Understanding alternative control methods and their mode of action for the control of outdoor biting mosquitoes

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Summary

Worldwide implementation of core vector control tools deployed for Anopheles mosquitoes consisting of indoor residual spraying (IRS) and insecticide-treated nets (ITNs). These control measures have contributed to approximately 70% reduction in global malaria between 2000 and 2015. However, there are concerns that progress has stagnated with the trend in malaria cases plateauing in several countries between 2015 and 2019. This situation is exacerbated by the increase in outdoor/daytime biting mosquitoes in settings where people spend a significant amount of time outside the house, the emergence of insecticide-resistant mosquitoes, and the limitations of the core interventions in certain settings. For example, the current tools do not provide complete protection against behavioral-resistant mosquitoes i.e. mosquitoes that bite outdoors, or physiological resistant i.e. mosquitoes that may survive lethal doses of insecticides.

For the control of arbovirus vectors, larval source management and application of insecticides on surfaces where mosquitoes rest with space spraying have been ongoing. However, there are concerns that the frequency of arboviral disease outbreaks is increasing. The reasons for this increase could be that the transportation of humans and materials has improved as well as the adaptation of the vectors in new areas. In addition, the ability of *Aedes* mosquitoes to breed in a wide range of small, transient and often hidden places has been implicated. The need for frequent reapplication using high levels of manpower impairs the effectiveness of these control tools against arbovirus vectors.

The most efficient vectors of malaria and arboviruses are strongly adapted to humans (synanthropic), and are therefore, most commonly encountered around human dwellings either indoors or in the peri-domestic space. Unfortunately, options for the delivery of effective vector control interventions against outdoor biting mosquitoes in the peri-domestic space are currently limited. Ideally, novel control interventions deployed in the peridomestic

space should prevent bites (personal protection) and kill mosquitoes (community protection). The efficacy of Volatile Pyrethroids (VP) and odor-baited traps (OBT) to provide protection against *Aedes* and Anopheles mosquitoes remains an outstanding research question and robust means to evaluate them are needed. Therefore, as proof of principle, this thesis aimed at conducting a semi-field evaluation of measures directed at protecting the peridomestic space based on the concept of the "Push Pull" strategy. Push refers to the use of VP to drive outdoor biting mosquitoes away from a treated area whereas pull is the use of OBT to attract and kill mosquitoes from a desired surrounding.

A series of iterative experiments were conducted in the semi-field system (SFS) in Tanzania as follows; 1) Comparison between Human Landing Catches (HLC) and exposure free Mosquito Electrocuting Trap (MET), and BG sentinel trap (BGS) for evaluation of spatial repellent against *Aedes aegypti* in a semi-field system. 2) Semi-field evaluation of freestanding transfluthrin passive emanators and the BG sentinel trap as a "push-pull control strategy" against *Aedes aegypti* mosquitoes 3) Transfluthrin Eave Positioned Targeted Insecticide (EPTI) for personal and community protection of malaria vectors in a semi-field simulated peridomestic space 4). Human landing catches (HLC) provide a useful measure of Protective Efficacy for evaluating volatile pyrethroid spatial repellents (VPSR). These experiments were conducted in the SFS using different species of disease-free laboratory-reared mosquitoes with a known physiological status. The outcomes of this work are presented in four different manuscripts encompassed in this thesis.

Chapter 1: Comparison between Human Landing Catches (HLC) and an exposure free Mosquito Electrocuting Trap (MET), and BG sentinel trap (BGS) for evaluation of spatial repellent against *Aedes aegypti* in a semi-field system

A choice and no choice experiments were conducted to understand how the presence of a human and behavioral modifying chemical in the surroundings affect the recapturing of mosquitoes using sampling methods evaluated here. In this experiment, the protective efficacy (PE) of freestanding transfluthrin passive emanators (FTPE) was measured using two types of mosquito traps (BGS+MET) as exposure-free sampling methods compared to HLC. The results showed that in a no-choice experiment, HLC, BGS, and MET measured similar PE whereas in a choice experiment, the PE varied considerably. The most important conclusion from this analysis is that these collection methods measured similar PE when independence was ensured, while in the choice experiment, the MET overestimated the PE while the BG underestimated the PE offered by the FTPE. Also, I observed that the emanator provides protection of around 50% to the person sitting 10 m from the emanator. Therefore, the evaluation of VP needs to be done in the SFS or if it is done in the field then the independence of these collection method traps should be ensured.

Chapter 2: Semi-field evaluation of freestanding transfluthrin passive emanators and the BG sentinel trap as a "push-pull control strategy" against *Aedes aegypti* mosquitoes

While previous studies demonstrated the efficacy of VP in preventing human vector contact, only studies with longer exposure or higher doses have shown additional mortality of exposed mosquitoes. In the field, where mosquitoes may move away after encountering sublethal insecticides, these findings may not reflect what is happening in real-life situations. Thus, the need to have another component that may kill repelled mosquitoes is important. Through mathematical simulation, it was shown that the efficacy of VP may be enhanced when used together with OBT in a "push-pull" control strategy. Odor-baited trap has been designed to attract and trap mosquitoes which could then be killed by starvation or contaminated with insecticides. Owing to the increase in the frequency of arboviral disease outbreaks, the need arises to evaluate these strategies for the control of Aedes mosquitoes.

An experiment was conducted against *Aedes aegypti* where the freestanding transfluthrin passive emanators (FTPE) were used to disperse transfluthrin (push) while BG sentinel trap was used to attract and catch mosquitoes (pull). The efficacy of these devices was evaluated individually and in combination with the "push-pull" concept. To know the duration with which the emanator "push" remains protective, the FTPE was evaluated at 0, 3, and 6 months after treatment. This study suggested that FTPE - the "push subunit" as well as FTPE combined with BGS- "push-pull" provided similar protection against the human landing rate of *Aedes aegypti* mosquitoes. This study concluded that push-alone and push-pull prevent humans from *Aedes aegypti* mosquitoes. However, the majority of the protection observed in the push-pull control strategy originated from the push subunit. Therefore, the use of push is sufficient to provide protection against the outdoor biting of *Aedes aegypti* mosquitoes. Also, this study showed that the FTPE remained protective for three months after impregnation.

Chapter 3; Transfluthrin Eave Positioned Targeted Insecticide (EPTI) for personal and community protection of malaria vectors in a semi-field simulated peridomestic space

The experiment on the evaluation of VP and OBT as push-pull control strategies has previously demonstrated that the majority of the protection in the push-pull control strategy originates from the push component. Thus, the push component was further evaluated against pyrethroid-resistant and susceptible malaria vectors to determine if resistance in mosquitoes is detrimental to the efficacy of transfluthrin a volatile pyrethroid that belongs to the same class of pyrethroids. Experiments were conducted using technical grade (TG) or emulsified concentrate (EC) transfluthrin-eave positioned targeted insecticides (EPTI) against various malaria vectors with various levels of insecticide resistance mechanisms including susceptible *Anopheles gambiae* (Kisumu and Ifakara strains), resistant *Anopheles gambiae* (Kisumu KDR) and resistant *Anopheles arabiensis* mosquitoes (Kingani and Mbita strain). EPTI-composed hessian strips made from *Corchorus olitorius* or jute were treated with transfluthrin 5.25g. The hessian fabric was used as previous work has shown that high cellulose content retains transfluthrin much longer than any other fabric. This study suggested that transfluthrin-treated EPTI reduces the landing of both resistant and susceptible malaria vectors. Also, I observed that mosquito landing is affected by mosquito species, human volunteers, and transfluthrin formulation. Therefore, in an area with low or highly-resistant malaria vectors, EC-transfluthrin-treated EPTI may be used to reduce human vector contact in an outdoor environment.

Chapter 4; Human landing catches (HLC) provide a useful measure of Protective Efficacy for evaluating volatile pyrethroid spatial repellents (VPSR).

Human landing catches (HLC) involve a human volunteer catching mosquitoes that land on them before they can bite. HLC is often used to measure the protective efficacy (PE) of bite prevention interventions. However, some repellents interfere with mosquito olfaction so that not all landed mosquitoes are able to bite. Also, in order to maximize the precision of estimating feeding success a cage mesh measured 6x6x2m to mimic the size of peridomestic space known as Ifakara ambient chamber test (I-LACT) was designed. This cage allowed the recapture of all released mosquitoes. Therefore, a comparison of PE of the volatile pyrethroid transfluthrin was conducted using either HLC or allowing landed mosquitoes to blood-feed, in order to measure whether HLC is a good proxy for bite-reduction.

The study was a fully balanced crossover design conducted in a 6x6x2 meter netted cage within a semi-field system (SFS). Three strains of Anopheles were used as well as *Aedes aegypti.* Mosquitoes were interacted with a volunteer for one hour in the cage. Transfluthrin-treated hessian at 5g, 10g, 15g, and 20g doses were evaluated with a paired negative control. Six replicates were performed per dose using each method. The number of landing or blood-fed mosquitoes was analyzed using negative binomial regression, and the agreement of PE by the method was compared by Bland-Altman.

HLC underestimated the feeding inhibition of transfluthrin, and there were species differences in the difference between landing and biting. However, findings demonstrate that the PE calculated by either method is closely agreed upon when tested by Bland Altman methods. Therefore, either method could be used interchangeably for assessing the personal protective efficacy of volatile pyrethroids. Taking into account the difficulties of measuring the number of fed mosquitoes in the field setting, the HLC could be used as the proxy of personal protective efficacy for evaluating volatile pyrethroids

Chapter 5: Semi-Field System and Experimental Huts Bioassays for the Evaluation of Spatial (and Topical) Repellents for Indoor and Outdoor Use

The considerations for the design of experiments to measure the protective efficacy of bite prevention tools against mosquito vectors. The chapter focuses on the evaluation of spatial repellents (specifically volatile pyrethroids) and topical repellents under semi-field conditions including a description of the semi-field system (SFS) and experimental huts (EH) used to simulate indoor and outdoor use settings. We also, explain the preparations needed for conducting an experiment in these bioassays and the limitations to allow reproducible data.

The book chapter concluded that during the planning of evaluation of spatial repellent or topical repellent, it is crucial to consider the following; the size of the facility, test system (resistance, age of mosquitoes, anthropophagy, climatic), and independence of observation.

List of Abbreviations

ACT	artemisinin-based combination therapy
BGL	BioGents Lure
BGS	BioGents sentinel trap
CI	Confidence interval
DEET	N, N-diethyl-meta- toluamide
DDT	Dichlorodiphenyltrichloroethane
DENV	Dengue virus
EC	Emulsified concentrate
EIR	entomological inoculation rate
EPTI	Eave positioned targeted insecticides
FTPE	freestanding transfluthrin passive emanators
GLMM	Generalized linear mixed model
GMEP	Global Malaria Eradication Program
HLC	human landing catches
I-LACT	Ifakara Large ambient chamber test
IHI	Ifakara health institute
IB	Ifakara blend
IRB	Institute review board
IRS	Indoor residual spraying
ITN	Insecticide-treated net
KDR	Knock down resistance
LLIN	Long lasting insecticides nets
MET	Mosquitoes electrocuting trap
GLMM	Generalized linear mixed model
NIMR	National Medical Research Institute
OBT	Odour baited trap
PE	Protective efficacy
SFS	semi-field system
TG	Technical grade
UV	Ultra violet
VP	Volatile pyrethroid
WHO	World Health Organization

Chapter 1: Introduction

1.1 Epidemiology of malaria

1.1.1 Global Burden of Malaria

Unfortunately, malaria continues to be a disease of public health importance due to continued transmission with associated morbidity and mortality (WHO, 2020a). Looking into the trend of malaria, the World Health Organization (WHO) reported that malaria cases dropped from 238 to 229 million over the last 20 years. Global malaria case incidence has decreased by 29% and mortality by 67% over the same period. During this time an estimated 1.5 billion cases and 7.6 million deaths of malaria have been prevented worldwide (WHO, 2020a). Also, no new cases were reported in the 38 countries that were already certified as malaria-free between 2000 and 2019 with new countries such as China and El Salvador planning to apply for certification as no malaria cases have been reported for the past three years (WHO, 2020a). This trend brings the hope that malaria may be eliminated from malaria-endemic areas.

However, there are concerns that progress has stagnated with the trend in malaria cases flattening out and malaria has increased in several countries between 2015 and 2019 (WHO, 2020a). In Africa where 94% of malaria cases occur, it is estimated that 35% of pregnancies exposed to malaria resulted in 822, 000 children with low birth weight between 2000-2019. There is also an increase in transmission in some areas where elimination was considered to be feasible (Geng et al., 2019). This rebound may have resulted from several factors including the incomplete coverage of the current vector control tools and the emergence of mosquitoes that are either physiologically or behaviorally resistant to the current core vector control tools (Monroe et al., 2015, Ranson and Lissenden, 2016, Monroe et al., 2019b).

1.1.2 The life cycle of the malaria parasite

The four main species of malaria parasites identified to cause human malaria are *Plasmodium falciparum*, *Plasmodium vivax, Plasmodium malariae,* and *Plasmodium ovale.* Of these parasites, *P. malariae* and *P. falciparum* are responsible for the majority of malaria cases in African countries (WHO, 2013b). *Plasmodium malariae* is widely distributed globally whereas *P. falciparum* is commonly found in Africa (WHO, 2013b). In sub-Saharan Africa, co-infection with two species of malaria parasite is very common. Due to cerebral involvement, *P. falciparum* causes a more severe form of malaria compared to other *plasmodium* species distributed in sub-Saharan Africa and results in higher mortality.

The life cycle of Malaria parasites is completed between humans and mosquitoes (Figure 1). During a blood meal on a human host, an infected female Anopheles mosquito injects a stage of malaria parasites called sporozoites from the salivary glands (WHO, 2013b). These are carried in the blood to the liver cells (hepatocytes). In the liver, the parasites grow, multiply, and transform into schizonts, which then burst and release numerous malaria parasites called merozoites (Tavares et al., 2013). The released merozoites then invade new red blood cells where the parasite multiply rapidly thereby rupturing the red blood cells to release another batch of merozoites. Signs and symptoms of malaria including fever and chills may occur when the parasites and their contents are released together with the merozoites. In response to various factors such as the availability of nutrients (Venugopal et al., 2020) and host immunity (Buckling and Read, 2001) some merozoites may switch to the sexual stage called gametocytes while others continue with the asexual life cycle. The gametocytes are haploid cells comprised of males and females which may be taken up by mosquitoes during the next blood meal.

When the gametocytes are ingested by the mosquitoes during feeding, the life cycle continues. In mosquitoes, the gametocytes mature due to the change in the pH, the presence of the mosquito-derived molecule xanthurenic acid (Garcia et al., 1998), and the drop in temperature (Aly et al., 2009). The male (microgametes) and female gametocytes (macrogametes) exit the red blood cell and fuse to form a zygote (Venugopal et al., 2020), which transforms into slender motile parasites stage called ookinetes. The ookinetes penetrate the midgut epithelial wall to the outer surface of the midgut, where it develops into an oocyst (Aly et al., 2009). Inside the oocysts which are located outside the gut membrane, several mitotic divisions occur to produce thousands of sporozoites that are released on maturation (Aly et al., 2009). Lastly, the sporozoites migrate towards the salivary glands and traverse the gland epithelium into the salivary gland lumen. In the lumen, further maturation of sporozoites occur before are become infective and pass to human during the next blood meal (Kojin and Adelman, 2019) and a cycle is completed. The time between infection to the maturation of sporozoites may vary considerably depending on the species of the malaria parasites whereas for *P. falciparum* it takes between 12 and 14 days.

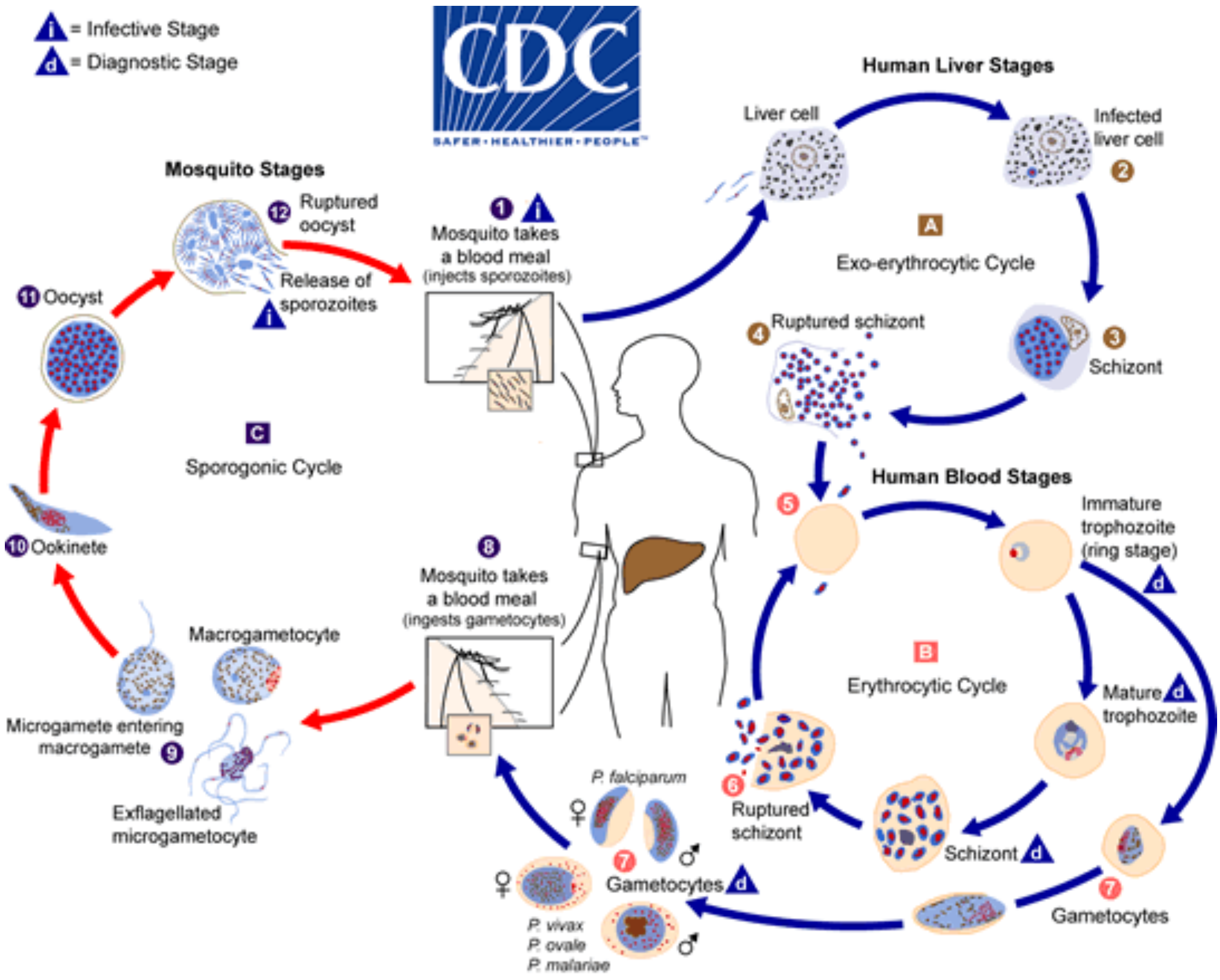

Figure 1.1 Life cycle of the *Plasmodium falciparum* in the human host and the mosquito vector. (CDC, 2021)(Figure reproduced from CDC's website for laboratory identification of parasites). In humans, the malaria parasite infects and multiplies in the liver cells and then the red blood cells for *P. falciparum* it takes about 13-14 days. The RBCs rapture to release merozoites which differentiate into gametocytes and are ingested by the mosquito during a blood meal. In mosquitoes, the sporogonic cycle takes 10-14 days – thus completing the malaria life cycle

1.2 Epidemiology of arbovirus

1.2.1 Global burden of arbovirus

Arboviral disease refers to infections in humans that are caused by a group of viruses (flavivirus and alphavirus) transmitted by the bite of infected arthropods (insects) such as mosquitoes and ticks. Varieties of arbovirus diseases have been reported to cause disease on humans for example yellow fever (Goldani, 2017, Kraemer et al., 2017), dengue virus

(DENV)(Mboera et al., 2016), Zika virus (Wikan and Smith, 2016) and others. Both arbovirus diseases have the same epidemiology, transmission, and clinical signs at onset. The Dengue virus described here represents other arboviral diseases as it carries the major burden of arboviral diseases (Wilder-Smith et al., 2017). Dengue fever is an acute and non-contagious infection caused by a dengue virus transmitted by the bite of Aedes mosquitoes. Recently, the dengue virus has been associated with several serious disease outbreaks in densely populated areas (Mboera et al., 2016). It is estimated that 2.5 billion people live in endemic areas, and 390 million are infected with DENV annually, resulting in 576,900 deaths (Wilder-Smith et al., 2017). The incidence of dengue is grown dramatically over the last 10 years due to urbanization, improved transportation, and the ability of the mosquito vector to colonize new environment (Jing and Wang, 2019).

Dengue virus belongs to the genus Flavivirus and the family Flaviridae exists in four serotypes DENV-1, DENV-2, DENV-3, and DENV-4 (Weaver and Vasilakis, 2009). These serotypes occur in the tropics across Africa, America, and Asia. Infection with one serotype provides long-term immunity against subsequent infection with the same serotype while cross immunity may lead to a severe form of the disease (WHO, 2009a).

1.2.2 Transmission and life cycle of the dengue virus

It has been suggested that for some arbovirus, disease transmission is maintained in the forest habitat between tree *Aedes* (i.e *Aedes africanus*) and non-human primates (monkey) known as the sylvatic cycle (Figure 1.2) (Valentine et al., 2019). Although this kind of transmission is common for yellow fever it is uncommon for dengue virus except for DENV-2 (Silva et al., 2020). *Aedes aegypti* mosquitoes are the primary vector that maintains transmission in urban and rural areas in an endemic area. These mosquitoes are highly anthropophilic and have a tendency to bite several people during the day making them highly efficient vectors (WHO, 2009a). *Aedes aegypti* is highly anthropophilic and tends to remain in the peridomestic space, thus major outbreak is fuelled by the movement of people between infected and uninfected areas. Outbreaks occur depending on herd immunity, social economic status climate variation, human migration, vertical transmission, and the presence of widespread asymptomatic but transmissible individuals (WHO, 2009a).

Humans acquire infection from the bite of infected *Aedes* mosquitoes (WHO, 2009a). The period between a mosquito's bites and the development of clinical signs is called the incubation period, which is between 3-12 days. A patient may be asymptomatic or showing some symptoms characterized by an abrupt onset of chills vomiting, fever, and muscle pain. During this time, the viruses circulate in the blood, when mosquitoes feed on this individual, they ingest viraemic blood and become infected (WHO, 2009a). In mosquitoes, the virus infects the mid-gut and then spreads systemically over a period of 8-12 days. After this time, the mosquitoes are infected forever and any subsequent blood meal, the virus can be transmitted to other humans and the cycle continues (WHO, 2009a). Also, mosquitoes can be infected through vertical transmission – where a mother passes the virus to the offspring.

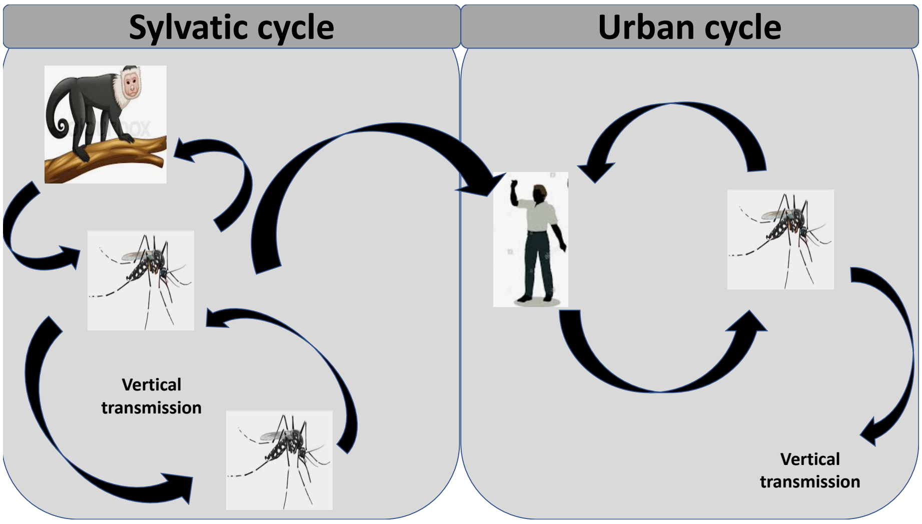

Figure 1.2 Life cycle of the arbovirus in the human host and the mosquito vector. In humans, the virus infects and multiplies cells then in blood. While sylvatic cycle occur when the viral transmission occurs in the forest between nonhuman primate and forest *Aedes* mosquitoes (*Aedes africanus, Aedes fulciperAedes aegypti formosus* etc) transmission in urban area is maintained between human and *Aedes aegypti* or *Aedes albopictus*.

1.3 The life cycle of the mosquito vectors

Understanding the life cycles of any vector is an important consideration in planning the control measures. For example, malaria was successfully eliminated in Europe following an understanding of the ecology of the Anopheles mosquito (Wilson et al., 2020). Both Anopheles and *Aedes* mosquitoes have similar stages of development (life cycle). To represent the vector life cycle, this section of the thesis will explain the life cycle of malaria vectors only (Figure 1).

Anopheles mosquitoes have four stages in their development namely eggs larvae, pupae, and adult, with the exception of the adult stage the rest are aquatic (WHO, 2013b). Adult Anopheles mosquitoes mate a few days after they emerge from the pupae. The sperm are stored in the spermatheca of the female mosquitoes and are enough to fertilize all the eggs during the female's lifetime (WHO, 2013b). Female mosquitoes feed on the different sources of glucose to obtain energy for dispersal and host-seeking as well as bite the host to obtain blood which contains nutrients necessary for egg development. Males mosquitoes do not bite instead they feed exclusively on plant liquids, including nectar, honeydew and fruit juices.

After taking a blood meal, 2-3 days later, female mosquitoes are ready to lay about 50-150 eggs during one oviposition. Eggs are boat-shaped and laid singly on the water surface which hatch into larvae within 2-3 days depending on the environmental conditions (WHO, 2013b).

The habitat for the larvae stages includes temporary or permanent groundwater bodies such as hoof-prints and rain pools to streams, swamps, canals, riverbeds, ponds, lakes, rice fields, and freshwater swamps (Paul et al., 2018). The larvae are surface feeders and feed on microorganisms, and decayed plant and animal material using filamentous mouth brushes. At a favorable condition the period from larvae to pupae takes about 8-10 days (Paul et al., 2018). The pupae that share the same habitat as larvae are comma-shaped lasting for 1-2 days (WHO, 2013b). The pupae do not feed and float on the surface of the water. Newly emerged adults rest on the surface of the water waiting for the wings to dry and further hardening of the body parts (WHO, 2013b). Adult mosquitoes live for approximately 3 weeks during which females may take a blood meal 4-7 times (WHO, 2013b).

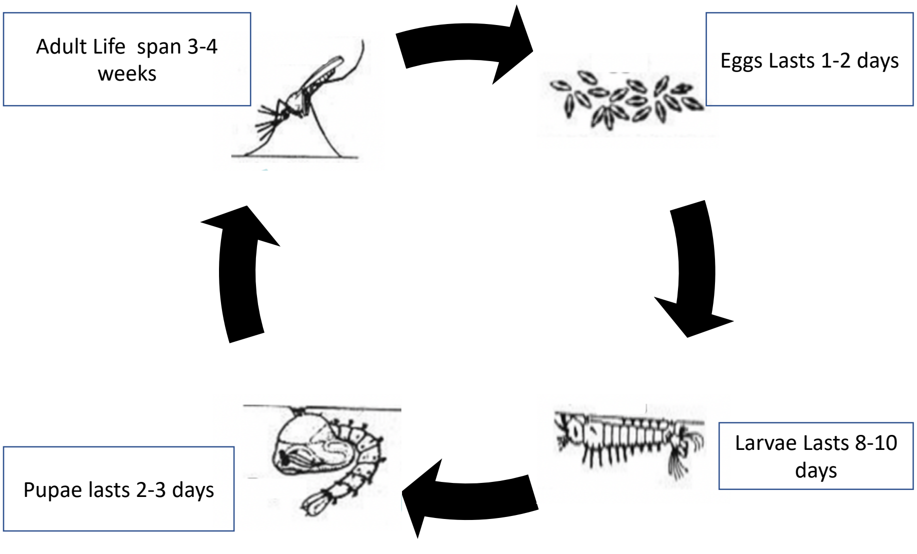

Figure 1.3: The life cycle of malaria vector. The adult mosquitoes lay eggs in water which hatches into larvae these develop into pupae then emerge as an adult. The adult female mosquitoes are found near the breeding site and not aquatic

1.4 Historical perspective of malaria vector control

As explained in the previous subsection vector control tool deployed against malaria mosquitoes may also affect *Aedes* mosquitoes, also in this section, I will focus only on Anopheles mosquitoes. The history of malaria vector control is shown in (Figure 1.4). During colonial times, malaria impaired the completion of various projects such as railway/road construction, hydroelectric power construction, and agricultural activities in several parts of the world including Brazil, the USA and Africa. In 1897, Ronald Ross began mosquito control after his discovery that malaria was transmitted by the bite of the female Anopheles mosquitoes (Panini M, 1923). Since then, the implementation of appropriate and effective vector control tools has become an integral component of the control of diseases transmitted by mosquitoes worldwide (Wilson et al., 2020). Historically, the control of malaria vectors may be divided into two phases namely non-insecticidal and insecticidal control methods.

1.4.1 Non–insecticide-based vector control methods

Vector control without using insecticides was the earliest effort implemented to fight against mosquitoes that transmits diseases to human. In the 1920s, several campaigns were implemented based on the ecological understanding of malaria vectors. At these times, extensive campaigns were done focusing on environmental management aiming at eliminating mosquito breeding sites (WHO, 1982). The mosquito control strategies were mainly implemented based on colonial interests (Griffing et al., 2015). For example, in Zambia copper belt mine, malaria was successfully controlled through the filling of ditches, house screening, modification of riverbanks, and oil application to open water bodies (Utzinger et al., 2002). Similar strategies were deployed during the exploration of the Tennessee Valley Authority for the production of hydroelectric power and agriculture activities in the USA as well as in the Sao Paulo railway construction and rubber industry in Brazil (Mukabana et al., 2002, Griffing et al., 2015). The control of malaria vectors using environmental management was very successful in Singapore (Watson M., 1921) and several other places in the world adopted a similar strategy in the fight against malaria disease (Wilson et al., 2020). However, malaria transmission resurged soon after these strategies were stopped (Nájera et al., 2011).

1.4.2 Insecticide-based vector control methods

The control of the larval stage of mosquitoes is the earliest method of vector control tool that used insecticides to combat malaria (Floore, 2006). In 1867, Paris green was already widely used in agriculture against beetles that destroyed potatoes in the United States of America (USA) (Cook, 1998, Floore, 2006). Due to its insecticidal effect on larvae of Anopheles mosquitoes, Paris green became the first insecticide to be used in the southeast USA and Italy for the control of malaria vectors in 1921 (Majori, 2012). It was however phased out due to the undesired side effects on non-targeted organisms such as fish, crabs as well as humans (Majori, 2012).

Concurrently, pyrethrum was in use for the control of adult mosquitoes in the 1930s, however, it was very unstable on sunlight exposure. World War II created the need for a new insecticide to control insects of public health importance following the massive suffering of soldiers in malaria-endemic areas. The invention of dichlorodiphenyltrichloroethane (DDT) insecticides by Paul Müller in 1940, renewed the use of insecticides for the control of malaria vectors (Casida and Quistad, 1998). Dichlorodiphenyltrichloroethane works by preventing the closure of the sodium-gated channel causing tremors and death on exposed mosquitoes. Five years after its discovery, DDT was widely used as an indoor-residual spray (IRS) targeting adult mosquitoes and successfully decreased the density of adult mosquitoes in Italy and the USA (Majori, 2012, Hays, 2000).

In 1955, the WHO recommended the use of DDT as an IRS to eliminate malaria through the Global Malaria Eradication Program (GMEP) (Sougoufara et al., 2017). While the use of DDT successfully eliminated malaria in some parts of the world such as those in high-income countries, it failed in low-income countries (African countries) due to the withdrawal of colonial powers (Wilson et al., 2020). The GMEP was phased out in 1969 as the result of undesired side effects of DDT on human ecology as well as the emergence of DDT-resistant mosquitoes, the (Berry-Cabán, 2011). In addition, the GMEP was halted due to a lack of funding as a result malaria control became the objective for each individual region. Between 1969 and the 1980s the responsibility of malaria control was left to individual countries.

Michael Elliot working at the Rothamsted Experimental Station, UK, discovered synthetic pyrethroids insecticides including permethrin, cypermethrin, and deltamethrin (Casida and Quistad, 1998). These chemicals were more stable compared to natural pyrethrum found in the daisy seed of *Tanacetum cineraria folium* (Asteraceae). This invention became very useful in agriculture, disease, and pest control. Concurrently, bed nets have been in use since ancient times in Egypt to protect individuals from mosquito bites (Wilson et al., 2020).

From 1970 to the 1980s, synthetic pyrethroids were the first insecticides used as IRS and coated on the net as insecticides treated net (ITNs) (Wilson et al., 2020). Insecticide-treated nets work by killing mosquitoes that contact the insecticide as well as protecting the person sleeping beneath the net as a physical barrier. Following promising findings from various studies conducted to determine the effect of ITNs on malaria prevalence, the WHO recommended the use of long-lasting insecticide-treated nets (LLINs) distributed after every 3 years. In 1990, LLINs were developed as cheap and safe tools to combat malaria. Because of their safeness on humans, pyrethroid insecticides have been the main insecticide class used in LLINs vector control (Zaim et al., 2000). Concurrently, between 1990-2000 funding was increased following the establishment of the agencies such as Roll Back Malaria (RBM), The Global Fund to Fight AIDS, Tuberculosis, and Malaria and the U.S. President's Malaria Initiative (PMI) that created the mechanism for resources mobilizing (Wilson et al., 2020). The invention of industrial insecticides impregnated net, long lasting insecticides treated nets (LLIN) coupled with the availability of funds, revolutionize the control of malaria strategies. In 2007, the WHO global malaria program (GMP) recommended that LLINs should be distributed to all community members for free or highly subsidized (WHO, 2020b). Over the last decade, LLINs and indoor residual spray has been the core vector control tool in different malaria-endemic areas (Bhatt et al., 2015).

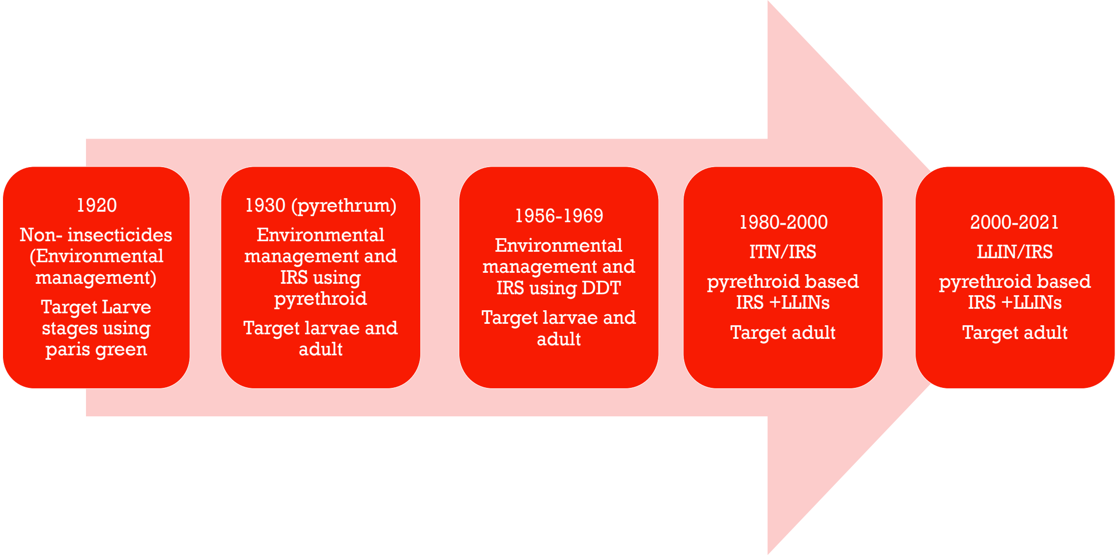

Figure 1.4 show the evolvement of strategies for vector control tools over the 100 years . This diagram is specifical for the vector of malaria.

1.5 Emerging of physiologically and behaviourally resistant mosquitoes against the current vector control tools

Despite well-planned vector control tools, malaria transmission has been found to persist even in settings where elimination was considered to be possible (Kleinschmidt, 2007). A recent resurgence of malaria transmission in endemic areas may be explained by several reasons including the development of physiological or behavioural resistance among mosquitoes.

Physiological resistance refers to the ability of the mosquitoes to survive an exposure to a lethal dose of insecticide (Ranson et al., 2011). Physiological resistance generally falls into two categories – metabolic and target site resistance (WHO, 2016c). Target site resistance is often caused by point mutations resulting in the transformation of insecticide binding sites, and thus the mosquito becomes less sensitive to that particular insecticide (Ranson et al., 2011). Metabolic resistance occurs when there is increased production of enzymes that break down insecticides. Another resistance mechanism known as cuticular resistance where the outer layer of the mosquitoes-cuticle is thickened and not allowing insecticides to pass through has been observed. Genes for resistance to the current insecticides used in vector control tools are widespread across different geographical locations in most malaria-endemic countries (Temu et al., 2012, Chouaibou et al., 2016). Resistance is developing because control applies selection pressure on malaria and arbovirus vectors. The use of pyrethroids in LLINs/ IRS and the exposure of mosquitoes to pyrethroids used in agriculture (Chouaibou et al., 2016) have contributed to the development of physiological resistance among malaria vectors. Resistance to compounds such as bendiocarb used for IRS is also emerging (Sande et al., 2015). Physiological resistance therefore greatly reduces the efficacy of IRS as a control tool while LLINs still provide some physical protection.

Before the implementation of the current vector control tools i.e. LLINs and IRS indoor, malaria transmission was mediated by high anthropophilic and endophilic *Anopheles gambiae* s.s. mosquitoes. These mosquitoes have a tendency to bite indoors and rest indoors with the biting peak between 23:00hrs and 02:00Hrs (Dambach et al., 2018). In response to the deployment of these tools which work inside the house behavioral resistance emerged where the mosquito avoids entering the house with insecticides. Recent data have shown a change in vector behavior following the introduction of malaria control interventions in certain locations (Moiroux et al., 2012, Govella and Ferguson, 2012, Reddy et al., 2011, Yohannes and Boelee, 2012, Wamae et al., 2015, Gatton et al., 2013, Sinka et al., 2016). For example, Matowo *et al*. showed that the optimal biting time of *An. arabiensis* mosquitoes coincide with when people are outside the house doing evening activities such as cooking, eating and story-telling (Matowo et al., 2016). These changes can include a change in species composition, change toward early evening and early morning biting, and outdoor resting and biting (Durnez and Coosemans, 2013, Matowo et al., 2015).

1.6 The role of volatile pyrethroid and odour baited trap to reduce mosquito borne diseases in peridomestic spaces

The most efficient vectors of malaria and arboviruses are strongly adapted to humans (synanthropic) and are therefore most commonly encountered around human dwellings either indoors (Bayoh et al., 2014) or in the peri-domestic space (Pollard et al., 2020). Peridomestic space refers to an extension of the verandah from the main house, a built-in space and a non-built-in space (Masalu et al., 2020). The primary vector control tools such as IRS and LLINs inside the house thus protecting mosquitoes that are biting inside leaving the peridomestic space unprotected. The role of unprotected peridomestic space for the ongoing malaria and arbovirus transmission has been very well explained (Carnevale and Manguin, 2021). Therefore, focusing on peridomestic spaces as an area for the delivery of vector control interventions against outdoor biting mosquitoes could help to close the gap in malaria and arbovirus transmission resulting from the inefficiency of the current vector control tools. Ideally, novel control interventions deployed in the peridomestic space should repel and kill mosquitoes to provide both personal and community protection for users and non-users (Magesa et al., 1991).

A potential tool that could help protect people in the peridomestic space is spatial repellents or/ odor-baited traps (Achee et al., 2012b, Johnson et al., 2017, Achee et al., 2012a). Volatile pyrethroids (VP) such as transfluthrin, metofluthrin, and allethrin are designed to release volatile chemicals into the air, creating a mosquito bite-free space (Achee et al., 2012a). Transfluthrin is a cheap pyrethroid spatial repellent, safe for humans (very low inhalation toxicity even after long-term exposure), and prevents mosquitoes from effectively locating a host (Ogoma et al., 2014b). For example, a field evaluation in Belize has shown that transfluthrin reduces house entry of *An. vestitipennis* by 60% (Wagman et al., 2015b) while Andres *et al.* showed a 69% reduction in *An. arabiensis* mosquito landings in Tanzania (Andrés et al., 2015). Findings from these experiments suggest that the efficacy of Volatile Pyrethroids (VP) to provide protection against mosquitoes in the peridomestic is very promising. Thus researchers have begun to investigate its efficacy in the peridomestic space but a lot more work is needed. In addition, robust methods/assays to evaluate these reported efficacies are needed.

Matowo *et al*., reported that mosquitoes that maintain malaria transmission in Kilombelo valley are resistant to traditional pyrethroids such as permethrin and deltamethrin (Matowo et al., 2017). Findings from the same area showed that transfluthrin-treated eave ribbon reduces mosquito landing rate (Mmbando et al., 2019). The explanation for this efficacy despite that transfluthrin belongs to the same class of insecticides (pyrethroid) could be that transfluthrin has a fluorine atom on the molecule interfering with mosquito detoxification mechanisms (Horstmann and Sonneck, 2016). While the results on the efficacy of transfluthrin against resistant mosquitoes are promising, the wide-scale presence of pyrethroid-resistant mosquitoes brings into question whether they can be realistically used as vector control tools. Transfluthrin has been tested on resistant mosquitoes, however, no studies have been conducted to determine if resistance in mosquitoes is detrimental to the efficacy of VP by directly comparing them to susceptible control. Due to the scarcity of susceptible mosquitoes in the field, the need to investigate this phenomenon in the SFS where peridomestic space can be simulated is necessary.

Odor-baited traps are designed to attract and kill or catch mosquitoes. Examples of these traps to target mosquito vectors include; the Suna trap evaluated in Kenya (Alexandra Hiscox, 2014), the Ifakara mosquito landing box in Tanzania (Matowo et al., 2016), the MM-X trap (Njiru et al., 2006) and BG sentinel trap (Krockel et al., 2006, Maciel-de-Freitas et al., 2006). The traps are commonly combined with odour lures that attract mosquitoes. Chemicals such as carbon dioxide, lactic acid, and 2- butanone simulate host odor and thus attract substantial numbers of disease-transmitting mosquitoes (Smallegange et al., 2011). A synthetic mosquito attractant, the Ifakara blend (IB), consisting of hydrous solutions of ammonia, L-lactic acid, aliphatic carboxylic acids, and carbon dioxide, was found to be more effective in attracting *An. gambiae s.l* compared to humans (Okumu et al., 2010). However, no wide-scale implementation of malaria control using trap alone, one study conducted in Kenya showed that Suna trap catching an average of 0.32 mosquitoes in a day was effective at reducing the prevalence of malaria in the intervention arm (Homan et al., 2016). Furthermore, odor-baited traps used alone may attract more mosquitoes into an area increasing bite to humans around.

It is known that VP may not kill mosquitoes when used alone meaning that mosquitoes may be disoriented or disarmed for some time (Denz et al., 2021). It is therefore suggested that volatile pyrethroid needs to be combined with odor baited trap. In agricultural pest-control strategies, the practice of repelling “push” insects from one area and attracting “pull” them to another area has been shown to be effective and consequently increases crop production (Cook et al., 2007). This strategy could be effective when deployed at the peridomestic area to control outdoor biting mosquitoes.

When VP and odor-baited trap are deployed together in a push-pull system, repellents ideally push mosquitoes from hosts to the pseudo host (odour-traps) that pull in and kill them. The two control methods may have a synergistic effect and models have predicted that the technology may reduce the entomological inoculation rate by 20-fold (Menger et al., 2015). For example, a semi-field evaluation using a spatial repellent and an odour-baited trap demonstrated up to 95% reduction in *An. gambiae* s.s. entering houses in Kenya (Menger et al., 2016). Another small-scale field evaluation of the combination of spatial repellent and BG malaria in Ifakara Tanzania showed a promising result in Ifakara by reducing human landing rate at the peridomestic space by more than 54% as compared to the control (Mmbando et al., 2019). Despite the increase in the frequency of arbovirus outbreaks, this control strategy has never been tested on *Aedes* mosquitoes. As the intervention can be deployed outside the house it may take control of both dengue and malaria mosquitoes.

1.7 Traps for the field evaluation of push pull control strategies

Previous studies have established that mosquito traps baited with odor lures that mimic human chemical attractants have the potential to be used as an alternative to human landing catches (HLC) for sampling mosquitoes (Williams et al., 2006, Tangena et al., 2015, Hawkes et al., 2017, Pombi et al., 2014). Although human landing catches continue to be performed in the field with wild mosquitoes (Mmbando et al., 2017), it is not ethically accepted due to the presence of potentially infected mosquitoes and may not apply where there is arboviruses outbreak thus odor baited traps become critical. Even if traps do not reflect the exact number of mosquitoes caught by HLC, for those that consistently catch lower or higher than HLC, correction factors can be used to obtain estimated counts. Similarly, for the testing of vector control tools in the field such as “push-pull”, traps do not necessarily have to catch exactly the same number of mosquitoes as HLC; however, it is vital that they accurately reflect the impact of the vector control intervention. While significant attention has been given to the use of odour baited trap for mosquitoes sampling, also known as the “pull” component, a significant gap remains in our understanding of how the odour-baited trap work in the presence of human volunteer and behavior-modifying compounds such as repellents (Okumu et al., 2009). If, as in the case of Okumu *et al.,* (Okumu et al., 2009), the repellent increases the attractiveness of the odour lure, then it will give an inaccurate picture of the efficacy of the repellent to reducing human–vector contact. There is an urgent need to understand how odor-baited traps work against *Aedes aegypti* mosquitoes in the presence of behavior-modifying compounds.

Owing to the increase in mosquitoes that are biting outdoors coupled with resistant mosquitoes, this thesis focuses mainly on the evaluation of push-pull technology using OBT and VP individually or in combination to reduce human landing rate in a simulated peridomestic space (Figure 1.5). The experiments in this thesis were conducted in the semi-field system as it was easier to use disease-free and laboratory-reared *Aedes aegypti* mosquitoes, and susceptible and resistant malaria vectors to allow comparison. This thesis contributes to the use of a volatile transfluthrin emanator as a “push” technology in the form of a freestanding transfluthrin emanator (FTPE) for the control of outdoor biting mosquitoes. This emanator is made up of aluminum material and measures 45cm high and 35cm in diameter attached inside are hessian strips from the *Corchorus olitorius* plant. This device is portable, does not need electricity or heat, and does not produce smoke during its operation which increases compliance to the user. The results of this work is presented in four manuscripts that have been published in scientific review journals.

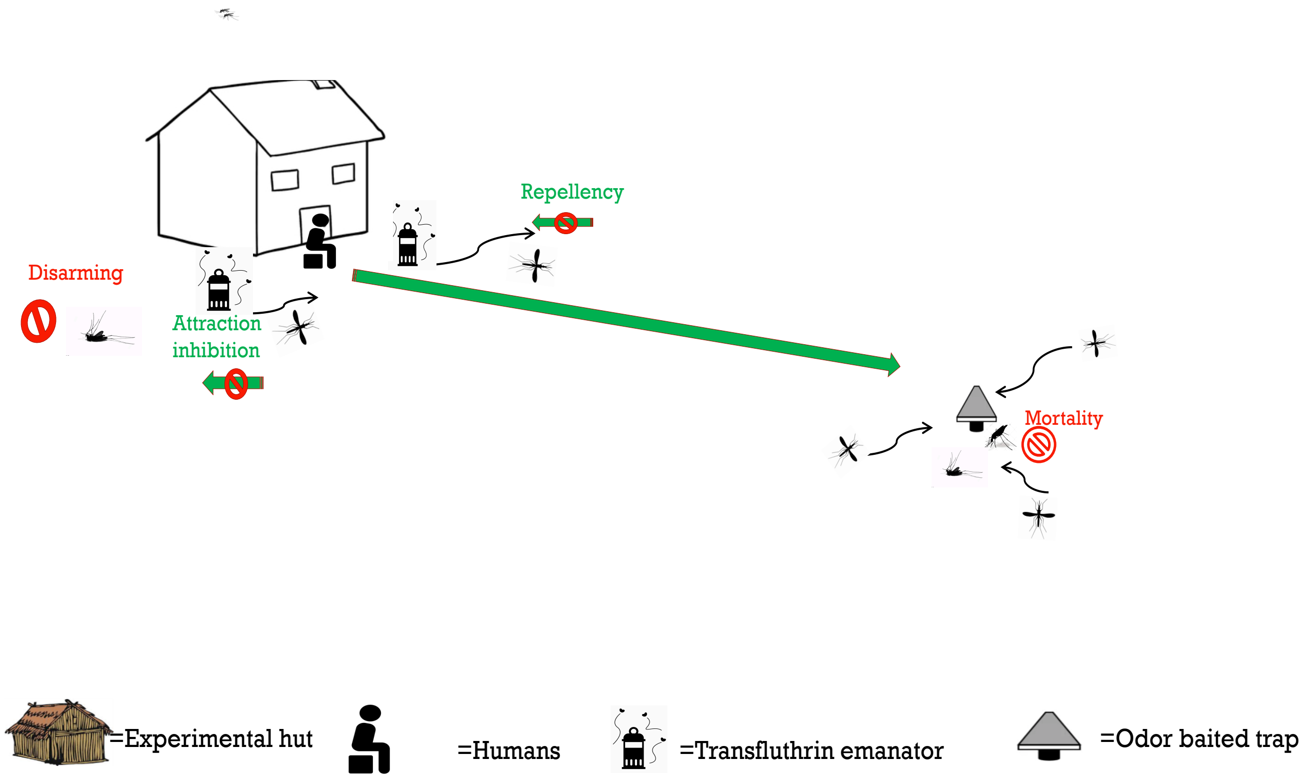

Figure 1.5. showing the concept of push-pull control strategy deployed in the peridomestic space where various activities are conducted before people are going inside the house where are protected. The peridomestic space is an appropriate place to deploy transfluthrin emanator as it has proved to produce various behavioral responses on exposed mosquitoes such as disarming (inability to bite until the next day), attraction inhibition, repellency(move away) and mortality (death 24 hours after exposure)

1.7 Thesis Aim and Objectives

The aim of this dissertation was to contribute to the understanding of the use of odour baited trap and volatile pyrethroid and their mode of action for the control of outdoor biting mosquitoes

Specific objectives include:

1. Comparison between an exposure-free Mosquito Electrocuting Trap (MET), Human Landing Catches (HLC) and BG sentinel trap (BGS) for evaluation of spatial repellents against *Aedes aegypti* in a semi-field system.
2. Semi-field evaluation of freestanding transfluthrin passive emanators and the BG sentinel trap as a "push-pull control strategy" against *Aedes aegypti* mosquitoes
3. Evaluate the efficacy of Transfluthrin Eave Positioned Targeted Insecticide (EPTI) to reduce the human landing rate of pyrethroid-resistant and susceptible malaria vectors in simulated peridomestic space of a semi-field system.
4. Human landing catches (HLC) provide a useful measure of Protective Efficacy for evaluating volatile pyrethroid spatial repellents (VPSR)
5. Semi-field system and experimental huts bioassays for the evaluation of spatial (and topical) repellents for indoor and outdoor use

Chapter 2: Semi-field evaluation of the exposure-free mosquito electrocuting trap and BG-Sentinel trap as alternative to the human landing catch for measuring the efficacy of transfluthrin emanators against *Aedes aegypti*

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2.1 Abstract

Background

The human landing catch (HLC) measures human exposure to mosquito bites and evaluates the efficacy of vector control tools. However, it may expose volunteers to potentially infected mosquitoes. The mosquito electrocuting trap (MET) and BG-Sentinel traps (BGS) represent alternative, exposure-free methods for sampling host-seeking mosquitoes. This study investigates whether these methods can be effectively used as alternatives to HLC for measuring the efficacy transfluthrin against *Aedes aegypti.*

Methods

The protective efficacy (PE) of freestanding passive transfluthrin emanators (FTPEs), measured by HLC, MET and BGS were compared in no-choice and choice tests. The methods were located 2 m from an experimental hut with FTPEs positioned 3 m on either side of them. For the choice experiment, a competitor HLC was included 10 m from the first collection point. One hundred laboratory-reared *Ae. aegypti* mosquitoes were released and collected for three consecutive hours.

Results

In the no-choice test, each method measured similar PE; HLC: 66% (95% confidence interval [CI]: 50–82), MET: 55% (95% CI: 48–63) and BGS: 64% (95% CI: 54–73). The proportion of mosquitoes recaptured was consistent between methods (20–24%) in treatment and varied (47–71%) in the control. However, in choice tests, the PE measured by each method varied; HLC: 37% (95% CI: 25–50%), MET: 76% (95% CI: 61–92) and BGS trap: 0% (95% CI: 0–100). Recaptured mosquitoes were no longer consistent between methods in treatment (2–26%) and remained variable in the control (7–42%). FTPE provided 50% PE to the second HLC 10 m away. In the control, the MET and the BGS were less efficacious in collecting mosquitoes in the presence of a second HLC.

Conclusions

Measurement of the PE in isolation was fairly consistent for HLC, MET and BGS. Because HLC is not advisable, it is reasonable to use either MET or BGS as a proxy for HLC for testing volatile pyrethroid (VP) in areas of active arboviruses endemic areas. The presence of a human host in close proximity invalidated the PE estimates from BGS and METs. Findings also indicated that transfluthrin can protect multiple people in the peridomestic area and that at short-range mosquitoes select humans over the BGS.

Keywords: mosquito electrocuting trap, human landing catches, BG-Sentinel, spatial repellent, *Ae. aegypti*

2.2 Background

Aedes aegypti (*Ae. aegypti*) and *Aedes albopictus* (*Ae. albopictus*) mosquitoes are responsible for the transmission of human arboviruses including dengue, yellow fever, chikungunya and zika viruses (Mboera et al., 2016, Gould et al., 2008, Wikan and Smith, 2016, WHO, 2009a). These mosquitos are well adapted to living in urban areas and bite during the daytime. The main vector control strategies deployed against *Aedes* vectors are larval source reduction, indoor residual spraying and space spraying (Wilson et al., 2020, WHO, 2009a). However, these control tools are labour intensive, costly and difficult to implement considering that *Ae. aegypti* mosquitoes can breed or rest in a wide range of small, transient and often cryptic places (WHO, 2009a). While these vector control approaches are useful, simpler and more cost-effective control strategies against *Ae. aegypti* mosquitoes are urgently needed due to the increased frequency of epidemics and the geographical spread of a number of arboviruses (Leta et al., 2018). Promising new strategies, including oviposition traps, transgenic mosquitoes, volatile pyrethroids (VP) and the use of *Wolbachia* spp., are currently under evaluation (Achee et al., 2012a, Ogoma et al., 2012b, Kamtchum-Tatuene et al., 2017).

The impact of new vector control strategies is measured through entomological indicators, including vector density and human exposure to mosquito bites (Schoeler et al., 2004, Naranjo-Diaz et al., 2013, Petrić et al., 2014), which have often been measured through human landing catch (HLC) (Sukkanon et al., 2021). Human landing catch is the gold standard measure of human–vector exposure whereby, using an aspirator, human volunteers collect host-seeking mosquitoes that land on the volunteers' exposed legs (Gimnig et al., 2013). The numbers of mosquitoes caught (the human landing rate) approximate the number of mosquitoes that would bite one person at a particular time and place (Schoeler et al., 2004, Briët et al., 2015). This is a simple method and a direct measure of human–vector contact for both indoor- and outdoor-biting mosquitoes.

However, ethical and technical concerns arise when HLC is performed in disease-endemic areas. Considering that no prophylaxis or vaccine is available for most arboviral diseases, with the exception of yellow fever, putting the catcher at risk of contracting an arboviral disease (Liang et al., 2016). Furthermore, differences in skills and motivation of the collectors may also introduce variation into the collected data. Human landing catches is often performed over several hours, so the quality of data obtained may decline over time as the collectors may get tired or lose concentration. These technical drawbacks can be improved through proper training and supervision of the collectors, but are unlikely to be eliminated. Thus, it might be difficult to standardize data collected through this method by different research institutions.

Previous studies have established that mosquito traps baited with odor lures that mimic human chemical attractants have the potential to be used as an alternative to HLC for sampling mosquitoes (Williams et al., 2006, Tangena et al., 2015, Hawkes et al., 2017, Pombi et al., 2014). Estimating human–mosquito contact accurately is vital for studies aiming to determine the disease risk of a certain area by calculating the entomological inoculation rate. Even if traps do not reflect the exact number of mosquitoes caught by HLC, for those that consistently catch lower or higher than HLC, correction factors can be used to obtain estimated counts. Similarly, for the testing of vector control tools, traps do not necessarily have to catch exactly the same number of mosquitoes as HLC; however, it is vital that they accurately reflect the impact of the vector control intervention. Several odor-baited traps have demonstrated to be an appropriate alternative to HLC for measuring mosquito densities of various species such as Anopheles and *Aedes* (Tangena et al., 2015, Hawkes et al., 2017). Knowledge of whether the presence in the environment of behaviour-modifying compounds such as repellents affects the relative efficacy of odor-baited lures is limited (Okumu et al., 2009). If, as in the case of Okumu et al (Okumu et al., 2009), the repellent increases the attractiveness of the odor lure, then it will give an inaccurate picture of the efficacy of the repellent in reducing human–vector contact.

Furthermore, when traps are used in the field, competing sources of host odor are present. It is therefore important to determine whether mosquitoes may be diverted from traps to other hosts and whether this diversion is exacerbated by the presence of a spatial repellent. It is not unforeseeable that if an odor lure is already weaker than a human at attracting mosquitoes (Krockel et al., 2006), then introducing a spatial repellent will mean any mosquitoes still host-seeking to go towards the stronger pull of the human. If this is the case, then the traps using odor lures may overestimate the efficacy of the spatial repellent. Traps with a human lure, such as the MET or the human-baited double net trap may therefore provide a more accurate measure of the efficacy of a spatial repellent (Govella et al., 2016, Maliti et al., 2015, Tangena et al., 2015).

While an enormous body of knowledge is available on the comparison of trap efficiency, no information is available on whether exposure-free methods (METs and the BGS trap) are suitable for testing spatial repellents such as volatile pyrethroids (VP). Therefore, the present study investigates three trapping methods — HLC, BGS and MET — for their ability to measure the protection provided by the VP transfluthrin against bites from *Ae. aegypti* mosquitoes and whether an alternative host can affect this protection. Two experiments were performed: (1) a no-choice experiment in which protective efficacy (PE) was measured with the traps used in isolation and (2) a choice test in which protective efficacy was measured with the traps used in the presence of a HLC.

2.3 Methods

Study Site

The experiment was conducted in the semi-field system (SFS) located in Bagamoyo, Tanzania, from January to June 2019. The SFS consists of large screened compartments that allow controlled experiments with disease-free laboratory-reared mosquitoes to be safely conducted under ambient climatic conditions (Ferguson et al., 2008). Experiments can be replicated within a short period of time by releasing the same number of laboratory-reared mosquitoes each time without bias introduced by natural daily heterogeneity in mosquito numbers that normally occurs in the field. The SFS is divided into two equal compartments, each measuring 9 m × 21 m (Fig. 2.1a &2.1b), which were used for the experiment with a middle corridor acting as a buffer. The walls of the middle corridor are made from heavy-duty polyethylene, thus preventing airflow between the chambers. This allowed the independent evaluation of the traps in the presence or absence of a spatial repellent to be conducted simultaneously. The mean temperature and relative humidity were 24 °C and 83%, respectively.

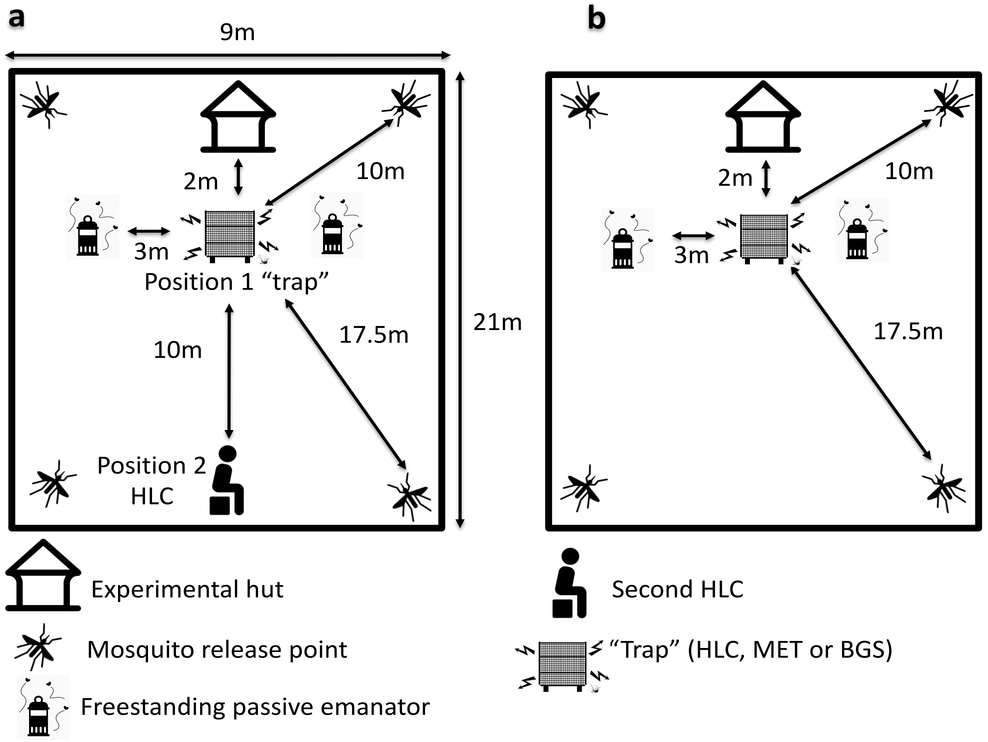


Figure 2.1. Experiment setup in the SFS (a) Schematic representation of the choice experiment with the HLC competitor. (b) Schematic representation of the no-choice experiment without the HLC competitor. To mimic outdoor conditions, the experiment was conducted outside the experimental hut fixed inside the compartment of the semi-field system. Shown at the corners are mosquito release points.

Mosquitoes

The experiments used laboratory-reared *Ae. aegypti* mosquitoes (Bagamoyo strain) originally colonized from Bagamoyo, Tanzania, and maintained at the Bagamoyo branch of the Ifakara Health Institute (IHI) since December 2015. The mosquitoes are susceptible to all classes of insecticides. The colony larvae were fed on Tropical fish flakes® until pupation, after which they were transferred to emergence bowls inside a 30 cm × 30 cm × 30 cm cage. The adult colony was fed on glucose 10% ad libitum, and cattle blood meals were given to adult females using membrane feeding for egg production on days 3, 6 and 9. The colony is maintained approximately at 12:12 (light:dark) natural light, 27 ± 2 °C and 80 ± 20% relative humidity.

For the purpose of this experiment, 3- to 8-day-old nulliparous female mosquitoes were used. Three cages of mosquitoes were sugar-starved for 12 hours before the experiments started. Two hours before the experiment started, active probing female mosquitoes were selected from the cages to ensure that only avid and fit mosquitoes were used. The mosquitoes were transferred to the SFS in smaller holding cages (10 cm × 10 cm × 10 cm with 25 mosquitoes each).

Collection methods

Human landing catches

Four male volunteers aged 25–35 years, experienced in conducting HLC, were recruited upon informed consent. Because observation in previous experiments showed that *Ae. aegypti* bite all over the body, volunteers were covered with net jackets to prevent bites on areas where HLC was not taking place (Fig. 2.2a). For HLC, the volunteers sat on chairs exposing their legs between the ankle and knee (Fig. 2.2a), aspirated any mosquito that landed, and gently expelled the mosquito into a paper cup.

Figure 2.2 Collection methods and FTPE used in the study (a) A volunteer conducting HLC. (b) A volunteer sitting on the chair with his leg inserted in an MET. (c) A BG-Sentinel trap with the battery and silicon tube supplying CO_2. (d) An FTPE device as a source of transfluthrin

Mosquito-electrocuting trap (MET)

Previous experiments have demonstrated that METs could be used for sampling Anopheles (Govella et al., 2016) and *Aedes* mosquitoes (Ortega-López et al., 2020). The MET consists of an electric grid and a power supply box. The electric grid is made up of four panels, each measuring 30 cm × 30 cm, in a square frame (Fig. 2.2b). Participants (in the present study, the same as those recruited for HLC) put their legs within the frame (in a similar fashion as for HLC), and host-seeking mosquitoes approaching the participants are intercepted and killed by the grids. In this way, participants are protected from mosquito bites and, consequently, from exposure to mosquito-borne infection. Preliminary testing of optimal voltage for electrocution of *Ae. aegypti* identified that 680 V is sufficient to kill mosquitoes (with the specimen remaining intact) without causing harm in accidental contact with the volunteer. The trap is designed such that electrocution occurs when a mosquito touches the two parallel wires of the electric grid (Maliti et al., 2015).

BG-Sentinel (BGS) trap

The BGS trap (Biogents AG, Regensburg, Germany) has been widely used as the standard trap for collection of adult *Aedes* mosquitoes (Krockel et al., 2006, Schoeler et al., 2004). It is used together with the BG-Lure (Figure 2.2c), a synthetic lure consisting of lactic acid, caproic acid, and ammonium bicarbonate dispensed via granules in the specified channel (Krockel et al., 2006). Despite being effective for five months, a new lure was used for each experimental round of eight days. Carbon dioxide was released from a pressurized cylinder at the rate of 500 mL/min, using an acrylic gas flow meter (Hangzhou Darhor Technology Co., Limited, China). The operation of the BGS trap has been explained elsewhere (Li et al., 2016, Maciel-de-Freitas et al., 2006).

Preparation of freestanding transfluthrin passive emanator (FTPE)

Previous work showed that transfluthrin freestanding passive emanators (FTPEs) used under simulated outdoor conditions could significantly reduce the human landing rate of *Ae. aegypti* (Tambwe et al., 2020). This device is a stool-like structure that supports hessian strips (made from plants of the species *Corchorus olitorius* or *C. capsularis*, also called jute, burlap or gunnysacks (Fig. 2d). The hessian strips were made from hessian sacks purchased locally, washed using well water and powder detergent (OMO®, Unilever, Nairobi, Kenya) and dried under direct sunlight. They were then cut into strips measuring 5 m × 10 cm and treated with

5.25 g of transfluthrin emulsified concentrate (EC; Bayothrin EC, Bayer AG, Monheim am Rhein, Germany). Two FTPEs with a total of 10.50 g (5.25 g each) of transfluthrin were used per experiment.

Experimental procedure

Experiments were conducted to compare the protective efficacies of HLC, MET, and BGS traps under no-choice (traps alone) and choice (with additional HLC). In the choice assay (Fig. 1), one type of sampling trap was allocated between the two chambers of the SFS with one as treatment and the other as control, and experimentation was conducted for 8 consecutive days before switching to another type of trap. The treated and untreated emanators once assigned in a particular chamber (whether treatment or control), remained there for four consecutive experimental days and were then exchanged between the chambers to minimize the potential bias between chambers which could arise due to variation in wind direction. In the treatment chamber, two treated FTPEs were placed 3m apart side-by-side of the trapping method while in the control similar fashion of placement of untreated FTPEs around the trap was employed (Fig. 1a & 1b). Two volunteers between the two chambers exchanged positions after every experimental day to account for potential bias due to differential attractiveness to mosquitoes between individuals (Lindsay et al., 1993).

The collection methods were conducted 2 m from an experimental hut inside the SFS to simulate an outdoor peridomestic setting (Fig. 1a and 1b). Experiments were conducted between 06:30 and 09:30 every day to reflect natural *Ae. aegypti* biting time. The experiment started when the volunteer sat on the chair and simultaneously released 100 mosquitoes (from four holding cages, each with 25 mosquitoes) per chamber on a signal from the team supervisor. HLC and MET collections were done continuously for 50 minutes with a 10-minute break at the end of each hour (WHO, 2013a). During the break, the MET was switched off to allow the collection of mosquitoes that had been trapped between stainless steel wires or had fallen on the ground due to electrocution. Because opening and closing the door for volunteers to break outside the SFS would cause mosquitoes to escape, during this time, volunteers remained inside with their trousers unfolded to restrict mosquito bites. For the same reason, the BGS trap was emptied after three hours. Collected mosquitoes were kept in waxed paper cups with net lids, labeled with time, date, and method of collection, and then transported to the insectary for counting and recording. After each experimental day, a thorough search within the SFS chambers were conducted, and all mosquitoes that were not recaptured were aspirated using a prokopack aspirator to avoid contamination of the subsequent replicates.

Under the "choice assay, or "competitive experimental assay" similar experimental procedures and trapping types as above were employed, with the exception that in addition to a trapping type assigned to a particular experimental day, a volunteer conducting HLC was added, and positioned 10 m away from FTPE in each chamber (Figure 2.1b). This was done to simulate the competition for mosquitoes that could happen when these collection methods are in the field. This setup also enabled the determination of whether mosquitoes in the presence of transfluthrin were diverted to the unprotected volunteer performing HLC and detect whether there was an increase in biting compared to the control

Sample size

Sample-size calculations were performed using simulation-based power analysis (Johnson et al., 2015) in R statistical software version 3.3 http://www.r-project.org with a significance level of 0.05 for rejecting the null hypothesis. The power to predict the 15% difference in mosquito landings between HLC, MET and BGS trap was estimated as the proportion of the 1000 simulated data sets in which the null hypothesis was rejected when the generalized linear mixed model (GLMM) was run. Inter-observational variance among daily experiments (0.5) was adapted from a previous study conducted in the semi-field. With our experimental design and a predicted 60% recapture of released mosquitoes by HLC in the control (reference method), there was 98% power to detect a difference.

Data analysis

Analyses of data were carried out in Stata 13 (StataCorp). Hourly data were collapsed to give the total of mosquitoes caught per replicate so that data for all three methods were comparable (additional file 1). Data analyses for the choice and no-choice experiments were performed separately. The mean percentage of recapture and confidence intervals (CI) were calculated for each collection method in the treatment and control in the no-choice and choice scenarios. The overall arithmetic mean PE and 95% CI for the experiment were calculated from the daily PE, which was measured by comparing the human landing rate on a volunteer with the intervention to the negative control using the following formula:

Protective efficacy = $[(C - T)/C] \times 100\%$,

where C stands for the number of mosquitoes landing in the control and T is the number of mosquitoes landing in the treatment.

Three generalised linear mixed models (GLMMs) with a binomial distribution with logit link were used to determine the following: (1) the ability of the traps to measure the protection conferred by the FTPE in a no-choice experiment, (2) the ability of the traps to measure the protection provided by the FTPE in a choice experiment and (3) the difference in the proportion of recaptured mosquitoes by HLC in position 2 (competitor HLC) when HLC was used. This allowed ascertaining of whether there was any diversion from the HLC in position 1 to the competitor HLC caused by the transfluthrin. Diversion was defined as the movement of mosquitoes from the HLC in position 1 to the HLC in position 2. The diversion of mosquitoes was evaluated by the odds of recapturing a mosquito in the competitor HLC (position 2) relative to position 1 for HLC only.

In all models, the independent variables included as fixed categorical effects were collection method, treatment (FTPE or control), temperature, and humidity, with an experimental day as a random effect. An interaction term between treatment and collection methods was introduced to determine if the reduction in landing caused by the VP was measured differently by the collection method.

Relative trap efficacy, that is, the ratio of mosquitoes recaptured in each trap relative to HLC, was calculated for the choice and no-choice experiments for both the transfluthrin and the control.

2.4 Results

Traps and HLC measure similar protective effects of transfluthrin in the no-choice test

When HLC, MET or BGS were used to collect mosquitoes with FTPE placed at 3 m on both sides of the collection method, approximately 22% of the mosquitoes were collected (MET: odds ratio [OR] 0.82 [95% CI: 0.69–1.14], *P* = 0.245; BGS: OR: 0.89 [95% CI: 0.64–1.24], *P* = 0.490; Table 1). In the control, similar proportions of mosquitoes (over 60%) were recaptured using HLC and BGS traps, although the MET showed lower trapping efficacy relative to HLC (MET: OR: 0.34 [0.25–0.46], *P* < 0.001; BGS: OR: 0.61 [95% CI: 0.45–0.83], *P* = 0.002; Table 1). This meant that while all collection methods measured that the FTPEs reduced mosquito landings, the reduction measured with the MET (OR: 0.29 [95% CI: 0.24–0.37], *P* < 0.001) was less than that measured with the BGS trap (OR: 0.18 [95% CI: 0.18–0.23], *P* < 0.001) and with HLC (OR: 0.12 [95% CI: 0.09–0.15], *P* < 0.001). The results of the interaction between collection method and treatment indicated that these differences were significant for MET (OR: 2.4 [95% CI: 1.75–3.03], *P* < 0.001) and for BGS (OR: 1.45 [95% CI: 1.05–1.98], *P* = 0.022).

When the protection provided by the FTPE was calculated using the PE, which is not adjusted for other sources of variation (such as location, day, and volunteer), all the collection methods measured a similar PE of approximately 60% (MET: 55% [95% CI: 48–63], HLC: 66% [95% CI: 50–82]; BGS: 64% [95% CI: 54–73]; Table 2)

Traps and HLC did not measure similar protective effects of transfluthrin in the choice test

In choice tests, the combined number of mosquitoes recaptured by both recapture methods was higher than a single trap in the no-choice tests however the presence of a second human substantially reduced mosquito numbers caught in all of the collection methods at position 1. In the treatment, 208 (52%), 22 (9%), and 38 (12%) mosquitoes were recaptured by HLC, MET and BGS trap, respectively; in the control, the corresponding numbers were HLC: 335 (47%), MET: 96 (20%) and BGS: 53 (9%; Table 1). In the treatment, the model showed that HLC in position 1 recaptured a significantly higher proportion of *Aedes* mosquitoes than either MET or BGS trap, MET [OR: 0.07 (95% CI: 0.04–0.13), $P < 0.0001$]; BGS [OR: 0.05 (95% CI: 0.02–0.13), $P < 0.0001$] (Table 2.1). A similar trend was observed when the trap performances were compared with the control MET [OR: 0.18 (95% CI: 0.13–0.25), $P < 0.0001$]; BGS [OR: 0.09 (95% CI: 0.05–0.15), $P < 0.0001$] (Table 1). When HLC is a reference, the model showed a significant interaction between HLC and MET but not with HLC and BGS. This indicated that the reduction in landing caused by the FTPE as measured by HLC and the BGS trap was not significantly different (OR: 1.44 [95% CI: 0.89–2.33], $P = 0.13$) but that of the MET measured higher protection than HLC (OR: 0.42 [95% CI: 0.25–0.71], $P < 0.001$). Significant reduction in the odds of landing of *Ae. aegypti* was observed using HLC (OR: 0.49 [95% CI: 0.39–0.60], $P < 0.001$), while MET measured slightly higher protection (OR: 0.20 [95% CI: 0.13–0.33], $P < 0.001$); protection could not be measured for the BGS trap as the confidence interval crossed 1 (OR: 0.70 [95% CI: 0.45–1.08], $P < 0.105$; Table 2.2).

When assessing the impact of the FTPE using PE, HLC in position 1 measured a PE of 37.2% (95% CI: 25.0–49.5), the MET overestimated PE at 75% (95% CI: 60.5–91.5) while the PE estimate for BGS was not measurable due to low attraction to the BGS trap (PE 0% (95% CI: 0–99.5); Table 2).

The proportion of recaptured mosquitoes for the second HLC sitting in position 2, located 10 m away in the treatment, ranged from 24% to 34%, whereas in the control the proportion of recaptured mosquitoes was 47% to 71% for all traps used. This means that FTPEs provided a consistent protection of about 50% to the second HLC sitting in position 2 independent of which mosquito collection method, HLC, MET or BGS, was used in position 1 (Table 2.2).

No evidence of mosquito diversion from HLC position 1 to HLC in position 2 at 10 metres in the presence of transfluthrin

Mosquito diversion was assessed from the relative proportion caught by HLC in position one, 3 m from the FTPE, and position two, 10 m from the FTPE, in the treatment. There was no diversion of mosquitoes from the HLC in position one to the HLC in position two in the presence of FTPE (OR: 0.87 [95% CI: 0.66–1.15], *P* = 0.324; Table 2.3). In both positions one and two, HLC captured similar proportions of mosquitoes in the presence of FTPE and of control, at a ratio of approximately 1:1 (Table 2.2).

Evidence that the presence of a human at 10 metres attracts all mosquitoes away from BGS trap and MET

In the presence of either FTPE or control, the relative recapture by the HLC in position 2 was higher in the presence of a BGS trap or MET (Table 2.4). In the control, it was observed that the HLC in position two caught 1.5 times more mosquitoes 565 (91%) in the presence of the BGS trap than in the presence of HLC 376 (53%); OR: 3.37 (95% CI: 2.35–4.85], *P* < 0.0001). A similar but less pronounced trend was observed in the presence of transfluthrin, with the HLC in position two receiving 1.39 times more mosquitoes than if a second HLC was being conducted 269 (88%) with BGS and 194 (48%) with HLC; OR=1.63 [95% CI: 0.79–3.34], *P* = 0.184; Table 4). With MET, more mosquitoes were recaptured by HLC than by the MET, but the number caught by HLC did not increase in either the control (OR: 1.06 [95% CI: 0.85–1.33], *P* = 0.593) or the treatment (OR: 1.15 [95% CI: 0.72–1.84], *P* = 0.547). Because recaptures did not increase using HLC, the lower proportion of mosquitoes recaptured by the MET is likely due to lower trapping efficiency, whereas humans were clearly more attractive than the BGS trap.

Relative trap efficiency in the absence of transfluthrin and competitor

In the experiments with the no-choice setup, in the control, it is possible to calculate the relative trapping efficiencies expected if the traps were used for monitoring human exposure to the *Ae. aegypti* and if no other kairomones were present. Both the MET and the BGS trap collected fewer mosquitoes than HLC. The recapture measured with the MET (OR: 0.34 [95% CI: 0.25–0.46], *P* < 0.001) and the BGS trap (OR: 0.61 [95% CI: 0.45–0.83], *P* = 0.002) differed from that of HLC. The MET collected approximately 37% fewer mosquitoes, and the BGS trap collected approximately 15% fewer mosquitoes (Table 2.1). Based on this data, for mosquito sampling as a measure of relative human exposure to *Ae. aegypti*, the BGS trap and the MET can be used with correction factors of 1.6 and 1.2, respectively.

Table 2.1. The relative trapping efficacy of human landing catch (HLC), mosquito-electrocuting traps (METs) and BG-Sentinel (BGS) traps evaluated individually in a no-choice test and in competition with HLC in a choice test, in the presence of two freestanding transfluthrin passive emanators (treatment) and with no transfluthrin (control)

Transfluthrin				Control					
HLC recapture position 2 (% recaptured)	Relative trapping efficacy[a]	OR (95% CI)[b]	*P*-value	Total recapture (% released)	"Trap" recapture position 1 (% recaptured)	HLC recapture position 2 (% recaptured)	Relative trapping efficacy[a]	OR (95% CI)[b]	*P*-value
–	1	1	–		571 (71)	–	1	1	–
–	0.85	0.82 (0.69–1.14)	0.245		372 (47)	–	0.63	0.34 (0.25–0.46)	< 0.001
–	0.91	0.89 (0.64–1.24)	0.490		487 (61)	–	0.85	0.61 (0.45–0.83)	0.002
194 (24)	1	1	–	711 (89)	335 (42)	376 (47)	1	1	–
210 (26)	0.11	0.07 (0.04–0.13)	< 0.001	480 (60)	96 (12)	384 (48)	0.29	0.18 (0.13–0.25)	< 0.001
269 (34)	0.18	0.05 (0.02–0.13)	< 0.001	618 (77)	53 (7)	565 (71)	0.16	0.09 (0.05–0.15)	< 0.001

The average proportion of released mosquitoes that were recaptured in the transfluthrin and control arm is presented as well as the total number of mosquitoes recaptured by each method out of 800 *Aedes aegypti* mosquitoes released over 8 replicates. [a]Relative trapping efficacy of METs and BGS traps is compared to HLC in no-choice and choice tests in both the treatment and the control arms (both in position 1). [b]The odds ratio (OR) estimates were derived from Stata output adjusted for temperature and humidity and presented with a 95% confidence interval.

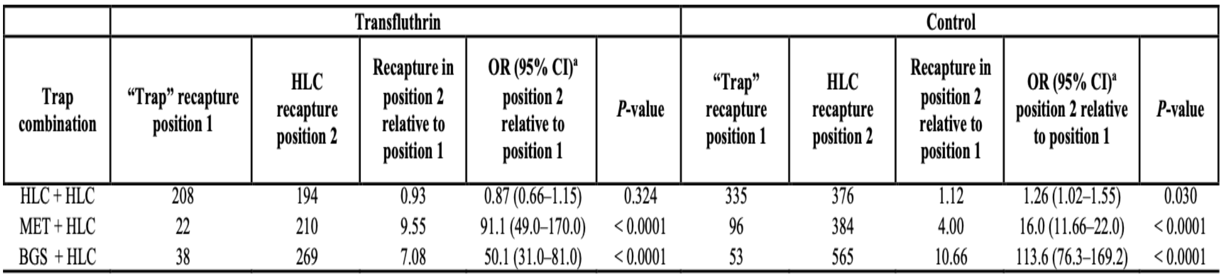
Table 2.2 The protective efficacy of freestanding transfluthrin passive emanators (FTPEs) measured using human landing catch (HLC), mosquito-electrocuting traps (METs) and BG-Sentinel (BGS) traps evaluated individually in a no-choice test and in competition with HLC in a choice test, in the presence of two FTPEs (treatment) and with no transfluthrin (control).

	Position 1					Position 2				
Trap Combination	% recapture Control (95% CI)	% recapture Treatment (95% CI)	%Protective efficacy (95% CI)	OR (95% CI)[a] treatment relative to the control	*P*-value	% recapture Control (95% CI)	% recapture Treatment (95% CI)	Protective efficacy (95% CI)	OR (95% CI)[a] treatment relative to the control	*P*-value
No-Choice										
HLC	71 (63–80)	24 (14–34)	66 (50–82)	0.12 (0.09–0.15)	< 0.0001					
MET	47 (41–52)	20 (18–23)	55 (48–63)	0.29 (0.24–0.37)	< 0.0001					
BGS	61 (53–68)	22 (17–27)	64 (54–73)	0.18 (0.15–0.23)	< 0.0001					
Choice										
HLC + HLC	42 (39–45)	26 (22–30)	37 (25–50)	0.49 (0.39–0.60)	< 0.0001	47 (43–51)	24 (14–34)	49 (30–68)	0.35 (0.28–0.43)	< 0.0001
MET + HLC	12 (7–17)	2 (1–4)	76 (61–92)	0.20 (0.13–0.33)	< 0.0001	48 (42–54)	26 (21–32)	44 (31–57)	0.38 (0.31–0.47)	< 0.0001
BGS + HLC	7 (4-9)	5 (0–10)	0 (0–100)	0.70 (0.45–1.08)	0.105	71 (65–76)	34 (26–41)	52 (40–64)	0.21 (0.17–0.26)	< 0.0001

The percentage of released mosquitoes recaptured by each method is presented out of 800 *Aedes aegypti* mosquitoes released over 8 replicates. The proportion recaptured is the arithmetic mean recaptured out of the total released.

[a]The odds ratio (OR) estimates were derived from Stata output of treatment relative to control from mosquitoes recaptured in position one in the presence (choice) and absence (no-choice) of additional HLC conducted in position two, adjusted for temperature and humidity and presented with a 95% confidence interval.

Table 2.3 Diversion of mosquitoes from human landing catch (HLC), mosquito electrocuting traps (METs) and BG-Sentinel (BGS) traps in position 1, located 3 metres from freestanding transfluthrin passive emanators (FTPEs) or control to a person conducting HLC in position 2, 10 metres from FTPEs or control.

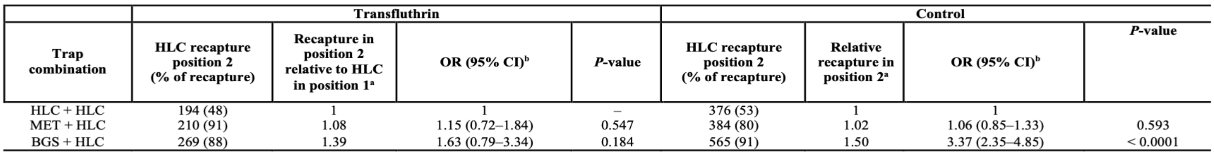


	Transfluthrin					Control				
Trap combination	"Trap" recapture position 1	HLC recapture position 2	Recapture in position 2 relative to position 1	OR (95% CI)[a] position 2 relative to position 1	*P*-value	"Trap" recapture position 1	HLC recapture position 2	Recapture in position 2 relative to position 1	OR (95% CI)[a] position 2 relative to position 1	*P*-value
HLC + HLC	208	194	0.93	0.87 (0.66–1.15)	0.324	335	376	1.12	1.26 (1.02–1.55)	0.030
MET + HLC	22	210	9.55	91.1 (49.0–170.0)	< 0.0001	96	384	4.00	16.0 (11.66–22.0)	< 0.0001
BGS + HLC	38	269	7.08	50.1 (31.0–81.0)	< 0.0001	53	565	10.66	113.6 (76.3–169.2)	< 0.0001

The percentage of released mosquitoes recaptured by each method is presented out of 800 *Aedes aegypti* mosquitoes released over 8 replicates. The proportion recaptured is the arithmetic mean recaptured out of the released mosquitoes.

[a] The odds ratio (OR) estimates were derived from the Stata output of position two relatives to position one in the presence of transfluthrin or control adjusted for the temperature, humidity, compartment, and volunteers and presented with a 95% confidence interval.

Table 2.4 The relative recapture of competing human landing catch (HLC) in the presence of HLC, mosquito-electrocuting traps (METs) and BG-Sentinel (BGS) traps in a choice test in the presence of two freestanding transfluthrin passive emanators (treatment) and with no transfluthrin (control)



	Transfluthrin				Control			
Trap combination	HLC recapture position 2 (% of recapture)	Recapture in position 2 relative to HLC in position 1[a]	OR (95% CI)[b]	*P*-value	HLC recapture position 2 (% of recapture)	Relative recapture in position 2[a]	OR (95% CI)[b]	*P*-value
HLC + HLC	194 (48)	1	1	–	376 (53)	1	1	
MET + HLC	210 (91)	1.08	1.15 (0.72–1.84)	0.547	384 (80)	1.02	1.06 (0.85–1.33)	0.593
BGS + HLC	269 (88)	1.39	1.63 (0.79–3.34)	0.184	565 (91)	1.50	3.37 (2.35–4.85)	< 0.0001

The average proportion of released mosquitoes that were recaptured by each method in position 1 and by HLC in position 2 is presented out of 800 *Aedes aegypti* mosquitoes released over 8 replicates.

[a] Relative recapture in HLC position 2 when HLC is conducted in position 1 compared to MET and BGS trap in position 1 in both the treatment and the control arms.

[b] The odds ratio (OR) estimates were derived from Stata output adjusted for temperature and humidity and presented with a 95% confidence interval

2.5 Discussion

Outdoor vector control tools such as spatial repellents, including VP, promise to be an important addition to the vector control toolbox because they protect multiple users within a defined space. The current study compared the efficacies of the gold standard, HLC, and two exposure-free mosquito-collection methods, MET and BGS trap in estimating the protective efficacy of the VP. The protective efficacy measured by each trapping method was evaluated either independently or in the presence of an additional HLC to simulate competition between blood hosts and its impact on mosquito behaviour (Krockel et al., 2006).

Traps and HLC measure similar protective effects of transfluthrin in the no-choice test

This study demonstrated that in the absence of an HLC competitor, the similar protective efficacy of VP was measured by BGS trap, MET, and HLC using the basic formula based on unadjusted mean mosquito landings. However, in the statistical model, a significant interaction between trap and treatment showed that MET and HLC measured the protective effect of the transfluthrin differently. The differences between the model estimates for the OR and the basic formula for PE may be explained by the fact that the model is adjusted to other variables. However, this difference between HLC/BGS and MET, MET being 10% lower than the others is too small for the basic PE formula to detect. Therefore, it can be inferred that field experiments to evaluate VP using exposure-free methods of *Ae. aegypti* collection is possible provided the experiments are sufficiently well-powered and are designed to ensure the independence of observations without the bias of alternative host cues. Because it is not ethical to measure PE in the viral endemic area using HLC, this small degree of error in estimating PE is acceptable. Furthermore, in field experiments, the incidence rate ratio will be calculated from mosquito count data adjusted for sources of variation, which allow estimation of the adjusted protective efficacy using IRR (Mmbando et al., 2017). In the current experiments, a binomial distribution was used because the data was collected from the semi-field system with a known number of released mosquitoes. The independence of observations is an essential consideration in the design of experiments, and field trials using METs or BGS traps, as a proxy for HLC must be conducted in locations away from competing sources of attraction. This result was encouraging because the use of METs or BGS traps would allow safe evaluation of VP in areas of active arbovirus transmission where HLC is not possible, although it must be understood that measures of protection are not exact due to the limitations of the traps used.

In the control, MET collected approximately half the number of mosquitoes caught by HLC, while the BGS trap caught about 15% fewer. Similar results have been seen repeatedly in other studies as traps generally provide some but not the complete suite of host cues required to maximize mosquito attraction. One exception is the host decoy trap (HDT), which provides whole-host odour, visual cues, and heat (Hawkes et al., 2017). Even so, the number of *Anopheles* mosquitoes caught by HLC was higher than that with HDT in the southeast Asia (Davidson et al., 2020) and compared to other human-baited traps, such as human double net trap in Laos (Tangena et al., 2015) and the MET in Tanzania (Le Goff G and Robert, 1975). A field study conducted in Ecuador showed that the mean *Ae. aegypti* collected when using MET or BGS were equally (Ortega-López et al., 2020), which contrasts with the current findings. This difference may be due to the closed SFS environment in which the traps were currently evaluated or due to the low density of *Ae. aegypti* captured in the Colombian study. Furthermore, in the Colombian study, *Culex quinquefasciatus* was highly abundant and the MET collected fewer of this species than did the BGS trap (Ortega-López et al., 2020).

The presence of host cues is an important consideration in testing repellents because it is known that molecules such as N, N-diethyl-3-methylbenzamide (DEET) interact with host odour receptors (Sparks and Dickens, 2017). As the MET and HLC methods use humans as bait, we would expect similar proportions of recaptured mosquitoes. The differences in catch size may be explained by the fact that day-active *Ae. aegypti* use visual cues to locate their host (Ray, 2015). It is, therefore, possible that they are more aware of the electric grid (Newland et al., 2015) or are unable to pick up as many short-range cues such as thermal and water-vapor cues (Liu and Vosshall, 2019, Cardé, 2015). Nonetheless, this finding warrants further comparison of BGS traps and METs under field conditions to confirm these promising SFS findings for monitoring *Ae. aegypti* in Tanzania. The advantages of using MET or BGS trap mosquito-collection methods as an alternative to HLC for monitoring human exposure to *Ae. aegypti* includes; it removes variation caused by individual skill and motivation to collect mosquitoes, it is far safer and it does not require extensive training to the user.

Traps and HLC did not measure similar protective efficacy of transfluthrin in the choice test

The presence of a person conducting HLC in the SFS strongly affected the estimated PE of the FTPE measured by the trap. It is difficult to interpret the results because very few mosquitoes were caught in the MET or BGS trap when there was a human competitor and therefore the power to measure the difference in treatment and control was very low. This result showed that human competitors could significantly affect the traps' collection ability. These experiments were conducted in the SFS, where the number of mosquitoes is limited to those released, and it may therefore be possible to increase the power to detect the difference by using more mosquitoes. Because space and host options for the mosquitoes are also limited, it would be useful to confirm if these results would be reflected in a field trial. However, there are ethical concerns in doing HLC in the field except in an area with no known arbovirus transmission.

A significant interaction between trap and treatment showed that METs, HLC and BGS traps measure the effect of transfluthrin differently. This was consistent even when the basic formula for PE was used to assess the efficacy of the collection methods in evaluating VP. The presence of a competitor HLC reduced the precision of METs and BGS traps to measure PE. However, this may reflect the true PE that could be measured in the field, where the possibility of finding someone in isolation is very small. The average PE was 62% in the no-choice experiments, which is consistent with other evaluations of FTPEs (Tambwe et al., 2020). However, in the choice tests, BGS traps measured a reduced PE and increased PE was measured by METs. This is explained by the presence of a second HLC, which introduces other cues, causing variability in the data. It is known that mosquitoes orient to carbon dioxide (CO_2) from over 20 m (Gillies and Wilkes, 1968) and select between hosts at distances of approximately 15 m (Ansell et al., 2002). Consequently, it is recommended that topical repellents be tested with individuals over 20 m apart (Maia and Moore, 2011) in no-choice tests (Moore et al., 2007) to ensure the independence of observations. The current data adds weight to this recommendation. It is consistent with observations that household mosquito densities are correlated with the number of occupants (Kaindoa et al., 2016). In addition, other studies of transfluthrin PE in semi-field systems demonstrated that the addition of a CO_2-baited Suna trap reduced transfluthrin PE and that the trap did not perform well in the presence of a human (Njoroge et al., 2021). This is consistent with the current findings that the protective efficacy of transfluthrin was lower, but not significantly so, in the presence of a second competitor HLC; BGS traps and METs collected substantially fewer mosquitoes.

No evidence of mosquito diversion from a protected individual to a second individual at 10 metres in the presence of transfluthrin

Spatial repellents, including VPs, are an important addition to the vector control toolbox because they protect multiple users within a defined space (Achee et al., 2012a). This study demonstrated that the presence of FTPEs in all of the experimental configurations (HLC, MET, and BGS trap) reduced the number of collected mosquitoes. The competitor HLC, located 10 metres from the FTPEs, also demonstrated approximately 50% PE. This is consistent with another study conducted against *Anopheles arabiensis* (*An. arabiensis*) *in* Tanzania, *An. harrisoni* and *An. minimus* in Thailand where the overall protective efficacy of 50% extended between 5 m and 10 m in an outdoor setting (Ogoma et al., 2017, Sukkanon et al., 2021). However, in the Thailand study both the treatment and the control were in the same compartment. Considering the mechanism of action of transfluthrin, with this experimental design the PE observed might be underestimated. Thus, the independency of the treatment arms is very important during the evaluation of volatile pyrethroid such as transfluthrin.

This study showed that VPs act on mosquitoes over distances of several metres with a non-contact (spatial) mode of action (Sukkanon et al., 2020). From a public health perspective, this is a useful characteristic robust of VPs used as spatial repellents because they can protect multiple users with no need for daily compliance, unlike topical repellents, which suffer from diversion of users to non-users (Moore et al., 2007) and extremely low daily compliance among users in endemic countries (Gryseels et al., 2015), travelers (Lalani et al., 2016) and military populations (Frances et al., 2003). Further testing of the usefulness of METs for the evaluation of topical repellents that act over distances of just a few centimetres (Riffell, 2019) is required to validate METs for evaluation of other bite prevention interventions, such as topical repellents and insecticide-treated clothing.

While there is some evidence that VPs can cause an increase in mosquito bites among non-repellent-using households in villages with incomplete coverage of VP (Maia et al., 2012a), it has also been observed that when applied at a large scale, transfluthrin VP can reduce malaria (Syafruddin et al., 2020). This is because transfluthrin has multiple modes of action. It can cause rapid knockdown and kill (Andrés et al., 2015) and feeding inhibition up to 12 hours post-exposure, referred to as “disarming” (Denz et al., 2021), as well as causing landing reduction, which is important when considering the use of this intervention at scale for public health (Mwanga et al., 2019). While diversion was not observed in this study, we cannot rule out the possibility of diversion occurring in other settings where an individual may be positioned outside the reach of the protective radius of transfluthrin.

Evidence that humans at 10 metres attract majority of mosquitoes in the presence of BGS traps

This study also observed that humans positioned 10 m away from a BGS trap received all the mosquito landings, similar to if they had been positioned alone. While the presence of transfluthrin did continue to protect the HLC participant in the presence of the BGS, in the control arm, mosquito landings substantially increased. This is unsurprising because mosquito sensitivity to skin odours has been shown to increase at least fivefold immediately following a brief encounter with a filament of CO_2 (Dekker et al., 2005). This mechanism may also explain the findings of a similar study in an SFS in Kenya, where transfluthrin showed lower PE in the presence of an odour-baited Suna trap than when used without the trap (Njoroge et al., 2021). However, the authors point out that the differing ambient temperatures, which may affect the release rates of VPs, may have confounded their data.

The same finding was observed in push–pull evaluations in Tanzania (Mmbando et al., 2019) in which increasing odour-baited trap density around houses increased landings on people conducting HLC while moving traps further away was protective (Mmbando et al., 2019). Therefore, the location of traps with CO_2 for *Ae. aegypti* surveillance should be carefully considered in areas of active arbovirus transmission to ensure that householders, where traps are located, do not experience increased bites. This finding has also been observed in Tanzania (Okumu et al., 2010), where odour-baited traps lured large numbers of mosquitoes from a distance but could not compete with humans at short range and actually resulted in increased landings for those sitting close to odour-baited traps. This causes difficulties: if the traps are moved out of peridomestic areas, they will likely no longer be able to measure the impact of peridomestic interventions such as VPs. So while odour-baited traps with CO_2 are being considered because of their safety for the HLC technicians, there may be unwanted side effects for community members.

Other considerations for repellent evaluations

In our study, the paired HLC captured similar proportions of mosquitoes in the absence of VPs, with a ratio of approximately 1:1. The participation of highly skilled technicians collecting over three hours allowed equivalent estimation of mosquito landings although the studies were performed at different times. This highlights the importance of training and supervision of staff involved in the conduct of entomological evaluations. The technical staff were highly motivated to perform the test accurately following discussion of the importance of the study and their role in the generation of accurate data (Begg et al., 2020). Also, it is important to highlight that during the evaluation of the spatial repellent, the number of mosquitoes collected using an odour-baited trap may be reduced. This may overestimate the efficacy of spatial repellent when odour-baited traps are used. Baseline information before the implementation of the trial must be conducted.

Study limitations

First, during collection, the BGS trap ran continuously for three hours while each hour a 10-minute break was provided for those conducting MET testing or HLC to stretch and to collect mosquitoes from the MET. Thus, the total sampling time for the BGS trap was three hours, whereas it was 2 hours 30 minutes for both HLC and the MET. Therefore, the number of mosquitoes caught by the BGS trap may be overestimated. Second, the volunteers observed that mosquitoes electrocuted by the MET occasionally recovered and flew away, which may contribute to a lower estimate of the mosquito landing rate. This study used 680 V generated by the MET, but for those experiment conducted in the SFS that do not need mosquito samples after electrocution, a higher voltage may be used. Third, the experiments were conducted in the semi-field system using laboratory-reared mosquitoes. Although the mosquitoes were recently colonised, it is possible that these results may not represent what would happen in a real-world situation with wild mosquitoes. In addition, the results may not be generalisable to all mosquito species. While the data were consistent with those from other experiments using a similar dose of transfluthrin, the relative efficacy of the BGS trap and the MET to estimate PE may vary according to transfluthrin concentration. Further experiments with varying doses of transfluthrin conducted in multiple settings would be useful to strengthen the findings of this study.

2.6 Conclusions

HLC, METs and BGS traps measured a consistent 60% PE of transfluthrin emanator in isolation from competing host cues, while PE estimated by each method was variable in the presence of an HLC competitor. Therefore, measurement of the PE, that is, reduction in landings of mosquitoes caused by VP spatial repellents, is possible using HLC, METs or BGS traps in no-choice tests. While HLC is probably a better measure of the PE offered by the volatile pyrethroid because the whole suite of medium- and short-range host cues is available to host-seeking mosquitoes, ethical concerns in arbovirus-endemic areas restrict its use in the field. This study suggests that estimation of the PE of VPs or other spatially acting compounds against anthropophilic mosquitoes such as *Ae. aegypti* could be evaluated in the field using either METs or BGS traps provided that independence of observations is ensured. Findings also indicate that transfluthrin can protect multiple people in the peridomestic area and that using a BGS trap close to people may increase their exposure to host-seeking mosquitoes that are attracted by CO_2 at long range then select humans at short range. This study needs to be repeated in other sites to confirm the findings.

2.7 Declaration

Ethics statement

The Ifakara Institute Review board (IHI-IRB) and the National Institute for Medical Research –Tanzania (NIMR), with approval numbers IHI/IRB/No: 024-2016 and NIMR/HQ/R.8a/Vol.IX/2381, consecutively approved this study. The volunteers recruited to perform either HLC or MET after a written informed consent was obtained. Also, volunteers consented for their photographs to appear in the manuscript. These experiments used mosquitoes that have been kept in the laboratory for over five years, this reduce the chance that they may transmits arboviruses to the volunteers. Female mosquitoes were fed on cow blood through membrane feeding, which further reduce the possibility of harboring viruses.

Consent for publication

Volunteers in the photographs consented for their image to appear in this publication.

Availability of data and materials

All data generated and analysed for this research article are included as additional files 1

Competing interest

The authors declare that they have no competing interest.

Authors' contributions

SJM conceived the study; MMT, AS and SJM designed the experiments. MMT and RM supervised data collection. MMT drafted the manuscript. MMT, AS, UAK and SJM conducted analysis and edited the manuscript. NJG and KK edited the manuscript. All authors read and approved the final manuscript.

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Chapter 3: Semi-field evaluation of freestanding transfluthrin passive emanators and the BG sentinel trap as a "push-pull control strategy" against *Aedes aegypti* mosquitoes

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3.1 Abstract

Background: The use of volatile pyrethroids aiming at repelling mosquitoes away and odor-baited traps that attract and kill mosquitoes in a push-pull system, has been shown to reduce house entry and outdoor bites for malaria vectors. This technology has the potential to control other outdoor biting mosquitoes such as *Aedes aegypti* that transmit arboviral diseases. In this study, semi-field experiments were conducted to evaluate whether a push-pull system could be used to reduce bites from *Aedes*.

Methods: The push and pull under investigation consisted of two freestanding transfluthrin passive emanators (FTPE), and a BG sentinel trap (BGS) respectively. The FTPE contained hessian strips treated with 5.25g of transfluthrin active ingredient. The efficacies of FTPE and BGS alone and in combination were evaluated by human landing catch in a large semi-field system in Tanzania. We also investigated the protection of FTPE over six months. The data was analyzed using generalized linear mixed models with binomial distribution.

Results: Two FTPEs provided a protective efficacy (PE) of 61.2% (95% Confidence interval (CI): 52.2-69.9) against human landing rate of *Aedes aegypti*. The BGS did not significantly reduce mosquito landings; the PE was 2.1% (95% CI: -2.9-7.2). The combination of FTPE and BGS (push-pull) provided a PE of 64.5% (95% CI: 59.1-69.9). However, there was no significant difference in the protective efficacy between the push-pull and the two FTPEs against *Ae. aegypti* (p=0.30). The FTPE offered significant protection against *Ae. aegypti* at month three, with a PE of 46.4% (95% CI: 41.1-51.8), but not at six months with a PE of 2.2% (95% CI: -9.0-14.0).

Conclusions: The protective efficacy of the FTPE and the full push-pull system are similar, indicative that bite prevention is primarily due to the activity of the FTPE. While these results are encouraging for the FTPE, further work is needed for a push-pull system to be

recommended for *Ae. aegypti* control. The three-month protection provided by the FTPE against *Ae. aegypti* bites suggests it would be a useful additional personal protection tool during dengue outbreaks, that does not require regular user compliance.

Keywords: spatial repellent, odor-baited trap, FTPE, push-pull, BG-sentinel trap, transfluthrin, *Aedes aegypti*

3.2 Background

The *Aedes aegypti* mosquito is the primary vector of many arboviral diseases of public health importance, including dengue, yellow fever, zika, and chikungunya (Mboera et al., 2016, Gould et al., 2008, Wikan and Smith, 2016). The risk of contracting an arboviral disease is increasing as the world becomes more urbanized, because *Ae. aegypti* thrive in verities of settlements (Messina et al., 2019). Dengue vector control is centered on larval source management, treatment of resting surfaces with insecticides and with space spraying as a response to disease outbreaks (Roiz et al., 2018). However, insecticides used for both space spraying and larviciding are short lasting and require a high frequency of reapplication to achieve sustained vector control. Larval habitat reduction is more sustainable but is not always practically or economically feasible in dengue endemic countries.

Personal protection measures are also recommended during disease outbreaks through the use of appropriate clothing or topical repellents (WHO, 2019). Topical repellents such as DEET (N, N-diethyl-m- toluamide) have demonstrated efficacy in reducing mosquito bites (Lupi et al., 2013), and are also recommended for arbovirus prevention among military personnel and travelers (PPAV Working Groups, 2011). However, there have been no studies to demonstrate their efficacy in reducing arboviral disease transmission. Topical repellents require frequent reapplication, which inevitably results in poor user compliance, and consequently coverage levels that are insufficient to interrupt disease transmission (Gryseels et al., 2015). Each of the current control tools for *Aedes* has clear limitations and therefore, the development of complimentary control tools to help fill these gaps is needed.

Spatial repellents (Ogoma et al., 2017, Achee et al., 2012a) and odor-baited traps (Okumu et al., 2010, Degener et al., 2014) have been suggested for the control of *Aedes* mosquitoes. Spatial repellents provide a bite-free space using repellent chemicals that passively evaporate at room temperature (emanators) or that are actively dispersed through heating coils, mats or vaporizers (Obermayr et al., 2015). By removing the need for individual application, higher coverage levels may be possible with spatial repellents compared to topical repellents. Furthermore, spatial repellents may last for days, even months, after one application reducing the hassle of re-application and potentially increasing the protection even further. Hessian strips treated with transfluthrin used as passive emanators have been shown to reduce human landing rates by >90% for *Culex* and Anopheles mosquitoes in both semi-field and field experiments for up to six months (Ogoma et al., 2017, Ogoma et al., 2012b).

Odor baited traps, such as the Biogents sentinel trap (BGS) (Rafael MF, 2006), have been used extensively for mosquito monitoring and have recently been found to have public health benefits by reducing the population density of malaria and dengue vectors when deployed at a large scale in sufficient numbers to ultimately decrease disease transmission (Homan et al., 2016, Degener et al., 2014). It has been shown that both spatial repellents and odor-baited traps used individually can be effective for the control of *Ae. aegypti*. These tools work in contrasting ways – one providing personal protection by reducing human-vector contact and the other providing community protection by reducing the mosquito population size.

The push-pull control strategy originated from studies of agricultural pests showing that the practice of repelling “pushing” insects from one area and attracting “pulling” them to another, increases crop production (Cook et al., 2007). The same strategy may be applied to control disease-transmitting mosquitoes of public health importance using spatial repellents and odor baited traps (Menger et al., 2015) and mathematical models have predicted that this control strategy may reduce entomological inoculation rate (EIR) by 20-fold for indoor biting malaria-transmitting mosquitoes(Menger et al., 2015). While push-pull control tools have also been tested against *Aedes*, successful laboratory results did not transfer to semi-field settings (Obermayr et al., 2015) with the researchers hypothesizing that the spatial repellent chemicals did not reach sufficient concentrations. In this study we investigate a new push-pull combination for *Aedes* using technologies that have individually proven successful under semi-field and field conditions. For the push component, transfluthrin treated hessian (Ogoma et al., 2012b) was adapted to make a freestanding transfluthrin passive emanator (FTPE). The widely studied BGS was selected as the pull component of the system. Here, we investigate the efficacy of push and pull separately and then in combination in a push-pull system to see if the combination provided better efficacy than either of the components used individually measured as reduction in mosquito landings. We also measured the duration of protective efficacy of the FTPE over a six-month period.

3.3 Methods

Study design

This study investigated the efficacy of the FTPE and BGS in a push-pull system to reduce human-landing rates compared to the control (no intervention). I also determined if the combination of FTPE and BGS was better than either FTPE or BGS alone whereby the following treatment arms were compared: 1) two FTPE versus negative control 2) BGS trap versus negative control 3) the combination of FTPE and BGS versus negative control. The study design was a randomised block design over 16 days per treatment arm. Each intervention and its control were assigned to one of two separate compartments in the semi-field system (SFS) for a block of four days, after which the treatment and its control were switched between compartments. Preliminary experiments showed that removing FTPE immediately after experiment and aired the compartment for 20 hours, was enough to prevent carry-over effect. In each block of four days, four volunteers rotated daily between chambers.

Study Site

The experiment was conducted in the SFS located in Bagamoyo-Tanzania, from January to December 2018 with on and off between experiments. The SFS measures 21 x 29 x 4.5 m with two compartments (21 x 9 m), separated by a corridor. A heavy-duty polyethene wall separates these compartments preventing air movement between the chambers and reducing any chance of cross-contamination when working with spatial repellents or other aerosols. The SFS allows for controlled experiments with set densities of disease-free mosquitoes to be conducted under field-like climatic conditions throughout the year (Ferguson et al., 2008).

Mosquitoes

Laboratory-reared, pyrethroid-susceptible *Ae. aegypti* (Bagamoyo strain) were used. Susceptibility bioassays performed prior the implementation of the experiment following the World Health Organization (WHO) guidelines (WHO, 2016b) showed that mortality of these mosquitoes was > 99% after exposure to all the pyrethroid insecticides tested (Deltamethrin (0.03%), Permethrin (0.25%) and Alpha-cypermethrin (0.03). These mosquitoes were colonized from Bagamoyo in December 2015. Larvae were fed on Tetramin® fish food and adult mosquitoes on 10% sucrose *ad libitum* and cow blood meals (heparinized) were given to adult females for egg production using a membrane-feeding assay. The colony is maintained at 27±5°C and 80±20% relative humidity.

For this experiment, 3–8 days old female mosquitoes, previously unfed with blood were used. These mosquitoes were sugar-starved for 12 hours before the start of experiments. Active probing female mosquitoes were selected from three different rearing cages and transferred to small releasing cages (10 x 10 x 10cm). Selection from cages was done by placing a hand close to the cage and choosing only aggressive host-seeking mosquitoes.

Preparation of the freestanding transfluthrin passive emanator (FTPE)

We designed a device that can easily be placed anywhere in the peri-domestic space (Figure 3.1 A-E). The emanator passively releases transfluthrin vapors into the surrounding area through evaporation. The device is a stool-like structure that supports hessian strips (made from plants of the genus *Corchorus olitorius* or *Corchorus capsularis* also called jute, burlap or gunny sacks), treated with the emulsifiable concentrate (EC) transfluthrin active ingredient (Bayothrin EC, Bayer AG Monheim am Rhein, Germany) as the push. The hessian fabrics were chosen as they have been shown to retain transfluthrin active ingredient for up to six months due to their high cellulose content (Ogoma et al., 2017, Ogoma et al., 2012b). The hessian fabrics were locally bought, washed with OMO® detergent powder (Unilever Kenya Limited, Kenya), and dried under direct sunlight. The fabrics were cut into strips with a surface area of 0.5m^2 (10cm x 5m) and treated with 5.25g of EC transfluthrin. To prevent photolysis of transfluthrin, the strips were left to dry under the shade in the SFS (Figure 3.1B-C). The strips were then wound around a pole into a spiral and sealed with outer wire mesh to prevent access to the treated hessian ribbon by children or animals (Figure 3.1 D). Two FTPEs with a total of 10.50 g (5.25g each) of transfluthrin were used per experiment.

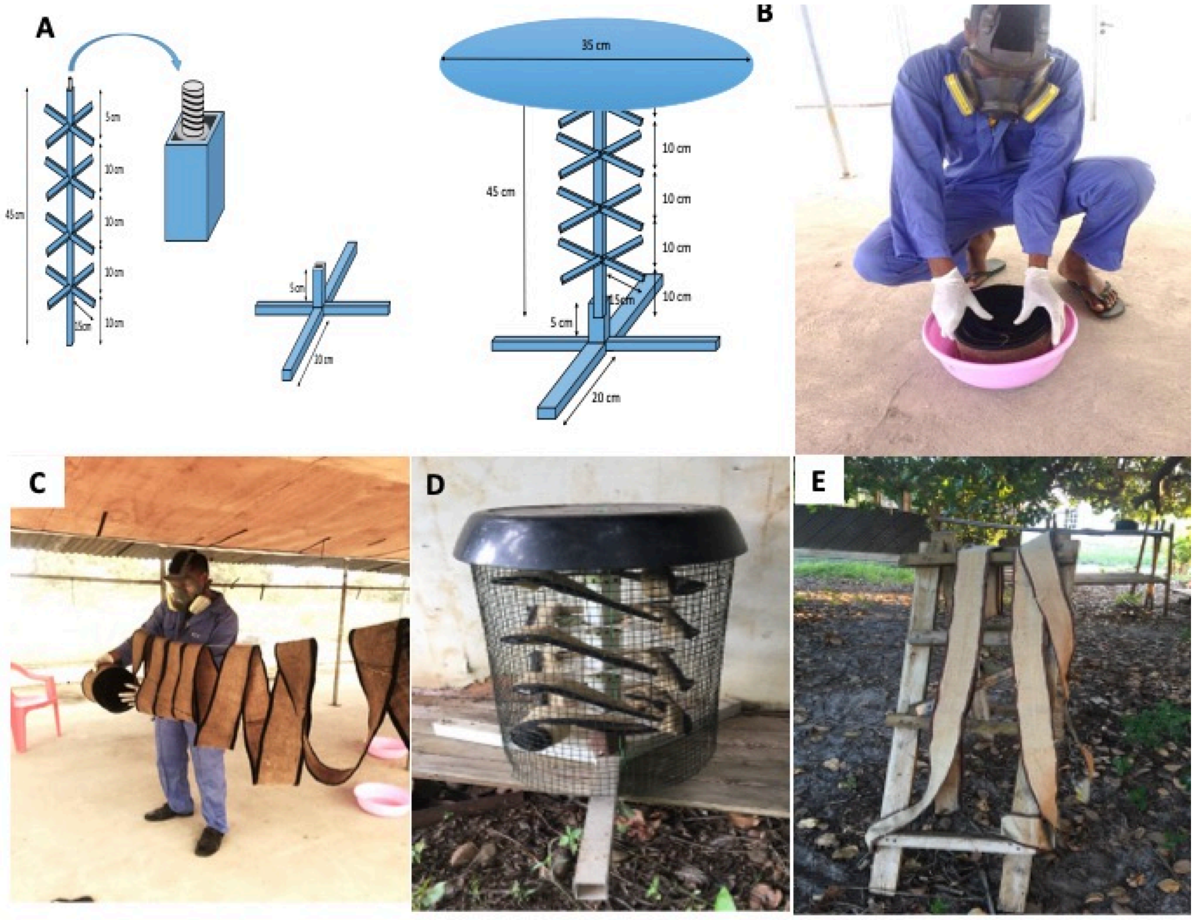

Figure 3.1 Preparation of the freestanding emanator (FTPE) "push". a Design of the freestanding emanator. The device measures 50 cm in height and 40 cm in diameter. It consists of three parts; the top cover, the central square pipe and a base. The central pipe rests on the base that supports the device. The pipe is divided into four portions 10 cm apart where small branches of an aluminium flat bar 15 cm long are attached. b, c Transfluthrin impregnation and drying of the hessian strips under the shade. d The FTPE (the hessian strip enclosed with the wire mesh). e The transfluthrin-treated hessian strips placed under the shade between the experiment for "field aging" for the duration of efficacy experiment

BG sentinel trap

The BGS (Biogents AG, Regensburg, Germany) has been widely used as the standard trap for the collection of adult *Aedes* mosquitoes (Schoeler et al., 2004, Krockel et al., 2006). The BGS was used with a Biogents-Lure (BGL) and carbon dioxide as a pull. The BGL is a synthetic lure consisting of lactic acid, caproic acid, and ammonium bicarbonate dispensed via granules (Krockel et al., 2006). The BG lure was used together with the BGS during the "pull" and "push-pull" experiments as per manufacturer instruction whereby a new cartilage was used for each experiment**.** Carbon dioxide was released from a pressurized cylinder at the rate of 500 ml/min, using an acrylic gas flow meter (Hangzhou Darhor, Technology Co., Limited, China).

Procedure to determine the protective efficacy of the FTPE and odour-baited trap

To simulate the peridomestic setting, human landing catches (HLC) were performed with a volunteer sitting 2 m from an experimental hut inside the SFS (Figure 3.2 A-C). For the "push" alone evaluation, two FTPEs were positioned six meters apart with the human volunteer sitting in between them conducting HLC (Figure 2A). During the evaluation of "pull" alone, the BG sentinel was placed 10 m away from the HLC (Figure 3.2B). For the "push-pull" evaluation, both FTPE and the BGS were used and positioned as described above in the "push" and "pull" alone setups (Figure 2C). In the control, untreated emanators and HLC were used.

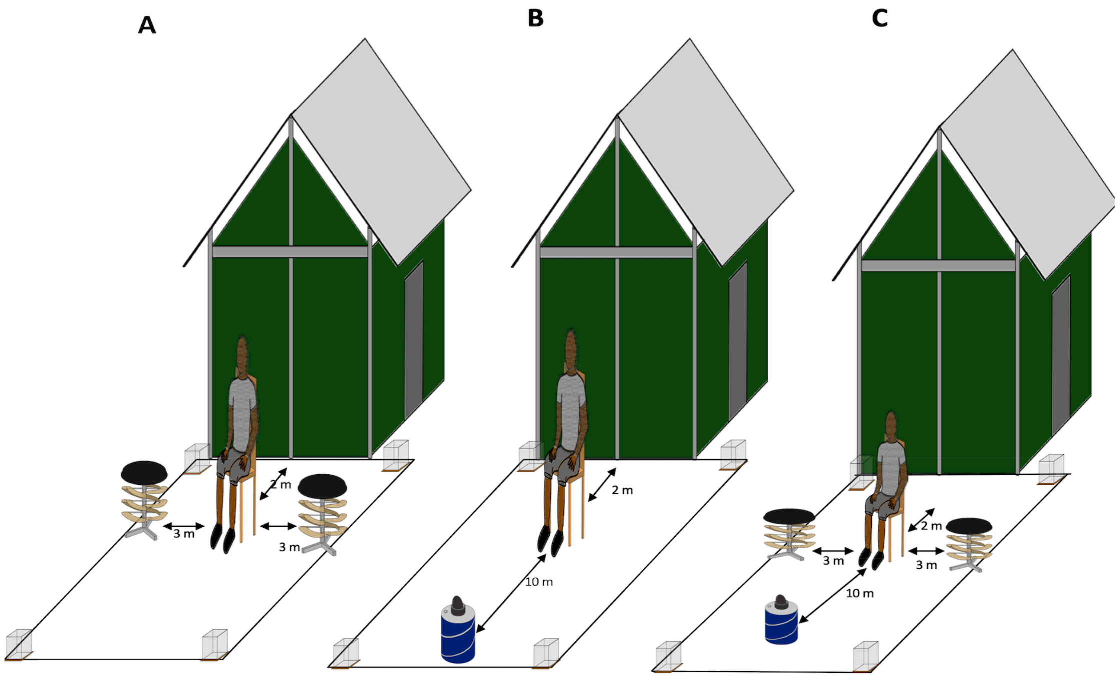

Figure 3.2. Schematic representation for the experiment in the SFS: A) **the arrangement of push intervention B) the BGS positioned 10m away from the human volunteer during the pull-alone evaluation C) the position of interventions during the push-pull evaluation. In each setup, a human volunteer performing HLC sat 2m away from the experimental hut and if push was involved, two FTPEs were positioned 3m on each side of the HLC volunteer. Small boxes at the corner represent the releasing cages positioned where mosquitoes were released.**

The FTPEs were positioned in the experimental chambers forty-five minutes before the experiment started to allow the release of active ingredients into the experimental space, and mosquitoes were transferred to the buffer chamber (corridor) of the SFS one hour before the experiment began to allow for acclimatization. During the acclimatization process, mosquitoes remained free from transfluthrin exposure. After the acclimatization period, cages with approximately 25 mosquitoes each were positioned in the four corners of both compartments (approximately 100 mosquitoes per compartment/treatment). Mosquitoes were released at 07:00hrs by a gentle pull of the strings connecting the releasing cages and the chair where volunteers were sitting. The experiment was conducted in the morning to reflect the natural biting time for *Aedes* mosquitoes (Ndenga et al., 2017).

The volunteers conducted HLC, collecting mosquitoes that landed on the area between the ankle and the knee for three consecutive hours (07:00hrs to 10:00hrs). All volunteers were males aged between 25-40. Volunteers were asked not to apply perfume, bathe using perfumed soap, smoke or drink alcohol during the experiments. During the experiment, volunteers wore shorts, covered shoes, and bug jackets to standardize the area available for mosquito landings. Mosquitoes were recaptured continuously for 50 minutes using a mouth aspirator. After 50 minutes the volunteers would take a break for 10 minutes, after which a new paper cup labeled with time and date were used. Collected mosquitoes were transferred to the insectary for sorting. After the experiment, mosquitoes that were not collected during the HLC were recaptured using Prokopack aspirators and killed to prepare the SFS for the next day's experiment. A Tinytag® view 2 data logger (model TV- 4500, Gemini data logger, United Kingdom) was placed inside the SFS throughout the experiment to record temperature and relative humidity.

Experiment to assess the longevity of the FTPE

To assess the longevity of FTPE protection, the devices were evaluated at zero-, three, and six months post-impregnation. The same setup as described previously (Figure 3.2A) was followed. Between the evaluations, the emanators were stored in an outdoor environment under the tree shade "field aged" to simulate aging on a verandah of a house, i.e. placed outdoors under ambient conditions, protected from direct sunlight and rain (Figure 3.1E).

Sample size

Sample size calculations were performed using a simulation-based power analysis (Johnson et al., 2015) in R statistical software version 3.02 http://www.r-project.org with a significance level of 0.05 for rejecting the null hypothesis. Analysis for experimental data was conducted using generalized linear mixed models (GLMMs). Therefore, one thousand simulations of generalized linear mixed models approximating those that will be used to analyse project data were run using the same experimental design. The power to predict the difference in mosquito landings between control and treatment was estimated as the proportion of the 1000 simulated data sets in which the null hypothesis was rejected when the generalized linear mixed model was run. Overdispersion parameters were set at 10% estimated variability between chambers, 10% variability between mosquito releases, and 10% variability between volunteers. Simulations indicated that with an estimated 100 mosquitoes released per night and 60% recapture of released mosquitoes in the control, there was 94% power (95% CI: 92 – 96%) to detect a 50% reduction in mosquito landings in the treatment arm after 16 nights of experimentation. Furthermore, there was 70% power (68% CI: 74 – 72%) to detect a 15% difference between the treatments.

Data analyses

Data were entered in Microsoft Excel 2010 and analyzed in Stata 13 (Stata Corp). The data were analyzed to determine the efficacy of each intervention (push-alone, pull alone, and push-pull) to reduce the human landing rate compared to the control. The arithmetic mean and 95% confidence interval percentage of recaptured mosquitoes in the intervention or negative control were calculated. Daily protective efficacy was measured by comparing the human landing rate on a volunteer with the intervention to the negative control using the following formula and then the mean for the experiment was calculated.

Protective efficacy= [(C-T)/C] x100%.

Where C stands for the number of mosquitoes landing in the control and T is the number of mosquitoes landing in the treatment. Then using the command, “mean PE” in stata enables to get the PE estimate with the 95% confidence interval (CI).

The effect of each intervention was determined by fitting a generalized linear mixed model (GLMM) with a binomial distribution and logit function. The binomial distribution was chosen, as the number of released mosquitoes (denominator) in a compartment was known before the HLC was performed. The dependent variable was the proportion of recaptured mosquitoes out of those released. Independent fixed effect categorical variables were treatment (intervention or control), compartment, volunteer, block (push, pull, push-pull), and an interaction term between treatment and block with day included as a random effect.

To determine the longevity of FTPE across six months after impregnation; a GLMM with a binomial distribution and logit function was also used. For this model, the dependent variable was again the proportion of recaptured mosquitoes. Independent fixed effect categorical variables were treatment (intervention or control), compartment, volunteer, month of testing (month 0, month 3, month 6), and an interaction term between treatment and months. This interaction was used to determine if the protective efficacy of FTPE changed between months. The day was included as a random effect.

During the evaluation, there was no significant association between humidity and temperature on the proportion of recaptured mosquitoes for all interventions, in all cases (P>0.05). Therefore, these variables were not included in the GLMMs. The average temperature during the experiment was 25.4°C (21.0°C - 26.0°C) and the average relative humidity was 90% (68%-100%).

3.4 Results

Protective efficacy of the push-alone, pull-alone and push-pull

During each experiment in each compartment total of 1600 mosquitoes were released for the duration of 16 days. Of which 439, 926, and 349 were recaptured by the HLC in the presence of FTPE, BGS, and push-pull respectively whereas in the control compartment, approximately 1000 mosquitoes were recaptured. The FTPE significantly reduced *Ae. aegypti* landings (Odds ratio (OR) =0.14 (95% CI: 0.12-0.16, p<0.0001) (Figure 3.3). The protective efficacy (PE) of FTPE against *Ae. aegypti* bites was 61% (95% CI: 52.18-69.91). The BGS did not reduce *Ae. aegypti* landings on a human volunteer sitting 10 meters away from the trap (OR=0.92, 95% CI: 0.81-1.08. p=0.371). The PE was 2.1% (95% CI: -2.9-7.2) (Figure 3.3). The combination of FTPE and the BGS significantly reduced *Ae. aegypti* landings (OR=0.16 (95% CI: 0.14-0.19, p<0.0001). The PE offered by this combination was 64.5% (95% CI: 59.1-69.9) (Figure 3.3). The proportion of mosquitoes caught by BGS during BGS alone or BGS and FTPE experiment showed no significant difference: 6.1% (95% CI: 5.1-6.1) and 6.1% (95% CI: 5.0-7.3) in the presence and absence of FTPE, respectively (p=0.34). This indicates that the push and pull components were not working synergistically with a majority of protection provided by the push alone.

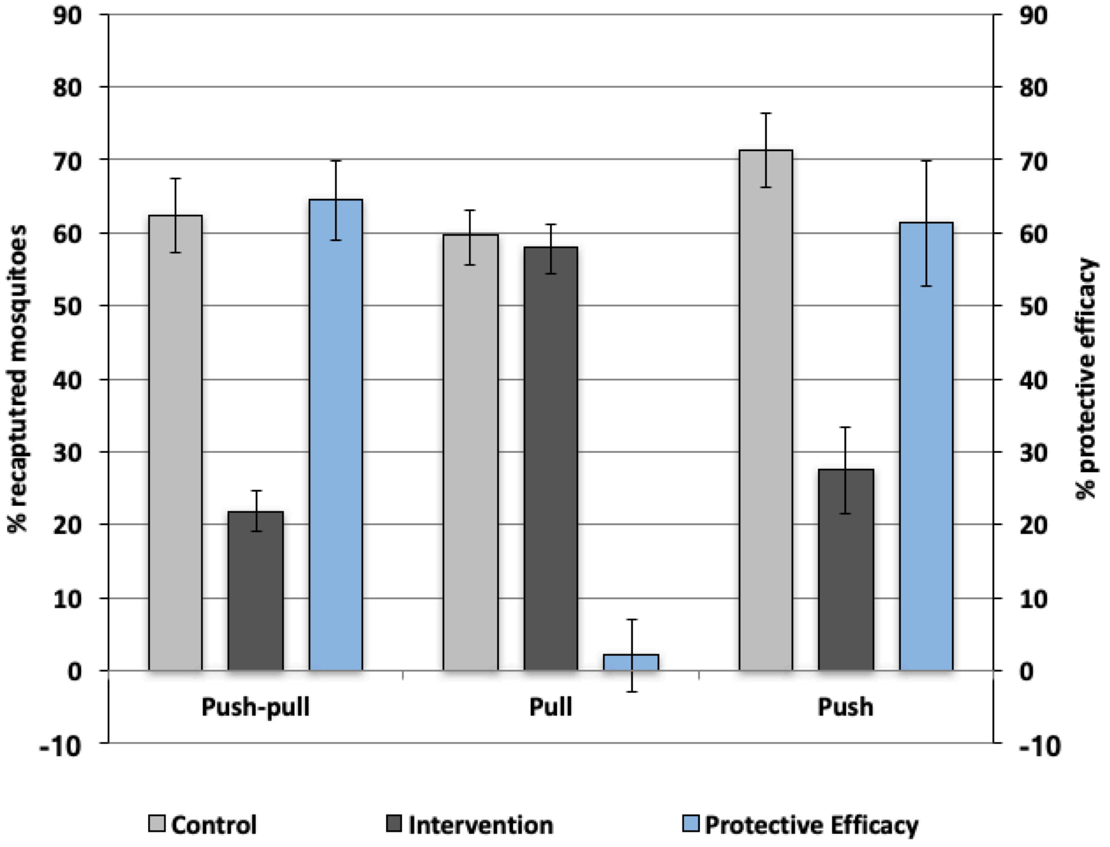

Figure 3.3. The % recaptured mosquitoes and protective efficacy. The arithmetic mean percentage of mosquitoes recaptured by HLC in the presence of the odour-baited trap (pull), spatial repellent emanator (push), spatial repellent emanator, and odour-baited trap (push-pull) compared to the control. The secondary axis shows the % protective efficacy of each intervention. Error bars represent the 95% confidence intervals.

Comparing the performance of the push-alone, pull-alone and push-pull

A significant interaction between block and treatment confirmed the protective efficacy of push-alone, as described above, was significantly greater than pull-alone ($p<0.001$). However, the protective efficacy of push-alone against *Ae. aegypti* was not significantly different from the full push-pull system ($p=0.29$). There was no significant difference in the compartment ($p=0.29$) or volunteers ($p>0.05$ for all volunteers) on the number of recaptured mosquitoes.

Protective efficacy of the push-alone over six months

There was a significant interaction between month and treatment showing that the PE of the FTPE decreased over time (p<0.001). At three months after impregnation, the FTPE was still providing significant 46.4% (95% CI: 41.1-51.8) protection against *Ae. aegypti* (OR=0.26, 95% CI: 0.22-0.29, p<0.001). However, when the FTPE were tested at month six after impregnation no significant protection was offered (OR= 0.91, 95% CI: 0.79-1.05, p=0.22), with protective efficacy dropping to 2.2% (95% CI: -9.0-14.0) (Figure 3.4).

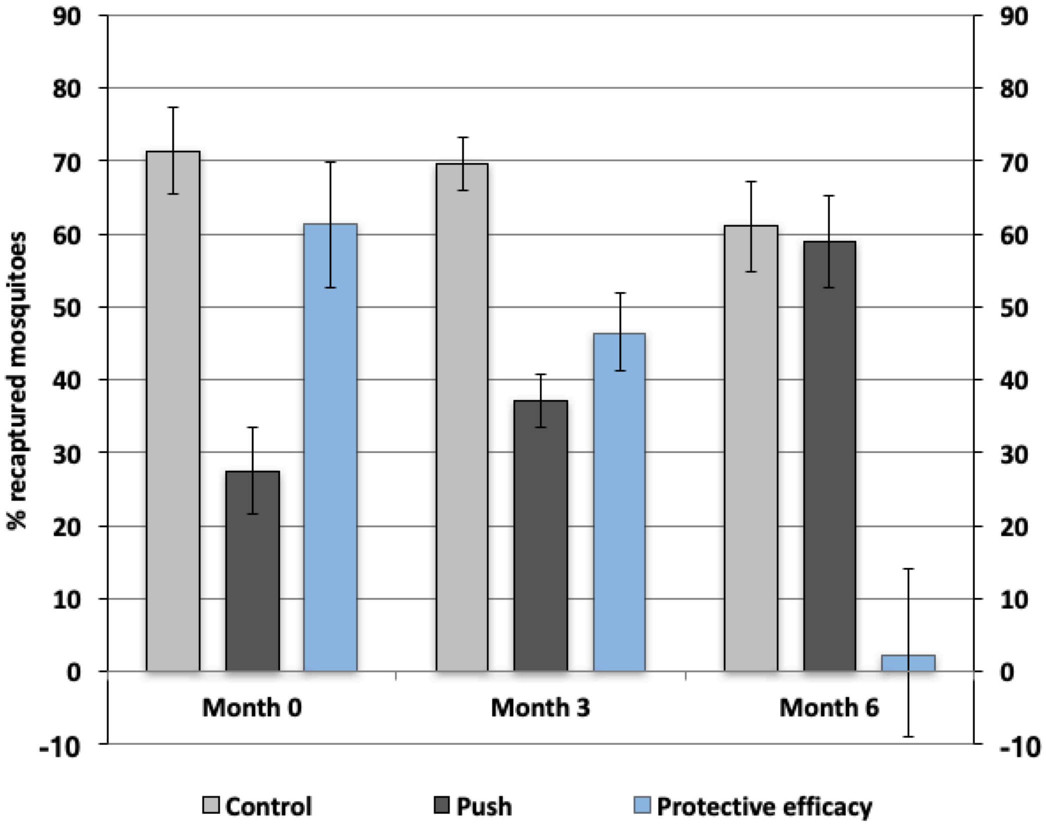

Figure 3.4. The duration of efficacy of the FTPE. The arithmetic mean percentage of mosquitoes recaptured by HLC in the compartment with FTPE compared to the control up to six months after treatment. The secondary axis represents the % protective efficacy of the push at each time point. Error bars represent the 95% confidence intervals

3.5 Discussion

The current vector control tools used against *Aedes* mosquitoes have several limitations, necessitating the development and testing of additional tools for proactive dengue prevention. This study demonstrated that while the push-pull system reduced human-vector contact of *Ae. aegypti* mosquitoes, the majority of protection was provided by the FTPEs. The likely reason for this is the poor response of mosquitoes to the BGS. Numerous previous studies on the use of push-pull technologies for the control of mosquitoes have demonstrated the higher efficacy of the push than the pull (Menger et al., 2016, Menger et al., 2015, Obermayr et al., 2015, Mmbando et al., 2017, Wagman et al., 2015b). While the push-pull system may need further development, the success of the FTPEs was encouraging and indicated their potential for the control of arboviral diseases.

The FTPEs remained protective for three months, therefore, it is possible to meet high levels of coverage in an urban setting with just one application, reducing the difficulties of re-application and user noncompliance. These promising results indicate that FTPEs could potentially be used to protect individuals in the peridomestic space longer than current personal protection methods (Roiz et al., 2018). This may be particularly useful during arboviral disease outbreaks that tend to coincide with the 3-5 month rainy season (Phanitchat et al., 2019).

Our finding on the efficacy of FTPEs as a spatial repellent is consistent with previous studies evaluating transfluthrin against *Anopheles arabiensis* and *Ae. aegypti* mosquitoes (Andrés et al., 2015, Amelia-Yap et al., 2018, McPhatter et al., 2017). However, we may not generalize that these transfluthrin-treated passive emanators provide protection in all geographical locations. In the field, it is important to consider environmental factors and the susceptibility status of the mosquitoes before implementing this control strategy. For example, in a windy environment, the active ingredient can be blown away, therefore, reducing the concentrations of active ingredients needed to repel mosquitoes. Temperature can affect the vaporization of transfluthrin and thereby its concentration and protective efficacy. It has been reported that the optimal temperature for a transfluthrin-treated emanator to provide maximum protection ranges between 21°C to 30°C, with a reduction in protection specifically in lower temperatures (Ogoma et al., 2017). This suggests that in geographical locations where the daytime ambient temperature is below 21°C the efficacy of these emanators for prevention of *Ae. aegypti* bites may be impaired. However, these experiments were conducted at a temperature ranging from 20.9-25.5°C, which is optimal for transfluthrin evaporation. Wagman *et al.* demonstrated that subsequent exposure of *Aedes Aegypti* to transfluthrin resulted in mosquitoes that were less likely to be repelled by transfluthrin passive emanator in the dual chamber test. Implies that the efficacy of these emanators may be impaired in the area with confirmed pyrethroid-resistant *Aedes* mosquitoes.

In this study, we have demonstrated that the BGS positioned 10 meters away did not significantly protect a person from mosquito bites. However, previous experiments have shown that the BGS used alone, is an effective trap for sampling *Ae. aegypti* (Maciel-de-Freitas et al., 2006, Mingote et al., 2013, Krockel et al., 2006). In this experiment, the BGS was placed near a human volunteer and they were the only "hosts" available. This demonstrated that the human cues were significantly more attractive to *Aedes* than the cues from the BGS. Because preliminary work in the semi-field system indicated that the BGS caught many *Aedes* in the absence of the human volunteers revealing that the efficacy of BGS is relative to the proximity and density of humans. This has also been observed in other studies with humans outcompeting traps at short-range (Okumu et al., 2010) and that whole human odour is optimally attractive to anthropophagic mosquitoes (Hawkes et al., 2017). While the BGS did not provide personal protection by reducing human-vector contract as the removal trap, it could still provide some level of community protection if used on a larger scale, although other traps such as the autocidal gravid trap may be more feasible for removal trapping (Barrera et al., 2014) as they don't require carbon dioxide.

The number of mosquitoes successfully caught by the BGS during the push-pull or the pull-only configuration was the same. While this showed that transfluthrin did not actively push mosquitoes into the trap it also indicated that transfluthrin exposure outdoors does not inhibit mosquitoes entering the BGS. This is contrary to Salazar *et al* who reported that exposing mosquitoes to transfluthrin significantly lowered trap catches (Salazar et al., 2013). Trap catches were not affected if the mosquitoes were allowed to recover for 12 h before BGS trap evaluation (Salazar et al., 2013). This suggests that the mode of action of transfluthrin is dose and distance-dependent. The use of a higher dose could be further optimized to prevent the diversion of repelled mosquitoes from repellent users to non-users in a community (Maia et al., 2016).

We have shown that FTPE remains protective for three months following impregnation. This is a relatively short duration compared to the previous studies which were conducted against malaria vectors demonstrating that transfluthrin-treated strips remain protective for up to six months against Anopheles mosquitoes (Ogoma et al., 2017, Ogoma et al., 2012b). A possible explanation for these differences could be due to the variation in transfluthrin dosage, mosquito species, and the distance from the emanator where HLC was performed. In the current study, 10.5 g of transfluthrin (5.25g on each of the two FTPEs) was used against *Ae. aegypti* and HLC conducted 3 meters from the emanators, whereas in the study by Ogoma *et al.,* the volunteer sat at 1 meter from a strip enclosing them on all four sides at an application of 15.1 g transfluthrin against Anopheles mosquitoes (Ogoma et al., 2017, Ogoma et al., 2012b). In general, the efficacy of the emanators in both studies decreases over time as a result of the loss of transfluthrin due to evaporation. To ensure the long-term efficacy of the FTPEs, a double layer of the hessian strips could be used or transfluthrin doses increased, provided they remain within the margin of safety for chronic inhalation exposure (WHO, 2006). The FTPEs is a simple proof of concept prototype and further work is required to develop a product for use as a public health intervention, including standardizing the release rate of the transfluthrin through standardization of the material upon which the transfluthrin is applied and improvement of the delivery unit to ensure it is cost-effective. This may also include the application of UV protection to the transfluthrin-treated material to prolong its efficacy outdoors (Maia et al., 2012b).

The use of FTPE improves user compliance, as they are movable and the replacement rate is every three months. This potentially avoids the problems associated with personal topical repellents that require a daily application but tend to be applied only when people notice mosquito bites (Lalani et al., 2016), resulting in a lack of public health benefit (Maia et al., 2018). As the device is intended to provide protection at the household level, it is likely to provide a convenient approach to bite prevention outside of sleeping hours and to be more acceptable among community members for the protection of the whole family (Sangoro et al., 2014a). While topical repellents are logistically prohibitive to use (Heng et al., 2015), the FTPEs are portable and easy to use which facilitates round-the-clock protection at the desired location in the peridomestic area. Therefore, they are suitable for targeted distribution among high-risk populations such as those reported to harbor *Aedes* breeding sites during the outbreak (Ali et al., 2003). Furthermore, the FTPEs do not produce smoke common to other methods of delivering transfluthrin such as mosquitoes coil or mat. Also, with FTPEs, ribbons that have been impregnated with transfluthrin cannot be accessed by children or animals.

Dengue tends to be focal in transmission with *Aedes* commonly having a short flight range although there are exceptions (Vavassori et al., 2019). Therefore, transmission is primarily mediated between locations by the movement of infected individuals (Stoddard et al., 2013). These devices are portable and may be deployed anywhere; they could be very useful if provided to those with confirmed dengue, deployed in entrance points (port and airport) where travelers are coming in from other countries or in places where new cases are suspected/outbreak is reported, such as markets (Mboera et al., 2016). The high mosquito toxicity of transfluthrin is an important feature of this tool, as it has the potential to kill a substantial proportion of mosquitoes that encounter the insecticide and reduce vector densities and vectorial capacity. Further work into the impact of such devices on the mortality of free-flying mosquitoes is recommended.

There were two limitations of this study including the use of laboratory-reared mosquitoes. Laboratory-reared mosquitoes may not represent what is happening in the field with wild mosquito populations therefore these findings may not applied to the real world. Also, the data for the longevity experiment were collected at 0, 3, and 6 months only. Whereas significant protective efficacy (44%) was observed up to three months after impregnation of the FTPEs. With this experimental design, we missed the exact time point (between 3 and 6 months) when the FTPEs stopped providing significant protection. We recommend that future studies conducting the same kind of experiment need to conduct weekly or monthly evaluations in order to provide a more precise estimate of efficacy over time, especially when testing the label claims of long-lasting spatial repellent products. Moreover, we only evaluated one distance setup for the use of the “push-pull” in the SFS. In the field, the positioning of this system may vary due to the local building layout resulting in varying protection levels. We recommend that further studies on push-pull should 1) focus on improving the attraction of pull components, since they need to outcompete humans and 2) explore optimal positioning of components to determine the effective distance at which the push and pull could work synergistically.

3.6 Conclusions

In this study, we have demonstrated that FTPEs have the potential to reduce bites from *Ae. aegypti* mosquitoes for up to three months. Using a combination of passively emanated transfluthrin and BGS as a push-pull did not provide any additional protection, with the majority of the protection originating from the push component. Additional work is needed in the field and through mathematical modeling to determine if the number of mosquitoes caught in the BGS would provide additional community protection. Also, as the protective efficacy of the FTPE and the push-pull are similar, it is convenient to use the push alone for the control of *Ae. aegypti* and other outdoor-biting mosquitoes. However, the use of push-pull in the community would be very advantageous as the pull component decreases the mosquito's density while the push component reduces the human landing rate. The FTPEs are portable and easy to use which facilitates round-the-clock protection at the desired location in the peridomestic area for much longer than most currently available personal protection methods.

3.7 Declarations

Ethics approval and consent to participate

The volunteers who participated in this experiment were IHI employees, fully trained and skilled in performing HLC. They were recruited voluntarily through written informed consent after the risks and benefits of the study procedures and their right to leave at any time during the study were clearly explained. All the mosquitoes used in this experiment were laboratory-reared and free from arboviral diseases. The study was approved by the IHI Institute Review

Board (IHI-IRB) and the National Institute for Medical Research –Tanzania (NIMR) with certificates number IHI/IRB/No: 024-2016 and NIMR/HQ/R.8a/Vol.IX/2381 respectively.

Additional file1

Dataset from the semi field evaluation of the push pull for the control of outdoor biting mosquitoes that support the conclusion of this article.

Consent for publication

Permission to publish this work was granted from the Director General of NIMR.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files]

Competing interest

The authors declare that they have no competing interests. SJM, JKS and CS conduct contract product evaluation of a number of vector control tools.

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Authors' contributions

MT, AS and SJM: conceived and designed the study. MT and HC: supervision of semi-field experiment, volunteers and data collection. MT AS, JKS, and SJM: data analysis. MT: draft the manuscript. AS, CS and SJM: revised the manuscript. CS: drew the schematic representation of the semi field system experiment. MMT and JDM: designing and drawing of the FTPE. All authors read and approved the final manuscript.

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Chapter 4: Transfluthrin Eave Positioned Targeted Insecticide (EPTI) reduces human landing rate of pyrethroid-resistant and susceptible malaria vectors in a semi-field simulated peridomestic space

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4.1 Abstract

Introduction: Volatile pyrethroids (VPs) are proven to reduce human–vector contact with mosquito vectors. With increasing resistance to pyrethroids in mosquitoes, the efficacy of VPs such as transfluthrin may be compromised. Therefore, experiments were conducted to determine if the efficacy of transfluthrin eave-positioned targeted insecticide (EPTI) depends on the resistance status of malaria vectors.

Methods: Ribbons treated with 5.25 g transfluthrin or untreated controls were used around the eaves of an experimental hut as EPTI inside a semi-field system. Mosquito strains with different levels of pyrethroid resistance were released simultaneously, recaptured by means of human landing catches (HLCs) conducted 2.5 m outside the hut, and monitored for 24-hour mortality. Technical-grade (TG) transfluthrin was used, followed by emulsifiable concentrate (EC) transfluthrin and additional mosquito strains. Generalized linear mixed models with binomial distribution were used to determine the impact of transfluthrin and mosquito strain on mosquito landing rates and 24-hour mortality.

Results: A significant interaction between strain and treatment indicated that the effect of the transfluthrin EPTI varied between the three strains under investigation ($P < 0.001$). Whether TG or EC, EPTI significantly reduced the odds of landing of pyrethroid-susceptible mosquitoes *Anopheles gambiae* (Ifakara) and *An. gambiae* (Kisumu) and of pyrethroid-resistant mosquitoes *An. arabiensis* (Mbita), *An. gambiae* Kisumu knockdown-resistant (Kisumu-kdr) and *An. arabiensis* (Kingani), with PE > 40% for all strains ($P < 0.001$). In the control, *An. gambiae* mosquitoes were more likely to land than *An. arabiensis* ($P < 0.05$).

Conclusions: This study confirms that the efficacy of EPTI was not dependent on mosquito pyrethroid resistance status. However, it remains unclear whether resistance to pyrethroids undermines the efficacy of transfluthrin for bite prevention. It is important to consider mosquito anthropophagy, strain, years of colonization and fitness when assessing vector control interventions. Overall, these findings suggest that transfluthrin-treated EPTI could be useful in areas with highly pyrethroid-resistant mosquitoes. At this dosage, transfluthrin EPTI cannot be used to kill exposed mosquitoes.

Keywords: volatile pyrethroid, transfluthrin, pyrethroid resistance, eave-positioned targeted insecticide, EPTI, *Anopheles gambiae* s.s., *Anopheles arabiensis*, semi-field system

4.2 Introduction

Indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) are currently the core mosquito vector control tools employed in national malaria control programs worldwide (Bhatt et al., 2015). Since 2000, global malaria incidence has decreased by 37% and mortality by 60% (Cibulskis et al., 2016), to which these tools have contributed approximately 70% of the reduction (Bhatt et al., 2015). However, there are concerns that progress has stagnated; the downward trend in malaria cases flattened and malaria increased in several countries between 2015 and 2019 (WHO, 2020b). Increased transmission in some areas where elimination was considered to be feasible has also been observed (Temu et al., 2012, Reddy et al., 2011). This increase is likely caused by insufficient coverage and use of core interventions, with fewer than half of households in sub-Saharan Africa owning enough nets for all occupants (WHO, 2020b). Progress may also be impeded by limitations of the core interventions and their effectiveness in certain settings. For example, the current tools do not provide complete protection outdoors in the peridomestic area, where humans and vectors frequently come into contact before bedtime (Monroe et al., 2019b). Furthermore, the development of physiological resistance (Hancock et al., 2018) in mosquito vectors may undermine the continued efficacy of IRS and LLINs (Sougoufara et al., 2017).

The development of alternative control strategies that cover the existing gaps and that complement core control tools remains necessary (Sougoufara et al., 2020). Proposed measures include spatial repellents (SR) (Ogoma et al., 2017, Masalu et al., 2017), genetically engineered mosquitoes (Benelli et al., 2016), attractive targeted (toxic) sugar bait (ATSB) (Marshall et al., 2013) and endectocides such as ivermectin (Chaccour et al., 2015). The focus of this study is SR from the pyrethroid class often referred to as volatile pyrethroids (VPs). VPs vaporize at room temperature and are dispersed into the surrounding area with the aim of creating a bite-free space (Achee et al., 2012a), and they can be used indoors and outdoors. Previous studies have demonstrated that VPs such as transfluthrin and metofluthrin are effective at reducing the human landing rate (HLR) of a range of mosquitoes (Bibbs and Kaufman, 2017). Passive emanators treated with transfluthrin or metofluthrin consistently demonstrated personal protective efficacy exceeding 50% in studies conducted in Cambodia (Liverani et al., 2017), Tanzania (Kawada et al., 2008), Belize (Wagman et al., 2015b) and Indonesia (Syafruddin et al., 2020). Transfluthrin applied to hessian strips as an eave-positioned targeted insecticide (EPTI) has provided over 68% reduction in human vector contact in semi-field studies (Ogoma et al., 2014b, Ogoma et al., 2017) and over 80% in field studies in Tanzania (Masalu et al., 2017, Ogoma et al., 2017). Volatile pyrethroids exhibit a dose-response, with lower concentrations eliciting behavioral effects that include deterrence, excito-repellency, and blood-feeding inhibition (Ogoma et al., 2014a) and with higher concentrations or longer exposure times increasing knockdown and mortality (Ten Bosch et al., 2018).

Pyrethroid insecticides have been the main class of insecticide used in LLINs and IRS (Zaim et al., 2000). Resistance to these insecticides is now widespread (Mitchell et al., 2012), which poses a threat not only to the efficacy of LLINs and IRS but potentially also to VPs. Furthermore, effective, long-lasting volatile insecticides of chemical classes other than pyrethroids are not yet available for public health use (Norris and Coats, 2017). It is necessary to know whether the efficacy of VPs may be compromised by pyrethroid resistance and, therefore, if VPs can be used in areas with existing pyrethroid-resistant mosquito populations. VPs are from the same chemical class, which would normally indicate cross-resistance; however, structural differences between transfluthrin and non-volatile pyrethroid indicate that cross-resistance may not occur (Horstmann and Sonneck, 2016). Therefore, the objectives of this study were to determine (1) the efficacy of transfluthrin applied as EPTI to reduce HLR of multiple strains of Afrotropical malaria vectors with varying levels of pyrethroid resistance and (2) delayed mortality induced by EPTI exposure.

4.3 Methods

Study Site

The experiment was conducted in a semi-field system (SFS) located in Bagamoyo, Tanzania, from March 2018 to October 2018 and from August 2019 to September 2019. The SFS measures 21 × 29 × 4.5 m and is divided into three compartments. Two heavy-duty polyethylene walls separate these compartments, preventing air movement between the chambers and reducing the chance of cross-contamination when working with VPs or other aerosols. The SFS allows for controlled experiments with disease-free mosquitoes to be conducted under field-like climatic conditions (Ferguson et al., 2008). In each compartment, an experimental hut (Okumu et al., 2012) was constructed, and tests were conducted outside the huts to simulate a peridomestic space.

Study Mosquitoes

Five laboratory-reared mosquito strains were used in these experiments: (1) pyrethroid-susceptible *Anopheles gambiae* s.s. (Kisumu strain) and (2) *An. gambiae* s.s. (Kisumu-kdr strain) with L1014S *kdr*, i.e., kdr-east resistance mechanism (Stump et al., 2004), both originating from Kisumu, Kenya; (3) pyrethroid-susceptible *An. gambiae* s.s. (Ifakara strain) originating from Ifakara, Tanzania, and in a colony at IHI since 1996; (4) pyrethroid-resistant *An. arabiensis* (Mbita strain) from the International Centre of Insect Physiology and Ecology (ICIPE), Kisumu, Kenya, expressing a moderate level of phenotypical resistance against permethrin and deltamethrin (the mechanism is likely metabolic but not confirmed); and (5) *An. arabiensis* (Kingani strain) originating from Ifakara and in the colony at Bagamoyo since 2015, expressing a high level of phenotypical resistance against permethrin and deltamethrin (Matowo et al., 2017). The two *An. arabiensis* strains have been tested and found to be free of *kdr* mutations (L1014F *kdr-west* and L1014S *kdr-east*) (unpublished data) commonly associated with pyrethroid resistance. It is likely that the metabolic resistance mechanism was responsible for their survival in the presence of pyrethroid insecticides.

Before the start of semi-field experiments, susceptibility tests were conducted for each mosquito strain using tube test bioassays performed following World Health Organization (WHO) guidelines (WHO, 2018). Non-blood-fed 3- to 5-day-old mosquitoes were exposed to insecticide-impregnated papers at the standard WHO discriminating dose for the pyrethroids permethrin (0.75%) and deltamethrin (0.05%). These insecticides were selected because they belong to the same chemical class as transfluthrin and are commonly used on LLINs.

All mosquito strains are maintained at the Bagamoyo branch of the Ifakara Health Institute (IHI) according to MR4 guidelines (MR4, 2009). Larvae are fed on fish food (TetraMin® tropical flakes) and adult mosquitoes on 10% sucrose *ad libitum*. Bovine blood meals are provided to adult females for egg production using membrane-feeding assay. The insectary is maintained at 27 ± 5 °C and 70–100% relative humidity with approximately 12:12 light: dark (ambient lighting).

The experiments used 3- to 8-day-old female mosquitoes that had never blood-fed. The mosquitoes were sugar starved for 6 hours prior to the experiment. Because more than one mosquito strain with the same morphology was released simultaneously, red and yellow fluorescent pigments (Swada, Cheshire, UK) were used to differentiate between strains. Mosquitoes were marked in a cup by dusting the mesh lid of the cup with a brush containing the colour pigment; thereby creating a cloud of pigment that was transferred to the mosquitoes in small amounts. Preliminary experiments indicated that the fluorescent pigments did not influence mosquito survival or feeding behaviors.

Preparation of transfluthrin eave-positioned targeted insecticide (EPTI)

Hessian material has proved very useful for the delivery of transfluthrin because it has a much slower release rate than other textiles and thus increases the longevity of the VP device (Ogoma et al., 2014b, Mmbando et al., 2018, Mmbando et al., 2019). Hessian sacks were purchased locally, washed using well water and powder detergent (OMO®, Unilever, Nairobi, Kenya), dried under direct sunlight and then cut into 21 m × 10 cm strips. The hessian was treated with either TG or EC transfluthrin formulations (Bayothrin EC, Bayer AG, Monheim am Rhein, Germany). The experiments were initially conducted using TG transfluthrin emulsified with 100 ml of Tween®20 (Sigma-Aldrich, CAS #9005-64-5). Bayer developed and introduced EC transfluthrin which was used for further experiments. In all experiments, with either formulation, 5.25 g of transfluthrin was impregnated into hessian equivalent to 2.5 g/m^2. Drying took place out of direct sunlight to protect the transfluthrin from photolysis by exposure to ultraviolet light (WHO, Horstmann and

Sonneck, 2016). For the control arms, the strips were prepared in the same manner as the treated strips but with only water. During the day, the treated hessian was kept out of direct sunlight at the ambient outdoor temperature (24–27.6 ºC) on a metal frame.

Experimental procedure

The primary aim of the study was to determine if pyrethroid resistance in mosquitoes has a negative impact on the efficacy of transfluthrin EPTI. To do this, the treated hessian was placed on the eaves gaps of experimental huts located in the SFS, out of direct sunlight (Fig. 4.1a). Applying insecticide in this targeted way exploits the natural movement of air rising inside houses and being funneled out through the eaves, over the treated hessian, and into the peridomestic space, helping to disperse insecticide.

Human landing catches (HLC) were conducted 2 m outside the experimental hut (Figure 4.1b and c) to mimic the peridomestic environment. Mosquitoes were released outside the experimental hut at every corner of the SFS compartment, eliminating directional bias in their approach to the human volunteer. Three separate experiments were conducted to evaluate the efficacy of (1) TG transfluthrin EPTI against Ifakara strain, Mbita strain and Kingani strain mosquitoes; (2) EC transfluthrin EPTI against Ifakara strain, Mbita strain and Kingani strain mosquitoes; and (3) EC transfluthrin EPTI against Kisumu strain and Kisumu-kdr strain mosquitoes.

During each experiment, either transfluthrin EPTI or the control (water-treated hessian) was assigned to one of two separate compartments of the SFS. The treatments remained fixed for a block of four days, after which they were rotated. HLC volunteers rotated between compartments daily. Four volunteers were recruited but only two were used each day. The experiment was conducted for 4 blocks over 16 days, after which each volunteer conducted HLC for each treatment 4 times in each compartment. The volunteers were rotated to control for any bias caused by individual attractiveness to mosquitoes [25]. Prior to the start of the experiment, for acclimatization, mosquitoes were transferred from the insectary to the middle compartment of the SFS 30 min before their release.

Each day 80 mosquitoes of each strain were introduced into each compartment. Mosquitoes were separated into batches of 20 per strain and placed into 4 release cages, one in each corner of each compartment. The mosquitoes were released remotely by gently pulling strings connecting the release cages to simulate mosquitoes approaching the peridomestic space from multiple directions.

Throughout the experiment, volunteers wore shorts, covered shoes, and bug jackets to standardize the area available for mosquito landings. Mosquitoes that landed on the area between the ankle and the knee were collected using mouth aspirators through HLC (Figure 4.1b). Mosquitoes were recaptured continuously for 50 minutes every hour for 4 consecutive hours between 18:30 and 22:30 hrs. Each hour, a new collection cup was used and labeled with the time and date. These mosquitoes were transferred to the insectary after 4 hours, supplied with 10% sucrose, and held for 24 hours to observe 24-hour mortality.

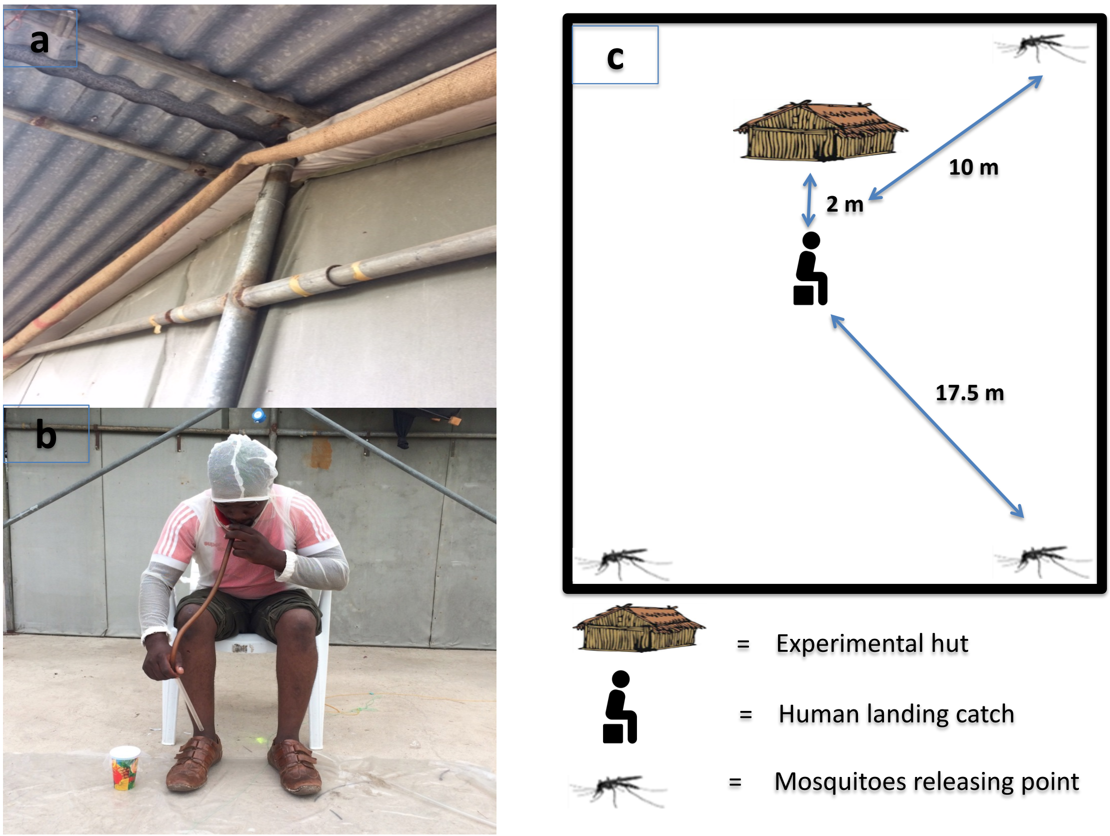

Figure 4.1: The evaluation of transfluthrin EPTI in the semi-field system (a) Yellowish strips represent transfluthrin hessian strip position on the eave " EPTI" (b) A volunteer sitting outside the experiment hut conducting HLC (c) The schematic representation of the experiment inside a compartment of the semi-field system.

Sample size

Sample-size calculations were performed using simulation-based power analysis [25] in R statistical software version 3.02 (http://www.r-project.org) with a significance level of 0.05 for rejecting the null hypothesis. Data analysis for experimental data was planned to be conducted using generalised linear mixed models (GLMMs) (Bates et al., 2015). Therefore, 1000 simulations of GLMMs approximating those used to analyse project data were run using a 2 × 2 Latin square design with volunteers rotating nightly.

The power to predict the difference in mosquito landings between control and treatment was estimated as the proportion of the 1000 simulated data sets in which the null hypothesis was rejected when the GLMM was run. The simulations indicated that with an estimated 80 mosquitoes released per compartment per night and 60% recapture of released mosquitoes, there was 100% chance of detecting a 50% reduction in mosquito landings in the treatment arm after 16 nights of experimentation. Inter-observational variance among daily experiments was set at 5%, and variability between times based on previous experiments was set at 25%.

Data Analysis

Data were recorded on paper forms and double-entered into Microsoft Excel. Cleaning and analysis were done in Stata 13 (StataCorp). For the WHO insecticide susceptibility tests, data were summarised as mean percentage (%) 24-hour mortality of the four replicates and reported with 95% confidence intervals.

Data for each experiment using each transfluthrin formulation (EC or TG) were analyzed separately.

The relative effect of transfluthrin on HLR and 24-hour mortality for different mosquito strains were investigated using GLMM with the binomial distribution. For HLR, the dependent variable was the proportion of released mosquitoes that were recaptured. For mortality, the dependent variable was the recaptured proportion that died. Treatment, mosquito strain, compartment, and volunteer were included as fixed categorical variables, with day included as a random effect. An interaction term between mosquito strain and treatment was included to determine if the effect of treatment varied between mosquito strains.

The protective efficacies of the transfluthrin EPTI against each mosquito strain were calculated as

Protective efficacy (PE) = $[(C - T)/C] \times 100\%$, where *C* stands for the number of mosquitoes landing in the control and *T* for the number of mosquitoes landing in the treatment. The PE was calculated for each day, and the mean proportion of mosquitoes landing was reported with 95% confidence intervals (CI). For 24-hour mortality, the control-corrected mortality was calculated as

Control mortality = $(T - C)/(1 - C) \times 100\%$,

where *C* and *T* represent the percentage mortality among mosquitoes landing in the control and treatment, respectively. The control-corrected mortality was calculated for each day, and the mean percentage dead was reported with 95% CI.

4.4 Results

WHO insecticide susceptibility tests

The susceptibility status of each mosquito strain to permethrin and deltamethrin is presented in Table 1. *An. gambiae* Ifakara and Kisumu strains were fully susceptible. *An. arabiensis* Kingani, *An. arabiensis* Mbita and *An. gambiae* kdr were found to be resistant to pyrethroid. *An. gambiae* kdr was susceptible to deltamethrin.

Table 4.1. KD and 24-hour mortality of the malaria vectors tested during the WHO insecticide susceptibility test

Mosquitoes	**Insecticides**	**Concentration** (%)	**24-hour mortality* (%)** (95% CI)
Kisumu susceptible	Permethrin	(0.75)	100 (100–100)
	Deltamethrin	(0.05)	100 (100–100)
Kisumu-kdr	Permethrin	(0.75)	98.9 (95.8–100)
	Deltamethrin	(0.05)	100 (100–100)
Ifakara strain	Permethrin	(0.75)	100 (100–100)
	Deltamethrin	(0.05)	100 (100–100)
Mbita strain	Permethrin	(0.75	72.6 (59.9–87.9)
	Deltamethrin	(0.05)	71.1 (53.1–95.2)
Kingani strain	Permethrin	(0.75)	19.7 (10.1–38.6)
	Deltamethrin	(0.05)	24.4 (13.5–44.8)

* 24-hour mortality is defined as the proportion of dead after 24 hours out of the total number of mosquitoes exposed. Proportion mortality is reported with 95% confidence interval.

The efficacy of the transfluthrin EPTI against different mosquito strains

In experiment 1 with TG transfluthrin, a significant interaction between strain and treatment was observed. This indicated that the effect of the transfluthrin EPTI varied between strains under investigation ($P < 0.001$; Table 4.2). The use of TG transfluthrin EPTI significantly reduced the odds of landing on pyrethroid-susceptible *An. gambiae* (Ifakara strain; OR = 0.22 [0.18 – 0.26], $P < 0.001$) and had a similar impact on the landing of highly pyrethroid-resistant *An. arabiensis* (Kingani; OR = 0.23 [0.19 – 0.27], $P < 0.001$; Table 4.3). However, while the TG transfluthrin EPTI reduced the landing of pyrethroid-resistant *An. arabiensis* (Mbita), it did so to a lesser extent (OR = 0.33 [0.28 – 0.39], $P < 0.001$; Table 4.3). When assessing the efficacy of the EPTI using PE, the PE was similar for susceptible Ifakara 46.2 (95% CI: 45.6–65.5), moderately resistant Mbita 46.4 (95% CI: 37.9–54.9) and the highly resistant Kingani strain 54.9 (95% CI: 41.6–64.1; Table 3.3). The binomial GLMM for TG transfluthrin indicated that both volunteers 3 and 4 and the compartment significantly influenced HLR (in both cases, $P < 0.05$; Table 2).

In experiment 2, using EC transfluthrin EPTI, there was again a significant interaction between strain and treatment, although a different trend was observed (Table 4.2). As with TG, the EC transfluthrin EPTI was observed to reduce the odds of landing for susceptible *An. gambiae* (Ifakara strain; OR = 0.17 [0.14 – 0.20], $P < 0.001$) and pyrethroid-resistant *An. arabiensis* (Mbita; OR = 0.23 [0.19 – 0.27], $P < 0.001$). However, EC transfluthrin showed lower efficacy against *An. arabiensis* (Kingani; OR = 0.57 [0.42 – 0.78], $P < 0.001$; Table 4.3). The model also indicated that the compartment significantly influenced the HLR of the mosquitoes (OR = 0.79 [0.71–0.87], $P < 0.001$). None of the volunteers influenced HLR ($P > 0.05$; Table 2).

Table 4.2. Generalised linear model output estimating the effect of EC/TG transfluthrin and mosquito strain on human landing rate in the semi-field system

	Experiment 1, TG transfluthrin- (5.25 g)		Experiment 2, EC transfluthrin (5.25 g)		Experiment 3, EC transfluthrin (5.25 g)	
Variables	**OR***	***P*-value**	**OR***	***P*-value**	**OR***	***P*-value**
Treatment						
Control	1		1		1	
Transfluthrin	0.22 (0.18–0.26)	< 0.001	0.10 (0.08–0.12)	< 0.001	0.14 (0.12–0.17)	< 0.001
Strain (In control)						
Ifakara strain (Susceptible)	1		1			
Mbita strain (Metabolic)	0.43 (0.32–0.57)	< 0.001	0.34 (0.26–0.46)	< 0.001	–	–
Kingani strain (Metabolic)	0.60 (0.45–0.80)	< 0.001	0.44 (0.33–0.59)	< 0.001	–	–
Kisumu susceptible	–	–	–	–	1	
Kisumu kdr	–	–	–	–	1.0 (0.86–1.17)	0.05
Volunteers						
Volunteer 1	1		1		1	
Volunteer 2	0.88 (0.69–1.14)	0.36	1.07 (0.84–1.39)	0.60	1.20 (0.99–1.46)	0.06
Volunteer 3	0.76 (0.59–0.98)	0.04	0.96 (0.74–1.24)	0.77	1.19 (0.98–1.44)	0.07
Volunteer 4	0.83 (0.72–0.95)	0.001	0.90 (0.78–1.04)	0.17	1.14 (0.94–1.36)	0.18
Compartment						
Compart 1	1		1		1	

Table 4.3. The adjusted odds ratio of mosquito landings and protective efficacy offered by EC and TG transfluthrin in the semi-field system, Bagamoyo

Transfluthrin EPTI	Mosquitoes	Landing in the presence EPTI		Landing in the control (reference)		
		n (% landing [95% CI])[a]	OR (95% CI)	*n* (%l anding [95% CI])[a]	OR (95% CI)	Protective efficacy (% [95% CI])
TG	Ifakara strain	500 (39.0 [32.9–45.2])	0.22 (0.18–0.26)*	939 (73.4 [66.9–79.8])	1	46.2 (45.6–65.5)
	Mbita arabiensis	370 (29.5 [24.4–34.7])	0.33 (0.28–0.39)*	706 (55.2 [51.7–58.6])	1	46.4 (37.9–54.9)
	Kingani strain	378 (28.9 [22.4–35.4])	0.23 (0.19–0.27)*	804 (62.8 [56.4–69.2])	1	54.9 (41.6–64.1)
EC	Ifakara strain	341 (26.6 [21.2–32.1])	0.17 (0.14–0.20)*	980 (76.6 [70.3–82.9])	1	65.0 (57.0–72.2)
	Mbita arabiensis	224 (17.5 [12.2–22.8])	0.23 (0.19–0.27)*	697 (54.5 [51.9– 57.0])	1	67.6 (57.6–77.6)
	Kingani strain	347 (27.1 [20.5–33.7])	0.57 (0.42–-0.78)*	774 (60.5 [56.6–64.4])	1	55.6 (45.6–65.5)
EC	Kisumu susceptible	166 (12.9 [9.6–16.3])	0.14 (0.11–0.17)*	647 (50.5 [50.0–51.0])	1	74.3 (67.7–80.9)
	Kisumu kdr	164 (12.8 [9.6–16.0])	0.14 (0.11–0.17)*	648 (50.6 [50.1–51.1])	1	75.1 (69.2–82.2)

[a] Numbers in the control and treatment refer to the total number of mosquitoes caught/released during each experiment; the percentage recaptured is in bracket. The percentage landing was calculated by dividing the number recaptured (*n*) by the total released (*N* = 1280). The OR is adjusted for temperature, humidity, compartment, volunteers and all other factors in the table.

Table 4.4. The adjusted odds ratio of mosquito landings and protective efficacy offered by EC and TG transfluthrin in the semi-field system, Bagamoyo

Transfluthrin EPTI	Mosquitoes	Landing in the presence of EPTI		Landing in the control	
		n (% landing [95% CI])[a]	OR (95% CI)	n (% landing [95% CI]) [a]	OR (95% CI)
TG	Ifakara strain	500 (39.0 [32.9–45.2])	1	939, (73.4 [66.9-79.8])	1
	Mbita arabiensis	370 (29.5 [24.4–34.7])	0.65 (0.49-0.86)*	706 (55.2 [51.7-58.6])	0.43 (0.32-0.57)*
	Kingani strain	378 (28.9 [22.4–35.4])	0.62 (0.47-0.83)*	804 (62.8 [56.4-69.2])	0.60 (0.45-0.80)*
EC	Ifakara strain	341 (26.6 [21.2–32.1])	1	980(76.6 [70.3-82.9)	1
	Mbita arabiensis	224 (17.5 [12.2–22.8])	0.58 (0.43-0.78)*	697 (54.5 [51.9- 57.0])	0.34 (0.25-0.46)*
	Kingani strain	347 (27.1 [20.5–33.7])	1.01 (0.76-1.38)	774 (60.5 [56.6-64.4])	0.44 (0.33-0.59)*
EC	Kisumu susceptible	166 (12.9 [9.6–16.3])	1	647 (50.5 [50.0-51.0])	1
	Kisumu kdr	164 (12.8 [9.6–16.0])	0.99 (0.78-1.24)	648 (50.6 [50.1-51.1])	1.00 (0.86-1.17)

[a] Numbers in the control and treatment refer to the total number of mosquitoes caught/released during each experiment; the percentage recaptured is in bracket. The percentage landing was calculated by dividing the number recaptured (*n*) by the total released (*N* = 1280). The ORs are adjusted for temperature, humidity, compartment, volunteers and all other factors in the table. * *P*-value < 0.05

Finally, in the analysis of the data from experiment 3, the interaction was not significant with Kisumu susceptible and kdr strains, indicating that the transfluthrin EPTI reduced landings of the two mosquito species in the same way (Table 2). The odds of landing of Kisumu susceptible and Kisumu kdr were equally reduced (OR = 0.14 [0.11 – 0.17], $P > 0.001$; Table 3).

During the experiments, the average temperature was 27.8 °C (23.8–31.5 °C) and average relative humidity (RH) was 76.5% (63.6–92%).

Effect of species on HLR in the control

The effects of mosquito species on HLR were examined in the control. The two species that originated from wild mosquitoes in Ifakara, Tanzania, were compared. In both experiments, consistently higher catches were observed with the Ifakara strain than with the Kingani strain. For example, in experiment 2, *An. gambiae* s.s. (Ifakara) showed a higher landing proportion, with an average of 76.6% (95% CI: 70.3–82.9), than did *An. arabiensis* (Kingani), with an average of 60.5% (95% CI: 56.6–64.4), and this difference was significant (OR = 0.5 [95% CI: 0.4–0.6], $P < 0.001$; Table 4.4).

Comparison of 24-hour mortality induced by transfluthrin-treated eave ribbon between mosquito strains.

At 5.25 g dosage, no significant difference in 24-hour mortality was observed in the presence of transfluthrin EPTI compared to the control across all mosquito strains ($P > 0.05$).

4.5 Discussion

The efficacy of EPTI to reduce HLR of malaria vectors

This study was conducted to determine if pyrethroid resistance in mosquitoes would have a negative impact on the efficacy of transfluthrin EPTI. Findings showed that *An. arabiensis* Kingani strain mosquitoes expressing high phenotypical resistance to pyrethroids were less repelled than the moderately resistant Mbita strain when using EC transfluthrin. However, Kingani, Mbita and Ifakara strains were equally repelled when using TG transfluthrin. It is therefore unclear how the different levels of metabolic resistance affect the efficacy of transfluthrin EPTI. TG was less effective against Mbita than against the susceptible Ifakara strain (*An. gambiae*), while EC was less effective against both the Mbita and the Kingani strains (*An. arabiensis*). This may indicate that metabolic resistance is indeed detrimental to the efficacy of transfluthrin; however, it is important to be cautioned when comparing species that have different levels of human biting preference (*An. gambiae*, *An. arabiensis*) because it is unknown how this variation affects the efficacy of transfluthrin. This study used *An. gambiae* s.s as a reference strain as the colonization of the susceptible *An. arabiensis* strain was not possible due to widespread resistance.

These results suggest that *kdr* target site mutations do not reduce the efficacy of transfluthrin. However, this finding must be interpreted with caution because the susceptibility test of the mosquitoes used revealed low levels of phenotypic resistance. What is clear from this study is that, compared to the control, transfluthrin EPTI can reduce the landings of resistant mosquitoes. These findings corroborate previous experiments conducted in field settings in Kilombero Valley, Tanzania (Mmbando et al., 2017, Masalu et al., 2017, Ogoma et al., 2017), in which transfluthrin applied to hessian in eaves (at concentrations higher than 5.25 g) significantly reduced HLR by over 80% and as well in the SFS, where the PE was over 68% (Andrés et al., 2015). Andres *et al.,* observed that transfluthrin-treated polyester strips provide significant protection in the semi-field using one species of mosquito that was moderately resistant to pyrethroid (Andrés et al., 2015). Furthermore, transfluthrin-treated eave ribbon provided protection in Kilombero Valley, where malaria transmission is transmitted by *An. arabiensis* and *An. funestus* mosquitoes (Lwetoijera et al., 2014), which were confirmed to be highly resistant to pyrethroid (Matowo et al., 2017). Methodologies used by these previous experiments were not designed to directly compare the differences in HLR between pyrethroid-susceptible and resistant mosquitoes. This study, however, provides a unique opportunity to compare the efficacy of transfluthrin applied as EPTI across different mosquito strains expressing different types and levels of insecticide resistance. Much more work is needed in this area, looking at a wider range of mosquito strains and resistance mechanisms.

It is known that the structural differences between VPs such as transfluthrin, which contain tetra fluoro benzyl alcohol, and non-VPs, such as permethrin, which contain phenoxy benzyl alcohol, may explain the efficacy of transfluthrin against resistant mosquitoes (Bohbot et al., 2011). Hortsman et al. observed that the enzyme responsible for the detoxification of non-VPs is unable to bind to the tetra fluoro benzyl moiety of VPs, leaving them active against resistant mosquitoes (Horstmann and Sonneck, 2016). Further work is needed to determine the mechanism that causes mosquitoes to be repelled by transfluthrin in order to ascertain whether cross-resistance is possible. On the other hand, combining multiple active ingredients in targeted eave applications may help to combat resistant mosquitoes. Strategies could also combine an SR with a chemical that has high-contact toxicity and thus kills those mosquitoes that are not repelled and that are attempting to enter through the eaves. It was observed that mosquitoes attempting to enter houses spend 80% of their time within 30 cm of the eave (Spitzen et al., 2016); thus, adding a second AI may enhance the control of resistant vectors. As has been observed in one study where the addition of the synergist piperonyl butoxide (PBO) can enhance knockdown by mosquito coils treated with a VP (Katsuda et al., 2008).

Despite the reduction of the HRL, inconsistent findings were observed when using PE for measuring efficacy compared to the OR estimates from the model. Such difference may be because OR from the GLMM contains additional explanatory variables that are not considered in calculating the PE. It is therefore suggested that for the evaluation of spatial repellent in the semi-field system, GLMM estimates should be presented rather than the calculated PE. The GLMM estimates are more robust as they account for other variables.

The effect of transfluthrin formulation on HLR

While the EC and TG formulations were not compared directly, the EC did produce higher reductions in HLR. This could be explained by formulation differences that may have resulted in higher release rates and thus in different amounts of transfluthrin available in the air. It is known that differential concentrations of transfluthrin will induce different behaviours, including avoidance, irritancy, knockdown, and mortality (Sukkanon et al., 2020). This dosage-dependent difference in mosquito behavioural response is also observed in other pyrethroid insecticides, including deltamethrin, cyphenothrin, d-tetramethrin, and tetramethrin (Mongkalangoon et al., 2009). The practical advantage of using EC was that it readily dissolves in water, making it more convenient to use, whereas TG transfluthrin required emulsification with detergent to mix with water. Further investigation into transfluthrin formulations is needed to fully inform the policymaker on which formulation should be used.

The influence of species and strain on HLR

In addition to resistance, mosquito landing (HLR) was likely to be influenced by other factors (Figure 4.2). In the absence of transfluthrin, this study observed differences in landing for the two different mosquito species. The Ifakara strain (*An. gambiae*) had a higher proportion of landing than did the Kingani strain (*An. arabiensis*) or the Mbita strain (*An. arabiensis*). Despite having been colonized for more than 10 years on particular Ifakara and Kingani strains, these mosquitoes demonstrated a behaviour seen in wild mosquitoes. Gilles *et al*. conducted an experiment in the field where they observed that *An. gambiae* s.s. were more likely than *An. arabiensis* strains to land on the person conducting HLC, indicating that species differences influence mosquito landing (Gillies, 1964, Curtis CF, 1987). The difference in landing between these mosquito species is caused by differences in attraction to human cues (Gillies, 1964). *An. arabiensis* feed on both human and animals (Mahande et al., 2007) depending on the relative abundance (Asale et al., 2017) or availability (Iwashita et al., 2014) of humans and animals, whereas *An. gambiae* s.s. feed exclusively on humans (Costantini et al., 1999). It is therefore suggested that the anthropophilic behaviour of *An. gambiae* s.s. may influence the landing of these mosquitoes compared to the more opportunistic *An. arabiensis.*

Furthermore, the response of different species to VPs is well documented, with higher doses of transfluthrin needed to elicit escape responses in robust species such as *Aedes aegypti* than in *Anopheles* mosquitoes (Sukkanon et al., 2020) and with different responses of members of the *An. minimus* complex to pyrethroids and DDT (Potikasikorn et al., 2005). It is also known that species vary in their sensitivity to topical repellents (Van Roey et al., 2014). Therefore, in evaluating the efficacy of volatile pyrethroids, it is important to investigate the species and strains that will ultimately be targeted.

The difference in behavioural response of mosquitoes in the presence of repellent may also be associated with age. Studies have demonstrated that younger mosquitoes showed a lower response to topical mosquito repellents (Xue and Barnard, 1996), with very old mosquitoes being more responsive to repellents (Mulatier et al., 2018). This study followed WHO guidance, using younger mosquitoes that are less likely to be affected by pyrethroid exposure (Aldridge et al., 2017). Because the use of young mosquitoes may underestimate the PE of the VP, it is therefore recommended that further work be carried out on the optimal physiological age of mosquitoes to be used in studies of VP.

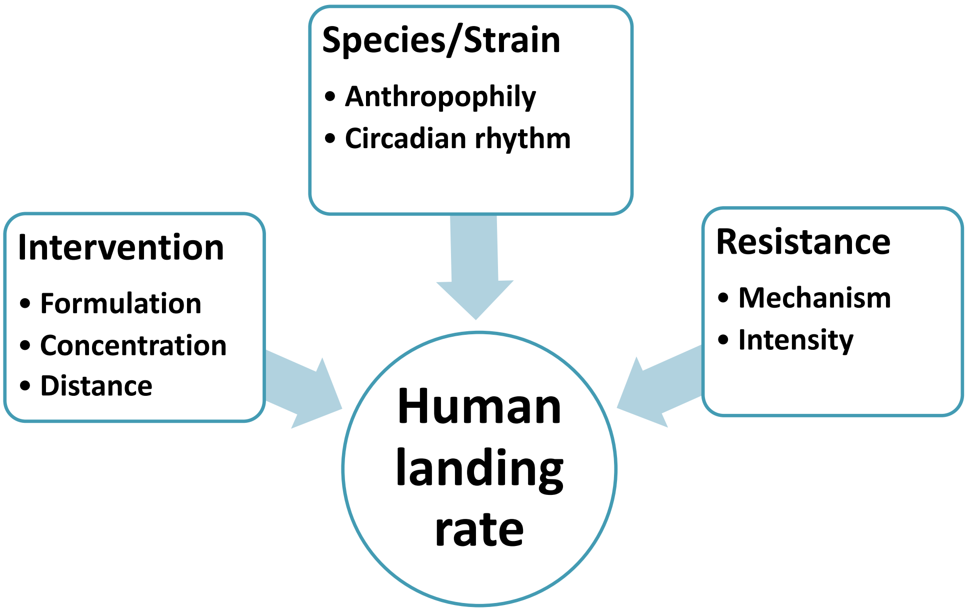

Figure 4.2: Factors shown to influence human landing rate and thus the protective efficacy of the EPTI

24-hour mortality of malaria vectors after exposure to transfluthrin

The transfluthrin dose used in this study did not induce mortality for any of the mosquito strains; therefore, we were unable to determine if there was cross-resistance between traditional pyrethroids and transfluthrin. Exposure to doses above 5.25 g of transfluthrin and long exposure have been associated with increased mortality in exposed mosquitoes (P.R., 1975, Ogoma et al., 2014a), so these higher doses would be required to determine if there is any difference between resistant and susceptible strains. Only those mosquitoes that were recaptured by HLC were examined for 24-hour mortality; therefore, the full impact of transfluthrin on mortality cannot be measured. It is possible that those that did not land may have received a higher and potentially more lethal dose of transfluthrin. While it is useful to know if a mosquito will survive after a bite (and thus potentially go on to transmit disease), a better picture of the efficacy of VPs would be achieved if all mosquitoes were accounted for.

4.6 Conclusion

Transfluthrin EPTI offered protection against all mosquito species regardless of the mosquitoes' level of resistance. However, the differences in effect observed in different mosquito species highlight the fact that resistance in mosquitoes may be detrimental to the efficacy of transfluthrin. These findings demonstrated that transfluthrin-treated EPTI could be used to control malaria in areas with pyrethroid-resistant mosquitoes. Although this study suggests that EPTI reduces human landing rate for both mosquitoes, additional evidence is needed to determine whether resistance in mosquitoes is detrimental to the efficacy of transfluthrin. This is particularly important in areas where transfluthrin will be considered for the control of mosquito vectors (Syafruddin et al., 2020).

4.7 Declarations

Ethics approval and consent to participate

Permission to conduct these experiments was granted by ethical review committees at Ifakara Health Institute (IHI/IRB/No: 024-2016) and the National Institute for Medical Research (NIMR/HQ/R.8a/Vol.IX/2381). The volunteers participating in these experiments were IHI employees skilled in performing HLC. They were recruited voluntarily with written informed consent after the risks and benefits of the study procedures and their right to leave at any time during the study was clearly explained. All mosquitoes used in this experiment were laboratory-reared with low risk of transmitting malaria parasite.

Consent for publication

The Director General of NIMR granted permission to publish this work.

Availability of data and materials

Data generated and analysed for this study are included in this article and its supplementary information files. (Additional file 1)

Competing interests

The authors declare that they have no competing interests. SJM and UAK conduct contract product evaluations of a number of vector control tools.

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Authors' contributions

SJM, AS and MMT conceived the study; AS and MMT performed the data collection; LH performed the molecular susceptibility assay for the *An. arabiensis* mosquitoes; AS, SJM, MMT and UAK performed data analysis; MMT wrote the manuscript; AS and SJM revised the manuscript. SJM and AS critically revised the final draft. All authors revised the final draft.

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Chapter 5: Human landing catches (HLC) provide a useful measure of Protective Efficacy for evaluating volatile pyrethroid spatial repellents (VPSR).

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5.1 Abstract

Background

Human landing catches (HLC), where human volunteers collect mosquitoes that land on them before they can bite, are used to quantify human exposure to mosquito disease vectors. Comparing HLC in the presence and absence of interventions such as repellents is often used to measure protective efficacy (PE). Some repellents have multiple actions including feeding inhibition so that mosquitoes may be unable to bite even if they find a host and land. Here, a comparison of PE of the volatile pyrethroid spatial repellent (VPSR) transfluthrin was conducted using either HLC "landing" or allowing landed mosquitoes to blood-feed "biting" to evaluate whether HLC estimates the personal protective efficacy of VPSR.

Method

A fully balanced, two-arm crossover design study was conducted in a 6x6x2 meter netted cage within a semi-field system (SFS). Transfluthrin-treated fabric at 5g, 10g, 15g, and 20g doses were evaluated against a paired negative control on three strains of laboratory-reared Anopheles and *Aedes aegypti* mosquitoes strain. Six replicates were performed per dose using either landing or biting method. The recaptured mosquitoes were analysed using negative binomial regression, and the agreement of PE by the two methods was compared using the Bland-Altman methods.

Result

For *Anopheles*, fewer mosquitoes blood-fed in the "biting" arm than landed in the "landing" arm (Incidence rate ratio (IRR)=0.87, 95% CI (confidence interval): 0.81-0.93, $P<0.001$). For *Ae. aegypti*, landing overestimated biting by around 37%, (IRR=0.63, 95% CI: 0.57-0.70, P=0.001). However, the PE calculated by either method is closely agreed when tested by Bland Altman methods.

Conclusions

HLC underestimated the feeding inhibition modality of transfluthrin, and there were species and dose-dependent differences in the relationship between landing and biting. However, estimated PE was consistent between methods. Considering the difficulties of measuring the number of blood-fed mosquitoes in the field setting, HLC can be used as the proxy of personal PE for evaluating VPSR.

KEYWORDS

ambient chamber, semi-field system, transfluthrin, volatile pyrethroid, passive emanator, *Aedes aegypti*, *Anopheles gambiae* s.s., *An. funestus* s.s., human landing catch, bioassay

5.2 Background

The implementation of appropriate and effective vector control tools is an integral component of mosquito-borne disease control programs worldwide (Wilson et al., 2020). However, incomplete coverage and poor compliance with vector control interventions remain a challenge for the control of malaria (WHO, 2020a) and arbovirus vectors (Achee et al., 2019). In addition, some malaria and arbovirus vector species are not completely controlled by current insecticidal tools because they are either behaviourally resistant (they avoid contact with insecticides through outdoor biting or resting, or bite during the day) or physiologically resistant (they are able to survive contact with insecticides) (Russell et al., 2011, Lwetoijera et al., 2014). The most efficient vectors of malaria and arboviruses are strongly adapted to humans (synanthropic) and are therefore most commonly encountered around human dwellings either indoors (Bayoh et al., 2014) or in the peri-domestic space (Pollard et al., 2020). Therefore, focusing on the peri-domestic space as an area for the delivery of vector control interventions for outdoor biting mosquitoes is an effective strategy. Ideally, novel control interventions deployed in the peridomestic space should prevent bites and kill mosquitoes to provide both personal and community protection for users and non-users (Magesa et al., 1991). The efficacy of Volatile Pyrethroids (VP) in providing protection against *Aedes* and Anopheles mosquitoes in the peridomestic space remains an outstanding research question and robust means to evaluate them are needed.

The semi-field system (SFS) was developed to evaluate the efficacy of vector control tools in a controlled disease-free environment (Ferguson et al., 2008). This bioassay provides a convenient alternative method to evaluate vector control tools, thereby eliminating the difficulties encountered in field trials such as variation in mosquito density, size, and design of houses (Okumu et al., 2012). The SFS has been used to demonstrate the efficacy of VP (Ogoma et al., 2014a, Sangoro et al., 2014b) through measurement of multiple outcomes including blood-feeding inhibition, delayed resumption of feeding (disarming), delayed mortality, deterrence, and fecundity reduction (Denz et al., 2021). However, in order to maximize the precision of measuring some endpoints such as blood-feeding inhibition collection of all released mosquitoes is important thus the I-LACT was developed. In this bioassay, outdoor vector control tools particularly those with multiple responses on exposed mosquitoes such as feeding success and induce sublethal incapacitation or delayed mortality may be adequately assessed.

Human landing catch (HLC) is a procedure whereby a human volunteer catches mosquitoes that land on them before the mosquito attempts to bite using a mouth aspirator (Gimnig et al., 2013). HLC is often used to measure the protective efficacy (PE) of bite prevention interventions (Andrés et al., 2015, Mmbando et al., 2017, Masalu et al., 2017). However, some repellents may interfere with mosquito olfaction (Riffell, 2019) so that not all mosquitoes that landed are able to bite. This could mean that HLC underestimates the full protective efficacy of a bite-prevention intervention that modulates mosquito host perception (Afify et al., 2019) or blood feeding (Bibbs and Kaufman, 2017). Therefore, a comparison of PE of the volatile pyrethroid transfluthrin was conducted using either HLC or allowing mosquitoes to freely interact with a volunteer and blood-feed in the I-LACT to allow recovery of all fed mosquitoes.

5.3 Methods

Mosquitoes

Four different species of laboratory-reared mosquitoes were used in these experiments including susceptible *An. gambiae* s.s (Ifakara strain), a pyrethroid-resistant strain with knockdown resistance *An. gambiae* s.s (KDR), a pyrethroid-resistant strain with metabolic resistance *An. funestus* (FUMOZ) and susceptible *Aedes aegypti* (Bagamoyo strain). These colonies are maintained according to MR4 guidance (MR4, 2009). Larvae are fed on Tetramin fish food (Tropical fish flakes), adults are fed on 10% sugar *ad libitum,* and females are membrane-fed on cow blood for egg production. The colonies are maintained approximately at 12:12 (light:dark) natural light, 27 ± 2 °C and 80 ± 20% relative humidity.

The experiments used nulliparous 3-8 day old mosquitoes. Avid mosquitoes were selected by placing a hand near the cage and mosquitoes that attempted to bite were aspirated to the releasing cages that measured 10cm x 10cm x 10cm. The mosquitoes were transferred from the insectary to the SFS in a black cloth bag to prevent them from damage that could be caused by the wind. *Aedes* mosquitoes were starved for 12 hours while Anopheles was starved for 6 hours prior to experiments. On each experimental day, mosquitoes were acclimatized for 45 minutes in the corridor of the SFS which is separated from the experimental space by polyurethane sheeting that prevents contact of the mosquitoes with insecticides.

Description of the Ifakara Large Ambient Chamber Test (I-LACT)

The Ifakara Large Ambient Chamber Test (I-LACT) is a polyester net cage measuring 6 x 6 x 2 m fixed inside the semi-field system (Figure 5.1). Its measurements were derived from the approximate size of the peri-domestic space where most activity occurs outside of rural Tanzanian homes (Masalu et al., 2020). This bioassay was designed to ensure maximum recovery of released mosquitoes when the SFS compartment is used for the evaluation of vector control tools. The sides and roof of the I-LACT are made up of white polyester net to allow airflow, while the floor is made up of white fabric. The cube seals with a zip to prevent mosquito loss, and white colour facilitates mosquito collection after the exposure as mosquitoes can be easily seen against the white background. In addition, the semi-field system is kept free of mosquito predators through daily clearing of spiders, and scavenging ants are minimised through the use of sugar spiked with boric acid. The I-LACT allows for controlled experiments with a simultaneous release of multiple laboratory mosquito strains. In addition, laboratory-reared mosquitoes are disease-free, so it is safe to conduct experiments with blood-feeding endpoints. For this experiment, two I-LACTs, one each for the treatment and control were used.

Figure 5.1. image of the Semi-field System with Large Ifakara ambient Chamber Test (I-LACT) measured (6x6x2 m). in each compartment

Preparation of transfluthrin passive emanator

Hessian sacks (made from plants of the species *Corchorus olitorius*) were purchased locally, washed using powder detergent (OMO®) and water and dried under the direct sunlight. A concentration series of Emulsified concentrate (EC) (Bayothrin EC, Bayer AG Monheim am Rhein, Germany) was prepared. Eave Positioned Targeted Insecticide (EPTI) were made up of hessian fabrics measured 4 m x 0.1 m hessian strip treated with 5g, 10g, 15g, 20g of transfluthrin (Mmbando et al., 2018) were used for Anopheles experiment while Freestanding Transfluthrin Passive Emanators (FTPEs) (Tambwe et al., 2020) consisted of hessian strips measured 5m x 0.1m treated with the same four doses of transfluthrin were used for *Aedes* mosquitoes. Controls were prepared in the same way using water.

Study procedure

Experimental design

A fully balanced cross-over dose-response experiment was conducted whereby mosquitoes were allowed to interact with humans for one hour in the presence of either treatment or control in the I-LACT (Figure 5.2). Each day, one replicate for biting and HLC were conducted. The biting or HLC was conducted 2 m from an experimental hut inside the I-LACT to simulate an outdoor peridomestic setting. Four doses of transfluthrin-treated emanators (5g, 10g, 15g, and 20g) were evaluated consecutively, with each tested for six replicates after which, the dose was increased to the next higher concentration. One replicate was considered to be one hour of exposure to either the treatment (transfluthrin-treated ribbon) or negative control.

Two male volunteers aged 25-40 were recruited on written informed consent. Volunteers were non-smokers and non-drinkers of alcohol and did not use perfumed cosmetics prior to testing to minimise heterogeneity in their attraction to mosquitoes (Shirai et al., 2002). To standardize the area available for mosquito biting (knee and ankles) volunteers wore closed shoes and a bug jacket (Figure 5.3). Volunteers were rotated between compartments after each replicate to account for potential bias due to differential attractiveness to mosquitoes between individuals (Lindsay et al., 1993). Temperature and humidity were recorded inside one of the I-LACT using a Tiny Tag Gemini Data Logger (Chichester, West Sussex, UK). To ensure evaporation of transfluthrin, the experiment was conducted at temperatures above 23ºC (Ogoma et al., 2017).

On each experimental day, the treated hessian and its untreated control were allocated to separate chambers of the I-LACT forty-five minutes before an experiment. This was done to allow emanation of transfluthrin into space. As previous experiments showed no differences in collected mosquitoes between the chambers, the treated and untreated emanators were fixed to the respective chambers for the duration of the experiment to avoid potential contamination. The experiment started when the volunteers sat on the chair and released mosquitoes inside the chamber of I-LACT (Figure 5.3).

Outcomes

The primary outcome was the difference in the number of landed or fed mosquitoes between the methods. The number of mosquitoes fed during the biting experiment and those caught during the landing experiment in the treatment and control were recorded. The secondary outcome was the protective efficacy (PE) which was measured by comparison of the number of mosquitoes landed or fed relative to the corresponding control.

Evaluation of different doses of Transfluthrin treated EPTI against Anopheles mosquitoes measured by HLC and biting method

For this experiment, the EPTI were mounted at the top of metal stands measuring 1.6 x 1.6 x 2m placed inside the cage to simulate the eave placement with a volunteer sitting 2m in front (Figure 5.3). Sixty mosquitoes with twenty mosquitoes from each of three strains: KDR resistant *Anopheles gambiae* (Kisumu strain), pyrethroid susceptible *Anopheles gambiae* s.s. (Ifakara strain) and *An. funestus* mosquitoes were released, per replicate (Figure 5.2). Three replicates of the biting experiment were conducted before the HLC between 18.30 hrs and 19.30 hrs and three were conducted after HLC between 20.30 hrs to 21.30 hrs. This was done to reduce temporal bias when comparing the two methods that could be affected by temperature and mosquito behaviour.

Evaluation of different doses of Transfluthrin treated FTPE against *Aedes aegypti* measured by HLC and biting method

The two FTPEs were positioned on the ground 2.5m on each side of the volunteer and 2 m from the back (Figure 5.3). Fifty pyrethroid susceptible *Ae. aegypti* mosquitoes (Bagamoyo strain), were released (Figure 5.2). A total of three replicates for the biting experiment and three for the landing method were conducted over 3 consecutive days, between 0630 and 0730 hours for the former and between 0830 and 0930 hours for the latter. This order was switched for the remaining 3 experimental days, with the landing method conducted first to control for temporal bias when comparing the results of the two methods, which could have been affected by temperature and mosquito circadian rhythm.

Biting experiment procedure

For the biting experiment, volunteers allowed mosquitoes to fly freely and feed in the area between the knee and ankle (WHO, 2013c). At the end of the exposure, mosquitoes were collected from within the netting chamber for 45-60 minutes. All knocked-down and resting mosquitoes were aspirated from the I-LACT chamber (floor and walls) using mouth aspirators and head torches (at night) and placed in paper cups with no more than 25 mosquitoes per cup to minimise mortality from mosquitoes interacting with one another as occurs at high densities. Mosquitoes were immediately transported to the insectary and scored as fed or unfed.

Landing experiment procedure

During the landing experiment, volunteers gently aspirate mosquitoes that land on the area between the knee and ankle using mouth aspirators through a procedure called HLC. Head torches were used when experiments were conducted in the evening with Anopheles mosquitoes. These mosquitoes were aspirated into a paper cup with a new cup used after every fifteen minutes. After collection, the paper cups were placed in a sealed plastic container to avoid transfluthrin exposure so they were effectively removed from the experiment upon collection. At the end of one hour, the experiment stopped, any remaining mosquitoes were collected and all the cups with mosquitoes were transported to the insectary for counting and recording.

Data analysis

Comparison between landing and biting methods for measuring protective efficacy

Analyses of the experimental data were done in Stata 14 (Stata Corp). Descriptive analyses were conducted to generate the mean proportion of fed or landing mosquitoes with the respective 95% confidence interval (CI) presented in graphs.

To compare the biting and landing in the treatment and control, the number of mosquitoes caught in the landing experiment and those fed in the biting experiment were merged making one variable called ”recapture”. The “recapture” mosquitoes were modelled using negative binomial probability distributions with logit link functions. The “recapture” mosquitoes were treated as dependent variables while the method of collection (landing vs biting), treatment, dose, volunteer, and mosquito species were treated as independent categorical fixed effects.

An interaction term between treatment and transfluthrin dosage was introduced to allow a comparison of the effect of transfluthrin on recaptured or fed mosquitoes as the dose increased. The protective efficacies were calculated from the relative risk (RR) using the formula (1− RR). Bland-Altman plots were used to assess the agreement between protective efficacy measured by the two collection methods and to examine any systematic difference (fixed bias) between the measurements [26]. The mean value of the difference was tested for significant difference from zero using a 1-sample t-test.

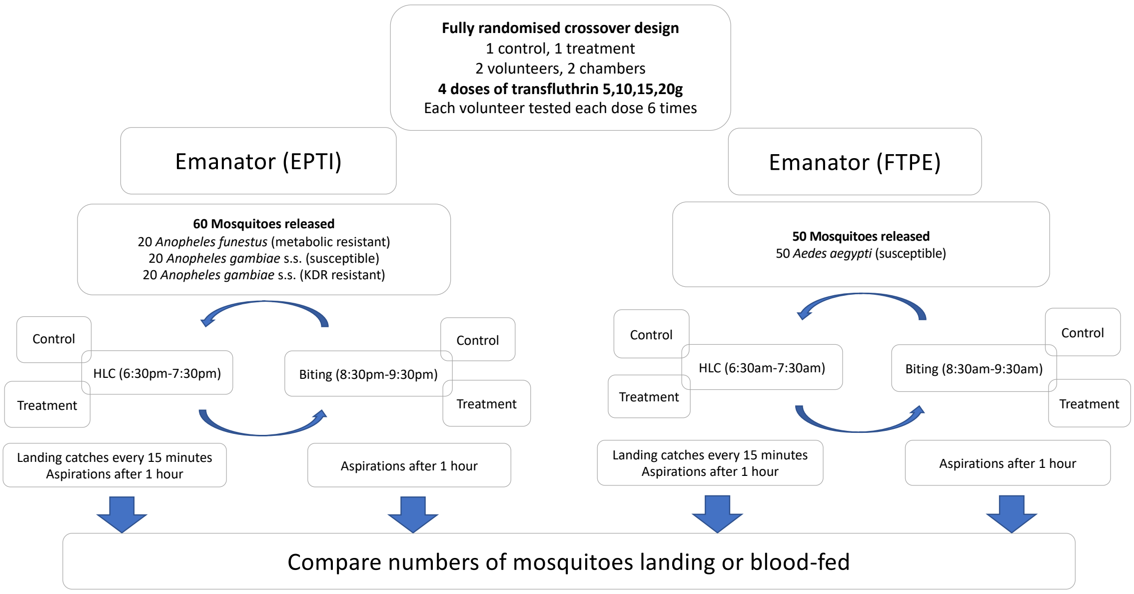

Figure 5.2. The flow chart showing various iteration of the experiments conducted in this study.

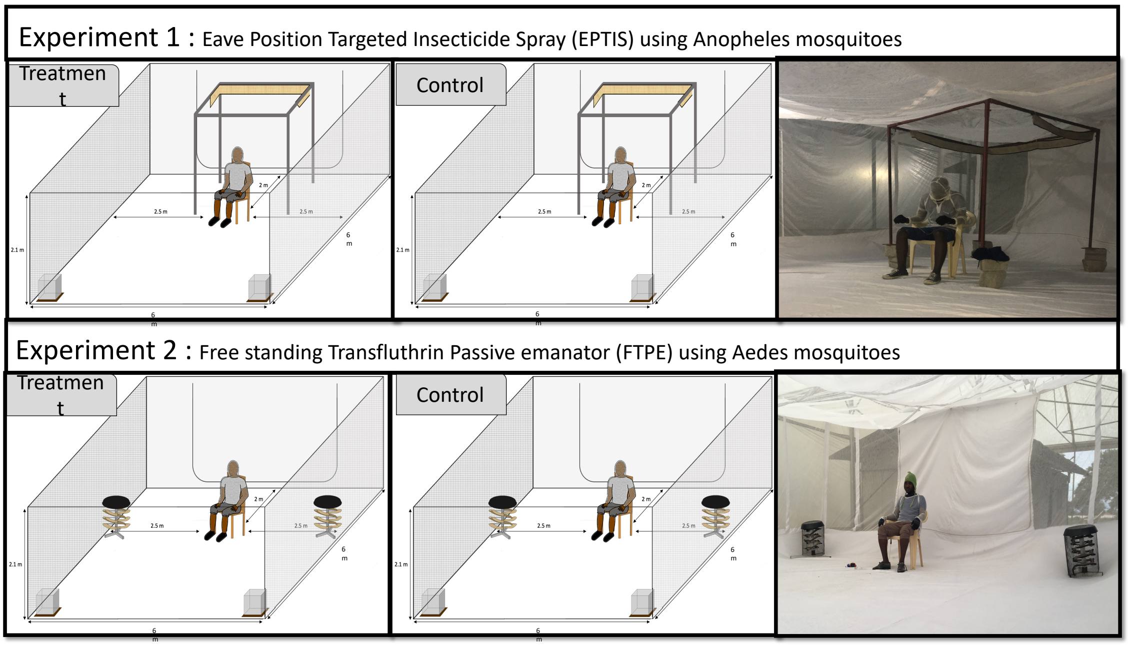

Figure 5.3. The I-LACT schematic representation for the experiments a) **The I-LACT arrangement for experiment with transfluthrin impregnated eave ribbon against Anopheles mosquitoes. b) Experiment with *Aedes aegypti* and freestanding transfluthrin passive emanators**.

5.4 Results

Comparison of the recaptured mosquitoes between landing and biting methods

For Anopheles mosquitoes, overall, the biting method measured significantly higher PE compared to the landing method (IRR=0.87, 95% CI (confidence interval): 0.81-0.93, P<0.0001). In the treatment, there was strong evidence to suggest that female Anopheles mosquitoes were less likely to feed using biting method compared to those caught during the landing method (IRR=0.82, 95% CI: 0.74-0.91, P<0.0001). This was greater than observed in the control (IRR=0.90, 95% CI: 0.82-0.97, P<0.001).

When the comparison between biting and landing methods was done across, species differences could be seen. In the treatment, compared to the landing method, biting was lower for *Anopheles gambiae* s.s. (IRR=0.77, 95% CI: 0.63-0.94, P=0.008) and *Anopheles funestus* (IRR=0.75, 95% CI: 0.63-0.89, P<0.0001) while no difference was observed for the *Anopheles gambiae* s.s. (KDR) (IRR=0.97, 95% CI: 0.80-1.17, P=0.69) (Table 5.1).

For *Ae. aegypti* mosquitoes, the overall PE measured by the biting method was significantly higher compared to the landing (IRR=0.63, 95% CI: 0.57-0.70, P=0.001). The landing and biting methods showed significantly different values for both the treatment (IRR=0.56, 95% CI: 0.46-0.67, P=0.01) and the control (IRR=0.70, 95% CI: 0.64-0.76), (Table 5.2).

Mosquitoes recaptured on exposed to different doses of Transfluthrin treated emanator against Anopheles and *Aedes* mosquitoes measured by landing and biting method

Results for the EPTI are illustrated in (Figure 5.4 and Table 5.1). Overall, higher recaptured was observed by the biting method than the landing method for *An. gambiae* s.s. *An. funestus* and *Ae. aegypti* but this difference became smaller at higher concentrations and no difference was seen between methods at 20g transfluthrin for *An. gambiae* s.s (KDR) and *Aedes aegypti*. There was a clear dose response seen with higher concentrations of transfluthrin providing greater protective efficacy. At 5g PE was 30 to 40% and this increased to 60-70% at 20g. At all doses transfluthrin significantly reduced both landings or blood-feeding for all species tested.

The Bland-Altman plot (Figure 5.5) showed that the data points were fairly evenly distributed around the mean (the central solid line) with the mean difference of -4.75 and limits of agreement between -25.57 and 16.07.

Recaptured mosquitoes in the I-LACT

A total of 1600 female *Aedes* mosquitoes and 480 female Anopheles mosquitoes were released into the control and treatment chambers of the I-LACT. For Anopheles mosquitoes, recapture ranged from 427/480 (89%) to 453/480 (95%) in the treatment and control for all species. For *Aedes aegypti* mosquitoes, a total of 1600 female mosquitoes were released into the control and treatment chambers of the I-LACT. The total number of collected mosquitoes in the control and treatment arms were 1565 (98%) and 1445 (90%) respectively.

Environmental conditions

During the experiment with Anopheles mosquitoes the average temperature was 25.5°C (24.5°C -27°C) while the average humidity was 70.2% (61.7%-76.1%). For the experiment with *Aedes* mosquitoes, the average temperature was 27.1°C (25.7°C-28.5°C) and average relative humidity (RH) was 90.0% (89.0%-90.8%). Using the anemometer at the site, the air flow could not be measured inside the I-LACT chamber.

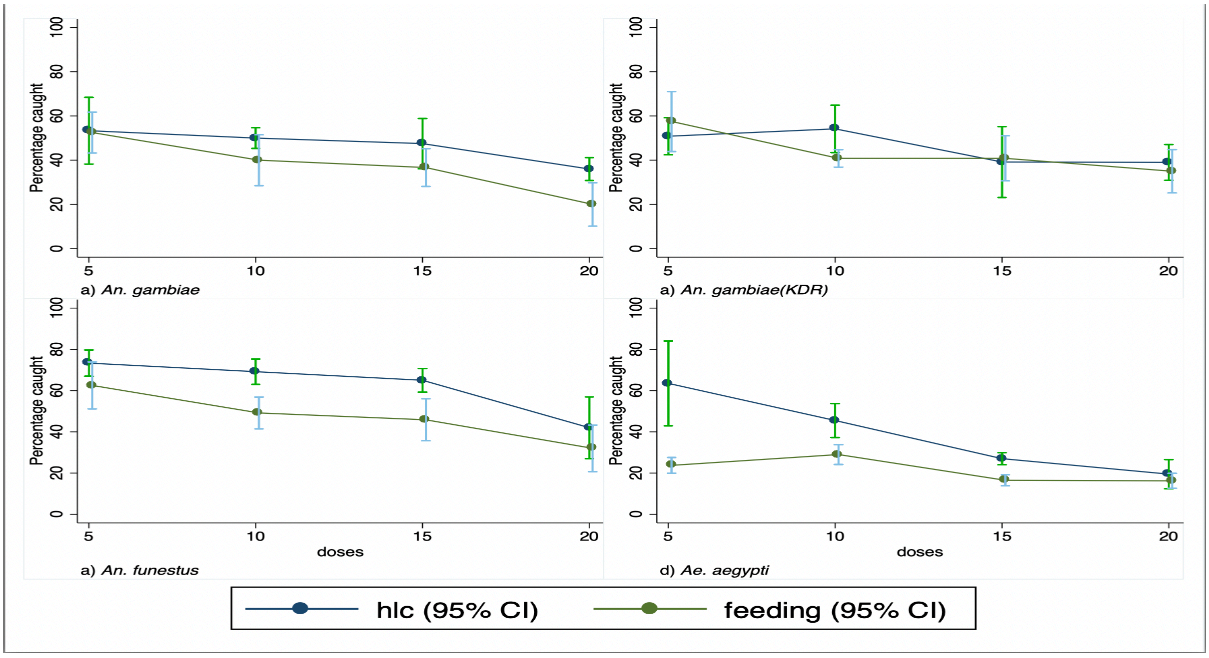

Figure 5.4. Graph showing the proportion of recaptured mosquitoes using either HLC or biting method for all mosquitoes species involved in this experiment

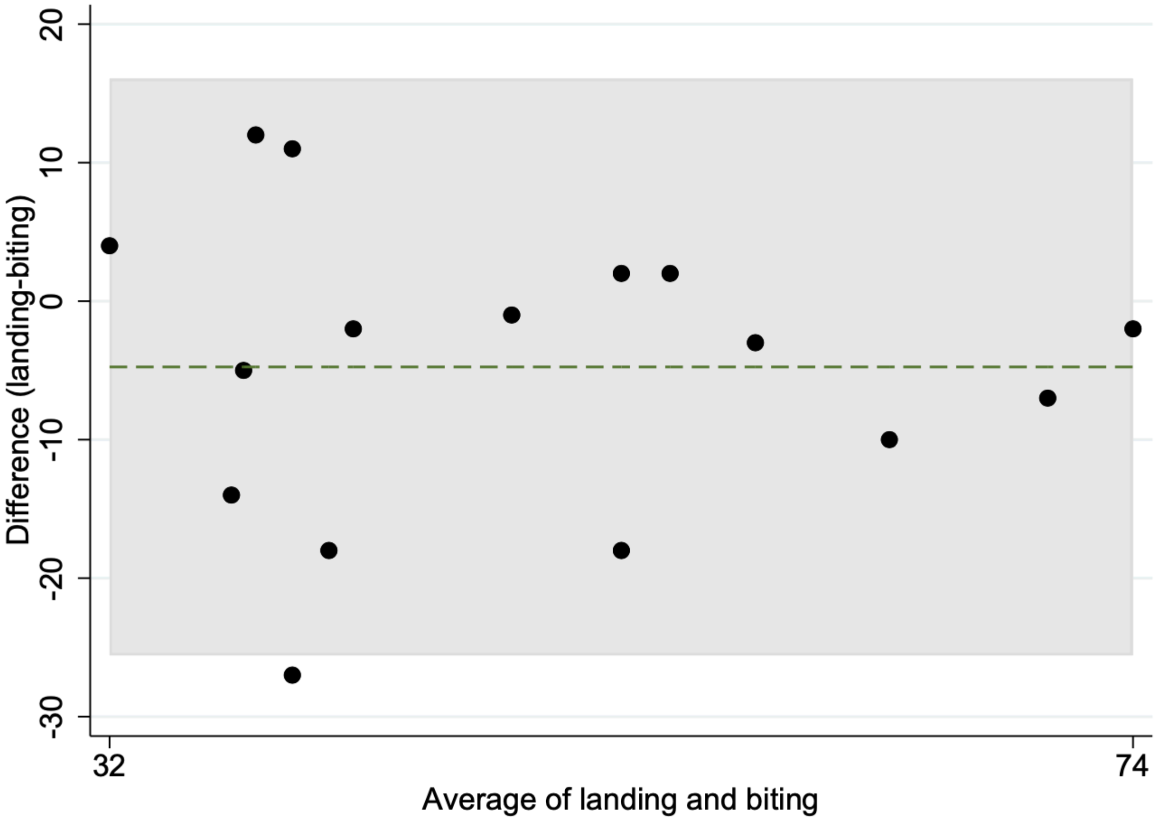

Figure 5.5 Figure 3. Bland and Altman graph to compare the efficacy of the HLC and biting method for Anopheles mosquitoes. The x and y-axes represent the ratio between the methods and mean respectively with 95% limit of agreement (dotted line). Solid line showed the mean bias between the two-method equal to -4.75. The data were logarithmic transformed to normalize the distribution.

Table 5.1. Summary of the results for the evaluation of different dose of transfluthrin emanators across different species of Anopheles mosquitoes in the I-LACT as measured by landing experiment (Human Landing Catch (HLC)) or biting experiment (blood feeding)

	IRR (95% CI) vs control	**IRR (95% CI) vs control**	**IRR (95% CI) vs control**	**IRR (95% CI) vs control**	**IRR(95% CI)**
	landing experiment	***PE***	**biting experiment**	***PE***	***IRR landing vs biting***
Overall					0.87 (0.81-0.93)***
Control					0.90 (0.82-0.97)**
Transfluthrin					0.82 (0.74-0.91)***
Anopheles gambiae					
5g	0.65(0.47-0.88)**	35	0.60(0.44-0.82)**	40	0.77 (0.63-0.94)**
10g	0.55(0.40-0.72)**	45	0.46(0.33-0.66)**	34	
15g	0.52(0.38-0.72)**	48	0.51(0.36-0.74)**	49	
20g	0.41(0.28-0.61)**	59	0.31(0.11-0.51)**	69	
Anopheles gambiae KDR					
5g	0.56(0.41-0.77)*	44	0.68(0.50-0.93)*	32	0.97 (0.80-1.17) N.S.
10g	0.59(0.43-0.81)**	41	0.57(0.41-0.81)**	43	
15g	0.46(0.32-0.65)**	54	0.48(0.34-0.69)**	52	
20g	0.44(0.32-0.64)**	56	0.46(0.31-0.69)**	54	
Anopheles funestus					
5g	0.76(0.57-1.00)*	34	0.70(0.53-0.95)*	30	0.75 (0.63-0.89)***
10g	0.70(0.52-0.92)**	30	0.56(0.40-0.77)**	44	
15g	0.68(0.50-0.90)**	32	0.50(0.36-0.69)**	50	
20g	0.43(0.30-0.62)**	57	0.40(0.26-0.60)**	60	

Landing experiment refers to the estimates on the comparison between the treatment and control for each dose for the Human Landing Catch (HLC) experiment while biting experiment refer to the estimate derived from the model from the mosquitoes were allowed to interact with a human volunteer and feed between the transfluthrin treatment or untreated control.

Incidence risk ratio (IRR) was adjusted for temperature, humidity, volunteer, and compartment. *p<0.05, **p<0.01, ***p<0.001, N.S. p>0.05

Table 5.2. Summary of the results for the evaluation of different dose of transfluthrin emanators across different species of Aedes mosquitoes in the I-LACT as measured by landing experiment (Human Landing Catch (HLC)) or biting experiment (blood feeding)

	IRR(95% CI)	**IRR(95% CI)**	**IRR(95% CI)**	**IRR(95% CI)**	**IRR(95% CI)**
	landing experiment	***PE***	**biting experiment**	***PE***	***IRR landing vs biting***
Aedes aegypti					
Overall					0.63 (0.57-0.70)**
Control					0.70 (0.64-0.76)**
Transfluthrin					0.56 (0.46-0.67)**
5g	0.74(0.63-0.87)**	26	0.47(0.32-0.60)**	53	0.63 (0.57-0.70)**
10g	0.66(0.52-0.85)**	44	0.38(0.31-0.47)**	62	
15g	0.37(0.28-0.49)**	67	0.24(0.18-0.31)**	74	
20g	0.27(0.19-0.36)**	73	0.25(0.19-0.32)**	75	

Landing experiment referred to the estimates on the comparison between the treatment and control for each dose for the Human Landing Catch (HLC) experiment while biting experiment refer to the estimate derived from the model from the mosquitoes were allowed to interact with a human volunteer and feed between the transfluthrin treatment or untreated control. Incidence risk ratio (IRR) was adjusted for temperature humidity, volunteer, and compartment. *p<0.05, **p<0.01, ***p<0.001, N.S. p>0.05

5.5 Discussion

Comparison between landing and biting for measuring the protective efficacy of volatile pyrethroid.

Human landing catches are the gold standard measure of human–vector exposure has been used for the evaluation of different vector control tools (Gimnig et al., 2013). The human landing rate approximates the number of mosquitoes that would bite one person at a particular time and place (Schoeler et al., 2004, Briët et al., 2015). For vector-borne pathogens, vector bites are critical for transmission, and host–vector contact rate in addition to daily mosquito mortality are consistently the most important parameters determining disease risk according to studies using mathematical models (Wallace et al., 2014). This study has shown that the HLC was a reasonable proxy for biting in the control.

While volatile pyrethroids interfere with the mosquito blood feeding response because the difference in biting compared to landing was greater in the transfluthrin arm than the control arm, the PE measured by landing or the biting method broadly agreed across all species and doses tested. Differences between the methods were smallest at the highest transfluthrin doses. While differences in measurement exist between landing and biting Bland Altman methods showed good agreement between the PE measured by either method so it can be concluded that HLC is a reasonable proxy for bite-prevention and can be used in field evaluations of volatile pyrethroids as a substitute for blood-feeding to limit risk of vector borne disease transmission (Harrington et al., 2020).

A higher number of Anopheles mosquitoes were caught when landing method was used compared to the biting. This was consistent when the methods were compared across the doses for *Aedes aegypti* mosquitoes. However, this reduction in blood-feeding was not observed with *Anopheles gambiae* KDR a pyrethroid-resistant mosquito. It has been observed in other studies that mosquitoes with the KDR mutation had higher blood-feeding success in the presence of pyrethroid insecticide-treated nets (ITNs) compared to pyrethroid-susceptible mosquitoes (Diop et al., 2020). In studies of pyrethroid ITNs, KDR-resistant mosquitoes were shown to tolerate longer contacts with pyrethroid-treated papers than susceptible mosquitoes and were more likely to pass through an ITN and successfully blood feed (Chandre et al., 2000). Resistant *Ae. aegypti* are also more likely to successfully feed through treated fabrics (Agramonte et al., 2017). This work adds to the body of evidence that indicates that KDR mutations can enhance mosquito feeding success when pyrethroids are presented in vapour form.

The differences between biting and landing observed for other mosquito vectors in the presence of transfluthrin may be due to behavioural modification so that mosquitoes may land but inhibited from feeding. Several authors have observed feeding inhibition induced by volatile pyrethroids (Ogoma et al., 2014b, Ritchie and Devine, 2013) and pyrethrum (Smith et al., 1971) and it has been hypothesized that volatile pyrethroids interact with olfactory sensors and alter a mosquitoes' ability to feed (Bibbs and Kaufman, 2017). Laboratory studies with membrane feeding have also shown significant reductions in host-seeking behaviours (landing, probing, and blood-feeding) of *Ae. aegypti* exposed to transfluthrin passive emanators (McPhatter et al., 2017). A recent room experiment with metofluthrin passive emanators showed a reduction in mosquito probing rates as a proxy for biting, which was dose-dependent (Darbro et al., 2017).

Use of I-LACT bioassay for measuring additional endpoints

The SFS provides a simulated user environment where the initial evaluation of both outdoor and indoor bite prevention interventions are performed (Tambwe et al., 2021c). Previous studies established that the recapture of the released mosquitoes in the semi-field system compartment is not 100% (Ponlawat et al., 2016, Ogoma et al., 2014a, Sukkanon et al., 2021, Njoroge et al., 2021). Clearly, some of the exposed mosquitoes are not recovered, therefore, not accounted for in the analysis which may bias the results. The I-LACT was designed in an attempt to address this challenge for the evaluation of outdoor vector control tools particularly those with multiple actions beyond reducing mosquito landings, including feeding inhibition, disarming, knockdown and delayed mortality. The I-LACT is constructed from white fabrics, which facilitate collection of mosquitoes. The use of net on the sides equalizes the climatic conditions between the inside and outside the chamber. The I-LACT dimension of 30m^2 is as equal to the peridomestic space (Masalu et al., 2020), which replicates the area within which the intervention will be deployed. Furthermore, the I-LACT is large enough to accommodate human volunteers, to allow human-mosquito interaction. This interaction is important as it mimics what happens during host searching, unlike the arm-in-cage experiment in which mosquitoes are placed close to an individual's arm or confined to small cage (Martin et al., 2020), which is most likely higher than if mosquitoes can fly away from the source of the pyrethroid. The I-LACT could be a useful bioassay for the evaluation of other outdoor vector control tools with multiple behavioural responses including knock-down, mortality, and blood-feeding inhibition. This is difficult to do in an outdoor field setting as mosquitoes in the vicinity of the ambient emanator may be knocked down or killed, but this information cannot be collected. It is also possible to use consistently high numbers of disease-free mosquitoes in semi-field experiments to ensure that studies are well powered.

The I-LACT bioassay demonstrated the recapture of >90% of released mosquitoes. This provides an opportunity to fully assess the multiple effects of volatile pyrethroids on exposed mosquitoes. Volatile pyrethroids exert several measurable outcomes on exposed mosquitoes including repellence (Achee et al., 2012b), blood-feeding inhibition (McPhatter et al., 2017), disarming (Denz et al., 2021), knock-down (sublethal incapacitation) (Ritchie and Devine, 2013) and mortality (Ritchie and Devine, 2013, Salazar et al., 2013). Of these outcomes, only repellence could be appropriately evaluated by HLC as landed mosquitoes are considered in the analysis. Other outcomes such as mortality or knocked down may not be fully assessed (Ponlawat et al., 2016, Tambwe et al., 2020) as mosquitoes will spend more time in contact with the treated device while blood-feeding, and blood-fed mosquitoes show enhanced survival of pyrethroid exposure (Machani et al., 2019). While these additional endpoints are routinely assessed in experimental hut trials of pyrethroids applied to bednets (WHOPES, 2013b), guidelines for ambient emanators and mosquito coils (WHOPES, 2009a) as well as spatial repellents (WHO, 2013a) focus on mosquito landing. The influence of the multiple endpoints of transfluthrin beyond bite prevention is demonstrated from a randomized control trial in Indonesia, where there was no significant protection from mosquito landings offered by transfluthrin emanator while malaria clinical cases were significantly reduced (Syafruddin et al., 2020). These findings suggest that there are some limitations on how the efficacy of volatile pyrethroid is evaluated in the field and further endpoints should be evaluated in randomised control trials of volatile pyrethroids including human blood index (Pappa et al., 2011) as a proxy for blood feeding inhibition and population survival estimates as a proxy for mortality (Matthews et al., 2020). A recent cluster randomised trial of a passive transfluthrin emanator in Iquitos, Peru demonstrated a reduction in arbovirus incidence as well as *Aedes aegypti* abundance and blood-fed abundance (Morrison et al., 2021) suggesting the importance of mortality and blood-feeding inhibition for public health applications of volatile pyrethroids.

Estimates of the protective efficacies at different doses measured by HLC or biting method

The I-LACT was used to evaluate a dose-response experiment to compare the protective efficacies of FTPE at different doses measured by either HLC or method methods. The experiment was conducted within a short exposure time to mimic what is happening in real life where mosquitoes are normally exposed for a short time before they elicit behavioural responses (Bibbs and Kaufman, 2017). This study showed no interaction between treatment and species, indicating that transfluthrin used in this experiment induced equal protection for all mosquito species, regardless of their resistance mechanism. This was consistent when the protective efficacy was measured using landing or biting. Therefore, in an area with resistant and susceptible outdoor biting malaria vectors as well as *Ae. aegypti* arbovirus mosquitoes any dose could be used for reducing human exposure to vector bites. Findings from this study corroborate with a field study in Tanzania by Ogoma *et al.* who showed that transfluthrin treated hessian ribbon between 5g and 15g reduced mosquito landings equally in the peridomestic space against several anopheles vector species (Ogoma et al., 2017). These results indicate that, in an area where mosquitoes are biting outdoors, the fabric treated with the lowest dose could be used to protect humans from mosquito bites and provide community protection, while maximising human safety. A consistent PE of 30% for several months will provide greater protection than a product with a higher PE but low compliance (Kiszewski and Darling, 2010).

Effect of surface area on the evaluation of volatile pyrethroid

In this experiment, we have measured a lower protective efficacy of around 30% offered by transfluthrin at the lowest dose of 5g emanators against *Aedes aegypti* and Anopheles compared to a previous experiment conducted in the entire compartment of the semi field system where the hessian strips treated with transfluthrin at the same dosage measured protective efficacy of around 60% (Tambwe et al., 2021a). This PE of 60% was replicated in Kenya (Njoroge et al., 2021). The differences in the protective efficacy could have been contributed by the differences in the volume of space between the I-LACT and the semi-field compartment. Using the I-LACT, mosquitoes were released in a compartment with a volume of 75.6m^3 while each semi-field compartment has a volume of 1228m^3 where mosquitoes can easily move away from the source of transfluthrin. Similarly, experiments conducted to measure the protective efficacy of a topical repellent in the semi-field system (here considered to be a small volume) and then repeated in the field (large volume) observed higher protective efficacy in the field trial (Sangoro et al., 2014b). Therefore, it is likely that in a large surface area, mosquitoes may move away from the host cues after contact with the intervention thereby reducing the chances of repeated biting. Suggesting that in a smaller space landing-inhibition will be underestimated and sublethal incapacitation and mortality may be overestimated as modes of action are dose dependent with mortality occurring at higher doses or longer exposure time (Bibbs and Kaufman, 2017).

Effect of climatic conditions on the efficacy of volatile pyrethroid

This study showed slightly higher protective efficacy when either of the methods was used in the experiment with *Aedes* mosquitoes compared to when the experiment was conducted with Anopheles mosquitoes. The differences in protection could have been attributed by the differences in temperature between the two experiments due to the time when the experiments were conducted. It was observed that during the nighttime experiment with Anopheles mosquitoes, the ambient temperature was slightly lower (25°C) compared to when the experiment was conducted in the morning against *Aedes* mosquitoes (27°C). However, a previous experiment has shown that the optimal performance of transfluthrin occurs when the temperature ranges between 21°C -30°C (Ogoma et al., 2017). Future studies should be designed to evaluate the efficacy of transfluthrin-treated emanators at different temperatures, and the environmental conditions should always be considered in the analysis. In the current experiment, the wind speed inside the semi-field system could not be measured because it was very low in a way that the instruments used could not detect it. Therefore, findings from this study may be interpreted with caution as in the area with high wind speed and low temperature the same emanator could be offered slightly lower protective efficacy compared to what has been observed in this experiment. Some investigators ensure consistency in the evaporation of volatile pyrethroids between replicates by using a fan to give a consistent airflow (Darbro et al., 2017) and this is an important consideration for future trials of ambient emanators.

5.6 Conclusions

The feeding inhibition of *An. gambiae* s.s., *An. funestus* and *Ae. aegypti* mosquitoes in the presence of transfluthrin was underestimated by the HLC method, and the magnitude of the difference between landing and biting varied among the species and doses of transfluthrin tested in this study. The PE calculated for the landing or biting methods did not show any systematic bias, and was generally in agreement when tested with the Bland–Altman plot, with better agreement at higher concentrations of transfluthrin, which also afforded greater PE. Therefore, either method can be used to assess the personal PE of volatile pyrethroids, with the caveat that results may vary due to the stochasticity inherent to entomological experiments, with greater variability occurring when interventions provide lower efficacy. The findings reported here indicate that HLC can be used as a proxy of personal PE for the evaluation of volatile pyrethroids, especially when the difficulties associated with counting fed mosquitoes in a field setting are taken into account.

Abbreviations

FTPE: Freestanding transfluthrin passive emanators; CI: Confidence interval; I-LACT; Ifakara Large ambient chamber test; RH relative humidity; EC: Emulsified concentrate; SFS: Semi field system; HLC: Human landing catches; PE: Protective efficacy; IHI: Ifakara Health Institute; IRB: Institutional Review Board; NIMR: National Medical Research Institute; WHO: World Health Organization.

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Authors' contributions

MMT and SJM: conceived and designed the study. MMT, OGO and RM: supervised semi-field experiment, volunteers and data collection. Performed susceptibility test. MMT, AS, UAK and SJM: data analysis. MMT: draft the manuscript. AS, and SJM: revised the manuscript. MMT and JDM: designed the FTPE and the I-LACT. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its additional file.

Ethics approval and consent to participate

The volunteers that participated in this experiment were IHI employees, fully trained and skilled in mosquito collection. They were recruited voluntarily through written informed consent after the risks and benefits of the study procedures and their right to leave at any time during the study was clearly explained.

All the mosquitoes used in this experiment were laboratory-reared and free from arboviral diseases. The study was approved by the IHI Institute Review Board (IHI-IRB) and the National Institute for Medical Research, Tanzania (NIMR) with certificate number IHI/IRB/No: 024-2016 and NIMR/HQ/R.8a/Vol.IX/2381, respectively.

Competing interest

The authors declare that they have no competing interests. SJM, UA and OGO conduct contract product evaluation of a number of vector control tools including volatile pyrethroids.

Chapter 6; Book Chapter ; Advances in arthropods repellents (chapter 10) Semi-Field System and Experimental Huts Bioassays for the Evaluation of Spatial (and Topical) Repellents for Indoor and Outdoor Use

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Chapter outline

3.7 Conclusion

6.1 Abstract

In this chapter, we describe the considerations for the design of experiments to measure the protective efficacy of bite prevention tools against mosquito vectors. The chapter focuses on the evaluation of spatial repellents (specifically volatile pyrethroids) and topical repellents under semi-field conditions including a description of the semi-field system (SFS) and experimental huts (EH) used to simulate indoor and outdoor use settings. We also, explain the preparations needed for conducting an experiment in these bioassays and limitations to allow reproducible data.

Bite prevention technologies are ultimately designed to prevent the transmission of pathogens by inhibiting the bite of an infected vector. We explain the primary outcomes used to measure the efficacy of repellents, analysis as well as data interpretation. We, also describe the relationship of data collected between the semi-field and field settings. The chapter ends with a brief description of using mathematical modeling to simulate the expected outcome when bite prevention tools are implemented alone or in combination with other vector control tools for the prevention of vector-borne disease.

Key Words: Semi-field system, Experimental hut, field experiment, volatile pyrethroid, topical repellent, mosquito, Anopheles, *Aedes*, mathematical model

6.2 Introduction

Vector-borne diseases account for more than 17% of all infectious diseases, causing more than 700,000 deaths annually (WHO, 2021). By far the most prevalent vector-borne diseases are malaria with 219 million cases and 40,000 deaths and dengue with 3.9 billion cases and 40,000 deaths, annually (WHO, 2021). Both of these diseases are transmitted by mosquitoes: a number of Anopheles species transmit malaria, whereas *Aedes aegypti* and *Ae. albopictus* are the principal vectors of the most common arboviruses including dengue, chikungunya, Zika, and yellow fever.

For public health, mosquito vector control tools using insecticides applied on surfaces/fabrics where vectors regularly rest or feed is used against adult vectors while modification of the environment is often deployed to control the aquatic stages (WHO, 2016a). These interventions have been widely used and have proved effective, in controlling malaria transmitted by indoor biting and resting vectors (Alonso et al., 2017). However, these interventions do not protect individuals against mosquito species that bite outside of sleeping hours, that bite outdoors, or that rest outdoors. Larval Source Management (LSM) is effective against indoor and outdoor biting mosquitoes and is recommended for community control of malaria (WHO, 2019) and dengue (WHO, 2012). In addition, the use of repellents and long clothing for personal protection against mosquito bites is recommended (WHO, 2016a).

The changing epidemiology of malaria and the global growth of dengue increasing the use of bite prevention techniques for the control of the vector-borne disease. Due to the enormous success in malaria control (WHO, 2020a), malaria is now increasingly focal and often clustered in subpopulations with similar social, behavioral, and geographical risk characteristics (Cotter et al., 2013) such as migrants (Kounnavong et al., 2017), forest workers (Sandfort et al., 2020) and people who work outdoors at night (Monroe et al., 2019b). In areas where vectors bite in the evening hours and rest outdoors peridomestic malaria transmission often occurs (Lana et al., 2021).

Arboviruses are likely to cause majority of the vector-borne diseases in the 21st century. These viruses are a growing threat worldwide due to the geographic expansion of vectors and viruses through globalization and urbanization (Brady and Hay, 2020). The mosquito *Ae. aegypti* is the primary vector, has evolved to mate, feed, rest, and lay eggs around urban human habitations: and flourishes in urban environments closely associated with humans (Powell and Tabachnick, 2013). It is a daytime feeder and its peak biting periods are early in the morning and before dusk in the evening. Female *Ae. aegypti* frequently bite multiple people during a single feeding period and dengue cases often cluster related to the presence of vectors (Liebman et al., 2012). The rapid spread of arboviral infections is a result of demographic and societal changes, importantly rural-urban migration leading to unplanned urban settlements and introducing viruses to new areas. Because the global urban population is set to rise to 5 billion by 2030 and land area with urban settlement to 1.2 million km^2 (Seto et al., 2012), it is unlikely that dengue will decline without sustained and effective control measures. This is of great concern, and in the absence of effective vaccines, interventions that reduce contact between humans and vectors (Achee et al., 2015a).

The ability of a mosquito to locate a human-host and blood-fed successfully plays an important role in the transmission of disease pathogens. Mosquitoes that feed primarily on humans are the most efficient vectors of human pathogens (Wynne et al., 2020, Ogoma et al., 2014b). These mosquitos detect and locate hosts principally through odorant cues released by hosts via their olfactory receptors that are located on their antennae, maxillary palps and labellum (Takken, 1991, Takken and Knols, 1999). At long range, carbon dioxide signals the presence of the host (Gillies, 1980), and sensitizes mosquito responses to host cues at shorter range (Webster et al., 2015). Mosquitoes use host cues to orient towards hosts using odours that are generated by the decomposition of skin secretions by skin microbiota (Takken and Verhulst, 2017) that are reliable cues for human hosts (Verhulst et al., 2018), in combination with heat and water vapor at close range (Wright and Kellogg, 1962). Visual cues are important particularly in diurnal species (Muir et al., 1992). Advancements in neurobiology and studies of insect olfactory systems have led to the identification and development of numerous behaviorally active compounds that can attract (Smallegange et al., 2011, Okumu et al., 2010, Verhulst et al., 2010) and repel mosquitoes (Rinker et al., 2012, Carey et al., 2010). These compounds can be applied topically (on the skin), on fabric/clothing, and or spatially (vapor phased from a point source).

While the effectiveness of topical repellents as public health tools is usually limited (Maia et al., 2018) because people often forget to regularly, or correctly apply them (Gryseels et al., 2015), they remain useful for at risk populations such as the military (Beiter et al., 2019) and non-immune travelers (Ahmed et al., 2020). Topical repellents are recommended by the WHO for bite prevention (WHO, 2019). There is now a growing body of evidence that spatial repellent on particular volatile pyrethroids have the potential to provide effective protection against malaria in areas where there is an early evening transmission (Syafruddin et al., 2020, Syafruddin et al., 2014, Hill et al., 2014) and arboviruses (Morrison et al., 2021). Due to this proven public health benefit, there is now a renewed research agenda to evaluate new iterations of bite prevention tools. Of particular importance is the development of longer-lasting volatile pyrethroids that can protect multiple users for many weeks. Such a mode of action ensures compliance, as little lifestyle modification is needed for an individual to receive protection from these chemicals. Having representative bioassays that allow cost-effective and precise estimates of efficacy evaluations is an extremely important component of the product development pathway.

Methods for testing repellents that do not require a human host include (1) synthetic human odor-baited traps (Salazar et al., 2013, Chauhan et al., 2012); (2) animals instead of human volunteers (Vatandoost and Hanafi-Bojd, 2008); (3) laboratory-based artificial blood feeding membrane systems (Debboun and Wagman, 2004); (4) olfactometer experiments (Bibbs et al., 2020) and (5) behavioral response screening systems (Thanispong et al., 2010). Although these methods are highly standardized and able to rapidly screen compounds and do not involve human volunteers, the test conditions are not fully representative of what occurs in real-life settings when humans use repellents. Because these systems do not emit the complete suite of host cues that are important for mosquito landing such as heat and water (Ray, 2015). Additionally, some repellents such as N, N-diethyl-3-methyl benzamide (DEET), ethyl butylacetylaminoproprionate (IR3535) and 2-(2-hydroxyethyl)-1-piperidine carboxylic acid 1-methylpropylester (Picaridin) exert their olfactory mode of action primarily by decreasing the number of volatile odorants reaching the odorant receptor neurons and therefore, their repellency is observable only in the presence of host odors (Afify et al., 2019). On the other hand, full-field experiments that are normally run to verify findings from laboratory tests, use human volunteers who are exposed to potentially infective mosquito bites and should be conducted when interventions are optimized because they have low throughput and relatively expensive (Harrington et al., 2020). Therefore, the efficacy of repellents against human host-seeking insects requires a combination of laboratory and field tests (WHO, 2009b, WHOPES, 2013a).

Using well-characterized bioassays in semi field systems (SFS) (Ogoma et al., 2014b) and experimental huts (EH) (Grieco et al., 2000), the efficacies of topical and spatial repellents can be more precisely evaluated against laboratory-reared and field mosquitoes (Figure 1). These bioassays have been proven to have the advantage of helping us to understand the behavioral responses of mosquitoes exposed to the repellents (Smith, 1963), and how to best link data from laboratory tests to that from field tests (Vontas et al., 2014). Here we describe consideration for the design and implementation of experiments to measure the protective efficacy of repellents in controlled experiments carried out in semi-field systems and experimental huts in the field.

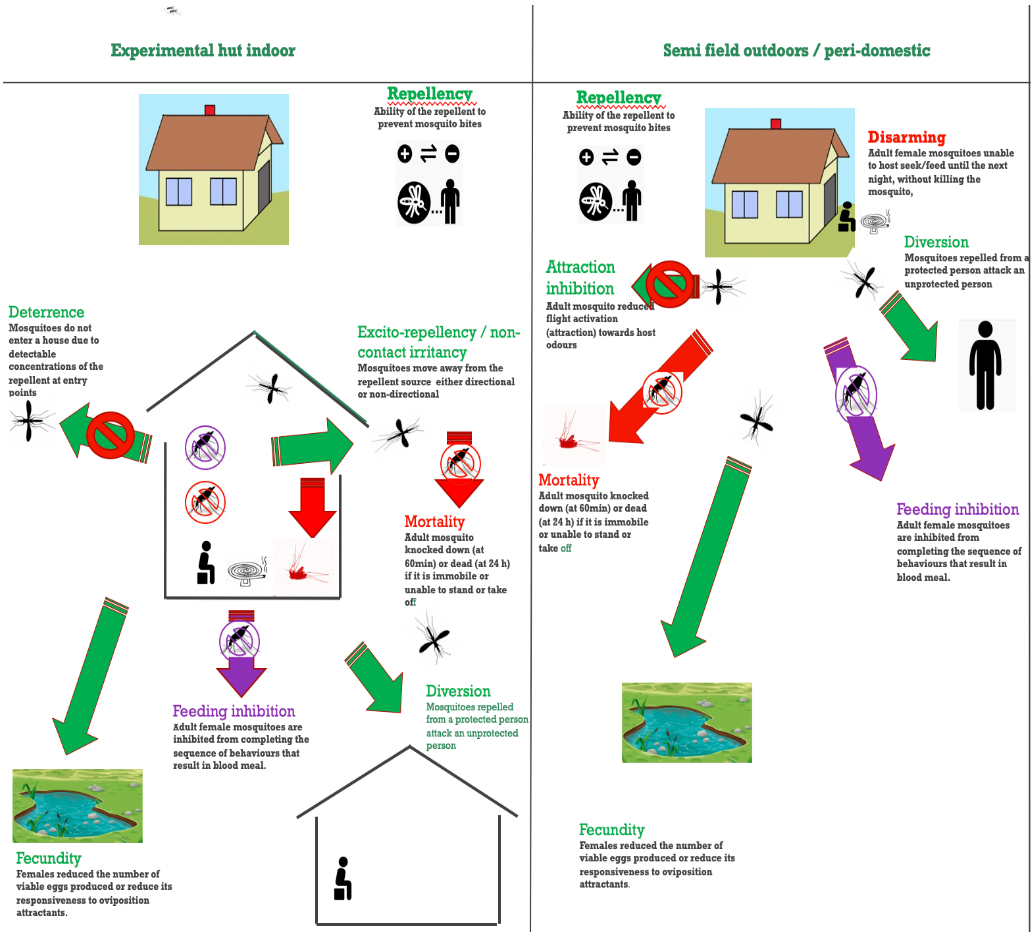


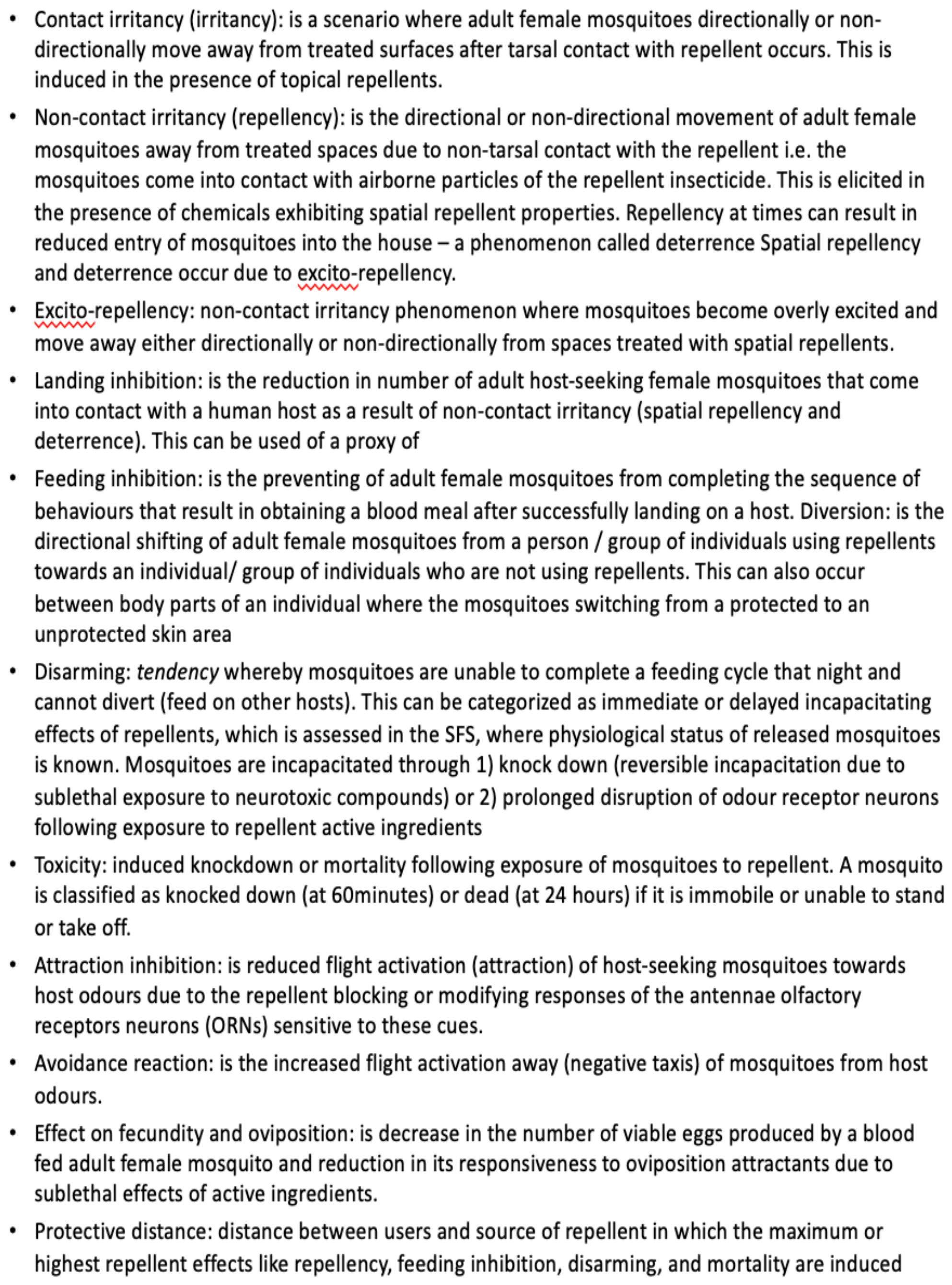
Figure 6.1. Outcomes measured when testing the mode of action of spatial and topical repellents and how they relate to product efficacy

6.3 Semi-field system (SFS) and experimental hut (EH) for evaluating repellents

Semi-field system (SFS) experiments were developed in an attempt to fill the gap between laboratory and field experiments for ecological studies (Ferguson et al., 2008) and for the evaluation of vector control tools such as indoor residual spraying (Silver and Service, 2008). They have proved extremely useful for the evaluation of behaviorally active odorants in mosquito traps (Schmied et al., 2008, Turner et al., 2011), topical repellents (Mbuba et al., 2020), repellent sandals (Sangoro et al., 2020) and spatial repellents (McPhatter et al., 2017).

Semi-field systems and EH evaluations are useful in the developmental pipeline of interventions such as repellent against mosquitoes transmitting diseases. Such bioassays allow indoor and outdoor evaluations of topical repellents (Sangoro et al., 2014b), and spatial repellents (Menger et al., 2014, Menger et al., 2015, Mmbando et al., 2018). They are useful methods to evaluate personal protection because protective efficacy estimations are similar for semi-field and full-field evaluations (Ogoma et al., 2017, Ogoma et al., 2012b). They allow experiments to be conducted at any time using disease-free insectary-reared mosquitoes of known physiological and insecticide resistance profiles making reliable and reproducible data. This provides an opportunity to evaluate the efficacy of repellents against both resistant and susceptible strains of the same species which might not be possible when conducting experiments in the field where most of the disease-vectoring mosquito species are likely to be of the same resistance phenotype (WHOPES, 2013a). Findings from semi-field systems could be satisfactorily used to extrapolate the efficacy of repellents when applied in the field while reducing the risk of acquiring mosquito-borne pathogens from field-testing (Sangoro et al., 2014b). Data generated from these studies can be used to improve the performance of repellents before further testing at the community level in randomized control trials as endpoints collected in these studies directly translate to impact on disease (Box with endpoints).

Box 6.1 Endpoints measured during the semi-field and experiment hut evaluation of spatial repellent and topical repellents



- Contact irritancy (irritancy): is a scenario where adult female mosquitoes directionally or non-directionally move away from treated surfaces after tarsal contact with repellent occurs. This is induced in the presence of topical repellents.
- Non-contact irritancy (repellency): is the directional or non-directional movement of adult female mosquitoes away from treated spaces due to non-tarsal contact with the repellent i.e. the mosquitoes come into contact with airborne particles of the repellent insecticide. This is elicited in the presence of chemicals exhibiting spatial repellent properties. Repellency at times can result in reduced entry of mosquitoes into the house – a phenomenon called deterrence Spatial repellency and deterrence occur due to excito-repellency.
- Excito-repellency: non-contact irritancy phenomenon where mosquitoes become overly excited and move away either directionally or non-directionally from spaces treated with spatial repellents.
- Landing inhibition: is the reduction in number of adult host-seeking female mosquitoes that come into contact with a human host as a result of non-contact irritancy (spatial repellency and deterrence). This can be used of a proxy of
- Feeding inhibition: is the preventing of adult female mosquitoes from completing the sequence of behaviours that result in obtaining a blood meal after successfully landing on a host. Diversion: is the directional shifting of adult female mosquitoes from a person / group of individuals using repellents towards an individual/ group of individuals who are not using repellents. This can also occur between body parts of an individual where the mosquitoes switching from a protected to an unprotected skin area
- Disarming: *tendency* whereby mosquitoes are unable to complete a feeding cycle that night and cannot divert (feed on other hosts). This can be categorized as immediate or delayed incapacitating effects of repellents, which is assessed in the SFS, where physiological status of released mosquitoes is known. Mosquitoes are incapacitated through 1) knock down (reversible incapacitation due to sublethal exposure to neurotoxic compounds) or 2) prolonged disruption of odour receptor neurons following exposure to repellent active ingredients
- Toxicity: induced knockdown or mortality following exposure of mosquitoes to repellent. A mosquito is classified as knocked down (at 60minutes) or dead (at 24 hours) if it is immobile or unable to stand or take off.
- Attraction inhibition: is reduced flight activation (attraction) of host-seeking mosquitoes towards host odours due to the repellent blocking or modifying responses of the antennae olfactory receptors neurons (ORNs) sensitive to these cues.
- Avoidance reaction: is the increased flight activation away (negative taxis) of mosquitoes from host odours.
- Effect on fecundity and oviposition: is decrease in the number of viable eggs produced by a blood fed adult female mosquito and reduction in its responsiveness to oviposition attractants due to sublethal effects of active ingredients.
- Protective distance: distance between users and source of repellent in which the maximum or highest repellent effects like repellency, feeding inhibition, disarming, and mortality are induced

6.3.1 Semi-field systems

An SFS is a large screened cage that facilitates controlled experiments with disease-free laboratory-reared mosquitoes under ambient climatic conditions (Ngowo et al., 2017, Ferguson et al., 2008). Generally, an SFS is made of several compartments with walls made of durable netting to approximate ambient microclimatic conditions (Figure 6.2) (Ferguson et al., 2008). These structures are found in many research institutes globally on all continents. The SFS structure can be mounted in a concrete base if the area is prone to flooding (Ferguson et al., 2008) and surrounded by a water channel (moat) that restricts entry of ants that would predate on mosquitoes during experiments. Standardized huts of similar construction materials and features to local houses that can be fitted with window and eave exit traps (Okumu et al., 2012) can be constructed within each SFS compartment for evaluations of indoor repellents or to simulate the peridomestic space.

The SFS can also be a long tunnels (semi-field tunnel (SFT)) (100m × 3.1m × 2.1m) made of mosquito netting. The SFT structure can also be mounted on a concrete base if the area is prone to flooding as well as have a water channel (moat) similar to SFS (Ogoma et al., 2014b).

Figure 6.2. image of the semi-field systems (SFS) located in Ifakara, Tanzania

6.3.2 Experimental huts (full-field testing)

Using local human houses to evaluate the efficacy of interventions for the control of arthropod vectors poses various limitations known to affect mosquito density and response to interventions. These limitations include (1) differences in the total number of individuals residing and the attractiveness of these occupants to mosquitoes (Mukabana W., 2002); (2) lack of uniformity in materials used to construct houses and furniture within (Kirby et al., 2008); (3) variation in size, number, and location of openings (Okumu et al., 2012); (4) variation in spatial location of homes in relation to larval habitats (Van Der Hoek et al., 2003); and (5) differences in the size of the house. In the home, it is almost impossible to find knocked down or dead mosquitoes because of the presence of scavenger insects such as ants. To standardize data collected during the evaluation of indoor vector control interventions such as indoor residual spray (IRS), researchers have designed and developed modified huts i.e. experimental huts which are similar in size to the house in the field (Okumu et al., 2012). The huts are constructed near natural mosquito larval habitats to increase the availability of mosquitoes in them (Van Der Hoek et al., 2003) and can be positioned inside the SFS (Figure 6.3). EHs have been widely used to study mosquito behavioral responses in the presence of interventions including repellents (Massue et al., 2016, Ogoma et al., 2014a). They allow mosquitoes to enter and then retain the mosquitoes that have entered so that mosquito behavior in response to repellent exposure can be evaluated.

Figure 6.3 Picture of experimental huts located in Ifakara, Tanzania.

6.4 Considerations for conducting semi-field system and experimental huts experiments

To ensure reproducible findings between SFS experiments in multiple sites under similar conditions, there are several considerations for SFS and EH studies that need to be harmonized before running the experiments (Figure 6.4). This will ensure that mosquitoes can exert their natural behavioral responses that they would occur under field conditions, while interacting with human hosts in the presence of repellents (Ogoma et al., 2012a). The factors can be organized into five main categories: (1) environmental conditions, (2) the product itself, (3) the bioassay, (4) test system (mosquito), and (5) host factors (Figure 6.4 and Table 6.1).

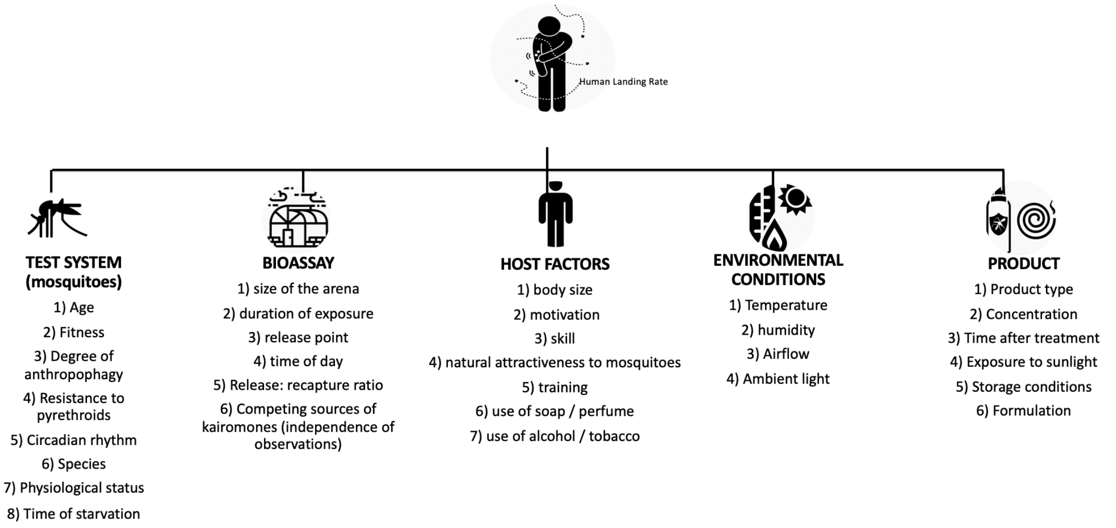


Figure 6.4. Factors influencing human landing rate that needs to be considered during the designing of an experiment to evaluate the protective efficacy of spatial repellents

6.4.1 Environmental conditions

Climatic conditions such as wind speed, temperature, and humidity in the treated space determine the performance of repellent (Kawada et al., 2005). Hoffman *et al.* (2002) demonstrated an increased efficacy of DEET when used in artificially windy conditions generated by a fan (Hoffmann and Miller, 2002). The temperature has been found to affect the vaporization of repellents hence, its concentration of the active ingredient available to mosquitoes. Volatile pyrethroids such as transfluthrin evaporate more readily at higher temperatures (Pettebone, 2014) and have been reported to provide higher protection at temperatures between 21°C and 30°C, with a reduction in protection specifically at lower temperatures (Ogoma et al., 2017). The toxicity (knockdown/mortality) of pyrethroids is also temperature-dependent(Glunt et al., 2018). Topical repellents evaporate more rapidly when participants are sweating and therefore less efficacious at high-temperature (Khan et al., 1973). When ambient temperatures are low, it is possible that the protective efficacy estimates of volatile actives may be low. These factors must be considered when planning experiments.

It is also known that differences in microclimatic conditions may affect mosquito behavior. The ability of the mosquito to search for human hosts is reduced at low humidity (<40%) for some species (Takken et al., 1997) which may overestimate the efficacy of repellents. It is, therefore, important to ensure that the micro-climatic conditions in the SFS and EH are within an acceptable range for the precision of estimated efficacy. The climatic condition underlines the usefulness of conducting controlled experiments under normal use conditions to give a more realistic estimate of efficacy. Micro-climatic data loggers can be placed inside the SFS during the evaluation of repellent to constantly monitor the temperature and humidity. During periods of the year when humidity is low, wetting the surface of SFS floor may help to increase humidity.

Generally, insectary mosquitoes are reared at 27± 5°C and 40-100% relative humidity with approximately 12:12 light-dark (ambient lighting) (MR4, 2009, Gerberg et al., 1994). Microclimatic differences between the inside and outside of houses have been found to influence mosquito abundance and biting behavior (Ngowo et al., 2017). Therefore, the sudden subjection of mosquitoes to a different microclimatic condition when taking them from the insectary to the SFS may alter their biting behavior (Kirby and Lindsay, 2004). To minimize this bias, laboratory-reared mosquitoes used in SFS experiments are transferred from the insectary to the SFS in the released cage or are released approximately 30–60 minutes before the experiment is initiated to allow them to get used to the environmental conditions (acclimatization).

6.4.2 Product type and handling

In this context, product type refers to the device itself, the type of repellent the device impregnated with, and/or the repellent formulation. Having this information helps in the correct experiment design to evaluate specific endpoints of the repellent (Table 6.2). To ensure a reproducible result it is important that the description of repellent to be evaluated is clearly described including the active ingredient (AI), formulation, loading dose or concentration, number of devices used, and method of use (topical application, mats, fabrics, and vaporizers).

Correctly handling repellent-containing bottles and treated devices ensures the true performance of that device. Storing repellents at 4 °C is often useful during product development to reduce evaporation of volatile components between tests until the product has been formulated to withstand room temperature storage. To correctly track the quality of the bottle containing repellents, it should be labeled with the day, month, and year when it is opened.

It is important to know how long the repellent-treated device remains protective. To do this correctly, storage of the treated devices between the experiments needs to be ensured, often in collaboration with the product manufacturer. At a minimum, treated devices should be stored in a shaded environment away from direct sunlight to represent normal “field age” storage.

Table 6.1. Factors influencing data collected in semi-field and experimental hut bioassays

Factors	**Consideration for testing**	**Recommendation**	**References**
Bioassay	Size of the arena	• Must be suitable for recapturing mosquitoes • Representative of the user case scenario • Baseline experiment needs to be conducted to ensure that a reasonable density of mosquitoes is available	(Ogoma et al., 2014b, Njoroge et al., 2021, Mmbando et al., 2018, Mmbando et al., 2015, Masalu et al., 2017, Mmbando et al., 2019)
	Duration of exposure	• Longer exposure should be avoided as they are not representing the field situation	
	Release point	• Remote release mechanism need to be in place • If several cages used, release mosquitoes simultaneously • Preferred at the four corners of the semi-field system (SFS) to mimic mosquitoes approaching a house from a different direction • If multiple hosts are used, must be positioned equidistant from mosquito release points	(Njoroge et al., 2021, Tambwe et al., 2020)
	Time of day	• Performed experiment under optimal climatic conditions for mosquitoes e.g., not at the hottest part of the day • Conducted experiment to coincide with the mosquito circadian rhythm	
	Release/recapture ratio	• Ensure that at least 50% of the mosquitoes are recaptured • For mortality measurement close to 100% recapture is optimal	(Tambwe et al., 2020)
	Independence of the observations	• Separate adjacent compartments with polyethylene fabric • Rotate treatments between huts or compartments if possible, to control for locational bias	(Andrés et al., 2015, Ferguson et al., 2008)

		• Use no-choice test set ups	
Product	Product type	• Select products proved to work against mosquitoes species in lab experiments • Secured in the proper supporting material according to user instructions • Protected from being accessed by the animal or children • Consider mode of action in bioassay design	(Njoroge et al., 2021, Lupi et al., 2013, WHOPES, 2013a)
	Concentration and formulation	• Select dose based on laboratory studies • Higher doses may be equal to low doses for bite prevention • Avoid switching between formulations or types of repellent in a single study • Select well-formulated products over poorly formulated ones for longer-lasting products • Formulation can also be used to boost volatility	(Tambwe et al., 2020) (Njoroge et al., 2021, Ogoma et al., 2017)
	Time after treatment	• Label treated emanator with day, month, and year of preparation because of decreased efficacy of spatial and topical repellents over time • Include data for time after deployment in statistical analysis	(Tambwe et al., 2020, Njoroge et al., 2021, Ogoma et al., 2017)
	Exposure to sunlight	• Devices should be stored or aged in a shaded location away from direct sun exposure	
	Storage conditions	• Store items according to manufacturer instructions • Between experiments, store the treated devices should be stored at room temperature in shaded under the shade to reflect field age	(Tambwe et al., 2020, Ogoma et al., 2017)
Climatic condition	Temperature	• Conduct experiments at the correct temperature for spatial repellent evaporation	(Njoroge et al., 2021, Martin et al., 2020)

		- Delay experiment until ambient temperature is within the range for repellent evaporation - Place a data logger in the SFS to measure/monitor temperature	
	Humidity	- Perform experiment when humidity is between 60–100% because of effect of humidity on mosquito host-searching behavior - Place a data logger in the SFS to measure humidity during the experiment	(Andrés et al., 2015)
	Wind	- Perform experiment when windy does not affect host-searching behavior of the mosquitoes - Measure wind speed and direction with a wind anemometer during the experiment because of effect of wind on amount of repellent received by mosquitoes	(WHOPES, 2013a)
	Ambient light	- Conduct experiments at the light to coincide with mosquito's ambient light for the biting	
Host factors	Body size	- Body size affect mosquitoes attraction to human due to amount of heat released. - Allow volunteer rotation to counter individual variation in body size that affects attraction to humans because of the heat release differences. - If possible, use individuals with similar body sizes.	(Ogoma et al., 2014a, Ogoma et al., 2017, Takken and Verhulst, 2017, Ray, 2015)
	Motivation	- Provide meals or drink before the experiment to motivate volunteers to complete data collection - Consider breaks during prolonged experiments	
	Skills	- Select skilled Individuals to perform human landing catches (HLCs) - Train all potential volunteer on HLC technique	(Ogoma et al., 2017)

		• Before the experiment, check that each volunteer is able to collect a given number of mosquitoes from a given surrounding; this should be done by the primary investigator	
	Natural attractiveness to mosquitoes	• Rotate volunteers between chambers to account for natural differences in attraction to mosquitoes	
	Use of soap, deodorant, alcohol and tobacco	• Volunteers should avoid using deodorant, soap, alcohol, and tobacco for the test days	(de Jong and Knols, 1995, Verhulst et al., 2016, Shirai et al., 2002, Jufri et al., 2016)
Test system	Age	• The World Health Organization recommends the use of 3- to 5-day-old mosquitoes. • 3- to 8-day-old mosquitoes may be used • Record mosquito age	(Tambwe et al., 2020, Ogoma et al., 2012b, Obermayr et al., 2015, WHOPES, 2013a)
	Fitness	• Regularly asses mosquito body size, i.e., wing size of laboratory-reared mosquitoes • Regularly conduct survival experiment • Conduct baseline experiments to assess mosquitoes' ability to search for the human host in the semi-field compartment • Ensure that mosquitoes are health by reducing the possibility of contamination (e.g. fungus, microsporidia) in the insectary to optimize fitness	(Njoroge et al., 2021)
	Anthropophagy	• Evaluate repellent using relevant anthropophilic mosquitoes to control for different mosquito host-feeding preferences, which affect human landing rate • If two morphologically identical species, e.g., *An. arabiensis* and *An. gambiae*, are used at the same time, mark them with fluorescent powder	(Gillies MT, 1970, WHOPES, 2013a)
	Resistance to pyrethroids	• Use mosquitoes of known insecticide resistance • Regularly conduct susceptibility tests	Tambwe et al unpublished

		• To compare landing between resistant and susceptible mosquitoes, use same-species mosquitoes with different resistance levels	
Test system	Circadian rhythms	• Conduct experiments during natural mosquito biting time (metabolism of insecticides and CYP450 modulators is regulated by circadian rhythms)	(Masalu et al., 2017, Ogoma et al., 2014a, Ogoma et al., 2017, Ogoma et al., 2014b)
	Physiological status	• Nulliparous and starved mosquitoes should be used for experiment	(Barnard, 1998)
	Time of starvation	• Anopheles mosquitoes should be starved for around 5-6 hours • *Aedes aegypti* mosquitoes may be starved for up to 12 hours.	(Fernandes and Briegel, 2005)

6.4.3 Bioassay

With the development of new tools, bioassays are needed to generate initial efficacy data that enable for the prediction of tools' impact on vectorial capacity (Figure 1). The SFS enables evaluation of the efficacy of vector control tools in a more controlled environment. In the SFS, it is possible to use laboratory-reared mosquitoes to overcome difficulties such as varying mosquito availability so that tests can be conducted all year round with a known number of mosquitoes. Experimental huts are designed to be proxies of local houses and are standardized to minimize heterogeneity in the size, materials, and openings of the EH that affect mosquito density, mosquito behavior, and the amount of AI that they encounter (Massue et al., 2016). Experimental huts have been extensively used to demonstrate the efficacy of vector control tools such as volatile pyrethroids in a variety of formats (Ogoma et al., 2014a, Sangoro et al., 2014b). Moreover, exposure to insecticide-treated bed nets or indoor residual spray with excite-repellent compounds such as DDT has been measured in EH (Grieco et al., 2000).

Results from the SFS and EH are highly dependent on several factors. The size of the arena (where the experiment is conducted) affects results because of the concentration-dependent effects of AI in spatial repellent tests (Achee et al., 2012b). Differences in mortality estimates have been observed between small arenas such as taxis boxes where mosquitoes are held close to the source of the repellent used (Martin et al., 2020) compared to a larger arena (Tambwe et al., 2021b). The duration of exposure may also affect the efficacy of repellents (Bernier et al., 2019). The use of a small space or longer exposure for assessment of 24-mortality following transfluthrin exposure may overestimate the result as transfluthrin increases mosquito activity due to excitation and mosquitoes will move away from the source of the transfluthrin under natural outdoor exposure conditions (Sukkanon et al., 2020). Larger chambers enable the use of free-flying mosquitoes and either provide space for the humans to either perform human landing catches (HLCs) or allow mosquitoes to feed in the presence of transfluthrin, which is more representative of what happens in the field (Tambwe et al., 2021b). Space and duration of exposures (Bernier et al., 2019) should be considered when designing the experiment to evaluate endpoints of the repellent, in particular, mortality.

Results are also affected by the accidental loss of mosquitoes through escape or scavenging ants, which leads to uncertainty of estimates, particularly mortality estimates (Nash et al., 2021). It is therefore important to design the arena to reduce accidental loss of insects (Massue et al., 2019) and to make them ant-proof. Regular monitoring of the proportion recaptured assists in maintaining the quality of bioassays.

The density of the mosquitoes plays an important role in the evaluation of spatial repellents. The rate at which mosquitoes bite human volunteers is density-dependent and mosquitoes can select between hosts at short range (Gillies and Wilkes, 1972, Okumu et al., 2010) when one of the hosts is protected by a repellent (Moore et al., 2007). In EH there is often extreme heterogeneity in mosquito densities between huts (Johnson et al., 2015) which can be overcome by rotating treatments and volunteers between huts for many nights. An EH, in particular, should be designed so that all the huts allow mosquitoes to enter and leave through the traps. This minimizes inter-house differences in mosquito densities during the evaluation (Okumu et al., 2012). The dimensions, structure, construction materials, and location of huts in relation to distance from larval habitats should also be considered. For semi-fieldwork, the relative positioning of mosquito release points may determine the number of mosquitoes captured by human volunteers conducting HLCs. Mosquitoes should be released at the nearest points and in all directions around the HLCs or hut (if it is positioned in the semi-field system) to reflect mosquitoes coming from different directions. If a topical repellent test is conducted by several people, positioning them at least 10 meters apart releasing mosquitoes equidistant, and rotating volunteers between collection locations can help to minimize these biases (Mbuba et al., 2020).

To effectively evaluate indoor vector control tools, enough mosquitoes must enter a hut. During dry seasons, mosquito densities are low and it may not be possible to conduct EH studies. Therefore, the huts can be used in the SFS. When the huts are used in the semi-field system, there is no need to be concerned about the quality and quantity of mosquitoes because the laboratory-reared released mosquitoes are always in the required quantity. However, when experiments are conducted in the field, the density of mosquitoes entering these huts must be optimized. The location of residential houses with respect to larval habitats is a significant factor affecting the density of mosquitoes inside human houses (Van Der Hoek et al., 2003, Okumu et al., 2012, Haddow, 1942). In field experiments, it is essential that the EHs placed at equidistance from the larval sites or in areas of high mosquito density. This placement ensures that a sufficient density of mosquitoes enters the huts because the emerging mosquitoes travel on average the same distance towards the huts from either the larval habitats or other surrounding locations.

Repellents, particularly spatial repellents work at a distance to exert behavioral changes on exposed mosquitoes (Achee et al., 2012a, Ogoma et al., 2014b). Considering this mode of action, it is important to ensure the independence of the SFS compartments when evaluation is performed (Moore et al., 2007). For example, heavy-duty polyethylene walls may be used to separate compartments, preventing air movement between them and reducing the chance of cross-contamination when working with repellents or other aerosols (Ferguson et al., 2008). Also, it is important to consider that there is a huge variation in climatic conditions over 24 hours. Normally, at mid-day, the temperature is very high accompanied by low humidity, which does not support conducting an experiment. It is therefore important to conduct experiments at the appropriate time of the day (Rund et al., 2016).

6.4.4 Test system

For efficacy evaluations of repellents in the SFS, anthropophilic, zoophillic, susceptible, and resistant strains of the local medically important must be considered (Besansky et al., 2004). This gives a conservative estimate of repellent efficacy before field trials, clinical trials, or policy recommendations (WHOPES, 2013a, WHOPES, 2009b).

Mosquitoes use different stimuli such as skin odor, water vapor, heat, and visual cues to locate potential blood sources (Tisgratog et al., 2011, Bibbs et al., 2018, Takken, 1991, Takken and Knols, 1999). The strength at which mosquitoes are attracted to human cues varies between mosquito species or strains. Differences in attraction to a human are attributed to species' anthropophilic and zoophilic behaviors which tend to be genetically fixed (Mahande et al., 2007). Anthropophilic species such as *An. gambiae* s.s. and *Anopheles funestus* are more likely to blood feed on humans (Costantini et al., 1999) depending on the relative abundance (Asale et al., 2017) or availability (Iwashita et al., 2014) compared to zoophilic species such as *An. arabiensis* (Orsborne et al., 2018). The differences in landing rate between these mosquito species is caused by differences in attraction to human cues (Gillies, 1964). *Ae. aegypti* also feeds almost entirely on humans (Scott et al., 2000). In addition, species vary in their sensitivity to repellents (Van Roey et al., 2014) which result in different doses of repellent active ingredients needed to elicit responses where among some of the robust species such as *Ae. aegypti* higher concentration of repellents are required (Sukkanon et al., 2020), whereas *Culex* mosquitoes are easier to repel (Lupi et al., 2013). Studies have demonstrated differences in the complete protection time of topical repellents (Schreck, 1977, Curtis CF, 1987). Data suggests that different species of mosquitoes have different behavioral responses to repellents due to differences in repellent-sensing neurons or olfactory neurons (Afify and Potter, 2020).

Previous studies have reported that parity, age, and feeding status can influence host-seeking behavior (Xue and Barnard, 1996). Removing these biases through a selection of appropriate mosquitoes is an essential step toward performing a high-quality experiment by ensuring that appropriately aggressive and fit mosquitoes are used (Table 6.2). Avid mosquitoes are selected by placing the palm or warm objects on the side of the cage and aspirating only mosquitoes that are probing (WHOPES, 2013a). Various factors may affect the avidity or fitness of adult mosquitoes. Firstly, during the larval stages, environmental variations between bowls such as density and amount of food dispensed potentially influence the fitness and therefore host-seeking behavior of adult mosquitoes (Araújo et al., 2012). Secondly, starved mosquitoes are more likely to be more aggressive than sugar-fed mosquitoes (Fernandes and Briegel, 2005). *Starving* refers to removing sugar solution from the cage containing adult mosquitoes before the evaluation of repellent to optimize the mosquitos' avidity and thereby host-searching behavior. Thirdly, mosquito avidity is related to age. Younger female mosquitoes have lower responses to topical mosquito repellents (Xue and Barnard, 1996), while older mosquitoes are more responsive to repellents (Mulatier et al., 2018). Therefore, younger mosquitoes are preferred because they are most likely to exhibit host-seeking behavior and are less likely to be affected by the repellents (Aldridge et al., 2017) giving the most conservative estimate of repellent activity.

To ensure that the mosquitoes used are as heterogeneous as possible, selection is done from different cages using a minimum of three cages. The WHO recommends that mosquitoes need to be nulliparous aged 3-5 days and starved for at least 6-8 hours before the experiment (WHOPES, 2013a). The number of mosquitoes to be selected depends on the number estimated by the sample size calculation, which is usually based on parameters measured in previous experiments such as variability between locations, daily variability in mosquito attack rate, and variability in volunteer attractiveness to mosquitoes (Johnson et al., 2014). Sangoro *et al.*, (2014) evaluated the efficacy of topical repellent when 300 mosquitoes were released for 3 hours inside the SFS to match biting pressures experienced in field trials (Sangoro et al., 2014b). In another study 100 *Ae. aegypti* mosquitoes were released inside an SFS during the evaluation of a transfluthrin-treated passive emanator with no concerns from the volunteer (Tambwe et al., 2020). However, in large SFS only 60% of the total released mosquitoes were recaptured by HLC (Njoroge et al., 2021).

Another factor for consideration is that mosquito species have different circadian rhythms. Although laboratory-reared mosquitoes may be adapted to bite at any time throughout the day, it is highly recommended that repellent evaluation be conducted to coincide with the mosquito's natural biting time. For example, in East Africa, the host-seeking activity of female Anopheles mosquitoes ranges from 18:00~06:00 (Moshi et al., 2017) which means that evaluation of repellent needs to be done between these times. In addition, circadian rhythms are an important determinant of the insecticide detoxification (Balmert et al., 2014) and will therefore impact responses to volatile pyrethroids (Tainchum et al., 2014).

Also, pyrethroids have been the main class of insecticide used in long-lasting insecticide-treated nets LLINs and IRS (Zaim et al., 2000). Resistance to these insecticides is now widespread (Mitchell et al., 2012), which poses a threat not only to the efficacy of the main vector control tool but potentially to repellents in particular spatial repellents as they belong to the same chemical class, which would normally indicate cross-resistance. Wagman *et al.* (2015) concluded that insensitivity to sub-lethal doses of transfluthrin against the dengue vector *Ae. aegypti* are heritable and correlate to reduced susceptibility to toxic doses of transfluthrin in CDC bottle (Wagman et al., 2015a). Therefore, it is necessary to know the insecticide susceptibility status of the mosquitoes to be used for the evaluation of volatile insecticides.

If multiple strains that are not morphologically distinguishable are used in semi-field experiments, mosquitoes can be marked with fluorescent colors to distinguish between strains. Mosquitoes are marked in a cup by dusting the mesh lid of the cup with a brush containing the color pigment thereby creating a cloud of pigment that is transferred to the mosquitoes in small amounts. Preliminary experiments have shown that the fluorescent pigments do not significantly influence mosquito survival or feeding behaviors and can easily identified using an infrared torch (Saddler et al., 2019).

6.4.5 Host factors

Variation in recaptured mosquitoes between human subjects has been demonstrated previously (Lindsay et al., 1993). Such differences affect the results of repellent evaluations and need to be considered during experimental design (Rutledge and Gupta, 1999).

Mosquitoes explore heat emitted by the human host (a short-range cues) to land and bite host vertebrates, including humans (Ray, 2015). The amount of heat emitted differs from one individual to another and is largely dependent on the body size, which affects individual attractiveness to host-seeking mosquitoes (Carnevale et al., 1978). To account for these variations, study designs should allow the rotation of treatments between volunteers (WHOPES, 2013a). Volunteer rotation between compartments can be straightforward, but treatment rotation may require knowledge from previous experiments or preliminary results. Repellents are known to have residual effects thus enough time is needed to allow diffusion of the residual repellent actives before rotation is done to avoid the occurrence of a carry-over effect (Ogoma et al., 2014a). Alternatively, in some circumstances, the treatment can remain fixed in one location over the duration of the experiment depending on the experimental design and the insecticides to be evaluated. For example, in a study by Ogoma *et al.* (2014) where the effect of DDT and airborne pyrethroids were evaluated on entomological parameters of malaria transmission, treatments were not rotated instead, the volunteer rotated between the treatments (Ogoma et al., 2014a). In contrast, both treatment and volunteer were rotated when Andres *et al.* (2015) evaluated the efficacy of transfluthrin in reducing human landing rate in the peridomestic space (Andrés et al., 2015).

The skill and motivation of mosquito collectors is known to cause variation in the number of mosquitoes collected. To account for this, experienced volunteers can be provided with proper training before the commencement of the experiment. Mosquito landing may also be affected by the use of soap (de Jong and Knols, 1995), deodorant (Verhulst et al., 2016), alcohol (Shirai et al., 2002) and tobacco (Jufri et al., 2016) prior to the experiment which may affect repellent endpoints. It is recommended that volunteers be educated in advance on how the use of these items may affect repellent evaluation.

6.5 Study Power

Study power refers to the calculation of the representative number of mosquitoes, number of replicates, and number of days required to detect a predefined difference between either the treatment and the control arms or different active formulations.

It is recommended that sample size calculations, for instance, generalized linear mixed models (GLMMs) using the lme4 package (Bates et al., 2011) be performed before an experiment, using a 1000 simulation-based power analysis (Johnson et al., 2015) in R statistical software version with at least >80% power and a significance level of 0.05 for rejecting the null hypothesis. Estimation of the variation among the locations, day, volunteers, mosquito density, and recapture rate should be considered in the simulation. These are usually estimated from previous evaluations of similar products. Other factors to consider are whether the study is measuring superiority, non-inferiority or equivalence between the treatment arms.

6.6 Primary outcomes measured in the semi-field system/experimental huts and computations

Repellents can induce various behavioral responses when exposed to mosquitoes depending on the dosage, distance from a point source, temperature, and airflow. Outcomes that result in the prevention of human-vector contact are defined in Box 1 and include repellency, irritancy, deterrence, attraction inhibition, feeding (biting) inhibition, toxicity (knockdown (KD) and mortality), disorientation or disarming and effects on fecundity (Sukkanon et al., 2020, Bibbs et al., 2020, Ogoma et al., 2014a, Achee et al., 2012a). These outcomes can be assessed in SFS and EH experiments (Table 6.2). In general, these outcome parameters measure the personal protection of the repellent user, while effects on fecundity, and toxicity (KD and mortality) can also measure the community protection of the repellents because both users and non-users benefit from the reduced size and survival of the mosquito population (Magesa et al., 1991).

Based on these outcomes, it is possible to measure the efficacy of repellents indoors and/or outdoors (in the peridomestic space) in both SFS and EH evaluations. The primary outcomes can be assessed using various study designs such as comparative cross-over designs with wash-out periods, choice and no-choice evaluations, and fully randomized and partially randomized Latin squares among others. The choice of the study design depends on the repellent being tested, the number of treatment arms, the purpose of the study, and the resources available. For the efficacy evaluations to be robust, it is important that control and treatment arms be present. The control arm is essential because it allows for the effects of any intervention (s) being tested to be distinguished from natural events that would have occurred even in the absence of intervention.

Formulae, classical and inferential statistical analysis can be used to estimate efficacy conferred by repellents. The analysis approach to be used depends on the study design and outcome of interest. Common classical analyses include; parametric and non-parametric t-tests, z-test and Analysis of variance (ANOVA). Inferential statistics such as generalized linear mixed model (GLMM), survival analysis, and binomial regression are usually applied to data generated from these evaluations accounting for temperature, humidity, volunteer attractiveness and treatment doses. The distribution to be used in the inferential analysis will depend on whether the proportions (Binomial) or actual numbers (Poisson) of the mosquitoes are being modeled. Additionally, it may be necessary to account for any overdispersion (variance > 2 times the mean) or the presence of zeros depending on the distribution of the data collected.

In regression analysis, the primary outcome effect of the repellent is fitted as the dependent variable, with treatment/volunteer/compartment/location and other factors as fixed independent variables while day can be included as a fixed or random effect. Basic formulas for protective efficacy (PE) can also be used in analysis. Inconsistencies in the PE estimates have been observed when using the basic formula and estimates from the model. The reason for this could be that the basic formula is not sensitive enough to capture small differences that may be attributed to other factors that are adjusted for in model estimates of PE. Estimates from the model are more reliable than the PE from the basic formula because they account for other variables in the experiment. The simplest way to generate model estimated PE is to use regressions for count data and to describe the PE as 1-the relative rate of mosquito recapture.

. Table 6.2. Outcomes during the evaluation of repellents in the semi-field and experimental huts

End points measured	Formula for measuring	Evaluation in Semi field system (SFS)	Evaluated in Experimental hut (EH)
Repellency	Protective efficacy (PE) = [(C – T)/C] × 100%, where C = proportion/number caught in control and T = proportion/number caught in treatment	Yes	Yes
Deterrence	Deterrence = [(C – T)/C] × 100% where C = number of fed mosquitoes in control and T = number of fed mosquitoes in treatment	Yes, if huts are inside the SFS	Yes
Excito-repellency	Excito-repellency= ((Te/Tt)-(Ce/Ct))/1-(Te/Tt) where Te = number of mosquitoes in exit trap in treatment, Tt = total number of mosquitoes in treatment hut, Ce = number of mosquitoes in exit trap in control, Ct = total number of mosquitoes in treatment hut	Yes, if huts are inside the SFS	Yes
Landing inhibition	Landing Inhibition (LI) = [(C – T)/C] × 100%, where C = proportion/number landed mosquitoes in human landing catch (HLC) in control and T = proportion/number landed mosquitoes in human landing catch (HLC) in treatment,	Yes	Yes
Feeding inhibition	Feeding Inhibition (FI) = [(C – T)/C] × 100%, where C = number of fed mosquitoes in control and T = number of fed mosquitoes in treatment	Yes	Yes

Diversion	Diversion= (T1/T2)/(C1/C2) Where T1= number of mosquitoes caught by HLC in position 1 near repellent T2= HLC in position 2 and number of mosquitoes caught by HLC in the control c1= HLC in position 1 and C2=HLC in position 2	Yes	Yes
Disarming	Disarming at 12 hours (D12) = [(CD12-TD12)/C D12] x100% Where C D12 =proportion of mosquitoes that successfully feed after exposure in the control , TD12 =proportion of mosquitoes that successfully feed after exposure in and treatment	Yes	Yes
Attraction inhibition	Attraction inhibition (AI)= activated mosquitoes (a) = [T/(T+A)] Where number of mosquitoes move toward=T and away=A	Yes	No
Avoidance reaction	Avoidance reaction (AR)= [(A/(T+A)] Where number of mosquitoes move toward=T and away=A	Yes	No
Mortality Corrected for the control	Corrected control mortality (CM24) = (T – C)/(1 – C) × 100%, where C = proportion/number of caught mosquitoes that died in control and T = proportion/number of caught mosquitoes that died in treatment	Yes	Yes
Fecundity	Fecundity= [(C – T)/C] × 100%, where T = median eggs per female in treatment and C = median eggs per female in control	Yes if mosquitoes feed	Yes if mosquitoes feed

6.6.1 Contact Irritancy

Irritancy is the ability of the repellent to induce directional or non-directional movements of the mosquitoes away from the treated surfaces resulting from tarsal contact irritancy. This can lead to the exiting of mosquitoes that had already entered a hut an occurrence referred to as excito-irritancy. This can be measured for topical repellents by comparing the relative rate of mosquitoes collected in experimental huts and SFS with treatment relative to an untreated (Table 6.2). Mosquitoes can be collected from HLC, indoor resting catches, or exit traps on the huts.

6.6.2 Non-contact irritancy (Repellency and deterrence)

Repellency (spatial repellency) is the ability of the repellent to keep mosquitoes away from the treated space that may occur through a variety of mechanisms (Ogoma et al., 2012a) (Box1) when the mosquitoes come into contact with airborne particles of repellent insecticides. The presence of repellents can reduce the entry of mosquitoes into the houses – a phenomenon called deterrence (Grieco et al., 2007, Kennedy, 1947, Ogoma et al., 2014b). Both repellency and deterrence occur in the presence of spatial repellents.

Repellency or deterrence is estimated by comparing the relative rate of mosquitoes collected in experimental huts with treatment relative to an untreated hut (Table 6.2). Mosquitoes can be collected from HLC, indoor resting catches, or exit traps on the huts (Figure 6.3). When risk ratios are used, repellency and its confidence intervals are estimated from the mosquito recapture counts in the control and treatment by replicate for instance for each day (Table 6.2).

6.6.3 Excito-repellency

Excito-repellency: non-contact irritancy phenomenon where mosquitoes become overly excited and move away either directionally or non-directionally from spaces treated with spatial repellents. This is estimated by comparing the number of mosquitoes collected in the treated huts relative to an untreated hut (Table 6.2). Mosquitoes can be collected from indoor resting catches and or exit traps on the huts

6.6.4 Landing inhibition

Landing inhibition refers to the reduction in the number of female mosquitoes that come in contact with the host. HLC remains the most accurate method for determining feeding/biting inhibition (WHOPES, 2009a) in both semi-field and full-field. The overall number of mosquitoes landing on the volunteers conducting HLC in the treatment relative to the control is determined. When risk ratios are used, landing inhibition and its confidence intervals are estimated from the mosquito recapture counts in the control and treatment by replicate (Table 6.2).

6.6.5 Feeding (biting) inhibition

Feeding (biting) inhibition refers to the ability of a repellent's AI to inhibit mosquitoes from feeding or biting even after landing on the potential host. Also, HLC remains the most accurate method for determining feeding/biting inhibition (WHOPES, 2009a) in both the semi-field and full-field and is safe in areas where there is no active transmission of vector-borne pathogens provided it is medically supervised (Achee et al., 2015b).

The feeding inhibition (FI) endpoint is determined by comparing the proportion of blood-fed mosquitoes between the control and treated arms. To directly measure this in the SFS, mosquitoes are released in the compartment and the volunteer remains in the chamber or room for the period of interest while allowing mosquitoes to bite. At the end of the experiment, all the mosquitoes are collected from the chamber and the proportion of fed mosquitoes in the treatment arm relative to the control is determined (Tambwe et al., 2021b).

In EH testing, the overall proportion of blood-fed mosquitoes caught inside the hut including the floor or wall or in the exit traps of the treatment hut relative to the control is determined. FI is calculated as personal protection (Table 6.2). It should be noted when running regression analysis, either the proportion fed or unfed out of the total recaptured, or the absolute numbers fed can be fitted in the model. When risk ratio/odds ratios are used, the FI and its confidence intervals are estimated (Table 6.2).

6.6.6 Diversion

In a scenario of incomplete coverage, that is incomplete application of a topical repellent or members of a group do not use repellent, mosquitoes that are repelled or inhibited from biting can switch to a nearby unprotected area of skin or another host (Moore et al., 2007). Diversion is measured by measuring the ratio of mosquito landings on a protected and unprotected individual in the treatment relative to landings on an unprotected individual in the control (Table 6.2).

6.6.7 Disarming

Disarming refers to mosquitoes that are unable to complete a feeding cycle that night and cannot divert (feed on other hosts). This can be categorized as immediate or delayed incapacitating effects of repellents, which is assessed in the SFS, where the physiological status of released mosquitoes is known. Mosquitoes are incapacitated through; (1) knockdown, which is a reversible incapacitation due to sublethal exposure to neurotoxic compounds, or (2) prolonged disruption of odor receptor neurons by repellents. This is important because it protects multiple individuals and not just users i.e., a community effect, and also reduces the vectorial capacity (Denz et al., 2021). Shorter-term feeding inhibition occurs when a mosquito can land but doesn't feed. This is commonly observed with repellents such as DEET that affect odor receptor neurons (DeGennaro, 2015). It is short lived and mosquitoes are able to divert to an unprotected host in the same feeding cycle. Disarmed mosquitoes return to host-seeking and start a new feeding cycle within one to three days.

Disarming is measured by collecting alive unfed mosquitoes, placing them in a holding cage or cups then observing the feeding success of the mosquitoes when they are offered a blood-meal source away from the source of repellent (Ogoma et al., 2014b) as soon as possible after exposure and counting the knocked down mosquitoes that are alive 24 hours later. It can also be determined using mathematical models of mosquito host-seeking and estimating the rates of mosquito feeding, repelling and disarming from HLC data collected in 15-minute intervals (Denz et al., 2021)

6.6.8 Toxicity (knockdown and mortality)

Toxicity is the measure of the degree of toxic effect of repellent on exposed mosquitoes. Knockdown (KD) and mortality are the two effects of repellent toxicity which depend on the initial loading dose on the substrate/surface, environmental factors including the volume of the treated space, distance from the repellent source, release rate, and degradation rates of the repellent. Knockdown is scored if a mosquito is unable to stand or fly in a coordinated manner within 60 minutes after exposure. Recaptured mosquitoes are placed in the netted cup supplied with sugar (10% sucrose or glucose) and taken to a climatic-controlled room for observation of delayed mortality. At 24 hours post-exposure, a mosquito should be re-examined and classified as knocked-down revived or knocked-down died (WHOPES, 2013a). Delayed mortality whether before (pre-prandial) or after (post-prandial) a successful blood meal measures the community or personal protection conferred by the repellent. This can be estimated by comparing the proportion of dead mosquitoes after 24 hours from those captured alive (fed or unfed) in the treatment arm relative to the control. To estimate toxicity in SFS and EH due to exposure to the repellent, the proportion of dead mosquitoes (fed and unfed) in the treatment arm corrected for mortality in the control arm is calculated for each experimental replicate (Table 6.2).

6.6.9 Attraction inhibition

Attraction inhibition refers to the reduced flight activation (attraction) of mosquitoes towards host odors. Attraction inhibition occurs mainly because repellents block or modify responses of the olfactory receptor neurons (ORNs) on mosquitoes' antennae; these receptors are sensitive to specific host attractants (Dickens et al., 2013, Davis and Sokolove, 1976). For example, the application of DEET has been found to reduce orientation of mosquitoes to lactic acid cues produced from human sweat by decreasing the sensitivity of ORN to lactic acid thus, reducing the attraction of mosquitoes towards human-host (Dickens et al., 2013). The same effect has also been reported for linalool, dehydrolinalool, catnip oil and citronella (Ogoma et al., 2014b, Bohbot and Dickens, 2010, Kuthiala et al., 1992). To estimate attraction inhibition numbers of mosquitoes that moved into the chamber closer or away to the stimulus in a taxis box can be recorded (Lorenz et al., 2013).

6.6.10 Avoidance reaction

Avoidance reaction refers to the induced flight activation (negative taxis) of mosquitoes away from host odors. To estimate avoidance, the numbers of mosquitoes moved into the chamber away from the stimulus in a taxis box is recorded (Lorenz et al., 2013).

6.6.11 Effect on fecundity and oviposition

Exposure to sublethal concentrations of repellents has been found to affect subsequent fecundity and oviposition behavior in mosquitoes by; (1) decreasing the number of viable eggs produced (Bibbs et al., 2018), and (2) reducing responsiveness of the ORN-sensitive to oviposition attractants (Kuthiala et al., 1992, Bibbs et al., 2018). This has been observed in *An. gambiae s.l.* mosquitoes when exposed to sublethal concentration of transfluthrin, where the number of eggs produced was reduced (Ogoma et al., 2014a). Similar effects were observed in *Ae. aegypti* and *Ae. albopictus* for which the number of viable eggs laid and their skip oviposition behavior were significantly declined when the mosquitoes were exposed to a repellent (Bibbs et al., 2018).

Fecundity in mosquitoes is measured by the proportion of viable eggs laid while oviposition is the measure of successful laying of viable eggs. The efficacy of repellents in reducing the number of viable eggs (fecundity) and oviposition success can be assessed by exposing adult females to a repellent during feeding then allowing them to oviposit and counting the number of eggs produced in the treatment arm relative to those from the control arm.

6.6.12 Protective distance

Protective distance is the distance between users and the source of the repellent in which repellents can confer repellency, feeding inhibition, disarm, knockdown, and kill. This depends on the initial loading dose on the substrate/surface, and environmental factors such as wind speed, release rate, and degradation rates of the repellent. Both SFS and EH experiments can be used to estimate the indoor and outdoor protective distance of a repellent product via HLCs, which can be achieved by having treatment arm/repellent users and control arm/nonusers apart at distinctly different distances. To ensure robustness, the volunteers should be rotated and adequate replication conducted to allow precise estimates of protection adjusting for volunteer, location, night and hour of collection. The distance after which an equal number of mosquitoes are captured in the control arms is determined to be the protective distance or radius of the repellent.

6.7 Use of SFS and experimental hut data for mathematical models

Mathematical models describe the underlying mechanisms that drive a system. They aim to represent a system based on assumptions of its dynamics with a simplified description of the mechanism using assumptions on parameters that often use estimates derived from collected data. The SFS and EHs are often used to parameterize mathematical models because they provide standardized estimates of effects that may not be measured under field conditions, or provide a more cost-effective means to provide model parameters. Models can be used to predict changes in vectorial capacity (Brady et al., 2016) in mosquitoes only as a proxy for transmission or impact on malaria using individual-based stochastic simulations of malaria epidemiology to predict the impacts of interventions on infection, morbidity, mortality, health services use and costs (Smith et al., 2008, Denz et al., 2021, Hellewell et al., 2021).

The two most important factors in determining the effectiveness of a vector control tool are feeding preference and average lifespan. A preference for feeding on humans increases the likelihood of parasite transmission. An adult mosquito's lifespan is also critical for malaria transmission as the mosquito must survive long enough for the parasite to complete the period of sporogonic development, which covers the period from the ingestion of gametocytes in the blood meal to the time when infectious sporozoites appear in the salivary glands. When evaluating the effects of repellents there are three main parameters measured in the semi-field/experimental huts:

1) Repellency/deterrence: The mosquito is unable to feed or enter a house when it wants to. However, it continues host-seeking through the night, either on the same host/household or it is diverted to a different host/household. At the end of the night, it may have successfully fed (on the same host/ household or a different host/household), it may have

died later in the night, or it may end the night unfed and alive. The repellency/deterrence is thus treated in the model as a reduction in the availability or the "attractiveness" of the human to the mosquito.

2) Disarmed: The mosquito is removed from the feeding cycle and no longer continues host-seeking that night. This is may be because the mosquito is (i) knocked down; (ii) no longer capable of biting (i.e it may appear to host-seek and may land on the host but would not bite); or enters the resting phase without having fed. The period of disarming would last for at least one night – but may be longer. At the end of the night, disarmed mosquitoes would either be knocked down (and alive 24 hours later) or recaptured unfed alive.
3) Dead: The mosquito is killed by the intervention, either immediately or after a short delay. These mosquitoes could be unfed dead (pre-prandial mortality), fed dead (post prandial mortality), or knocked down (and dead after 24 hours).

Using these relationships described through equations, mathematical models can predict the community-level impact of a repellent by incorporating the outcome parameters measured from the SFS or EH experiments. Outcomes can be used to parameterize models with different species characteristics such as levels of anthropophagy or exophily and how a repellent with a specific mode of action e.g., landing inhibition, knock down or repellency may affect malaria or other vector-borne diseases when the tool is applied to an individual (personal protection) or a community (community protection) at different coverage levels.

The sensitivity of effect sizes to proportional changes in the parameters comprising vectorial capacity shows that protecting people from bites through the use of personal protection has a second-order effect because it appears twice in the VC equation (Brady et al., 2016). Therefore reducing 50% of mosquito bites would result in VC that is ½ x ½ = ¼ i.e.

only 25% of VC would remain giving a 75% reduction. Importantly, by reducing the human biting rate, especially for a feeding cycle (disarming), fewer mosquitoes will be able to develop eggs and this will have a knock on effect on VC. As fewer eggs means fewer mosquitoes – this is a first order effect similar to that seen with larval source management, and combined with the second order effect of reducing bites an intervention with 50% efficacy would result in ½ x ½ x ½ =12.5% giving a 87.5% reduction in VC. However, the greatest impact of VC is through reducing a mosquito life span. Reducing mosquito daily survival impacts the probability that the mosquito will survive the intrinsic incubation period as well as the number of eggs that the mosquito will lay in its lifetime (lifetime fecundity). It reduces adult density that means that the next generation also contains fewer adults. Therefore, if it is assumed that the effect on oviposition and the next generation is also 50% the effect is 4th order, therefore for an intervention that kills 50% of mosquitoes the effect on VC would be ½ x ½ x ½ x ½ = 6.25% giving a 92.7% reduction in VC. New vector control tool that prevent bites but also stop mosquitoes from feeding as many times in their lifetime through protecting multiple users in a space, disarming mosquitoes from feeding on other hosts post exposure and that cause mosquito mortality are therefore likely to have far greater impact on disease when utilized for public health than traditional topical repellents that prevent bites for a single user.

6.8 Conclusions

Spatial repellents are an important addition to the vector control toolbox because they protect multiple users within a defined space. Numerous products that are effective for use indoors or outdoors are coming to the market. Repellents in particular volatile pyrethroid spatial repellents exert many behavioral effects on exposed mosquitoes resulting in multiple outcome parameters to be assessed. These outcome parameters require standardized bioassays so that the data are reliable and reproducible, which helps to generate the robust data sets required to recommend new repellents for use as consumer products or public health tools. Concurrently, robust data analysis is important for the interpretation of the data. Data from the SFS and EH experiments can be used to extrapolate the effect of repellent at the community level through mathematical models and provides a useful arena for controlled evaluation of formulated products that gives realistic estimations of the field efficacy and that can be used to rapidly and economically measure multiple endpoints relevant to public health, such as mosquito disarming and mortality that are not easily captured in full field experiments.

Chapter 7: Discussion

Vector-borne diseases such as malaria and arbovirus continue to cause morbidity and mortality regardless of the advances in the vector control arsenal. Malaria transmission is ongoing regardless of well-designed and implemented vector control programs (Carnevale and Manguin, 2021) with an increased frequency of epidemics and the geographical spread of a number of arboviruses (Leta et al., 2018). To maintain the gain resulting from the current control tool for malaria and arboviruses prevention it is critical to focus on the measures that may provide effective protection on the peridomestic spaces. Moving forward with how to deploy these tools it is important to understand how they work, the species of mosquitoes that they affect, and where can be deployed for maximum efficacy. This thesis, focuses on determining how transfluthrin “push “ and odor-baited trap “pull” may be evaluated in the field, whether push and pull are needed, and designing additional bioassay to adequately evaluate the effect of transfluthrin. This work helps to improve our understanding of the use of alternative vector control tools against outdoor biting mosquitoes. This Ph.D. thesis contributes to the pipeline for the development of transfluthrin and other volatile pyrethroids.

7.1 The exposure-free methods for evaluation of spatial repellent

The results presented in Chapter 2 where an experiment was designed to compare the protective efficacy of VP as measured by HLC compared to the exposure-free methods including MET and BG sentinel demonstrated that these methods could be used interchangeably. This is the first experiment designed to compare how the traps measure the protective efficacy of transfluthrin relative to human landing catches. These findings further proved that in the field, odour baited trap may be used to evaluate the efficacy of VP only if isolation is ensured. This is possible only in the semi-field system using laboratory-reared mosquitoes. Previous experiment which compare the efficacy of the HLC against BGS has also reported that the presence of an alternative host in the surrounding reduces the efficacy of the BGS trap in catching *Aedes aegypti* (Krockel et al., 2006). Furthermore, it was demonstrated that in the presence of an HLC competitor, the measured protective efficacy varied considerably. This is a reason for the variable protective efficacy across the traps resulting from the number of mosquitoes that were recaptured using exposure-free methods. It is known that humans present a full suit of cues needed by the mosquitoes to locate, land, and bite the host (Ray, 2015) however this was not true for the BG sentinel except for the human decoy trap which was not tested in this experiment. Therefore, it can be inferred that field experiments evaluate transfluthrin and other VP using exposure-free methods of *Ae. aegypti* collection is possible provided the experiments are sufficiently well-powered and are designed to ensure the independence of observations without the bias of alternative host cues.

In the control, MET collected approximately half the number of mosquitoes caught by HLC, and the BGS trap about 15% fewer. Similar results have been repeatedly observed in other studies with different traps because traps generally do not provide the complete suite of host cues required to maximise mosquito attraction. One exception is the host decoy trap (HDT), which provides whole-host odour, visual cues and heat (Hawkes et al., 2017). Even so, the number of *Anopheles* mosquitoes caught by HLC was higher than that with HDT in the southeast Asia (Davidson et al., 2020) and compared to other human-baited traps, such as human double net trap in Laos (Tangena et al., 2015) and the MET in Tanzania (Le Goff G and Robert, 1975, Abreu et al., 2020, Maliti et al., 2015).

Furthermore, I observed that the use of BG sentinel trap closer to the human increases bites to the human in the vicinity. This is because BG lures activate the mosquitoes from far as they approach the trap, the mosquitoes do not receive enough short-range cue such as heat and sweat (Ray, 2015) thus opting to bite a human in the vicinity. Similar findings were observed with the suna trap baited with MB5 and carbon dioxide in Kenya where the trap collect <1% of the released mosquitoes when the human is present in the same compartment (Njoroge et al., 2021). I concluded that, the BG sentinel trap and MET cannot be recommended for estimating protective efficacy in the presence of alternative human in the semi field system.

7.2 Is push or pull or the combination needed for the control of *Aedes aegypti* mosquitoes?

Evidence from this study presented in chapter 3 demonstrated that while the push-pull system reduced human-vector contact of *Ae. aegypti* mosquitoes, the majority of protection was provided by the FTPE (push component). The likely reason for this finding was that the pull component (BGS) was very weak in attracting mosquitoes in the presence of humans. This is not strictly for *Aedes* mosquitoes, similar findings were observed in the experiments with Anopheles mosquitoes (Obermayr et al., 2015, Mmbando et al., 2019). While the push-pull system may need further improvement, the success of the FTPE was encouraging and indicated their potential for the control of arboviral diseases. In this study, we have demonstrated that the BGS positioned 10 meters away did not significantly protect a person from mosquito bites.

Menger *et al.* reported that the use of repellent and odor-baited trap in a “push-pull” system could significantly reduce the burden of malaria disease by 20-fold (Menger et al., 2015). Another study conducted in Belize reported that push-pull is very effective in reducing the human landing rate (Wagman et al., 2015b). However, findings from this study showed that there is no additional value of “pull” to the “push-pull” against *Aedes* mosquitoes this was not only for *Aedes aegypti* consistent findings were observed from the studies in Kenya and Tanzania against Anopheles mosquitoes (Njoroge et al., 2021, Mmbando et al., 2019, Denz et al., 2021). As the majority of protection in a “push-pull” originates from the push, for convenience reason push alone is enough to reduce the human landing rate for outdoor biting mosquitoes.

The replacement of transfluthrin emanator after every three months avoids the problems associated with topical repellents that require daily application, but tend to be applied only when people notice mosquito bites (Lalani et al., 2016) , resulting in a lack of public health benefit (Maia et al., 2018). As the device potentially provides protection to multiple people without the need for personal reapplication, it is likely a convenient approach to bite prevention outside of sleeping hours and to be more acceptable among community members for the protection of the whole family (Sangoro et al., 2014a). Therefore, transfluthrin emanators are suitable for targeted distribution among high-risk populations such as those reported to harbor *Aedes* breeding sites during the outbreak (Ali et al., 2003).

7.3 Transfluthrin Eave Positioned Targeted Insecticide (EPTI) reduces human

Due to the increasing insecticide resistance levels in malaria vectors, an experiment was conducted in the semi-field system to compare the landing rate between susceptible and pyrethroid-resistant malaria vectors. Findings presented in chapter 4 of this thesis demonstrated that the resistance mechanism in mosquitoes is not detrimental to the efficacy of VP. This finding concur with the result conducted by Bohbot *et al.* which explained the reason behind the efficacy of VP against resistant mosquitoes. Bohbot *et al.* linked the presence of tetrafluorobenzyl alcohol meioty on VPs such as transfluthrin making it effective to repel pyrethroid-resistant mosquitoes compared to non-VPs, such as permethrin, which contain phenoxybenzyl alcohol (Bohbot et al., 2011). Furthermore, Horstman explained that enzyme responsible for the detoxification of non-VPs are unable to bind to the tetrafluorobenzyl moiety of VPs, leaving them active against resistant mosquitoes (Horstmann and Sonneck, 2016).

A study conducted in Kenya observed that transfluthrin-treated emanator outperform other microencapsulated citridiol suggesting that transfluthrin is a promising emanator to reduce human landing rate of outdoor biting mosquitoes (Njoroge et al., 2021).

Recent work has demonstrated that traditional pyrethroids combined with PBO have the ability to kill strongly resistant Anopheles mosquitoes with either metabolic or KDR resistance mechanism (Oumbouke et al., 2019). This is very encouraging information which highlight the importance of synergism in the fighting against malaria. Thus future studies should investigate the use of more than insecticides for the control of malaria vector. However, yet it is of no advantageous for the mosquitoes which are biting outdoor.

This work has also demonstrated that in the absence of transfluthrin there was a considerable differences in mosquitoes landing. Suggesting that not only insecticides, mosquito landing may also be affected by several other factors including host, rearing conditional, and mosquito species. Previous experiment has demonstrated that mosquitoes landing varies considerably depending on species (Gillies, 1964) host specific cues (Mukabana et al., 2002) and environments factors such as temperature and humidity (Kirby and Lindsay, 2004, Hawkes et al., 2017)

7.4 Is human landing catches a proxy of biting rate?

Human landing catches have been used to estimate the protection offered by the behavioral modifying insecticides. However, this is not an adequate measure of protection for VP as mosquitoes may land without an intention to feed which emphasizes that biting method measure is important. In Chapter 5, we conducted an experiment to determine if HLC is the proxy of mosquito feeding during the evaluation of volatile pyrethroid such as transfluthrin. This study showed that a relatively higher number of Anopheles mosquitoes were caught when the landing method was used compared to the biting. This was consistent when the methods were compared across the doses for Anopheles and *Aedes aegypti* mosquitoes. There was evidence of feeding inhibition caused by transflurthin as the difference in biting compared to landing was greater in the transfluthrin arm than the control arm.

The differences between biting and landing observed may be due to behavioural modification so that mosquitoes may land but be inhibited from feeding. Several authors have observed feeding inhibition induced by volatile pyrethroids (Ogoma et al., 2014b, Ritchie and Devine, 2013) and pyrethrum (Smith et al., 1971) and it has been hypothesised that volatile pyrethroids interact with olfactory sensors and alter mosquitoes' ability to feed (Bibbs and Kaufman, 2017). Despite of the differences in the number of recaptured mosquitoes, findings demonstrated that the protective efficacy calculated by either method closely agreed when tested by Bland Altman methods. Therefore, the two methods could be used interchangeably.

7.5 Practical application of the "push-pull" control strategies

Evidence from this work can be used to control mosquitoes that are biting outdoors. This includes mosquitoes that are resistant to insecticides. In an area where malaria transmission is mediated by outdoor biting mosquitoes or arbovirus transmission is ongoing the push component may be deployed in many houses in particular those with high mosquito density. These components release active ingredients that will prevent human mosquitoes contact hence mosquitoes may not be able to do host seeking behavior on that particular day. As explained above the pull component is not so important when thinking of this technology. But considering that push-pull does not work in synergism, the pull component may be deployed far away in a few areas for example near the breeding sites. This will be a useful approach as mosquitoes tend to search for sugar and then host for blood meals. Thus the presence of a pull component in some areas near the breeding site might reduce the number of host seeking mosquitoes. The push component using transfluthrin has an added advantage as it can be made in movable components and protect multiple people in a given environment.

Chapter 8: Conclusions

The identification of alternative control strategies and the development of robust evaluation may help to maintain the gain in malaria and arbovirus prevention. This thesis focuses on understanding how the traps can be used to evaluate the outdoor intervention, address whether "push" or "push-pull" control strategies are needed for the control of *Aedes* mosquitoes, provide evidence for the use of transfluthrin against resistant mosquitoes, and highlighted a better way to measure outdoor intervention with multiple outcomes.

This study has demonstrated that the evaluation of volatile pyrethroids can be done accurately only in isolation. We also observed that transfluthrin treated emanator may be used to protect individuals from multiple species of mosquitoes for a period of three months. In addition to that transfluthrin provide protection to multiple users.

While significant protection can be achieved using LLIN and IRS if applied at high access, still people will be exposed to mosquitoes that bite outdoors during early morning or evening hours. I observed that a transfluthrin treated emanator may be used in an area with both susceptible and resistant outdoor biting mosquitoes. Finally, I conclude that although human landing catches caught more mosquitoes compared to the biting method used, it is still sufficient to be used for the evaluation of volatile pyrethroids. This thesis contributed to an improved understanding of malaria and arboviruses control using a transfluthrin-treated emanator deployed at the peridomestic space.

References

ABREU, F. V. S., FERREIRA-DE-BRITO, A., AZEVEDO, A. S., LINHARES, J. H. R., DE OLIVEIRA SANTOS, V., HIME MIRANDA, E., NEVES, M., YOUSFI, L., RIBEIRO, I. P., SANTOS, A., DOS SANTOS, E., SANTOS, T. P. D., TEIXEIRA, D. S., GOMES, M. Q., FERNANDES, C. B., SILVA, A., LIMA, M., PAUPY, C., ROMANO, A. P. M., ANO BOM, A. P. D., OLIVEIRA-PINTO, L. M., MOUTAILLER, S., MOTTA, M. A., CASTRO, M. G., BONALDO, M. C., MARIA BARBOSA DE LIMA, S. & LOURENÇO-DE-OLIVEIRA, R. 2020. Survey on Non-Human Primates and Mosquitoes Does not Provide Evidences of Spillover/Spillback between the Urban and Sylvatic Cycles of Yellow Fever and Zika Viruses Following Severe Outbreaks in Southeast Brazil. *Viruses,* 12.

ACHEE, N. L., BANGS, M. J., FARLOW, R., KILLEEN, G. F., LINDSAY, S., LOGAN, J. G., MOORE, S. J., ROWLAND, M., SWEENEY, K., TORR, S. J., ZWIEBEL, L. J. & GRIECO, J. P. 2012a. Spatial repellents: from discovery and development to evidence-based validation. *Malar J,* 11**,** 164.

ACHEE, N. L., GOULD, F., PERKINS, T. A., REINER, R. C., JR., MORRISON, A. C., RITCHIE, S. A., GUBLER, D. J., TEYSSOU, R. & SCOTT, T. W. 2015a. A Critical Assessment of Vector Control for Dengue Prevention. *PLOS Neglected Tropical Diseases,* 9**,** e0003655.

ACHEE, N. L., GRIECO, J. P., VATANDOOST, H., SEIXAS, G., PINTO, J., CHING-NG, L., MARTINS, A. J., JUNTARAJUMNONG, W., CORBEL, V., GOUAGNA, C., DAVID, J. P., LOGAN, J. G., ORSBORNE, J., MAROIS, E., DEVINE, G. J. & VONTAS, J. 2019. Alternative strategies for mosquito-borne arbovirus control. *PLoS Negl Trop Dis,* 13**,** e0006822.

ACHEE, N. L., MASUOKA, P., SMITH, P., MARTIN, N., CHAREONVIRYIPHAP, T., POLSOMBOON, S., HENDARTO, J. & GRIECO, J. P. 2012b. Identifying the effective concentration for spatial repellency of the dengue vector *Aedes aegypti*. *Parasit Vectors,* 5**,** 300.

ACHEE, N. L., YOUNGBLOOD, L., BANGS, M. J., LAVERY, J. V. & JAMES, S. 2015b. Considerations for the use of human participants in vector biology research: a tool for investigators and regulators. *Vector Borne Zoonotic Dis,* 15**,** 89-102.

AFIFY, A., BETZ, J. F., RIABININA, O., LAHONDÈRE, C. & POTTER, C. J. 2019. Commonly Used Insect Repellents Hide Human Odors from Anopheles Mosquitoes. *Current Biology,* 29**,** 3669-3680.e5.

AFIFY, A. & POTTER, C. J. 2020. Insect repellents mediate species-specific olfactory behaviours in mosquitoes. *Malar J,* 19**,** 127.

AGRAMONTE, N. M., BLOOMQUIST, J. R. & BERNIER, U. R. 2017. Pyrethroid resistance alters the blood-feeding behavior in Puerto Rican *Aedes aegypti* mosquitoes exposed to treated fabric. *PLoS Negl Trop Dis,* 11**,** e0005954.

AHMED, S., REITHINGER, R., KAPTOGE, S. K. & NGONDI, J. M. 2020. Travel Is a Key Risk Factor for Malaria Transmission in Pre-Elimination Settings in Sub-Saharan Africa: A Review of the Literature and Meta-Analysis. *Am J Trop Med Hyg,* 103**,** 1380-1387.

ALDRIDGE, R. L., KAUFMAN, P. E., BLOOMQUIST, J. R., GEZAN, S. A. & LINTHICUM, K. J. 2017. Application Site and Mosquito Age Influences Malathion- and

Permethrin-Induced Mortality in *Culex quinquefasciatus* (Diptera: Culicidae). *J Med Entomol,* 54**,** 1692-1698.

ALEXANDRA HISCOX, B. O., ANTHONY KIBET, COLLINS K MWERESA, PHILEMON OMUSULA, MARTIN GEIER, ANDREAS ROSE, WOLFGANG R MUKABANA AND WILLEM TAKKEN 2014. Development and optimization of the Suna trap as a tool for mosquito monitoring and control. *Malar J***,** 257.

ALI, M., WAGATSUMA, Y., EMCH, M. & BREIMAN, R. F. 2003. Use Of A Geographic Information System For Defining Spatial Risk For Dengue Transmission In Bangladesh: Role For *Aedes Albopictus* In An Urban Outbreak. *Am. J. Trop. Med. Hyg.,* 69**,** 634–640.

ALONSO, P., ENGELS, D. & REEDER, J. 2017. Renewed push to strengthen vector control globally. *Lancet,* 389**,** 2270–2271.

ALY, A. S. I., VAUGHAN, A. M. & KAPPE, S. H. I. 2009. Malaria Parasite Development in the Mosquito and Infection of the Mammalian Host. *Annual Review of Microbiology,* 63**,** 195-221.

AMELIA-YAP, Z. H., CHEN, C. D., SOFIAN-AZIRUN, M., LAU, K. W., SUANA, I. W., H., SYAHPUTRA, E., RAZAK, A. & LOW, V. L. 2018. Efficacy of Mosquito Coils: Cross-resistance to Pyrethroids in *Aedes aegypti* (Diptera: Culicidae) From Indonesia. *J Econ Entomol,* 111**,** 2854-2860.

ANDRÉS, M., LORENZ, L. M., MBELEYA, E. & MOORE, S. J. 2015. Modified mosquito landing boxes dispensing transfluthrin provide effective protection against *Anopheles arabiensis* mosquitoes under simulated outdoor conditions in a semi-field system. *Malar J,* 14**,** 255.

ANDRIESSEN, R., SNETSELAAR, J., SUER, R. A., OSINGA, A. J., DESCHIETERE, J., LYIMO, I. N., MNYONE, L. L., BROOKE, B. D., RANSON, H., KNOLS, B. G. J. & FARENHORST, M. 2015. Electrostatic coating enhances bioavailability of insecticides and breaks pyrethroid resistance in mosquitoes. *Proceedings of the National Academy of Sciences,* 112**,** 12081-12086.

ANSELL, J., HAMILTON, K. A., PINDER, M., WALRAVEN, G. E. & LINDSAY, S. W. 2002. Short-range attractiveness of pregnant women to *Anopheles gambiae* mosquitoes. *Trans R Soc Trop Med Hyg,* 96**,** 113-6.

ARAÚJO, M., GIL, L. H. & E-SILVA, A. 2012. Larval food quantity affects development time, survival and adult biological traits that influence the vectorial capacity of *Anopheles darlingi* under laboratory conditions. *Malar J,* 11**,** 261.

ASALE, A., DUCHATEAU, L., DEVLEESSCHAUWER, B., HUISMAN, G. & YEWHALAW, D. 2017. Zooprophylaxis as a control strategy for malaria caused by the vector *Anopheles arabiensis (*Diptera: Culicidae): a systematic review. *Infect Dis Poverty,* 6**,** 160.

BABER, I., KEITA, M., SOGOBA, N., KONATE, M., DIALLO, M. B., DOUMBIA, S., TRAORÉ, S. F., RIBEIRO, J. M. C. & MANOUKIS, N. C. 2010. Population size and migration of *Anopheles gambiae* in the Bancoumana region of Mali and their significance for efficient vector control. *PLoS ONE,* 5.

BALMERT, N. J., RUND, S. S., GHAZI, J. P., ZHOU, P. & DUFFIELD, G. E. 2014. Time-of-day specific changes in metabolic detoxification and insecticide resistance in the malaria mosquito *Anopheles gambiae*. *J Insect Physiol,* 64**,** 30-9.

BARNARD, D. R. 1998. Mediation of deet repellency in mosquitoes (Diptera, Culicidae) by species, age and parity. *Journal of Medical Entomology,* 35**,** 340-3.

BARRERA, R., AMADOR, M., ACEVEDO, V., CABAN, B., FELIX, G. & MACKAY, A. J. 2014. Use of the CDC autocidal gravid ovitrap to control and prevent outbreaks of *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol,* 51**,** 145-54.

BATES, D., MÄCHLER, M., BOLKER, B. & WALKER, S. 2015. Fitting Linear Mixed-Effects Models Usinglme4. *Journal of Statistical Software,* 67.

BATES, D., MAECHLER, M. & BOLKER, B. 2011. lme4: Linear mixed-effects models using S4 classes. R package version 0999375–39. http://cran.r-project.org/web/packages/lme4/index.html

BATY, F., RITZ, C., CHARLES, S., BRUTSCHE, M., FLANDROIS, J.-P. & DELIGNETTE-MULLER, M.-L. 2015. A Toolbox for Nonlinear Regression in R: The Package nlstools. *Journal of Statistical Software,* 66**,** 1-21.

BAYOH, M. N., WALKER, E. D., KOSGEI, J., OMBOK, M., OLANG, G. B., GITHEKO, A. K., KILLEEN, G. F., OTIENO, P., DESAI, M., LOBO, N. F., VULULE, J. M., HAMEL, M. J., KARIUKI, S. & GIMNIG, J. E. 2014. Persistently high estimates of late night, indoor exposure to malaria vectors despite high coverage of insecticide treated nets. *Parasites & Vectors,* 7**,** 380.

BEGG, S., WRIGHT, A., SMALL, G., ABDOULAYE, D., KISINZA, W., KOUDOU, B., MOORE, S., MOSHA, F., EDI, C., KIRBY, M., KIJA, P., MALIMA, R., MOORE, J. & BATES, I. 2020. Ripple effects of research capacity strengthening: a study of the effects of a project to support test facilities in three African countries towards Good Laboratory Practice certification [version 1; peer review: awaiting peer review]. *Gates Open Research,* 4.

BEITER, K. J., WENTLENT, Z. J., HAMOUDA, A. R. & THOMAS, B. N. 2019. Nonconventional opponents: a review of malaria and leishmaniasis among United States Armed Forces. *PeerJ,* 7**,** e6313.

BENEDICT, M., CHARLWOOD, J., HARRINGTON, L., PHILIP LOUNIBOS, L., REISEN, W. & TABACHNICK, W. 2018. Guidance for Evaluating the Safety of Experimental Releases of Mosquitoes, Emphasizing Mark-Release-Recapture Techniques. *Vector-Borne and Zoonotic Diseases,* 18**,** 39-48.

BENELLI, G., JEFFRIES, C. L. & WALKER, T. 2016. Biological Control of Mosquito Vectors: Past, Present, and Future. *Insects,* 7.

BERNIER, U. R., KLINE, D. L., VAZQUEZ-ABAD, A., PERRY, M., COHNSTAEDT, L. W., GURMAN, P., D’HERS, S. & ELMAN, N. M. 2019. A combined experimental-computational approach for spatial protection efficacy assessment of controlled release devices against mosquitoes (Anopheles). *PLOS Neglected Tropical Diseases,* 13**,** e0007188.

BERRY-CABÁN, C. B. S. 2011. DDT and Silent Spring: Fifty Years After. *J Mil Veterans Health,* 19.

BESANSKY, N. J., HILL, C. A. & COSTANTINI, C. 2004. No accounting for taste: host preference in malaria vectors. *Trends Parasitol,* 20**,** 249-51.

BHATT, S., WEISS, D. J., CAMERON, E., BISANZIO, D., MAPPIN, B., DALRYMPLE, U., BATTLE, K., MOYES, C. L., HENRY, A., ECKHOFF, P. A., WENGER, E. A., BRIËT, O., PENNY, M. A., SMITH, T. A., BENNETT, A., YUKICH, J., EISELE, T. P., GRIFFIN, J. T., FERGUS, C. A., LYNCH, M., LINDGREN, F., COHEN, J. M.,

MURRAY, C. L. J., SMITH, D. L., HAY, S. I., CIBULSKIS, R. E. & GETHING, P. W. 2015. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature,* 526**,** 207-211.

BIBBS, C. S., HAHN, D. A., KAUFMAN, P. E. & XUE, R. D. 2018. Sublethal effects of a vapour-active pyrethroid, transfluthrin, on *Aedes aegypti* and *Ae. albopictus* (Diptera: Culicidae) fecundity and oviposition behaviour. *Parasit Vectors,* 11**,** 486.

BIBBS, C. S. & KAUFMAN, P. E. 2017. Volatile Pyrethroids as a Potential Mosquito Abatement Tool: A Review of Pyrethroid-Containing Spatial Repellents. *J. Integr. Pest Manag,* 8**,** 1–10.

BIBBS, C. S., KLINE, J., KLINE, D. L., ESTAVER, J., STROHSCHEIN, R., ALLAN, S. A., KAUFMAN, P. E., XUE, R. D. & BATICH, C. D. 2020. Olfactometric Comparison of the Volatile Insecticide, Metofluthrin, Through Behavioral Responses of *Aedes albopictus* (Diptera: Culicidae). *J Med Entomol,* 57**,** 17-24.

BOHBOT, J. D. & DICKENS, J. C. J. P. O. 2010. Insect repellents: modulators of mosquito odorant receptor activity. 5**,** e12138.

BOHBOT, J. D., FU, L., LE, T. C., CHAUHAN, K. R., CANTRELL, C. L. & DICKENS, J. C. D. 2011. Multiple activities of insect repellents on odorant receptors in mosquitoes. *Medical and Veterinary Entomology* 25,**,** 436–444.

BRADY, O. J., GODFRAY, H. C., TATEM, A. J., GETHING, P. W., COHEN, J. M., MCKENZIE, F. E., PERKINS, T. A., REINER, R. C., JR., TUSTING, L. S., SINKA, M. E., MOYES, C. L., ECKHOFF, P. A., SCOTT, T. W., LINDSAY, S. W., HAY, S. I. & SMITH, D. L. 2016. Vectorial capacity and vector control: reconsidering sensitivity to parameters for malaria elimination. *Trans R Soc Trop Med Hyg,* 110**,** 107-17.

BRADY, O. J. & HAY, S. I. 2020. The Global Expansion of Dengue: How *Aedes aegypti* Mosquitoes Enabled the First Pandemic Arbovirus. *Annu Rev Entomol,* 65**,** 191-208.

BRIËT, O. J., HUHO, B. J., GIMNIG, J. E., BAYOH, N., SEYOUM, A., SIKAALA, C. H., GOVELLA, N., DIALLO, D. A., ABDULLAH, S., SMITH, T. A. & KILLEEN, G. F. 2015. Applications and limitations of Centers for Disease Control and Prevention miniature light traps for measuring biting densities of African malaria vector populations: a pooled-analysis of 13 comparisons with human landing catches. *Malar J,* 14**,** 247.

BUCKLING, A. & READ, A. F. 2001. The effect of partial host immunity on the transmission of malaria parasites. *Proc Biol Sci,* 268**,** 2325-30.

BUONACCORSI, J. P., HARRINGTON, L. C. & EDMAN, J. D. 2003. Estimation and Comparison of Mosquito Survival Rates with Release-Recapture-Removal Data. *Journal of Medical Entomology,* 40**,** 6-17.

CARDÉ, RING T. 2015. Multi-Cue Integration: How Female Mosquitoes Locate a Human Host. *Current Biology,* 25**,** R793-R795.

CAREY, A. F., WANG, G., SU, C.-Y., ZWIEBEL, L. J. & CARLSON, J. R. 2010. Odorant reception in the malaria mosquito *Anopheles gambiae*. *Nature,* 464**,** 66-71.

CARNEVALE, P., FRÉZIL, J. L., BOSSENO, M. F., LE PONT, F. & LANCIEN, J. 1978. [The aggressiveness of *Anopheles gambiae* A in relation to the age and sex of the human subjects]. *Bull World Health Organ,* 56**,** 147-54.

CARNEVALE, P. & MANGUIN, S. 2021. Review of Issues on Residual Malaria Transmission. *The Journal of Infectious Diseases,* 223**,** S61-S80.

CARROLL, S. P. 2007. Evaluation of topical insect repellents and factors that affect their performance. *Insect repellents : principles, methods, and uses.* CRC Press: Boca Raton

CASIDA, J. E. & QUISTAD, G. B. 1998. Golden age of insecticide research: past, present, or future? *Annu Rev Entomol,* 43**,** 1-16.

CDC 2021. Life cycle of malaria parasite. https://www.cdc.gov/malaria/about/biology/index.html.

CHACCOUR, C., BARRIO, A., GIL ROYO, A. G., MARTINEZ URBISTONDO, D., SLATER, H., HAMMANN, F. & DEL POZO, J. L. 2015. Screening for an ivermectin slow-release formulation suitable for malaria vector control. *Malar J,* 14**,** 102.

CHANDRE, F., DARRIET, F., DUCHON, S., FINOT, L., MANGUIN, S., CARNEVALE, P. & GUILLET, P. 2000. Modifications of pyrethroid effects associated with kdr mutation in *Anopheles gambiae*. *Medical and Veterinary Entomology,* 14**,** 81-88.

CHARLWOOD, J. D., VIJ, R. & BILLINGSLEY, P. F. 2000. Dry season refugia of malaria-transmitting mosquitoes in a dry savannah zone of east Africa. *American Journal of Tropical Medicine and Hygiene,* 62**,** 726-732.

CHAUHAN, K. R., ALDRICH, J. R., MCCARDLE, P. W., WHITE, G. B. & WEBB, R. E. 2012. A field bioassay to evaluate potential spatial repellents against natural mosquito populations. *J Am Mosq Control Assoc,* 28**,** 301-6.

CHOUAIBOU, M. S., FODJO, B. K., FOKOU, G., ALLASSANE, O. F., KOUDOU, B. G., DAVID, J. P., ANTONIO-NKONDJIO, C., RANSON, H. & BONFOH, B. 2016. Influence of the agrochemicals used for rice and vegetable cultivation on insecticide resistance in malaria vectors in southern Cote d'Ivoire. *Malar J,* 15**,** 426.

CIBULSKIS, R. E., ALONSO, P., APONTE, J., AREGAWI, M., BARRETTE, A., BERGERON, L., FERGUS, C. A., KNOX, T., LYNCH, M., PATOUILLARD, E., SCHWARTE, S., STEWART, S. & WILLIAMS, R. 2016. Malaria: Global progress 2000 - 2015 and future challenges. *Infect Dis Poverty,* 5**,** 61.

CIOTA, A. T., DRUMMOND, C. L., RUBY, M. A., DROBNACK, J., EBEL, G. D. & KRAMER, L. D. 2012. Dispersal of Culex Mosquitoes (Diptera: Culicidae) From a Wastewater Treatment Facility. *Journal of Medical Entomology,* 49**,** 35-42.

CONTRIBUTORS", O. 2019. OpenStreetMap.

COOK, G. M. 1998. "Spray, Spray, Spray!": insecticides and the making of applied entomology in Canada, 1871-1914. *Sci Can,* 22**,** 7-50.

COOK, S. M., KHAN, Z. R. & PICKETT, J. A. 2007. The use of push-pull strategies in integrated pest management. *Annu Rev Entomol,* 52**,** 375-400.

COSTANTINI, C., LI, S. G., DELLA TORRE, A., SAGNON, N., COLUZZI, M. & TAYLOR, C. E. 1996. Density, survival and dispersal of *Anopheles gambiae* complex mosquitoes in a west African Sudan savanna village. *Med Vet Entomol,* 10**,** 203-19.

COSTANTINI, C., SAGNON, N., DELLA TORRE, A. & COLUZZI, M. 1999. Mosquito behavioural aspects of vector-human interactions in the *Anopheles gambiae* complex. *Parassitologia,* 41**,** 209-17.

COTTER, C., STURROCK, H. J., HSIANG, M. S., LIU, J., PHILLIPS, A. A., HWANG, J., GUEYE, C. S., FULLMAN, N., GOSLING, R. D. & FEACHEM, R. G. 2013. The changing epidemiology of malaria elimination: new strategies for new challenges. *Lancet,* 382**,** 900-911.

CURTIS CF, L. J., IJUMBA J, CALLAGHAN A,' HILL N, KARIMZA MA 1987. The relative efficacy of repellents against mosquito vectors of disease. *Medical and Veterinary Entomology,* 1**,** 109-119.

CURTIS, C. F. & RAWLINGS, P. 1980. A preliminary study of dispersal and survival of *Anopheles culicifacies* in relation to the possibility of inhibiting the spread of insecticide resistance. *Ecological Entomology,* 5**,** 11-17.

DAMBACH, P., SCHLEICHER, M., KORIR, P., OUEDRAOGO, S., DAMBACH, J., SIÉ, A., DAMBACH, M. & BECKER, N. 2018. Nightly Biting Cycles of Anopheles Species in Rural Northwestern Burkina Faso. *Journal of Medical Entomology,* 55**,** 1027-1034.

DARBRO, J. M., MUZARI, M. O., GIBLIN, A., ADAMCZYK, R. M., RITCHIE, S. A. & DEVINE, G. J. 2017. Reducing biting rates of *Aedes aegypti* with metofluthrin: investigations in time and space. *Parasit Vectors,* 10**,** 69.

DAVIDSON, J. R., BASKIN, R. N., HASAN, H., BURTON, T. A., WARDIMAN, M., RAHMA, N., SAPUTRA, F. R., AULYA, M. S., WAHID, I., SYAFRUDDIN, D., HAWKES, F. M. & LOBO, N. F. 2020. Characterization of vector communities and biting behavior in South Sulawesi with host decoy traps and human landing catches. *Parasit Vectors,* 13**,** 329.

DAVIS, E. E. & SOKOLOVE, P. G. J. J. O. C. P. 1976. Lactic acid-sensitive receptors on the antennae of the mosquito, *Aedes aegypti*. 105**,** 43-54.

DE JONG, R. & KNOLS, B. G. J. 1995. Selection of biting sites on man by two malaria mosquito species. *Experientia,* 51**,** 80-4.

DEBBOUN, M., FRANCES, S. P. & STRICKMAN, D. 2014. *Insect repellents handbook*, CRC Press.

DEBBOUN, M. & WAGMAN, J. 2004. In vitro repellency of N,N-diethyl-3-methylbenzamide and N,N-diethylphenylacetamide analogs against *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). *J Med Entomol,* 41**,** 430-4.

DEBBOUN, S. J. M. A. M. 2006. History of Insect Repellents. *Insect Repellents: Principles, Methods, and Uses.*

DEGENER, C. M., EIRAS, A. E., AZARA, T. M., ROQUE, R. A., ROSNER, S., CODECO, C. T., NOBRE, A. A., ROCHA, E. S., KROON, E. G., OHLY, J. J. & GEIER, M. 2014. Evaluation of the effectiveness of mass trapping with BG-sentinel traps for dengue vector control: a cluster randomized controlled trial in Manaus, Brazil. *J Med Entomol,* 51**,** 408-20.

DEGENNARO, M. 2015. The mysterious multi-modal repellency of DEET. *Fly (Austin),* 9**,** 45-51.

DEKKER, T., GEIER, M. & CARDE, R. T. 2005. Carbon dioxide instantly sensitizes female yellow fever mosquitoes to human skin odours. *J Exp Biol,* 208**,** 2963-72.

DENZ, A., NJOROGE, M. M., TAMBWE, M. M., CHAMPAGNE, C., OKUMU, F., VAN LOON, J. J. A., HISCOX, A., SADDLER, A., FILLINGER, U., MOORE, S. J. & CHITNIS, N. 2021. Predicting the impact of outdoor vector control interventions on malaria transmission intensity from semi-field studies. *Parasit Vectors,* 14**,** 64.

DICKENS, B. L. & BRANT, H. L. 2014. Effects of marking methods and fluorescent dusts on *Aedes aegypti* survival. *Parasit Vectors,* 7**,** 65.

DICKENS, J. C., BOHBOT, J. D. J. P. B. & PHYSIOLOGY 2013. Mini review: Mode of action of mosquito repellents. 106**,** 149-155.

DIOP, M. M., CHANDRE, F., ROSSIGNOL, M., PORCIANI, A., CHATEAU, M., MOIROUX, N. & PENNETIER, C. 2020. Sub-lethal insecticide exposure affects host biting efficiency of Kdr-resistant *Anopheles gambiae bioRxiv***,** 653980.

DURNEZ, L. & COOSEMANS, M. 2013. Residual Transmission of Malaria: An Old Issue for New Approaches.

ENDO, N. & ELTAHIR, E. A. B. 2018. Modelling and observing the role of wind in Anopheles population dynamics around a reservoir. *Malaria Journal,* 17.

EPOPA, P. S., MILLOGO, A. A., COLLINS, C. M., NORTH, A., TRIPET, F., BENEDICT, M. Q. & DIABATE, A. 2017. The use of sequential mark-release-recapture experiments to estimate population size, survival and dispersal of male mosquitoes of the *Anopheles gambiae* complex in Bana, a west African humid savannah village. *Parasit Vectors,* 10**,** 376.

FERGUSON, H. M., NG'HABI, K. R., WALDER, T., KADUNGULA, D., MOORE, S. J., LYIMO, I., RUSSELL, T. L., URASSA, H., MSHINDA, H., KILLEEN, G. F. & KNOLS, B. G. 2008. Establishment of a large semi-field system for experimental study of African malaria vector ecology and control in Tanzania. *Malar J,* 7**,** 158.

FERNANDES, L. & BRIEGEL, H. 2005. Reproductive physiology of *Anopheles gambiae* and *Anopheles atroparvus*. *J Vector Ecol,* 30**,** 11-26.

FILLINGER, U., SOMBROEK, H., MAJAMBERE, S., VAN LOON, E., TAKKEN, W. & LINDSAY, S. W. 2009. Identifying the most productive breeding sites for malaria mosquitoes in The Gambia. *Malaria journal,* 8**,** 62.

FINDA, M. F., MOSHI, I. R., MONROE, A., LIMWAGU, A. J., NYONI, A. P., SWAI, J. K., NGOWO, H. S., MINJA, E. G., TOE, L. P., KAINDOA, E. W., COETZEE, M., MANDERSON, L. & OKUMU, F. O. 2019. Linking human behaviours and malaria vector biting risk in south-eastern Tanzania. *PLoS One,* 14**,** e0217414.

FLOORE, T. G. 2006. Mosquito Larval Control Practices: Past and Present. *J. Am. Mosq. Control Assoc.,* 22**,** 527-533.

FRANCES, S. P., AULIFF, A. M., EDSTEIN, M. D. & COOPER, R. D. 2003. Survey of personal protection measures against mosquitoes among Australian defense force personnel deployed to East Timor. *Mil Med,* 168**,** 227-30.

FRANCES, S. P., DEBBOUN, M. J. I. R. P., METHODS, & USES 2007. User acceptability: public perceptions of insect repellents. 397-404.

GARCIA, G. E., WIRTZ, R. A., BARR, J. R., WOOLFITT, A. & ROSENBERG, R. 1998. Xanthurenic acid induces gametogenesis in Plasmodium, the malaria parasite. *J Biol Chem,* 273**,** 12003-5.

GATTON, M. L., CHITNIS, N., CHURCHER, T., DONNELLY, M. J., GHANI, A. C., GODFRAY, H. C. J., GOULD, F., HASTINGS, I., MARSHALL, J. & RANSON, H. 2013. The importance of mosquito behavioural adaptations to malaria control in Africa. *Evolution: international journal of organic evolution,* 67**,** 1218-1230.

GENG, J., MALLA, P., ZHANG, J., XU, S., LI, C., ZHAO, Y., WANG, Q., KYAW, M. P., CAO, Y., YANG, Z. & CUI, L. 2019. Increasing trends of malaria in a border area of the Greater Mekong Subregion. *Malaria Journal,* 18**,** 309.

GERBERG, E. J., BARNARD, D. R. & WARD, R. A. 1994. *Manual for mosquito rearing and experimental techniques, revised edn.,* Lake Charles, American Mosquito Control Association, Inc.

GILLIES, M. T. 1961. Studies on the dispersion and survival of Anopheles gambiae Giles in East Africa, by means of marking and release experiments. *Bulletin of Entomological Research,* 52**,** 99.

GILLIES, M. T. 1964. Selection for host preference in *Anopehles gambiae Nature,* 203**,** 852-854.

GILLIES, M. T. 1980. The role of carbon dioxide in host-finding by mosquitoes (Diptera, Culicidae): A review. *Bull Entomol Res,* 70**,** 525-532.

GILLIES, M. T. & WILKES, T. J. 1968. A comparison of the range of attraction of animal baits and of carbon dioxide for some west African mosquitoes. *Bull Entomol Res,* 59**,** 441-446.

GILLIES, M. T. & WILKES, T. J. 1972. The range of attraction of animal baits and carbon dioxide for mosquitoes. Studies in a freshwater area of West Africa. *Bull Entomol Res,* 61**,** 389-404.

GILLIES MT, W. T. 1970. The range of attraction of single baits for some West African mosquitoes. *Bull. ent. Res.,* 69**,** 225-235.

GIMNIG, J. E., WALKER, E. D., OTIENO, P., KOSGEI, J., OLANG, G., OMBOK, M., WILLIAMSON, J., MARWANGA, D., ABONG'O, D., DESAI, M., KARIUKI, S., HAMEL, M. J., LOBO, N. F., VULULE, J. & BAYOH, M. N. 2013. Incidence of malaria among mosquito collectors conducting human landing catches in western Kenya. *Am J Trop Med Hyg,* 88**,** 301-8.

GLUNT, K. D., OLIVER, S. V., HUNT, R. & PAAIJMANS, K. P. 2018. The impact of temperature on insecticide toxicity against the malaria vectors *Anopheles arabiensis* and *Anopheles funestus*. *Mal J,* 17.

GOLDANI, L. Z. 2017. Yellow fever outbreak in Brazil, 2017. *Braz J Infect Dis,* 21**,** 123-124.

GOODYER, L., LAWRENCE, J., FELLER, C. A., DEBOUN, M., FRANCES, S. & STRICKMAN, D. 2014. Marketing for Efficacy, Acceptability, Safety and Health. *Insect Repellents Handbook.* CRC Press Boca Raton.

GOULD, L. H., OSMAN, M. S., FARNON, E. C., GRIFFITH, K. S., GODSEY, M. S., KARCH, S., MULENDA, B., EL KHOLY, A., GRANDESSO, F., DE RADIGUES, X., BRAIR, M. E., BRIAND, S., EL TAYEB EL, S. M., HAYES, E. B., ZELLER, H. & PEREA, W. 2008. An outbreak of yellow fever with concurrent chikungunya virus transmission in South Kordofan, Sudan, 2005. *Trans R Soc Trop Med Hyg,* 102**,** 1247-54.

GOVELLA, N. J. & FERGUSON, H. 2012. Why use of interventions targeting outdoor biting mosquitoes will be necessary to achieve malaria elimination. *Frontiers in Physiology,* 3.

GOVELLA, N. J., MALITI, D. F., MLWALE, A. T., MASALLU, J. P., MIRZAI, N., JOHNSON, P. C., FERGUSON, H. M. & KILLEEN, G. F. 2016. An improved mosquito electrocuting trap that safely reproduces epidemiologically relevant metrics of mosquito human-feeding behaviours as determined by human landing catch. *Malar J,* 15**,** 465.

GRIECO, J. P., ACHEE, N. L., ANDRE, R. G. & ROBERTS, D. R. 2000. A comparison study of house entering and exiting behavior of *Anopheles vestitipennis* (Diptera: Culicidae) using experimental huts sprayed with DDT or deltamethrin in the southern district of Toledo, Belize, CA. *J Vector Ecol,* 25**,** 62-73.

GRIECO, J. P., ACHEE, N. L., CHAREONVIRIYAPHAP, T., SUWONKERD, W., CHAUHAN, K., SARDELIS, M. R. & ROBERTS, D. R. J. P. O. 2007. A new classification system for the actions of IRS chemicals traditionally used for malaria control. 2**,** e716.

GRIFFING, S. M., TAUIL, P. L., UDHAYAKUMAR, V. & SILVA-FLANNERY, L. 2015. A historical perspective on malaria control in Brazil. *Mem Inst Oswaldo Cruz,* 110**,** 701-18.

GRYSEELS, C., UK, S., SLUYDTS, V., DURNEZ, L., PHOEUK, P., SUON, S., SET, S., HENG, S., SIV, S., GERRETS, R., THO, S., COOSEMANS, M. & PEETERS GRIETENS, K. 2015. Factors influencing the use of topical repellents: implications for the effectiveness of malaria elimination strategies. *Sci Rep,* 5**,** 16847.

GUERRA, C. A., REINER, R. C., JR., PERKINS, T. A., LINDSAY, S. W., MIDEGA, J. T., BRADY, O. J., BARKER, C. M., REISEN, W. K., HARRINGTON, L. C., TAKKEN, W., KITRON, U., LLOYD, A. L., HAY, S. I., SCOTT, T. W. & SMITH, D. L. 2014. A global assembly of adult female mosquito mark-release-recapture data to inform the control of mosquito-borne pathogens. *Parasit Vectors,* 7**,** 276.

HADDOW, A. J. B. O. E. R. 1942. The mosquito fauna and climate of native huts at Kisumu, Kenya. 33**,** 91-142.

HANCOCK, P. A., WIEBE, A., GLEAVE, K. A., BHATT, S., CAMERON, E., TRETT, A., WEETMAN, D., SMITH, D. L., HEMINGWAY, J., COLEMAN, M., GETHING, P. W. & MOYES, C. L. 2018. Associated patterns of insecticide resistance in field populations of malaria vectors across Africa. *Proc Natl Acad Sci U S A,* 115**,** 5938-5943.

HARRINGTON, L. C., FOY, B. D. & BANGS, M. J. 2020. Considerations for Human Blood-Feeding and Arthropod Exposure in Vector Biology Research: An Essential Tool for Investigations and Disease Control. *Vector Borne Zoonotic Dis,* 20**,** 807-816.

HAWKES, F. M., DABIRE, R. K., SAWADOGO, S. P., TORR, S. J. & GIBSON, G. 2017. Exploiting Anopheles responses to thermal, odour and visual stimuli to improve surveillance and control of malaria. *Sci Rep,* 7**,** 17283.

HAYS, C. W. 2000. The United States Army and malaria control in World War II. *Parassitologia,* 42**,** 47-52.

HELLEWELL, J., SHERRARD-SMITH, E., OGOMA, S. & CHURCHER, T. S. 2021. Assessing the impact of low-technology emanators alongside long-lasting insecticidal nets to control malaria. *Philos Trans R Soc Lond B Biol Sci,* 376**,** 20190817.

HENG, S., DURNEZ, L., GRYSEELS, C., VAN ROEY, K., MEAN, V., UK, S., SOVANNAROTH, S., GRIETENS, K. P., SOCHANTHA, T., COOSEMANS, M. & SLUYDTS, V. 2015. Assuring access to topical mosquito repellents within an intensive distribution scheme: a case study in a remote province of Cambodia. *Mal J,* 14**,** 468.

HILL, N., ZHOU, H. N., WANG, P., GUO, X., CARNEIRO, I. & MOORE, S. J. 2014. A household randomized, controlled trial of the efficacy of 0.03% transfluthrin coils alone and in combination with long-lasting insecticidal nets on the incidence of *Plasmodium falciparum* and *Plasmodium vivax* malaria in Western Yunnan Province, China. *Mal J,* 13**,** 208.

HOFFMANN, E. J. & MILLER, J. R. 2002. Reduction of mosquito (Diptera: Culicidae) attacks on a human subject by combination of wind and vapor-phase DEET repellent. *J Med Entomol,* 39**,** 935-8.

HOMAN, T., HISCOX, A., MWERESA, C. K., MASIGA, D., MUKABANA, W. R., ORIA, P., MAIRE, N., PASQUALE, A. D., SILKEY, M., ALAII, J., BOUSEMA, T., LEEUWIS, C., SMITH, T. A. & TAKKEN, W. 2016. The effect of mass mosquito trapping on malaria transmission and disease burden (SolarMal): a stepped-wedge cluster-randomised trial. *The Lancet,* 388**,** 1193-1201.

HON, K. L., TSANG, Y. C., PONG, N. H., LEE, V. W., LUK, N. M., CHOW, C. M. & LEUNG, T. F. 2015. Patient acceptability, efficacy, and skin biophysiology of a cream and cleanser containing lipid complex with shea butter extract versus a ceramide product for eczema. *Hong Kong Med J,* 21**,** 417-25.

HORSTMANN, S. & SONNECK, R. 2016. Contact Bioassays with Phenoxybenzyl and Tetrafluorobenzyl Pyrethroids against Target-Site and Metabolic Resistant Mosquitoes. *PLoS One,* 11**,** e0149738.

HOTHORN, T., BRETZ, F. & WESTFALL, P. 2008. Simultaneous Inference in General Parametric Models. *Biometrical Journal,* 50**,** 346-363.

IWASHITA, H., DIDA, G. O., SONYE, G. O., SUNAHARA, T., FUTAMI, K., NJENGA, S. M., CHAVES, L. F. & MINAKAWA, N. 2014. Push by a net, pull by a cow: can zooprophylaxis enhance the impact of insecticide treated bed nets on malaria control? *Parasit Vectors,* 7**,** 52.

JAMES, S., COLLINS, F. H., WELKHOFF, P. A., EMERSON, C., GODFRAY, H. C. J., GOTTLIEB, M., GREENWOOD, B., LINDSAY, S. W., MBOGO, C. M., OKUMU, F. O., QUEMADA, H., SAVADOGO, M., SINGH, J. A., TOUNTAS, K. H. & TOURE, Y. T. 2018. Pathway to Deployment of Gene Drive Mosquitoes as a Potential Biocontrol Tool for Elimination of Malaria in Sub-Saharan Africa: Recommendations of a Scientific Working Group. *American Journal of Tropical Medicine and Hygiene,* 98**,** 1-49.

JING, Q. & WANG, M. 2019. Dengue epidemiology. *Global Health Journal,* 3**,** 37-45.

JOHNSON, B. J., RITCHIE, S. A. & FONSECA, D. M. 2017. The State of the Art of Lethal Oviposition Trap-Based Mass Interventions for Arboviral Control. *Insects,* 8.

JOHNSON, P. C., BARRY, S. J., FERGUSON, H. M. & MÜLLER, P. 2015. Power analysis for generalized linear mixed models in ecology and evolution. *Methods Ecol Evol,* 6**,** 133-142.

JOHNSON, P. C. D., BARRY, S. J. E., FERGUSON, H. F. & MULLER, P. 2014. Power analysis for generalized linear mixed models in ecology and evolution. *Methods in Ecology and Evolution*.

JUFRI, M., IRMAYANTI, E. & GOZAN, M. 2016. Formulation of Tobacco Based Mosquito Repellent to Avoid Dengue Fever. *International Journal of PharmTech Research,* 9**,** 140-5.

KAINDOA, E. W., MKANDAWILE, G., LIGAMBA, G., KELLY-HOPE, L. A. & OKUMU, F. O. 2016. Correlations between household occupancy and malaria vector biting risk in rural Tanzanian villages: implications for high-resolution spatial targeting of control interventions. *Malaria Journal,* 15**,** 1-12.

KAMTCHUM-TATUENE, J., MAKEPEACE, B. L., BENJAMIN, L., BAYLIS, M. & SOLOMON, T. 2017. The potential role of Wolbachia in controlling the transmission of emerging human arboviral infections. *Curr Opin Infect Dis,* 30**,** 108-116.

KANG, S. Y., BATTLE, K. E., GIBSON, H. S., COOPER, L. V., MAXWELL, K., KAMYA, M., LINDSAY, S. W., DORSEY, G., GREENHOUSE, B., RODRIGUEZ-BARRAQUER, I., REINER, R. C. J., SMITH, D. L. & BISANZIO, D. 2018. Heterogeneous exposure and hotspots for malaria vectors at three study sites in Uganda. *Gates Open Res,* 2**,** 32.

KATSUDA, Y., LEEMINGSAWAT, S., THONGRUNGKIAT, S., KOMALAMISARA, N., KANZAKI, T., WATANABE, T. & KAHARA, T. 2008. Control of mosquito vectors of tropical infectious diseases: (1) Bioefficacy of mosquito coils containing several pyrethroids and a synergist. *Southeast Asian J Trop Med Public Health,* 39**,** 48-54.

KATZ, T. M., MILLER, J. H. & HEBERT, A. A. 2008. Insect repellents: historical perspectives and new developments. *J Am Acad Dermatol,* 58**,** 865-71.

KAWADA, H., TEMU, E. A., MINJAS, J., MATSUMOTO, O., IWASAKI, T. & TAKAGI, M. 2008. Field Evaluation of Spatial Repellency of Metofluthrin-Impregnated Plastic Strips Against *Anopheles gambiae* Complex in Bagamoyo, Coastal Tanzania. *JAMCA,* 24**,** 404-9.

KAWADA, H., YEN, N. T., HOA, N. T., SANG, T. M., N, V. A. N. D. & TAKAGI, M. 2005. Field evaluation of spatial repellency of metofluthrin impregnated plastic strips against mosquitoes in Hai Phong City, Vietnam. *Am J Trop Med Hyg,* 73**,** 350-3.

KENNEDY, J. S. 1947. The excitant and repellent Effects on Mosquitos of sub-lethal Contacts with DDT. *Bulletin of Entomological Research*.

KHAN, A. A., MAIBACH, H. I. & SKIDMORE, D. L. 1973. A study of insect repellents 2. Effect of temperature on protection time. *J Econ Entomol,* 66**,** 437-9.

KIRBY, M. J., GREEN, C., MILLIGAN, P. M., SISMANIDIS, C., JASSEH, M., CONWAY, D. J. & LINDSAY, S. W. 2008. Risk factors for house-entry by malaria vectors in a rural town and satellite villages in The Gambia. *Malar J,* 7**,** 2-2.

KIRBY, M. J. & LINDSAY, S. W. 2004. Responses of adult mosquitoes of two sibling species, *Anopheles arabiensis* and *A. gambiae* s.s. (Diptera: Culicidae), to high temperatures. *Bull Entomol Res,* 94**,** 441-8.

KISZEWSKI, A. E. & DARLING, S. T. 2010. Estimating a mosquito repellent’s potential to reduce malaria in communities. *J Vector Borne Dis,* 47**,** 217-221.

KLEINSCHMIDT, I., MIGUEL, TORREZ., M., SCHWABE, C., BENAVENTE L, SEOCHARAN I, JITUBOH D, NSENG G, SHARP B 2007. Factors influencing the effectiveness of Malaria control in Bioko Island, Equitorial Guinea. *The American Society of Tropical Medicine and Hygiene,* 76**,** 1027–1032.

KOJIN, B. B. & ADELMAN, Z. N. 2019. The Sporozoite's Journey Through the Mosquito: A Critical Examination of Host and Parasite Factors Required for Salivary Gland Invasion. *Frontiers in Ecology and Evolution,* 7.

KOUNNAVONG, S., GOPINATH, D., HONGVANTHONG, B., KHAMKONG, C. & SICHANTHONGTHIP, O. 2017. Malaria elimination in Lao PDR: the challenges associated with population mobility. *Infect Dis Poverty,* 6**,** 81.

KRAEMER, M. U. G., FARIA, N. R., REINER, R. C., GOLDING, N., NIKOLAY, B., STASSE, S., JOHANSSON, M. A., SALJE, H., FAYE, O., WINT, G. R. W., NIEDRIG, M.,

SHEARER, F. M., HILL, S. C., THOMPSON, R. N., BISANZIO, D., TAVEIRA, N., NAX, H. H., PRADELSKI, B. S. R., NSOESIE, E. O., MURPHY, N. R., BOGOCH, I. I., KHAN, K., BROWNSTEIN, J. S., TATEM, A. J., DE OLIVEIRA, T., SMITH, D. L., SALL, A. A., PYBUS, O. G., HAY, S. I. & CAUCHEMEZ, S. 2017. Spread of yellow fever virus outbreak in Angola and the Democratic Republic of the Congo 2015–16: a modelling study. *The Lancet Infectious Diseases,* 17**,** 330-338.

KREPPEL, K. S., JOHNSON, P. C. D., GOVELLA, N. J., POMBI, M., MALITI, D. & FERGUSON, H. M. 2015. Comparative evaluation of the Sticky-Resting-Box-Trap, the standardised resting-bucket-trap and indoor aspiration for sampling malaria vectors. *Parasites & vectors,* 8**,** 462.

KROCKEL, U., ROSE, A., EIRAS, A. E. & GEIER, M. 2006. New tools for surveillance of adult yellow fever mosquitoes: comparison of trap catches with human landing rates in an urban environment. *J Am Mosq Control Assoc,* 22**,** 229-38.

KUTHIALA, A., GUPTA, R. K. & DAVIS, E. J. J. O. M. E. 1992. Effect of the repellent deet on the antennal chemoreceptors for oviposition in *Aedes aegypti* (Diptera: Culicidae). 29**,** 639-643.

KWEKA, E. J., ZHOU, G., LEE, M.-C., GILBREATH, T. M., MOSHA, F., MUNGA, S., GITHEKO, A. K. & YAN, G. 2011. Evaluation of two methods of estimating larval habitat productivity in western Kenya highlands. *Parasites & vectors,* 4**,** 110.

LALANI, T., YUN, H., TRIBBLE, D., GANESAN, A., KUNZ, A., FAIRCHOK, M., SCHNAUBELT, E., FRASER, J., MITRA, I., KRONMANN, K. C., BURGESS, T., DEISS, R. G., RIDDLE, M. S. & JOHNSON, M. D. 2016. A comparison of compliance rates with anti-vectorial protective measures during travel to regions with dengue or chikungunya activity, and regions endemic for *Plasmodium falciparum* malaria. *J Travel Med,* 23**,** 1-7.

LANA, R., NEKKAB, N., SIQUEIRA, A. M., PETERKA, C., MARCHESINI, P., LACERDA, M., MUELLER, I., WHITE, M. & VILLELA, D. 2021. The top 1%: quantifying the unequal distribution of malaria in Brazil. *Malar J,* 20**,** 87.

LE GOFF G, C. P. F. E. & ROBERT, V. 1975. Comparison of three sampling methods of man-biting Anophelines in order to estimate the malaria transmission in a village of south Cameroon. *parasite*.

LENGELER, C. 2004. Insecticide-treated bed nets and curtains for preventing malaria. *Cochrane Database Syst Rev***,** Cd000363.

LETA, S., BEYENE, T. J., DE CLERCQ, E. M., AMENU, K., KRAEMER, M. U. G. & REVIE, C. W. 2018. Global risk mapping for major diseases transmitted by *Aedes aegypti* and *Aedes albopictus*. *Int J Infect Dis,* 67**,** 25-35.

LI, Y., SU, X., ZHOU, G., ZHANG, H., PUTHIYAKUNNON, S., SHUAI, S., CAI, S., GU, J., ZHOU, X., YAN, G. & CHEN, X. G. 2016. Comparative evaluation of the efficiency of the BG-Sentinel trap, CDC light trap and Mosquito-oviposition trap for the surveillance of vector mosquitoes. *Parasit Vectors,* 9**,** 446.

LIANG, H., LEE, M. & JIN, X. 2016. Guiding dengue vaccine development using knowledge gained from the success of the yellow fever vaccine. *Cell Mol Immunol,* 13**,** 36-46.

LIEBMAN, K. A., STODDARD, S. T., MORRISON, A. C., ROCHA, C., MINNICK, S., SIHUINCHA, M., RUSSELL, K. L., OLSON, J. G., BLAIR, P. J., WATTS, D. M., KOCHEL, T. & SCOTT, T. W. 2012. Spatial dimensions of dengue virus transmission across interepidemic and epidemic periods in Iquitos, Peru (1999-2003). *PLoS Negl Trop Dis,* 6**,** e1472.

LILLIE, T. H., MARQUARDT, W. C. & JONES, R. H. 1981. The Flight Range of Culicoides Variipennis (Diptera: Ceratopogonidae). *The Canadian Entomologist,* 113**,** 419-426.

LIN, T. K., ZHONG, L. & SANTIAGO, J. L. 2017. Anti-Inflammatory and Skin Barrier Repair Effects of Topical Application of Some Plant Oils. *Int J Mol Sci,* 19.

LINDSAY, S. W., ADIAMAH, J. H., MILLER, J. E., PLEASS, R. J. & ARMSTRONG, J. R. 1993. Variation in attractiveness of human subjects to malaria mosquitoes (Diptera: Culicidae) in The Gambia. *J Med Entomol,* 30**,** 368-73.

LINES, J. D., LYIMO, E. O. & CURTIS, C. F. 1986. Mixing of indoor- and outdoor-resting adults of *Anopheles gambiae* Giles s.l. and *A. funestus* Giles (Diptera: Culicidae) in coastal Tanzania. *Bulletin of Entomological Research.*

LIU, M. Z. & VOSSHALL, L. B. 2019. General Visual and Contingent Thermal Cues Interact to Elicit Attraction in Female *Aedes aegypti* Mosquitoes. *Curr Biol,* 29**,** 2250-2257.e4.

LIVERANI, M., CHARLWOOD, J. D., LAWFORD, H. & YEUNG, S. 2017. Field assessment of a novel spatial repellent for malaria control: a feasibility and acceptability study in Mondulkiri, Cambodia. *Mal J,* 16.

LORENZ, L. M., KEANE, A., MOORE, J. D., MUNK, C. J., SEEHOLZER, L., MSEKA, A., SIMFUKWE, E., LIGAMBA, J., TURNER, E. L., BISWARO, L. R., OKUMU, F. O., KILLEEN, G. F., MUKABANA, W. R. & MOORE, S. J. 2013. Taxis assays measure directional movement of mosquitoes to olfactory cues. *Parasit Vectors,* 6**,** 131.

LORENZ, L. M., OVERGAARD, H. J., MASSUE, D. J., MAGENI, Z. D., BRADLEY, J., MOORE, J. D., MANDIKE, R., KRAMER, K., KISINZA, W. & MOORE, S. J. 2014. Investigating mosquito net durability for malaria control in Tanzania - Attrition, bioefficacy, chemistry, degradation and insecticide resistance (ABCDR): Study protocol. *BMC Public Health,* 14**,** 1-11.

LUPI, E., HATZ, C. & SCHLAGENHAUF, P. 2013. The efficacy of repellents against Aedes, Anopheles, Culex and Ixodes spp. - a literature review. *Travel Med Infect Dis,* 11**,** 374-411.

LWETOIJERA, D. W., HARRIS, C., KIWARE, S. S., DONGUS, S., DEVINE, G. J., MCCALL, P. J. & MAJAMBERE, S. 2014. Increasing role of *Anopheles funestus* and *Anopheles arabiensis* in malaria transmission in the Kilombero Valley, Tanzania. *Malar J,* 13**,** 331.

MACHANI, M. G., OCHOMO, E., SANG, D., BONIZZONI, M., ZHOU, G., GITHEKO, A. K., YAN, G. & AFRANE, Y. A. 2019. Influence of blood meal and age of mosquitoes on susceptibility to pyrethroids in *Anopheles gambiae* from Western Kenya. *Malar J,* 18**,** 112.

MACIEL-DE-FREITAS, R., EIRAS, A. E. & LOURENÇO-DE-OLIVEIRA, R. 2006. Field evaluation of effectiveness of the BG-Sentinel, a new trap for capturing adult *Aedes aegypti* (Diptera: Culicidae). *Mem Inst Oswaldo Cruz,* 101**,** 321-5.

MAGESA, S. M., WILKES, T. J., MNZAVA, A. E., NJUNWA, K. J., MYAMBA, J., KIVUYO, M. D., HILL, N., LINES, J. D. & CURTIS, C. F. 1991. Trial of pyrethroid impregnated bednets in an area of Tanzania holoendemic for malaria. Part 2. Effects on the malaria vector population. *Acta Tropica,* 49**,** 97-108.

MAHANDE, A., MOSHA, F., MAHANDE, J. & KWEKA, E. 2007. Feeding and resting behaviour of malaria vector, *Anopheles arabiensis* with reference to zooprophylaxis. *Malar J,* 6**,** 100.

MAIA, M., SANGORO, P., THELE, M., TURNER, E. & MOORE, S. 2012a. Do topical repellents divert mosquitoes within a community? *Malaria Journal,* 11**,** P120.

MAIA, M. F., ABONUUSUM, A., LORENZ, L. M., CLAUSEN, P. H., BAUER, B., GARMS, R. & KRUPPA, T. 2012b. The effect of deltamethrin-treated net fencing around cattle enclosures on outdoor-biting mosquitoes in Kumasi, Ghana. *PLoS One,* 7**,** e45794.

MAIA, M. F., KLINER, M., RICHARDSON, M., LENGELER, C. & MOORE, S. J. 2018. Mosquito repellents for malaria prevention. *Cochrane Database of Systematic Reviews*.

MAIA, M. F., KREPPEL, K., MBEYELA, E., ROMAN, D., MAYAGAYA, V., LOBO, N. F., ROSS, A. & MOORE, S. J. 2016. A crossover study to evaluate the diversion of malaria vectors in a community with incomplete coverage of spatial repellents in the Kilombero Valley, Tanzania. *Parasit Vectors,* 9**,** 451.

MAIA, M. F. & MOORE, S. J. 2011. Plant-based insect repellents: a review of their efficacy, development and testing. *Malaria Journal,* 10**,** S11.

MAJORI, G. 2012. Short History of Malaria and its Eradication in Italy with Short Notes on the Fight against the Infection in the Mediterranean Basin. *Mediterr J Hematol Infect Dis,* 4.

MALITI, D. V., GOVELLA, N. J., KILLEEN, G. F., MIRZAI, N., JOHNSON, P. C., KREPPEL, K. & FERGUSON, H. M. 2015. Development and evaluation of mosquito-electrocuting traps as alternatives to the human landing catch technique for sampling host-seeking malaria vectors. *Malar J,* 14**,** 502.

MARSHALL, J. M., WHITE, M. T., GHANI, A. C., SCHLEIN, Y., MULLER, G. C. & BEIER, J. C. 2013. Quantifying the mosquito's sweet tooth: modelling the effectiveness of attractive toxic sugar baits (ATSB) for malaria vector control. *Malar J,* 12**,** 291.

MARTIN, N. J., NAM, V. S., LOVER, A. A., PHONG, T. V., TU, T. C. & MENDENHALL, I. H. 2020. The impact of transfluthrin on the spatial repellency of the primary malaria mosquito vectors in Vietnam: Anopheles dirus and *Anopheles minimus*. *Malar J,* 19**,** 9.

MASALU, J. P., FINDA, M., KILLEEN, G. F., NGOWO, H. S., PINDA, P. G. & OKUMU, F. O. 2020. Creating mosquito-free outdoor spaces using transfluthrin-treated chairs and ribbons. *Malar J,* 19**,** 109.

MASALU, J. P., FINDA, M., OKUMU, F. O., MINJA, E. G., MMBANDO, A. S., SIKULU-LORD, M. T. & OGOMA, S. B. 2017. Efficacy and user acceptability of transfluthrin-treated sisal and hessian decorations for protecting against mosquito bites in outdoor bars. *Parasit Vectors,* 10**,** 197.

MASSUE, D. J., KISINZA, W. N., MALONGO, B. B., MGAYA, C. S., BRADLEY, J., MOORE, J. D., TENU, F. F. & MOORE, S. J. 2016. Comparative performance of three experimental hut designs for measuring malaria vector responses to insecticides in Tanzania. *Malar J,* 15**,** 165.

MASSUE, D. J., LORENZ, L. M., MOORE, J. D., NTABALIBA, W. S., ACKERMAN, S., MBOMA, Z. M., KISINZA, W. N., MBUBA, E., MMBAGA, S., BRADLEY, J., OVERGAARD, H. J. & MOORE, S. J. 2019. Comparing the new Ifakara Ambient

Chamber Test with WHO cone and tunnel tests for bioefficacy and non-inferiority testing of insecticide-treated nets. *Malar J,* 18**,** 153.

MATOWO, J., KITAU, J., KAAYA, R., KAVISHE, R., WRIGHT, A., KISINZA, W., KLEINSCHMIDT, I., MOSHA, F., ROWLAND, M. & PROTOPOPOFF, N. 2015. Trends in the selection of insecticide resistance in *Anopheles gambiae* s.l. mosquitoes in northwest Tanzania during a community randomized trial of longlasting insecticidal nets and indoor residual spraying. *Med Vet Entomol,* 29**,** 51-9.

MATOWO, N. S., KOEKEMOER, L. L., MOORE, S. J., MMBANDO, A. S., MAPUA, S. A., COETZEE, M. & OKUMU, F. O. 2016. Combining Synthetic Human Odours and Low-Cost Electrocuting Grids to Attract and Kill Outdoor-Biting Mosquitoes: Field and Semi-Field Evaluation of an Improved Mosquito Landing Box. *PLoS One,* 11**,** e0145653.

MATOWO, N. S., MUNHENGA, G., TANNER, M., COETZEE, M., FERINGA, W. F., NGOWO, H. S., KOEKEMOER, L. L. & OKUMU, F. O. 2017. Fine-scale spatial and temporal heterogeneities in insecticide resistance profiles of the malaria vector, *Anopheles arabiensis* in rural south-eastern Tanzania. *Wellcome Open Res,* 2**,** 96.

MATTHEWS, J., BETHEL, A. & OSEI, G. 2020. An overview of malarial Anopheles mosquito survival estimates in relation to methodology. *Parasit Vectors,* 13**,** 233.

MBOERA, L. E., MWEYA, C. N., RUMISHA, S. F., TUNGU, P. K., STANLEY, G., MAKANGE, M. R., MISINZO, G., DE NARDO, P., VAIRO, F. & ORIYO, N. M. 2016. The Risk of Dengue Virus Transmission in Dar es Salaam, Tanzania during an Epidemic Period of 2014. *PLoS Negl Trop Dis,* 10**,** e0004313.

MBUBA, E., ODUFUWA, O. G., TENYWA, F. C., PHILIPO, R., TAMBWE, M. M., SWAI, E. S., MOORE, J. D. & MOORE, S. J. 2020. MAÏA® Topical Repellent Ointment Provides Long-Lasting Protection Against *Anopheles gambiae* s.s., *Anopheles arabiensis* and *Aedes Aegypti* Under Semi-Field Conditions in Tanzania. *Mal J,* In press.

MCCALL, P. J., MOSHA, F. W., NJUNWA, K. J. & SHERLOCK, K. 2001. Evidence for memorized site-fidelity in *Anopheles arabiensis*. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 95**,** 587-590.

MCPHATTER, L. P., MISCHLER, P. D., WEBB, M. Z., CHAUHAN, K., LINDROTH, E. J., RICHARDSON, A. G. & DEBBOUN, M. 2017. Laboratory and semi-field evaluations of two (transfluthrin) spatial repellent devices against *Aedes aegypti* (L.) (Diptera: Culicidae). *US Army Med Dep J***,** 13-22.

MENGER, D. J., OMUSULA, P., HOLDINGA, M., HOMAN, T., CARREIRA, A. S., VANDENDAELE, P., DERYCKE, J. L., MWERESA, C. K., MUKABANA, W. R., VAN LOON, J. J. & TAKKEN, W. 2015. Field evaluation of a push-pull system to reduce malaria transmission. *PLoS One,* 10**,** e0123415.

MENGER, D. J., OMUSULA, P., WOUTERS, K., OKETCH, C., CARREIRA, A. S., DURKA, M., DERYCKE, J. L., LOY, D. E., HAHN, B. H., MUKABANA, W. R., MWERESA, C. K., VAN LOON, J. J., TAKKEN, W. & HISCOX, A. 2016. Eave Screening and Push-Pull Tactics to Reduce House Entry by Vectors of Malaria. *Am J Trop Med Hyg,* 94**,** 868-78.

MENGER, D. J., OTIENO, B., MARJOLEIN DE RIJK, W., VAN LOON, J. J. & TAKKEN, W. 2014. A push-pull system to reduce house entry of malaria mosquitoes. *Malaria Journal,* 13**,** 119.

MESSINA, J. P., BRADY, O. J., GOLDING, N., KRAEMER, M. U. G., WINT, G. R. W., RAY, S. E., PIGOTT, D. M., SHEARER, F. M., JOHNSON, K., EARL, L., MARCZAK, L. B., SHIRUDE, S., DAVIS WEAVER, N., GILBERT, M., VELAYUDHAN, R., JONES, P., JAENISCH, T., SCOTT, T. W., REINER, R. C., JR. & HAY, S. I. 2019. The current and future global distribution and population at risk of dengue. *Nat Microbiol,* 4**,** 1508-1515.

MIDEGA, J. T., MBOGO, C. M., MWNAMBI, H., WILSON, M. D., OJWANG, G., MWANGANGI, J. M., NZOVU, J. G., GITHURE, J. I., YAN, G. & BEIER, J. C. 2007. Estimating dispersal and survival of *Anopheles gambiae* and *Anopheles funestus* along the Kenyan coast by using mark-release-recapture methods. *Journal of medical entomology,* 44**,** 923-9.

MINGOTE, T., ÁZARA, F. D., DEGENER, C. M., ROQUE, R. A., OHLY, J. J., GEIER, M. & EIRAS, Á. E. 2013. The impact of CO 2 on collection of *Aedes aegypti* (Linnaeus) and *Culex quinquefasciatus* Say by BG-Sentinel ® traps in Manaus , Brazil. 108**,** 229-232.

MITCHELL, S. N., STEVENSON, B. J., MULLER, P., WILDING, C. S., EGYIR-YAWSON, A., FIELD, S. G., HEMINGWAY, J., PAINE, M. J., RANSON, H. & DONNELLY, M. J. 2012. Identification and validation of a gene causing cross-resistance between insecticide classes in *Anopheles gambiae* from Ghana. *Proc Natl Acad Sci U S A,* 109**,** 6147-52.

MMBANDO, A. S., BATISTA, E. P. A., KILALANGONGONO, M., FINDA, M. F., MWANGA, E. P., KAINDOA, E. W., KIFUNGO, K., NJALAMBAHA, R. M., NGOWO, H. S., EIRAS, A. E. & OKUMU, F. O. 2019. Evaluation of a push-pull system consisting of transfluthrin-treated eave ribbons and odour-baited traps for control of indoor- and outdoor-biting malaria vectors. *Malar J,* 18**,** 87.

MMBANDO, A. S., NGOWO, H., LIMWAGU, A., KILALANGONGONO, M., KIFUNGO, K. & OKUMU, F. O. 2018. Eave ribbons treated with the spatial repellent, transfluthrin, can effectively protect against indoor-biting and outdoor-biting malaria mosquitoes. *Malar J,* 17**,** 368.

MMBANDO, A. S., NGOWO, H. S., KILALANGONGONO, M., ABBAS, S., MATOWO, N. S., MOORE, S. J. & OKUMU, F. O. 2017. Small-scale field evaluation of push-pull system against early- and outdoor-biting malaria mosquitoes in an area of high pyrethroid resistance in Tanzania. *Wellcome Open Res,* 2**,** 112.

MMBANDO, A. S., OKUMU, F. O., MGANDO, J. P., SUMAYE, R. D., MATOWO, N. S., MADUMLA, E., KAINDOA, E., KIWARE, S. S. & LWETOIJERA, D. W. 2015. Effects of a new outdoor mosquito control device, the mosquito landing box, on densities and survival of the malaria vector, *Anopheles arabiensis*, inside controlled semi-field settings. *Malar J,* 14**,** 494.

MNZAVA, A. E., RWEGOSHORA, R. T., WILKES, T. J., TANNER, M. & CURTIS, C. F. 1995. *Anopheles arabiensis* and *An. gambiae* chromosomal inversion polymorphism, feeding and resting behaviour in relation to insecticide house-spraying in Tanzania. *Medical and veterinary entomology,* 9**,** 316-324.

MOIROUX, N., GOMEZ, M. B., PENNETIER, C., ELANGA, E., DJENONTIN, A., CHANDRE, F., DJEGBE, I., GUIS, H. & CORBEL, V. 2012. Changes in *Anopheles funestus* biting behavior following universal coverage of long-lasting insecticidal nets in Benin. *J Infect Dis,* 206**,** 1622-9.

MONGKALANGOON, P., GRIECO, J. P., ACHEE, N. L., SUWONKERD, W. & CHAREONVIRIYAPHAP, T. 2009. Irritability and repellency of synthetic pyrethroids on an *Aedes aegypti* population from Thailand. *Journal of Vector Ecology,* 34**,** 217-224.

MONROE, A., ASAMOAH, O., LAM, Y., KOENKER, H., PSYCHAS, P., LYNCH, M., RICOTTA, E., HORNSTON, S., BERMAN, A. & HARVEY, S. A. 2015. Outdoor-sleeping and other night-time activities in northern Ghana: implications for residual transmission and malaria prevention. *Malar J,* 14**,** 35.

MONROE, A., MIHAYO, K., OKUMU, F., FINDA, M., MOORE, S., KOENKER, H., LYNCH, M., HAJI, K., ABBAS, F., ALI, A., GREER, G. & HARVEY, S. 2019a. Human behaviour and residual malaria transmission in Zanzibar: findings from in-depth interviews and direct observation of community events. *Malar J,* 18**,** 220.

MONROE, A., MOORE, S., KOENKER, H., LYNCH, M. & RICOTTA, E. 2019b. Measuring and characterizing night time human behaviour as it relates to residual malaria transmission in sub-Saharan Africa: a review of the published literature. *Malar J,* 18**,** 6.

MOORE, S. J., DAVIES, C. R. & CAMERON, M. M. 2007. Are mosquitoes diverted from repellent-using individuals to non-users? Results of a field study in Bolivia. *Trop Med Int Health,* 12**,** 1-8.

MORRIS, C. D., LARSON, V. L. & LOUNIBOS, L. P. 1991. Measuring mosquito dispersal for control programs. *Journal of the American Mosquito Control Association*.

MORRISON, A. C., REINER, R. C., ELSON, W. H., ASTETE, H., GUEVARA, C., DEL AGUILA, C., BAZAN, I., SILES, C., BARRERA, P., KAWIECKI, A. B., BARKER, C. M., VASQUEZ, G. M., ESCOBEDO-VARGAS, K., FLORES-MENDOZA, C., HUAMAN, A. A., LEGUIA, M., SILVA, M. E., JENKINS, S. A., CAMPBELL, W. R., ABENTE, E. J., HONTZ, R. D., PAZ-SOLDAN, V. A., GRIECO, J. P., LOBO, N. F., SCOTT, T. W. & ACHEE, N. L. 2021. Efficacy of a Spatial Repellent for Control of Aedes-Borne Virus Transmission: A Cluster Randomized Trial in Iquitos, Peru. *medRxiv***,** 2021.03.03.21252148.

MOSHI, I. R., MANDERSON, L., NGOWO, H. S., MLACHA, Y. P., OKUMU, F. O. & MNYONE, L. L. 2018. Outdoor malaria transmission risks and social life: a qualitative study in South-Eastern Tanzania. *Malar J,* 17**,** 397.

MOSHI, I. R., NGOWO, H., DILLIP, A., MSELLEMU, D., MADUMLA, E. P., OKUMU, F. O., COETZEE, M., MNYONE, L. L. & MANDERSON, L. 2017. Community perceptions on outdoor malaria transmission in Kilombero Valley, Southern Tanzania. *Malar J,* 16**,** 274.

MR4. 2009. Methods in Anopheles Research Manual https://www.beiresources.org/Publications/MethodsinAnophelesResearch.aspx

MR4 2014. Methods in Anopheles Research Manual.

MUIR, L. E., KAY, B. H. & THORNE, M. J. 1992. *Aedes aegypti* (Diptera: Culicidae) Vision: Response to Stimuli from the Optical Environment. *Journal of Medical Entomology,* 29**,** 445-450.

MUKABANA, W., TAKKEN, W., COE, R. & KNOLS, B. 2002. Host-specific cues cause differential attractiveness of Kenyan men to the African malaria vector *Anopheles gambiae*. *Mal J,* 1.

MUKABANA W. 2002. PhD thesis Differential attarctiveness of humans to the African malaria vector *Anopheles gambiae* Giles Effect of host characteristic and parasite infection.

MULATIER, M., PORCIANI, A., NADALIN, L., AHOUA ALOU, L. P., CHANDRE, F., PENNETIER, C., DORMONT, L. & COHUET, A. 2018. DEET Efficacy Increases With Age in the Vector Mosquitoes *Anopheles gambiae* s.s. and *Aedes albopictus* (Diptera: Culicidae). *J Med Entomol,* 55**,** 1542-1548.

MUSIIME, A. K., SMITH, D. L., KILAMA, M., REK, J., ARINAITWE, E., NANKABIRWA, J. I., KAMYA, M. R., CONRAD, M. D., DORSEY, G., AKOL, A. M., STAEDKE, S. G., LINDSAY, S. W. & EGONYU, J. P. 2019. Impact of vector control interventions on malaria transmission intensity, outdoor vector biting rates and Anopheles mosquito species composition in Tororo, Uganda. *Malar J,* 18**,** 445.

MWANGA, E. P., MMBANDO, A. S., MROSSO, P. C., STICA, C., MAPUA, S. A., FINDA, M. F., KIFUNGO, K., KAFWENJI, A., MONROE, A. C., OGOMA, S. B., NGOWO, H. S. & OKUMU, F. O. 2019. Eave ribbons treated with transfluthrin can protect both users and non-users against malaria vectors. *Malar J,* 18**,** 314.

MWANYIKA, G., MBOERA, L. E. G., RUGARABAMU, S., LUTWAMA, J., SINDATO, C., PAWESKA, J. T. & MISINZO, G. 2019. Co-circulation of Dengue Virus Serotypes 1 and 3 during the 2019 epidemic in Dar es Salaam, Tanzania. 763003.

NÁJERA, J. A., GONZÁLEZ-SILVA, M. & ALONSO, P. L. 2011. Some lessons for the future from the Global Malaria Eradication Programme (1955-1969). *PLoS Med,* 8**,** e1000412.

NARANJO-DIAZ, N., ROSERO, D. A., RUA-URIBE, G., LUCKHART, S. & CORREA, M. M. 2013. Abundance, behavior and entomological inoculation rates of anthropophilic anophelines from a primary Colombian malaria endemic area. *Parasit Vectors,* 6**,** 61.

NASH, R., LAMBERT, B., N'GUESSAN, R., N'GUFOR, C., ROWLAND, M., OXBOROUGH, R., MOORE, S. J., TUNGU, P., SHERRARD-SMITH, E. & CHURCHER, T. S. 2021. Systematic review of the entomological impact of insecticide-treated nets evaluated using experimental hut trials in Africa. *Biorxiv*.

NDENGA, B. A., MUTUKU, F. M., NGUGI, H. N., MBAKAYA, J. O., ASWANI, P., MUSUNZAJI, P. S., VULULE, J., MUKOKO, D., KITRON, U. & LABEAUD, A. D. 2017. Characteristics of *Aedes aegypti* adult mosquitoes in rural and urban areas of western and coastal Kenya. *PLoS One,* 12**,** e0189971.

NEWLAND, P. L., GHAMDI, M. S. A., SHARKH, S., AONUMA, H. & JACKSON, C. W. 2015. Exposure to static electric fields leads to changes in biogenic amine levels in the brains of Drosophila. *Proceedings of the Royal Society B: Biological Sciences,* 282**,** 20151198.

NGOWO, H. S., KAINDOA, E. W., MATTHIOPOULOS, J., FERGUSON, H. M. & OKUMU, F. O. 2017. Variations in household microclimate affect outdoor-biting behaviour of malaria vectors. *Wellcome Open Res,* 2**,** 102.

NIEBYLSKI, M. L. & CRAIG, G. B. 1994. Dispersal and survival of *Aedes albopictus* at a scrap tire yard in Missouri. *Journal of the American Mosquito Control Association,* 10**,** 339-43.

NIEBYLSKI, M. L. & MEEK, C. L. 1989. A self-marking device for emergent adult mosquitoes. *Journal of the American Mosquito Control Association,* 5**,** 86-90.

NJIRU, B. N., MUKABANA, W. R., TAKKEN, W. & KNOLS, B. G. 2006. Trapping of the malaria vector *Anopheles gambiae* with odour-baited MM-X traps in semi-field conditions in western Kenya. *Malar J,* 5**,** 39.

NJOROGE, M. M., FILLINGER, U., SADDLER, A., MOORE, S., TAKKEN, W., VAN LOON, J. J. A. & HISCOX, A. 2021. Evaluating putative repellent 'push' and attractive 'pull' components for manipulating the odour orientation of host-seeking malaria vectors in the peri-domestic space. *Parasites & Vectors,* 14**,** 42.

NORRIS, E. J. & COATS, J. R. 2017. Current and Future Repellent Technologies: The Potential of Spatial Repellents and Their Place in Mosquito-Borne Disease Control. *Int J Environ Res Public Health,* 14.

OBERMAYR, U., RUTHER, J., BERNIER, U. R., ROSE, A. & GEIER, M. 2015. Evaluation of a Push-Pull Approach for *Aedes aegypti* (L.) Using a Novel Dispensing System for Spatial Repellents in the Laboratory and in a Semi-Field Environment. *PLoS One,* 10**,** e0129878.

OGOMA, S. B., LORENZ, L. M., NGONYANI, H., SANGUSANGU, R., KITUMBUKILE, M., KILALANGONGONO, M., SIMFUKWE, E. T., MSEKA, A., MBEYELA, E., ROMAN, D., MOORE, J., KREPPEL, K., MAIA, M. F. & MOORE, S. J. 2014a. An experimental hut study to quantify the effect of DDT and airborne pyrethroids on entomological parameters of malaria transmission. *Malar J,* 13**,** 131.

OGOMA, S. B., MMANDO, A. S., SWAI, J. K., HORSTMANN, S., MALONE, D. & KILLEEN, G. F. 2017. A low technology emanator treated with the volatile pyrethroid transfluthrin confers long term protection against outdoor biting vectors of lymphatic filariasis, arboviruses and malaria. *PLoS Negl Trop Dis,* 11**,** e0005455.

OGOMA, S. B., MOORE, S. J. & MAIA, M. F. 2012a. A systematic review of mosquito coils and passive emanators: defining recommendations for spatial repellency testing methodologies. *Parasit Vectors,* 5**,** 287-287.

OGOMA, S. B., NGONYANI, H., SIMFUKWE, E. T., MSEKA, A., MOORE, J. & KILLEEN, G. F. 2012b. Spatial repellency of transfluthrin-treated hessian strips against laboratory-reared *Anopheles arabiensis* mosquitoes in a semi-field tunnel cage. *Parasit Vectors,* 5**,** 54.

OGOMA, S. B., NGONYANI, H., SIMFUKWE, E. T., MSEKA, A., MOORE, J., MAIA, M. F., MOORE, S. J. & LORENZ, L. M. 2014b. The mode of action of spatial repellents and their impact on vectorial capacity of *Anopheles gambiae* sensu stricto. *PLoS One,* 9**,** e110433.

OKUMU, F. O., KILLEEN, G. F., OGOMA, S., BISWARO, L., SMALLEGANGE, R. C., MBEYELA, E., TITUS, E., MUNK, C., NGONYANI, H., TAKKEN, W., MSHINDA, H., MUKABANA, W. R. & MOORE, S. J. 2010. Development and field evaluation of a synthetic mosquito lure that is more attractive than humans. *PLoS One,* 5**,** e8951.

OKUMU, F. O., MOORE, J., MBEYELA, E., SHERLOCK, M., SANGUSANGU, R., LIGAMBA, G., RUSSELL, T. & MOORE, S. J. 2012. A modified experimental hut design for studying responses of disease-transmitting mosquitoes to indoor interventions: the Ifakara experimental huts. *PLoS One,* 7**,** e30967.

OKUMU, F. O., TITUS, E., MBEYELA, E., KILLEEN, G. F. & MOORE, S. J. 2009. Limitation of using synthetic human odours to test mosquito repellents. *Malar J,* 8**,** 150.

ORSBORNE, J., FURUYA‐KANAMORI, L., JEFFRIES, C. L., KRISTAN, M., MOHAMMED, A. R., AFRANE, Y., O'REILLY, K., MASSAD, E., DRAKELEY, C., WALKER, T. & YAKOB, L. 2018. Using the human blood index to investigate host biting plasticity: a systematic review and meta-regression of the three major African malaria vectors. *Mal J,* 17**,** 479.

ORTEGA-LÓPEZ, L. D., PONDEVILLE, E., KOHL, A., LEÓN, R., BETANCOURTH, M. P., ALMIRE, F., TORRES-VALENCIA, S., SALDARRIAGA, S., MIRZAI, N. & FERGUSON, H. M. 2020. The mosquito electrocuting trap as an exposure-free method for measuring human-biting rates by Aedes mosquito vectors. *Parasites & Vectors,* 13**,** 31.

OUMBOUKE, W. A., ROWLAND, M., KOFFI, A. A., ALOU, L. P. A., CAMARA, S. & N'GUESSAN, R. 2019. Evaluation of an alpha-cypermethrin + PBO mixture long-lasting insecticidal net VEERALIN® LN against pyrethroid resistant *Anopheles gambiae* s.s.: an experimental hut trial in M'bé, central Côte d'Ivoire. *Parasit Vectors,* 12**,** 544.

P.R., C. 1975. The activity of some pyrethroids, DDT and lindane in smoke from coils for biting inhibition, knockdown and kill of mosquitoes (Diptera, Culicidae). *Bull. ent. Res.,* 65**,** 97-107.

PANINI M, M. G., MOORES GD, MAZZONI E 1923. The Memoirs of Sir Ronald Ross. *The Indian medical gazette,* 58**,** 481-484.

PAPPA, V., REDDY, M., OVERGAARD, H., ABAGA, S. & CACCONE, A. 2011. Estimation of the human blood index in malaria mosquito vectors in Equatorial Guinea after indoor antivector interventions. *Am J Trop Med Hyg,* 84.

PAUL, P., KANGALAWE, R. Y. M. & MBOERA, L. E. G. 2018. Land-use patterns and their implication on malaria transmission in Kilosa District, Tanzania. *Tropical Diseases, Travel Medicine and Vaccines,* 4**,** 6.

PETRIĆ, D., BELLINI, R., SCHOLTE, E. J., RAKOTOARIVONY, L. M. & SCHAFFNER, F. 2014. Monitoring population and environmental parameters of invasive mosquito species in Europe. *Parasit Vectors,* 7**,** 187.

PETTEBONE, M. S. 2014. Characterisation of transfluthirn emissions over time in an enclosed space over a range of discreet temperatures*.* Uniformed Services University of the Health Sciences.

PHANITCHAT, T., ZHAO, B., HAQUE, U., PIENTONG, C., EKALAKSANANAN, T., AROMSEREE, S., THAEWNONGIEW, K., FUSTEC, B., BANGS, M. J., ALEXANDER, N. & OVERGAARD, H. J. 2019. Spatial and temporal patterns of dengue incidence in northeastern Thailand 2006-2016. *BMC Infect Dis,* 19**,** 743.

POLLARD, E. J. M., MACLAREN, D., RUSSELL, T. L. & BURKOT, T. R. 2020. Protecting the peri-domestic environment: the challenge for eliminating residual malaria. *Scientific Reports,* 10**,** 7018.

POMBI, M., JACOBS, F., VERHULST, N. O., CAPUTO, B., TORRE, A. & TAKKEN, W. 2014. Field evaluation of a novel synthetic odour blend and of the synergistic role of carbon dioxide for sampling host-seeking *Aedes albopictus* adults in Rome, Italy. *Parasit Vectors,* 7**,** 580.

PONLAWAT, A., KANKAEW, P., CHANAIMONGKOL, S., PONGSIRI, A., RICHARDSON, J. H. & EVANS, B. P. 2016. Semi-Field Evaluation of Metofluthrin-Impregnated

Nets on Host-Seeking *Aedes aegypti* and *Anopheles dirus*. *J Am Mosq Control Assoc,* 32**,** 130-8.

POTIKASIKORN, J., CHAREONVIRIYAPHAP, T., BANGS, M. J. & PRABARIPAI, A. 2005. Behavioral responses to DDT and pyrethroids between *Anopheles minimus* species A and C, malaria vectors in Thailand. *Am J Trop Med Hyg,* 73**,** 343-9.

POWELL, J. R. & TABACHNICK, W. J. 2013. History of domestication and spread of *Aedes aegypti*--a review. *Mem Inst Oswaldo Cruz,* 108 Suppl 1**,** 11-7.

PPAV WORKING GROUPS 2011. Personal Protection Against Vectors. *Parasite,* 18.

QUALLS, W. A., MULLER, G. C., TRAORE, S. F., TRAORE, M. M., ARHEART, K. L., DOUMBIA, S., SCHLEIN, Y., KRAVCHENKO, V. D., XUE, R. D. & BEIER, J. C. 2015. Indoor use of attractive toxic sugar bait (ATSB) to effectively control malaria vectors in Mali, West Africa. *Malaria Journal,* 14.

QUIÑONES, M. L., LINES, J. D., THOMSON, M. C., JAWARA, M., MORRIS, J. & GREENWOOD, B. M. 1997. *Anopheles gambiae* gonotrophic cycle duration, biting and exiting behaviour unaffected by permethrin-impregnated bednets in The Gambia. *Medical and veterinary entomology,* 11**,** 71-78.

RAFAEL MF, A. L. E., RICARDO LO 2006. Field evaluation of effectiveness of the BG-Sentinel, a new trap

for capturing adult *Aedes aegypti* (Diptera: Culicidae). *Mem Inst Oswaldo Cruz, Rio de Janeiro,* 101**,** 321-325.

RANSON, H. & LISSENDEN, N. 2016. Insecticide Resistance in African Anopheles Mosquitoes: A Worsening Situation that Needs Urgent Action to Maintain Malaria Control. *Trends Parasitol,* 32**,** 187-196.

RANSON, H., N'GUESSAN, R., LINES, J., MOIROUX, N., NKUNI, Z. & CORBEL, V. 2011. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? *Trends Parasitol,* 27**,** 91-8.

RAWLINGS, P., CURTIS, C. F., WICKRAMASINGHE, M. B. & LINES, J. 1981. The influence of age and season on dispersal and recapture of *Anopheles culicifacies* in Sri Lanka. *Ecological Entomology,* 6**,** 307-319.

RAY, A. 2015. Reception of odors and repellents in mosquitoes. *Curr Opin Neurobiol,* 34**,** 158-64.

REDDY, M. R., OVERGAARD, H. J., ABAGA, S., REDDY, V. P., CACCONE, A., KISZEWSKI, A. E. & SLOTMAN, M. A. 2011. Outdoor host seeking behaviour of *Anopheles gambiae* mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea. *Malar J,* 10**,** 184.

RIFFELL, J. A. 2019. Olfaction: Repellents that Congest the Mosquito Nose. *Current Biology,* 29**,** R1124-R1126.

RINKER, D. C., JONES, P. I., PITTS, R. J., RÜTZLER, M., CAMP, G. J., SUN, L., XU, P., DORSET, D., WEAVER, D. & ZWIEBEL, L. J. 2012. Novel high-throughput screens of *Anopheles gambiae* odorant receptors reveal candidate behaviour-modifying chemicals for mosquitoes. *Physiol Entomol,* 37**,** 33-41.

RITCHIE, S. A. & DEVINE, G. J. 2013. Confusion, knock-down and kill of *Aedes aegypti* using metofluthrin in domestic settings: a powerful tool to prevent dengue transmission? *Parasit Vectors,* 6**,** 262.

ROIZ, D., WILSON, A. L., SCOTT, T. W., FONSECA, D. M., JOURDAIN, F., MULLER, P., VELAYUDHAN, R. & CORBEL, V. 2018. Integrated Aedes management for the control of Aedes-borne diseases. *PLoS Negl Trop Dis,* 12**,** e0006845.

RUND, S. S., O'DONNELL, A. J., GENTILE, J. E. & REECE, S. E. 2016. Daily Rhythms in Mosquitoes and Their Consequences for Malaria Transmission. *Insects,* 7.

RUSSELL, T. L., GOVELLA, N. J., AZIZI, S., DRAKELEY, C. J., KACHUR, S. P. & KILLEEN, G. F. 2011. Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. *Malar J,* 10**,** 80.

RUTLEDGE, L. C. & GUPTA, R. K. 1999. Variation in the protection periods of repellents on individual human subjects: an analytical review. *J Am Mosq Control Assoc,* 15**,** 348-55.

SADDLER, A., KREPPEL, K. S., CHITNIS, N., SMITH, T. A., DENZ, A., MOORE, J. D., TAMBWE, M. M. & MOORE, S. J. 2019. The development and evaluation of a self-marking unit to estimate malaria vector survival and dispersal distance. *Malar J,* 18**,** 441.

SALAZAR, F. V., ACHEE, N. L., GRIECO, J. P., PRABARIPAI, A., OJO, T. A., EISEN, L., DUREZA, C., POLSOMBOON, S. & CHAREONVIRIYAPHAP, T. 2013. Effect of *Aedes aegypti* exposure to spatial repellent chemicals on BG-Sentinel™ trap catches. *Parasit Vectors,* 6**,** 145.

SANDE, S., ZIMBA, M., CHINWADA, P., MASENDU, H. T., MAZANDO, S. & MAKUWAZA, A. 2015. The emergence of insecticide resistance in the major malaria vector *Anopheles funestus* (Diptera: Culicidae) from sentinel sites in Mutare and Mutasa Districts, Zimbabwe. *Malar J,* 14**,** 466.

SANDFORT, M., VANTAUX, A., KIM, S., OBADIA, T., PEPEY, A., GARDAIS, S., KHIM, N., LEK, D., WHITE, M., ROBINSON, L. J., WITKOWSKI, B. & MUELLER, I. 2020. Forest malaria in Cambodia: the occupational and spatial clustering of *Plasmodium vivax* and *Plasmodium falciparum* infection risk in a cross-sectional survey in Mondulkiri province, Cambodia. *Malar J,* 19**,** 413.

SANGORO, O., KELLY, A. H., MTALI, S. & MOORE, S. J. 2014a. Feasibility of repellent use in a context of increasing outdoor transmission: a qualitative study in rural Tanzania. *Malar J,* 13**,** 347.

SANGORO, O., LWEITOJERA, D., SIMFUKWE, E., NGONYANI, H., MBEYELA, E., LUGIKO, D., KIHONDA, J., MAIA, M. & MOORE, S. 2014b. Use of a semi-field system to evaluate the efficacy of topical repellents under user conditions provides a disease exposure free technique comparable with field data. *Malar J,* 13**,** 159.

SANGORO, O. P., GAVANA, T., FINDA, M., MPONZI, W., HAPE, E., LIMWAGU, A., GOVELLA, N. J., CHAKI, P. & OKUMU, F. O. 2020. Evaluation of personal protection afforded by repellent-treated sandals against mosquito bites in south-eastern Tanzania. *Malar J,* 19**,** 148.

SCHMIED, W. H., TAKKEN, W., KILLEEN, G. F., KNOLS, B. G. & SMALLEGANGE, R. C. 2008. Evaluation of two counterflow traps for testing behaviour-mediating compounds for the malaria vector *Anopheles gambiae* s.s. under semi-field conditions in Tanzania. *Malar J,* 7**,** 230.

SCHOELER, G. B., SCHLEICH, S. S., MANWEILER, S. A. & SIFUENTES, V. L. 2004. Evaluation of surveillance devices for monitoring *Aedes aegypti* in an urban area of northeastern Peru. *J Am Mosq Control Assoc,* 20**,** 6-11.

SCHRECK, C. E. 1977. Techniques for the evaluation of insect repellents: a critical review. *Annu Rev Entomol,* 22**,** 101-19.

SCOTT, T. W., AMERASINGHE, P. H., MORRISON, A. C., LORENZ, L. H., CLARK, G. G., STRICKMAN, D., KITTAYAPONG, P. & EDMAN, J. D. 2000. Longitudinal studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: blood feeding frequency. *J Med Entomol,* 37**,** 89-101.

SERVICE, M. 1993. Mosquito Ecology: field sampling methods. 2nd ed.

SETO, K. C., GUNERALP, B. & HUTYRA, L. R. 2012. Global forecasts of urban expansion to 2030 and direct impacts on biodiversity and carbon pools. *Proc Natl Acad Sci U S A,* 109**,** 16083-8.

SHERRARD-SMITH, E., SKARP, J. E., BEALE, A. D., FORNADEL, C., NORRIS, L. C., MOORE, S. J., MIHRETEAB, S., CHARLWOOD, J. D., BHATT, S., WINSKILL, P., GRIFFIN, J. T. & CHURCHER, T. S. 2019. Mosquito feeding behavior and how it influences residual malaria transmission across Africa. *Proc Natl Acad Sci U S A,* 116**,** 15086-15095.

SHIRAI, O., TSUDA, T., KITAGAWA, S., NAITOH, K., SEKI, T., KAMIMURA, K. & MOROHASHI, M. 2002. Alcohol ingestion stimulates mosquito attraction. *J Am Mosq Control Assoc,* 18**,** 91-6.

SILVA, N. I. O., SACCHETTO, L., DE REZENDE, I. M., TRINDADE, G. S., LABEAUD, A. D., DE THOISY, B. & DRUMOND, B. P. 2020. Recent sylvatic yellow fever virus transmission in Brazil: the news from an old disease. *Virol J,* 17**,** 9.

SILVER, J. B. & SERVICE, M. W. 2008. Mosquito ecology : field sampling methods.**,** 1494.

SINKA, M. E., GOLDING, N., MASSEY, N. C., WIEBE, A., HUANG, Z., HAY, S. I. & MOYES, C. L. 2016. Modelling the relative abundance of the primary African vectors of malaria before and after the implementation of indoor, insecticide-based vector control. *Malaria journal,* 15**,** 142.

SLUYDTS, V., DURNEZ, L., HENG, S., GRYSEELS, C., CANIER, L., KIM, S., VAN ROEY, K., KERKHOF, K., KHIM, N. & MAO, S. J. T. L. I. D. 2016. Efficacy of topical mosquito repellent (picaridin) plus long-lasting insecticidal nets versus long-lasting insecticidal nets alone for control of malaria: a cluster randomised controlled trial. 16**,** 1169-1177.

SMALLEGANGE, R. C., VERHULST, N. O. & TAKKEN, W. 2011. Sweaty skin: an invitation to bite? *Trends Parasitol,* 27**,** 143-8.

SMITH, A. 1963. Principles in Assessment of Insecticides by Experimental Huts. *Nature,* 198**,** 171.

SMITH, A., HUDSON, E. J. & ESOZED, S. 1971. Trials with pyrethrum mosquioto coils against *Anopheles gambiae* Gillies, *Mansonia uniformis* Theo. and *Culex fatigans* Wied. entering verandah-trap huts. *Pyrethrum Post***,** 111-115.

SMITH, T., MAIRE, N., ROSS, A., PENNY, M., CHITNIS, N., SCHAPIRA, A., STUDER, A., GENTON, B., LENGELER, C., TEDIOSI, F., DE SAVIGNY, D. & TANNER, M. 2008. Towards a comprehensive simulation model of malaria epidemiology and control. *Parasitology,* 135**,** 1507-16.

SOUGOUFARA, S., DOUCOURÉ, S., SEMBÉNE, P., HARRY, M. & SOKHNA, C. 2017. Challenges for malaria vector control in sub-Saharan Africa: Resistance and behavioral adaptations in Anopheles populations. *J Vector Borne Dis,* 54**,** 4-15.

SOUGOUFARA, S., OTTIH, E. C. & TRIPET, F. 2020. The need for new vector control approaches targeting outdoor biting Anopheline malaria vector communities. *Parasit Vectors,* 13**,** 295.

SPARKS, J. T. & DICKENS, J. C. 2017. Mini review: Gustatory reception of chemicals affecting host feeding in aedine mosquitoes. *Pesticide Biochemistry and Physiology,* 142**,** 15-20.

SPITZEN, J., KOELEWIJN, T., MUKABANA, W. R. & TAKKEN, W. 2016. Visualization of house-entry behaviour of malaria mosquitoes. *Malaria Journal,* 15**,** 1-10.

STODDARD, S. T., FORSHEY, B. M., MORRISON, A. C., PAZ-SOLDAN, V. A., VAZQUEZ-PROKOPEC, G. M., ASTETE, H., REINER, R. C., JR., VILCARROMERO, S., ELDER, J. P., HALSEY, E. S., KOCHEL, T. J., KITRON, U. & SCOTT, T. W. 2013. House-to-house human movement drives dengue virus transmission. *Proceedings of the National Academy of Sciences of the United States of America,* 110**,** 994-999.

STUMP, A. D., ATIELI, F. K., VULULE, J. M. & BESANSKY, N. J. 2004. Dynamics of the pyrethroid knockdown resistance allele in western Kenyan populations of *Anopheles gambiae* in response to insecticide-treated bed net trials. *Am J Trop Med Hyg,* 70**,** 591-6.

SUKKANON, C., NARARAK, J., BANGS, M. J., HII, J. & CHAREONVIRIYAPHAP, T. 2020. Behavioral responses to transfluthrin by *Aedes aegypti*, *Anopheles minimus*, *Anopheles harrisoni*, and *Anopheles dirus* (Diptera: Culicidae). *PLOS ONE,* 15**,** e0237353.

SUKKANON, C., TISGRATOG, R., MUENWORN, V., BANGS, M. J., HII, J. & CHAREONVIRIYAPHAP, T. 2021. Field Evaluation of a Spatial Repellent Emanation Vest for Personal Protection Against Outdoor Biting Mosquitoes. *J Med Entomol,* 58**,** 756-766.

SYAFRUDDIN, D., ASIH, P. B. S., ROZI, I. E., PERMANA, D. H., NUR HIDAYATI, A. P., SYAHRANI, L., ZUBAIDAH, S., SIDIK, D., BANGS, M. J., BØGH, C., LIU, F., EUGENIO, E. C., HENDRICKSON, J., BURTON, T., BAIRD, J. K., COLLINS, F., GRIECO, J. P., LOBO, N. F. & ACHEE, N. L. 2020. Efficacy of a Spatial Repellent for Control of Malaria in Indonesia: A Cluster-Randomized Controlled Trial. *Am J Trop Med Hyg,* 103**,** 344-358.

SYAFRUDDIN, D., BANGS, M. J., SIDIK, D., ELYAZAR, I., ASIH, P. B., CHAN, K., NURLEILA, S., NIXON, C., HENDARTO, J., WAHID, I., ISHAK, H., BOGH, C., GRIECO, J. P., ACHEE, N. L. & BAIRD, J. K. 2014. Impact of a Spatial Repellent on Malaria Incidence in Two Villages in Sumba, Indonesia. *Am J Trop Med Hyg*.

TAINCHUM, K., RITTHISON, W., SATHANTRIPHOP, S., TANASILCHAYAKUL, S., MANGUIN, S., BANGS, M. J. & CHAREONVIRIYAPHAP, T. 2014. Influence of Time of Assay on Behavioral Responses of Laboratory and Field Populations *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae) to DEET. *J Med Entomol,* 51**,** 1227-36.

TAKKEN, W. 1991. The role of olfaction in host-seeking of mosquitoes: a review. *International Journal of Tropical Insect Science,* 12**,** 287-295.

TAKKEN, W., CHARLWOOD, J. D., BILLINGSLEY, P. F. & GORT, G. 1998. Dispersal and survival of *Anopheles funestus* and *A. gambiae s.l.* (Diptera: Culicidae) during the rainy season in southeast Tanzania. *Bulletin of Entomological Research.*

TAKKEN, W., KNOLS, B., OTTEN, H. J. A. O. T. M. & PARASITOLOGY 1997. Interactions between physical and olfactory cues in the host-seeking behaviour of mosquitoes: the role of relative humidity. 91, S119-S120.

TAKKEN, W. & KNOLS, B. G. 1999. Odor-mediated behavior of Afrotropical malaria mosquitoes. *Annu Rev Entomol,* 44, 131-157.

TAKKEN, W. & VERHULST, N. O. 2017. Chemical signaling in mosquito-host interactions: the role of human skin microbiota. *Curr Opin Insect Sci,* 20, 68-74.

TAMBWE, M. M., MOORE, S., HOFER, L., KIBONDO, U. A. & SADDLER, A. 2021a. Transfluthrin eave-positioned targeted insecticide (EPTI) reduces human landing rate (HLR) of pyrethroid resistant and susceptible malaria vectors in a semi-field simulated peridomestic space. *Malar J,* 20, 357.

TAMBWE, M. M., MOORE, S. J., CHILUMBA, H., SWAI, J. K., MOORE, J. D., STICA, C. & SADDLER, A. 2020. Semi-field evaluation of freestanding transfluthrin passive emanators and the BG sentinel trap as a "push-pull control strategy" against *Aedes aegypti* mosquitoes. *Parasit Vectors,* 13, 392.

TAMBWE, M. M., SADDLER, A., MOORE, J. D., KIBONDO, U. A., ODUFUWA, O. G., STICA, C. & MOORE, S. J. 2021b. The use of Ifakara large ambient chamber test (I-LACT) as a modified peri-domestic space bioassay for measuring feeding success and 24-hour mortality of volatile insecticides in the semi field system. *Biorxiv*.

TAMBWE, M. M., SWAI, J. K. & MOORE, S. J. 2021c. Semi-field Bioassays for the Evaluation of Spatial (and Topical) Arthropod Repellents for Indoor and Outdoor Use. *In:* COATS, J., CORONA, C. & DEBBOUN, M. (eds.) *Advances in Arthropod Repellents.* Elsevier.

TANGENA, J. A., THAMMAVONG, P., HISCOX, A., LINDSAY, S. W. & BREY, P. T. 2015. The Human-Baited Double Net Trap: An Alternative to Human Landing Catches for Collecting Outdoor Biting Mosquitoes in Lao PDR. *PLoS One,* 10, e0138735.

TAVARES, J., FORMAGLIO, P., THIBERGE, S., MORDELET, E., VAN ROOIJEN, N., MEDVINSKY, A., MÉNARD, R. & AMINO, R. 2013. Role of host cell traversal by the malaria sporozoite during liver infection. *The Journal of experimental medicine,* 210, 905-915.

TEMU, E. A., MAXWELL, C., MUNYEKENYE, G., HOWARD, A. F., MUNGA, S., AVICOR, S. W., POUPARDIN, R., JONES, J. J., ALLAN, R., KLEINSCHMIDT, I. & RANSON, H. 2012. Pyrethroid resistance in *Anopheles gambiae*, in Bomi County, Liberia, compromises malaria vector control. *PLoS One,* 7, e44986.

TEN BOSCH, Q. A., CASTRO-LLANOS, F., MANDA, H., MORRISON, A. C., GRIECO, J. P., ACHEE, N. L. & PERKINS, T. A. 2018. Model-based analysis of experimental data from interconnected, row-configured huts elucidates multifaceted effects of a volatile chemical on *Aedes aegypti* mosquitoes. *Parasit Vectors,* 11, 365.

THANISPONG, K., ACHEE, N. L., GRIECO, J. P., BANGS, M. J., SUWONKERD, W., PRABARIPAI, A., CHAUHAN, K. R. & CHAREONVIRIYAPHAP, T. 2010. A high throughput screening system for determining the three actions of insecticides against *Aedes aegypti* (Diptera: Culicidae) populations in Thailand. *Journal of medical entomology,* 47, 833-41.

THERNEAU, T. M. 2018. coxme: Mixed Effects Cox Models.

TISGRATOG, R., TANANCHAI, C., BANGS, M. J., TAINCHUM, K., JUNTARAJUMNONG, W., PRABARIPAI, A., CHAUHAN, K. R., POTHIKASIKORN, J. & CHAREONVIRIYAPHAP, T. 2011. Chemically induced behavioral responses in *Anopheles minimus* and *Anopheles harrisoni* in Thailand. *Journal of Vector Ecology,* 36**,** 321-331.

TOURÉ, Y. T., DOLO, G., PETRARCA, V., TRAORÉ, S. F., BOUARÉ, M., DAO, A., CARNAHAN, J. & TAYLOR, C. E. 1998. Mark-release-recapture experiments with *Anopheles gambiae* s.l. in Banambani village, Mali, to determine population size and structure. *Medical and Veterinary Entomology,* 12**,** 74-83.

TURNER, S. L., LI, N., GUDA, T., GITHURE, J., CARDE, R. T. & RAY, A. 2011. Ultra-prolonged activation of CO2-sensing neurons disorients mosquitoes. *Nature,* 474**,** 87-91.

UTZINGER, J., TOZAN, Y., DOUMANI, F. & SINGER, B. H. 2002. The economic payoffs of integrated malaria control in the Zambian copperbelt between 1930 and 1950. *Tropical Medicine & International Health,* 7**,** 657-677.

VALENTINE, M. J., MURDOCK, C. C. & KELLY, P. J. 2019. Sylvatic cycles of arboviruses in non-human primates. *Parasit Vectors,* 12**,** 463.

VAN DER HOEK, W., KONRADSEN, F., AMERASINGHE, P. H., PERERA, D., PIYARATNE, M. K. & AMERASINGHE, F. P. 2003. Towards a risk map of malaria for Sri Lanka: the importance of house location relative to vector breeding sites. *Int J Epidemiol,* 32**,** 280-5.

VAN ROEY, K., SOKNY, M., DENIS, L., VAN DEN BROECK, N., HENG, S., SIV, S., SLUYDTS, V., SOCHANTHA, T., COOSEMANS, M. & DURNEZ, L. 2014. Field Evaluation of Picaridin Repellents Reveals Differences in Repellent Sensitivity between Southeast Asian Vectors of Malaria and Arboviruses. *PLos NTD,* 8**,** e3326.

VATANDOOST, H. & HANAFI-BOJD, A. A. 2008. Laboratory evaluation of 3 repellents against *Anopheles stephensi* in the Islamic Republic of Iran. *East Mediterr Health J,* 14**,** 260-7.

VAVASSORI, L., SADDLER, A. & MULLER, P. 2019. Active dispersal of Aedes albopictus: a mark-release-recapture study using self-marking units. *Parasit Vectors,* 12**,** 583.

VAZQUEZ-PROKOPEC, G. M., GALVIN, W. A., KELLY, R. & KITRON, U. 2009. A New, Cost-Effective, Battery-Powered Aspirator for Adult Mosquito Collections. *Journal of Medical Entomology,* 46**,** 1256-1259.

VENUGOPAL, K., HENTZSCHEL, F., VALKIUNAS, G. & MARTI, M. 2020. Plasmodium asexual growth and sexual development in the haematopoietic niche of the host. *Nat Rev Microbiol,* 18**,** 177-189.

VERHULST, N. O., ANDRIESSEN, R., GROENHAGEN, U., BUKOVINSZKINE KISS, G., SCHULZ, S., TAKKEN, W., VAN LOON, J. J., SCHRAA, G. & SMALLEGANGE, R. C. 2010. Differential attraction of malaria mosquitoes to volatile blends produced by human skin bacteria. *PLoS One,* 5**,** e15829.

VERHULST, N. O., LOONEN, J. A. & TAKKEN, W. 2013. Advances in methods for colour marking of mosquitoes. *Parasites & Vectors,* 6**,** 200.

VERHULST, N. O., UMANETS, A., WELDEGERGIS, B. T., MAAS, J. P. A., VISSER, T. M., DICKE, M., SMIDT, H. & TAKKEN, W. 2018. Do apes smell like humans? The role of skin bacteria and volatiles of primates in mosquito host selection. *J Exp Biol,* 221.

VERHULST, N. O., WELDEGERGIS, B. T., MENGER, D. & TAKKEN, W. 2016. Attractiveness of volatiles from different body parts to the malaria mosquito *Anopheles coluzzii* is affected by deodorant compounds. *Sci Rep,* 6**,** 27141.

VONTAS, J., MOORE, S., KLEINSCHMIDT, I., RANSON, H., LINDSAY, S., LENGELER, C., HAMON, N., MCLEAN, T. & HEMINGWAY, J. 2014. Framework for rapid assessment and adoption of new vector control tools. *Trends Parasitol,* 30**,** 191-204.

WAGMAN, J. M., ACHEE, N. L. & GRIECO, J. P. 2015a. Insensitivity to the spatial repellent action of transfluthrin in *Aedes aegypti:* a heritable trait associated with decreased insecticide susceptibility. *PLoS Negl Trop Dis,* 9**,** e0003726.

WAGMAN, J. M., GRIECO, J. P., BAUTISTA, K., POLANCO, J., BRICENO, I., KING, R. & ACHEE, N. L. 2015b. The field evaluation of a push-pull system to control malaria vectors in northern Belize, Central America. *Malar J,* 14**,** 184.

WALLACE, D. I., SOUTHWORTH, B. S., SHI, X., CHIPMAN, J. W. & GITHEKO, A. K. 2014. A comparison of five malaria transmission models: benchmark tests and implications for disease control. *Mal J,* 13**,** 268.

WAMAE, P. M., GITHEKO, A. K., OTIENO, G. O., KABIRU, E. W. & DUOMBIA, S. O. 2015. Early biting of the *Anopheles gambiae* s.s. and its challenges to vector control using insecticide treated nets in western Kenya highlands. *Acta Trop,* 150**,** 136-42.

WATSON M. 1921. The prevention of malaria in the federated Malay states; a record of twenty years progress.

WEAVER, S. C. & VASILAKIS, N. 2009. Molecular evolution of dengue viruses: contributions of phylogenetics to understanding the history and epidemiology of the preeminent arboviral disease. *Infect Genet Evol,* 9**,** 523-40.

WEBSTER, B., LACEY, E. S. & CARDE, R. T. 2015. Waiting with Bated Breath: Opportunistic Orientation to Human Odor in the Malaria Mosquito, *Anopheles gambiae*, is Modulated by Minute Changes in Carbon Dioxide Concentration. *J Chem Ecol,* 41**,** 59-66.

WHO WHO specification and evaluations for public health pesticides, tranfluthrin.

WHO. 2006. WHO specifications for pesticides used in public health - transfluthrin http://www.who.int/whopes/quality/Transfluthrin_eval_only_Nov2006.pdf.

WHO 2009a. Dengue guidelines for diagnosis, treatment, prevention and control : new edition. https://apps.who.int/iris/handle/10665/44188.

WHO 2009b. Guidelines for efficacy testing of mosquito repellents for human skin.

WHO 2012. Global strategy for dengue prevention and control 2012-2020 WHO/HTM/NTD/VEM/2012.5. Geneva: World Health Organisation.

WHO 2013a. Guidelines for efficacy testing of spatial repellents Geneva: World Health Organisation.

WHO 2013b. Malaria entomology and vector control guide for participants

WHO 2013c. World Health Organization, WHO Pesticide Evaluation Scheme - 2013 - Guidelines for Efficacy Testing of Spatial Repellents-annotated.

WHO 2016a. Global vector control response 2017–2030. Geneva: World Health Organisation.

WHO 2016b. Test proceduresfor insecticide resistance monitoring in malaria

vector mosquitoes.

WHO 2016c. Test procedures for insecticide resistance monitoring in malaria

vector mosquitoes.

WHO 2017. Design of epidemiological trials for vector control products, Report of a WHO Expert Advisory Group, Chateau de Penthes, Geneva, 24–25 April 2017. WHO/HTM/NTD/VEM/2017.04. Geneva: World Health Organization.

WHO 2018. Test procedure for insecticides resistance monitoring in malaria vector mosquitoes https://apps.who.int/iris/bitstream/handle/10665/250677/9789241511575-eng.pdf?sequence=1.

WHO 2019. Guideline for malaria vector control. Geneva: World Health Organization.

WHO 2020a. The World Malaria Report 2020. Geneva: World Health Organization.

WHO. 2021. Vector Borne Disease Fact Sheet. [Accessed 11/03/2021].

WHO, W. H. O. 1982. Manual on environmental mangement for mosquitoes control.

WHOPES 2009a. Guidelines for efficacy testing of mosquito repellents for human skin. World Health Organization.

WHOPES. 2009b. *Guidelines for efficacy testing of mosquito repellents for human skin WHO/HTM/NTD/WHOPES/2009.4 http://whqlibdoc.who.int/hq/2009/WHO_HTM_NTD_WHOPES_2009.4_eng.pdf* [Online]. Geneva: World Health Organisation. [Accessed].

WHOPES 2013a. Guidelines for efficacy testing of spatial repellents.

WHOPES 2013b. Guidelines for laboratory and field testing of long-lasting insecticidal nets WHO/HTM/NTD/WHOPES/2013.3. Geneva: World Health Institution.

WIKAN, N. & SMITH, D. R. 2016. Zika virus: history of a newly emerging arbovirus. *Lancet Infect Dis,* 16**,** e119-e126.

WILDER-SMITH, A., GUBLER, D. J., WEAVER, S. C., MONATH, T. P., HEYMANN, D. L. & SCOTT, T. W. 2017. Epidemic arboviral diseases: priorities for research and public health. *Lancet Infect Dis,* 17**,** e101-e106.

WILLIAMS, C. R., LONG, S. A., RUSSELL, R. C. & RITCHIE, S. A. 2006. Field efficacy of the BG-Sentinel compared with CDC Backpack Aspirators and CO2-baited EVS traps for collection of adult *Aedes aegypti* in Cairns, Queensland, Australia. *J Am Mosq Control Assoc,* 22**,** 296-300.

WILSON, A. L., COURTENAY, O., KELLY-HOPE, L. A., SCOTT, T. W., TAKKEN, W., TORR, S. J. & LINDSAY, S. W. 2020. The importance of vector control for the control and elimination of vector-borne diseases. *PLoS Negl Trop Dis,* 14**,** e0007831.

WRIGHT, R. H. & KELLOGG, F. E. 1962. Response of *Aedes aegypti* to moist convection currents. *Nature,* 194**,** 402-3.

WYNNE, N. E., LORENZO, M. G. & VINAUGER, C. 2020. Mechanism and plasticity of vectors' host-seeking behavior. *Curr Opin Insect Sci,* 40**,** 1-5.

XUE, R. D. & BARNARD, D. R. 1996. Human host avidity in *Aedes albopictus*: influence of mosquito body size, age, parity, and time of day. *J Am Mosq Control Assoc*.

XUE, R. D., BARNARD, D. R. & SCHRECK, C. E. 1995. Influence of body size and age of *Aedes albopictus* on human host attack rates and the repellency of deet. *J Am Mosq Control Assoc,* 11**,** 50-3.

YOHANNES, M. & BOELEE, E. 2012. Early biting rhythm in the Afro-tropical vector of malaria, *Anopheles arabiensis*, and challenges for its control in Ethiopia. *Med Vet Entomol,* 26**,** 103-5.

ZAIM, M., AITIO, A. & NAKASHIMA, N. 2000. Safety of pyrethroid-treated mosquito nets. *Med Vet Entomol,* 14**,** 1-5.

ANNEX 1

The development and evaluation of a self-marking unit to estimate malaria vector survival and dispersal distance

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Abstract

Background

A clear understanding of mosquito biology is fundamental to the control efforts of mosquito-borne diseases such as malaria. Mosquito mark-release-recapture (MMRR) experiments are a popular method of measuring the survival and dispersal of disease vectors; however, examples with African malaria vectors are limited. Ethical and technical difficulties involved in carrying out MMRR studies may have held back research in this area and therefore, we have developed and evaluated a device that marks mosquitoes as they emerge from breeding sites to overcome the problems of MMRR.

Methods

A modified self-marking unit that marks mosquitoes with fluorescent pigment as they emerge from their breeding site was developed based on a previous design for Culex mosquitoes. The self-marking unit was first evaluated under semi-field conditions with laboratory-reared *Anopheles arabiensis* to determine the marking success and impact on mosquito survival. Subsequently, a field evaluation of MMRR was conducted in Yombo village, Tanzania, to examine the feasibility of the system.

Results

During the semi-field evaluation the self-marking units successfully marked 86% of emerging mosquitoes and there was no effect of fluorescent marker on mosquito survival. The unit successfully marked wild male and female *Anopheles gambiae* s.l. in sufficiently large numbers to justify its use in MMRR studies. The estimated daily survival probability of *Anopheles gambiae* s.l. was 0.86 (95% CI: 0.68-0.98) and mean dispersal distance was 579m (95% CI: 521- 636m).

Conclusions

This study demonstrates the successful use of a self-marking device in an MMRR study with African malaria vectors. This method may be useful in investigating population structure and dispersal of mosquitoes for deployment and evaluation of future vector control tools, such as gene drive, and to better parameterise mathematical models.

Keywords: Mark-release-recapture, Anopheles, Mosquito, Vector, MMRR, release-recapture, dispersal, survival

Background

A clear understanding of mosquito biology is fundamental to the effective control of mosquito-borne diseases including malaria, dengue, Zika and lymphatic filariasis. While novel vector control tools such as spatial repellents (Maia et al., 2018, Achee et al., 2012a), attractive toxic sugar baits (ATSBs) (Marshall et al., 2013, Qualls et al., 2015) and gene-drive systems (James et al., 2018), continue to be developed, our understanding of several key aspects of mosquito biology remains limited It is difficult to predict how these novel tools will function in real world settings. Planning and evaluation of these new tools, in particular gene drives, require a detailed understanding of mosquito dispersal and survival of both male and female mosquitoes (Epopa et al., 2017).

Mosquito mark-release-recapture (MMRR) studies have been one of the most widely used ways to obtain field estimates of daily mosquito survival, population size, duration of the gonotrophic cycle and dispersal distances(Service, 1993, Guerra et al., 2014), since tracking individual mosquitoes over distances larger than a few meters remains infeasible. A review in 2014 of MMRR studies with female mosquitoes identified 774 separate MMRR experiments covering 58 mosquito species that are of importance for human disease transmission (Guerra et al., 2014). However, there is a paucity of studies on African malaria vectors with only 11 studies on *Anopheles gambiae* s. l. identified. Daily survival is the most studied parameter in MMRR studies on malaria vectors (Gillies, 1961, Takken et al., 1998, Touré et al., 1998, Midega et al., 2007), however, population estimates (Lines et al., 1986, Touré et al., 1998, Baber et al., 2010, Epopa et al., 2017) and behavioural studies (Mnzava et al., 1995, Quiñones et al., 1997, McCall et al., 2001) are also conducted. Valuable studies that described detailed analysis of dispersal distances include the seminal study by Gillies (1961) with An. gambiae in Tanzania (Gillies, 1961) and a study in Sudan with An. gambiae s.l. by Costantini et al (1996) (Costantini et al., 1996). Considering the impact of malaria across Africa and the variety of ecological settings across the continent there is a surprisingly limited number of field-estimates of key entomological parameters, which are essential for optimising the implementation of focused vector control (Kang et al., 2018) and designing effective randomised control trials (WHO, 2017).

The ethical and technical difficulties involved in carrying out MMRR studies are described in in-depth reviews of MMRR methodology by Silver and Service (Service, 1993, Silver and Service, 2008) and guidance on the safety of MMRR studies (Benedict et al., 2018). The methods used in each step of an MMRR study from, marking, capturing, releasing and sourcing mosquitoes, has the potential to disrupt the normal behaviour and survival of the wild mosquitoes and thus provide misleading results. Visual markers such as fluorescent powders and paints have been the main method to mark anopheline mosquitoes (Silver and Service, 2008, Guerra et al., 2014) but reports on their impact on mosquito survival are inconclusive (Curtis and Rawlings, 1980, Silver and Service, 2008, Verhulst et al., 2013, Dickens and Brant, 2014). This variation in results may be caused by species or environmental differences but is most likely due to the different application methods, the amount of mosquito handling needed and the different brands of fluorescent pigment (Dickens and Brant, 2014). It is therefore important to measure the impact of the marking method on survival when using a new marker and species combination.

At the forefront of ethical concerns is the risk of releasing highly competent, potentially disease transmitting mosquitoes and so careful consideration is needed for a study to be carried out safely (Benedict et al., 2018). It could be argued that as MMRR studies capture far more mosquitoes than they release they mitigate any risk of increasing the local vector population in the short-term (Benedict et al., 2018). However, the MMRR studies that release laboratory strains of mosquitoes have potential longer-term risks. Laboratory strains are often deliberately or inadvertently bred for increased longevity, strong human feeding preference or high susceptibility to parasite infection. These characteristics could dramatically increase vector competence if expressed in wild vector populations through inter-breeding with released individuals. In addition to the ethical concerns, using laboratory reared mosquitoes with different survival rates and dispersal behaviours to their wild counterparts will misrepresent the very parameters that are being estimated (Rawlings et al., 1981).

Sourcing mosquitoes from wild populations is preferable, and is in fact the most common method used in MMRR studies with Anopheles (Guerra et al., 2014), but it has its own limitations. Collection of large numbers of mosquitoes is required for MMRR studies, but capturing adult mosquitoes and holding them before marking may cause stress and thus impact on survival and dispersal. Furthermore, information on mosquito age or infection status is unknown at the time of marking and may influence results.

In order to overcome these disadvantages we optimised a self-marking unit first developed by Niebylski and Meek (1989) to mark Culex quinquefasciatus (Niebylski and Meek, 1989) that marks wild mosquitoes as they emerge from the breeding sites.

Methods

Study design

The self-marking unit was first evaluated under semi-field conditions with laboratory-reared *Anopheles arabiensis* to determine the marking success and impact of marking on mosquito survival. Subsequently, a field evaluation of MMRR was conducted in Yombo village, Tanzania, to examine the feasibility of the system.

Study site

The marking unit was first evaluated in the Ifakara Tunnel at the Bagamoyo branch of the Ifakara Health Institute, Tanzania (Lorenz et al., 2014). The tunnel provides ambient environmental conditions where experiments can be conducted safely with disease-free laboratory-reared mosquitoes. Field evaluations were conducted at Yombo village, Tanzania (6°35'01.0"S, 38°50'48.4"E, Figure 1) located approximately 17 km south of Bagamoyo town and 5 km east of the Ruvu river in the Pwani region of Tanzania. Bagamoyo district experiences an annual rainfall of 800mm-1000mm and an average temperature of 28°C. Two rainy seasons replenish permanent breeding sites such as streams and ponds and create temporary breeding sites such as puddles. Malaria is endemic in Bagamoyo and the main vectors are *Anopheles arabiensis*, *An. funestus*, and *An. gambiae* s.s.

Experimental design

Experiments to optimise the self-marking unit were conducted under semi-field conditions to determine (i) the efficiency of the self-marking unit measured as the percentage of marked mosquitoes; (ii) if marking had an impact on survival; and (iii) if pigment transfer to unmarked mosquitoes occurred during mosquito collection. Experiments were conducted using *Anopheles arabiensis* (Ifakara strain) reared under standard laboratory conditions previously described here (Andrés et al., 2015).
This was followed by preliminary field experiments in breeding sites located close to the IHI, Bagamoyo Branch to measure the number of mosquitoes marked by the self-marking units. Marking units covering natural breeding sites were compared to units which had additional pupae placed underneath them from the surrounding breeding site. Finally, to demonstrate the feasibility of the self-marking unit and possible applications in studies with wild malaria vectors, we conducted a small MMRR in Yombo Village.

Self-marking unit design

The core component of the self-marking unit is the marking grid containing cloth impregnated with fluorescent pigment (Figure 2). As adult mosquitoes emerge from pupae and take their first flight, they are forced to pass through the layers of impregnated cloth and are marked with the pigment. To allow changing of the colour on different days, the unit is designed so that the marking grid can easily be removed and replaced with a new grid containing a different colour of fluorescent pigment. Five colours from the A series range of fluorescent pigments were selected for the study: Laser Red 3, Flame Orange 4, Solar Yellow 7, Stellar Green 8 and Comet Blue 80 (SWADA, Cheshire, United Kingdom). For the remainder of the manuscript the colours will be referred to as pink, orange, yellow, green and blue respectively. White cloth was purchased from a local fabric store and cut into 50 x 50 cm squares. The cloth was placed in a large plastic bag with half a cup (approx. 120ml) of fluorescent pigment and shaken until an even coating of colour was achieved.

Marking grid dimensions

The marking grid was made from 2 x 2 cm wide, square metal tubing and measured 54.5 x 45 cm with interspersed metal rods at 5 cm intervals spanning the 45cm width. Impregnated cloth was attached to the grid by looping a 50 x 50 cm piece of cloth around one rod and by stapling the ends of the cloth together at the bottom – this was done for all eight rods of the marking grid. The frame to hold the marking grid was also made from 2 x 2 cm square metal tubing and measured 59 x 49.5cm with 62 cm long legs. An inner lip of metal sheeting measuring 2 cm (Figure 2) allowed the marking exit grid to sit snugly within the frame without the use of tools for attachment. A local tradesman conducted all metal work and soldering.

Black cloth was attached with Velcro to all sides of the frame to enclose the unit and ensuring the only exit was up through the impregnated cloth. Preliminary experiments indicated that dark cloth that fitted tightly around the frame increased exiting rates of laboratory reared An. arabiensis from the units compared to netting or a loose-fitting funnel shape. The dimensions of the marking units allowed the attachment of exit traps previously designed for trapping mosquitoes exiting windows in hut studies(Okumu et al., 2012) (Figure 2,3). The exit traps were used in both semi-field and field experiments to capture mosquitoes exiting the marking units and thus enabling mosquito collection and the examination of pigment transfer between mosquitoes.

Marking success and survival of laboratory reared *Anopheles arabiensis*

Six self-marking units with exit traps attached were placed in a large experimental chamber (5 m x 3 m x 2.1 m) under semi-field conditions. Each unit contained a different colour of fluorescent marker (orange, blue, yellow, green, pink) or a pigment-free cloth as a control (Figure 3). At 17:00hrs East African Time (EAT), 60 laboratory reared *An. arabiensis* pupae in a bowl with water were placed under the self-marking unit and left overnight to emerge. At 10:00hrs EAT the following day, mosquitoes that had emerged and had been captured in the exit traps were transferred to holding cups and provided with cotton wool soaked in 10% sugar solution. Any pupae that had not emerged and remained in the bowl were left for a further 24hrs for a second collection from the exit traps. The marking process was repeated four times over eight days. Holding cups were given an individual identification number that was used to identify the exit trap, colour pigment and date of emergence. Each holding cup contained a maximum of twenty mosquitoes was immediately transferred to a screened laboratory that was subject to fluctuations in local temperature and humidity. Survival of the mosquitoes was recorded daily until all mosquitoes were dead. Each day all dead mosquitoes were removed and checked for florescent powder using a UV-torch and microscope.

Pigment Transfer during recapture of laboratory *Anopheles arabiensis* using CDC light traps or aspiration

The self-marking device relies on mosquitoes picking up fluorescent pigment when they come into contact with impregnated cloth. It is therefore reasonable to assume that unmarked mosquitoes could also pick up the pigment when they come into contact with mosquitoes that already have been marked. Traps and collection tools often force mosquitoes into confined spaces where they may come into close contact with each other. We examined three common methods of mosquito sampling to determine if pigment transfer can occur: the Centers for Disease Control and Prevention light trap (CDC-LT), the battery powered Prokopack aspirator (Vazquez-Prokopec et al., 2009) and standard mouth aspiration.

Five CDC-LTs were hung individually in five large cages (120 × 120 × 120 cm). At 18:00hrs EAT, 20 mosquitoes were introduced to each cage: 10 mosquitoes that were marked using the self-marking unit and 10 unmarked mosquitoes. The traps were left to run overnight and the number of mosquitoes with colour pigment was assessed the following day (if there were more than 10 marked mosquitoes then it was deemed pigment transfer had taken place). Due to the size of the cages, the CDC-LTs did not use an odour-lure as the phototactic response was sufficient to attract mosquitoes to the trap. However, any mosquitoes not in the trap and still in the cage were taken into account when recording results. Five replicates of each of the five colours were conducted over five nights.

A similar method was used to examine pigment transfer while using a Prokopack or manual aspiration, however, only five marked mosquitoes and five unmarked were released into the large cages. Rather than waiting overnight the collections were conducted ten minutes after the mosquitoes were released. Two manual aspiration methods were examined; (i) aspiration of mosquitoes individually but transferred to the same cup (ii) group aspiration (3-5 mosquitoes at a time) before transferring to the same cup. Again five replicates were conducted for each colour pigment. All replicates were completed in a single day after which they were transferred to the laboratory for counting (approximately 1-2hrs after collection).

Field testing

Preliminary trials in natural breeding sites and the development of pupae collection method

The original self-marking device for Culex quinquefasciatus mosquitoes was designed to be used over natural breeding sites (Niebylski and Meek, 1989). As the breeding sites of mosquito species can vary significantly, we developed several different prototypes to cover the breeding sites of *Anopheles arabiensis*, An. funestus, and An. gambiae s.s.. The basic marking unit covers a breeding site of 55cm x 45cm and therefore it is able fit over small temporary breeding sites, such as filled hoof prints or puddles, typical of An. gambiae. The Velcro side panels were removed and prototypes with tarpaulin skirt extensions were made to cover ditches and larger breeding sites. A floating unit was designed in order to mark An. arabiensis, which is often found in rice paddies, and An. funestus, often located in more permanent water bodies like swamps and ponds. We also tested a method to increase the mosquito numbers passing through the marking unit by collecting pupae and stage four larvae from the surrounding breeding site and placing them under the unit.

Initial trials of these designs and methods were conducted to determine the number of mosquitoes passing through and if this could be increased through manipulation of pupae. Five of the basic marking units were deployed for ten days over a breeding site close to IHI Bagamoyo Branch and trapped emerging mosquitoes in attached exit traps (Figure 4). For five of these days, the units were deployed on natural breeding sites and for the other five days, the devices were deployed in the same area but contained small bowls underneath where pupae and stage four larvae were placed after collection by the field team.

MMRR field trial

Estimation of released mosquitoes

A productive breeding site for Anopheles mosquitoes was identified in Yombo and one marking unit was placed adjacent to the breeding site for five days. On each day, a marking grid with a new colour was introduced. Trained technicians collected pupae from an area within 20m of the marking unit for approximately one hour each day. As species identification of pupae can be difficult, breeding sites were sampled where predominantly Anopheles larvae were found. The collected pupae were counted and placed in a bowl under the marking system at 18:00hrs EAT. The following day, the pupae remaining in the bowl were counted and subtracted from the previous day’s total to calculate the number of mosquitoes that had emerged through the marking grid.

Recapture methods

Adult mosquito collections were conducted for 12 days following the first marking day among thirty houses upon written informed consent of the household head. GPS coordinates of each household were taken to calculate the distance and direction of the household in relation to the marking unit using QGIS software version 3.6.0 (2019). No other household information was recorded. To maximise recapture probability, we focused recapture in an area within 1km of the breeding site (Figure 5). Outdoor resting mosquitoes were sampled from all 30 households with resting buckets (RBu) (Kreppel et al., 2015) and indoor host seeking mosquitoes were sampled among 20 of the 30 households using CDC-LTs.

Ten RBu were deployed at each sampling site and were placed facing the household roughly five meters away in all directions. Mosquitoes were collected at 06:00 each morning using a Prokopack aspirator (Vazquez-Prokopec et al., 2009). The sum of mosquitoes caught in the ten buckets was considered the RBu catch for one day for that household. CDC-LTs were deployed from between approximately 18:00 and 06:00 every night by hanging them approximately 1.5 m above ground and close to the foot of a bed in which an individual slept under an ITN. CDC-LT catch bags were collected in the morning shortly after the resting buckets were sampled. Mosquito identification and inspection of each mosquito for fluorescent pigment was performed on a daily basis using UV-torch and microscope.

Data management and Statistical Analysis

All data were collected first by hard copy and then transferred into Excel using double entry. Analyses were carried out with R statistical software v3.5.2 .

Survival analysis of laboratory *An. arabiensis*

Mosquito survival was measured in days and analysed with a mixed-effects Cox model using the "coxme" package in R (Therneau, 2018). Colour was included in the model as a fixed factor with 6 levels (5 colours and control). Night of emergence and mosquito sex were also included as fixed factors. Round and cup ID number were included as random factors. From the mosquitoes that passed through the coloured marking grids, only mosquitoes identified as with a colour pigment were included in the survival analysis.

Marking success of laboratory An. arabiensis

Marking success was determined by the percentage of mosquitoes marked after passing through the marking unit and was analysed using binomial Generalised Linear Mixed Effects Models (GLMM). The colour of the pigment, mosquito sex and emergence day were treated as fixed factors and round of experiment was included as a random factor. An individual random effect was included in the model to account for overdispersion after it was identified in the initial models. The analysis was carried out using the "lme4" package (Bates et al., 2015). Post hoc pairwise comparisons using Tukey contrasts were preformed between each colour pigment using the "multcomp" package (Hothorn et al., 2008).

MMRR Field Trial

Summary statistics were used to describe the number of mosquitoes marked by the units and the total number of marked and unmarked mosquitoes captured during the trapping. Data from the mark-recaptured mosquitoes was used to calculate daily survival rates and mean distance travelled (MDT) by *Anopheles gambiae s.l.*

The MDT was calculated using a correction factor that takes account of uneven sampling effort over distance (Lillie et al., 1981, Morris et al., 1991). Briefly, we divided the sampling area into four concentric annuli separated by 200m. For each annulus the number of traps and area were used to calculate a correction factor. The correction factor was then applied to the observed recapture numbers in order to calculate the estimated recapture per annulus and overall MDT. The MDT was first calculated using recaptured mosquitoes from all 12 days of recapture; we then calculated a "first flight" MDT using only mosquitoes recaptured in the 3 days following marking. Survival rates were estimated using the Buonaccorsi nonlinear model, which adjusts for mosquito removal as a result of recapture (Buonaccorsi et al., 2003). Confidence intervals of the survival estimate were calculated by bootstrap (1000 repeats) using the "nlstools" package in R (Baty et al., 2015). Average life expectancy (ALE) was derived from the survival estimate (Niebylski and Craig, 1994). In order to make comparisons to previous studies we also calculate daily survival using an exponential model (Gillies, 1961).

Results

Survival of laboratory An. arabiensis

The daily survival of marked mosquitoes in the laboratory was not significantly different from unmarked mosquitoes independent of the pigment colour; Blue (HR = 1.12, 95% CI: 0.81-1.43, p=0.48), Green (HR = 1.34, 95% CI: 0.98-1.68, p=0.10), Orange (HR=1.02, 95% CI: 0.71-1.35, p=0.91), Pink 0.97, 95% CI: 0.64-1.30, p=0.87) and Yellow (HR=1.19, 95% CI:0.86-1.52, p=0.30) (Figure 6). With a hazard ratio of 1.67 (95% CI: 1.51-1.83, $p<0.001$), male mosquitoes were found to be 67% more likely to die than females each day. Mosquitoes that emerged on the second night of marking also had increased daily mortality risk (HR = 1.52, 95% CI: 1.32-1.72, $p < 0.001$).

Marking success of laboratory An. arabiensis

On average, 85.9% of An. arabiensis emerging in the laboratory experiments were marked. Yellow pigment was successfully transferred to the 98.3% mosquitoes passing through the marking grid compared to 88.6% with green pigment, 84.8% with orange pigment, 82.8% with blue pigment and 75% with pink pigment. Tukey contrast indicated the only significant differences in the marking success were between yellow and pink ($p<0.001$), yellow and orange ($p=0.011$), and yellow and blue ($p=0.004$). Mosquitoes emerging on the second day had a higher overall marking success with 87.9% marked compared with 82.4% on the first night ($p=0.011$). The mosquitoes emerging on the second day may have spent longer under the marking unit, which could explain the higher marking rate. There was no difference in the marking rate of male (85.1%) and female mosquitoes (86.5 %) ($p=0.52$).

The number of mosquitoes marked during the MMRR field trial was calculated using a correction factor based on the average marking success in this semi-field experiment. While an individual correction factor could be applied for each colour, we decided to use the average of all colours (85.9%) as only the yellow pigment showed differences in marking success in select comparisons.

Pigment transfer experiments

During the controlled pigment transfer experiment, transfer of pigment was not observed when collecting mosquitoes with CDC light traps (0/25 trials), Prokopacks

(0/25 trials) or when aspirating mosquitoes individually (0/25 trials). However, pigment transfer was observed in 2/25 trials (8%) when aspirating mosquitoes in groups. Of the two trials where pigment transfer was observed only one additional mosquito contained colour pigments.

Natural breeding sites vs pupae collection

The basic units over natural breeding sites marked an average of 0.6 *Anopheles gambiae* s.l per trap per day, with 15/15 mosquitoes being marked. The marking units that contained collected pupae marked an average of 4.4 *Anopheles gambiae* s.l per day, with 110/110 being marked. The latter method was therefore taken forward to the **MMRR trial.**

MMRR Field Trial

Trapping

A total of 5,116 mosquitoes were caught and identified during the 12 days of trapping. Table 1 summarises the breakdown by trap type, mosquito family and sex. Of the 770 Anopheles mosquitoes captured, 8 were morphologically identified as *Anopheles funestus* s.l. and the remaining were *Anopheles gambiae* s.l. Figure 5 shows Anopheline numbers by trap.

Marking and Recapture

502 mosquitoes emerged from the marking unit over five marking days (Table 2). A correction factor of 0.86, based on the average marking success in the semi-field experiment, was used to predict the number of marked mosquitoes. Of the 432 predicted to be marked, 41 were recaptured giving an overall recapture rate of 9.5%. If a 50:50 sex ratio is assumed, then the recapture rate for females was 16.7% but only

1.9% for males. There were variations between the colour cohorts ranging from a 4.1% recapture success with the orange cohort to 30.9% recapture success with the green cohort.

Data from each colour cohort was combined to estimate the daily survival probability of the local female An.gambiae s.l. population. Insufficient data were available to measure male survival. The nonlinear Buonaccorsi model was fitted to the number of marked mosquitoes recaptured against the days after marking (Figure 7). The daily survival probability of female An. gambiae s.l. was 0.86 (95% CI: 0.68-0.98). This equates to a life expectancy of 6.6 days. The probability of daily survival calculated with the log-linear model produced a very similar estimate of 0.85.

The distribution of recaptured mosquitoes by distance is shown in figure 8. The MDT, which accounts for sampling effort by distance was 579m (95% CI: 521- 636m) for female An. gambiae s.l of any age. Using only female An. gambiae s.l. recaptured up to 3 days after marking, the “first flight” MDT travelled was 597m (95% CI: 509-685 m). Insufficient data were available to measure male MDT, however, the maximum male flight distance observed was 645m.

Discussion

This study demonstrates the successful use of a self-marking device in an MMRR study with African malaria vectors. We believe the self-marking method described here has the potential to be a useful tool for measuring the dispersal and survival of wild African malaria vectors. Studies of this kind will provide much needed parameter estimates for malaria transmission models (Endo and Eltahir, 2018) and allow the assessment of novel control tools. While many other methods for MMRR studies are available, this method minimises the effect of human interference on the survival and dispersal of wild Anopheles mosquitoes.

Pigment load and survival

In the current study, we did not observe a negative effect of the pigment on the survival of laboratory-reared anopheles mosquitoes which is in agreement with Niebylski and Meek's observation when using a self-marking device for Culex mosquitoes (Niebylski and Meek, 1989). They attributed this to the fact the self-marking unit results in a relatively low pigment load on the mosquito (5-15 pigment particles) and mainly on the legs, abdomen and thorax area – again we observed similar pigment loads on our laboratory-reared and wild Anopheles mosquitoes. This is in contrast to traditional dusting methods that apply excessive pigment, often covering wings and the sensory organs on the head, potentially impacting survival (Service, 1993). Fluorescent pigments could also impact a mosquito's host-seeking response and while the current evidence suggest the behaviour of *An. gambiae* is not affected by marking powders (Verhulst et al., 2013), further studies in this area are needed. The drawback to the lighter pigment load is that it is less obvious to the naked eye and it is more difficult to distinguish between the different pigment colours. UV torches and microscopes are therefore essential in identifying marked mosquitoes, which increases time and workload for identification.

Benefits of the self-marking unit

A self-marking unit has clear benefits of reducing the man-power involved in marking and eliminates any human-handling that may be detrimental to mosquito survival or natural behaviour. Ethically, it is a preferable method because there are no additional mosquitoes being added to the population and no additional genetic material. By marking field caught mosquitoes as they emerge from pupae with the current device we are able to know the exact age of a marked mosquito when it is recovered providing information that is rarely known in MMRR studies with wild mosquitoes. Release methods may have important implications for MMRR studies but are often overlooked. It has been suggested that some mosquitoes memorize their home range and establish flight paths (Charlwood et al., 2000, McCall et al., 2001) and therefore using laboratory-reared mosquitoes or releasing adult-caught mosquitoes in areas away from their origin, may misrepresent true dispersal. Allowing mosquitoes to emerge close to their breeding site and disperse in their own time removes this stress factor and any arbitrary effects of release point and time on their behavior.

Study limitations

In our semi-field experiments around 14% of mosquitoes emerging from the device did not pick up the fluorescent pigment. We were able to correct for this during analysis, however, complete marking success would be preferred. Niebylski and Meek (Niebylski and Meek, 1989) observed 100% marking success with their device and this may be due to the cloth used: they impregnated cheesecloth with pigment whereas in the current study, a white cotton fabric was used as it was available locally. Modifications to the device could be made using materials to increase pigment transfer, for example, electrostatic gauze has been previously shown to mark mosquitoes with pigment after brief contact (Andriessen et al., 2015).

During the semi-field experiments, we observed pigment transfer between mosquitoes when manually aspirating them in groups. Due to a small diameter of the aspirator, mosquitoes occasionally get bunched together which may have caused the transfer of pigment. CDC-LT or Prokopack aspirators have much larger trapping containers and so mosquitoes are less likely to come into direct contact with each other and for pigment transfer to occur. Low levels of pigment transfer occur when dusted mosquitoes are held together (Service, 1993), but transfer has not been observed between mating pairs (Niebylski and Meek, 1989, Service, 1993). Here we show that recapture methods can also cause pigment transfer and so should be considered when selecting recapture methods in a MMRR trial. During the trapping phase of the MMRR study we observed mosquitoes carrying the same colour pigment in the same trap on the same night on four occasions. This indicates the mosquitoes emerging on the same night arrived at the same house independently, however, contamination cannot be ruled out. In the semi-field experiments a small number of control mosquitoes were identified to be carrying colour pigment, which could be due to contamination through forceps or microscope. It is therefore important to keep equipment scrupulously clean during mark-recapture experiments. It was also observed that swapping the colour grids each day was quite messy and a small amount of pigment from the previous day remained on the marking frame and surrounding area. This could be overcome by making more marking frames to keep the colours independent. On the rare occasion were two colour pigments on the same mosquito were observed, it was assumed that the mosquito emerged on the day of the most recent colour.

It was previously noted that a limitation of the self marking device over a natural breeding site was that the number of mosquitoes emerging and the time of emergence could not be determined (Ciota et al., 2012). However, it is possible to place pupae collected from several closely located breeding sites underneath the marking device and count pupal emergence as was done in this study and in a separate study with

Aedes albopictus, to gain accurate estimates of the numbers emerging from the device (Niebylski and Craig, 1994). Preliminary evaluations of the units indicated it was impractical to mark our vectors of interest emerging from their natural breeding site. Our basic units covered a breeding site of 0.25m^2 and marked 0.6 mosquitoes per day and so, assuming an equal emergence rate across the breeding site, an area roughly 20.8 m^2 would need to be covered by emergence markers (84 self-marking units) to mark a minimum of 50 mosquitoes a day. This was not feasible in the breeding site under investigation and is unlikely for other malaria vector breeding sites were emergence rates are lower. The weekly emergence rate of anopheline mosquitoes in The Gambia has been estimated as 0.56 mosquitoes per m^2 per week (Fillinger et al., 2009) and a study in the western Kenyan highlands estimated the emergence of An. gambiae as 1.82 per m^2 per week (Kweka et al., 2011). In our study, collecting pupae and stage four larvae from the surrounding area was very successful; however, the methodology is still dependent on there being relatively productive breeding sites. While this is certainly a limitation in areas with low mosquito numbers, overall the self-marking system provides a useful non-invasive alternative for measuring wild mosquito bionomics.

Mosquito survival estimates

Due to the small sampling area and clustering of houses in the MMRR study, the distance and survival estimates calculated have to be interpreted with caution. MDT estimates are highly correlated to sampling area in MMRR studies (Guerra et al., 2014) and survival estimates are influenced by mosquitoes leaving the study area. Despite this, our estimates for female An. gambiae s.l. survival are similar to that found in previous studies. Our daily survival estimates were similar whether estimating using the log-linear model of Gilles (Gillies, 1961), giving a daily survival of 0.85, or the nonlinear model of Morris (Morris et al., 1991), giving a daily survival estimate of 0.86. Gilles estimated the daily survival of An. gambiae to be 0.841 in an area of Tanzania slightly further inland. Other studies predicted daily survival to be 0.80-0.88 in Burkina Faso (Costantini et al., 1996), 0.80 in Mali (Touré et al., 1998) and estimates ranging from 0.78 in another Tanzanian study (Takken et al., 1998) up to 0.95 in coastal Kenya (Midega et al., 2007). The few estimates for An. funestus are more widely dispersed and range from a daily survival rate of 0.63 (Takken et al., 1998) to 0.837 (Charlwood et al., 2000) and up to 0.96 (Midega et al., 2007).

Mosquito dispersal studies

Of the studies that previously measure dispersal in An. gambiae s.l., Costantini et al sampled an area of similar size to the current study and estimated the daily dispersal of female An. gambiae s.l. to be 350-650m which is line with 579m overall MDT and 597m “first flight” MDT measured here. Gilles on the other hand sampled up to 3.62 km away from the release point and estimated the mean dispersal distance (unadjusted) of female mosquitoes to be 1.02 and 1.58km depending on their release point, in the centre or periphery of a village respectively. These distances were calculated over 23 days and therefore could include back and forth flight. To account for this, Gilles also looked at the dispersal after one day and found it to be 720m in the central area which is similar to the estimates of Costantini *et al*., (1996) and those observed in the current study. The current study was restricted in size; however, the self-marking unit has since been used in a large scale MMRR study that will further add to the mosquito biology knowledge base.

Conclusions

Despite the importance of mosquitoes for the transmission of malaria, there are relatively few empirical studies investigating key entomological parameters in wild mosquitoes. MMRR studies still have an important role in obtaining field estimates; and although there are a variety of methods to mark mosquitoes, the self-marking unit described here has several logistical, ethical and biological benefits. The unit successfully marked wild *Anopheles gambiae* s.l. males and females in sufficiently large numbers to justify its use in MMRR studies. The estimated daily survival probability of *Anopheles gambiae* s.l. was 0.86 and mean dispersal distance was 597m. We hope these benefits will encourage further MMRR studies, allowing more accurate modelling and localised predictions of malaria transmission. In addition, the technique is simple enough to be used in studies where population age and dispersal are important, including testing new vector control tools such as spatial repellents, gene drive mosquitoes and ATSB.

Declarations

Ethics approval and consent to participate

Full ethical approval was obtained from ethical review committees at Ifakara Health Institute (IHI/IRB/No: 016-2016) and the National Institute for Medical Research (NIMR/HQ/R.8c/Vol. IX/2392). Households used for mosquito collections were recruited upon written informed consent of the household head.

Consent for publication

All authors read and approved the final version of the manuscript.

Availability of data and materials

Data generated and analysed during this study are included in this published article and its supplementary information files (Additional file 1)

Competing interests

The authors declare no conflict of interest.

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Authors’ contributions

SJM, TAS, NC conceived the study; AS, JDM designed the modified marking unit; AS, MMT, KSK performed the data collection; AS wrote the manuscript; KSK, SJM critically revised the manuscript.

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Tables

	Anopheline				Culicine			
	CDC-LT		RBu		CDC-LT		RBu	
	Male	Female	Male	Female	Male	Female	Male	Female
Total	7	632	60	71	1027	2412	594	313
Total/Trap	0.32	31.60	2.00	2.37	51.35	120.60	19.80	10.43
Total/Trap/Night	0.02	1.66	0.11	0.12	2.70	6.35	1.04	0.55

Table 1 – Summary of the trapping results during the MMRR study.

	Pupae emerged and approximate number of mosquitoes marked					Number and proportion of marked recaptured mosquitoes					
Dates	Colour	Number emerged	Number marked	Male*	Female	Total	%	Female	%	Male	%
29/03/17	Pink	30	26	13	13	4	15.38	4	30.77	0	0
30/03/17	Blue	165	142	71	71	9	6.34	7	9.86	2	2.82
31/03/17	Orange	200	172	86	86	7	4.07	4	4.65	2	2.33
01/04/17	Green	64	55	27.5	27.5	17	30.91	17	61.82	0	0
02/04/17	Yellow	43	37	18.5	18.5	4	10.81	4	21.62	0	0
	Totals	502	432	216	216	41	9.49	36	16.67	4	1.85

Table 2: Summary of the marking and recapture data. The number of mosquitoes emerged through the unit was calculated from the number of pupae placed underneath the marking device and removing the number that remained the following day. To calculate the number marked a correction factor of 0.86 was applied to account for the marking success of the unit

as observed in previous semi-field studies. * We assume a 50:50 sex ratio of pupae to estimate the number of mosquitoes of each sex marked. An overall marking rate is given as well as data for each colour pigment for both sexes.

Figure 1: Map highlighting Yombo village (6°35'01.0"S, 38°50'48.4"E) the site for the MMRR study. Yombo is approximately 17 km south of Bagamoyo town and 5 km east of the Ruvu river in the Pwani region (in green) of Tanzania. The Bagamoyo branch of Ifakara Health Institute, where the semi-field work was conducted, is based to the west of Bagamoyo town centre. Base maps were provided by Open Street Map Contributors (Contributors", 2019) through the QGIS plugin . Map data copyrighted by OpenStreetMap contributors and available from https://www.openstreetmap.org.

Figure 2:The self-marking unit adapted from Niebylski (1989). Left panel: 3D model of the marking unit indicating the key components. A – An exit trap used previously for hut trials as a window trap [31]. A slit in the netting allows mosquitoes to pass through in one direction thus collecting the mosquitoes after they have passed through the marking unit. The exit traps were only used when marked mosquitoes need to be retained as in the semi-field experiments, B – Cloth impregnated with fluorescent pigment, C – Black cloth side panels attached to the frame with Velcro, D –Detachable marking grid from which the impregnated cloth hangs. It can be removed without tools and replaced with another grid containing a new colour E – Frame to hold the marking grid made from 2 x 2 cm square metal tubing. Right panel: Side view of the unit to show the frames' internal lip on which the marking grid sits. The path of an emerging mosquito is shown passing through the marking grid and picking up fluorescent pigments.

Figure 3: Evaluation of the self-marking units under semi-field conditions.

Left panel: An open side panel showing a bowl of An. arabiensis pupae underneath. The side panel is closed for the experiment and emerging adult mosquitoes fly and bump their way through the layers of cloth to exit the marking grid and into the exit trap. Adults are collected from the exit trap by aspiration through the cloth sleeve on top of the trap. Right panel: The units set-up as intended for field use with side panels closed and exit traps removed. Five units each containing a different colour marker used in the study.

Figure 4: Self-marking units in the field with exit traps to measure exiting rates of mosquitoes from natural breeding sites and sites were the numbers of pupae were manipulated. Top left: Self-marking unit with tarpaulin skirt extension to cover a ditch where Anopheles larvae were found. Right: Five basic self-marking units containing the five pigments used in the study (pink, orange, yellow, green and blue). Bottom Left: Pupae collection with dippers. Larval dippers were first used to identify productive breeding sites and then to collect pupae to be placed under the self-marking devices.

Figure 5: Distribution of marked and unmarked female Anopheline mosquitoes caught by CDC light traps (top) or resting bucket traps (bottom). Size and colour of circles indicate the total number of female anopheline mosquitoes (unmarked and marked) caught in each trap for the duration of the trapping (12 days). Marked and recaptured mosquitoes are indicated by lines dispersing from the self marking unit - also indicating the total number of marked mosquitoes caught at the final trap location.

Figure 6: Kaplan-meier survival curves of laboratory reared An. arabiensis mosquitoes marked with the self-marking unit. Five colours (blue, pink, yellow, orange and green) were examined for their impact on mosquito survival. The colours of the lines represent mosquitoes marked with that colour. The black survival curve is from unmarked controls. There was no significant impact of individual colours or marking as a whole.

Figure 7: Daily survival of female An. gambiae s.l. in Yombo, Tanzania. Left:Number of recaptured female An. gambiae s.l. by days after marking. The fitted nonlinear Buonaccorsi model (Buonaccorsi et al., 2003) gave the daily survival probability of female An.gambiae s.l. as 0.86. Right: Exponential model predicting a daily survival probability of 0.85.

Figure 8: Boxplots visualising the distribution of recaptured mosquitoes by distance. All mosquitoes captured (n=41) are represented in the top boxplot with only mosquitoes ages 3-days or less (n=15) represented in the bottom boxplot. Dots indicate individual mosquitoes.

ANNEX 2.

MAÏA® topical repellent ointment provides long-lasting protection against *Anopheles gambiae s.s., Anopheles arabiensis,* and *Aedes aegypti* under semi-field conditions in Tanzania

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Abstract

Background: DEET topical mosquito repellents are effective complementary personal protection tools against mosquito bites that cannot be prevented by core interventions. However, DEET repellents tend to have low consumer acceptability, hence more attractive formulations are needed to encourage regular compliance. We evaluated the protective efficacy and duration of protection of a new topical repellent ointment, MAÏA® compared to 20% DEET in ethanol using malaria and dengue mosquito vectors in Tanzania.

Methods: Fully balanced 3x3 Latin square design studies were conducted in large semi-field chambers using laboratory strains of *Anopheles gambiae* s.s*, Anopheles arabiensis* and *Aedes aegypti.* Human volunteers applied either MAÏA® ointment, 20% DEET or ethanol to their lower limbs. Approximately 100 mosquitoes per replicate per strain were released inside each chamber, with 25 mosquitoes released at regular intervals during the collection period to maintain adequate biting pressure throughout the test. Volunteers recaptured mosquitoes landing on their lower limbs for six hours over a period of six to twelve hours post-application of repellents. Data analysis was conducted using mixed-effects logistic regression.

Results: The protective efficacy of MAÏA® was not statistically different from 20% DEET in each of the mosquito strains: 95.9% vs 97.4% against *An. gambiae* (OR=1.53 [95% CI: 0.89–2.66] p=0.128); 97.2% vs 96.8% against *An. arabiensis* (OR =1.08 [95% CI: 0.57–2.04] P=0.809); 93.1% vs 94.6% against *Ae. aegypti* (OR=0.72 [95% CI: 0.32-1.59] p=0.418). Average complete protection time (CPT) of MAÏA® and that of DEET was similar for each of the mosquito strain: 571.6 minutes (95% CI: 558.3-584.8) vs 575.0 minutes (95% CI:

562.1-587.9) against *An. gambiae*; 585.6 minutes (95% CI: 571.4-599.8) vs 580.9 minutes (95% CI: 571.1-590.7) against *An. arabiensis*; 444.1 minutes (95% CI: 401.8-486.5) vs 436.9 minutes (95% CI: 405.2- 468.5) against *Ae. aegypti.*

Conclusions: MAÏA® repellent ointment provides complete protection for 9 hours against Afrotropical Anopheles and 7 hours against *Aedes aegypti* similar to 20% DEET (in ethanol). MAÏA® repellent ointment can be recommended as a tool for prevention of outdoor biting mosquitoes in tropical locations with a suggested reapplication time of 6 hours.

***Keywords**:* Malaria, Mosquito, topical repellent, ointment, protective efficacy, complete protection time, CPT.

Background

The use of insecticide-treated bed nets and Indoor Residual Sprays (IRS) has almost halved malaria burden throughout sub-Saharan Africa (Lengeler, 2004, Bhatt et al., 2015). However, a considerable proportion of malaria transmission occurs outside of sleeping hours when ITNs are not in use (Sherrard-Smith et al., 2019). Deployment of topical repellents for malaria prevention is not recommended by the World Health Organisation as an intervention with public health value; however, topical repellents may be used to provide personal protection against malaria (WHO, 2019). Repellents are one of the oldest methods used for vector control globally (Debboun, 2006, Katz et al., 2008) and are useful for people who spend time outdoors, sometimes late in the evening or overnight for occupational, household or social activities (Monroe et al., 2015, Monroe et al., 2019a, Moshi et al., 2018, Gryseels et al., 2015). However, inconsistent repellent compliance is a major barrier to repellents providing significant disease prevention because few people use repellents often enough to protect them from the vector-borne disease (Gryseels et al., 2015).

According to Carroll *et al*, “a good repellent ointment should be effective against target vector strain, easy to apply and has a nice odour and the residual feeling after application”

(Carroll, 2007). To obtain better compliance, consideration of human customs and behaviour that may encourage consistent use of repellents is essential. In the study conducted in Burkina Faso, about 91% of children under 5–years are bathed in the evening and 80% of them receive ointment on their skin after bathing and before bedtime (Traoré *et al; manuscript under preparation*). During this time, mosquitoes are actively seeking and interacting with humans outdoors (Finda et al., 2019, Maia et al., 2016, Musiime et al., 2019). Therefore, a well-formulated topical mosquito repellent for daily use after bathing may improve user compliance as well as protect against vector-borne diseases such as dengue and malaria when people are outdoors in the early evening.

The Maïa Africa SAS , a company based in Burkina Faso has developed a MAÏA® repellent ointment formulated with petroleum jelly, shea butter, cotton oil, beeswax, fragrance, and 15% DEET. The company has developed MAÏA® with local mothers to combine in a single product an attractive moisturizing ointment with a long-lasting mosquito protection period. Shea butter-based ointments are widely used for skin softening purposes (Hon et al., 2015, Lin et al., 2017) and MAÏA® repellent is designed to repel mosquitoes as well as softening the human skin. In this study, the protective efficacy and duration of repellency of MAÏA® repellent ointment were evaluated in comparison to the gold standard, unformulated 20% DEET against *Anopheles* and *Aedes* mosquitoes under semi-field system conditions in Tanzania. The semi-field evaluation of topical repellents generates data that are comparable to a full-field evaluation (Sangoro et al., 2014b).

Methods

Study area

This study was conducted under ambient conditions in a semi-field system (SFS) measuring 29 x 21 meters built from a fabricated greenhouse frame modified to make two compartments with a central corridor and an opaque polyethylene roof for rain protection (Figure 1) (Sangoro et al., 2014b) . The SFS is located at 6° 8' S, 30° 37' E at the Ifakara Health Institute in Bagamoyo district in Tanzania. Bagamoyo district experiences annual rainfall between 800 mm and 1000 mm, temperature between 22ºC and 33ºC, and mean relative humidity of 73%. This evaluation followed the WHO Guidelines for efficacy testing of topical repellents (WHOPES, 2009a).

Study design

This study was divided into two parts to accommodate the circadian rhythm of mosquitoes investigated. *Anopheles gambiae* s.s. (Kisumu) and *Anopheles arabiensis* (Kingani) were tested at night and *Aedes aegypti* in the early morning. A pilot study for the first six hours after repellent application was conducted and found that a longer testing period was required. The final study tested the repellent from six to twelve hours post-application.

A study for Anopheles

In the pilot study of Anopheles strains repellents were applied at 17:45 hours and mosquito collection started at 18:00 hours to 00:00 hours (Fig. 2a). In the final study, repellents were applied at 17:45 hours and mosquito collection started at 00:00 hours up to 06:00 hours (i.e. 6-12 hours after the application of repellents) (Figure 2B).

Both pilot and final study consisted of two fully balanced (3x3) Latin squares (LS) design conducted in two chambers of the SFS simultaneously over nine nights using six volunteers. In each LS, three volunteers rotated sequentially between the three mosquito collections positions daily in each chamber and swapped repellents after every 3-days interval. After nine days of the study period, each volunteer had tested each of the repellent at each of the three mosquito collection positions inside three times. The study flow plan for *Anopheles gambiae* s.s. (Kisumu) and *Anopheles arabiensis* (Kingani) is shown in (Figure 2).

A study for Aedes

For *Aedes aegypti* repellents were applied at 05:50 hours in the pilot study and at 23:45 hours in the final study. Mosquito collection was conducted from 06:00 hours to 10:00 hours in the pilot and final study. The pilot study consisted of two fully balanced (3x3) LS conducted in two chambers of the semi-field system simultaneously over nine nights using six volunteers. In the final study, one fully balanced (3x3) LS was conducted in one chamber over nine nights using three new volunteers. In both studies, volunteers rotated sequentially between three collection positions each day inside the chamber of the SFS and swapped repellents after every 3-days interval. After nine days of the study period, each volunteer had tested each of the repellents at each of the three mosquito collection positions inside the SFS chamber three times. The study flow plan for *Aedes aegypti* is shown in (Figure 3).

Mosquito strains

We used nulliparous female laboratory-reared mosquitoes, aged 6 days old, sugar starved for 8 hours and reactive to human odour on the day of the experiment. Mosquito strains used were the pyrethroid-resistant (20% mortality with pyrethroid) *Anopheles arabiensis* (Kingani), fully pyrethroid susceptible *Anopheles gambiae* (Kisumu) and fully pyrethroid susceptible *Aedes aegypti* (Bagamoyo). Mosquitoes were reared following MR4 guideline (MR4, 2014). Before the experiment, *Anopheles arabiensis* were lightly marked by placing them in a cup coated with a fluorescent dye to make them distinguishable from the morphologically identical *An. gambiae*. By very lightly marking the mosquitoes there is no significant effect on their fitness nor host preference (Saddler et al., 2019). Mosquitoes were then sugar-starved for 8-hours. About 30 minutes before the experiment, we selected 100 female mosquitoes that were responsive to human odour and transported them in boxes to the SFS chambers to acclimatize with the ambient environmental conditions.

Repellents tested

MAÏA® ointment 600ml and 20% DEET (N,N-Diéthyl-3-methylbenzamide) 97% (reference number 26028, lot number 2436308) were shipped to Ifakara Health Institute (IHI) Vector Control Product Testing Unit (VCPTU) in plastic jars by MAÏA Africa SAS . The repellents were received at IHI on 24th September 2019 and stored the same day at room temperature between 25ºC and 29ºC until they were used in the experiment. The 20% DEET in ethanol (V/V) was prepared in-house before the experiment.

Volunteers

Nine male volunteers aged between 24 to 30 years were recruited after signing informed consent forms written in Swahili. All volunteers were tested for malaria parasite infection using SD BIOLINE Malaria Ag P.f rapid malaria diagnostic kits before participating in the study and once per week during the study period as part of IHI health and safety procedures

Allocation of volunteers

At the beginning of the study (pilot study), six male volunteers were assigned into two groups of 3 volunteers. One group was assigned to chamber '1' and the other in chamber '2' of the SFS (Figure 1). Inside each chamber, three mosquito recapture positions and mosquito releasing point measured nine apart were marked (Figure 4). Each volunteer was assigned to one of the three mosquito recapture positions inside one chamber. After nine days of experiments, It was discovered that the complete protection time of both repellents is above six hours. Therefore the final study of was set up for twelve hours recapture period with an additional three volunteers recruited to run the *Aedes aegypti* experiment separately due to differences in time of activity.

Application of repellents

Volunteers washed their lower limbs using water without soap before starting the experiment. They wore shorts, closed shoes and a mesh bug jacket to standardize an area lower limbs accessed by mosquitoes. Volunteers were non-smoker, and were requested to not drink alcohol or use perfumed soaps or ointment during the study period. We calculated a lower limb-skin surface area for each volunteer using the following formula at the beginning of the study;

Area = ½ (K+A) x L

Where "L" represents the leg length between the knee and the ankle and "K" represents the circumference at the knee and "A" represents the circumference of the ankle area. The average lower limb skin-surface area of volunteers was 1259.2 $cm^{2.}$ All repellents were measured using Ohaus CS200 weighing scale (Ohaus Corporation, CITY, USA). The average amount of MAÏA® repellent ointment applied per limb was 2.52 grams corresponding to a target dose of 2 mg/cm^2. All volunteers applied repellents using latex-gloved hands to minimize absorption onto the hands. Empty plastic cups that contained MAÏA® ointment were weighed to determine the amount left in the cups after application. Repellents for the Anopheles study were applied at 17:45 hours and for the Aedes study at 05:50 hours during the pilot study, while during the final study, repellents were applied at … for the Anopheles study and at …… for the Aedes study. After repellents application, participants were asked to rest with their trousers rolled up to prevent abrasion of the repellents until mosquito collection at 06:00 hours.

Duration of the study

Six-hour pilot test: During the first 9 days, 50 *An. arabiensis* (Kingani) and 50 *An. gambiae* (Kisumu) were released in each of the SFS chambers and testing proceeded for 6 hours. During the 6 hours recapture period, no confirmed mosquitoes were recaptured by volunteers who applied DEET and MAÏA®. Therefore, we extended the recapture period to 12-hours in order to confidently determine the duration of complete protection of 20% DEET and MAÏA® repellent ointment.

Twelve-hour test: During the LS with 12-hours of recapture period, the same volunteers, chambers and rotation schedule was used for Anopheles experiment: 100 *An. arabiensis* (Kingani) and 100 *An. gambiae* (Kisumu) were released (25 per release) starting at 00:00 hours. Three additional volunteers were recruited and were assigned to the *Aedes aegypti* experiments in which 100 *Aedes aegypti* were released with 25 released every hour between 06:00 and 10:00hrs.

Mosquito recapture: Volunteers recorded the time of a first mosquito recapture of each experiment (Anopheles or Aedes) and placed in a separate cup labelled with the time of recapture, volunteer's code, position and repellent (treatment) code. Volunteers collected subsequent mosquitoes that landed from 6 hour up to 12-hours post-application of repellent, with cups labelled with repellent code, position and time. Cups were changed after every hour. At the end of recapture time, mosquitoes were killed by refrigeration at $^{-}4^{o}C$ for about 40 minutes and then sorted to species type. If mosquitoes recaptured by a negative control volunteer were less or equal to 50% of the total recapture, the data were discarded and the experiment was repeated. During the experiment temperature, relative humidity was recorded using data logger Tinytag® view 2 data logger (model TV- 4500; Gemini data logger, Chichester, UK) and wind speed was recorded using anemometer.

Data management and statistical analysis

Data were recorded in paper forms and then double entered and cleaned in the Microsoft Excel 2016. Data analyses were performed in Stata 15.1 (Stata Corp, USA). Descriptive analyses of mosquitoes recaptured by repellents was performed. The mean complete protection time (CPT) of each repellent using the Kaplan-Meier survival analysis curve for each mosquito strain was estimated. The protective efficacy (PE) was established for the data collected up to 12-hours and calculated using this formula; $P = ((C - T)/C)*100$;

Where P represents the percentage protection, C represents the number of mosquitoes recaptured on the negative control (ethanol) and T represents the number of mosquitoes recaptured on volunteer's lower limbs treated with either MAÏA® or 20% DEET. Statistical analysis was performed using a mixed-effects binary logistic regression to compare the protective efficacy of MAÏA® to 20% DEET (as the reference in the statistical model). Several models were tested using recaptured mosquitoes as the outcome variable and repellent type (treatments), mosquito strain, volunteer, position of the volunteer and time of recapture as fixed effects. The best-fit model was determined using the Aikaike's Information Criterion (AIC) and the model with the smallest AIC value was selected.

Results

General test conditions

Environmental conditions for Anopheles experiment was 26.5°C (95% CI: 26.4–26.6) temperature, 82.96% (95% CI: 82.4–83.5) relative humidity, and 0.36 m/s (95% CI: 0.3–0.4) wind speed, and for *Ae. aegypti* experiment, temperature was 24.6°C (95% CI: 24.5–24.7), relative humidity was 59.45% (95% CI: 55.7–63.2), and wind speed was 0.00 m/s (95% CI: 0.0–0.0). All tests was conducted with recapture rate in the negative control arm exceeding 50%.

Descriptive analysis

The percentage recapture for *An. gambiae* (Kisumu) was 69.9% (1258/1800) and that of *An. arabiensis* (Kingani) was 75.4% (1358/1800) during the 12-hour tests. The percentage recapture for *Ae. aegypti* was 88.9% (800/900) (Table 1). All volunteers preferred using MAÏA® repellent ointment than 20% DEET and absolute ethanol.

The geometric mean (GM) hourly landings of *An. gambiae* mosquitoes on volunteers was 1.57 (95% CI: 1.3–1.9) for those who applied MAÏA® in comparison to 1.05 (95% CI: 1.3–1.4) for those who applied 20% DEET and 9.46 (95% CI: 8.4 – 10.6) for those who applied ethanol. , For *An. arabiensis* mosquitoes, it was 1.52 (95% CI: 1.1–2.0) for MAÏA® in comparison to 1.40 (95% CI: 1.2–1.7) for 20% DEET and 10.2 (95% CI: 9.0–11.5) for absolute ethanol, and for *Ae. Aegypti*, it was 2.47 (95% CI: 1.7–3.5)for MAÏA® in comparison to 3.36 (95% CI: 2.4–4.8) for 20% DEET and 10.0 (95% CI: 8.3–12.1) for absolute ethanol (Table 1).

The protective efficacy of MAÏA® and 20% DEET

In the 6-12 hours post-application experiment, both MAÏA® and 20% DEET provided above 93% protective efficacy against all strains (Table 1). There was no significant difference in the protective efficacy (PE) between the unformulated DEET and MAÏA® over the 12 hour test period for any of the mosquito strains tested. For *An. gambiae* s.s. DEET PE was 97.4% (95% CI: 97.1-97.6) and MAÏA® PE was 95.9% (95% CI: 95.4 - 96.3), OR= 1.53 [95% CI: 0.89 – 2.66] p=0.128. For *An. arabiensis* DEET PE was 97.2% (95% CI: 96.9-97.4) and MAÏA® PE was 96.8% (95% CI: 96.3 - 97.3), OR =1.08 [95% CI: 0.57 – 2.04] p=0.809. For

Ae. aegypti 20% DEET PE was 93.1% (95% CI: 92.2- 94.1) and MAÏA® PE was 94.6% (95% CI: 93.8-95.4), OR=0.72 [95% CI: 0.32 - 1.59] p=0.418 (Table 1).

Complete protection time (minutes) of MAÏA® and 20% DEET

MAÏA® and unformulated 20% DEET had similar complete protection time (CPT) exceeding 9 hours (Table 1, Figure 5). The average CPT in minutes of MAÏA® was 571.6 (95% CI: 558.3-584.8) and 20% DEET was 575.0 (95% CI: 562.1-587.9) against *An. gambiae* s.s. (Kisumu). Average CPT (minutes) of MAÏA® was 585.6 (95% CI: 571.4-599.8) and 20% DEET was 580.9 (95% CI: 571.1-590.7) for *An. arabiensis* (Kingani). Average CPT (minutes) was 444.1 (95% CI: 401.8-486.5) and 20% DEET was 436.9 (95% CI: 405.2-468.5) for *Ae. aegypti*, (Figure 5). The CPT for absolute ethanol (control) was 0 minutes (not shown in the results).

Table 1: The percentage (%) recapture, geometric mean of hourly mosquito landings, % protection, CPT and odds ratio between 20% DEET, MAÏA$^{®}$ repellent ointment and Absolute ethanol.

Test systems (mosquito strain)	**Test items (repellents)**	**Percentage recapture**	**Geometric mean hourly landings**	**Percentage protection and 95% CI**	**Odds Ratio**	**Z value**	**P value**	**95% CI**	**Mean CPT in minutes (95% CI)**
Susceptible *An. gambiae* (Kisumu)	20% DEET	2.5% (31/1258)	1.21 (1.05–1.40)	97.4% (97.1-97.6)	1	-	-	-	575.0 (562.1-587.9)
	MAÏA$^{®}$ Ointment	3.9% (49/1258)	1.57 (1.28–1.94)	95.9% (95.4-96.3)	**1.53**	**1.52**	**0.128**	**0.89 - 2.66**	571.6 (558.3-584.8)
	Absolute Ethanol	93.6% (1178/1258)	9.46 (8.43–10.60)	-	62.63	20.68	<0.0001	42.31-92.70	-
Resistant *An. arabiensis* (Kingani)	20% DEET	2.7% (36/1357)	1.40 (1.15–1.71)	97.2% (96.9-97.4)	1	-	-	-	580.9 (571.1-590.7)
	MAÏA$^{®}$ Ointment	3% (40/1357)	1.52 (1.18–1.96)	96.8% (96.3-97.3)	**1.08**	**0.24**	**0.809**	**0.57-2.04**	585.6 (571.4-599.8)
	Absolute Ethanol	94.4% (1281/1357)	10.17 (9.01–11.48)	-	58.93	18.68	<0.0001	38.43-90.38	-
Aedes aegypti	20% DEET	4.8% (38/800)	3.36 (2.35–4.82)	93.1% (92.2-94.1)	1	-	-	-	436.9 (405.2-468.5)
	MAÏA$^{®}$ Ointment	6% (48/800)	2.47 (1.7–3.5)	94.6% (93.8-95.4)	**0.72**	**- 0.81**	**0.418**	**0.32-1.59**	444.1 (401.8-486.5)
	Absolute Ethanol	89.3% (714/800)	10.0 (8.30–12.12)	-	11.56	8.57	<0.0001	6.61-20.23	-

Discussion

Topical mosquito repellents provide personal protection against human-biting insects including African mosquitoes of medical importance including the malaria vectors such as *Anopheles gambiae* and *An. arabiensis* and the dengue vector *Aedes aegypti* strain. In this study, we evaluated the protective efficacy and duration of protection of a formulated DEET product: MAÏA® repellent ointment comparing to unformulated 20% DEET in ethanol under semi-field conditions. Sangoro *et al*, demonstrated that a semi-field evaluation of topical mosquito repellent gives similar results to field studies but is far safer as only disease-free laboratory-reared mosquitoes are used (Sangoro et al., 2014b). This is especially important in the case of *Aedes aegypti* evaluations in areas of active dengue transmission such as Tanzania (Mwanyika et al., 2019). Moreover, semi-field environments allow volunteers to be accessible to a known number of mosquitoes of known age, physiological status, and avidity and it minimizes heterogeneity in the data, allowing a more precise estimation of true repellent efficacy. The study is also conservative as it used young, never blood-fed mosquitoes raised under optimal conditions to maximize body size, which are less repelled by DEET than older or smaller mosquitoes (Xue et al., 1995).

The study results demonstrated that MAÏA® repellent ointment is comparable to unformulated 20% DEET in terms of mean repellency over twelve hours as well as the duration of complete protection time against both malaria and dengue vectors. The study demonstrates that MAÏA® repellent ointment and 20% DEET are comparable in terms of duration of CPT: above 9-hours against *An. gambiae* (Kisumu) and *An. arabiensis* (Kingani) and more than 7-hours for *Ae. aegypti* post-application of the repellents. These results are similar to another study which evaluated the effectiveness MAÏA® repellent ointment under field conditions in Burkina Faso in which authors concluded that MAÏA® and 20% DEET are comparable in terms of duration of complete protection time against Anopheles strain and *Aedes aegypti* (Guelbeogo *et al.*, *Manuscript under preparation*).

The study results indicated that MAÏA® repellent ointment is an effective mosquito repellent suitable for the use even under high mosquito biting pressure. According to Goodyer *et al,* an ideal mosquito repellent should provide CPT above 6-hours in the highest mosquito biting pressure (Goodyer et al., 2014). Our study fulfilled this characteristic by demonstrating the mean CPT above 9-hours against *An. gambiae* (Kisumu) and *An. arabiensis* (Kingani) and above 7-hours against *Ae. aegypti* for MAÏA® with an average of 10 mosquito landings per hour in the control.

User compliance is a major limitation of most topical insect repellents (Maia et al., 2018, Gryseels et al., 2015, Sluydts et al., 2016) and since the repellent is applied to the skin most users prefer insect repellents which are cosmetically pleasant in terms of odour and feel on the skin in addition to providing protection from biting insects (Debboun et al., 2014, Carroll, 2007, Frances et al., 2007). The study's volunteers preferred to use MAÏA® repellent ointment compared to 20% DEET and ethanol because MAÏA® repellent ointment felt better on their skin. This confirms than some users are influenced by product characteristics such as texture, skin feel, and odour as previously reported (Frances et al., 2007). Therefore, the use of MAÏA® repellent ointment may be a suitable alternative to less cosmetically appealing DEET-based formulations. However, more studies are required to specifically assess user acceptability comparing between MAÏA® repellent ointment and other formulated products available on the market.

After the initial nine days of the experiment, we extended the study for another nine days in order to confidently determine the CPT of MAÏA® repellent ointment and 20% DEET. Ideally, the experiment would use more volunteers to capture the repellent efficacy against a wide range of people (Rutledge and Gupta, 1999), however the study recruited few people. The study did not asses the efficacy of the repellent against nuisance mosquitoes such as *Culex quinquefasciatus*, which may also be important driver of consumer acceptance of repellents.

Conclusion

In conclusion, MAÏA® repellent ointment is comparable to unformulated 20% DEET under high biting pressure. Therefore, it may be recommended for use in disease-endemic areas. We recommend a reapplication interval of six hours based on the shortest complete protection time of 7 hours observed for *Aedes aegypti* in this study. It is a cosmetically appealing mosquito bite protection tool that also nourishes and moisturizes the skin, which may improve consumer acceptability and fit into daily life if used every evening after bathing.

Declarations

Ethical approval and volunteer protection

This study was approved by the Institutional Review Board of Ifakara Health Institute with approved certificate number: IHI/IRB/No: 38 – 2019. Also, the National Institute for Medical Research ethical clearance committee approved this study with certificate number NIMR/HQ/R.8a/Vol. IX/ 3445. All volunteers involved in this study were recruited upon a written informed consent form and they were informed that they are free to leave the study at any time without any repercussion.

Consent for publication

Permission to publish these findings was granted by the National Institute of Medical Research, Tanzania.

Competing interests

All authors work in the Vector Control Product Testing Unit (VCPTU), in Bagamoyo Tanzania and conduct product evaluations for a number of companies.

Funding source

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Author's contributions

EM, OGO, MMT, FT, KS, JM, SM designed the study. EM wrote the study protocol and supervised the experiment. EM, SM conducted data analysis and wrote the manuscript. FT, RP, OGO, MM, KS, JM, SM reviewed the manuscript. All authors gave permission to publish.

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Figures

Figure 1: A semi-field system (SFS)

A semi-field system (SFS) with 29 x 21 meters built from a fabricated greenhouse frame modified to make two chambers with a central corridor and an opaque polyethylene roof for rain protection.

Figure 2: Study flow for Anopheles

Study flow for *Anopheles gambiae* s.s. (Kisumu) and *Anopheles arabiensis* (Kingani) conducted for 6 hours and 12-hours of recapture period. Two semi-field system (SFS) chambers (1 & 2) were used and two fully balanced Latin square (LS) design (3x3) were conducted simultaneously using six human volunteers. Mosquito recapture started immediately after application of repellent in a pilot study (Figure 2A) and started 6-hours post-application of repellents in a final study (Figure 2B**).** Total mosquitoes of each strains released were 50 (25 per release) and 100 (25 per release) in a pilot and final study, respectively.

Figure 3: Study flow for *Aedes aegypti*

Study flow plan for *Aedes aegypti* experiment conducted for 6 hours and 12 hours of recapture period. Two semi-field system (SFS) chambers (1 & 2) were used simultaneously and two fully balanced Latin square (LS) design (3x3) were conducted using six human volunteers in a pilot study. Mosquito recapture started immediately after application of repellent in a pilot study (Figure 3A) and started 6 hours post-application of repellents in a final study (Figure 3B). Total female *Aedes aegypti* released were 50 (25 per release) and 100 (25 per release) in the pilot and final study, respectively.

Figure 4: **An experimental set up in a semi-field system**

A schematic diagram of a semi-field system showing two chambers (A & B), three mosquito collection positions and one mosquito releasing point inside each chamber. The distance between releasing point and mosquito collection positions was nine meters.

Figure 5: Kaplan meier graph Complete protection time (CPT) of MAÏA® repellent ointment and 20% DEET.

The CPT of MAÏA® repellent ointment and 20% DEET against laboratory-reared mosquito strains. **(A)** Probability of no *An. gambiae* (Kisumu) landing on lower limbs of volunteers treated with MAÏA® repellent ointment (green) and 20% DEET (red). **(B)** Probability of no *An. arabiensis* (Kingani) landing on lower limbs of volunteers treated with MAÏA® repellent ointment (green) and 20% DEET (red). **(C)**The probability of no *Ae. aegypti* landing on lower limbs of volunteers treated with MAÏA® repellent ointment (green) and 20% DEET (red).

Curriculum Vitae

I am a Research Scientist at the Ifakara Health Institute (IHI) in Tanzania with over a decade of experience working with Spatial repellent, transmission-blocking intervention, and establishing testing facilities. I lead the repellent testing subunit of the Vector Control Product Testing Unit (VCPTU), which helps to bring new vector control products to the market more rapidly and cost-efficiently through the conduct of rigorous evaluations.

In recent years, I led the establishment of a category II transmission facility capable of evaluating the endpoints of transmission-blocking interventions that block the malaria parasites from transmitting from humans to mosquitoes. As proof of principle, we conducted several mosquito-feeding assays using wild malaria parasites recruiting participants from the community that donated gametocytemic blood. I also contributed to the establishment of a world-class biosafety level three laboratory which is focusing on producing and testing mosquitoes that cannot transmit malaria parasites. I also have experience with designing and implementing studies that measure the efficacy of odor-baited traps and conduct robust data analysis. My work, therefore, spans designing studies, establishing testing facilities, and conducting entomological analysis. Some of my studies also involve working closely with community members, which can be applied to implementation science research as well.

EDUCATION

2021	*Ph.D.,* Epidemiology, University of Basel, Basel, Switzerland. Thesis: "*Understanding alternative control methods and their mode of action for the control of outdoor biting mosquitoes*" Committee; Prof Marcel Tanner, Prof Christian Lengeler (Chair), Prof Neil Lobo, Dr. Sarah Moore.
2011	*MSc.,* Parasitology and Entomology, Muhimbili University of Health and Allied science, Dar es salaam, Tanzania. Thesis: "*Prevalence and Risk Factors Associated with Schistomiasis Infection among Primary School Children in Kigogo Ward, Kinondoni District"* Committee: Prof C. Kihamia, Prof M. Matee, and Dr. D. Tarimo
2005	*B.Sc.,* Bachelor of Science in Nursing, Muhimbili University College of Health Sciences (MUCHS), Dar es salaam, Tanzania. Thesis: "*Patient's participation in Informed Consent Form and post operative nursing care in Muhimbili National Hospital, Dar es Salaam."*

RESEARCH EXPERIENCE

2015-Present	Senior research scientist (entomologist), Vector Control Product Testing Unit VCPTU, Environmental Health and Ecological Sciences (EHES), Bagamoyo, Tanzania

2010-2014 Research nurse, phase I clinical trial facility, Bagamoyo, Tanzania

TEACHING EXPERIENCE

Ifakara Health Institute ,
Masters Students Advised/Supervised

- **Masudi Suleiman,** Evaluation of MTego trap for sampling Anopheles and Aedes mosquitoes in the semi-field system in Bagamoyo-On going
- **Tunu Mwamlima,** Understanding the role of serological and clinical data on assessing the dynamic of malaria transmission; a case study of Bagamoyo district, Tanzania
- **Dismas Kamande**, Modified WHO tunnel test for high throughput evaluation of insecticide-treated nets considering the effect of hosts, exposure time and mosquito density
- **Shamim Msangi,** Evaluation of potential risk of mosquito borne diseases at Dar es salaam University College of Education (DUCE)

Ministry of Health and social welfare, School of pediatric nursing KCMC, Moshi, Tanzania, June 2005 – 2010
Nurse tutor

- Student guidance
- Teaching of subjects (leadership and management, pharmacology, parasitology and physiology)
- Prepare, administer, and invigilate examinations and grade the results.
- Participating in curriculum review and development

HONOUR AND AWARDS

Award

Awarded Rudolf Geigy Award 2023: Establishment of the platform to evaluate transmission-blocking interventions.

Grant

Year: September 2023 – December 2026
Title: Development of second-generation passive emanators to reduce mosquito biting behavior
Value: US$300,00
Involvement: Principal Investigator

MEMBERSHIP IN PROFESSIONAL SOCIETIES

1. University Nursing Student Association of Tanzania (UNSATA) 2004 to date
2. Tanzania registered nurses association(TARENA) 2007 to date

PROFESSIONAL SERVICE

Peer review for Malaria journal.
Peer review for Parasite and vector.

PROFESSIONAL DEVELOPMENT TRAINING

1. Training on parasite culture, mosquito feeding assay and dissection at Tres Cantos, Spain organized by medicine for malaria venture (MMV) September 7-28, 2014.

2. Training workshop on the evaluation of transmission-blocking intervention in mosquitoes in Africa organized by the Institut de recherche. Yaounde, Cameroun 13th-17th October 2014**.**

3. Teaching Methodology workshop at Mirembe Mental Health Nursing School, Dodoma organized by Ministry of Health and Social Welfare in Dodoma, July 2022.

4. Internship Training Programme at Amana Hospital – Ilala Municipality, Dar es Salaam. March2006-March2007

5. Workshop on Scientific Research Methodology, Research Ethics and Research Findings Communication Skills conducted National Institute of Medical Research in collaboration with MUCHS. April 2005

PRESENTATION

Oral presentation

Tambwe MM, Moore S, Hofer L, Kibondo U, Saddler A. Transfluthrin eave-positioned targeted insecticide (EPTI) reduces human landing rate (HLR) of pyrethroid-resistant and susceptible malaria vectors in a semi-field simulated peridomestic space. , Entomology Society of America (ESA) meeting, October 2021.

Tambwe, M.M., Moore, S.J., Chilumba, H. Swai, J. K. Moore, J. D. Stica, C. Saddler, A. Semi-field evaluation of freestanding transfluthrin passive emanators and the BG sentinel trap as a “push-pull control strategy” against *Aedes aegypti* mosquitoes. Oral presentation, PAMCA Meeting, September 2019.

Poster presentation

Tambwe, M.M., Saddler, A., Kibondo, U.A. *et al.* Semi-field evaluation of the exposure-free mosquito electrocuting trap and BG-Sentinel trap as an alternative to the human landing catch for measuring the efficacy of transfluthrin emanators against *Aedes aegypti*. Oral presentation, PAMCA Meeting, September 2021

Mgeni M Tambwe, Ummi A. Kibondo, Olukayode G. Odufuwa, Jason Moore, Ahmed Mpelepele, Rajabu Mashauri, Adam Saddler and Sarah J Moore. Human landing catches (HLC) provide a useful proxy of Protective Efficacy for evaluating volatile pyrethroid spatial repellents (VPSR), PAMCA Meeting, September 2021

PUBLICATIONS

1. Fillinger, U. and Denz, A. and Njoroge, M. M. and **Tambwe, M. M**. and Takken, W. and van Loon, J. J. A. and Moore, S. J.and Saddler, A. and Chitnis, N. and Hiscox, A.. (2023) *A randomized, double-blind placebo-control study assessing the protective efficacy of an odour-based 'push-pull' malaria vector control strategy in reducing human-vector contact.* Sci Rep,

2. Kweyamba, P.A., Hofer, L.M., Kibondo, U.A Ummi A. Kibondo, Stutz S, Austin, J.W, Moore SJ, Müller P., **Tambwe M.M**. Sub-lethal exposure to chlorfenapyr reduces the probability of developing *Plasmodium falciparum parasites* in surviving Anopheles mosquitoes. *Parasites Vectors* **16**, 342 (2023). https://doi.org/10.1186/s13071-023-05963-2

3. Hofer, L.M., Kweyamba, P.A., Sayi, R.M., Chabo M.S., Maitra S.L., Moore S.J., **Tambwe MM**. Malaria rapid diagnostic tests reliably detect asymptomatic *Plasmodium falciparum* infections in school-aged children that are infectious to mosquitoes. *Parasites Vectors* **16**, 217 (2023). https://doi.org/10.1186/s13071-023-05761-w
4. Maasayi, M.S., Machange, J.J., Kamande, D.S. **Tambwe MM** The MTego trap: a potential tool for monitoring malaria and arbovirus vectors. *Parasites Vectors* **16**, 212 (2023). https://doi.org/10.1186/s13071-023-05835-9
5. **Tambwe MM**, Kibondo UA, Odufuwa OG, Moore J, Mpelepele A, Mashauri R, Saddler A, Moore SJ. Human landing catches provide a useful measure of protective efficacy for the evaluation of volatile pyrethroid spatial repellents. Parasit Vectors. 2023 Mar 7;16(1):90. doi: 10.1186/s13071-023-05685-5. PMID: 36882842; PMCID: PMC9993701.
6. Tunu G. Mwamlima, Solomon M. Mwakasungula, Catherine G. Mkindi, **Mgeni Tambwe,** Sarah Mswata, Stephen G. Mbwambo, Michael F. Mboya, Simon J. Draper, Sarah E. Silk, Maxmillian Mpina, Sr. John-Mary Vianney and Ally Olotu. Understanding the role of serological and clinical data on assessing the dynamic of malaria transmission; a case study of Bagamoyo district, Tanzania. https://doi:10.11604/pamj.2022.43.60.35779
7. **Tambwe MM,** Moore S, Hofer L, Kibondo UA, Saddler A. Transfluthrin eave-positioned targeted insecticide (EPTI) reduces human landing rate (HLR) of pyrethroid resistant and susceptible malaria vectors in a semi-field simulated peridomestic space. Malaria Journal. 2021;20(1):357.
8. **Tambwe MM**, Swai JK, Moore SJ. Semi-field Bioassays for the Evaluation of Spatial (and Topical) Arthropod Repellents for Indoor and Outdoor Use. In: Coats J, Corona C, Debboun M, editors. Advances in Arthropod Repellents: Elsevier; 2021.
9. **Tambwe MM,** Saddler A, Kibondo UA, Mashauri R, Kreppel KS, Govella NJ, et al. Semi-field evaluation of the exposure-free mosquito electrocuting trap and BG-Sentinel trap as an alternative to the human landing catch for measuring the efficacy of transfluthrin emanators against *Aedes aegypti*. Parasit Vectors. 2021;14(1):265.
10. Denz A, Njoroge MM, **Tambwe MM**, Champagne C, Okumu F, van Loon JJA, et al. Predicting the impact of outdoor vector control interventions on malaria transmission intensity from semi-field studies. *Parasit Vectors.* 2021;14(1):64.
11. Mbuba E, Odufuwa OG, Tenywa FC, Philipo R, **Tambwe MM,** Swai ES, et al. MAÏA® Topical Repellent Ointment Provides Long-Lasting Protection Against *Anopheles*

gambiae s.s., *Anopheles arabiensis* and *Aedes aegypti* Under Semi-Field Conditions in Tanzania. *Mal J*. 2020;20(12):https://doi.org/10.1186/s12936-020-03461-9.

12. **Tambwe MM**, Moore SJ, Chilumba H, Swai JK, Moore JD, Stica C, et al. Semi-field evaluation of freestanding transfluthrin passive emanators and the BG sentinel trap as a "push-pull control strategy" against *Aedes aegypti* mosquitoes. *Parasit Vectors.* 2020;13(1):392.
13. Saddler A, Kreppel KS, Chitnis N, Smith TA, Denz A, Moore JD, **Tambwe MM**, Moore SJ. The development and evaluation of a self-marking unit to estimate malaria vector survival and dispersal distance. Malar J. 2019;18(1):441.
14. **Tambwe MM,** Mbeyela EM, Massinda BM, Moore SJ, Maia MF. Experimental hut evaluation of linalool spatial repellent agar gel against *Anopheles gambiae* sensu stricto mosquitoes in a semi-field system in Bagamoyo, Tanzania. *Parasit Vectors*. 2014;7:550.
15. Thiévent K, Hofer L, Rapp E, **Tambwe MM**, Moore S, Koella JC. Malaria infection in mosquitoes decreases the personal protection offered by permethrin-treated bednets. *Parasit Vectors.* 2018;11:284.

In preparation or accepted for publication

16. Lorenz Hofer, Prisca A. Kweyamba, Rajabu M. Sayi, Mohamed S. Chabo, Rehema Mwanga, Sonali L. Maitra, Mariam M. Somboka, Annina Schnoz, Pierre Schneeberger, Christian Nsanzabana, Sarah J. Moore, **Mgeni M. Tambwe**. The impact of a blood meal on the development and genetic diversity of *Plasmodium falciparum* field-isolates in the malaria vector *Anopheles gambiae* Under review

BOOK CHAPTER

1. **Mgeni Mohamed Tambwe**, Swai Kyeba and Sarah J Moore (2021) Semi-field system and experimental huts bioassays for the evaluation of spatial (and topical) repellents for indoor and outdoor use in Advances in Arthropod Repellents Editors Mustapha Debboun and Larry Goodyer, Elsevier