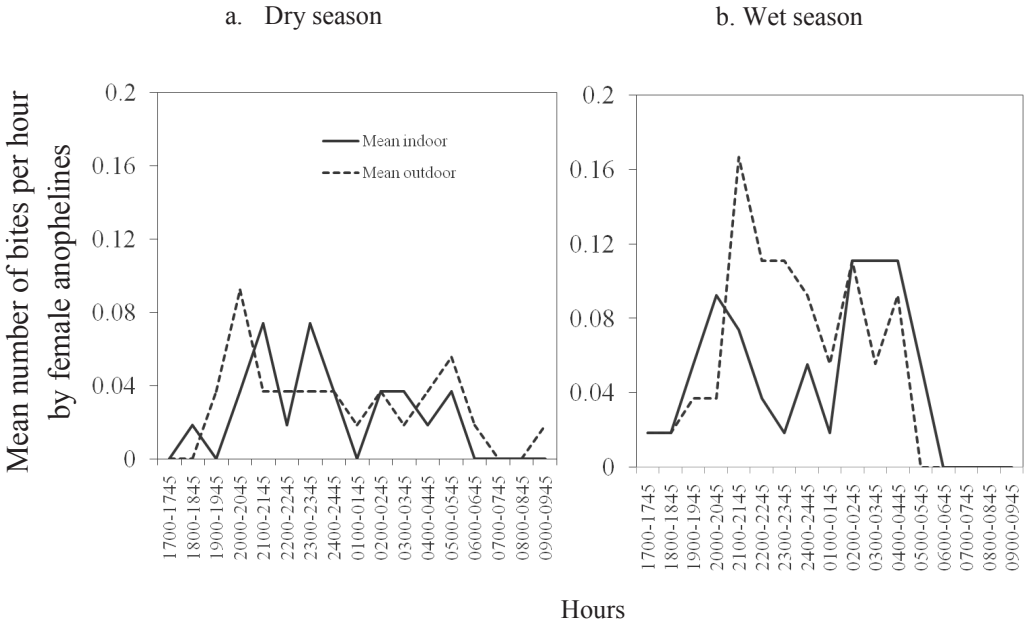


Figure 2: Mean number of bites per hour by female anophelines both indoors and outdoors during the dry and wet seasons.

The biting activity of female culicines was lower in the dry season than in the wet season (RR = 0.65, CI = [0.60-0.71], P = 0.001). Indoor culicine biting rates were lower than the outdoor biting rates in the dry (RR = 0.85, CI = [0.74-0.97], P = 0.014) and wet (RR = 0.8, CI = [0.72-0.89], P = 0.001) seasons (Figure. 4).

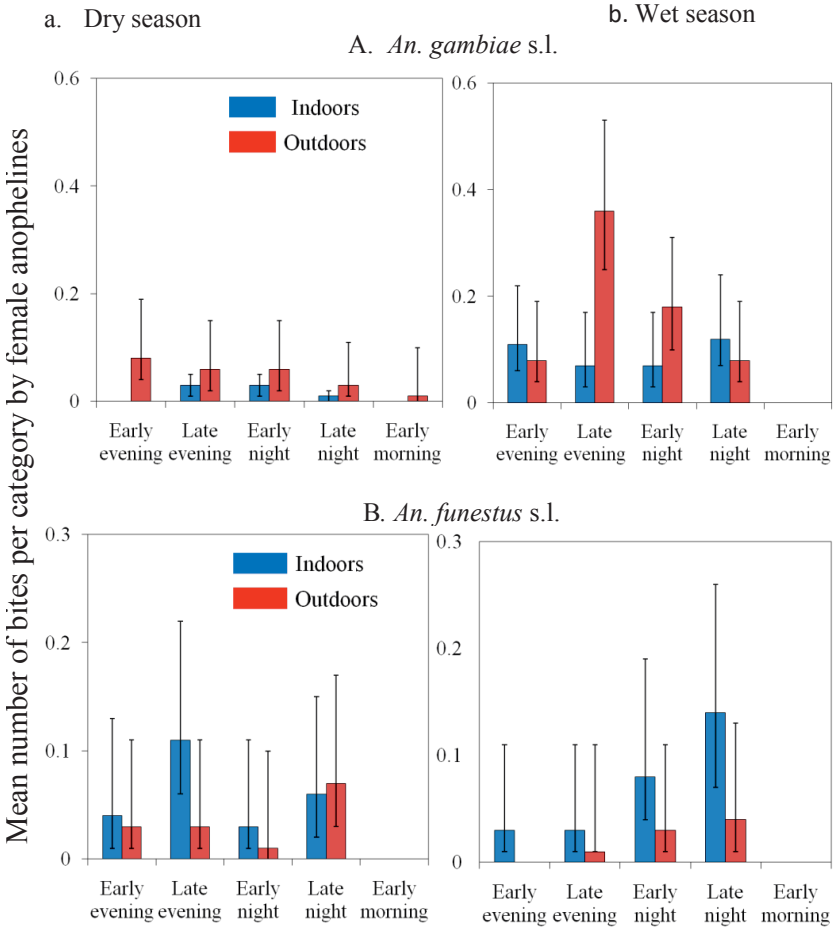


Figure 3: Mean number of bites (95% CI) per category by female *An. gambiae* s.l. (A) and *An. funestus* s.l. (B) both indoors and outdoors during the dry and wet seasons.

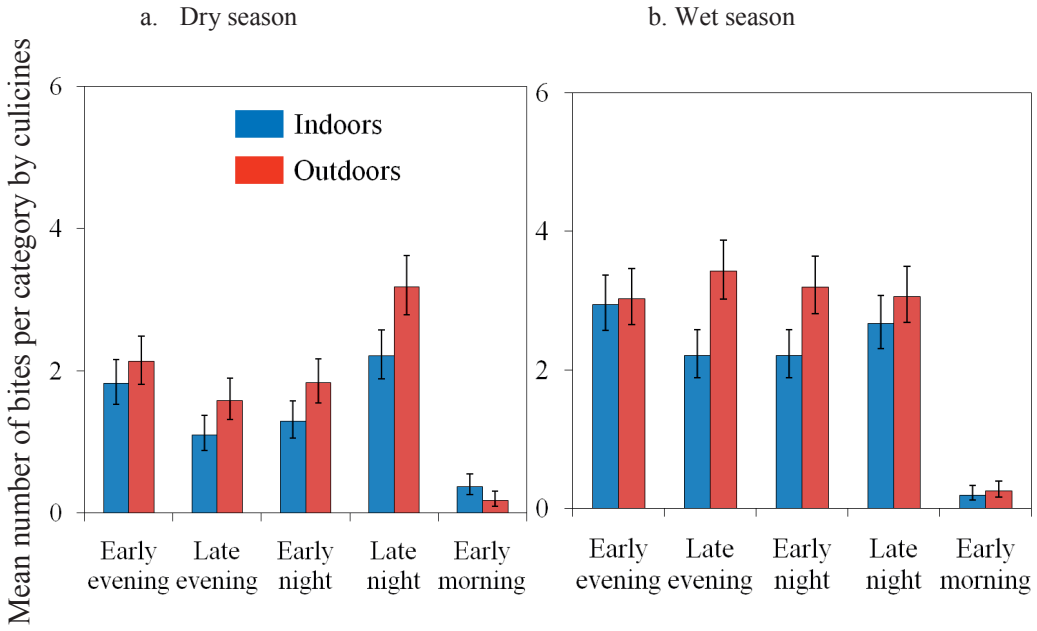


Figure 4: Mean number of bites (95% CI) per category by female culicines both indoors and outdoors during the dry and wet seasons.

**Discussion**

The malaria vectors identified in this study were *An. gambiae* s.l. (primarily *An. arabiensis*) and *An. funestus* s.l. (exclusively *An. funestus* s.s.). Whereas the density of *An. funestus* s.s. was constant in both seasons of this study, the density of *An. gambiae* s.l. was higher in the wet season than in the dry season. In the dry season, the biting activity of *An. gambiae* s.l. was constant across the categorized hours, outdoors, but highest in the late evening hours (21:00 h to 23:45 h) during the wet season. During the dry season, the biting activity of *An. funestus* s.s. was highest in the late evening hours, while in the wet season, the peak biting activity of this species was in the late night hours (03:00 h to 05:45 h). From these results, we conclude that malaria vectors in this region bite

people during times when many individuals may still be active and therefore unprotected by LLINs, which would clearly impact malaria transmission in this region. Furthermore, *An. gambiae* s.l., i.e. *An. arabiensis*, was more likely to bite outdoors than indoors in both seasons, indicating that outdoor biting likely plays a role in malaria transmission in this region.

Previous studies in this region of Malawi conducted in the early 2000s identified three species of malaria vectors: *An. funestus* s.s., *An. gambiae* s.s. and *An. arabiensis* (Spiers et al. 2002, Mzilahowa et al. 2012). The current study identified these same three species, but *An. gambiae* s.s. accounted for only 2% and 10% of the malaria vectors collected in the dry and wet seasons, respectively. This low density of *An. gambiae* s.s. relative to that of *An. arabiensis* and *An. funestus* s.s. agrees with other recent studies in this area (Kabaghe et al. 2018) and warrants further investigation.

The biting activity by *An. tenebrosus* in both seasons was surprising, as little is known about this species. This species has not been incriminated as a malaria vector (Gillies and De Meillon 1968), though it is closely related to *An. coustani* (Gillies and Coetzee 1987*)*. However, in Tanzania, *An. tenebrosus* was reported with infective larvae of *Dirofilaria immitis* (Gillies and Coetzee 1987*)* and therefore, it may be a species of medical importance.

Currently, *An. arabiensis* and *An. funestus* s.s. may be considered the primary malaria vectors in southern Malawi. Furthermore, the density of *An. gambiae* s.l. was higher during the wet season than in the dry season, while that of *An. funestus* s.l. was constant in both seasons, similar to previous studies from Mozambique, Malawi and Tanzania (Mendis et al. 2000, Mzilahowa et al. 2012, Finda et al. 2018), and highlighting the different impacts of seasonality on the abundance of different mosquito species. In the case of *An. gambiae* s.l. and *An. funestus* s.s., this difference may

reflect differences in the preferred larval habitats of each species. While *An. funestus* s.s. typically inhabits more permanent water bodies during its immature stages, *An. gambiae* s.l. is able to use the more temporary larval habitats that occur more often in the wet season (Gimnig et al. 2001, Mutuku et al. 2009).

*Anopheles arabiensis* was more likely to bite outdoors than indoors in this study, both in the dry and wet season. This species is considered as a dominant malaria vector in neighbouring southern Zambia (Kent et al. 2007, Fornadel et al. 2010a) and has been associated with outdoor biting in other regions (Mendis et al. 2000, Tirados et al. 2006, Geissbühler et al. 2007, Oyewole et al. 2007, Russell et al. 2011). The biting densities of *An. funestus* s.s. were higher indoors than outdoors in the wet season, confirming that this species is predominantly endophagic (Awolola et al. 2003, Antonio-Nkondjio et al. 2006, Mwangangi et al. 2013). However, in the dry season, there was no difference between the indoor and outdoor biting densities of *An. funestus* s.s. In other regions, outdoor biting has been associated with the relative availability of hosts outdoors, when they were sleeping in the courtyards or on the verandas of their houses (Faye et al. 1997). Although the current study did not quantify host availability, some people in the region sleep outdoors during the dry season because of higher temperatures as compared to the wet season. During the rainy season, most people in this region sleep indoors, when many are protected by LLINs. Their exposure to mosquito bites would, therefore, occur mostly at times when they are outdoors in the early evening hours. In this context, outdoor biting activities by both *An. arabiensis* and *An. funestus* s.s. are important factors to consider when selecting and planning malaria control interventions. Because LLINs and IRS target indoor biting vectors, there is a need for additional tools that can provide protection against outdoor biting (Govella and Ferguson 2012, Russell et al. 2013, Killeen et al. 2016).

Studies prior to the large-scale introduction of bed nets in Africa found that the major malaria vectors, *An, gambiae* s.s., *An. arabiensis* and *An. funestus*, are nocturnal with peak biting activity occurring in the late night hours (usually from 23:00 h or 24:00 h to 06:00 h) (Fontenille et al. 1990, Githeko et al. 1996b, Fontenille et al. 1997, Pates and Curtis 2005). We refer to this biting as the historic biting time of malaria vectors. These historic biting times coincide with hours that people are usually asleep, which is integral to the effectiveness of LLINs to protect sleepers from infectious bites by malaria vectors. However, some studies have found peak biting activity of malaria vectors outside of these historic biting times. For example, the peak biting activity of *An. arabiensis* in Ethiopia was reported in the early evening hours (19:00 h to 20:00 h), both before and after the implementation of LLINs (Yohannes et al. 2005, Yohannes and Boelee 2012). Such variation in the historic biting times may be explained by regional differences. More recently, in some regions the peak biting times of malaria vectors have been observed outside of the historic biting times, with biting in the early evening (Reddy et al. 2011, Russell et al. 2011) or morning hours (Reddy et al. 2011, Moiroux et al. 2012b, Sougoufara et al. 2014) . Most of these studies lack data on the biting times of malaria vectors in their specific study sites before the implementation of LLINs (Reddy et al. 2011, Sougoufara et al. 2014) but the high levels of reported LLIN use support the hypothesis that it is possible for malaria vector populations to shift peak biting times to avoid LLINs. In the present study, the biting activities of *An. gambiae* s.l. in the early and late evening hours in the dry and wet season, respectively, and *An. funestus* s.l. in the dry season, also differ from the historic biting times of malaria vectors but are similar to results from studies in Ethiopia (Yohannes and Boelee 2012), Mozambique and Tanzania (Mendis et al. 2000, Geissbühler et al. 2007, Russell et al. 2011) . One potential explanation for the observed peak biting time could be that the temperatures are cooler in the late evening hours in this part of

Malawi compared to regions closer to the equator, resulting in the activation of the mosquitoes' host-seeking behaviour (Silver 2008). On the other hand, it could be that *An. gambiae* s.l. had limited access to humans at times when people are protected by LLINs as observed in other regions (Charlwood and Graves 1987, Yohannes and Boelee 2012). Regardless of the explanation, our finding of outdoor biting has implications for malaria control in the region because the biting coincides with the times at which many individuals may still be active and therefore unprotected by LLINs. While the observed biting activity of *An. funestus* s.l.in the early night hours during the wet season suggests that LLIN use still provides significant protection from malaria transmission, the reported levels of insecticide resistance in *An. funestus* populations in Malawi (Riveron et al. 2015, Mzilahowa et al. 2016) raises further concerns about the long-term effectiveness of LLINs as an intervention.

The biting activity of female culicines was constant from the early evening hours to the late night hours both indoors and outdoors. These mosquitoes are a nuisance and have been implicated as vectors of other diseases. In the present study area, filariasis is prevalent (Nielsen et al. 2002, Ngwira et al. 2007) and culicine species have been reported with infective filarial larvae (Merelo-Lobo et al. 2003) highlighting the need for vector control tools that can also target these mosquitoes.

The use of LLINs would be effective against the indoor biting that occurred in the early and late night hours as many individuals are likely to be asleep. However, the observed biting in the early and the late evening hours before people would be under LLINs, both indoors and outdoors, is a major concern. Changes in the biting behaviour from late night to early evening has been associated with the prolonged use of LLINs (Reddy et al. 2011) and studies suggest that due to limited access to hosts by the vectors, the vectors may prefer to bite either in the early

evening/morning or change their biting preferences from indoors to outdoors, where individuals are most likely not protected by LLINs. Complementary tools are then required to tackle this early biting both indoors and outdoors. These tools have been highlighted by Ferguson et al. (2010) and Williams et al. (2018). For instance, house improvement protects all individuals in a house equally. This is being assessed in a number of regions (Killeen et al. 2017b, McCann et al. 2017b) as well as the use of insecticide-impregnated tubes along the eaves, which are the preferred entry points for mosquitoes (Knols et al. 2016, Sternberg et al. 2016, Oumbouke et al. 2018). The development of protective measures that divert malaria vectors from human beings to alternative hosts like cattle is important, especially for species with an opportunistic host-feeding behaviour such as *An. arabiensis*. However, such measures would still sustain the densities of biting malaria vectors and therefore, as suggested by Killeen et al. (2017a), the use of insecticide-treated cattle could be more effective in reducing the density of biting malaria vectors. Other complementary measures that would reduce the densities of biting malaria vectors significantly include the use of insecticide-treated clothes (Kimani et al. 2006, Banks et al. 2014), larval source management and the 'push-pull' approach, which is directed at adult vectors and can be implemented either by the of use of attractive toxic sugar baits (Müller et al. 2010, Beier et al. 2012) or by use of attractants and repellents in traps (Menger et al. 2014a, Menger et al. 2016).

**Conclusion**

The observed biting peaks in the early and late evening hours, both indoors and outdoors, coincide with the times at which individuals may still be awake and physically active, and therefore unprotected by LLINs. A large fraction of biting by anopheline mosquitoes occurred outdoors in

the evening hours. These findings imply that LLINs would only provide partial protection from malaria vectors, which would affect malaria transmission in this area. Therefore, it is important to consider this early biting both indoors and outdoors, as well as the site of biting, when selecting and planning malaria control interventions. Because LLINs and IRS target indoor biting vectors, there is a need for additional tools that can provide protection against outdoor biting (Govella and Ferguson 2012, Russell et al. 2013, Killeen et al. 2016).

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## Chapter 4

# Assessment of the Suna trap for sampling mosquitoes indoors and outdoors

Monicah M. Mburu, Kennedy Zembere, Alexandra Hiscox, Jomo Banda, Kamija S. Phiri, Henk van den Berg , Themba Mzilahowa, Willem Takken and Robert S. McCann

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Chapter 4

**Abstract**

Entomological monitoring is important for public health because it provides data on the distribution, abundance and host-seeking behaviour of disease vectors. Various methods for sampling mosquitoes exist, most of which are biased towards, or specifically target, certain portions of a mosquito population. This study assessed the Suna trap, an odour-baited trap for sampling host-seeking mosquitoes both indoors and outdoors. Two separate field experiments were conducted in villages in southern Malawi. The efficiency of the Suna trap in sampling mosquitoes was compared to that of the human landing catch (HLC; indoors and outdoors) and the Centers for Disease, Control and Prevention Light Trap (CDC-LT; indoors). Potential competition between two Suna traps during simultaneous use of the traps indoors and outdoors was assessed by comparing mosquito catch sizes across three treatments: one trap indoors only; one trap outdoors only; and one trap indoors and one trap outdoors at the same house. The efficiency of the Suna trap in sampling female anophelines was similar to that of HLC indoors ($P = 0.271$) and HLC outdoors ($P = 0.125$) but lower than that of CDC-LT indoors ($P = 0.001$). Anopheline catch sizes of the Suna trap indoors were similar to those that were caught indoors when another Suna trap was simultaneously present outdoors ($P = 0.891$). Similarly, catch sizes of female anophelines with the Suna trap outdoors were similar to those that were caught when another Suna trap was simultaneously present indoors ($P = 0.731$). The efficiency of the Suna trap in sampling mosquitoes was equivalent to that of the HLC. Whereas the CDC-LT was more efficient in collecting female anophelines indoors, the use of this trap outdoors is limited given the requirement of setting it next to an occupied bed net. As demonstrated in this research, outdoor collections are also essential because they provide data on the relative contribution of outdoor biting to malaria transmission. Therefore, the Suna trap can serve as a better alternative

to the HLC and the CDC-LT because it does not require the use of humans as natural baits, allows equal sampling conditions across sampling points, and can be used outdoors. Furthermore, using two Suna traps simultaneously indoors and outdoors does not interfere with the sampling of either trap, which saves a considerable amount of time, energy, and resources compared to setting the traps indoors and then outdoors in two consecutive nights.

**Keywords:** Anophelines, Culicines, CDC-LT, HLC, Suna trap, Simultaneous use, Sampling, efficiency, Indoors, Outdoors.

## Introduction

Control of adult malaria mosquitoes in Africa has been primarily based on the use of insecticides applied either on the inner walls of houses (indoor residual spraying (IRS)) or by impregnating bed nets. As a result, significant reductions in malaria cases have been achieved (Bhatt et al. 2015). However, there are concerns on the long-term effectiveness of such tools because, since the introduction of these chemicals for malaria control, widespread resistance by anopheline mosquitoes has been reported (Chandre et al. 1999, Ranson et al. 2009, Ranson et al. 2011, Riveron et al. 2015, Mzilahowa et al. 2016, Ranson and Lissenden 2016, Wiebe et al. 2017). Furthermore, changes in the biting behaviour of malaria vectors have been reported following the use of long-lasting insecticidal nets (LLINs) (Reddy et al. 2011, Russell et al. 2011, Sougoufara et al. 2014, Meyers et al. 2016).

When assessing the impact of vector control tools on malaria vector populations, entomological monitoring provides important data on the species composition of mosquito communities, the abundance of each species contributing to malaria transmission in a region, the biting behaviour

of these mosquitoes, and the susceptibility of mosquitoes to insecticides. A variety of methods for sampling mosquitoes exist, most of which are biased towards, or specifically target, certain portions of a mosquito population (e.g. host-seeking females or resting mosquitoes). Therefore, it is important to understand the strengths and weaknesses of any sampling method to determine whether it is appropriate for addressing a specific question about the behaviour of malaria vectors (Mboera et al. 1998, Mboera 2005).

Host-seeking females are considered the most epidemiologically relevant portion of a mosquito population because they are directly responsible for disease transmission through blood-feeding (Takken and Knols 1999, Smith et al. 2006). The gold standard for measuring host-seeking malaria mosquitoes (*Anopheles* spp.) has traditionally been the human landing catch (HLC), whereby mosquitoes are captured as they land to feed on a human host (Service 1993b). The HLC method directly estimates the peak biting times for vectors, the vectors' indoor/outdoor biting preferences and the number of infectious bites that a single individual can receive per unit time. One limitation of HLC is that it is labour intensive, requiring collectors to be alert and active throughout each night of the sampling period. Additionally, standardization of HLC across sampling points is restricted by differences among people in their attractiveness to mosquitoes and ability to collect mosquitoes. Concerns about exposing HLC volunteers to malaria during sampling have also been raised, but providing collectors with a prophylactic drug during the sampling period significantly minimizes the risk of malaria infection (Gimnig et al. 2013).

Alternatively, mechanical traps targeting host-seeking female *Anopheles* have been developed as potential substitutes to HLC (Rubio-Palis et al. 2012). These include the CDC-LT, which is usually placed next to a person sleeping under a bed net (Lines et al. 1991), whereby the person acts as an attractive stimulant for mosquitoes (Garret-Jones and Magayuka 1975), but the

mosquitoes are not able to reach the person because of the bed net. Mosquitoes are subsequently caught by the fan-driven suction system of the CDC-LT as they fly near the filament bulb lighting the trap, though the benefit of adding light to the trap beyond the attraction of the human host may be limited (Garret-Jones and Magayuka 1975, Costantini et al. 1998b). While CDC-LT requires less labour than HLC, variation among humans in their attractiveness to mosquitoes (Verhulst et al. 2011) still inhibits standardization of the CDC-LT across sampling points. Comparisons of the sampling efficiency of the CDC-LT relative to that of the HLC have given variable results in different regions (Lines et al. 1991, Mathenge et al. 2004, Overgaard et al. 2012b, Wong et al. 2013, Kenea et al. 2017), indicating that a more standardized method for sampling host-seeking *Anopheles* is needed. Furthermore, CDC-LT sampling of *Anopheles* is primarily designed for indoor sampling, given the requirement of setting it next to an occupied bed net. When used outdoors, the CDC-LT generally collects very few *Anopheles* (Costantini et al. 1998b). Therefore, a better alternative to HLC than CDC-LT is needed.

Other mechanical traps target host-seeking female *Anopheles* using chemical baits composed of volatiles found on human skin (Kline et al. 1990, Njiru et al. 2006, Qiu et al. 2007, Okumu et al. 2010b, Menger et al. 2014a), which are attractive to host-seeking *Anopheles*. For instance, the Suna trap is an odour-baited trap that has recently been developed to collect host-seeking mosquitoes both indoors and outdoors (Hiscox et al. 2014). To attract mosquitoes, it uses a synthetic blend of chemicals found on human skin (Mukabana et al. 2012, Menger et al. 2014b) and carbon dioxide ($CO_2$) produced through a process of yeast and molasses fermentation (Mweresa et al. 2014b). The odour blend is standardized, allowing for reliable comparisons among trapping locations. It does not require any human interaction between trap set up in the afternoon and collecting the mosquitoes from the trap the next morning. The minimal labour

requirements, the ability for use outdoors, and capacity for standardization make the Suna trap a promising alternative for large-scale monitoring of *Anopheles* populations.

The positioning of traps during entomological monitoring is also important (Mboera et al. 1998). As the Suna trap can be used for collection of mosquitoes both indoors and outdoors, considerable time could be saved if the trap could be used simultaneously indoors and outdoors. However, there are concerns about possible competition between traps under such arrangements, whereby the presence of one trap may affect the catch of the other trap. Therefore, the objectives of this study were to compare the efficiency of the Suna trap in sampling mosquitoes relative to the HLC and the CDC-LT and to assess the effect of the simultaneous use of the Suna trap indoors and outdoors on the collection of mosquitoes in each trap.

## Methods

### Study site

Two separate field experiments were conducted in rural villages of Chikwawa District, in southern Malawi. The villages lie along the lower Shire valley and experience a single rainy season from November through April. The main malaria vectors in the region are *Anopheles gambiae* s.s., *An. funestus* and *An. arabiensis* (Spiers et al. 2002, Mzilahowa et al. 2012). Malaria transmission occurs throughout the year with the rates intensifying during the rainy season. The region is characterized by subsistence farming, and most of the houses have mud or clay-brick walls with grass-thatched or iron-sheet roofs. Each study was conducted in villages that were part of the Majete Malaria Project (MMP), a cluster-randomised malaria control trial which has been described in detail by McCann et al. (2017b). The experiment assessing the efficiency of

the Suna trap in sampling mosquitoes, took place in two neighbouring villages, namely Tsekera (-15.985, 34.78) and Chipula (-15.990, 34.78) in 2014 before the MMP trial activities began. The study on the simultaneous use of the Suna trap indoors and outdoors took place in Chigwata II (-16.02, 34.52) and Kalonga (-16.02, 34.51) villages in 2017. These two villages were under the control arm of MMP (i.e. no larval source management or house improvement was implemented in these two villages).

**Comparing the efficiency of the Suna trap in sampling mosquitoes**

In Tsekera and Chipula, ten houses representative of the local setting were selected as locations for sampling mosquitoes based on the following criteria: houses with open eaves, grass thatched roofs, mud walls, three to five people sleeping in the house each night, and the residents did not normally cook inside the house or on the veranda. All houses were of a similar size and were at least 50m from each other**.**

Mosquitoes were sampled using three methods: the Suna trap, CDC-LT and HLC. Two of these methods (Suna trap and HLC) were used both indoors and outdoors. The CDC-LT was only used for indoor sampling based on previous studies (Costantini et al. 1998b). Thus, there were five treatments in the experimental design: Suna trap indoors or outdoors; HLC indoors or outdoors; and CDC-LT indoors. Mosquitoes were sampled five nights per week for 8 weeks from 7 July to 29 August 2014, except for one night missed due to field supervisor illness, resulting in 39 sampling nights. The five treatments were rotated through ten houses according to a Williams design (Additional file 1) to control for any potential effects of the sequence in which they were used at a house (Williams 1949).

A solar power system was set up at each house to run the Suna trap and CDC-LT. A solar panel was set on the roof and connected to a controller, battery and timers to run the traps. The timers were set to turn on a trap at 1700h the day it was scheduled to run according to the study design (Additional file 1) and turn off at 0700h the following morning. The CDC-LT were suspended 50 cm above the floor in the bedroom, at the foot of a bed where a resident of the house slept under their own insecticide-treated bed net (Lines et al. 1991). Suna traps were suspended with the entry 30 cm above ground level (Hiscox et al. 2014). For outdoor sampling, a Suna trap was hung at the side of the house from an overhanging eave. For indoor sampling, a Suna trap was hung in the sitting room. Suna traps were baited with the MB5 blend of attractants (Mukabana et al. 2012, Menger et al. 2014b). The medium for dispensing the MB5 blend was similar to that of Mweresa et al. (2014a), which consists of an absorbent layer (95% cellulose and 5% sodium polyacrylate fibres) of a disposable menstrual sanitary pad (unscented Always ultra thin, ultra-fine Gel-X, FabricadonaEgiptopor, EG Procter & Gamble Company, Egypt). Suna traps were supplied with $CO_2$ produced through a process of yeast and molasses fermentation (Mweresa et al. 2014b) prepared each night of sampling. Household residents were informed about the operation of the traps and instructed not to interfere with the traps or solar power system.

For HLC, eight collectors were recruited from the study villages. Prior to the study, all collectors were trained in the HLC technique and tested for malaria using a rapid diagnostic test (RDT). Collectors with a positive RDT were treated with lumefantrine-artemether (LA) according to current national treatment guidelines. All collectors were given malaria prophylaxis with doxycycline at a daily dose of 100mg for the duration of exposure (8weeks) and for 30 days thereafter. A team of two people worked at each house assigned for HLC, working one-after-the-other in two 7-hour shifts. The first shift was from 1700 hrs to midnight and the second from

midnight to 0700 hrs. Each hour, collectors worked for 45 minutes followed by a 15-minute break. A field supervisor did sporadic spot checks to ensure HLC collectors were following protocol. For indoor sampling, the collector sat in the sitting room of the house. For outdoor sampling, the collector sat about 1-3m from the front door of the house. Collectors sat with their legs exposed from their knees down and collected mosquitoes that landed on their legs using a mouth aspirator. Mosquitoes were then gently blown into a paper cup that had been pre-labeled with the house number, date and hour of collection.

**Assessing the simultaneous use of Suna trap indoors and outdoors**

Twelve houses that had the following criteria were recruited for the study in Kalonga and Chigwata II villages: open eaves, grass-thatched roofs, houses that were ≥25m apart and at least 100m away from any mosquito breeding habitat. Placement of Suna traps comprised of: (a) a single trap indoors, (b) a single trap outdoors and (c) two traps (one indoors and one outdoors) at the same house, simultaneously (Additional file 2). Mosquito sampling was carried out four nights per week from 23 March to 19 May 2017. To rule out order effects, a 12 × 12 experimental design was adopted (Additional file 3). The set-up of the Suna traps was similar to that described above with the following exceptions: the medium for dispensing the MB5 blend was a manufactured cartridge (BG-MB5 blend dispenser, Biogents, Regensburg, Germany); and the batteries were charged at an MMP research station and moved to the study houses each afternoon.

**Identification of mosquitoes and detection of *Plasmodium falciparum* DNA**

All mosquitoes were taken to the laboratory for processing. They were identified using the protocol by Gillies and Coetzee (1987). All *Anopheles* were identified as either *An. gambiae* s.l.,

*An. funestus* s.l., *An. coustani* or *An. tenebrosus* and the abdominal status was recorded. There was no further classification of culicines beyond the subfamily level. Female *An. gambiae* s.l. and *An. funestus* s.l. were further identified to species level using PCR. The head and thoraces of all female *An. gambiae* s.l. and *An. funestus* s.l. were tested for the presence of *P. falciparum* DNA using qPCR (Perandin et al. 2004) with a Ct value ≤ 37.0 as the cut-off for *P. falciparum* positive.

**Data analysis**

For both studies, generalized linear models with a Poisson distribution and a log link function were used to assess differences among the treatments in the number of female anophelines and culicines collected per night. For each set of analyses, the two outcomes assessed were the number of female anophelines and the number of female culicines caught per house, per night. Generalized estimating equations were used to account for repeated measures by house. The three sampling methods used indoors in the first study (Suna trap, HLC and CDC-LT) were compared in one set of analyses, while the two methods used outdoors (Suna trap and HLC) were compared in a separate set of analyses. For the study assessing whether the simultaneous use of Suna traps in the same house leads to competition between the traps, three comparisons were made: (1) the numbers of mosquitoes collected indoors (without another trap outdoors) were compared to the numbers collected indoors when a trap was used simultaneously outdoors; (2) the numbers of mosquitoes collected outdoors (without another trap indoors) were compared to the numbers collected outdoors when a trap was used simultaneously indoors; and (3) the numbers of mosquitoes collected indoors (combined across treatments) were compared to the numbers of mosquitoes collected outdoors (also combined across treatments). A number of

variables were included as covariates in these models: the number of people that slept in the house the previous night, wall type, use of bed net, cooking location and kind of livestock that stayed within 20m of the house the previous night. Wall type was categorized as mud, fire-baked bricks and sun-dried bricks. Cooking locations were: inside the house, on the veranda, outside the house but within 2m, and outside more than 2m from the house. Livestock comprised of cattle, goats, and chickens. Floor and door types were not included as covariates because all the floors and doors were made of mud and wood, respectively. All analyses were performed using IBM SPSS statistics, version 20.0.

**Results**

In the experiment comparing the efficiency of the Suna trap in sampling mosquitoes relative to the HLC and the CDC-LT, a total of 2,458 mosquitoes were collected. Of these, 8% were female anophelines, 87% female culicines, 1% male anophelines and 4% male culicines. Of the female anophelines, catches comprised of *Anopheles gambiae* s.l. (59%; n = 116) and *An. funestus* s.l. (41%; n = 82). Out of the 198 female anophelines, 189 were analysed molecularly using PCR. Of the 189, 115 were identified as *An. arabiensis*, 50 as *An. funestus* s.s. and 5 as *An. parensis*. Nineteen of the anophelines could not be identified further because they failed to amplify. Most of the female anophelines were unfed, but some fed, half-gravid or gravid female anophelines were also collected (Table 1).

Table 1: Mosquitoes collected across all methods by species/subfamily/abdominal status and sex for the study on the efficiency of the Suna trap in sampling mosquitoes

| | Unfed | Fed | Half gravid | Gravid | Undetermined | Total females | Male |
|---|---|---|---|---|---|---|---|
| *An. gambiae* s.l. | | | | | | | |
| CDC-LT indoors | 53 | 19 | 6 | 8 | 2 | 88 | 4 |
| HLC Indoors | 3 | 5 | 0 | 0 | 0 | 8 | 1 |
| HLC outdoors | 6 | 1 | 0 | 0 | 0 | 7 | 0 |
| Suna indoors | 3 | 4 | 0 | 0 | 0 | 7 | 0 |
| Suna outdoors | 6 | 0 | 0 | 0 | 0 | 6 | 2 |
| *An. funestus* s.l. | | | | | | | |
| CDC-LT indoors | 29 | 22 | 3 | 2 | 0 | 56 | 11 |
| HLC Indoors | 0 | 4 | 0 | 0 | 0 | 4 | 0 |
| HLC outdoors | 3 | 0 | 0 | 0 | 0 | 3 | 0 |
| Suna indoors | 6 | 0 | 0 | 0 | 1 | 7 | 1 |
| Suna outdoors | 9 | 2 | 0 | 0 | 1 | 12 | 2 |
| Culicines | | | | | | | |
| HLC Indoors | 567 | 26 | 2 | 10 | 0 | 605 | 13 |
| HLC outdoors | 966 | 47 | 3 | 20 | 0 | 1036 | 16 |
| Suna indoors | 49 | 0 | 0 | 0 | 0 | 49 | 4 |
| Suna outdoors | 169 | 2 | 0 | 1 | 0 | 172 | 7 |

Of the 189 female anophelines tested for the presence of *P. falciparum* DNA, 34 (30 *An. arabiensis* and 4 *An. funestus* s.s.) were positive for *P. falciparum* DNA, indicating a sporozoite rate of 18% across sampling methods and locations. Of these, 27 were from the CDC-LT (sporozoite rate of 19%), 4 were from HLC indoors (33%), 2 were from HLC outdoors (20%) and 1 was collected indoors with the Suna trap (7%).

Twenty-one house-nights were excluded from the analysis due to incomplete sampling effort (e.g. dead battery or the owner of the house was unavailable), resulting in 369 total house-nights analysed instead of 390.

Indoors, catches of female anophelines with Suna traps were similar to those of the HLC (RR = 0.66, 95% confidence interval (CI) 0.32-1.38, P = 0.271) but lower than those of the CDC-LT (RR = 8.18, 95% CI 4.95-13.53, P = 0.001). For outdoor sampling, catches with Suna trap were similar to those of the HLC (RR = 0.54, 95% CI 0.25-1.19, P = 0.125; Fig. 1).

For female culicines, indoor collections with the Suna trap were lower than those of the HLC (RR = 3.27, 95% CI 2.76-3.87, P = 0.001) and the CDC-LT (RR = 1.59, 95% CI 1.32-1.92, P = 0.001). Likewise, outdoor collections of female culicines with the Suna trap were lower than those of the HLC (RR = 5.51, 95% CI 4.69-6.47, P = 0.001; Fig. 2).

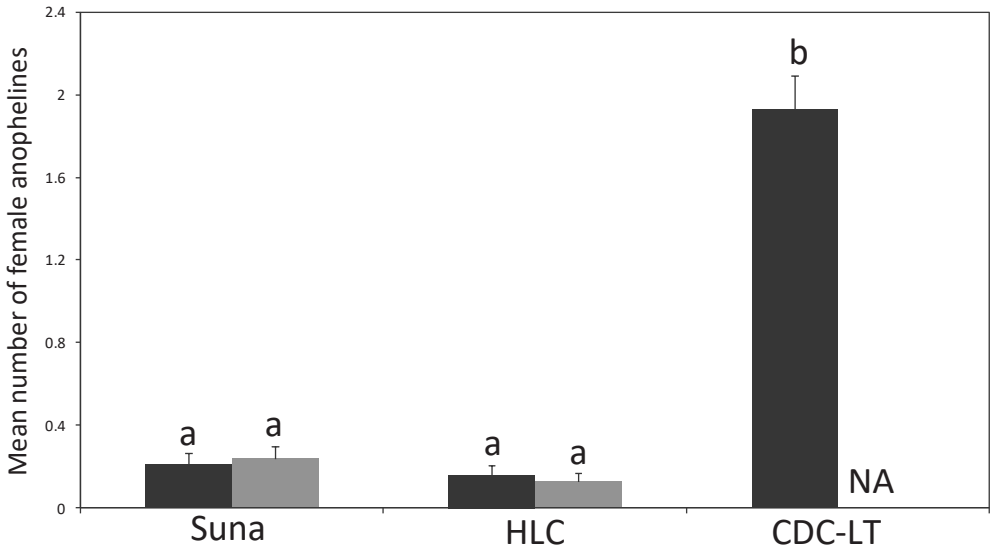



Figure 1: Mean number (±SE) of female anophelines caught indoors (black bar) and outdoors (grey bar) with Suna traps, HLC and CDC-LT. NA indicates the absence of a trap in a different location. Bars with different letters denote differences within location (i.e. indoors/outdoors).

For the study assessing the simultaneous use of the Suna trap indoors and outdoors, the total number of mosquitoes caught was 328. Of these, 3% were males (n=10) and 97% were females (n=318). The male mosquito catches comprised of *An. gambiae* s.l. (n = 3) and culicines (n = 7). The female catches comprised of *An. gambiae* s.l. (40.3%; n = 128), *An. coustani* (0.3%; n = 1), *An. tenebrosus* (0.6%; n = 2) and culicines (58.8%: n = 187). Of the 128 female *An. gambiae* s.l., 117 were identified as *An. arabiensis* and 11 as *An. gambiae* s.s.. Twenty-seven (all *An. arabiensis*) were positive for *P. falciparum* DNA, indicating a sporozoite rate of 21%. Most of the female anophelines were unfed (n = 124) while the rest were either half gravid (n = 3) or fed (n = 2).

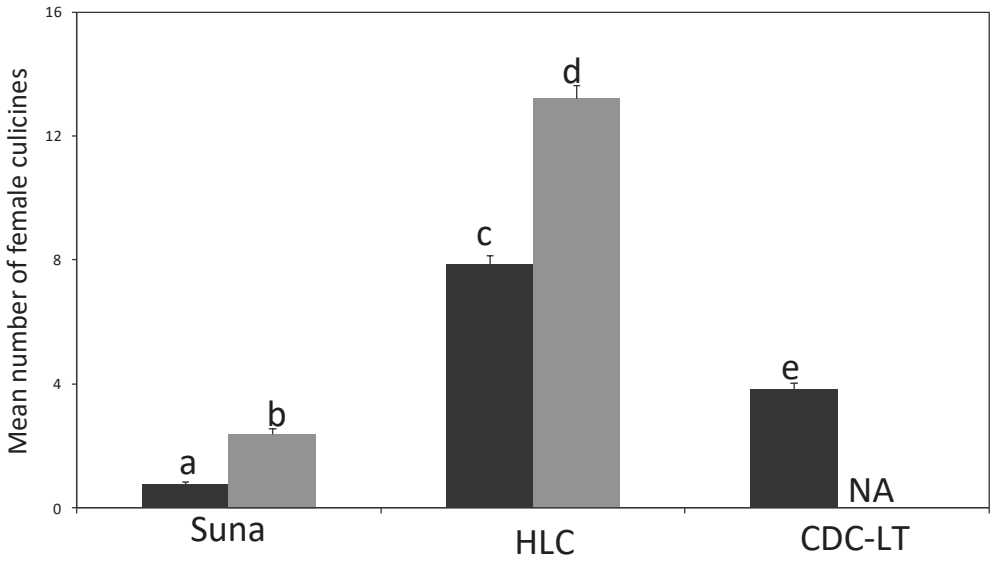



Figure 2: Mean number (±SE) of female culicines caught indoors (black bar) and outdoors (grey bar) with Suna traps, HLC and CDC-LT. NA indicates the absence of a trap in a different location. Bars with different letters denote differences within location (i.e. indoors/outdoors).

Sixteen house-nights were excluded from the analysis due to incomplete sampling effort (e.g. dead battery or the owner of the house was unavailable), resulting in 368 total house-nights analysed instead of 384.

Of the total female anophelines, 29 were caught indoors (without another trap outdoors) and 34 were caught outdoors (without another trap indoors). When the indoor and outdoor traps were run simultaneously, the indoor and outdoor catches of female anophelines were 28 and 38, respectively. There were no differences in the number of female anophelines that were caught indoors (without another trap outdoors) and in those that were caught indoors when a trap was used simultaneously outdoors (RR = 1.04, CI = 0.61-1.76, P = 0.891; Fig. 3A). Similarly, the number of female anophelines that were caught outdoors (without another trap indoors) were

similar to those that were caught outdoors when a trap was used simultaneously indoors (RR = 0.92, CI = 0.57-1.48, P = 0.731; Fig. 3B). Pooling across all indoor and outdoor collections irrespective of the simultaneous use of a trap, the catches of female anophelines were similar indoors and outdoors (RR = 0.78, 95% CI = 0.55-1.11, P = 0.162; Fig. 3C).

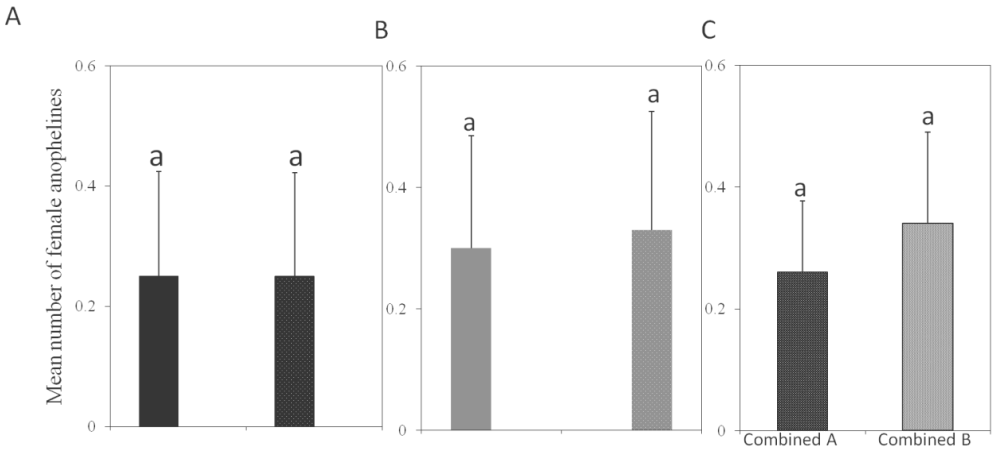



Figure 3: Mean number (±SE) of female anophelines caught with Suna traps A. indoors (black bar), indoors with another trap outdoors (black hatched bar), B. outdoors (grey bar), outdoors with another trap indoors (grey hatched bar), and C. Combined A and B indicate all female anophelines catches indoors and outdoors, respectively, irrespective of the simultaneous use of trap in either location. Bars with same letters denote the similar number of mosquitoes trapped.

The number of female culicine mosquitoes caught indoors (without another trap outdoors) and outdoors (without another trap indoors) were 44 and 54, respectively. When the indoor and outdoor traps were run simultaneously, the indoor catches were 45 and outdoor catches were 44. There were no differences in the number of female culicine mosquitoes caught indoors (without another trap outdoors) and in those caught indoors when a trap was used simultaneously outdoors

(RR = 0.97, CI = 0.64-1.48, P = 0.889; Fig. 4A). Likewise, the mosquitoes that were caught outdoors (without another trap indoors) were similar to those that were caught outdoors when a trap was used simultaneously indoors (RR = 1.24, CI = 0.83-1.86, P = 0.302; Fig. 4B). Pooling across all indoor and outdoor collections irrespective of simultaneous use of trap, the catches of female culicines were similar (RR = 0.92, 95% CI = 0.69-1.23, P = 0.591; Fig. 4C).

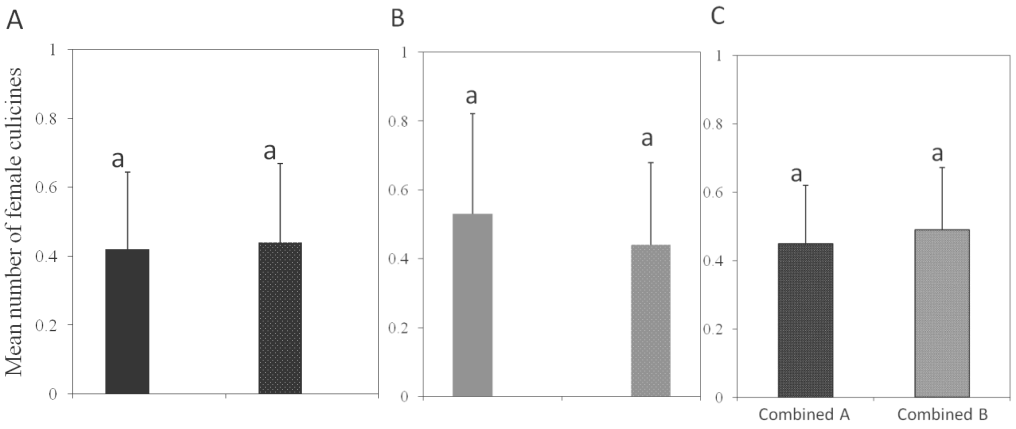



Figure 4: Mean number (±SE) of female culicines caught with Suna traps A. indoors (black bar), indoors with another trap outdoors (black hatched bar), B. outdoors (grey bar), outdoors with another trap indoors (grey hatched bar), and C. Combined A and B indicate all female culicines catches indoors and outdoors, respectively, irrespective of the simultaneous use of trap in either location. Bars with same letters denote the similar number of mosquitoes trapped.

Cooking on the veranda was positively associated with female anophelines when the trap was set indoors (without another trap outdoors) and indoors with simultaneous use of another trap outdoors (RR = 3.71, CI = 1.42-9.71, P = 0.007). The number of people that slept in the house the previous night was positively associated with the number of female anophelines that were caught outdoors (without another trap indoors) and those that were caught outdoors when a

simultaneous trap was used indoors (RR = 1.53, CI = 1.12-2.09, P = 0.007). The wall type, use of bed nets rate, presence of cattle, goats, and chickens did not have an effect on the number of female anophelines caught in either location ($P \geq 0.05$) (Table 2).

There is some evidence that the presence of cattle within 20m of the house the previous night reduced the catches of female culicines when the trap was set indoors (without another trap outdoors) and indoors with the simultaneous use of a trap outdoors (RR = 0.12 CI = 0.02-0.96, P =0.04). The number of people that slept in the house the previous night, wall type, use of bed nets, presence of goats, and chickens did not have an effect on the number of female culicines caught in either location ($P \geq 0.05$) (Table 2).

**Discussion**

These studies describe the use of Suna traps for sampling mosquitoes. Comparing the efficiency of the Suna trap relative to the HLC, similar numbers of female anophelines were collected using each method both indoors and outdoors. When assessing whether the simultaneous use of the Suna trap indoors and outdoors in a house leads to competition between the two traps, the results demonstrate that the simultaneous use does not affect the catch size in either location. In addition, the observations on the abdominal status showed that most of the female anophelines caught with the Suna trap were unfed, supporting the hypothesis that the Suna trap catches the host-seeking fraction of the anopheline population. Finally, the catch sizes of female anophelines in all indoor collections were similar to those of all outdoor collections in these studies, highlighting the importance of sampling for malaria vectors outdoors in addition to indoors. This sampling can provide data on the relative contribution of indoor and outdoor biting vectors to malaria transmission.

Table 2: Effect of treatment, use of bed net, livestock, cooking locations, wall type and the number of people that slept in the house the previous night on the catch sizes of anophelines and culicines for the study on the simultaneous use of Suna trap indoors and outdoors.

| **Treatment** | Indoors[a] | | Outdoors[b] | | Combined[c] | |
|---|---|---|---|---|---|---|
| | **RR** | **95% CI** | **RR** | **95% CI** | **RR** | **95%CI** |
| **Female anophelines** | 1.04 | 0.61–1.76 | 0.92 | 0.57–1.48 | 0.78 | 0.55-1.11 |
| People that slept in the house the previous night | 1.23 | 0.87–1.75 | 1.53 | 1.12–2.09 | 1.39 | 1.10-1.74 |
| Wall type fire baked bricks | 0.31 | 0.04-2.57 | 1.15 | 0.41-3.21 | 0.82 | 0.34-1.99 |
| Wall type mud bricks | 1.59 | 0.79-3.18 | 1.47 | 0.76-2.83 | 1.52 | 0.95-2.45 |
| Wall type sun-dried bricks | Ref | – | Ref | – | Ref | - |
| Mosquito control-bed-net | 0.86 | 0.43-1.72 | 1.25 | 0.69-2.26 | 1.03 | 0.66-1.60 |
| Mosquito control-none | Ref | – | Ref | – | Ref | - |
| Cooking inside the house | 2.77 | 0.84–9.17 | 2.35 | 0.48-11.43 | 2.50 | 0.99-6.31 |
| Cooking on the veranda | 3.71 | 1.42–9.71 | 1.30 | 0.63–2.70 | 2.01 | 1.14-3.55 |
| Cooking outside, within 2m of the house | 0.83 | 0.27–2.62 | 0.91 | 0.36–2.28 | 0.88 | 0.44-1.78 |
| Cooking outside, more than 2m from the house | Ref | – | Ref | – | Ref | - |
| Cow | 1.21 | 0.21–6.95 | 0.14 | 0.02-1.21 | 0.40 | 0.12-1.46 |

Table 2 Continued

| **Treatment** | Indoors[a] | | Outdoors[b] | | Combined[c] | |
|---|---|---|---|---|---|---|
| | **RR** | **95% CI** | **RR** | **95% CI** | **RR** | **95%CI** |
| Goat | 2.31 | 0.84–6.36 | 1.02 | 0.48–2.20 | 1.42 | 0.78-2.57 |
| Chicken | 1.07 | 0.36–3.23 | 0.38 | 0.08–1.70 | 0.70 | 0.30-1.65 |
| | | | | | | |
| **Female culicines** | 0.97 | 0.64-1.48 | 1.24 | 0.82-1.86 | 0.92 | 0.69-1.23 |
| People that slept in the house the previous night | 1.18 | 0.91-1.54 | 1.02 | 0.78-1.34 | 2.0 | 0.91-1.32 |
| Wall type fire baked bricks | 0.51 | 0.19-1.42 | 0.43 | 0.12-1.53 | 0.49 | 0.22-1.07 |
| Wall type mud bricks | 0.92 | 0.50-1.67 | 1.18 | 0.69-2.02 | 1.05 | 0.71-1.57 |
| Wall type sun-dried bricks | Ref | – | Ref | – | Ref | - |
| Mosquito control-bed-net | 0.87 | 0.51-1.49 | 0.77 | 0.48-1.25 | 0.85 | 0.59-1,21 |
| Mosquito control-none | Ref | – | Ref | – | Ref | - |
| Cooking inside the house | 0.58 | 0.15-2.18 | 0.54 | 0.16-1.88 | 0.55 | 0.23-1.36 |
| Cooking on the veranda | 1.0 | 0.53-1.90 | 0.60 | 0.33-1.10 | 0.75 | 0.49-1.15 |
| Cooking outside, within 2m of the house | 1.61 | 0.77-3.38 | 0.85 | 0.39-1.88 | 1.19 | 0.70-2.03 |
| Cooking outside, away from 2m of the house | Ref | – | Ref | – | Ref | - |
| Cow | 0.12 | 0.02-0.96 | 0.30 | 0.09-1.09 | 0.21 | 0.07-0.61 |

Table 2 Continued

| **Treatment** | Indoors[a] | | Outdoors[b] | | Combined[c] | |
|---|---|---|---|---|---|---|
| | **RR** | **95% CI** | **RR** | **95% CI** | **RR** | **95%CI** |
| Goat | 0.75 | 0.38-1.46 | 0.64 | 0.36-1.16 | 0.67 | 0.43-1.04 |
| Chicken | 1.15 | 0.45-2.92 | 1.41 | 0.61-3.23 | 1.27 | 0.69-2.34 |

[a]Description of column A - Suna trap indoors (only) and indoors (with another trap outdoors)
[b]Description of column B - Suna trap outdoors (only) and outdoors (with another trap indoors)
[c]Description of column C - Suna trap indoors and outdoors (with or without another trap indoors or outdoors)

This is the first study of which we are aware comparing the sampling efficiency of the Suna trap with that of HLC, and similar numbers of female anophelines were collected using each method both indoors and outdoors. The Suna trap was designed to mimic a human host, using both $CO_2$ and a synthetic odour bait to attract host-seeking mosquitoes. The bait used in the Suna trap was composed of five volatiles normally found on human skin (Mukabana et al. 2012, Menger et al. 2014b), which, when compared to humans, is equally attractive to female anophelines (Okumu et al. 2010b, Mukabana et al. 2012). While further studies are needed to assess the effect of different environmental conditions on the comparability of the two methods, the results presented here suggest that sampling with the Suna trap can approximate the human biting rate of anophelines in this region.

When compared to the CDC-LT, the Suna trap showed lower efficiency in sampling both anopheline and culicine mosquitoes. This contrasts with findings from western Kenya where the

indoor catches with the Suna trap were similar to those of the CDC-LT in a semi-field experiment (Hiscox et al. 2014). One possible explanation could be that the placement of traps relative to sleepers may affect the mosquito catches (Okumu et al. 2010b). Hiscox et al. (2014) placed both the Suna trap and CDC-LT next to a person sleeping under a bed-net in a single-room house constructed within a screen house. In southern Malawi, where the present study was conducted, houses are typically divided into at least two rooms (a bedroom and a sitting room), and the CDC-LT was set in the bedroom next to a person sleeping under a bed-net, following the standard for this sampling method in Africa (Lines et al. 1991). The indoor Suna trap sampling, however, took place in the sitting room to match the standard protocol of the HLC method. Differences in the concentrations of odours provided by human hosts between the bedroom and sitting room could have attracted more mosquitoes to the former, where the CDC-LT was located. Further studies on the placement of the Suna trap relative to sleepers are needed. Secondly, differences in sampling efficiencies could be explained by differences in the mosquito species being observed. In their semi-field comparison of the Suna trap and CDC-LT, Hiscox et al. (2014) used laboratory reared *An. gambiae* s.s. while in the present study, the most abundant species were *An. arabiensis* and *An. funestus*. It is possible that these two species respond differently to the CDC-LT and/or the Suna trap. Thirdly, the two studies used different media for dispensing the odour baits from the Suna trap. Hiscox et al. (2014) used nylon strips (Okumu et al. 2010a), and the present study used a sanitary pad absorbent layer (Mweresa et al. 2014a). However, this third explanation is unlikely, given that Mweresa et al. (2014a) collected more anophelines in odour-baited traps using the sanitary pad absorbent layer than nylon strips.

When compared with the HLC indoors, the CDC-LT was more effective in collecting female anophelines in this study, which is in line with findings from Tanzania (Govella et al. 2009),

Kenya (Wong et al. 2013) and Zambia (Fornadel et al. 2010b). However, most studies demonstrate that the two methods collect similar numbers of anophelines (Lines et al. 1991, Mbogo et al. 1993, Mathenge et al. 2005, Ndiath et al. 2011, Sikaala et al. 2013), while others report that the efficiency of the HLC in sampling host-seeking anophelines is higher than that of the CDC-LT (Mbogo et al. 1993, Govella et al. 2011, Overgaard et al. 2012b). A comprehensive review looking at paired mosquito collections of the HLC and the CDC-LT found that the sampling efficiencies of the two methods vary, in that, the CDC-LT catches are either similar to those of the HLC, higher or lower than those of the HLC (Briet 2002). Therefore, it is possible that the local environmental conditions affect the efficiency of both sampling methods and may explain the observed differences in catch size.

The HLC and CDC-LT both collected more female culicines than the Suna trap. Moreover, the culicine catch sizes of the HLC were more than those of the CDC-LT, which is consistent with a study from Zambia (Sikaala et al. 2013) but in contrast with that of Mweresa (2014). Mweresa (2014) suggested that the CDC-LT caught more culicines because of the presence of multimodal stimuli (human bait + light). As with anophelines, it is likely that the efficiency of these two methods varies with local mosquito species and environmental conditions.

Combined across the two experiments presented here, the infection rate of *An. arabiensis* with *P. falciparum* was higher than that of *An. funestus.* Though *An. arabiensis* is often seen as a less efficient vector, the abundance of this species, together with its relatively high infection rate, confirms that this species is important as a malaria vector and that it contributes significantly to transmission of malaria in southern Malawi. The absence of *An. funestus* in one of the two studies presented here is most likely explained by seasonal fluctuations. The assessment of simultaneous Suna trap use indoors and outdoors was conducted during the rainy season when

densities of *An. arabiensis* are generally higher in this region, while those of *An. funestus* tend to increase at the end of the rainy season and at the beginning of the dry season (Mzilahowa et al. 2012, Kabbale et al. 2013). Recent findings have shown that this species is still abundantly present in the region (Kabaghe et al. 2018). In addition to *An. arabiensis* and *An. funestus, An. gambiae* s.s. was previously common in this region (Spiers et al. 2002, Mzilahowa et al. 2012), but the current study and others (Kabaghe et al. 2018) have found very few *An. gambiae* s.s. relative to other anopheline species. The apparent decline of *An. gambiae* s.s. in southern Malawi warrants further investigation, as similar declines in East Africa have been associated with the long-term use of bednets (Bayoh et al. 2010).

In assessing the simultaneous use of the Suna trap indoors and outdoors, the results demonstrate that the trap can be used simultaneously in both locations without any competition. This would save a considerable amount of time, energy and resources when monitoring the abundance of malaria vectors indoors and outdoors, compared to using the trap indoors only and then outdoors only, for two consecutive days. Furthermore, the catch sizes of female anophelines collected indoors (with or without the simultaneous use of the trap outdoors) were similar to those that were collected outdoors (with or without the simultaneous use of the trap indoors). This can be explained by the predominance of *An. arabiensis* during this study, as the species exhibits both indoor and outdoor host-seeking behaviour (Mendis et al. 2000). While indoor mosquito collections are important for assessing vector control programmes, outdoor collections are also essential, in particular with the potential shift towards outdoor biting in some anopheline populations (Reddy et al. 2011, Russell et al. 2011, Killeen et al. 2016). Therefore, methods for assessing outdoor host-seeking mosquito densities relative to indoor host-seeking mosquito densities are required. The Suna trap addresses this need as a method that provides equal

sampling conditions both indoors and outdoors. The Suna trap also requires less labour than the HLC or CDC-LT because it does not rely on the use of humans as baits. The use of a standard synthetic bait in the Suna trap also provides equal sampling conditions across sampling locations, unlike the HLC and CDC-LT, which are subject to differences in attractiveness to mosquitoes among human volunteers (Verhulst et al. 2011).

**Conclusion**

The efficiency of the Suna trap in sampling host-seeking anopheline mosquitoes was equivalent to that of the HLC. Whereas the CDC-LT was more efficient in collecting female anophelines indoors, the use of the CDC-LT outdoors is limited given the requirement of setting it next to an occupied bed net. As demonstrated in this study, outdoor collections are also essential because they provide data on the relative contribution of outdoor biting to malaria transmission. Therefore, the Suna trap can serve as a better alternative to the HLC and CDC-LT because it does not require the use of humans as natural baits, allows equal sampling conditions across sampling points, and can be used outdoors. Furthermore, using two Suna traps simultaneously indoors and outdoors does not interfere with the sampling of either trap, which saves a considerable amount of time, energy, and resources compared to setting the traps indoors and then outdoors in two consecutive nights.

**Acknowledgements**

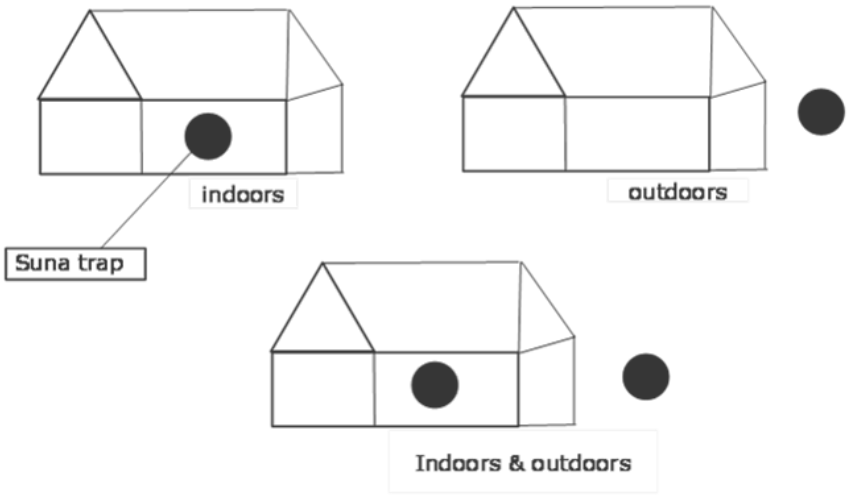
We acknowledge the local leaders and residents of Tsekera, Chipula, Kalonga and Chigwata II for allowing us to work in their houses and their cooperation. Benjamin Nyoni and human volunteers are thanked for helping out with the human landing collections. We thank the

laboratories of Malaria Alert Centre and Blantyre Malaria Project (BMP) where we did the molecular identification of female anophelines. We also extend our thanks to Majete Malaria Project (MMP) team. The study was funded by Dioraphte Foundation, the Netherlands.

Additional file 1: Nightly schedule of sampling methods used at each house, repeated for eight weeks.

| House | Sunday | Monday | Tuesday | Wednesday | Thursday |
|---|---|---|---|---|---|
| 1 | CDC-LT (In) | Suna (In) | HLC (In) | Suna (Out) | HLC (Out) |
| 2 | Suna (In) | Suna (Out) | CDC-LT (In) | HLC (Out) | HLC (In) |
| 3 | Suna (Out) | HLC (Out) | Suna (In) | HLC (In) | CDC-LT (In) |
| 4 | HLC (Out) | HLC (In) | Suna (Out) | CDC-LT (In) | Suna (In) |
| 5 | HLC (In) | CDC-LT (In) | HLC (Out) | Suna (In) | Suna (Out) |
| 6 | HLC (Out) | Suna (Out) | HLC (In) | Suna (In) | CDC-LT (In) |
| 7 | HLC (In) | HLC (Out) | CDC-LT (In) | Suna (Out) | Suna (In) |
| 8 | CDC-LT (In) | HLC (In) | Suna (In) | HLC (Out) | Suna (Out) |
| 9 | Suna (In) | CDC-LT (In) | Suna (Out) | HLC (In) | HLC (Out) |
| 10 | Suna (Out) | Suna (In) | HLC (Out) | CDC-LT (In) | HLC (In) |

Additional file 2: Schematic drawing of Suna trap replacement for the study on the simultaneous use of the Suna trap.




Additional file 3: Nightly schedule of Suna trap placement at each house, for six weeks.

| | Days | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| **Hse** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** |
| **1** | in | out | in/out | in/out | in | out | out | in/out | in | in | out | in/out |
| **2** | out | in/out | out | in/out | in/out | in | out | in | in/out | in | out | in |
| **3** | in/out | out | in | out | out | in/out | in | in/out | out | in/out | in | in |
| **4** | in/out | out | in | in | in/out | in | in/out | out | in | out | in/out | out |
| **5** | in | in/out | out | out | in | out | in | in/out | in/out | in | out | in/out |
| **6** | out | in/out | out | in | out | in/out | in/out | out | in | in/out | in | in |
| **7** | out | in | in/out | out | out | in/out | in | in | in/out | in/out | in | out |
| **8** | in/out | in | in/out | in | in/out | in | out | in | out | in/out | out | out |
| **9** | in | in | out | in/out | in/out | out | out | out | in/out | in | in | in/out |
| **10** | in | in/out | in | out | in | in | in/out | out | out | out | in/out | in/out |
| **11** | out | out | in | in/out | in | out | in/out | in/out | in | out | in/out | in |
| **12** | in/out | in | in/out | in | out | in/out | in | in | out | out | in/out | out |

## Chapter 5

# Impact of cattle on the resting behaviour of malaria vectors in southern Malawi

Monicah M. Mburu, Kennedy Zembere, Themba Mzilahowa, Anja Terlouw, Tumaini Malenga, Henk van den Berg, Willem Takken and Robert S. McCann

To be submitted in a modified form

Chapter 5

**Abstract**

Zooprophylaxis is a promising complementary strategy for malaria control. For instance, the presence of cattle has been associated with fewer *Anopheles arabiensis* in houses in close proximity to cattle than in houses where cattle are absent. However, the current evidence for the effectiveness of this strategy is weak because the presence of cattle may enhance the availability of blood meals. As a result, infectious mosquitoes may survive longer thereby increasing the risk of malaria transmission. This study assessed the effect of presence and distribution of cattle on the indoor and outdoor resting malaria vectors. Houses with and without cattle were selected in Chikwawa district, southern Malawi for indoor and outdoor sampling of resting malaria vectors. Prokopack aspirators and claypots were used for indoor and outdoor sampling, respectively. Each house was sampled in two consecutive days. For houses with cattle, the number of cattle and the distances from the house to where the cattle were corralled the previous night were recorded. All data were analyzed using a generalized linear model fitted with Poisson distribution. The malaria vectors caught resting indoors were mostly *Anopheles gambiae* s.l. (n= 33, 76% of which were *An. arabiensis*) and *An. funestus* s.l. (n=30, 97% of which were *An. funestus* s.s.). Outdoor collections consisted primarily of *An. arabiensis* (n=11). The catch sizes of indoor resting *An. gambiae* s.l. were similar in houses with and without cattle (Risk ratio (RR) = 0.69, Confidence interval (CI) = (0.35-1.37), P = 0.29). The presence of cattle near a house was associated with a reduction in the abundance of indoor resting *An. funestus* s.l. (RR= 0.43, CI = (0.21-0.90), P = 0.03). This effect was strongest when cattle were ≤ 15 m away from the houses: compared to houses without cattle, the presence of cattle at an average distance of 1-15m significantly reduced the number of indoor resting *An. funestus* s.l. (RR = 0.19, CI = (0.04-0.81), P = 0.03). Therefore, zooprophylaxis would have an impact on densities of *An. funestus*, but not on *An. arabiensis* in southern Malawi.

Chapter 5

**Keywords:** Anopheline mosquitoes, malaria, resting behaviour, indoors, outdoors, cattle

## Introduction

Current methods of malaria vector control implemented by national control programmes rely on the use of insecticides. While significant reductions in malaria prevalence and incidence have been achieved (Bhatt et al. 2015), there is a need for the development of complementary tools. This is due to the growing concern of (a) resistance of mosquitoes to insecticides which may limit the effectiveness of long-lasting insecticide treated nets (Hemingway et al. 2016, Ranson and Lissenden 2016) and (b) variations in the vectors' biting behaviour from predominantly indoors to increasingly outdoors (Reddy et al. 2011, Russell et al. 2011, Overgaard et al. 2012a, Meyers et al. 2016), and from throughout the night to the early evening/morning hours (Reddy et al. 2011, Moiroux et al. 2012b, Sougoufara et al. 2014). One of the potential complementary tools is zooprophylaxis, which is defined as "the use of wild or domestic animals, which are not the reservoir hosts of a given disease, to divert the blood-seeking mosquito vectors from the human hosts of that disease" (WHO 1982). While the association of livestock with malaria transmission is complex, clear differences among vector species in host preference exist (White et al. 1974, Takken and Verhulst 2013). Of the dominant malaria vector species in Africa, *Anopheles gambiae* s.s. and *An. funestus* are highly anthropophagic, while *An. arabiensis* is more variable in its feeding behaviour, readily feeding on cattle in addition to humans (Ralisoa and Coluzzi 1987, Fontenille et al. 1992, Habtewold et al. 2004, Mahande et al. 2007a). A number of studies have supported the hypothesis that the presence of cattle around human dwellings provides a protective effect against biting by malaria vectors (Kirnowordoyo and Supalin 1986, Seyoum et al. 2004, Mahande et al. 2007a, Bulterys et al. 2009, Yamamoto et al. 2009, Maia et al. 2012,

Franco et al. 2014, Iwashita et al. 2014, Donnelly et al. 2015, Massebo et al. 2015, Donnelly et al. 2016) and mostly when the cattle are distanced from human dwellings (Kirnowordoyo and Supalin 1986, Seyoum et al. 2004). Conversely, conflicting results have been reported in other studies. For instance, in Pakistan (Bouma and Rowland 1995), The Gambia (Bøgh et al. 2001, Bøgh et al. 2002), Ethiopia (Tirados et al. 2011) and Lao PDR (Hiscox et al. 2013), the presence of cattle was associated with more malaria vectors and higher risk of malaria (Ghebreyesus et al. 2000). Additionally, a review by Donnelly et al. (2015) clearly demonstrates that zooprophylaxis would be effective in regions where the dominant vectors do not prefer to feed on human hosts and where livestock are kept at a distance away from humans at night. A model has also suggested that the presence of cattle near human dwellings would provide sufficient blood meals for the vectors, a phenomenon that would enhance the reproductive success of malaria vectors, thereby increasing the abundance of malaria vectors and the risk of malaria transmission (Sota and Mogi 1989).

Therefore, more studies are needed to evaluate: (a) whether the presence of cattle would have an impact on the abundance and feeding behaviour of malaria vectors and (b) the distances at which livestock should be corralled to promote zooprophylaxis and prevent zoopotentiation (Donnelly et al. 2015). The present study aimed at assessing the effect of cattle presence and distribution on the abundance, resting behaviour, and blood-meal hosts of malaria vectors.

## Methods

### Study site

The study was conducted in eight villages in Chikwawa district, southern Malawi, a low-lying

region with high rates of malaria transmission (Kazembe et al. 2006, Kabaghe et al. 2018). Most of the houses are made of sun-dried or fire-baked bricks with grass thatched or corrugated iron-sheet roofs. Residents of this region engage in subsistence farming with maize and millet as the main crops.

**Selection of households**

The villages in this study were part of a cluster-randomised trial assessing the effects of larval source management and house improvement on malaria transmission (McCann et al. 2017b). An inclusion criterion was applied to allow for uniformity across the houses. The criterion included: houses with open eaves, houses that were ≥ 25m apart, houses more than 100m from any mosquito breeding habitat. From these eligible houses, houses with and without cattle were selected. The first house at the start of the study was purposefully selected by a member of the research team. For the subsequent selections, the owner of each house would randomly select the next house to be sampled by selecting a piece of paper from an envelope that had several pieces of papers that had been folded and pre-labeled indicating 'cattle' or 'no cattle'. Depending on the result (cattle or no cattle), the next nearest house that fit the criterion would be chosen.

**Mosquito sampling**

Mosquito sampling was done from November 2016 through March 2017. The sampling included indoor and outdoor resting collections in 100 houses, 40 of which had no cattle and 60 of which had cattle. Clay pots (Odiere et al. 2007) were used for the outdoor collections, whereby three pots were set outside, 1m away from the wall of the house, in the previous evening till the following morning (Figure 1). The mosquitoes resting in the clay pots were collected the following morning by covering the pot with a cotton cloth and dropping a cotton ball soaked with chloroform to

anesthetize the mosquitoes. After 4-5 min, the mosquitoes were collected from the clay pots and placed in 1.5ml Eppendorf tubes that were then placed in containers with a desiccant. Prokopack aspirators (Vazquez-Prokopec et al. 2009) were used for the indoor collections. These collections were conducted from 07:00 hrs to 10:00 hrs, on the same morning as mosquitoes were collected from the clay pots, by actively searching mosquitoes in all the rooms for a maximum of 10 minutes per house. The containers were assigned a unique code to distinguish the indoor and outdoor collections, the day of collection and the specific house. In houses with cattle, the number of cattle and the distances from the house to where the cattle were corralled the previous night were recorded. Each house was sampled on two consecutive days, resulting to 200 sampling days. Furthermore, brief interviews were conducted with householders to obtain data on house parameters such as the number of people that occupied the house the previous night, wall type, floor type, door type and cooking locations. The following represent the categorizations: door type as wood and reed; floor type as dirt/mud/dung/sand; wall type as sun-dried bricks and fire-baked bricks; cooking location as inside the house, on the veranda, outside but within 2m of the house and outside more than 2m away from the house.

**Mosquito identification**

In the laboratory, all mosquitoes were identified morphologically using the guide from Gillies and Coetzee (1987). All anophelines were classified as *An. gambiae* s.l., *An. funestus* s.l. or *An. coustani*. There was no further classification of the culicines beyond the subfamily level. Females from the *An. gambiae* species complex and *An. funestus* species group were further identified to species level using polymerase chain reaction (PCR) (Scott et al. 1993, Koekemoer

et al. 2002, Cohuet et al. 2003), respectively. For the *An. gambiae* species complex, the PCR included species-specific primers for *An. gambiae* s.s., *An. arabiensis*, and *An. quadriannulatus*. For the *An. funestus* species group, the PCR included species-specific primers for *An. funestus* s.s., *An. vandeeni*, *An. rivulorum*, *An. rivulorum-like*, *An. parensis*, and *An. leesoni*. The heads and thoraces of all female *An. gambiae* s.l. and *An. funestus* s.l. were tested for the presence of *P. falciparum* DNA using real-time polymerase chain reaction (RT-PCR) (Perandin et al. 2004) with a Ct value ≤ 37.0 as the cut-off for *P. falciparum* positive. The abdomens of all fed and half gravid female *An. gambiae* s.l. and *An. funestus* s.l. were analysed using PCR to identify the blood-meal host (Kent and Norris 2005). The PCR included species-specific primers for cow, goat, human, pig and dog, as well as general primers designed for mammal and avian hosts (Hamer et al. 2008) when species-specific primers did not amplify.

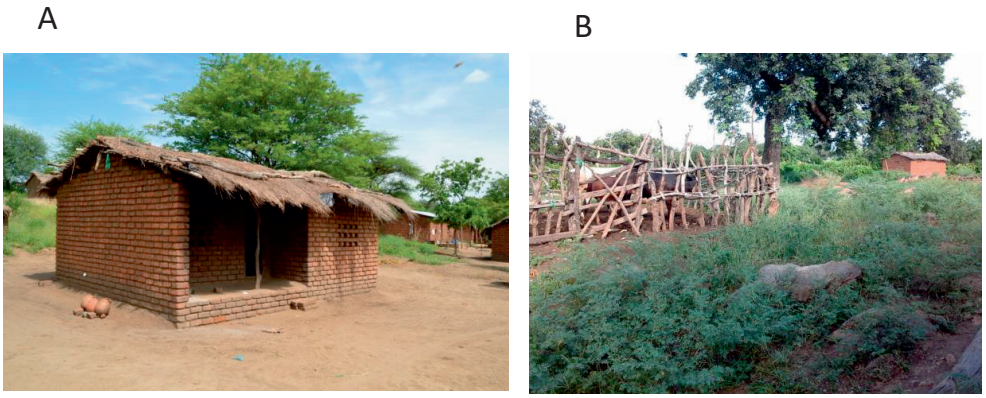



Figure 1. Typical house in the study region with (A) three clay pots set outdoors on the left side of the house and (B) cattle in a cattle-kraal.

**Data analysis**

Generalized linear models fitted with Poisson distribution were used to calculate: (a) the mean catches of mosquitoes per night in houses with and without cattle and (b) the average distances from the house to where the cattle were corralled the previous night. Catches of female *An. gambiae* s.l., *An. funestus* s.l. and culicines were treated as dependent variables in separate fitted models. Average distances were calculated by multiplying each cow/bull near a house with its own distance from the house i.e. if two animals were near house x; then for this specific house x, we calculated animal #1 by distance 1=M; and animal #2 by distance 2=N; and then calculated the averages [(M+N)/2]. The cooking locations, number of people that slept in the house the previous night and the use of bed-net were included as covariates in each of the models. Doors were not included in the analysis because all the doors were made of wood. The datasets were analysed using SPSS Version 20.0. Furthermore, a regression model was fitted in R (version 3.5.1) to assess the effect of the number of cattle on the abundance of indoor resting malaria vectors.

**Results**

Combined across all locations, a total of 571 mosquitoes was collected. Of these, 300 were males (anophelines: 13 indoors and 5 outdoors; culicines: 278 indoors and 4 outdoors) and 271 were females. Of the 271 females, 190 were culicines (179 indoors, 11 outdoors) and 81 were malaria vector species (63 fed, 13 half-gravid, 3 gravid and 2 unfed). Of the 81 malaria vectors, 48 were *An. gambiae* s.l. (33 indoors and 15 outdoors), 32 were *An. funestus* s.l. (30 indoors and 2 outdoors) and *An. coustani* (1 outdoors; Table 1). Of the 63 malaria vectors caught indoors, 60 were identified by PCR as *An. arabiensis* (n=25), *An. gambiae* s.s. (n=6) and *An. funestus* s.s.

(n=29). The DNA of three of the sixty-three malaria vectors caught indoors failed to amplify (2 *An. gambiae* s.l.; 1 *An. funestus* s.l.). Of the 17 *An. gambiae* s.l. and *An. funestus* s.l. caught outdoors, 13 were identified by PCR as *An. arabiensis* (n=11), *An. rivulorum-like* (n=1) and *An. funestus* s.s. (n=1). The DNA of four of the seventeen vectors caught outdoors failed to amplify (4 *An. gambiae* s.l.).

Table 1. Mosquito collections in houses with and without cattle.

| **Mosquito resting collection** | **Indoors** | **Outdoors** | **Totals** |
|---|---|---|---|
| **No. of nights** | 200 | 200 | 400 |
| *An. gambiae* s.l. | 33 | 15 | 48 |
| *An. funestus* s.l. | 30 | 2 | 32 |
| *An. coustani* | 0 | 1 | 1 |
| Female culicines | 179 | 11 | 190 |
| Male Anophelines | 13 | 5 | 18 |
| Male Culicines | 278 | 4 | 282 |

Of the 80 malaria vectors tested for the presence of *P. falciparum* DNA, only one was positive for *P. falciparum* (*An. arabiensis,* indoor, fed on human blood).

Of the 81 malaria vectors, 75 (62 fed and 13 half -gravid) were tested for presence of blood meals and 42 were positive as shown: cow (n=22; 18 *An. arabiensis*,1 *An. gambiae* s.s., 2 *An. gambiae* s.l. and 1 *An. funestus* s.s.); goat (n=2; 1 *An. arabiensis*, 1 *An. rivulorum-like*), human (n=1; *An. arabiensis*) and mammal (not human, not cow) (n= 17; 1 *An. gambiae* s.s., 5 *An. arabiensis*, 11 *An. funestus* s.s.). Thirty-three of the seventy-five blood meals failed to amplify (Table 2).

Table 2. Blood meal analysis results.

| | Cow | Goat | Mammal | Human | Totals | No amplifications |
|---|---|---|---|---|---|---|
| *An. gambiae* s.l. | 2 | 0 | 0 | 0 | 2 | 4 |
| *An. funestus* s.l. | 0 | 0 | 0 | 0 | 0 | 1 |
| *An. arabiensis* | 18 | 1 | 5 | 1 | 25 | 10 |
| *An. gambiae* s.s. | 1 | 0 | 1 | 0 | 2 | 3 |
| *An. funestus* s.s. | 1 | 0 | 11 | 0 | 12 | 15 |
| *An. rivulorum like* | 0 | 1 | 0 | 0 | 1 | 0 |

The abundance of *An. gambiae* s.l. resting indoors was similar in houses with and without cattle (Risk ratio (RR) = 0.69, Confidence interval (CI) = (0.35-1.37), P = 0.29). The abundance of *An. funestus* s.l. resting indoors was lower in houses with cattle than in houses without cattle (RR = 0.43, CI = (0.21-0.90), P = 0.03) (Figure. 2). The number of cattle did not have an effect on the abundance of the indoor resting malaria vectors (P = 0.29).

Compared to houses without cattle, the presence of cattle at various distances did not have an impact on catch sizes of *An. gambiae* s.l.: 1-15m (RR = 0.42, CI = 0.14-1.27, P = 0.13); 15.01-30m (RR = 0.67, CI = 0.26-1.70, P = 0.39) and 30.01-50m (RR = 1.14, CI = 0.47-2.77, P = 0.78) (Figure. 3). However, compared to houses without cattle, there was a reduction in the catch sizes of indoor resting *An. funestus* s.l. when cattle were present at average distances of 1-15m (RR = 0.19,

CI = (0.04-0.81), P = 0.03). As the average distances increased, the catch sizes of this species were similar to those of houses without cattle: average distances of 15.01-30m (RR = 0.59, CI = 0.24-1.49, P = 0.26) and 30.01-50m (RR = 0.58, CI = 0.20-1.71, P = 0.32) (Figure. 4). Because of the low catch sizes of malaria vectors resting outdoors in clay pots (n = 18), a statistical analysis comparing houses with and without cattle was not done.

For the indoor resting female culicines, the catch sizes of these mosquitoes were lower in houses with cattle than in houses without cattle (RR = 0.62, CI = 0.46-0.82, P = 0.001; Figure. 5).

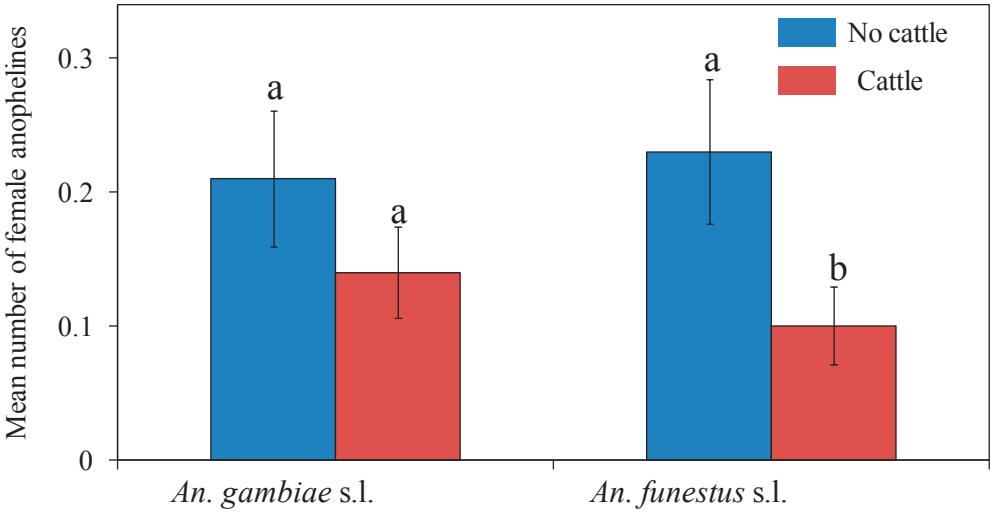



Figure 2. Effect of cattle presence or absence on female anophelines caught resting indoors.

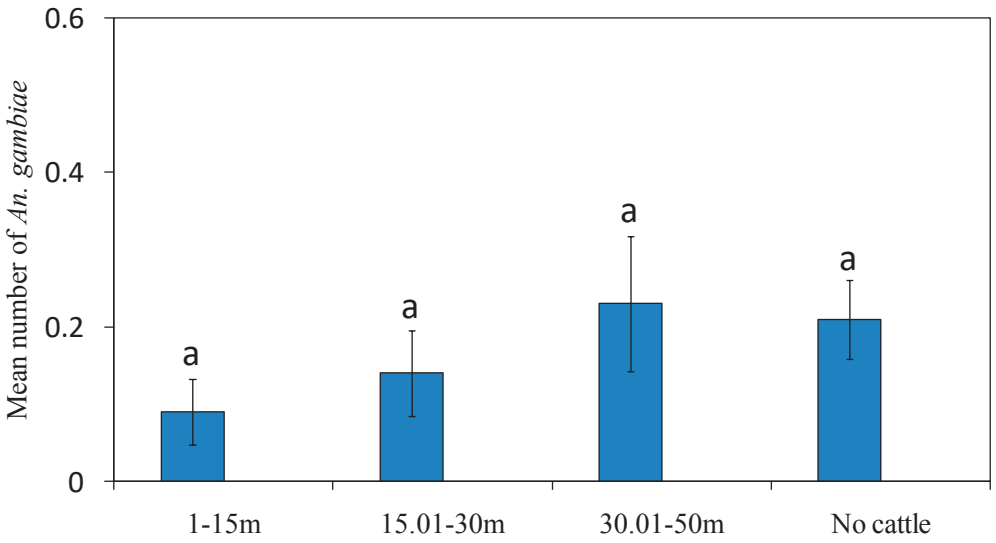



Figure 3. Effect of the presence of cattle at various distances on female *An. gambiae* s.l. resting indoors.

Additionally, compared to houses without cattle, the presence of cattle reduced the abundance of indoor resting culicines at an average distance 1-15m (RR = 0.39, CI = (0.24-0.62), P = 0.001); and 30.01-50m (RR = 0.45, CI = (0.27-0.77), P = 0.003). However, compared to houses without cattle, the catches of indoor resting culicines were similar to those caught near houses with cattle at an average distance of 15.01-30m (RR = 0.96, CI = (0.68-1.36), P = 0.83; Figure. 6). Interestingly, the catch sizes of indoor resting female culicines (n = 179) were lower than those of the male culicines (n = 278) indoors (Table 1).

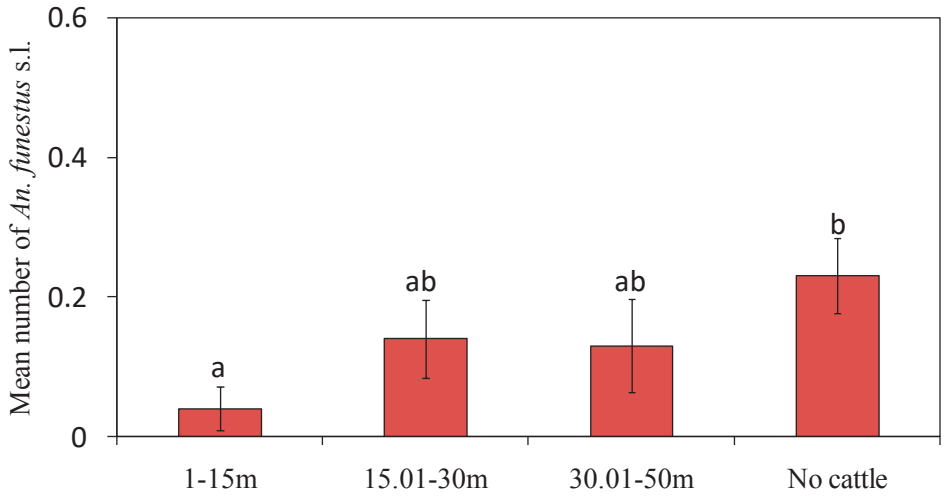



Figure 4. Effect of the presence of cattle at various distances on female *An. funestus* s.l. resting indoors.

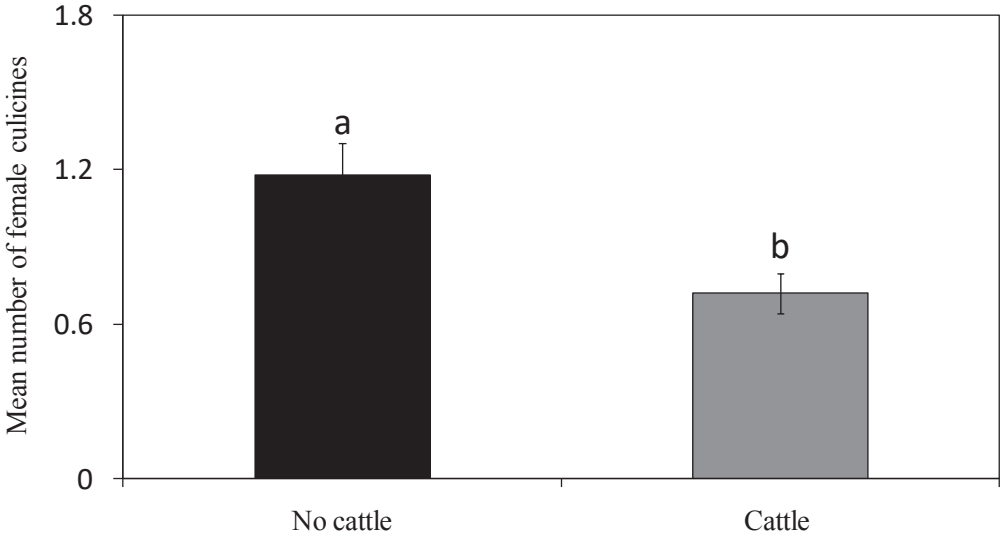



Figure 5. Effect of cattle presence or absence on female culicines caught resting indoors.

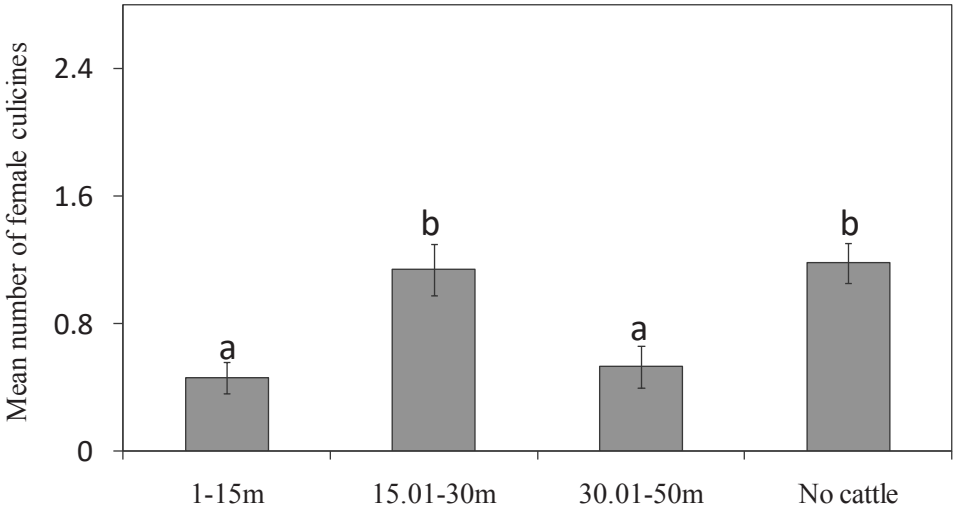



Figure 6. Effect of the presence of cattle at various distances on female culicines resting indoors.

**Discussion**

The most abundant vectors caught resting indoors were *An. gambiae* s.l. (primarily *An. arabiensis*) and *An. funestus* s.l. (primarily *An. funestus* s.s.). For the outdoor collections, the most abundant vector was *An. arabiensis*. The presence of cattle reduced the abundance of *An. funestus* s.l. mosquitoes that were resting indoors, but not that of *An. gambiae* s.l.. Furthermore, the presence of cattle at average distances of 1-15m away from the house significantly reduced the abundance of indoor resting *An. funestus* s.l. but not that of *An. gambiae* s.l. (i.e. *An. arabiensis*). Whereas most of the *An. arabiensis* mosquitoes were positive for cattle blood, *An. funestus* s.s. mosquitoes were mainly positive for mammalian blood other than from a human or cattle. The species' origin of these blood meals could not be identified further. Only one *An. arabiensis* was shown to have fed

on human blood. These results demonstrate that presence of cattle near a house impacts on the *An. arabiensis* and *An. funestus* in a different manner: *An. arabiensis* feeds on cattle and this species, when blood-fed, may use the house as a resting site. By contrast, when cattle are near the house, fewer *An. funestus* enter the house and this species switches to hosts other than cattle or humans.

*Anopheles funestus* is highly anthropophagic (Gillies and De Meillon 1968, Highton et al. 1979, Githeko et al. 1994, Githeko et al. 1996b, Antonio-Nkondjio et al. 2002, Awolola et al. 2003, Mwangangi et al. 2003, Wanji et al. 2003, Temu et al. 2007, Dabire et al. 2008, Seyoum et al. 2012, Dadzie et al. 2013) and the finding that this species was reduced in houses near cattle was unexpected. One potential reason could be that the odours from the cattle had a deterrent effect on this species. A high degree of aversion to cattle odour has been reported for *An. gambiae* s.s. which is also anthropophagic (Pates et al. 2007) and a study in Ghana also found a similar finding for *An. gambiae* s.s. (Maia et al. 2012). The possibility that *An. funestus* may have been deterred by the cattle odours is seconded by the finding that, compared to houses without cattle, the presence of cattle at average distances of 1-15m was associated with reduced abundance of indoor resting *An. funestus*. A model by Hassanali et al. (2008) showed that as the distance between animals and human locations increased, the number of bites by mosquitoes reduced followed by an increase in the number of bites. Therefore, it is possible that when cattle are close to a house in this region of southern Malawi, their odours cause aversion of this species and these mosquitoes feed on other hosts other than humans or cattle. On the other hand, *An. arabiensis* has an opportunistic feeding behaviour, whereby this species feeds on cattle or humans indiscriminately (Takken and Verhulst 2013). It is therefore not surprising that the abundance of this species indoors was not affected by the presence of cattle because cattle are suitable hosts. Conversely, in other regions, the presence of cattle has been associated with a reduction in the abundance of *An. arabiensis* (Kaburi et al.

2009, Mayagaya et al. 2015) and a model by Killeen et al. (2001) shows that zooprophylaxis would be effective in regions where *An. arabiensis* is the primary vector. The variable resting behaviours between the two species in houses with and without cattle in this region of southern Malawi warrants further investigation as this may have implications on malaria risk because whereas, *An. funestus* avoids resting indoors when a cow is near a house, *An. arabiensis* remains unaffected. The reduction of *An. funestus* in houses near cattle has a possible application value as zooprophylaxis to reduce malaria transmission. However, studies incorporating the vectors' host-seeking behaviour/human biting rates are recommended to fully support this finding.

Although the DNA from some blood-fed mosquitoes did not amplify, identification of the blood meals demonstrated that in the study area, only one *An. arabiensis* fed on humans. Most of the *An. arabiensis* mosquitoes were positive for cattle blood and *An. funestus* s.s. mosquitoes were mainly positive for mammalian blood that could not be identified further. With only one blood meal identified from a human host, humans do not seem to be a favoured host by *An. arabiensis* in southern Malawi. Though the present study did not look at the biting rates, the finding agrees with that of Iwashita et al. (2014) in western Kenya, where human blood-feeding was reduced when an animal was tethered near a house. Furthermore, the reduction in human biting rates has been observed in households with cattle (outdoors) (Seyoum et al. 2004, Kaburi et al. 2009) in Ethiopia and Kenya, respectively. However, the finding disagrees with that of Tirados et al. (2006) where, despite the high ratio of cattle to humans in a region in southern Ethiopia, *An. arabiensis* was highly anthropophagic. This can be explained by the fact that many people slept outdoors close to cattle and therefore, the mosquitoes were more likely attracted to the human odour than to the cattle odour. Additionally, in Senegal, there was an increase in human blood meals after the rainy

season where the authors suggest that cattle were kept away from the houses (Lemasson et al. 1997).

*Anopheles funestus* s.s. mosquitoes found resting indoors, were mainly positive with mammalian blood which could not be identified further. This finding suggests that these mosquitoes may have fed elsewhere but rested indoors. Therefore, there is a possibility of the exophagic-endophilic behaviour of this species in this region. In Tanzania, similar observations on the exophagic-endophilic behaviour were made where *An. funestus* s.s. and *An. rivulorum* were positive for goat blood (Temu et al. 2007). The failure of some blood meals in our study to amplify for identification of the blood source was unexpected. Studies conducted in Tanzania (Temu et al. 2007) and Zambia (Kent et al. 2007) experienced the same challenge when quite a number of the samples failed to amplify. These authors suggest that there was a possibility that the vectors (i) either fed on other hosts that could not be detected by the available primers in their studies, (ii) had incomplete blood meals or (iii) that the DNA of the blood-meal host was degraded. In our study, mosquitoes were collected early in the morning, at a time when blood meals are still relatively "fresh", a condition for the process of identification of the host species. We conclude that in our study area an important part of the *An. funestus* population fed on mammals other than humans, cattle, goats and pigs, which may indicate that *An. funestus* is less anthropophagic than currently understood as was previously reported from Madagascar (Fontenille et al. 1990).

The number of cattle did not have an effect on the abundance of indoor resting malaria vectors, indicating that in this region, most likely, the presence or absence of cattle influences the abundance of resting malaria vectors independent of cattle density.

More male culicines were caught resting indoors than male anophelines. This may be explained by a finding from Mozambique, where the results showed that male anophelines exit houses earlier (Charlwood 2011). Therefore, it could be that male culicines exit houses later and hence were more abundant in our resting collections.

Although the outdoor collections with clay pots yielded lower catches of resting malaria vectors, which is contrary to other findings (Odiere et al. 2007, Dandalo et al. 2017, Debebe et al. 2018), the most abundant vector was *An. arabiensis*. This is in agreement with a study from Tanzania where this species was caught more outdoors owing to the fact that these mosquitoes fed on cattle outdoors and sought to rest near the cattle sheds (Mayagaya et al. 2015). However, outdoor collections are more prone to predation than indoor collections (Sikaala et al. 2013). In the present study, the clay pots were dusted every day to remove any webs or insects and therefore predation of mosquitoes from the clay pots was unlikely. Most likely, the mosquitoes sought to rest in alternative sites outdoors. Therefore, this raises the need for the development of tools that can be effective in collecting outdoor resting mosquitoes such as the recently developed host decoy trap with cattle odour (Abong'o et al. 2018). Additionally, tools that can target different outdoor sites are also highly recommended as recent studies have shown that mosquitoes mostly prefer to rest in shady sites (Debebe et al. 2018).

**Conclusion**

In southern Malawi, variations in the resting behaviour of *An. arabiensis* and *An. funestus* were found. This may have implications for malaria risk because whereas *An. funestus* avoids resting indoors when a cow is in close proximity to a house, *An. arabiensis* remains unaffected. The

reduction of *An. funestus* in houses near cattle has a possible application value as zooprophylaxis to reduce malaria transmission. However, studies incorporating the vectors' host-seeking behaviour/human biting rates are recommended to fully support this finding.

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**Chapter 6**

# Impact of partially and fully closed eaves on house entry rates by mosquitoes

Monicah M. Mburu, Malou Juurlink, Jeroen Spitzen, Paula Moraga, Alexandra Hiscox, Themba Mzilahowa, Willem Takken, Robert S. McCann

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**Abstract**

Most people infected with malaria acquire the infection indoors from mosquito vectors that entered the house through open eaves, windows and doors. Structural house improvement (e.g. closed eaves; screened windows) is an established method of reducing mosquito entry. It could be complementary to other interventions such as insecticide-treated bed nets (ITNs) for malaria control because it covers and protects all individuals in a house equally. However, when implemented at a large scale, house improvement may not be employed optimally. It is therefore critical to assess whether partial house improvement will have any effect on mosquito house entry. We investigated the effect of partial and complete eave closure on the house-entry rates of malaria vectors and other mosquitoes in southern Malawi.

The study was conducted for 25 nights in May-June 2016. Twenty-five traditional houses were modified according to five treatments: fully closed eaves, three different levels of partially closed eaves, and open eaves. All houses had fully screened windows and closed doors. Host-seeking mosquitoes were sampled inside these houses using Centers for Disease Control and Prevention (CDC) light traps. The effect of open eaves versus partial or complete eave closure on the number of mosquitoes trapped inside the house was estimated using a generalized linear mixed model fitted with Poisson distribution and a log-link function.

House entry by malaria vectors was 14 times higher in houses with fully open eaves compared to houses with fully closed eaves adjusting for wall-type, number of people that slept in the house the previous night, cooking locations and presence of livestock. Houses with four small openings had 9 times more malaria vectors compared to houses with fully closed eaves. The catches of culicine mosquitoes caught in houses with fully closed eaves were not different from those caught in houses with the other treatments.

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Closed eaves resulted in fewer malaria vectors in houses, with differences depending on the degree of eave closure. The ability of malaria vectors to locate any remaining entry points on improved houses, as demonstrated here, suggests that quality control must be an important component of implementing house improvement as an intervention. The lack of effect on culicine mosquitoes in this study could reduce acceptance of house improvement, as implemented here, by household residents due to continued nuisance biting. This limitation could be addressed through community engagement (e.g. encouraging people to close their doors early in the evenings) or improved designs.

**Keywords:** House improvement, Eaves, Malaria vectors, House entry, *Anopheles*, Culicines, Vector control.

## Introduction

Malaria continues to place a heavy burden on communities living in malaria endemic areas, in spite of promising declines in malaria globally due to the use of insecticide-treated bed nets (ITNs), indoor residual spraying (IRS) and effective drug therapy (Bhatt et al. 2015). In endemic regions of Africa, where 90% of cases and deaths from malaria occur (WHO 2017), indoor biting by malaria vectors still plays a prominent role in malaria transmission (Huho et al. 2013, Bayoh et al. 2014, Killeen et al. 2017b) and the structural design of houses affects the entry of malaria vectors into residences. Houses with modern features (e.g. closed eaves, screened doors and windows, and ceilings) can provide the first line of defense against bites from infected malaria vector mosquitoes, whereas houses without these features have been associated with increased numbers of mosquitoes indoors (Lindsay et al. 2003, Ogoma et al. 2010, Mutuku et al. 2011) and

higher levels of malaria (Ghebreyesus et al. 2000, Lindsay et al. 2002, Tusting et al. 2015, Wanzirah et al. 2015). Open eaves are significant entry points into houses for malaria vector species in Africa (Snow 1987, Lindsay and Snow 1988, Njie et al. 2009) and are therefore recognized as a risk factor for malaria.

Most studies looking at house design and mosquito entry (or malaria) have been observational studies of incremental improvements in house design that occur coincidentally with socioeconomic improvements over time (Tusting et al. 2015). In addition to those studies, others have tested the effect of deliberate structural modifications, also known as house improvement, as a direct intervention to block mosquito entry using materials such as netting, papyrus reeds, sand, rubble and concrete. These studies have associated house improvement with fewer mosquitoes entering homes (Atieli et al. 2009, Kirby et al. 2009, Kampango et al. 2013) and reduced anaemia prevalence in children (Kirby et al. 2009).

Modern house features have been viewed favourably by residents because of their perception that these features reduce mosquito bites (Atieli et al. 2009, Ogoma et al. 2009, Kirby et al. 2010, von Seidlein et al. 2017), with the primary concerns being the costs of these features and the potential for increased indoor temperatures (Atieli et al. 2009, Ogoma et al. 2009). Additional benefits of house improvement as an intervention against malaria include: equal protection is offered to all individuals in a house, no daily action from the end user is required, it is technologically simple and it does not require insecticides in principle.

These advantages, together with the spread of insecticide resistance threatening the efficacy of ITNs and IRS (Hemingway et al. 2016, Kisinza et al. 2017), have led to a renewed interest in the broad concept of house improvement as an intervention and a need to address key questions about specific aspects of the intervention related to the effectiveness of particular features,

safety, acceptability and implementation (RBM 2015b, Tusting et al. 2016). As with any health intervention, measuring the percentage of the population effectively covered by house improvement will be important for understanding the effectiveness of the intervention in both trial settings (McCann et al. 2017b) and on a larger scale (e.g. as programmes implement house improvement at a district or national scale). Here, we refer to the malERA Consultative Group on Health Systems and Operational Research and their definition of effective coverage, which goes beyond simple access to an intervention to also include provider compliance and client adherence (The mal and Operational 2011, The mal and Policy 2017). In the context of house improvement, compliance could be measured in terms of the number and size of any remaining gaps in the housing structure following implementation. While the goal of implementation would be to leave zero gaps for mosquito entry, in real-world settings this would not be the case for 100% of houses with access to house improvement. Therefore, it will be important to understand the extent to which houses with remaining gaps for mosquito entry following implementation of these modifications would still provide any effective protection from mosquito bites compared to fully improved houses. The aim of this study was to assess differences in partial or complete closure of the eaves on house-entry rates by anopheline and culicine mosquitoes in a randomized field experiment.

## Materials and methods

### Study site

The study was conducted in Chikwawa District, southern Malawi, which lies along the lower Shire valley. This area experiences a single rainy season from November through to April. The main malaria vectors prevalent in the region are *Anopheles gambiae* s.s., *An. funestus* and *An.*

*arabiensis* (Spiers et al. 2002, Mzilahowa et al. 2012). Malaria transmission occurs throughout the year with rates intensifying during the rainy season. Malaria parasitemia in children under five years of age in this region varies seasonally from 11% to 40% (Kabaghe et al. 2017).

Four neighboring villages in Chikwawa District (Fombe, Jacobo I, Jacobo II and Semu) were identified for the study (Fig. 1) to allow for random selection of houses separated by a distance of 25m away from each other. The combined population of the villages was 4,740 (*personal communication, secretary-group village head)*. The area is relatively flat (i.e. little topographic relief), with two seasonal streams. Farming subsistence crops and small-scale cash crops is the primary means of occupation in the study area. Houses in the selected villages are typical for the region. The general house design consists of four walls in a rectangular arrangement with a two-sided roof oriented along the long axis of the house (Fig. 2). House walls are typically constructed with either sun-dried or fire-baked bricks, and roofs are made with either grass thatch or corrugated sheet metal. Most houses have one door, two to four square windows, and either open or closed eaves.

**House selection**

The study included 25 houses. The local leaders (i.e. village chiefs) provided a list of 100 houses across the four villages separated by a distance of ≥ 25 m that fit the following criteria: open eaves, open windows, gaps around the doors, and grass thatched roofs. From these, twenty-five houses were randomly selected for enrollment into the study (6 from Fombe, 7 from Jacobo I, 7 from Jacobo II, and 5 from Semu). Prior to enrollment, we applied further inclusion criteria such that every house would be at least 20 m away from cattle sheds and within a range of 100 m from any mosquito breeding habitat. The houses that did not meet the inclusion criteria were replaced

with the nearest neighboring house that met all inclusion criteria. The geo-location of each house was recorded at enrollment.

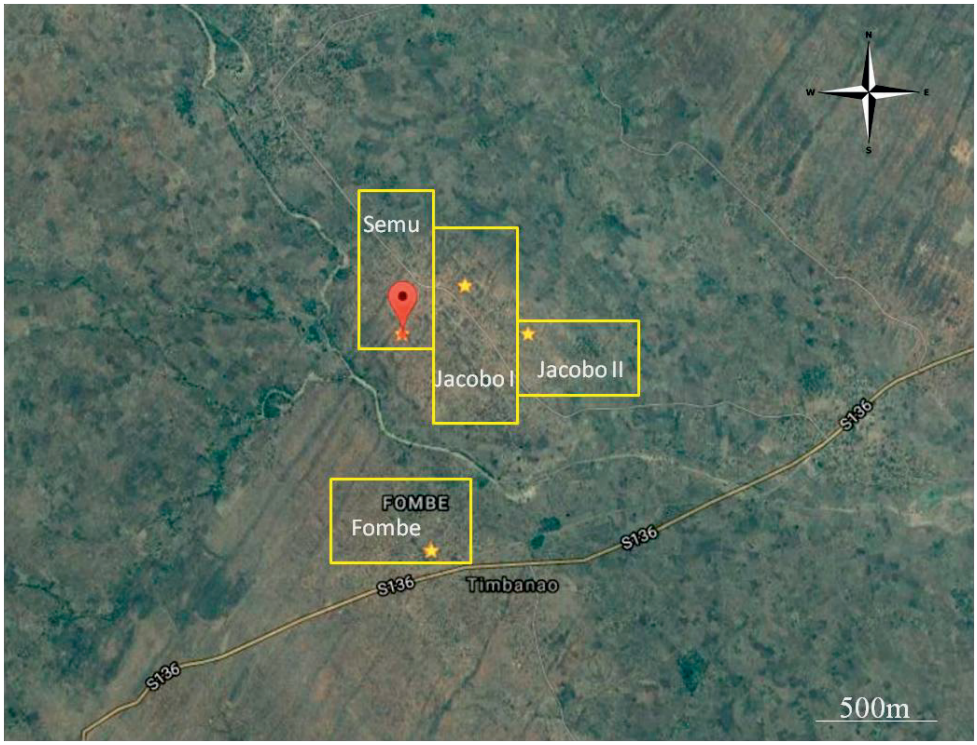


Figure 1: Geo-location of the four villages selected for the study.
Fombe:16.06962496, 34.73430784; Jacobo I: 16.05327887, 34.7365262; Jacobo II: 16.05628311, 34.74051196; Semu: 16.05625729, 34.73250272.

Figure 2: Photograph of a typical house in the study area.

**Treatments**

The five treatments in this study were: fully closed eaves, eaves with a single 5cm × 1cm opening (hereafter referred as a single small opening), eaves with four 5cm × 1cm openings (hereafter referred as four small openings), eaves with two long sides open and houses in which the eaves were open on all four sides (Fig. 3). Treatments were assigned randomly to each house using a random number generator in Microsoft Excel, with five houses being assigned to each treatment. For all 25 houses, all the gaps in walls were closed with muddy soil, gaps in the doors were closed with wooden planks and windows were closed with wire gauze. Small apertures between the window frame and the wall were filled in with mud. For houses with partial and complete eave closure, a combination of bricks and muddy soil were used for eave closure. Local builders

and carpenters were hired to perform the house modifications which were checked for quality by the researchers at completion. The householders provided muddy soil, while the researchers provided the wire gauze for screening the windows and some bricks to close the larger openings. From our observations, the grass thatched roofs were intact, with the exception of one house where the roof had some openings. The owner of this house repaired the roof by filling in the openings with more grass.

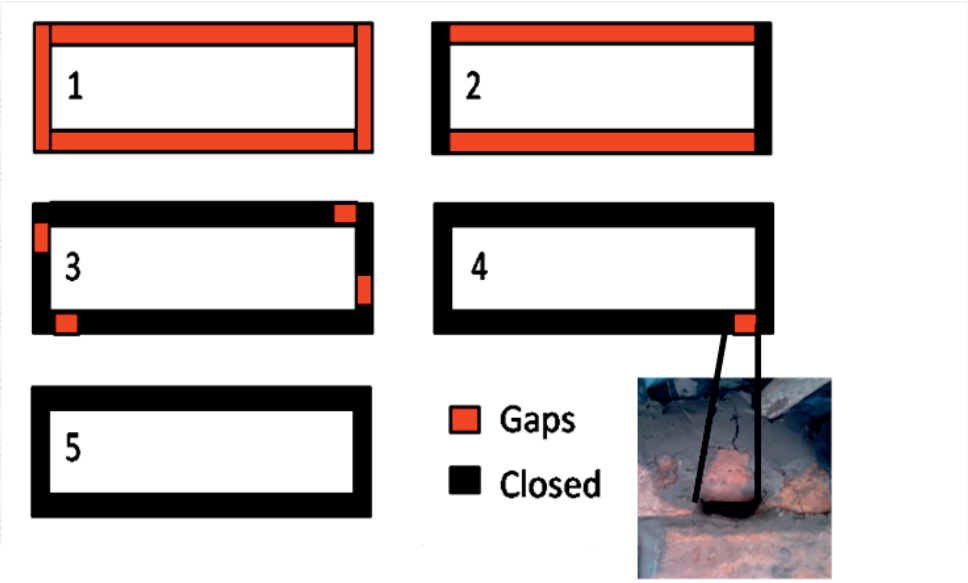


Figure 3: Design of the five treatments assigned to the sets of five houses

**Mosquito sampling**

Mosquito sampling was carried out for four nights a week for a total of 25 nights from 12 May to 24 June 2016. Centers for Disease Control and Prevention (CDC) light traps were used to sample the mosquitoes inside all houses. The traps were powered by 6V batteries and operated from 17:00 h (15 min before sunset at this time of year) until 7:00 h (1 hour after sunrise). In each house, the trap was hung with the fan at 150cm above the ground, at the foot end of a bed in

which a person was sleeping under a bed net (Lines et al. 1991, Mboera et al. 1998). The bed nets were owned by the household. Every morning after a night of sampling, chloroform was used to immobilize the mosquitoes caught in the traps. The mosquitoes were then transferred into an Eppendorf tube containing a silica gel desiccant, and transported to the laboratory for morphological identification.

During mosquito collections, brief interviews were conducted with householders to obtain data on house parameters such as the number of people that occupied the house the previous night, livestock that stayed within 20 m of the house the previous night, wall type, floor type, door type and cooking locations. The following represent the categorizations: door type as wood and reed; floor type as dirt/mud/dung/sand; wall type as sun dried bricks and fire baked bricks; cooking location as inside the house, on the veranda, outside but within 2m of the house and outside more than 2m away from the house. Data were recorded on a tablet computer using Open Data Kit (Hartung et al. 2010).

**Mosquito identification**

All mosquitoes were identified morphologically as either anophelines or culicines. Anophelines were further classified as either *Anopheles gambiae* s.l., *An. funestus* or *An. coustani* using the dichotomous key published by Gillies and Coetzee (Gillies and Coetzee 1987). There was no further classification of the culicines beyond the subfamily level. Females from the *An. gambiae* s.l. species complex were further identified to species level using polymerase chain reaction (PCR) (Scott et al. 1993).

**Data analysis**

The effect of eave closure on the number of mosquitoes caught indoors was tested using a generalized linear mixed model fitted with a Poisson distribution and a log-link function. House identification number was included as a random effect in the model to account for the repeated measures by house. The kind of livestock that stayed within 20m of the house the previous night, the cooking location, wall type and the number of people who slept in the house the previous night were included as covariates in the model. Livestock comprised of cattle, goats, sheep, chicken and pigs. Sheep and pigs were excluded from the analysis because of the low number of houses with either animal (≤6). Similarly, floor and door types were excluded from the analysis because all the floors were made of mud; doors were made of wood in 24 houses while in the remaining house the door was made of reed. All analyses were performed using R, version 3.3. The primary outcome was the number of female malaria mosquitoes (hereafter referred as anophelines) caught with a CDC light trap per house, per night. Due to the low number of anophelines caught, count data for all anopheline species were pooled per treatment and day for statistical analysis. Secondary outcomes were the number of culicine females and the number of culicine males caught per house, per night. Fully closed eaves served as the reference in our analysis. Pairwise comparisons were performed with the Dunnett's test to compare each of the treatments to the reference treatment, fully closed eaves.

**Results**

Combined across all treatments, a total of 777 mosquitoes were collected over 625 trap-nights. Of these, 48 were female anophelines, 6 were male anophelines, 466 were female culicines, 248 were male culicines and 9 were unidentifiable. Of the female anophelines, 47 were *An. gambiae*

s.l. and one *An. coustani*. Thirty-six and two of the female *An. gambiae* s.l. mosquitoes were identified to species level as *An. arabiensis* and *An. gambiae* s.s., respectively. The remainder (n = 9) could not be identified further because they failed to amplify. Abdominal status of the female anophelines included: unfed 85.42% (n = 41) and fed 14.58% (n = 7). No gravid or semi-gravid malaria vectors were caught. Abdominal status of female culicine mosquitoes trapped included: unfed 97.21% (n = 453), fed 2.58% (n = 12) and semi gravid 0.21% (n = 1).

The catches of female anophelines per treatment were: fully closed eaves, 4.16% ($n$ = 2); eave with a single small opening, 12.5% ($n$ = 6); eave with four small openings, 27.08% ($n$ = 13); eave with two long sides open, 12.5% ($n$ = 6); and open eaves: 43.75% ($n$ = 21). Catches in houses with fully closed eaves were significantly lower than catches in houses with four small openings (Risk ratio, RR = 8.83, 95% CI: 1.16–67.14, $Z$ = 2.105, $P$ = 0.035), and with completely open eaves (RR = 14.16, 95% CI: 2.05–97.91, $Z$ = 2.687, $P$ = 0.007). Catch sizes of female anophelines caught in houses with fully closed eaves were similar to those in houses with a single small opening in the eave (RR = 4.38, 95% CI: 0.59–32.46, $Z$ = 1.444, $P$ = 0.149) and two long sides open (RR = 5.41, 95% CI: 0.72–40.40, $Z$ = 1.645, $P$ = 0.10) (Fig. 4). Pairwise comparisons between houses with fully open eaves and fully closed eaves showed that the female anopheline catches were different ($Z$ = 2.687, Adjusted $P$ = 0.022).

The catches of female culicine per treatment were: fully closed eaves, 15.02% ($n$ = 70); eave with a single small opening, 12.66% ($n$ = 59); eave with four small openings, 25.11% ($n$ = 117); eave with two long sides open, 21.67% ($n$ = 101); and open eaves, 25.54% ($n$ = 119). Catch sizes of female culicines in houses with fully closed eaves were similar to those in houses with a single small opening in the eave (RR = 0.86, 95% CI: 0.40–1.88, $Z$ = -0.371, $P$ = 0.711), fully open eaves (RR = 1.14, 95% CI: 0.52–2.52, $Z$ = 0.333, $P$ = 0.739), four small openings (RR =

1.17, 95% CI: 0.52–2.62, $Z$ = 0.377, $P$ = 0.706) and two long sides open (RR = 1.28, 95% CI:0.60–2.75, $Z$ = 0.637, $P$ = 0.524) (Fig. 5). Pairwise comparisons did not provide evidence that catch sizes of female culicines caught in houses with fully closed eaves were different from those caught in houses with a single small opening in the eave ($Z$ = -0.371, Adjusted $P$ = 0.987), fully open eaves ($Z$ = 0.333, Adjusted $P$ = 0.991), four small openings ($Z$ = 0.377, Adjusted $P$ = 0.986) and two long sides open ($Z$ = 0.637, Adjusted $P$ = 0.915).

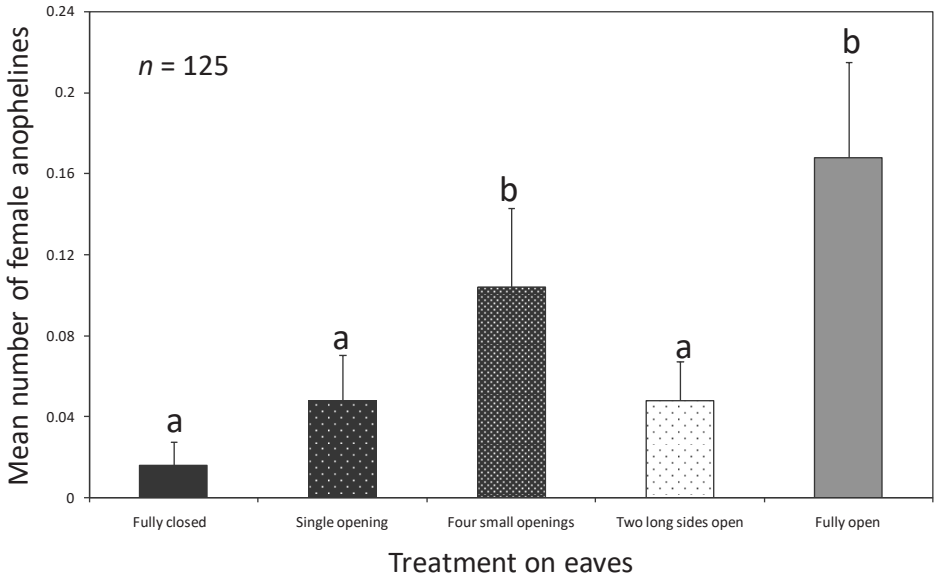


Figure 4: Mean number of female anophelines caught indoors with CDC light traps in houses where eaves: were fully closed, had a single small opening, four small openings, two long sides open and fully open. Bars with different letters denote significant differences in the number of mosquitoes trapped. N = trap nights for each treatment.

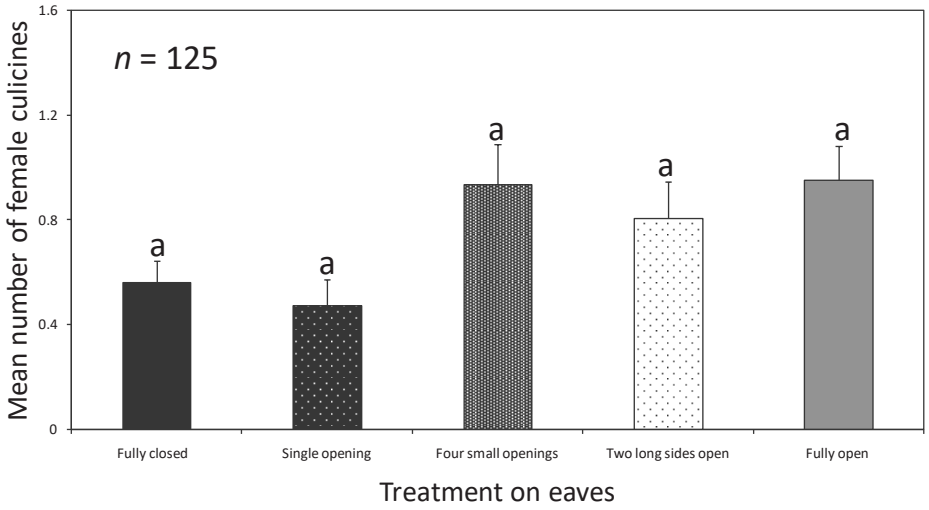


Figure 5: Mean number of female culicines caught indoors with CDC light traps in houses where eaves: were fully closed, had a single small opening, four small openings, two long sides open and fully open. Bars with same letters denote similarities in the number of mosquitoes trapped. N = trap nights for each treatment.

The proportions of male culicine mosquitoes caught per treatment were: fully closed eaves, 18.95% ($n$ = 47); eave with a single small opening, 6.85% ($n$ = 17); eave with four small openings, 29.44% ($n$ = 73); eave with two long sides open, 8.06% ($n$ = 20); and open eaves, 36.69% ($n$ = 91). There was no evidence to indicate a significant difference between catch sizes of male culicine mosquitoes in houses with fully closed eaves and houses with other treatments. Pairwise comparison showed that catch sizes of male culicine mosquitoes in houses with fully closed eaves were similar to those in houses with an eave that had one small opening ($Z$ = -1.135, Adjusted $P$ = 0.609), two long sides open ($Z$ = -0.884, Adjusted $P$ = 0.785), four small openings ($Z$ = -0.370, Adjusted $P$ = 0.988) and fully open eaves ($Z$ = 0.040, Adjusted $P$ = 1.0) (Fig. 6).

Wall type did not have an effect on the number of female anophelines (RR = 0.57, 95% CI: 0.11–2.86, $Z$ = -0.681, $P$ = 0.496) but the presence of chickens and the number of people who slept in the house the previous night were significantly and positively associated with catches of female anophelines (RR = 4.15, 95% CI: -2.04–8.42, $Z$ = 3.938, $P$ = 0.001 and RR =1.27, 95% CI: 1.03–1.56, $Z$ = 2.263, $P$ = 0.024, respectively) (Table 1). The number of people that slept in the house the previous night ranged from one to eight (mean ± SE, 3.41± 0.063). The presence of goats near a house was negatively associated with female culicine catches (RR = 0.70, 95% CI: 0.52–0.94, $Z$ = -2.385, $P$ = 0.017). Catches of female culicines in houses where people cooked outside, 2m away from the house, were different from those in houses where people cooked on the veranda (RR = 0.63, 95% CI: 0.46–0.87, $Z$ = -2.816, $P$ = 0.005), but similar to those where people cooked within 2m of the house (RR = 0.80, 95% CI: 0.62–1.02, $Z$ = -1.819, $P$ = 0.069) and inside the house (RR = 1.46, 95% CI: 0.98–2.17, $Z$ = 1.841, $P$ = 0.066). The presence of chickens was negatively associated with the male culicine catches (RR = 0.56, 95% CI: 0.32–0.99, $Z$ = -2.002, $P$ = 0.045) (Table 1).

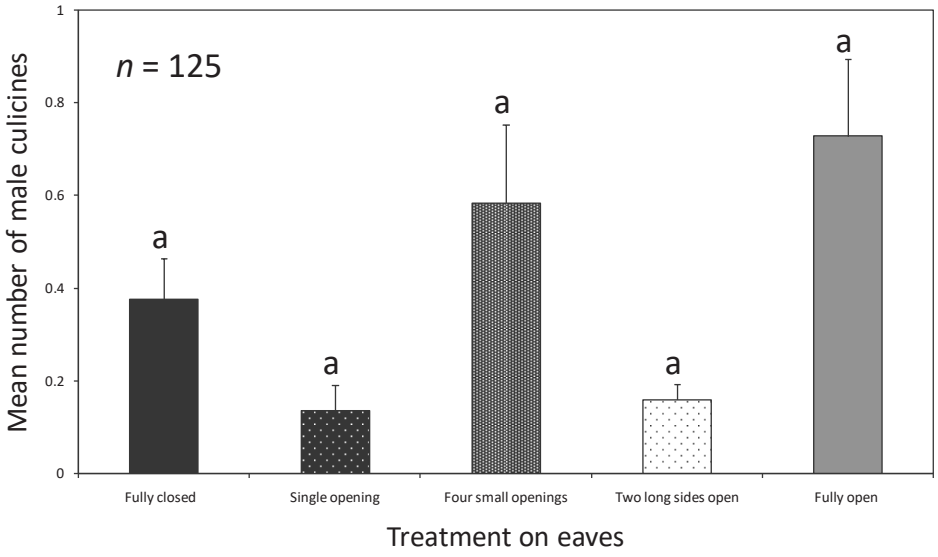


Figure 6: Mean number of male culicines caught indoors with CDC light traps in houses where eaves: were fully closed, had a single small opening, four small openings, two long sides open and fully open. Bars with same letters denote similarities in the number of mosquitoes trapped. N = trap nights for each treatment.

Table 1: Effect of treatment, livestock, cooking locations, wall type and the number of people that slept in the house the previous night on the catch sizes of anophelines and culicines. The risk ratios (RR) and 95% confidence intervals (CI) are shown.

| **Treatment** | **Female anophelines** | | **Female culicines** | | **Male culicines** | |
|---|---|---|---|---|---|---|
| | **RR** | **95% CI** | **RR** | **95% CI** | **RR** | **95% CI** |
| Open eaves | 14.16 | 2.05–97.91 | 1.14 | 0.52–2.52 | 1.03 | 0.21–5.12 |
| Eaves with two long sides open | 5.41 | 0.72–40.40 | 1.28 | 0.60–2.75 | 0.49 | 0.10–2.37 |
| Eaves with four small openings | 8.83 | 1.16–67.14 | 1.17 | 0.52–2.62 | 0.73 | 0.14–3.82 |
| Eaves with a single small opening | 4.38 | 0.59–32.46 | 0.86 | 0.40–1.88 | 0.40 | 0.08–1.94 |
| Fully closed eaves | Ref | – | Ref | – | Ref | – |
| People that slept in the house the previous night | 1.27 | 1.03–1.56 | 1.07 | 0.99–1.15 | 0.98 | 0.90–1.07 |
| Cow | 0.46 | 0.10–2.15 | 1.29 | 0.96–1.73 | 0.68 | 0.44–1.05 |
| Goat | 1.16 | 0.49–2.77 | 0.70 | 0.52–0.94 | 1.04 | 0.70–1.55 |
| Chicken | 4.15 | 2.04–8.42 | 0.95 | 0.69–1.32 | 0.56 | 0.32–0.99 |
| Cooking inside the house | 2.20 | 0.66–7.36 | 1.46 | 0.98–2.17 | 0.98 | 0.52–1.85 |
| Cooking on the veranda | 2.34 | 0.78–7.05 | 0.63 | 0.46–0.87 | 0.85 | 0.52–1.39 |
| Cooking outside, within 2m of the house | 1.04 | 0.43–2.50 | 0.80 | 0.62–1.02 | 1.13 | 0.80–1.60 |
| Cooking outside, away from 2m of the house | Ref | – | Ref | – | Ref | – |
| Wall type fire baked bricks | 0.57 | 0.11–2.86 | 1.83 | 0.89–3.75 | 1.73 | 0.42–7.13 |
| Wall type sun-dried bricks | Ref | – | Ref | – | Ref | – |

Chapter 6

**Discussion**

Houses with fully closed eaves had reduced rates of house entry by anopheline mosquitoes compared to houses with fully open eaves, similar to findings from other regions in Africa (Kirby et al. 2009, Ogoma et al. 2010, Menger et al. 2016). The reduced number of anophelines indoors suggests that a house improvement package that includes fully closed eaves could serve as an effective malaria intervention by reducing vector-human contact. Houses with fully closed eaves also had fewer malaria mosquitoes than houses with four small openings in the eaves, indicating that the latter group of houses would not provide the same level of protection against bites from malaria vectors as would houses with fully closed eaves. Malaria vectors were likely able to locate the small gaps in the eaves (i.e. the experimental sub-optimal modifications) due to the concentration of airflow and host odours emanating through such small gaps (Kampango et al. 2013). In fact, the ability of mosquitoes to readily find these holes is being exploited by studies looking at the impact of eave tubes on mosquito populations, whereby small sections of PVC tubing fitted with electrostatic netting that is treated with powdered insecticide or entomopathogenic fungi are inserted along closed eaves (Andriessen et al. 2015, Waite et al. 2016). Small, uncovered openings in the eaves, such as those used as experimental treatments in the current study, may reduce the effectiveness of house improvement as a malaria intervention because malaria mosquitoes would still find their way into the house (Mnyone et al. 2012).

While fully-closed eaves clearly reduced the number of mosquitoes in the house, we still collected a few malaria vectors, and a considerable number of culicines, in those houses. The most probable explanation is that the mosquitoes entered through the doors (Njie et al. 2009). While the doors on all of the houses were modified so that mosquitoes could not enter when the doors were closed, we could not control when the doors were closed. Many residents shut their

doors late in the evening, facilitating the entry of mosquitoes, especially for crepuscular species. Further research is needed into the behaviour of mosquitoes around doors, and the effect of door modifications for vector-borne disease control.

The study was carried out in traditional houses spread across four villages (about 2 km) allowing for comparisons among different levels of eave closure under natural conditions. While inclusion criteria were used to increase comparability among the houses, we included in our analysis additional factors that may have influenced the entry of mosquitoes into houses. Similar to previous studies, the number of people who slept in the house the previous night was associated with significantly higher numbers of female malaria vectors indoors (Mbogo et al. 1999, Haddow 2009, McCann et al. 2017a). In the current study, the presence of chickens within 20m of the house was also associated with more female anophelines indoors, most of which were *Anopheles arabiensis*. This concurs with the findings of a semi-field study using chicken odour in Kenya (Busula et al. 2015), but differs with the findings of Jaleta et al. (2016), who found that chickens or chicken volatiles reduced the catches of female *An. arabiensis* mosquitoes. The relationship between chicken odours and anopheline mosquitoes warrants further investigation.

Presence of goats and cooking on the veranda was associated with reduced female culicines. Interestingly, cooking on the veranda was also associated with reduced male culicine catches. Male mosquitoes feed on sugar and do not seek hosts for blood, but this factor was also associated with the female culicines. It is possible that the males could have been using these odour cues to locate likely presence of female culicines, an area that needs further investigation.

The relatively low number of mosquitoes collected during this study can probably be attributed to two factors. First, the rainy season prior to the study (November 2015 to April 2016) was relatively dry, with drought conditions throughout the region, and Chikwawa District specifically

receiving extremely below average rainfall (Nations 2016). Additionally, the National Malaria Control Programme in Malawi conducted a mass distribution of ITNs in April 2016. Both factors likely reduced the mosquito populations in the study area.

Observational studies assessing the impact of housing on malaria have consistently found that people living in houses with modern features, such as closed eaves, have lower odds of malaria infection (Tusting et al. 2016), even when accounting for ITN use (Tusting et al. 2017). These findings have increased international interest in house improvement as a deliberate intervention against malaria (Tusting et al. 2016). House improvement covers and protects all individuals sleeping in a house equally, and its impact should not be affected by insecticide resistance. Still, observational studies are considered low-quality evidence with a high risk of bias. An ongoing trial in The Gambia aims to assess the impact of house improvement on the incidence of clinical malaria using a randomised design (Pinder et al. 2016). An ongoing cluster randomised trial in Malawi is evaluating the impact of house improvement, using a community-led implementation approach, on malaria transmission (McCann et al. 2017b). The results of the current study indicate that the quality of eave closure will be one of the important coverage indicators for understanding the effects of house improvement in these ongoing trials.

**Conclusions**

Our study adds to the evidence that house improvement, including fully closed eaves, reduces the number of malaria vectors indoors and, therefore, shows promise as a complementary tool for malaria control. While further research is necessary to understand the behaviour of malaria vectors around house entry points, the results of this study demonstrate the ability of malaria vectors to locate any remaining entry points on improved houses, suggesting that quality control

must be an important component of implementing house improvement as an intervention (WHO 2017). The lack of effect on culicine mosquitoes in this study could reduce acceptance of house improvement, as implemented here, by household residents due to continued nuisance biting. This limitation could be addressed through community engagement (e.g. encouraging people to close their doors early in the evenings) or improved designs.

**Acknowledgements**

We acknowledge the local leaders and residents of Fombe, Semu, Jacobo I and II for allowing us to work in their houses and their cooperation. Michael Chipeta is thanked for valuable discussions on data analysis. We thank laboratory of Blantyre Malaria Project (BMP) where we did the molecular identification of female anophelines. We also extend our thanks to Majete Malaria Project (MMP) team. The study was funded by Dioraphte Foundation, the Netherlands.

# Chapter 7

# General discussion

Chapter 7

## Introduction

This thesis focused on the biting behaviour of malaria vectors, both indoors and outdoors in southern Malawi, to assess the risk of malaria transmission in time and space. The findings demonstrate that in southern Malawi, the major malaria vectors are *Anopheles arabiensis* and *Anopheles funestus* s.s., and both species contribute mostly to the outdoor and indoor malaria transmission, respectively. The biting activity in the early and late evenings has an impact on malaria risk because it coincides with the times that individuals may be active while they are unprotected by bed nets. This research has shown that a structural house improvement strategy that includes the closure of eaves can significantly reduce house entry by malaria vectors. However, outdoor protective measures remain a challenge given that *An. arabiensis* was more likely to bite outdoors than indoors. Tools for monitoring both indoor and outdoor biting are also important in understanding the dynamics of malaria transmission. This research has shown that the Suna trap, an odour-baited trap, has the potential to sample host-seeking mosquitoes both indoors and outdoors and that the simultaneous use of this trap indoors and outdoors does not affect the catch sizes of either trap. As many traps can be used concurrently indoors and outdoors, surveillance for malaria vectors can be conducted reliably over a large area within the same time period, which is useful for malaria epidemiological studies.

The main objectives, findings and recommendations for future research are discussed below:

## Biting patterns of mosquitoes of the lower Shire valley, southern Malawi

Since 2000, the primary malaria vector control tools in Africa have been the use of long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS) (WHO 2006, 2018). The effectiveness of these tools is dependent on the biting patterns of malaria vectors and insecticide-

induced mortality (Lengeler 2004, Le Menach et al. 2007). These tools are effective based on the knowledge that malaria vectors bite primarily at night and predominantly indoors when people are asleep. However, biting activities in the early evenings (Reddy et al. 2011) and partly in the mornings (Reddy et al. 2011, Moiroux et al. 2012b, Sougoufara et al. 2014), as well as increasingly outdoors (Reddy et al. 2011, Russell et al. 2011, Meyers et al. 2016), have been reported in some regions. As a result, the effectiveness of the primary tools for vector control remains a concern. Therefore, assessment of the biting activities of malaria vectors, spatially and temporally, is crucial because it provides the times at which different malaria control interventions would be effective. In Chapter 3, we assessed the biting patterns of mosquitoes in southern Malawi. The research showed that in the present study region, *An. arabiensis* and *An. funestus* s.s. bites predominantly outdoors and indoors, respectively. This confirms previous findings of the exophagic behaviour of *An. arabiensis* (Mendis et al. 2000, Tirados et al. 2006, Oyewole et al. 2007, Russell et al. 2011), and the endophagic behaviour of *An. funestus* (Awolola et al. 2003, Antonio-Nkondjio et al. 2006, Mwangangi et al. 2013). Furthermore, the temporal distributions of *An. arabiensis* and *An. funestus* s.s. showed unimodal patterns. Whereas *An. arabiensis* showed a major biting peak in late evening hours, outdoors during the wet season, that of *An. funestus* was in the early evenings and late night hours, mostly indoors, during the dry and wet seasons, respectively. The variability of the biting peaks of *An. funestus* s.s according to season may reflect the effect of peoples' behaviour during the dry and wet seasons. In other regions, individuals spend most their time and sleep outdoors during the dry season because of high temperatures at night whereas, during the rainy seasons, individuals mostly sleep indoors (Binka and Adongo 1997, Frey et al. 2006, Ritmeijer et al. 2007).

The ability of the vectors to engage in biting activities in the early and late evening hours, both indoors and outdoors, may enhance malaria transmission. This is because these biting activities coincide with the times that individuals are still active, exposed to mosquito bites while unprotected by bed nets. Biting in the early evening has been reported in other regions (Mendis et al. 2000, Geissbühler et al. 2007, Reddy et al. 2011, Yohannes and Boelee 2012) and most of this biting has been associated with long-term use of LLINs. It is difficult to draw a conclusion whether the biting activity of these vectors in the present study area is a result of the use of LLINs because data on the abundance of malaria vectors before and after LLINs distribution is lacking. The considerable amount of outdoor biting by *An. arabiensis* raises the need for the development of vector control tools that can target these vectors. Furthermore, the development of vector control tools that can tackle the biting activity in the early and late evening hours, both indoors and outdoors, is highly recommended because the current primary indoor-based tools provide only partial protection against the bites by malaria vectors. Management of water bodies (Mutero et al. 2000) would be one approach, whereby, communities would engage in activities such as draining or refilling stagnant water bodies. This strategy may help because my study has shown that the abundance of *An. arabiensis* was higher during the wet seasons than in the dry seasons, a finding which is likely to be associated with the availability of larval habitats. Therefore, programmes that would focus on managing the availability of larval habitats for mosquitoes would help to reduce the emergence of adult mosquitoes. Such management activities would likely translate into fewer bites by malaria vectors. Other methods include the use of insecticide-treated clothes (Kimani et al. 2006) for use during the times when people are still active and unprotected by bed nets although the resistance of mosquitoes to such insecticides remains a challenge (Strode et al. 2014, Hemingway et al. 2016)

Chapter 7

**Assessment of the Suna trap in sampling mosquitoes indoors and outdoors**

With the growing concern of the residual transmission of malaria, there is a need for monitoring and assessing the vectors sustaining indoor malaria transmission and the vectors contributing to outdoor transmission. Different tools for monitoring malaria vectors exist and the advantages and disadvantages of these tools vary. For instance, the use of human landing catches (HLCs) directly estimates the number of infectious bites that a person can receive, as mosquitoes are collected by a volunteer as they land on his or her legs. However, the method is labour intensive and expensive on large scale implementation. Moreover, volunteers vary in their individual attractiveness to mosquitoes (Mukabana et al. 2002). The Centers for Diseases Control and Prevention Light Trap (CDC-LT) is another tool that is usually placed next to a person sleeping under a bed net whereby, the person acts as the bait (Lines et al. 1991, Mboera et al. 1998). However, the performance of this tool outdoors is limited Costantini et al. (1998b) and when compared to the HLC in different regions, varying results of its effectiveness have been reported (Briët et al. 2015). The Suna trap, a recently developed odour-baited trap, has the potential to sample mosquitoes both indoors and outdoors (Hiscox et al. 2014). The trap uses synthetic odour baits that mimic human odours (Menger et al. 2014a, Mweresa et al. 2014b). However, it has been unclear whether the simultaneous use of this trap both indoors and outdoors would lead to competition between the two traps. The research in Chapter 4 assessed the use of the Suna trap in sampling mosquitoes indoors and outdoors. The findings show that the use of the Suna trap both indoors and outdoors equals that of the HLC but catches were lower than those of the CDC–LT. The efficiency of the CDC-LT may be dependent on the placement of the trap next to an individual sleeping under a bed net. Mosquitoes have been shown to have a higher contact with the top of a bed net (Parker et al. 2015), which roughly may coincide with the optimal height

(150cm above the ground) for the CDC-LT and may explain why the catches were higher with CDC-LT than with the Suna trap which was placed in the sitting room and not in the bedroom, with the opening at 30cm above the ground. However, the effectiveness of the Suna trap in sampling mosquitoes when placed either indoors, outdoors or simultaneously indoors and outdoors is the same (Chapter 4). This finding makes the Suna trap more efficient than the CDC-LT because the use of the latter trap is seemingly dependent on the attractiveness of the individual sleeping under the bednet and the use of the CDC-LT outdoors catches fewer malaria vectors (Costantini et al. 1998b). Additionally, the Suna trap can serve as a substitute for the HLC for estimating the biting rates.

A review by Takken and Knols (2009) provides a variety of alternative tools for vector control. One of the tools involves disruption mechanisms by use of odour baits in traps to reduce the abundance of mosquitoes. Recently, mass trapping of mosquitoes led to a reduction of malaria vectors and a significant reduction in malaria prevalence in a stepped-wedge cluster-randomised trial (Homan et al. 2016). The Suna trap, an odour baited trap, may therefore, be a promising alternative tool for vector control (Killeen 2016). The development and use of odour baits look promising for lure and kill (push-pull) strategies to suppress populations of malaria vectors (Menger et al. 2014a, Menger et al. 2016) thereby reducing the malaria burden. Push-pull systems (Cook et al. 2007) have already been highly successful in agricultural practices, whereby plants such as Napier grass serve as trap plants that attract (pull) pests that could otherwise attack crop plants such as maize. Other intercrops such as *Desmodium* species repel (push) the pests, thereby increasing the yields of these plants (Khan et al. 2000). Push-pull systems have the additional advantage that they do not depend on insecticides for pest control, and hence provide a sustainable alternative to insecticide-based vector control interventions.

Chapter 7

**Impact of cattle on the resting behaviour of malaria vectors**

The resting behaviour of both *An. arabiensis* and *An. funestus* s.s. varied when cattle were in close proximity to a house (Chapter 5). Whereas the presence of cattle has been associated with zooprophylaxis against infectious bites by *An. arabiensis*, this effect was absent in the present study. This can be explained by the opportunistic feeding behaviour of this species whereby it bites humans or cattle indiscriminately (Takken and Verhulst 2013) and therefore, the abundance of this species was similar in houses with and without cattle. Of the positive blood meal samples, the majority of *An. arabiensis* fed on cattle (n = 18, whereby 7 fed in houses without cattle and 11 fed in houses where cattle were present). This demonstrates the exophagic-endophilic behaviour of this species and the ability to feed elsewhere (where cattle were absent) but still use a house as a resting site. In other regions, the exophagic-endophilic behaviour of *An. arabiensis* has been associated with the availability of hosts (Faye et al. 1997). For *An. funestus*, the presence of cattle near a house would provide a zooprophylactic effect in this region because our results show that the abundance of this species was reduced in houses where cattle were near a house compared to houses where cattle were absent. Most of the blood meals that amplified for this species were mainly identified as mammalian (n=11; i.e. 8 in houses without cattle and 3 in houses where cattle were near) other than humans, cow, pigs, dogs or goats. This implies that the presence of cattle near a house may have diverted this species to houses where cattle were absent, possibly due to the deterrent effect of cow odour. A high degree of aversion to cow odour has been reported for the highly anthropophilic *An. gambiae* s.s. (Costantini et al. 1998a, Pates et al. 2007) and this behaviour may also be present in the anthropophilic *An. funestus*. Further exploration of the odours that have a repellent effect on malaria vectors can help in developing synthetic repellents for use either in traps as discussed in Chapter 4 or when people are not

protected by LLINs. For example, Chapter 3 shows the biting activities in the early and late evening hours when people may not be protected by LLINs and would benefit from any form of a volatile repellent.

The unidentified hosts that *An. funestus* may have fed on in my study (Chapter 4) were either indoors or outdoors. Chapter 3 found that some biting-peaks of *An. funestus* were at the late night hours most likely, when people are asleep protected by bed nets. Therefore, it is possible that due to the inaccessibility of human hosts by *An. funestus*, these mosquitoes fed on any other available mammalian host indoors such as rodents. On the other hand, there is a possibility that these mosquitoes fed on other mammalian hosts outdoors, such as monkeys, given that the study region is in close proximity to a wildlife reserve. After feeding, the mosquitoes still used the house as a resting site. Whereas these are speculations, the finding warrants further investigation because it could be that a fraction of *An. funestus* in this region exhibits a zoophagic-anthropophagic trait similar to a previous finding in Madagascar (Fontenille et al. 1990). Overtime, due to insecticide pressure, a fraction of *An. funestus* in the study region may become zoophagic as seen in Madagascar and western Kenya (Fontenille et al. 1990, Githeko et al. 1996b).

**Impact of fully and partially closed eaves on house entry rates by mosquitoes**

With the growing concern of malaria vectors resistant to insecticides that are impregnated in LLINs or sprayed on walls of houses, one of the potential complementary tools is house improvement. House improvement protects all individuals in a house equally and is not threatened by insecticide resistance. Structural house improvement has been associated with fewer mosquitoes entering the house (Atieli et al. 2009, Kirby et al. 2009, Kampango et al. 2013)

and reduced malaria prevalence/incidence (Gamage-Mendis et al. 1991, Bradley et al. 2013) and anaemia (Kirby et al. 2009). However, during large-scale implementations, the quality of structural house improvement especially on the eaves, which are the preferential entry points for mosquitoes into houses (Snow 1987, Lindsay and Snow 1988, Njie et al. 2009), may differ. Therefore, Chapter 6 assessed the impact of fully and partially closed eaves on house entry by malaria vectors. It was found that houses with fully closed eaves and those with one gap on the eaves significantly reduced the house entry of malaria vectors compared to houses with the other two levels of partially closed eaves (four gaps on the eaves and two long sides open on the eaves) or fully open eaves (Mburu et al. 2018). With partially-closed eaves, mosquitoes were still able to locate the remaining small gaps in the eaves. For instance, entry by mosquitoes into houses with four small gaps in the eaves was higher than into houses with two small gaps or two long sides open in the eaves. The ability of the vectors to locate the remaining entry points on the eaves has an implication for malaria transmission and demonstrates that quality control is an important component when implementing structural house improvements. The results of Chapter 3 show that *An. funestus* s.s. is more likely to bite indoors than outdoors in the present study region and therefore, a house improvement strategy that includes the closure of eaves may be a complementary tool for vector control that protects against biting by *An. funestus* which is responsible for the indoor biting in southern Malawi (Chapter 3). In other regions, structural house improvement that includes the use of tubes impregnated with insecticides along the eaves has been associated with a reduction in house entry by mosquitoes (Sternberg et al. 2016, Oumbouke et al. 2018).

Overall, the research described in this thesis was conducted in a variety of villages. The findings show that the sporozoite rates of malaria mosquitoes varied among the villages. The study

regions described in Chapter 4 had higher densities of malaria vectors and sporozoite rates than the study regions that are described in Chapters 3 and 5. Although the major vector species in the study regions were mostly the same, the variations in sporozoite rates may be explained by other factors such as local environmental conditions and unequal risks of exposure/contact with the vectors within villages in a region. When linked to malaria transmission, the variation may explain the fact that malaria transmission varies across regions (Burkot 1988, Gamage-Mendis et al. 1991, Beier 1998). Furthermore, in western Kenya, variations in malaria parasitaemia among the population were associated with locations whereby a higher parasitaemia was recorded on the western than on the eastern locations (Olanga et al. 2015). Such variations would affect the infection rate of malaria mosquitoes, which is dependent on the malaria prevalence of the population on which they feed.

**Recommendations for future research**

To achieve a better understanding of the biting behaviour of mosquitoes in southern Malawi, further research is recommended on:

1. Incorporating studies on the behaviour of people when assessing the biting patterns of mosquitoes. My study has shown that a considerable amount of biting occurs outdoors, with peaks in the late evening hours. Additionally, indoor biting was prevalent in the late evening hours as well during the dry season. Comparing this biting behaviour with that of people would help to understand whether there is a close association between the two behaviours. This is because the behaviour of people varies across regions whereby some people will spend more time indoors or outdoors. This behaviour is usually dependent on seasons, economic and social

activities and may enhance malaria transmission because based on such factors, individuals may not use protective measures as shown by Pulford et al. (2011). For instance, in regions where individuals rely on fishing as a source of livelihood, most likely these individuals will spend their time outdoors engaging in fishing activities. Such activities have been associated with risks for malaria transmission (Rajagopalan et al. 1986) as individuals are prone to receiving infectious bites because they are unprotected by bed nets. In other regions, the sleeping patterns of individuals vary during the dry and wet seasons. For instance, during the dry season, most individuals will sleep outdoors, unprotected by LLINs due to high temperatures during the night and the perceived lower densities of vectors during this season than in the rainy seasons (Binka and Adongo 1997, Frey et al. 2006, Ritmeijer et al. 2007).

2. There is the need for conducting studies that assess the host-seeking and resting behaviours of malaria vectors simultaneously. Such studies are crucial for estimating the number of bites and relating these numbers to the sources of the blood meal hosts of the malaria vectors. For example, my data on blood meal sources of *An. funestus* showed that many of them had fed on non-human hosts, but the origin of the blood meals was not revealed. Whereas Chapter 5 has shown that the Suna trap has the potential for sampling mosquitoes indoors and outdoors, tools for monitoring mosquitoes resting outdoors remain a challenge. Development of such tools is highly recommended as recent studies have shown that malaria vectors prefer to rest in shady sites mostly outdoors (Debebe et al. 2018).

3. With the early and late evening biting activities in the present study area, there is a need for developing vector control strategies that can provide protection against the vectors biting at these times. Although the use of repellents or insecticide-treated clothes (Kimani et al. 2006) seem to be a viable solution, the resistance of these vectors to insecticides remains a challenge.

4. Given the low densities of malaria vectors in the study area in southern Malawi, the infectious reservoir of malaria parasites should be assessed to understand the hidden risk of malaria transmission. A study in Burkina Faso had low mosquito catches but the human biting index remained high (Pombi et al. 2018). This finding may reflect on the possibility of mosquitoes obtaining blood meals from asymptomatic individuals harbouring gametocytes (Busula et al. 2017). Such studies may be designed by collaborating with local health centers whereby the detection of positive malaria cases could form a basis for following up the individuals in their homes. With more screening and with the help of molecular techniques, the infectivity of mosquitoes by asymptomatic individuals can be evaluated. Such studies have been conducted in the neighbouring country, Zambia (Stresman et al. 2010).

**Conclusions**

This thesis has shown that (i) *An. arabiensis* and *An. funestus* s.s. are the primary malaria vectors in the present study region, (ii) considerable proportion of anopheline bites occurs in the late evening hours both indoors and outdoors. The biting activity coincides with the times that individuals are still active, exposed to mosquito bites while unprotected by bed nets, (iii) the presence of a cow near a house reduces the abundance of indoor resting *An. funestus*, (iv) the simultaneous use of the Suna trap both indoors and outdoors in the same house and the same night does not lead to competition between the two traps, and (v) a house improvement strategy that includes fully closed eaves significantly reduces house entry by malaria vectors.

Future research should be directed at (a) incorporating the behaviour of people and bed net usage in studies assessing the biting patterns of mosquitoes, (b) developing more tools for

collecting outdoor biting mosquitoes and for targeting different sites, (c) developing tools/measures that would protect individuals from mosquito bites outdoors and at times when they are not protected by bed nets, (d) assessing the effect of other preferential entry points for malaria mosquitoes and studies comparing both the indoor and the outdoor mosquito densities in regions where structural house improvement is implemented, and (e) assessing the infectious reservoirs of malaria parasites.

# General summary

General summary

Current methods of malaria vector control implemented by national control programmes rely mainly on the use of insecticides. These include the use of long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS). The success of LLINs and IRS is underpinned by the protection from infectious mosquito bites provided to individuals and the reduction in mosquito population size caused by sufficient contact of mosquitoes with the insecticide in the nets or on the walls of the houses. The high degree of endophily (resting indoors) and endophagy (feeding indoors) exhibited by the dominant African malaria vectors has been, therefore, a key component of that success.

However, in recent years in some regions, the biting behaviour of the African malaria vectors, both indoors and outdoors and during a wider range of times than previously recognized, has been reported. This has an implication on malaria control because individuals are at risk of receiving infectious bites from vectors that are biting either outdoors or indoors at times when people are not protected by the primary control tools. Additionally, resistance of mosquitoes to these insecticides exacerbates the risk for malaria transmission. Therefore, understanding the degree of endophagy/exophagy of the vectors, when or where humans are exposed to mosquito bites, entry points for malaria vectors into houses and biological factors enhancing malaria transmission in a region is important. The collective information from studying these natural behavioural aspects of mosquitoes will help in designing interventions that protect individuals from infective mosquito bites, thereby reducing malaria transmission and disease burden.

The research described in this thesis focused on the biting behaviour of malaria vectors in and around houses in southern Malawi. Chapter 2 provides an overview of the biting times of malaria vectors in Africa, both historically and currently. Our literature search showed that the biting behaviour of mosquitoes both indoors and outdoors was common but the biting peaks vary across

and within regions. We explored the factors that may be associated with the variations in the biting behaviour of the vectors. We found that the availability of hosts is one of the potential factors. Furthermore, there is a likelihood that the prolonged use of LLINs may lead to variations in the biting behaviour of malaria vectors although in some regions where such variations have been reported, they rely on data after the implementation of LLINs only. In Chapter 3, the biting patterns of mosquitoes were assessed both indoors and outdoors and during the wet and dry seasons. We found that the major malaria vectors were *Anopheles arabiensis* and *An. funestus*. Whereas *An. arabiensis* was more likely to bite outdoors than indoors, *An. funestus* was more likely to bite indoors than outdoors. During the dry season, the biting activity of *An. gambiae* s.l. was constant outdoors across the time of observation (18:00 h to 08:45 h), but highest in the late evening hours (21:00 h to 23:45 h) during the wet season. The biting activity of *An. funestus* s.l. was highest in the late evening hours (21:00 h to 23:45 h) during the dry season and in the late night hours (03:00 h to 05:45 h) during the wet season. Biting activities that occurred in the late evening hours, both indoors and outdoors, coincided with the times at which individuals may still be awake and physically active, and therefore unprotected by LLINs. Additionally, a substantial number of anopheline bites occurred outdoors. These findings imply that LLINs would only provide partial protection from malaria vectors, which would affect malaria transmission in this area. Therefore, protection against bites by malaria mosquitoes in the early and late evening hours is essential and can be achieved by designing interventions that reduce vector-host contacts during this period.

Results of Chapter 3 highlight the need for effective tools for sampling mosquitoes indoors and outdoors. Chapter 4 compares the efficiency of the Suna trap, an odour baited trap, to that of the human landing catch (HLC) and Centers for Disease Control Light Trap (CDC-LT). We found

that use of the Suna trap both indoors and outdoors compares well with that of the HLC. This implies that since the HLC method is labour intensive and expensive at large scale implementations, the Suna trap can serve as a substitute for the HLC for estimating the biting rates. On the other hand, the mosquito catches with the Suna trap were lower than those of the CDC–LT. The effectiveness of the Suna trap in sampling mosquitoes when placed either indoors, outdoors or simultaneously indoors and outdoors is the same (Chapter 4). This finding makes the Suna trap more efficient than the CDC-LT because the use of the latter trap is seemingly dependent on the attractiveness of the individual sleeping under the adjacent bed net and the use of this trap outdoors yields fewer malaria vectors. Additionally, the Suna trap uses synthetic odour baits and does not rely on use of humans as baits as with the HLC or CDC-LT methods.

Biological factors such as the presence of cattle around houses has been associated with either a protective effect against bites by vectors as these vectors are diverted to other blood meal hosts such as cattle rather than humans or with more bites as the vectors have sufficient blood meal hosts (humans and cattle). Therefore, Chapter 5 describes the results from an assessment of the impact of cattle on the resting behaviour of malaria vectors. The presence of cattle near a house significantly reduced the abundance of indoor resting *An. funestus* but not *An. arabiensis*. This implies that the reduction of the former species was possibly due to the deterrent effect of cow odours. These data suggest that repellents around a house disrupt the host-seeking behaviour of malaria vectors. When combined with attractant traps, the resulting push-pull system would lead to reduction of malaria vectors and hence, malaria transmission.

In Chapter 6 the impact of fully and partially closed eaves on house entry rates mosquitoes was studied. We compared mosquitoes in houses with fully closed eaves, open eaves and three levels of partially closed eaves. It was found that fully closed eaves and houses with one small opening

on the eave significantly reduced house entry of malaria vectors compared to partially and fully open eaves. The mosquitoes were able to locate the remaining entry points on the eaves, a finding which has an implication on malaria transmission. Therefore, quality control is an important component when implementing structural house improvements. The results of Chapter 3 showed that *An. funestus* s.s. was more likely to bite indoors than outdoors in the present study region and therefore, a house improvement strategy that includes the closure of eaves may be a complementary tool for vector control that protects against biting by *An. funestus* which is responsible for the indoor biting in southern Malawi (Chapter 3).

In Chapter 7, the general discussion interprets the key findings and links these to the implications for malaria control. Furthermore, the findings described in this research provide recommendations for future research.

It is concluded that in southern Malawi, the major malaria vectors are *An. arabiensis* and *An. funestus* contributing to outdoor and indoor malaria transmission, respectively. Development of tools that can target the biting activity of these vectors both indoors and outdoors at times when individuals are not under bet nets is highly recommended. Furthermore, a house improvement strategy that includes closure of eaves can significantly reduce house entry by malaria vectors. Additionally, the use of odour-baited traps looks promising as tools for sampling malaria vectors both indoors and outdoors as well as tools for mass trapping of mosquitoes to reduce malaria vectors thereby reducing malaria transmission and burden.

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List of abbreviations

| | |
|---|---|
| *An.* | *Anopheles* |
| CDC-LT | Centers for Disease Control and Prevention Light Trap |
| $CO_2$ | Carbon dioxide |
| CI | Confidence interval |
| DNA | Deoxyribonucleic acid |
| HLC: | Human landing catch |
| IRS | Indoor residual spraying |
| ITNs | Insecticide treated nets |
| LA | Lumefantrine-artemether |
| LLINs | Long-lasting insecticide treated nets |
| MMP | Majete Malaria Project |
| mRDT | Malaria rapid diagnostic test |
| *P. falciparum* | *Plasmodium falciparum* |
| PCR | Polymerase chain reaction |
| qPCR | Real time polymerase chain reaction |
| RBM | Roll Back Malaria |
| RR | Risk ratio |
| s.l. | sensu lato |
| spp. | Species |
| s.s. | sensu stricto |
| SUFI | Scaling up for impact |
| WHO | World Health Organization |

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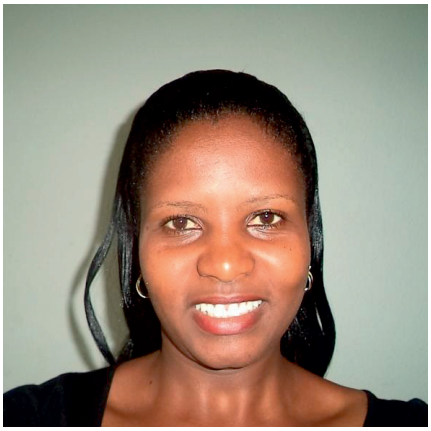
# Curriculum vitae

Curriculum vitae



Monicah M. Mburu was born on 23rd September 1983 in Nairobi, Kenya. After completing her primary and secondary school education, she joined the University of Nairobi (UON) in 2004-2008, where she pursued a Bachelor of Science degree in Botany and Zoology. She did an internship at the Kenya Industrial Research and Development Institute (KIRDI) where she worked on the utilization of rice straw in mushroom production and the chemical/microbiological analysis of water samples and various raw/processed foods. In 2011-2013, she did a Master of Science (MSc.) degree in Applied Parasitology at UON. During her MSc. she joined the International Centre of Insect Physiology and Ecology, Kenya *(icipe*-Mbita, western Kenya) as a graduate research intern under the Dissertation Research Internship Programme. During this period, she evaluated the use of 2-butanone as a mimic for carbon dioxide in synthetic attractants for malaria mosquitoes. In 2014, she was employed as a junior researcher at *icipe* on the SolarMal project which aimed at reducing the prevalence of malaria by mass trapping of mosquitoes in western Kenya. In 2015, Monicah started her Ph.D. at Wageningen University & Research, The Netherlands and College of Medicine, University of Malawi. Her research focused on the indoor and outdoor biting behaviour of malaria vectors in southern Malawi. The results of the research work are presented in this thesis. She also gave lectures in research methods at the College of Medicine, University of Malawi, and was actively involved in forums such as the Malawi March for Science, which advocates for 'equitable evidence-based policies that serve all communities'. Monicah's ambition is to continue conducting research on the development of novel technologies for control of malaria vectors and other neglected tropical diseases.

List of publications

**Mburu MM**, Zembere K, Hiscox A, Banda J, Phiri KS, van den Berg H, Mzilahowa T, Takken W and McCann RS 2019. Assessment of the Suna trap for sampling mosquitoes indoors and outdoors. Malaria Journal, 18:51

**Mburu MM,** Juurlink M, Spitzen J, Moraga P, Hiscox A, Mzilahowa T, Takken W, McCann RS. 2018. Impact of partially and fully closed eaves on house entry rates by mosquitoes. Parasites & Vectors, 11:383.

Kabaghe AN, Chipeta MG, Gowelo S, **Mburu MM,** Truwah Z, McCann RS, van Vugt M, Grobusch MP, Phiri KS. 2018. Fine-scale spatial and temporal variation of clinical malaria incidence and associated factors in children in rural Malawi: a longitudinal study. Parasites & Vectors, 11:129.

Van den Berg H, van Vught M, Kabaghe AN, Nkalapa M, Kaotcha R, Truwah Z, Malaenga T, Kadama A, Banda S, Tizifa T, Gowelo S, **Mburu MM**, Phiri KS, Takken W, McCann RS. 2018: Community-based malaria control in southern Malawi: a description of experimental interventions of community workshops, house improvement and larval source management. Malaria Journal, 17:266.

McCann RS, van den Berg H, Diggle PJ, van Vugt, M, Terlouw DJ, Phiri KS, Pasquale AD, Maire N, Gowelo S, **Mburu MM**, Kabaghe AN, Mzilahowa T, Chipeta MG, Takken W. 2017: Assessing the effect of larval source management and house improvement on malaria transmission when added to standard malaria control strategies in southern Malawi: study protocol for a cluster-randomised controlled trial. BMC Infectious Diseases, 17:639.

**Mburu MM**, Mzilahowa T, Amoah B, Chifundo D, Phiri KS, van den Berg H, Takken W and McCann RS. Biting patterns of malaria vectors of the lower Shire valley, southern Malawi (submitted).

# PE & RC Training and Education Statement

PE & RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

**Review of literature (4.5 ECTS)**

- Indoor and outdoor biting behaviour of the human malaria vectors *An. gambiae* sensu stricto, *An. coluzzii, An. arabiensis and An. funestus* s.s. in Africa- A review.

**Writing of Project proposal (4.5 ECTS)**

- Indoor and outdoor biting behaviour of malaria vectors and the potential risk factors that enhance malaria in southern Malawi.

**Post-graduate courses (3 ECTS)**

- Protecting Human Research Participants; National Institutes of Health (2015)
- Good Clinical Practice (GCP); University of Malawi (2016)
- Introduction to R; DataCamp (2018)

**Laboratory training and working visits (4.5 ECTS)**

- Analysis of blood meals for malaria vectors; Blantyre Malaria Project (2016)

**Deficiency, refresh, brush-up courses (21 ECTS)**

- Ecological methods; WUR (2015)
- Ecological aspects of biological interactions; WUR (2015)
- Molecular aspect of biological interactions; WUR (2015)
- Analysis and prevention of health risks in the tropics; WUR (2015)

**Competence strengthening / skills courses (3.9 ECTS)**

- Essentials of scientific writing & presenting; WUR (2015)

- Information literacy including Endnote; WUR (2015)
- Leadership and management; Malawi Liverpool Wellcome Trust (2016)
- Scientific writing; Malawi Liverpool Wellcome Trust (2017)
- Critical thinking and argumentation; WUR (2018)

**PE&RC Annual meetings, seminars and the PE&RC weekend (1.8 ECTS)**

- PE&RC Weekend (2015)
- PE&RC Day (2018)
- PE&RC Weekend (2018)

**Discussion groups / local seminars / other scientific meetings (4.7 ECTS)**

- PhD lunch group discussion; Laboratory of Entomology (2015, 2016, 2018)
- One health meetings; Laboratory of Entomology (2015, 2016, 2018)
- Journal club; Liverpool Wellcome Trust, Malawi (2016-2018)
- College of Medicine Research and Dissemination Conference; Malawi (2017-2018)

**International symposia, workshops and conferences (6 ECTS)**

- Entomological Society of America; poster presentation (2018)
- American Society of Tropical Medicine and Hygiene, 67$^{th}$ annual meeting; oral presentation and poster presentation; New Orleans, Louisiana (2018)

**Lecturing / Supervision of practicals / tutorials (0.3 ECTS):**

- Research methods; College of Medicine, University of Malawi (2018)

**Supervision of MSc students (9 ECTS)**

- Effect of partial house improvement on house entry by mosquitoes
- Assessment of the Suna trap for sampling mosquitoes
- Assessment of the preferential entry points for malaria vectors

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