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Host location by Exophagic African Malaria vectors:

Evaluating the performance of a novel exposure-free human-baited mosquito trap and studies on the perception of indoors/outdoors by malaria vectors in southern Mozambique

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Dedication

To my beloved

and inspiring

family members

whom have suffered

from my absences

and

to my late brother Filipe

who is always in my memory

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Abstract

Malaria is, preferentially, transmitted by mosquitoes that bite indoors or outdoors. To date, the most successful vector control methods, namely: indoors residual spraying (IRS) and long lasting insecticide treated bed nets (LLINs) have targeted indoor species. Outdoor biters (exophagic) are difficult to target and no satisfactory control methods exist. Already common outside Africa, outdoor-transmitted malaria is growing in importance within Africa. To target exophagic mosquitoes we need to understand more how/why they choose to enter or to avoid houses, *i.e.* what do mosquitoes perceive as indoors or outdoors?

Field experiments were undertaken in Massavasse, a village located in the eastern region of Chóckwè district, Gaza province, southern Mozambique, to investigate that question and addressing two objectives:

1. To design, build and evaluate an electric net trap (ENT), named the "Shock-wè (SHK-wè) trap" for reliable sampling of mosquitoes responding to human bait, indoors and outdoors;

2. To determine the contribution of simple elements of a house (*i.e.* roof, walls, partial walls) to occurrence and entry rates of host seeking mosquitoes.

To accomplish the first objective, a series of paired field tests were performed to evaluate the performance of SHK-wè trap compared to Human landing catch (HLC) method. Results showed that SHK-wè trap is a safe and reliable sampling and surveillance tool for African malaria vector populations. The trap performed well indoors and outdoors and is potentially a robust and safe practical replacement for the conventional HLC method.

To accomplish the second objective, SHK-wè trap was used in a series of randomized treatment-assigning experiments to determine the response of local mosquitoes populations to presence of structural components of a purpose-built experimental house. The experiment consisted on randomly dismantling or reassembling an experimental house into its basic elements (*i.e.* a frame on a base, lower walls, upper walls and roof). Results from this experiment suggest that, contrarily to current belief,

the roof of the house was a key component that triggered endophagic malaria vectors, such as members of *A. gambiae* complex, to enter. Data indicated that the probability of *A. gambiae s.l.* entering an experimental house increased 4.5 [IRR = 1.50 (0.63 – 2.37); p = 0.001)] times when the roof was in place compared to other types of treatments.

Key-words: Mosquitos, malaria vectors, perception, Shock-wè trap

Resumo

A malária é transmitida por mosquitos que, preferencialmente, picam no interior (endofágicos) ou no exterior (exofágicos) das habitações. Atualmente, os métodos mais sucedidos no controlo vectorial são a pulverização residual intra-domiciliar (PIDOM) e as redes mosquiteiras tratadas com insecticidas de longa duração (REMILD), que têm como alvo principal os vectores endofágicos. No entanto, as espécies que picam no exterior (exófagicas) têm sido as mais difíceis de controlar e, atualmente, não existem métodos satisfatórios para o controlo de populações destes vectores.

A transmissão da malária feita por vectores exofágicos é comum fora de África; contudo, o fenómeno vem, progressivamente, ganhando importância também no continente Africano. Daí que, para que os vectores exofágicos sejam controlados de forma efetiva, é necessário entender melhor *como* ou *porquê* alguns vectores preferem procurar refeições sanguíneas em ambientes fechados, enquanto os outros evitam entrar em tais compartimentos? Ou seja, como é que um vector localiza e reconhece um ambiente fechado (ou intra-domiciliar) ou aberto (extra-domiciliar)?

Com vista a obter respostas à tais questões, varias experiências de campo foram realizadas na aldeia de Massavasse, localizada na região leste do distrito de Chóckwè, província de Gaza, sul de Moçambique. As experiências assentaram-se em dois objetivos principais:

1. Projetar, construir e avaliar a performance de uma armadilha de grelha elétrica, apelidada de armadilha Shock-wè (SHK-wè), que poderá ser utilizada como uma alternativa fiável às colheitas com isca humana, na amostragem de vectores da malária que procuram um hospedeiro humano em ambientes interiores ou exteriores.

2. Determinar a contribuição dos elementos estruturais básicos que compõe uma habitação (ou seja, tecto, paredes, pilares, etc.) para a ocorrência e entrada de mosquitos que procuram uma refeição sanguínea em casas experimentais.

Para cumprir o primeiro objectivo, foram feitas colheitas emparelhadas de mosquitos através de armadilhas SHK-wè e colheitas com isca humana (CIH) durante 35 noites

consecutivas. Os resultados indicam que a SHK-wè é uma ferramenta segura e fiável para a amostragem e vigilância das populações de vetores importantes da malária em África. SHK-wè mostrou um bom desempenho tanto no interior como no exterior e, portanto, é potencialmente um substituto prático, robusto e seguro para as capturas CIH convencionais.

Para cumprir o segundo objetivo, foram igualmente utilizadas armadilhas SHK-wè numa série de experiências de tratamentos randomizados para determinar a resposta das populações de mosquitos locais à presença de componentes estruturais de uma casa experimental construída para o efeito. A experiência foi realizada durante 50 noites consecutivas e constituiu em montagens e desmontagens aleatórias da casa experimental nos seus elementos básicos (isto é, o suporte de metal, paredes inferiores e superiores e o tecto). Os resultados desta experiência sugerem que, contrariamente ao que se conhecia, o tecto da casa é um componente chave para o reconhecimento e entrada dos vectores endofágicos do complexo *A. gambiae s.l* em ambientes intra-domiciliares. Os dados indicaram que a probabilidade de *A. gambiae s.l.* entrar numa casa experimental aumenta 4.5 vezes [IRR = 1.50 (0.63 – 2.37; p = 0.001) quando o tecto está presente em comparação com outros tipos de tratamentos.

Palavras-chave: Mosquitos, vectores de malária, percepção, armadilha SHK-wè

List of abbreviations

CDC – Centre for Disease Control and Prevention

DDT − Dichlorodiphenyltrichloroethane

EIR − Entomological Inoculation Rate

GAM – Generalized addictive models

GEE – Generalized estimating equations

GLM – Generalized linear models

HLC − Human Landing Catch

IHMT – Institute of Hygiene and Tropical Medicine

INS – Instituto Nacional de Saúde

IRS – Indoor residual spraying

ITN – Insecticide treatment nets

LLINs – Long lasting insecticide treated bed nets

LSM – Larval source or environmental management

LSTM – Liverpool School of Tropical Medicine

ma – Human biting rate

MISAU – Ministério da Saúde

PNCM − Programa Nacional de Controlo da Malária

SHK-wè − Shock-wè trap

WHO − World Health Organization

R^2_{adj} = R^2 adjusted

Contents

1. General introduction

1.1. Malaria: global epidemiology and trends

Malaria has long been one of the most important global infectious diseases, particularly in tropical and subtropical countries. The disease is actively transmitted in 95 countries and territories worldwide with 3.2 billion individuals, approximately the half population of the world's population, at risk of infection. The most vulnerable groups are young children, pregnant women, those living with HIV or affected by humanitarian emergencies and natural disasters, and non-immune travellers moving into endemic areas (1, 2).

Malaria is caused by five Protozoan parasites of the genus *Plasmodium*: *Plasmodium* (*Laverania*) *falciparum*, *Plasmodium (Plasmodium) vivax*, *P. (P.) malariae* and *P. (P.) ovale* (3, 4) occur in humans only and a fifth, *P*. (*P*.) *knowlesi*, is found in primates and humans (5-9). *Plasmodium vivax* is the most widespread species and is rarely fatal (10-12). *Plasmodium falciparum* is the most pathogenic species (13, 14) and is confined mainly to tropical regions (Figure 1), most notably in Africa where it can be responsible for 80% of all malaria cases and 90% of deaths (15-19). In 2015, 214 million cases, leading to 438,000 deaths, were recorded globally (2). This enormous loss of life and the effects on population growth, productivity, education and investment all contribute to malaria's socio-economic burden on affected countries (20-23). Since 2000, the average annual cost of malaria cases management in Africa countries has been estimated to be nearly US$300 million (2).

In recent years, malaria has declined in many endemic regions, particularly in sub-Saharan Africa (2), where a 50% reduction was attributed to the scaling up of vector control, mainly insecticide treated nets (ITNs) (24). However, sustaining these recent gains is threatened by increasing resistance in both malaria vectors and *P. falciparum* to most classes of insecticides and antimalarial drugs approved, respectively (25-27).

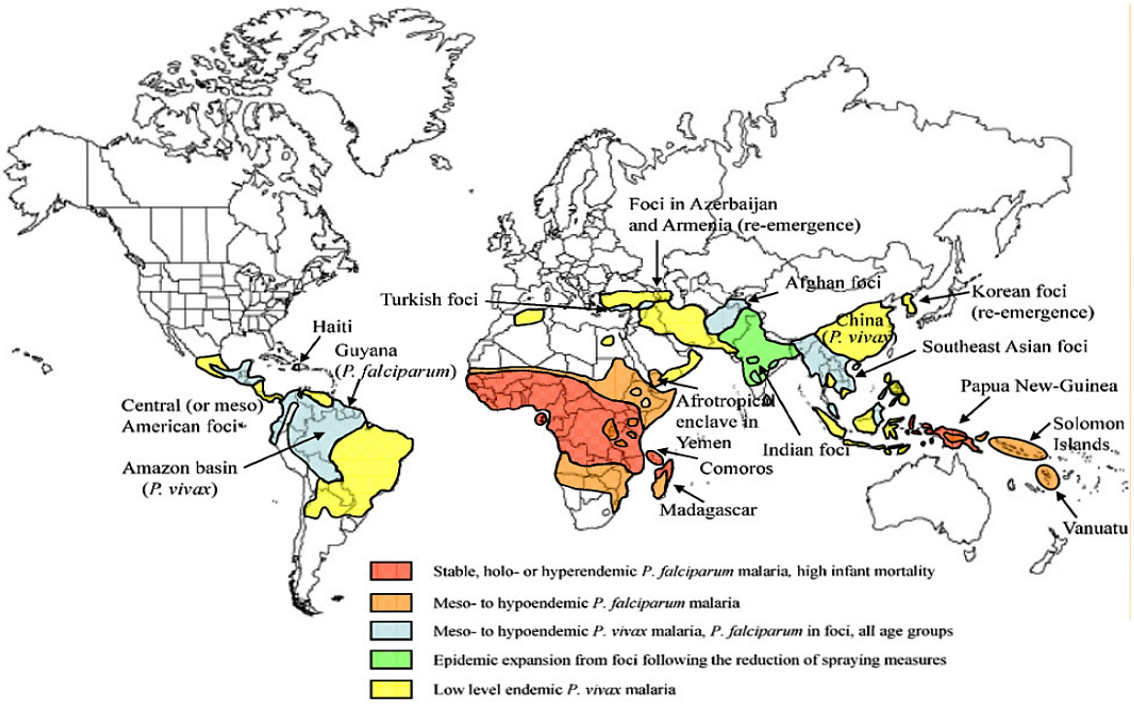


Figure 1. World distribution of *Plasmodium falciparum* and *P. vivax* malaria endemicity. Source: reproduced from Manguin *et al*., (8).

1.2. Malaria transmission natural history

1.2.1. *Plasmodium* life cycle in the mosquito

Human malaria parasites are transmitted by mosquitoes of the genus *Anopheles* (3, 28, 29). In Africa, *Anopheles gambiae sensu lato* and *A. funestus s.l* are the two most important malaria vector groups (28, 30).

In nature, the parasites life cycle inside the invertebrate host starts with the ingestion of blood by an unfed female *Anopheles* mosquito from a sick person (Figure 2). One female mosquito can ingests an average of 10^3 gametocytes in an infected blood meal that develop into 50 – 100 ookinetes but only around five ookinetes survive into oocysts (31). The mosquito requires a blood meal fundamentally for egg production and maturation (32). The ingested gametocytes undergo maturation inside the lumen of the midgut, few minutes after ingestion, generating male (micro) and female (macro) gametocytes. The male gamete exflagellates, giving rise to microgametes that seek out and fertilize the female gamete to form a zygote. The zygote undergoes meiosis and differentiates into an elongate and mobile form of the parasite, known as an ookinete.

The ookinete fixes itself in the epithelial lining of the midgut wall, and matures into oocyst (33, 34). After 4-15 days, depending on environmental temperature (32, 35), the oocyst matures and releases thousands of the sporozoites into mosquito hemocoel. The sporozoites then, migrate toward mosquito´s head and penetrate the salivary glands. At this stage, mosquito is infectious so it can now transmit the parasites to a new or same host in the next blood meal. Malaria vectors are gonotrophically concordant, which means they usually take blood meal after each oviposition cycle, and the interval between one blood meal to the next one vary between 2 and 3 days (32).

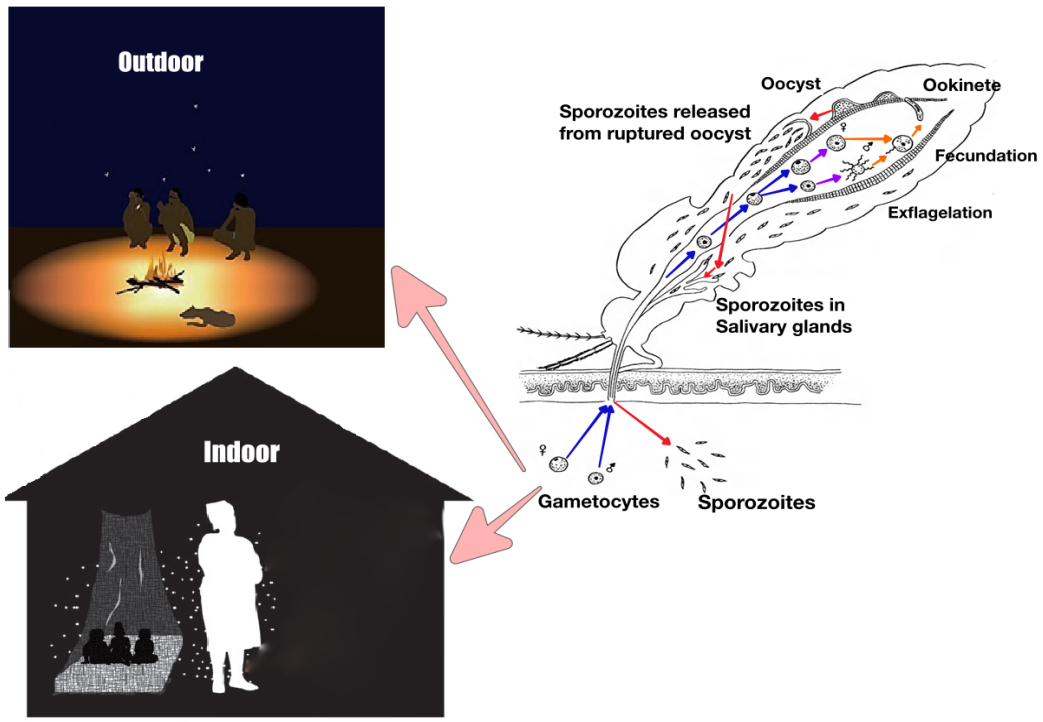


Figure 2. *Plasmodium spp* extrinsic life cycle inside mosquito. Mosquito get infect while blood feeding on an infected host either indoor or outdoor. Coloured arrows inside mosquito highlight the key phases of the parasite evolution, from ingestion of gametocytes (blue arrow) until vector becomes infective and being able to transmit the parasites to susceptible host in subsequent blood meals (red arrows). Image of *Plasmodium sp* life cycle inside mosquito adapted from Burkot *et al.,* (36).

1.2.2. Vector blood feeding cycle

The life cycle of haematophagous mosquitoes is strongly related to the feeding (or gonotrophic) cycle (Figure 3). During the life cycle, mosquito passes through three distinct aquatic phases (i.e., egg, four larval instars and pupa) and one terrestrial (adult) phase. Usually, after emergence, adult *Anopheles* females engages into reproductive

activity, at dusk, and is attracted by males into a swarming arena in which copulation occurs (37, 38). After copula, inseminated female engages into host-seeking activity in order to find suitable source of blood used for eggs maturation (39, 40). If successfully blood-fed, engorged female seeks suitable place, either indoor or outdoor, to rest until the blood is entirely digested and eggs fully matured. The rate of blood digestion is highly dependent on the environmental temperature at the resting place; in the Afro–tropical region, it usually takes, in average, 2 to 3 days (41). *Anopheles* females usually feed once per gonotrophic cycle (32, 42, 43). However, under poorly understood circumstances, newly emerged virgin females of both *Anopheles funestus* and *A. gambiae s.l* can take several blood meals before mating then becoming pre-gravid (44-46). Some authors argue that non-insemination is the reason females take at least two blood meals during their first gonotrophic cycle (47-49).

Following eggs maturation, gravid female seeks for a suitable place to lay eggs; a gravid anopheline female can lay between 300 and 500 eggs (50) which, depending on the physical, chemical and environmental condition of the breeding site (51), will once again pass through the aforementioned stages of the life cycle until emerge into adult nulliparous females again.

After oviposition, a female mosquito usually searches for a new blood meal either in the same night or it can postpone the blood finding activity to the following night, depending on the environmental factors such as moon phases as documented in *Anopheles farauti s.l* (52) in *Anopheles funestus s.s* (53) and, if it was infected with human malaria *Plasmodium spp* during the first blood meal, the vector can transmit the parasites to a susceptible host during the second or other subsequent blood feeding and the cycle repeats again during vector´s life time (Figure. 3).

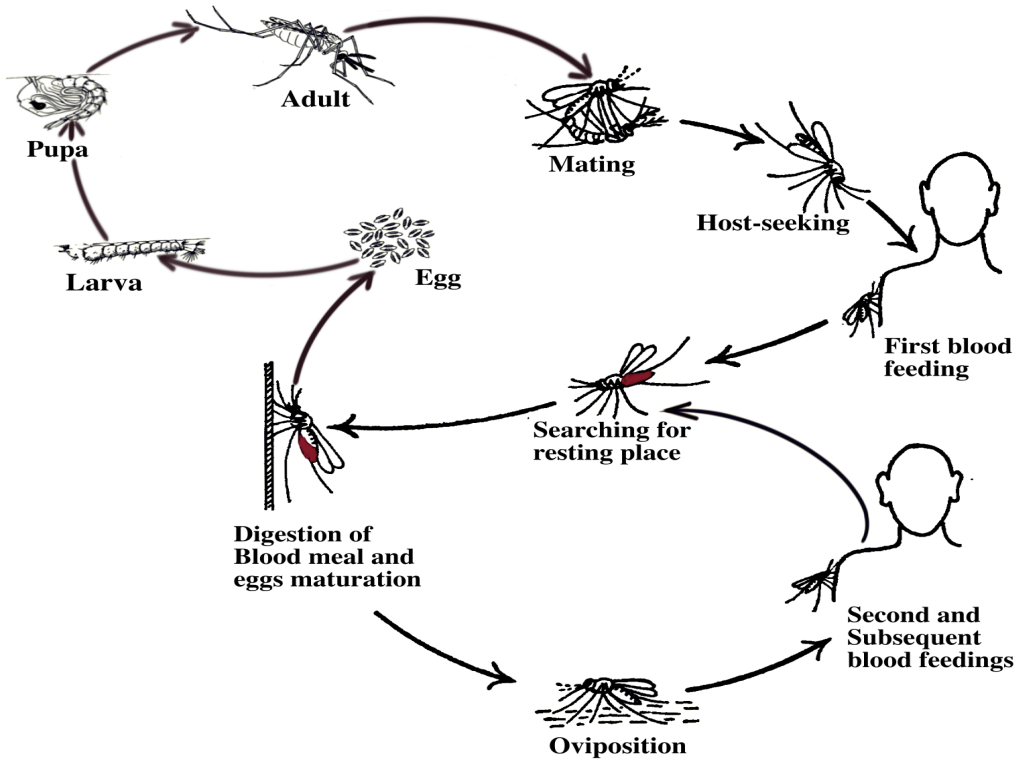


Figure 3. Relationship between mosquito life and feeding (gonotrophic) cycles. Source: recreated after Mattingly (4).

1.3. Determinants of malaria transmission

Malaria transmission is non-randomly distributed across time and space; and in any endemic area, there will be sites of higher ("hotspots") or lower ("coldspots") transmission intensities (54-59). The magnitude and timing of malaria transmission depends on the interplay of numerous factors including vector susceptibility and competence to transmit the parasite, character of local parasite, human host and parasite populations, and climatic and environmental conditions (60, 61). The main factors governing malaria transmission can be divided into intrinsic (or direct) and extrinsic (or indirect) factors and it is the interactions between them that shape malaria transmission in any given area (62).

1.3.1. Intrinsic factors

Intrinsic or direct factors influence directly the malaria transmission process and can result in an outbreak or an epidemic by affecting any one of the 3 living elements

needed for the transmission cycle, *i.e.* the mosquito, parasite or human (35, 61-65). They are divided into:

1) Entomological factors

a) Vector density in relation to humans

b) Daily vector survival rate

c) Length of extrinsic development of the parasite inside the infected mosquito (sporogonic cycle). The entire sporogonic cycle usually takes 8 to 15 days depending on *Plasmodium* species (63, 66)

d) Proportion of mosquitoes infective (*i.e.* with sporozoites in salivary gland)

2) Parasitological factors

a) Parasite load in the infected human host

b) Proportion of hosts that are infectious to the mosquito, *i.e.* carrying gametocytes in their blood stream (60, 62, 67).

3) Host factors

Human populations vary in their susceptibility to infection by malaria parasites and severity of illness. The immune status of the individual and population plays the most important role in the clinical response to infection and transmission intensity (68). Lower immune status of a community can favour the resurgence of malaria, whereas concomitant immunity produced by epidemics may partially suppress transmission (60).

1.3.2. Extrinsic factors

Extrinsic or indirect factors usually affect malaria transmission by influencing any of the direct factors above. The most important extrinsic factors are:

2) Climatic factors

a) *Rainfall* - Moderate to heavy rainfalls increase the size and number of suitable breeding sites, for temporary and sunlit water pool breeders such as *Anopheles*

gambiae s.l (69, 70) which, in turn, would directly affect transmission intensity by increasing the density of vector populations (71)

b) *Temperature and humidity* - Both survival and longevity of an adult mosquito depend on temperature and humidity. The longer a mosquito lives, the greater the likelihood of transmission of the parasites in the next blood meal (32, 60). Furthermore, temperature is the main factor determining the rate of *Plasmodium* sporogony (Nikolaev, 1935) cited in (32).

2) Environmental factors

Environmental changes, many caused by human activities, have been linked to unexpected increases in malaria vectors and expansion beyond the normal or historic ranges (62, 72). Many activities can cause significant ecological or landscape alterations creating suitable novel biotypes for some vectors (73, 74). Introduction of non-immune hosts and new parasites as consequence of mass population movement, civil wars and natural disasters can greatly affect the pattern of malaria transmission in those areas were populations have been moved (68, 75).

1.4. Malaria control: a global perspective

Malaria control programmes typically involve diagnosis and treatment of malaria cases, personal protection against mosquito bites and control of vector populations by mean of indoor residual spraying (IRS), long lasting insecticide treated nets (LLINs), or through the use of larvicides and/or breeding source management (2, 76-78). The main objectives of all control programmes is to reduce the disease burden and maintain it at a reasonably low level, with the longer term aim of eliminating the disease from a defined geographical area and, ultimately, eradicating it globally (79).

Antimalarial drugs continue being one of the most powerful tools in malaria control; they contribute significantly for reduction of morbidity and mortality by terminating malaria infection in a patient and curtail malaria transmission by diminishing the parasite reservoir (24, 79). However, chemo-prophylactic approaches are considered unsustainable, logistically and financially in very-low-income countries for a number of reasons (79). These include, increasing widespread of parasite resistances, particularly

Plasmodium falciparum, to almost all commonly available antimalarial drugs, including artemisinin and its derivatives (27, 80-82); the lack of effective vaccines capable of inducing long lasting immunity against infections; the relatively short shelf-lives of nearly all available antimalarial drugs; the elevated cost of management and treatment of malaria cases (83-85). Therefore, vector control approaches have been widely adopted, being more cost-effective and economically feasible when implemented at large scale compared to antimalarial medicines (85).

1.5. Methods of malaria vectors control

The control of malaria vectors is usually directed to target both adult and larval populations and to reduce man-vector contact. The methods are usually classified into chemical, biological and environmental, depending on whether the control of vectors is attempted through the use of chemicals or biological agents, or by management of the environment (86-89). They may be integrated in a balanced combination to suit local conditions, needs, and resources and to ensure the maximum cost-effectiveness and benefit (86). A brief account on the main methods applied to control malaria vectors is described in the following sections.

1.5.1. Chemical control of adult mosquitoes

Malaria vector control was triggered by the discovery in 1897 of the role of mosquito as the main transmitter of malaria parasites. However, it was not until after 1939, when the insecticidal properties of the first synthetic insecticide DDT was discovered, that the Global Malaria Eradication Programme was launched. Methods of chemical control that target adult vectors are intended to impact on mosquito densities, longevity, and survivorship and, reduce transmission (90, 91). There are, currently, twelve types of insecticides approved for chemical control of adult mosquito; they are divided into four main groups, according to their chemical structure, viz.: organochlorine, organophosphate, carbamate and pyrethroid insecticides (88, 92). The four groups of the aforementioned insecticides have been deployed over the past 5 decades, by means of (indoor/outdoor) residual spraying (all four groups), or on insecticide treated nets and curtains (only pyrethroids).

1) Indoor residual spraying

Indoor residual spraying (IRS) is considered one of the most effective and feasible chemical interventions for reducing and interrupting malaria transmission (79, 92). Many important malaria vectors feed and rest inside human dwells and animal, shelters and IRS involves spraying the insecticides, singly or in combination, on all surfaces that mosquitoes might land on (92) . Thus it is expected to reduce the life span of the vector below that of the sporogonic cycle, reducing both malaria transmission and vector density (92). The residues persist from a few weeks to over a year, depending on the type of insecticide, formulation, the dosage applied, the type of surfaces sprayed, and climatic conditions (92). Although effective, IRS requires stringent planning, management and supervision and can be delivered only by well-staffed and well-equipped vector control services, which do not exist in many endemic countries (92). Issues such as community fatigue, financial and operational logistic constraints, as well as insecticide resistance all limit its sustainability and success (79, 90)

2) Insecticide treated nets

Bed nets have been used for many centuries for protection of people against host-seeking mosquitoes and other arthropods that bite during the night (76, 93). The development of bed nets impregnated with pyrethroids insecticides (ITNs) in the 20th century supplemented their protective role with a lethal effect on mosquitoes (78). Since the development of Long-lasting Insecticide treated nets (LLINs) in the 1990s (77), their use greatly increased worldwide. In Africa in particular, the percentage of nets usage was estimated to be around 74% (2), and responsible for approximately two-thirds of the reductions in malaria mortality and morbidity since 2000 (24).

LLINs act by both reducing vector longevity through the lethal effect of the insecticide and as physical barrier against mosquito bites (as long as, the physical integrity of the bed net has not been severely compromised) (79, 94). LLINs can be as effective for community protection as indoors residual spraying only if $\geq$ 80% of the population is sleeping under the bed net protection (95-98), a level that is rarely achieved, for many reasons (83-85).

Pyrethroids are the only class of insecticides currently permitted for treatment of bed nets (92) for further details regard type and insecticide formulations. However, emerging insecticide resistances in African vectors is a major threat to their future of pyrethroid-based vector control (26).

1.5.2. Chemical control of mosquito immature stages

Until the advent of adult mosquito control in the 1950s, mosquito larvae and pupae were the primary targets for malaria control (99). To ensure success, areas receiving larvicidal interventions should have the following key conditions (90, 99, 100):

a) Human-vector contact should occur at low density

b) Breeding sites must be well known, accessible to treatment, few in number and relatively limited in size

These criteria are unlikely to be fulfilled by most rural areas throughout sub-Saharan Africa, where the breeding sites are numerous, particularly in the rainy season. The second condition can be met only in small urban areas (90, 99, 100). Therefore, larval source control is currently recommended as a supplement to IRS and LLIN.

1.5.3. Biological methods to control vector populations

Biological control approaches target mosquito immature stages by introducing natural enemies, parasitic or predatory animals that feed upon mosquitoes, such as insects, viruses, bacteria, protozoa, fungi, plants, nematode worms and fish (93, 101). An updated list of both available and WHO recommended biological control agents is reviewed in (101). The most frequently used agents are larvivorous fish, entomopathogenic fungi, bacterias, virus, nematodes and plants (92, 101).

One stated advantage of biological control over chemical control is that vectors will rarely develop resistance to biological agents (93). However, despite many efforts over the past 50 or more years there is no consistent evidence to link the use of biological control agents, with the reduction of malaria prevalence (102).

1.5.4. Larval source management

Larval source or environmental management (LSM) (99), is defined as "the planning, organization, carrying out, and monitoring of activities for the modification and/or manipulation of environmental factors or their interaction with humans with a view to preventing or minimizing vector propagation and reducing human-vector-pathogen contact" (86). LSM can involve environmental modification, manipulation as well as community education and awareness promotion. There is evidence that, when properly implemented in areas where a sufficient proportion of larval habitats can be targeted and malaria transmission remains unstable, environmental management can enable 80-90% reductions in both malaria incidence and parasite prevalence, in a cost-effective manner (103, 104).

1.6. Current situation and knowledge of malaria transmission in Mozambique

Malaria is by far the most important cause of morbidity and mortality in Mozambique (105). There were around 6,481,516 cases and a total of 2,465 deaths recorded in the country in 2015. The disease burden remains high with parasite prevalence estimate to be approximately 35% in 2015 (105). Malaria is responsible for nearly 45% of hospital outpatients and approximately 56% of cases in paediatric wards, with high maternal mortalities (1.500 per 100.000 births). The case fatality rate is thought to vary between 1.8% and 9.9%, depending on the level of the health facility (105-107). Parasite prevalences are higher in the central and northern provinces, which account for more than 67%% of malaria cases recorded (Figure 4) (105, 108-110). Malaria transmission is perennial with peaks occurring during after the rainy season (November to April) (105). *Plasmodium falciparum* is the predominant parasite, accounting for 90% of all infections, followed by *P. malariae* (9%) and *P. ovale* (1%) (105, 107, 110, 111). Currently, there has been emergence of malaria cases due to *P. vivax*, particularly in the central and northern regions (112).

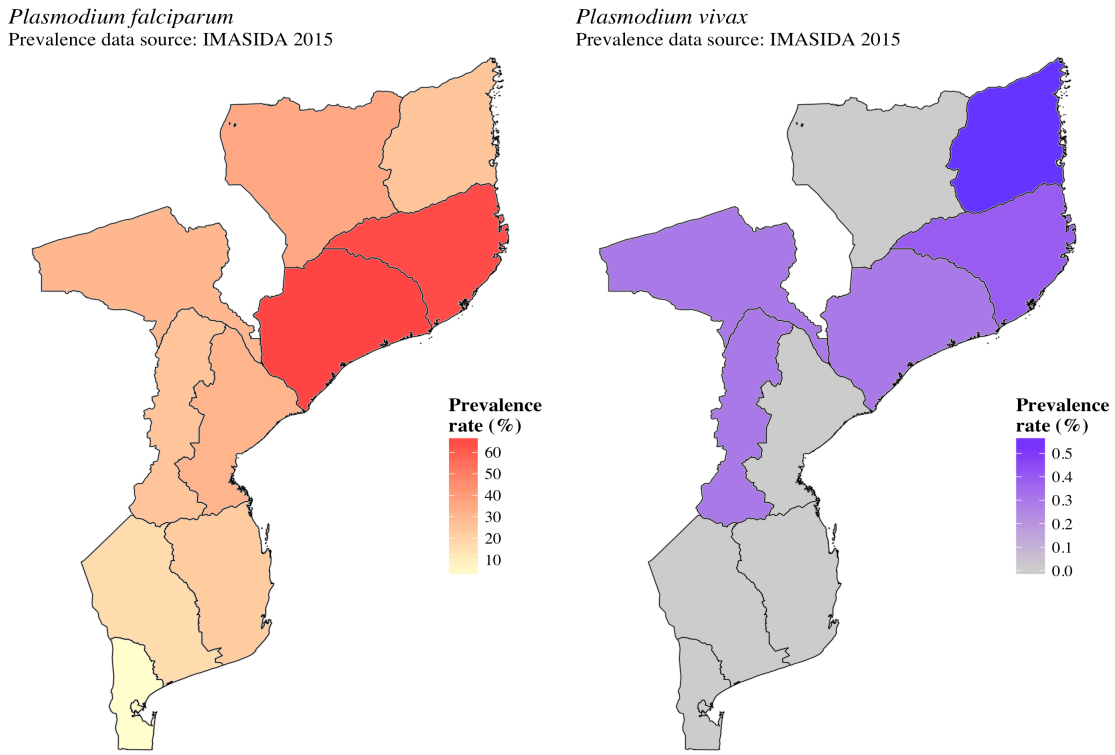


Figure 4 Prevalence of *Plasmodium falciparum* and *P. vivax* malaria in Mozambique. Source: Maps were built based on annual report published by National Malaria Control Program (PNCM) (105) and The national Survey on Indicators of Immunization, Malaria and HIV/AIDS (IMASIDA) (110).

In general, the southern regions of the country are considered mesoendemic, whereas those of central and northern are hyperendemic (107, 112). The parasitaemia risk in the latter regions can be as higher as 95% (109).

1.6.1. Malaria control in Mozambique: Past and present

Malaria control in Mozambique was firstly introduced around 1907 during Portuguese colonial administration. It was confined to the former Lourenço Marques city (now Maputo city), financial and human workforce limitation hindered the expansion of anti-vectorial campaigns to other parts of the territory. The control measures consisted basically in anti-vectorial approaches, such as elimination of breeding sites an application of oils with larvicide effect (113).

Activities later extended to Beira city (in 1946 and 1948) and campaigns consisting mainly in IRS with DDT, pyrethrum and Benzene-Hezachloride (BHC), and larviciding

using kerosene and oils; spatial fogging was also used in Maputo city (112). From 1976, malaria eradication campaigns remained non-operational for sixteen years due the escalation of a civil war and were not reactivated again until the 1990s, when IRS campaigns began in suburban areas of the provincial capital cities (114). In 2000, IRS campaigns with the carbamate bendiocarb was re-introduced in the rural parts of Maputo province as part of the Lubombo Spatial Development Initiative (LSDI), an initiative that involved three countries, viz: Mozambique, Swaziland and South Africa (115). Currently, malaria control strategies in Mozambique consist of three basic elements:

a) Early diagnosis and treatment of malaria cases

b) Community health education and social mobilisation

c) Application of measures to reduce human-vector contact, (LLINs and IRS).

LLINs and IRS are presently the main measures of vector control implemented in Mozambique. Periodically LLINs are freely provided as part of antenatal and universal access campaigns and it has been estimated that more than 7.6 million of LLINs have been distributed by the Ministry of Health and partners in rural settings throughout the country since the introduction of mass distribution campaigns in 2000. Indoor residual spraying operations take place once a year and usually occur before December, when the main rain season starts. Spraying has usually been performed to 50 kilometres radius around the major populations centres (111)

1.7. Bionomics of adult malaria vectors

1.7.1. Classification of malaria vectors

There are 484 formally recognised *Anopheles* species (28, 29, 116), 70 of which are capable of successfully transmitting *Plasmodium* to a susceptible host (8, 30). Of these, 41 have been considered vectors of public health importance (69, 117, 118). There are at least thirty species of *Anopheles* found naturally infected with human *Plasmodium* species in sub-Saharan regions (69, 117, 118). An updated list of the most common African malaria vectors, and their key behavioural aspect relevant for malaria transmission is shown in table 1.

Table 1. Summary of distribution, feeding preference and biting habits of malaria vectors of regional importance in sub-Saharan regions. Symbols indicate that vector species frequently shows this (XX) preference or habit than otherwise (X).

Specie	Distribution	Feeding preference		Biting habit		References
		Anthro - pophilic	Zoo - philic	Endo - phagic	Exo - phagic	
Anopheles funestus	Widespread	XX		X		(121)
Anopheles arabiensis	Widespread	X	X	X	XX	(141-146)
Anopheles gambiae s.s	Widespread	XX		X		(141-146)
Anopheles pharoensis	Widespread	X	X		X	(169, 319)
Anopheles melas	Western coastal regions	X	X		X	(169)
Anopheles merus	Eastern coastal regions	X	X		X	(150)
Anopheles nili s.s	Western and Central Africa	XX	X	XX	X	(169, 170)
Anopheles moucheti	Western and Central Africa	XX	X	XX	X	(168, 171)
Anopheles ovegensis	Western and Central Africa	X	XX		X	(169)
Anopheles hanckoki	Western and Central Africa	X	XX		X	(169)
Anopheles hargraevesi	Western and Central Africa	X	XX		X	(119)
Anopheles paludis	Central and Eastern Africa	X	XX		X	(169)
Anopheles ziemanni	Central and Eastern Africa	X	XX		X	(319, 320)

1.7.2. Bionomics and distribution of the main Afro-tropical malaria vectors

The intensity of malaria transmission is geographically heterogeneous and it is influenced by spatial and temporal distribution of vector populations and the variation of climatic and environmental variables (54, 57, 119, 120). Therefore, an in-depth knowledge of the distribution and bionomics of the local malaria vectors is crucial before the implementation of any vector control measures. The bio-ecology and distribution of the five most important Afro-tropical malaria vectors is described.

1.7.2.1. The *Anopheles gambiae* complex

Anopheles gambiae complex comprises some of the most important African malaria vectors (121). Earlier behavioural and cytogenetical observations and laboratory crossing experiments revealed the presence of eight sibling species belonging to the complex, namely: *Anopheles gambiae s.s*, *A. arabiensis*, *A. melas*, *A. merus*; *A. quadriannulatus sp.* A (122-127); *A. bwamba*e (128), *A. quadriannulatus sp.* B (129), later named as *A. amharicus* (130). *Anopheles gambiae s.s* has now been recognised as two molecular forms: *Anopheles gambiae* (formerly known as S from) and *A. coluzzii* (formerly known as M form) (130-132).

Anopheles gambiae s.s and *A. arabiensis* are widespread throughout all sub-saharan countries (30, 118, 124, 133, 134) and, with a few exceptions in the central equatorial region where *A. arabiensis* is virtually absent (135), the two species occur sympatrically over extensive areas. Sympatric occurrences have been recorded between all the members of the complex, with the exception of *A. melas* and *A. merus*, which are mutually allopatric and confined to the West and East African coasts respectively (121, 125, 136, 137). Larvae of all species rapidly occur in natural or artificial, temporary and sunlit breeding sites, ranging from rice paddies to tyre tracks, hoof prints, small depressions or excavations along irrigation channels and ditches. The population density increases significantly following the onset of the rainy season (70).

Anopheles gambiae s.s (henceforth termed *A. gambiae*) is the most important malaria vector from the complex. It is highly anthropophilic with the human blood indices (HBI) usually ranging from 80% - 96%, depending on the availability of human hosts

relatively to alternative mammals (138-140). It also shows high longevity in the field and is one of the most efficient of all malaria vectors (118, 141).

During the dry season, when the availability of suitable breeding sites for *A. gambiae* tends to reduce in number and size, *Anopheles arabiensis* often exceeds it in terms of numbers and vectorial importance (142-144). *Anopheles arabiensis* usually shows opportunistic anthropophlic/zoophilic feeding habits, with host choice usually proportional to host density (118, 140, 145). Nonetheless, the entomological inoculation rate can be as higher than 85% with this species (146).

Anopheles merus, *A. melas* and *A. bwambae* are partially zoophilic and exophilic, and of secondary importance as malaria vectors; often they are responsible for local outbreaks in unstable transmission settings, particularly when the density of the main vectors is low (117, 137, 147, 148).

1.7.2.2. *Anopheles funestus* group

Though first described in 1900, it was until the 1930s that *Anopheles funestus* was recognised as a series of morphologically overlapping individual species, with limited differences visible only at the immature stages (69, 117, 149, 150), but with distinct behavioural and vectorial capacity (69, 118, 150).

The *Anopheles funestus* group comprises one of the most diverse and widely distributed group of malaria vectors. Currently the group has 23 recognised species, divided into 3 main groups: Funestus (6 species), Rivulorum (4 species) and Minimus groups (13 species), which in turn are divided into five subgroups, that is; Funestus, Rivulorum, Minimus, Aconitus and Culicifacies, currently known as the African/Asian Funestus Group (151-153).

The African members of *A. funestus* group have discrete distribution throughout sub-Saharan region; *An. funestus*, *An. lessoni* and *An. rivulorum* are widely spread across the continent. The Northernmost limit of their distribution starts from Morocco, Niger and Ethiopia, to the countries in the Southern part of the continent; Botswana, South Africa and Mozambique (30, 69, 150). The distribution of other African *funestus* siblings, namely: *A. confusus* and *A. parensis* , *A. aruni; A. fuscivenosus; A. brucei; An.*

vaneedeni; A. funestus-like and *A. rivulorum*-like is localized or uncertain (118, 154, 155).

Anopheles funestus is the most anthropophilic and endophilic member of Funestus group. It is the most efficient malaria vector along its distribution (118). The index of anthropophagy can be as high as 91-100%, even in the presence of alternative host (139, 156). The species can exceed *A. gambiae* in terms of its abundance and its ability to transmit malaria parasites, in several parts of southern and east Africa (53, 149, 157-159) and in west Africa (160, 161).

In some regions of southern Mozambique, both *A. arabiensis and A. funestus* can be equally important at maintaining malaria transmission (162).

1.7.2.3. Other important African malaria vectors

***Anopheles nili* complex**

The *Anopheles nili* group comprises a group of four morphologically overlapped species: *Anopheles nili, A. somalicus* and *A. carnevalei* (118, 135, 163) and *A. ovengensis* (164). Among them, *Anopheles nili s.s* (hereinafter *Anopheles nili*) is the most widely distributed throughout sub-Saharan Africa (30, 117, 118). All members of the complex, with the exception of *A. somalicus* which is virtually zoophilic, are efficient vectors of regional importance, particularly in forested areas of western and central Africa (135, 165, 166). *Anopheles nili s.s* is the most important vector of the complex; usually it is highly anthropophilic and feeds indoors and outdoors with sporozoites rates of up to 3% recorded (135, 165-167).

Anopheles moucheti group

The *Anopheles moucheti* group constitutes a group of three morphologically similar species, *Anopheles moucheti moucheti sensu stricto* (hereinafter, *A. moucheti s.s.), A. moucheti nigeriensis* and *A. moucheti bervoetsi,* reported only from Congo (135). Extensively distributed throughout central and west Africa, particularly in forested regions, *A. moucheti s.s.* is anthropophilic, endophilic, with sporozoite rates of up to 5% (117, 166) and the entomological inoculation rate can reach 300 infective

bites/person/night (168). *A. moucheti nigeriensis* can be a vector of local epidemiology importance in Lagos (Nigeria), where sporozoite rate can reach 1% (169).

1.8. Malaria vectors in Mozambique

Twenty-two *Anopheles* species have been recorded in Mozambique (170, 171). The *Anopheles gambiae* complex and *A. funestus* groups are the most common anthropophilic anophelines, with *A. gambiae* (mainly *A. arabiensis*) and *A. funestus s.s* are the most important and widely distributed species (53, 158, 159, 162, 172-174). *Anopheles arabiensis* is considered the primary malaria vector and is mainly responsible for seasonal epidemic transmission, while *A. funestus* is responsible for perennial transmission and the main vector where the annual mean temperature is below 21ºC (111). The sporozoite rate in *A. funestus s.s* can vary from 4.3% to 22.0%, while in *A. gambiae s.l* can vary from 0.1 to 14.7%, depending on the season (159, 175).

In some coastal regions where alternative hosts are rarely available, *A. arabiensis* can be as efficient as *A funestus* (162). *Anopheles merus* may be a secondary vector in some coastal regions (147, 159) and *A. pharoensis* and *A. ziemanni* may contribute to maintaining malaria transmission in some regions of southern Mozambique (Kampango et al., *in preparation*).

1.9. Host searching and location behaviour by malaria vectors

The mechanisms governing the process of host location and recognition by malaria vectors remain poorly understood. As anophelines are nocturnally active, the majority of detailed studies on the main malaria vectors have been laboratory based and mainly with *A. gambiae*. The responses obtained by laboratory experiments, in which the main factors governing host-location are singly studied may be an oversimplification of what really occur in nature, potentially limit the interpretation of currently available findings. However, a number of important field studies exist, such as those made by Bertram and McGregor (176); Gillies (177); Gillies and Wilkes (178, 179); Snow (180), with *A. funestus* and *A. gambiae s.l.* An overview is presented here of current knowledge of the mechanisms involved in host location by mosquitoes, with particular emphasis on African malaria vectors.

1.9.1. Orientation toward source stimuli

Malaria vectors and other haematophagous mosquitoes usually engage into periodical and rhythmic searching activity in order to locate distant source of sugar, in the form of nectar (40, 181, 182) or blood for survival and reproduction, respectively. The onset of such resource-finding flights is triggered by responses to complex and poorly understood internal and external stimuli whose expression is modified by microclimatic and environmental factors, especially air temperature, relative humidity, light intensity and wind speed and direction (183-187). These environmental factors can maintain, inhibit or affect the timing of the activity, as well as determine the extent to which insect perceives surrounding stimulants (183, 188). Insects have to employ different sensory modalities to get appropriate cues and fine-tuned information to optimize the likelihood of finding the sources of signal while also minimizing the energy cost and risk of predation (189).

The accuracy of the terms used to describe insect stimulus-orientation mechanisms has long been controversial (190-193). However, while suitable designations are still awaited, it is widely accepted that an insect can orientate itself toward a source of stimulant by means of random undirected manoeuvres, not necessarily motivated by external stimuli; a type of orientation known as **taxis**. However, the same insect can switch to an orientated and predictable flight manoeuvres upon encountering a stimulus, an orientation type called **kinesis** (193). Kinesis usually involves flying straight and at a high rate when the stimulant trails are encountered (183, 194). As such, the insect can reach the source by modulating the velocity of its displacement (orthokinesis), or by modulating its tendency to turn (klinokinesis**)** (187, 194).

Taxis orientation is termed according to the sensory modality; thus, if mosquitoes orientate to the stimulant by following wind current, it is anemotaxis. Similarly, responses to odour/taste, pressure, sound, light and water are termed respectively, chemotaxis, mechanotaxis, phonotaxis, phototaxis, rheotaxis, etc (191, 194, 195). Taxis represent basic orientation mechanism and, differently from kinaesthetic orientation, that is greatly controlled by external stimuli (194).

Nature is undoubtedly a “mesocosm of stimuli” and a flying mosquito constantly has to

process and integrate a vast array of signals emanating from multiple sources. Therefore, efficient orientation would need to involve a combination of different navigational and sensory modalities to maximise the chance and probability of rapidly encountering and discriminating suitable sources of sugar, blood or shelter (187, 193, 196). Hence, where known, both day or night flying mosquitoes also combine information gathered by chemical, visual and tactile sensors and use the product to better orientate toward the source of stimuli (196, 197).

There is some limited field evidence to suggest that, like many social insects (198, 199), malaria vectors may use experiences or a memory in resource location. This type of behaviour has been also reported in *A. arabiensis* (200) and *A. farauti* (201) and in several other important disease vectors, such as vectors of Chaga disease (202).

1.9.2. Response to host stimuli

Humans, like other endothermic vertebrates emit a vast number of physic-chemical cues many of which might function as signals to haematophagous insects. For instance, the eccrine and apocrine glands distributed over the human body produce a myriad of sweat compounds whose decomposition products following bacterial action give rise to several volatile substances known to elicit responses from mosquitoes (203-206). A hypothetical sequence in which host stimuli are presented to the vectors is depicted in figure 5. Mosquito sensory systems have evolved to be very selective and sensitive in detecting and processing cues emitted by potential hosts (185, 187, 189, 207). The main type of stimuli that have been known to be involved in host location and recognition by disease vectors are odour, carbon dioxide, heat, moisture and visual cues (183, 185, 187, 204). Responses of vectors to some or combinations of these factors can trigger, even under laboratory conditions, a sequence of flexible behaviour phases, recognized across different groups of blood-sucking Diptera. These are activation, appetitive search, host detection, host finding and host contact (187, 208-210). Most of the phases identified were defined according to the particular behavioural characteristics of the species being studied (187). The behaviour patterns involved are not required to occur in a strict and rigid hierarchy, arguably allowing a flexible response by the insect to the differing circumstances in which it will encounter hosts (209).

A. *Activation* —spontaneous non-oriented behaviour induced by hunger and regulated by a circadian clock; random flight manoeuvres probably bring the mosquito into contact with stimuli derived from a potential host within range. This phase of host-location behaviour can be unimodal, occurring at specific part of the day or bimodal (*e.g.* crepuscular) (209).

2) *Appetitive searching* — upon encountering host stimuli, non-oriented movements give way to oriented movements that enhance the probability of the individual to find the trails left by stimulant factors. Most flying insects calibrate their trajectory to the source according the direction of the wind (211, 212). Thus, if the wind direction is fairly constant, that is, not differing by more than 30º each direction, insect tends to move crosswind. Otherwise, when the direction of wind current is greater than 30º, it alternates orientation by moving either upwind or downwind the cues transported by the wind (184)

3) *Host detection* — when the stimulus (or stimuli) gradient is intense enough to allow the insect to determine whether the signals belong to a suitable host, it shifts to a more direct flight trajectory by moving faster and scanning a wider area (187)

4) *Host finding and contact* —the final approach to the host, when the stimuli, particularly odour-laden airstreams, gradually become more concentrated and the chemical gradients are more easily perceived. Odour becomes more useful for directional orientation but, at the same time, it is gradually replaced by the most effective stimuli at short range, such as heat, water vapour, visual contrast, host contours and movements. These signals bring the insect in to land or into contact with the host (178, 195, 213).

1.9.3. Long and short range stimuli

As pointed above, the human host produces and releases several types of attractants most of which might equally be produced by a large number of other endothermic vertebrates (203, 205, 214). Therefore, anthropophagic mosquitoes must be capable of accurately filtering and selecting only those signals belonging to the right host or group of hosts. Some signals may be strong enough to stimulate vector sensory organs at long

distance, whereas detection of others, possibly more specific stimuli depends on how close the mosquito is to the host. Gillies and Wilkes arbitrarily divided the stimuli released by hosts into long-range and short-range attractants (178). Although in nature the scenario seems to be more complex, see (215, 216), this classification (Figure 5) is convenient and widely used.

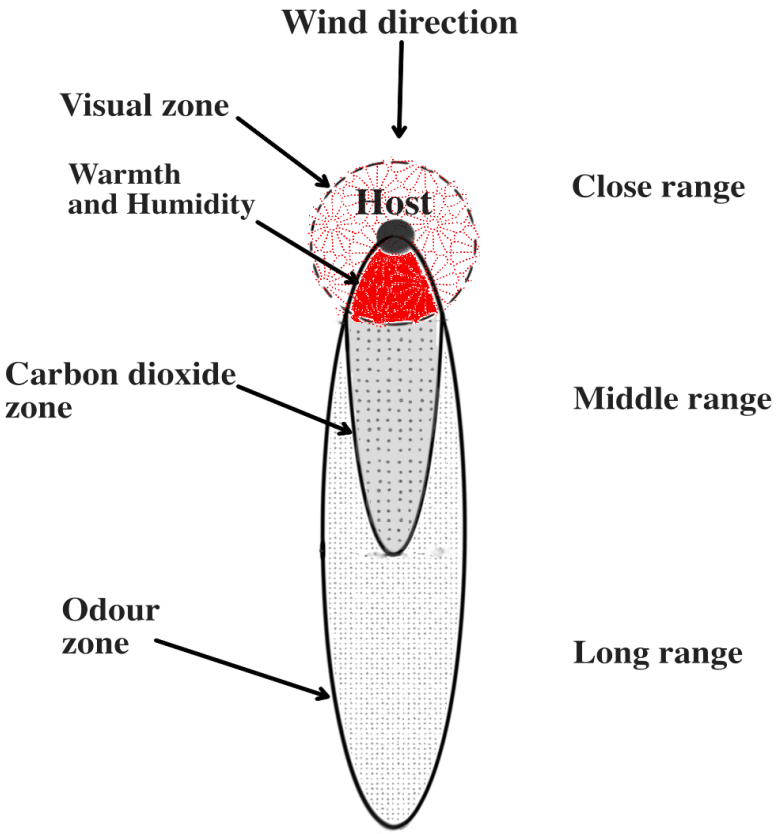


Figure 5. Hypothetical sequences of presentation of stimuli to host-seeking vector approaching upstream stationary host. Adapted after Gillies and Wilkes (217)

1.9.3.1. Long-range stimuli

Long-range attractants are volatile olfactory stimulants constantly released by hosts that can easily be transported by air currents. Hundreds of constituents of human sweat, breath and urine can bring mosquitoes to the vicinity of potential hosts (204, 205, 218-221). Carbon dioxide (CO_2) has been the most studied component of the human breath. Although, virtually all haematophagous mosquito species show chemotactic orientations toward a source of CO_2, identifying its precise experimentally is controversial, with disagreement over whether it is an activator or an attractant (177,

180, 222-224). Gillies (177) concluded that CO_2 alone may act as long-range attractant but, in close contact with host, it is effective only in combination with host warmth current and moisture (Figure 5). Smallegange and colleagues (225) laboratory experiments seem to corroborate Gillies findings, reporting that, CO_2 is not an indispensable stimulus in host seeking by *A. gambiae*. It has been argued that long-range attractants, such as CO_2, stimulate the mosquito to embark on upwind host-seeking flight while also increasing mosquito sensitivity and responsiveness to the array of short-range stimuli (178, 183, 185, 196, 197). However, the efficiency of wind as mean of transportation of odour plume over long distances is greatly influenced by wind speed and direction. Variation in wind speed can affect the shape and structure of odour plume and the extent to which a mosquito perceives an olfactory signal (185).

1.9.3.2. Short-range attraction

Short-range attractant stimuli modulate mosquito behaviour near the host and play a decisive role in the final phase of host-location flight, during host recognition, selection and on alighting and probing specific host body site. Very few field-based reports exist but laboratory evidence confirms earlier observations that mosquitoes can also be attracted to different gradients of heat currents, moisture, as well as, show visual sensitivity to colours contrast and contour of objects (226-228). Growing evidence also shows that, convection thermal currents from host body play key role as directional attractant and possible also in the discrimination and selection of preferred host individuals at close range (197, 229). Several mosquito species land and probe warmed objects or hands compared to cooler controls (197, 229-231). At the time of writing, no field studies existed demonstrating a role for moisture as an attractant although laboratory evidences suggested that water vapour can significantly enhance the response of haematophagous insects to thermal stimuli (222, 232). Hence, warm moist air currents might be important at close range (233).

The role of vision in host location by night flying haematophagous mosquito remain a poorly studied topics. However, existing laboratory reports indicate that the compound eyes of nocturnal mosquitoes have low visual acuity, *Anopheles gambiae s.s* for instance is less to 40º (185); but the eyes of nocturnal mosquitoes are also highly sensitivity to light and shade, even in one-quarter star-light condition (207, 234).

Recent study with *A. coluzzii* may combine both visual and olfactory stimuli to make final decision to land on a potential host (235). Furthermore, field evidence also suggests that visual cues have indirect role on host-seeking activity of nocturnal anophelines, including important malaria vector such as *A. gambiae s.l* (176, 236). It has been argued that this enhanced light sensitivity allows mosquitoes to follow host-odour plumes even at low light intensities by optomotor anemotaxis, as initially demonstrated by Kennedy (195), with day light flying mosquito *Ae. aegypti*.

1.10. Sampling host-seeking mosquitoes

1.10.1. Human landing catches (HLC)

The human landing catch method is considered the most reliable method to obtain direct estimations of the two most important and commonly used malaria transmission indices: the human biting rate and the entomological inoculation rate. The human biting rate (ma) is defined as the product of adult mosquito density in relation to the human population and the proportion of mosquitoes feeding on humans. The entomological inoculation rate (EIR) is defined as the estimated number of potentially infective bites delivered by the vector population, and is calculated as the product of the human biting rate and the proportion of mosquitoes carrying *Plasmodium* sporozoites in their salivary glands (61, 66, 67). HLC also enables estimates of distribution of bites per hour of the night, crucial for the selection and application of control measures to reduce man-vector contact (61).

Human landing catches were introduced for the first time by J. A. Kerr (237), in the earlier 1930s, in Lagos, Nigeria, West Africa, as a tool for routine sampling and estimation of mosquito feeding habits. Five years later, the procedure was improved and standardized in South America (238, 239). While performing landing collections, the collectors, usually one to three seated adults (Figure 6), may act as bait and collector, attempting to aspirate all the hungry females mosquito that alight to feed on the lower limbs, during a given interval of time.

Figure 6. Photograph showing a collector performing night-time landing collections inside an experimental hut in Massavasse village in 2016. Source: Author´s photo.

Despite being a very reliable sampling method, HLC has several operational drawbacks: it is physically demanding and its accuracy greatly depends on the collector's stamina and strength, as well as their competence, experience (240, 241) and inherent attractiveness (242-245). In some situations, when the rate of recruitment of newly emerged females increases, there may be such a high number of mosquitoes biting that collections have to be conducted at shorter intervals with intermittent resting periods throughout the night. Finally there is a serious risk of disease transmission by the range of mosquitoes or other vectors that are attracted to the collector (240). Due to practical limitations inherent of HLC, several alternative trapping methods have been proposed and are widely used today.

1.11. Alternative approaches to human landing catches

1.11.1. CDC Light trap

Centre of Disease Control and Prevention light trap (CDC light trap), developed by Sudia and Chamberlain (246), is an improvement of the battery-operated New Jersey

light trap firstly developed in the 1940s (247, 248), see figure 7. Due to its simplicity the CDC light trap has gained acceptance and, it is probably the most widely used mosquito-trapping device to date. The trap captures mosquitoes when the air current generated by the fan draws insects that are lured close to the fan by the light, into the catching bag. The trap has been employed for monitoring and sampling both indoors and outdoors host-seeking mosquitoes. It is usually hung at a height of approximately 130 to 150 centimetres above the floor to prevent catches from being destroyed by ants or other scavenger. When used indoors, the trap is often suspended beside a bed whose occupants, protected by untreated bed net, act as an additional bait (246). When employed outdoors synthetic attractants, generally carbon dioxide or 1-octen-3-ol, have been used with some success (239).

Figure 7. Details of a standard CDC Light trap hung outdoor. Source: Author´s photo.

CDC light-traps can be used for routine entomological surveillance and, when calibrated with reference methods, the trap can be used to make an indirect estimate of some important entomological indexes, such as biting activity and EIR (249).

However, CDC Light traps have many operational limitations, mainly when used outdoors (250, 251). Since mosquito show differential responsiveness to visual stimuli (227), some may be less attracted to the yellow light than others. Increasing field evidence indicates that light traps collect fewer mosquito numbers than HLC (252-257). In fact, only a single study has shown proportionality between HLC and CDC light trap catches (258). Moreover, light traps also attract numerous insects from other groups, particularly Lepidoptera and nocturnal Diptera that can inflict serious morphological damage to collected mosquitoes. In Venezuela, for instance, approximately 20% of mosquitoes captured could not be identified to species level because of damage (253).

1.11.2. Net traps

Baited animal or human net traps (Figure 8) have been tested as an alternative to HLC, mainly for sampling outdoor host-seeking mosquitoes (259). The procedure usually involves suspending a transparent netting material, usually rectangular, by four poles firmly fixed into the ground around an animal or human bait sleeping inside the suspended net. Typically, the human bait is protected by a second inner untreated bed net to avoid disease transmission, see Fig. 8A, (239, 240, 259). The outer net is raised, ideally not higher than 8 cm (260), from floor or can have one or two panels rolled back or horizontal slits or tears to allow hungry mosquitoes to enter (240). Depending on the study goals, mosquitoes trapped within the spaces between the outer and inner nets can be collected either by the person acting as bait or by other personnel at intervals throughout the night or, even more, by performing single collection in the early morning (240). Several types of modifications of the first used net trap (261) have been proposed (260, 262-264), but conflicting results have raised questions over how representative catches actually are.

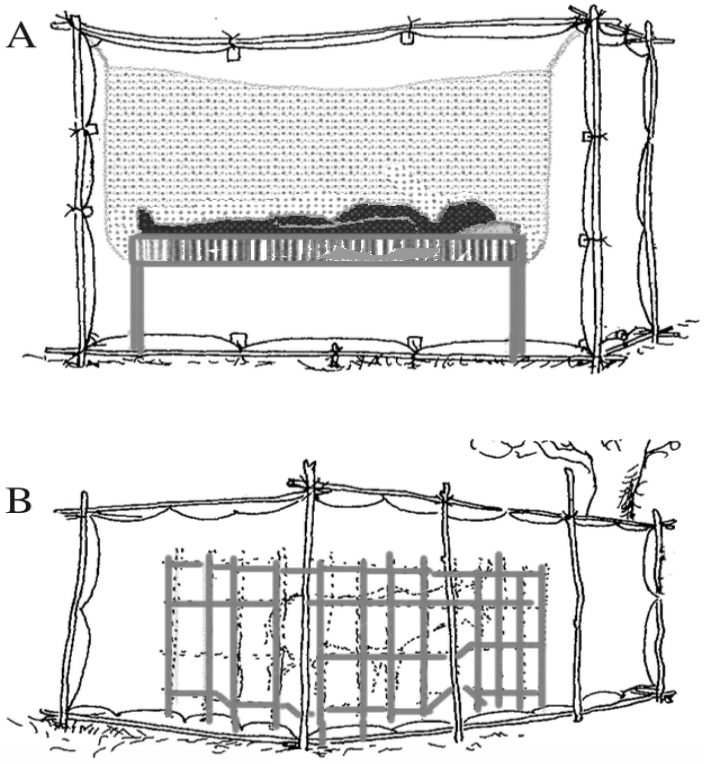


Figure 8. Common deployment of human (A) and animal-baited (B) net traps. Source: Adapted from WHO (259).

As with baited CDC light traps, the main advantage of net traps over HLC is that they are labour-saving, since a single person can be employed as bait throughout the night (240). Studies have shown that the proportion of parous and nulliparous females collected by net traps is statistically similar to HLC (265). However, the number of mosquitoes sampled by net traps is usually lower than HLC, probably because some mosquitoes escape from the net trap particular when the bait is protected by a bed net or the entry opening is too large (266).

Several other alternative to HLC have been developed for sampling Afro-tropical host-seeking mosquitoes outdoors, and include electrocution grid devices (267-269), odour-baited traps (OBET) (270), the Furvela trap (271), the Mbita trap (272), Mosquito Magnet X traps® (273), Ifakara tent traps (266) and the Ifakara odour-baited station (274). However, to date few of these have been tested widely enough for conclusions to be drawn about their reliability and potential.

2. Research question and hypothesis

A range of anopheline mosquitoes that bite humans outdoors or indoors, termed exophagic or endophagic respectively, transmits malaria. After feeding, these mosquitoes will rest either outdoors or indoors (termed exophilic or endophilic, respectively). To date, the most successful vector control methods have targeted the indoor species, mainly using residual insecticides delivered either on bed nets or on the walls (24). Outdoor-active species are much more difficult to target and no satisfactory control methods exist. In fact, outdoor residual malaria transmission has been considered as one of the main causes for the collapse of what was considered a prototype of successful malaria control program in Africa, *i.e.* the Garki Project (275). Outdoor-transmitted malaria is most common outside Africa, but it is growing in importance within Africa since intensive and effective indoor vector control has revealed the extent and importance of outdoor malaria transmission (276-281).

Currently, there are no appropriate effective methods for targeting exophilic or exophagic malaria vectors. Expert committees have highlighted the importance of outdoor biting and the major challenge it presents to control efforts, particularly where malaria elimination is being considered (98).

The present study tackles the challenge of outdoor vector biting by seeking to understand better what determines why or how malaria vectors bite outdoors or indoors. There have been numerous studies on outdoor mosquito behaviour over many decades including many attempts to develop effective traps. However, we are aware of only one that has sought to answer the questions being examined in the present study. Snow (282) studied the house-entering habits of mosquitoes in The Gambia, West Africa in experiments with prefabricated huts with varied wall apertures. However, while that study used partially constructed structures similar to those used here, Snow investigated primarily *how* mosquitoes *entered* houses (*i.e.* through which apertures) rather than an investigation into which structural elements influenced entry preference. The present study asks: what do mosquitoes perceive as indoors or outdoors and hypothesises that the signals used to recognize a three-dimensional structures as being ‘indoors’ are likely to be similar in those species or populations that preferentially enter as well as those that avoid human-made housing. Identifying those signals would provide

unprecedented insight into fundamental mosquito behaviour, with a high potential for exploitation in the design of novel control tools.

The project basically aims to test the hypothesis that both endophagic and exophagic host-seeking mosquitoes recognise physical structures or structural elements of housing (including possible non-visual cues associated with micro-climatic conditions within them) and in response, are more or less likely to blood feed in the presence or absence of those signals, respectively.

3. Objectives of the study

3.1. General objectives

The overall objective of the study was to test in the field a novel exposure-free man-baited trap (Shock-wè trap) and try to understand what a malaria vector may perceive as indoor environ.

3.2. Objective specifics

1) To evaluate the performance of Shock-wè trap as alternative devise for sampling indoors/outdoors host-seeking mosquitoes, with emphasis to malaria vectors

2) To determine whether different physical structural elements of a human habitation are associated with the presence/absence of endophilic/ exophilic mosquitoes

4. Material and Methods

4.1. Description of study site

The study was conducted in Massavasse village (24° 62' S; 33° 108' E). The village, previously described by Charlwood *et al*., (283), is approximately 2x2 km in area and is located in the Chókwè district, administrative post of Lionde (Fig. 9), southwest Gaza Province, and southern Mozambique. The climate has two seasons, one hot and wet (from October - April) and another dry and cold (May - September). Mean temperature range between 25ºC to 34ºC in the summer and from 22ºC to 16ºC during the winter; the maximum average annual rainfall is 600 mm, usually observed in the summer.

Massavasse village is administratively divided into six neighbourhoods and, data from 2007 population census indicated that the village is inhabited by at least 4711 individuals divided in, at least, 989 households (Massavasse leader *personal communication*). The Limpopo river is the only river bordering the village to the North side. Four main irrigations channels supply water to and are responsible for the numerous water bodies that support mosquito breed all year round. The soils have high clay content and low quantities of rain or irrigation water often accumulates for days or weeks. *Anopheles* species belonging to *Anopheles funestus* group, *Anopheles gambiae* complex and other important anophelines including *A. tenebrosus*, *A. pharoensis* (283) and *A. ziemanni* (Kampango et al., *in preparation*) and more than 15 species of culicines are found in the village (283) and Kampango *et al*., (*in preparation*). *An. funestus* and *An. arabiensis* are the two main important malaria vectors identified. However, the role of other common anophelines found in Massavasse is yet to be determined.

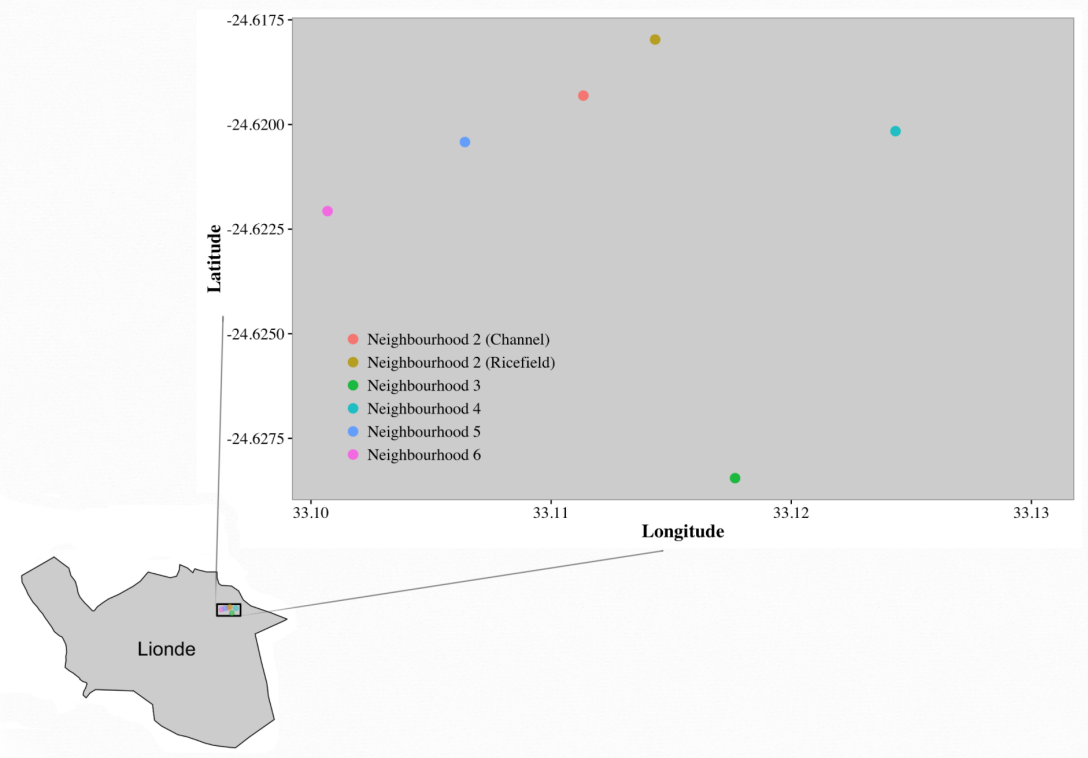


Figure 9. Map of administrative post of Lionde showing the geographical location of Massavasse village and the five studied neighbourhoods. In the neighbourhood 2, the experiments were conducted in two distinct biotopes, viz.: near one of the main irrigation channel (locally known as ditch 10) and close to one of the main rice fields active during the period of field experiments. Source: Maps were built using R v.3.3.1 software (284). The polygon shape files of Lionde administrative boarders were obtained from (http://www.diva-gis.org/gdata).

4.2. Experimental designs

Paired nightly mosquito collections by Human-landing catch and Shock-wè trap (see description of Shock-wè trap below) were performed both indoors and outdoors. Indoor collections were performed inside a portable experimental hut (described in section 4.2.2).

4.2.1. Description of Shock-wè trap

The Shock-wè trap (Figure 10) comprises a metal frame, 200 cm in length, 100 cm wide at the base, 70cm wide at the top and 65cm in height from the trap base to the roof (equivalent to the maximum flight height reported for several *Anopheles* species (220, 222). The base of the trap is 10 cm from the floor, corresponding to the reported flight height of several mosquito species (Haddow *et al*., 1968 cited in (183). A modified insect electrocution grid device (Bower Products, London; www.bower.co.uk), delivering 3800 volts at 9 miliampers (input of 230v) at the electric grid, is deployed on top of the frame. The device was fully compliant with EU safety specifications for use (BS EN 60335F2F59 and the European EMC directive). The electrocution grid measures approximately 65 x 68 cm (16 cm deep) and the area of the active electric net is 493 x 500 mm. A grounded outer aluminium mesh of a size that allows mosquito entry but prevents any human body parts from contacting the grid protects the live wires. The electric grid was positioned on the frame to be above the head and torso region of a supine adult lying on the base beneath, as this is where the majority of both *Anopheles* (and *Culex spp.*) approach the host at the top surface of the net, directly above the human bait (285, 286), who is protected by an untreated bed net within the trap frame (Figure 10A). The human bait sleeps inside the trap under the protection of a customized, untreated, bed net whose the roof region, immediately beneath the bottom of the electrocution grid was adapted to work also as mosquito collecting sack (Fig 10B).

The electricity supply incorporated a circuit breaker (a residual current device or RCD) and, in the event of a short circuit, the circuit would cut out instantly, significantly reducing the risk of serious injury.

In the field, the trap was powered by 7.5kW 4-stroke Ryobi petrol generator (https://www.ryobitools.com/outdoor/products/list/category/generators), providing an uninterrupted alternate electric current (AC) supply to the traps for approximately 11 hours.

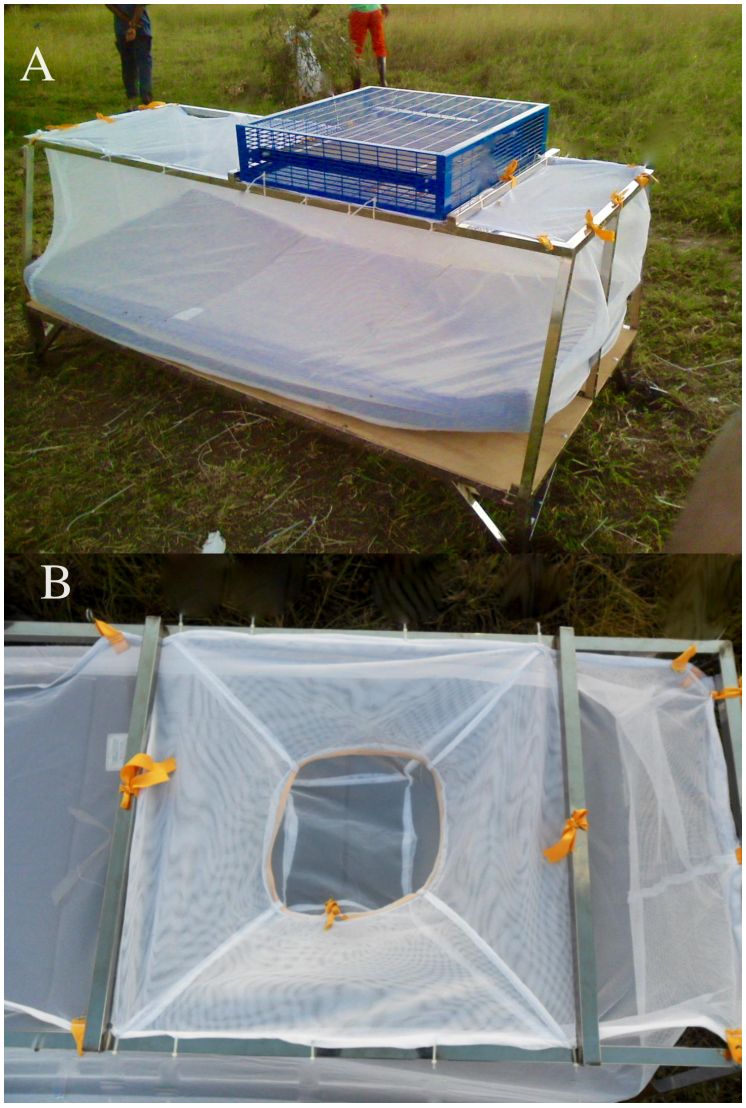


Figure 10. Fully assembled Shock-wè trap in an outdoor location at the field site (A), showing its main components: the electrocution grid within the blue metal frame, above the bed nets where the human volunteer slept and, the details of mosquito collecting sack (B). Source: Author´s photo

4.2.2. Description of Experimental huts

The simple and portable experimental hut measured 290 cm x 264 cm x 270 cm aluminium frame. The walls had no windows and comprised a high-density polyethylene shade cloth, manufactured by (Knittex® South Africa; http://multiknit.co.za/knittex/). The shade cloth reportedly blocks 98% ultraviolet rays and 95% of visible light. Previous experiment with the same type of material (158) showed that it prevented 99% of air currents. On this basis, it was assumed that the

only possible escape of odour plumes from the hut would be through 15 cm eaves apertures between the walls and the roof.

The roof of the hut was covered by 100% waterproof canvas manufactured by Sombra Matsinhe® (http://www.sombramatsinhe.co.mz). The walls were affixed with plastic cable ties to facilitate assembling and disassembling. The roof was fixed to the ground with guy lines, to prevent movement in strong winds. The average temperature inside the experimental hut was 27±2ºC.

Figure 11. Complete experimental hut used as indoors sentinel collection point. Source: Author´s photo

4.3. Determination of sample size and statistical power

4.3.1. Statistical power and sample size to determine the performance of Shock-wè trap

The sample size and statistical power for this experiment was estimated as follows; paired collections were planned to be undertaken in at least five (k = 5**)** neighbourhoods of Massavasse village and, in each neighbourhood, seven (n=5**)** consecutives replicates nights of mosquito collections would be obtained, giving a total

sample size of N = 5x5 = 25 replicate nights.

The statistical power of a total sample size of N = 25 replicates was estimated assuming that the regression tests and correlation would be the main statistical test to be used to detect whether there was significant agreement between paired catches from HLC and Shock-wè trap. The effect size expected the sample to have enough power to detect was the average correlation coefficient of r = 0.5, estimated based on data published by Charlwood *et al*., (283) and Kampango *et al*., (53) reported a minimum correlation coefficient between population of the most common mosquito‘ species found outdoors vs. indoors in some regions of southern Mozambique. Therefore, the power analysis was performed to determine whether a sample size of N = 25 replicates would be able to detect a minimum effect size of $|\rho| \geq 0.5$ if the correlation analysis was undertaken at a significance level of $\alpha = 5\%$.

The sample size and statistical power was calculated applying the method suggested by Cohen (287, 288). Cohen‘s method was implemented using the software G*Power v.3.1 (289) and the package pwr v.1.2. (290), run in R v.3.3.1 (284), respectively and, can be briefly described as follow: To test the null (H_o) hypothesis of correlation between mosquitoes’ replicate counts (i.e. HLC vs. SHK-wè) being equal to zero (H_o: $\rho = 0$), at the significance level of $\alpha = 5\%$ and sample size of **N**. The Power (1-β) (or the probability) of correctly reject the null hypothesis (of no correlation) when in fact the correlation coefficient is different from zero, i.e. H_1: $\rho \neq$, can be determined by using the Fisher’ z transformation of the value of previous reported correlation coefficient *r* (in our case r = 0.5) and the critical value of sample correlation coefficient, $r_{\alpha\nu_{(n-2)}}$, the critical value of sample $r_{\alpha\nu_{(n-2)}}$, that is:

$$Z_\beta = (z - r) \times \sqrt{n - 3}; \text{ where:} \quad (1)$$

Z_β = the value for the probability (either one or two tail) of fail to reject the null hypothesis when in fact it is false (β). Zar (291) provides a table (see appendixes in his book) that can be used to convert Z_β value into the probability of falsely not reject the null hypothesis (β) (i.e. probability of committing type II error).

z = Fisher‘ z transformation of estimated sample correlation coefficient (r); determined by the equation:

$$z = 0.5ln\times\left(\frac{1+r_{\alpha\upsilon}}{1-r_{\alpha\upsilon}}\right);\text{ where:} \quad (2)$$

$r_{\alpha v}$ = critical value of sample correlation coefficient, estimate by:

$$r_{\alpha\upsilon} = \sqrt{\frac{t^2_{\alpha\upsilon}}{t^2_{\alpha\upsilon}+\upsilon}};\text{ where:} \quad (3)$$

$t^2_{\alpha\upsilon}$ - is the critical value of t student distribution at α=5% and υ = n-2 is degrees of freedom (df). Then, υ = 25-2=23 df and, the critical value for t student distribution at 5% of significance is1.65

n = is the sample size of which power we want to determine (that is n=25).

The power analysis was performed, based on above described Cohen‘s method, using the software G*Power v3.1 (289) and the packages pwr v.1.2 (290), run in R v3.3.1 (284), to determine the probability (1-β) the minimum sample size of N = 25 has to reject the null hypothesis H_o: ρ=0, when the correlation between replicate mosquito counts is H_1: |ρ| ≥ 0.5, at a significance level of α = 0.05. Applying the aforementioned equations, the critical value of sample correlation coefficient (r=0.5) was $r_{0.05(23)}$ = 0.32533; Fisher‘s transformation of r = 0.5 was z = 0.549306, then the estimated value of Z_β = 1.050529. So, the probability of committing type II error associated to Z_β is β = 0.11192. Thus, the power a sample size of N = 25 replicates has to detect changes at correlation coefficient |ρ|≥0.5 and at a significance level of α = 0.05% is p (1 - 0.11192) = 0.88807 ≈ 88.8%. Therefore, n=25 replicates is statistically the ideal sample size required to accurately analysis the data collected from this experiment (Figure 12).

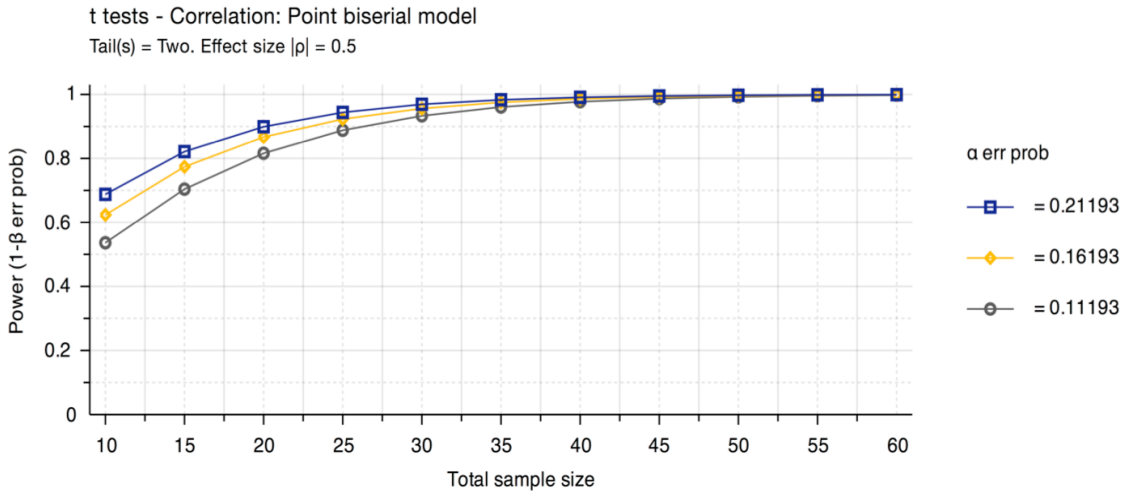


Figure 12. Result of estimation of statistical power to determine the performance of Shock-wè trap. The graphs show that the sample size of 25 replicates would have a statistical power of 88.8%.

4.3.2. Determination of sample size and statistical power to determine the response of malaria vectors to house structures

The sample size to address the hypothesis raised in this experiment was estimated assuming that the experiment was to be undertaken in two (k=2) different neighbourhoods (blocks) of Massavasse villages. In each block, five types of treatment (t = 5, see section 4.4.2 below) would be testes at least five times each one (r = 5). So the final minimum sample size to undertake the experiment was 5x5x2 = 50 night replicates (25 night replicates per experimental block). The statistical power of the number of replicates (**n=50)** was calculated using the software G*Power v.3.1 (289). The effect size, i.e. the minimum difference between mosquito density capable of being detected by the sample size of 50 replicates) was estimate according to Cohen`s effect size **(f)** for analysis of variances (287, 288), described by the equation:

$$f = \sqrt{\frac{\sum_k (x_{ij} - X)^2}{ks}}$$; where: (4)

xij - is the mean values of specie *i* in the group *j*;

X - is the overall grand mean for all groups being compared;

k - is the number of groups and

s - is the standard deviation within groups

The effect size of f = 0.36 ≈ 0.4, was estimated based on the data provided by Charlwood *et al*., (283), table 4 in their paper; about the spatial and temporal variations of mosquito density in Massavasse village. Additionally, based on a previous field experiment (53, 158) involving the same mosquito species analysed by Charlwood and colleagues (283) it was assumed that, the minimum amount of serial correlation (r_{sc}) of repeated measures between levels of manipulations expected to be detected by the sample size would be r_{sc} = 0.5. Thus, having an estimate of the effect size of f = 0.4; serial correlation r_{sc} = 0.5; a total number of repeated measure of n = 50 replicate-nights (i.e. 5 levels x 5 measures per level x 2 experimental sites, see details above about the experimental design). The estimated power a sample size N=50 had to detect a minimum difference of mosquito abundance of 0.4 when mosquito counts are being analysed by ANOVA with repeated measures or by Generalized linear models, at significance level of α=0.05 is p(1-0.1938380) = 0.8061620≈ 80.6%, as estimated by G*Power v.3.1 (289). So, the statistical power for 50 replicate-nights is approximately 80.6% (Figure 13).

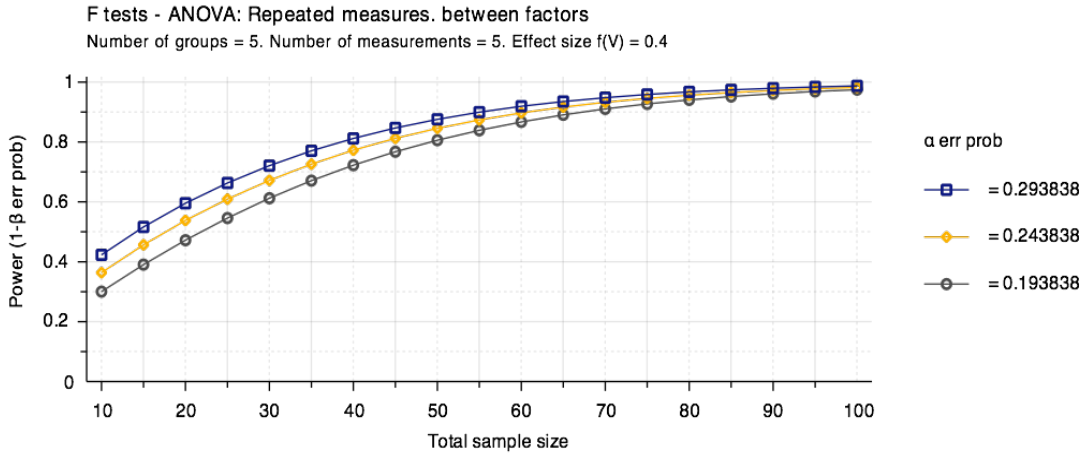


Figure 13. Result of estimation of statistical power of the sample size need to determine the response of mosquito to house structures. The graphs show that 50 replicates would a statistical power of 80.6%.

4.4. Sampling design

4.4.1. Sampling design to determine the performance of Shock-wè trap

A series of paired collections were conducted in five selected neighbourhoods of Massavasse village, separated by at least 350m. In each location, four experienced male collectors (of 18-50 years old) were randomly assigned to four randomly selected sentinel posts to perform indoors and outdoors pairs of human-landing catches (HLC x 2) or Shock-wè trap (SHK-wè x 2) collections (see figure 14). The sentinel posts were located between 12 to 20 metres apart, *i.e.* the maximum attraction range of several African mosquitoes (217, 292) to prevent any possible interference. The night collections ran for eleven hours (limited by the generator), from 18:30 to 05:30 hrs. The collections were divided into one-hour periods and at the end of each period, volunteers were swapped between HLC and SHK-wè to minimize any effect caused by differential attractiveness or catching ability of the human baits/ collectors. The experiment was performed during 35 days, from 15th Feb to 09th April 2016, spending at least 5 days in each one of the 5 selected Massavasse neigbouhoods (see map on Fig. 9, section 4.1, for details).

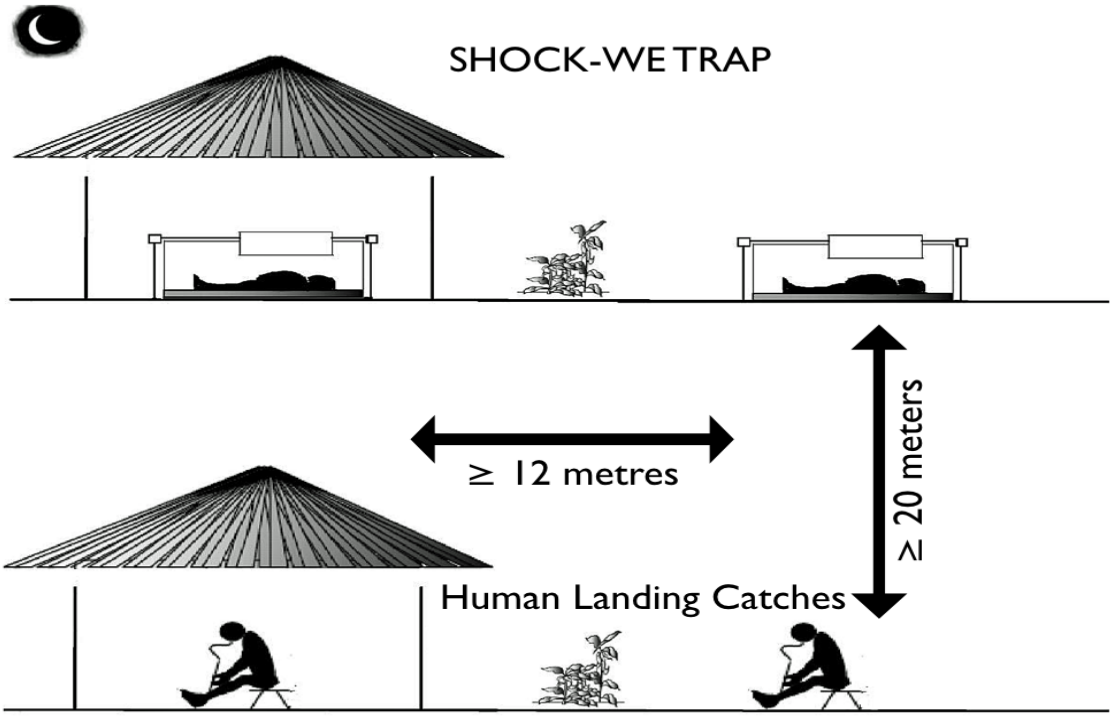


Figure 14. Illustrative scheme of adopted sampling design to determine the performance of Shock-wè trap. See text above for further details.

4.4.2. Sampling design to determine the response of malaria vectors to house structural elements

Two neighbourhoods, namely neighbourhoods 5 and 6, at which high numbers of both endophagic and exophagic malaria vectors were recorded in the previous experiments were selected as the experimental sites. In each site, three mosquito collection sentinel points, located between 12 to 16 metres apart, were randomly selected by coin toss. In one of the three sentinel points, a complete fully assembled experimental hut (see description section 4.4.1 regarding comparative performance of Shock-wè trap and HLC) was built to act as indoor environment for a Shock-wè trap (Indoor control), while at the second point, the Shock-wè trap was deployed in the open air (Outdoor control). In the third sentinel point, one of the following five types of manipulations was deployed (Figure 15, 16):

a) **No treatment** (Level 0) - the Shock-wè trap was deployed outdoor at the sentinel post, without any further type of intervention, excepting the volunteer sleeping inside it. This level was identical to the outdoor control

b) **Lower walls** (level 1) - Consisted of walls covering 50% of the hut from the ground, *i.e.* raised to a height of 135 cm from the floor.

c) **Walls only, no roof** (Level 2) - Consisted of walls covering 95% of the hut, *i.e.* raised to a height of 255 cm (135+120), excluding 15 cm of eave opening.

d) **Roof only** (Level 3)

e) **Complete hut** (Level 4) - House fully assembled; identical to the indoor control hut

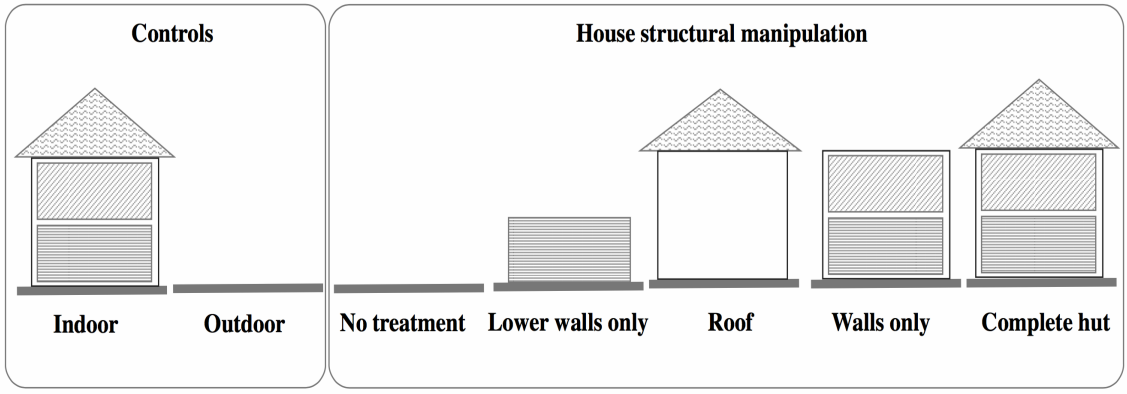


Figure 15. Schematic representation of different types treatment levels tested. The treatments assignment followed a completely randomized treatment-assigning scheme

The five levels of treatments were randomly assigned to the experimental hut on a daily basis following a completely randomized treatment-assigning scheme using agricolae v. 1.2-4 package (293); implemented using the statistical software R v. 3.3.1 (284). Each treatment level was replicated five times and the treatment levels were completely randomly assigned to control for any possible bias in collection.

Three volunteers from the previous experiment (see section 4.4.1) were also randomly assigned to sleep inside the Shock-wè traps and mosquito collections were undertaken over approximately 11 hours, from 18:30 pm to 05:30 am. The volunteers and the electrocution grids associated to each of the three Shock-wè traps were all rotated between the sentinel points daily. The experiments were conducted over 50 days, between 11th April and 31st May 2016, spending 25 days at each neighbourhood.

Figure 16. Panoramic view of the experimental setup at neighbourhood 5 of the Massavasse village. The labels denote (A) the experimental huts chosen to receive treatments, (B) indoor catch control and (C) the Shock-wè trap deployed alone outdoors. The sentinel sites were located between 12 and 16 metres apart.

4.5. Data entry, validation and eligibility for further analysis

Data were entered into an Excel database and each field form was given a single page number. Comparisons between the information contained in each field form with that in the database was used to validate the consistency of the entered data. The author undertook the process of validation every time each batch of data was introduced.

Mosquito catches were organized by date (or week) of collection, region and species collected. Mosquito catches that were less than 5% of the total catch for a given species were described only as means with supporting descriptive statistics, such as standard deviation or confidence interval or, if it was a member of species complex/group, it was then pooled with its siblings and further analysed as a group/complex. Species whose collections exceeded 5% of the total collected for a given group were used for further statistical analysis.

4.6. Mosquito samples processing and identification

Adult mosquitoes were sorted by sex and genus. *Anopheles* mosquitoes were morphologically identified to species according to taxonomic keys from Gillies & De

Meillon (118) and Gillies & Coetzee (294), whilst the non-*Anopheles* mosquitoes were identified using taxonomic keys proposed by Edwards (295); Jupp (296); Harbach (297) and Service (298). The identification of members of *Anopheles gambiae* complex and *An. funestus* group were confirmed by molecular analysis (PCR) through the protocols proposed by Scott *et al*., (299) and Koekemoer *et al*., (300), respectively.

4.7. Estimation of relevant entomological indexes

4.7.1. Mosquito abundance and composition

The relative abundance of mosquitoes collected at each site or treatment level was expressed in terms of Williams's geometric mean (±95% Confidence Interval). Mosquito catch distribution was firstly log-transformed log(x+1) and the Williams's mean was obtained by calculating the mean of the log-transformed log(x+1) catches, followed by back-transformation of the estimated log mean by calculating the anti-logarithm of the log-transformed mean.

4.7.2. Estimation of man-biting rate

The man-biting rate, that is, the average number of bites received per person per night by a vector species was estimated by dividing the total number of mosquitoes collected either indoors or outdoors by total number of individuals that performed collection, per total number of collection nights (35 nights). Since there was hourly rotation between collection points, then, all four collectors alternatively undertook both indoors vs. outdoors. The estimate of man-biting rate was weighed having in account the nighttime habits of the local populations, that is, the mean length of time spent both outdoor versus indoors by Massavasse residents. This was estimated by response to structured questionnaires administrated to 312 randomly chosen households from all five neighbourhoods studied. Thus, the average number of bites received by unprotected individual outdoors and indoors was estimated as follows (301):

Mean nº of bites received by unprotected individuals outdoors (**B_{out}**)

$B_{out} = \frac{ty}{uTc_y}$; where: (5)

T- number of hours from dusk until the time when all villagers are indoors

t - average number of hours spent by each villager outdoors after dusk.

y - number mosquitoes collected outdoors during period T

c_y – number of collectors outdoors

u - number of nights of collection

Mean nº. of bites received by unprotected individual indoors (**B_{ind}**):

$$B_{ind} = \frac{\left(1-\frac{t}{T}\right)\times x_1 + x_2}{uc_x}; \text{ where:} \quad (6)$$

T - number of hours from dusk until the time when all villagers are indoors

t - average time spent by each villager outdoors after sunset

x_1- number mosquitoes collected indoors during period T

x_2 – number of mosquitos collected indoor after period T

c_x – number of collectors indoor

u - number of nights of collection

Then, the total man biting rate (B_T) was estimated by the sum of **B_{out}** + **B_{ind}**

4.8. Data processing and statistical analysis

4.8.1. Determination of agreement between Shock-wè trap and Human landing catches

The quantification of the level of agreement between SHK-wè trap catches and HLC was done using the method of Bland & Altman (302-304). The Bland and Altman quantify the sampling bias methods by determine the interval of agreement, plotting the difference of the measurements taken on the same individual by two paired methods, in our case (SHK-wè - HLC), against the mean of the two measurements, *i.e.* (SHK-wè + HLC)/2. Daily mosquito catches arranged by neighbourhoods were log-transformed by applying log(x+1) to normalize the data or to remove excessive variance, since the method only allows estimation of valid confidence intervals of agreement under normal distribution assumptions (302-304).

To assess the agreement between *Anopheles* catches the difference (or the ratio) of log-transformed catches were plotted against the mean of the paired catches, *i.e.* log[(SHK-wè + HLC)/2] *vs*. log(SHK-wè - HLC). The bias of the candidate method compared to the existing or reference method was estimating by calculating the mean of the differences, i.e. mean [log(SHK-wè - HLC)], which is also, the mean ratio of log(SHK-wè) to log(HLC) catches.

The lower and upper 95% agreement interval was estimated by subtracting/adding the mean difference by 1.96 plus the standard deviation of the difference, *i.e.* Lower = D – 1.96xSD; Upper = D – 1.96xSD. This is the interval that would be expected if most of the difference between the paired catches would lie, if the difference were normally distributed. The normal distribution of the difference was verified by applying the Shapiro-Wilk test under the null hypothesis Ho = the data is normally distributed at a significance level of 5%. A regression line was drawn to detect any proportional trend or density dependence in the efficiency of the candidate trap. Student's *t*-test was used to determine the significance of the change of the slope of the regression line at 5% significance level.

Two-way analysis of variance was applied to determine whether there was species and neighbourhood dependence of SHK-wè efficiency, estimated by the ratio log(SHK-wè/HLC). TukeyHSD pairwise comparisons were performed to identify significantly different pairs at the 5% significance interval using the multcomp v.1.4-4 packages (305). The statistical analyses were conducted using R version 3.3.1 (284).

The possible influence of environmental and climate factors, such as variations of air temperature, wind speed and direction and, change of moon phases was determine by Generalized additive models (GAM) (306, 307) or Generalized linear models (GLM) (308), according to the pattern, that is, non-linearity or linearity, respectively, of predictor variables

4.8.2. Determination of response of malaria vectors to house structures

Associations between the presence of one of the five types of treatments and abundance of each mosquito species were determined by fitting each treatment separately to mosquito counts using Generalized Estimating Equations (GEE) with a negative binomial error distribution, and exchangeable correlation structure, to account, respectively, for over-dispersion of mosquito counts and any possible serial correlation between repeat catches over time and space (309). Hence, the variable 'time' expressed in weeks was chosen as subject, and region as a within-subject factor. The magnitude of the association of each intervention and mosquito presence at the manipulated sentinel hut was estimated in terms of incidence rate ratio (IRR) of number of mosquitoes collected when there was a treatment compared to the reference level (*i.e.* no treatment or level 0). IRR was calculated by exponentiation of coefficients obtained via GEE models. The GEE models were fitted using SPSS® v. 20 (310).

4.8.3. Effect of climate and environmental factors on the efficiency of Shock-wè trap and response of malaria vectors to house structures

The significance of the influence of environmental and climate data such as, air temperature, wind speed and direction and changes of moon phases on SKH-wè trap catching performance and response of host-seeking mosquito to house structure was determined by mean of either Generalized Linear Models (GLM), for linear association between predictor and response variables, or Generalized Addictive Models (GAM), if non-linear association was found. Air temperature and wind data for Lionde administrative post were obtained via AccuWeather™ website (http://www.accuweather.com). Moon phase data was obtained via Garmin Extrex 10 portable GPS (http://www.garmin.com/). All statistical analyses assumed a significance level of 5%.

4.9. Ethical considerations

The major ethical issue in this study was use of human landing catches (HLC) as described in objective specific 1. Increasingly, HLC has been viewed negatively over the past decade, despite evidence indicating that it does not increase the risk of infection

in those carrying out this activity if proper chemoprophylaxis measures are taken (311). The use of HLC is justified in this study, as a primary objective is the evaluation of an alternative method.

4.9.1. Potential risks, discomfort for participants and measures for minimising its occurrence

Though protected within a bed net during the hours of the study, SHK-wè trap-volunteers are also at risk of malaria, at times before and after entering the net, or if infected mosquitoes inadvertently enter the net. For volunteer sleepers, there is a minor risk of electric shocks from the SHK-wè trap. Sleeping within a bed net can be uncomfortable, though no different to the conditions experienced by populations living in traditional dwellings across Africa.

To avoid or reduce the likelihood of any eventual risk to occur, all volunteers acting as attractant baits (both HLC and SHK-wè) during the field studies were provided with anti-malaria prophylaxis, with Fasidar® (Sulfadoxine-Pyrimethamine), in accordance with the recommendations of the Mozambique National Malaria Control Program and World Heath Organization. A recent study showed that this practice reduced 96.6% of malaria incidence risk in HLC volunteers than in non-volunteers in similar studies in Africa (311). In addition, the SHK-wè trap was never switched on unless the volunteer was within the bed net, and therefore unable to contact the SHK-wè trap live parts. Risks of electric shock are minimised by the protective outer casing that completely prevents contact the naked electric grid within. The possible discomfort that might be felt by a volunteers sleeping inside bed net during the experiment will not differ from that usually felt by the local villager under bed nets inside their house.

All members of the team that spent periods of the night in the village were at risk of malaria and may also experience discomfort caused by irregular sleep patterns or mosquito bites. To minimise these risks, all team members involved were required to use anti-malaria prophylaxis (in accordance with the recommendations of the Mozambique National Malaria Control Program). Team members not actively performing a role, as volunteers, will be permitted to wear repellents.

Risks of electric shock were minimised by the protective outer casing that completely prevents contact the naked electric grid within. SHK-wè trap handlers were trained in operation of the trap and good practice and volunteer sleepers also trained in basic good safe practices prior to use. SHK-wè trap has a built-in RCD circuit breaker to prevent shocks and all equipment will be labelled prominently with warning and safety signs. The numbers of successive nights spent working in the field was restrict with intermittent days off, to avoid team fatigue.

The research did not pose any risk for any members of the public, apart from those described above, and nor impacted the normal activity of the local health services.

4.9.2. Privacy and Confidentiality

Other than the identity of the individuals acting as human baits in different tests, no personal data were collected and stored. Individuals (including name, sex and age) will be identified by code, and subsequently referred to by these codes only.

Written informed consent in Portuguese was presented to volunteers for signing (see copy in Annexe I).

4.9.3. Ethical clearance

The study received ethical approval from *Comité Nacional de Bioética para Saúde de Moçambique* (CNBS), reference **208/CNBS/15** and the Liverpool School of Tropical Medicine Ethics Committee, Research Protocol **n. º 14.055** (See Annexes II and III).

5. Results

5.1. Mosquito abundance and catch composition

A total of 35 nights of paired HLC and SHK-wè collections resulted in 96,696 mosquitoes comprising twenty different species in five genera (Tab. 2). *Anopheles* were identified as *Anopheles gambiae s.l* (Theobald), *Anopheles pharoensis* (Theobald)*, Anopheles tenebrosus* (Dönitz)*, Anopheles ziemanni* (Grünberg) and *Anopheles funestus* (Giles), and accounted for 17.9% (12,451/69,546) of the total by HLC (combined indoor and outdoor) and 5.3% (1,452/27,150) of the total by and SHK-wè, respectively. Eight *Culex* species, were collected by HLC [31.8% (22,085/69,546)] and SHK-wè trap [15.45% (4,196/27,150)], respectively and two *Mansonia* species, *Mansonia africana* (Theobald) and *Mansonia uniformis* (Theobald) totalled 50.22% (34,923/69,546) and 78.68% (21,361/27,150) of all mosquitoes captured by HLC and SHK-WE, respectively. The genus *Aedes*, comprised two species only collected by HLC [0.01% (7/69,546)]. The mosquitoes of genus *Ochlerotatus* were also collected by both methods, that is, HLC [0.10% (68/69,546)] and SHK-wè [0,52% (141/27.150), though specific identification of was not possible yet. Finally, *Coquilletidea aurites* (Theobald) and *Coquilletidea sp,* represented 0.018% (12/69,546) and 0.015% (4/27,156) of the total mosquito yield, by HLC and SHK-wè, respectively.

Table 2. Total number of adult female mosquito collected indoors and outdoors by paired HLC and SHK trap from February to April 2016 in five neighbourhoods of Massavasse village.

Species	Human landing catch		Total	Shock-we Trap		Total
	Indoors	Outdoor		Indoors	Outdoor	
Anopheles gambiae s.l	3,431	4,617	8,048	725	147	872
Anopheles pharoensis	30	1,889	1,919	126	338	464
Anopheles tenebrosus	27	1,024	1,051	1	38	39
Anopheles ziemanni	46	1,327	1,373	0	65	65
Anopheles funestus	32	28	60	11	1	12
Culex tritaeniorhynchus	992	7,157	8,149	547	1,963	2,510
Culex pipiens	1,346	5,282	6,628	353	275	628
Culex poicilipes	1,318	4,286	5,604	0	664	664
Culex antennatus	199	1,445	1,644	91	282	373
Culex quinquefasciatus	41	9	50	0	3	3
Culex bitaeniorhynchus	2	3	5	1	11	12
Culex sitiens	1	3	4	0	1	1
Culex sp	1	0	1	0	1	1
Mansonia africana	1,730	2,462	4,192	510	857	1,367
Mansonia uniformis	11,005	19,726	30,731	5,467	14,527	19,994
Coquilletidea aurites	5	6	11	4	0	4
Coquilletidea sp	0	1	1	0	0	0
Ochlerotatus sp	4	64	68	14	127	141
Aedes subargenteus	0	2	2	0	0	0
Aedes sudanensis	1	4	5	0	0	0
Total	20,211	49,335	**69,546**	7,850	19,300	**27,150**

Both methods detected nearly the same number of species (Tab 2. Fig. 17). The genera *Culex*, *Anopheles*, were the most diverse with 8, 5 species respectively. *M. uniformis* was by far the most common mosquito species found in all five regions (Fig. 17), whilst *Cx. tritaeniorhynchus* (Giles), *Cx. poicilipes* (Theobald) and *Cx. pipiens sp* (Linneaus) were the most common culicines. *Anopheles gambiae s.l*, was the common malaria vector, *A. funestus* the least. *Aedes* (*Stegomya*) *subargenteus* (Edwards) and *Aedes* (*muscidus*) *sudanensis* (Theobald) was only collected by HLC; the former species only in neighbourhood 6 and the latter in neighbourhood 3, 4 (at the south-eastern area of the village) (Fig. 17).

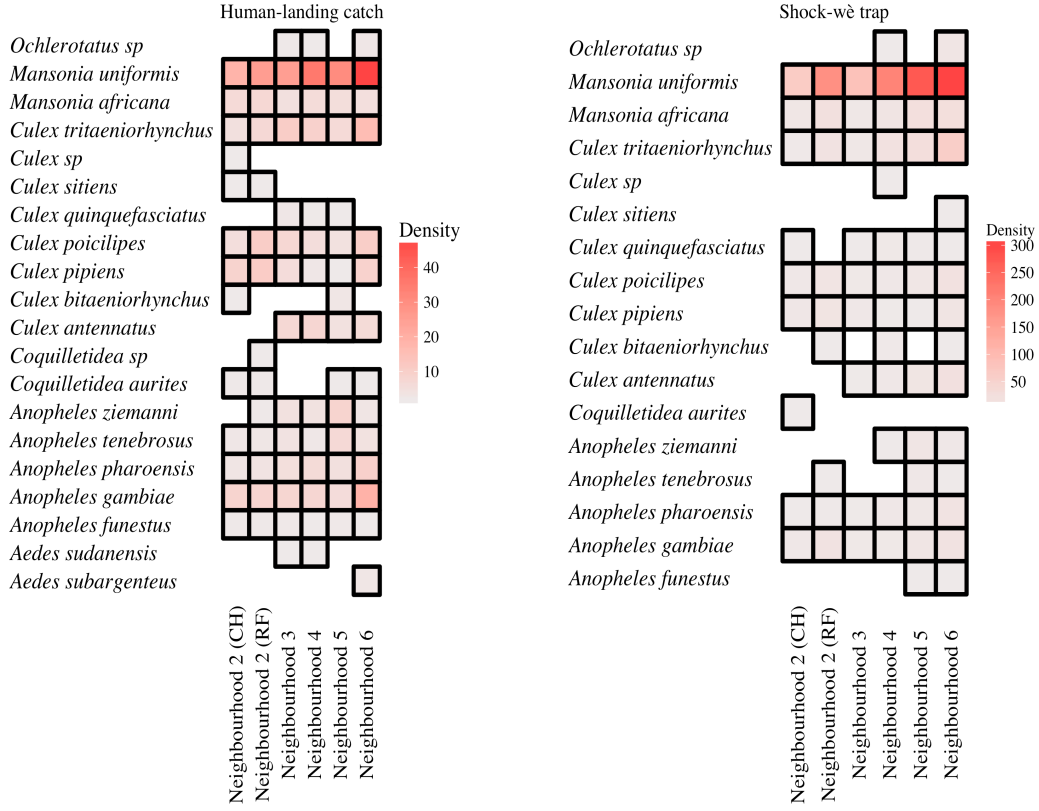


Figure 17. Heat map showing the spatial distribution and density (express in terms of Williams mean) of mosquito species collected by paired HLC and SHK-wè trap in Massavasse village, from February to April 2016. Empty spaces indicate that the species were not detected by either method in particular region.

5.2. Human sleeping habits and mosquito feeding times

A survey of 312 randomly selected households (corroborated by observations made by the team during the study) (Fig. 18) indicated that most inhabitants stay outdoors for no more than 6 hours after dusk and all members of the community have retired to bed by

23:00hrs. Outdoor activities begin at 03:00 - 04:00hrs with most people emerging to go to work by 05:00 - 06:00hrs. It was estimated that, on average, individuals spent at least one hour outdoors after dusk before retiring indoors until the next morning.

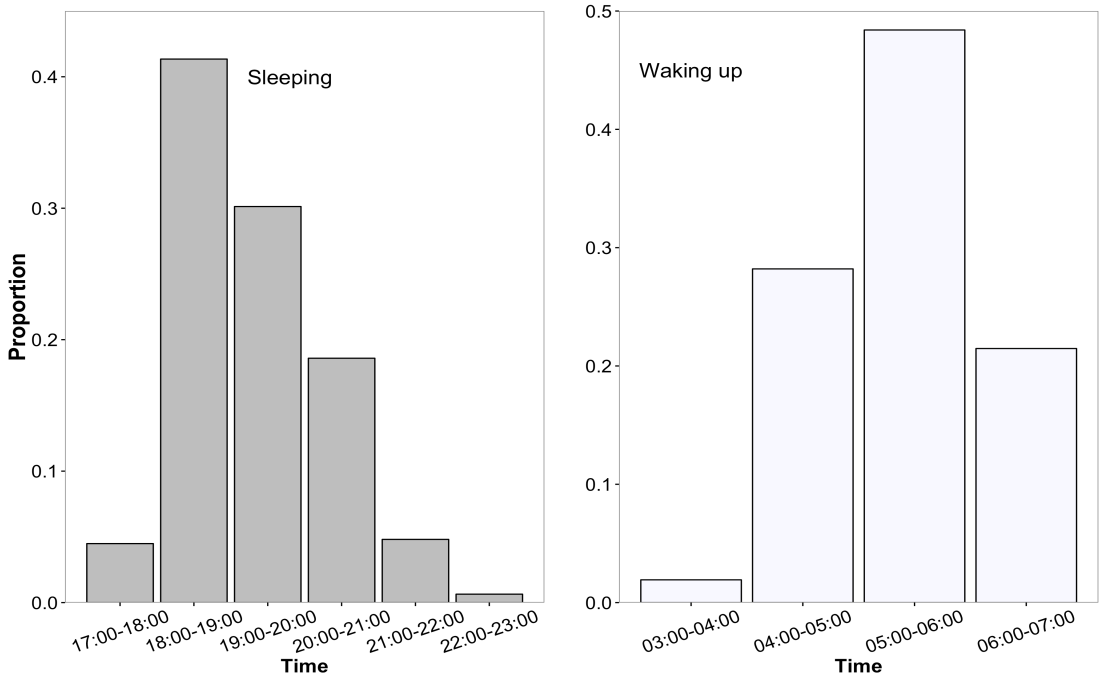


Figure 18. Sleeping and waking patterns of Massavasse villagers in Feb-Apr 2016.

5.3. Biting activity of Anopheles mosquitoes

Figure 19 depicts the biting activity of the most common *Anopheles spp*. collected indoors and outdoors in Massavasse village from 15th February to 09th April 2016. *A. gambiae s.l* had the earliest biting activity outdoors, beginning immediately after sunset, with two distinct activity peaks, the first at 22:00 to 23:00hrs, and the second at 03:00-04:00hrs. Indoors, biting activity of this species progressively increased after 21:00hrs, with a single peak at 02:00-03:00hrs. The estimated number of bites by *A. gambiae s.l.* received by an unprotected individuals, outdoor (17:00 - 23:00) and indoor (17:00 - 06:00), during the study was, respectively, B_{out} = 98.65 and B_{ind} = 8.54. So, the total man-biting rate (B_{out}+B_{ind}) by this vector species during the study was 107.19 (98.65+8.54) bites/person/night.

Anopheles pharoensis also showed an evident bimodal biting pattern outdoors with two distinct activity peaks (Fig 19). Regarding indoors, the vectors bit nearly at same proportion, from sunset to dawn. Overall, the results suggest that *A. pharoensis* showed

higher biting outdoor compared to indoor. The estimated man-biting rate for *A. pharoensis* was 5.52 (4.34 +1.18) bites/person/night.

Anopheles tenebrosus and *A. ziemanni* are important secondary malaria vectors within the *Anopheles coustani* group, and showed very similar biting pattern. Indoors, both did not start biting until 2-3 hours after dusk though *An. ziemanni* continued biting until close to dawn. *A. tenebrosus* and *A. ziemanni* equally showed exophagic preferences; the total man-biting rate (B_{out}+B_{ind}) estimated for *A. tenebrosus* was 2.71 (1.87+0.84), whereas, for *A. ziemanni*, it was 3.64 (2.11+1.52) bites/person/night.

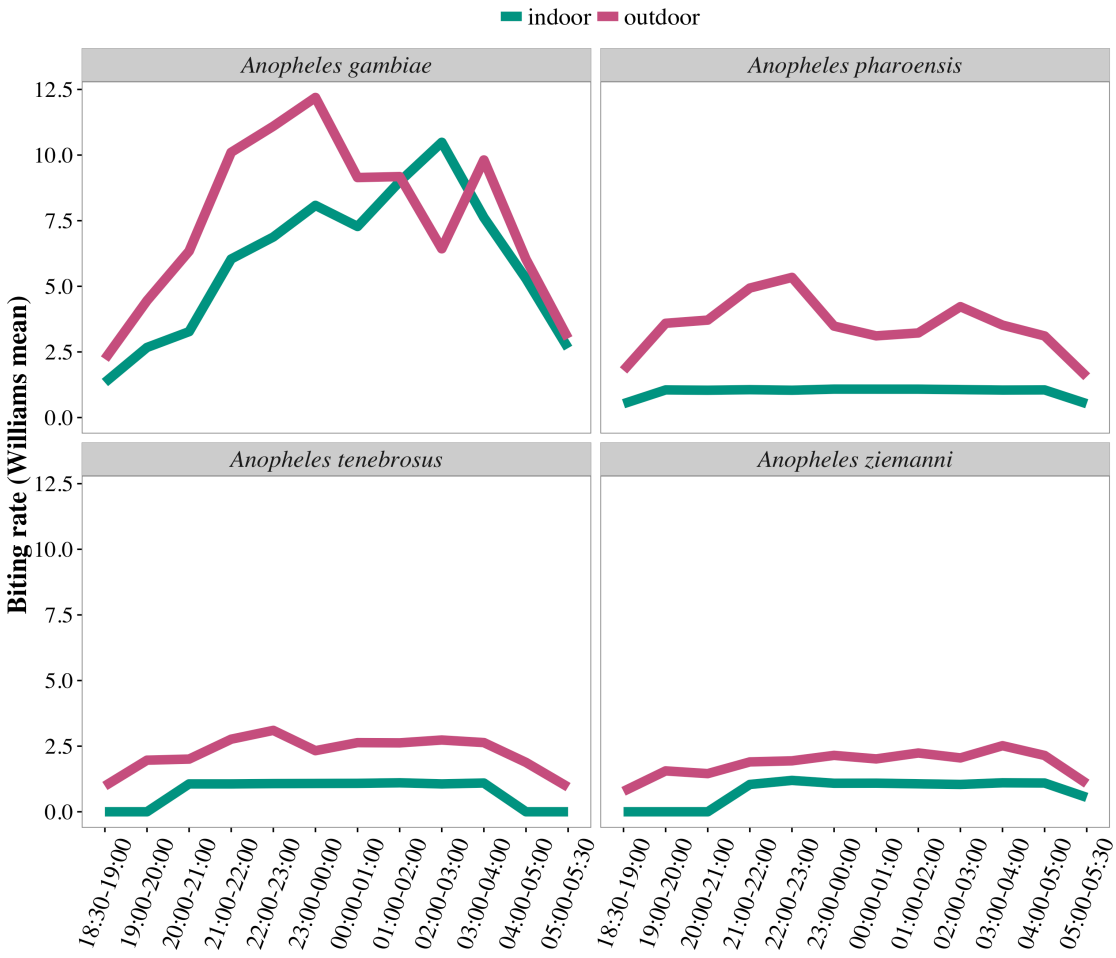


Figure 19. Biting activity patterns of the most common *Anopheles* species found in Massavasse village. Biting rate expresses in terms of Williams mean (Xw), estimated by calculating the antilog of the log-transformed mean catches.

5.4. Comparative performance of Shock-wè trap versus Human Landing Catch experiment

5.4.1. Efficiency of Shock-wè on sampling malaria vectors indoors

Figure 20 shows the degree of agreement between (HLC) and SHK-wè trap in sampling malaria vectors indoors. Significant linear correlation (R^2_{adj} = 0.2701, p = 0.00081)) was observed between daily paired catches of *A. gambiae s.l* obtained by HLC and SHK-wè trap (Fig 20, left side). However, estimation of the global agreement (± 95% agreement interval) between the two methods, (obtained by calculating the antilog of the mean difference (*i.e.* the efficiency ratio), indicated that HLC captured -2.29 (-1.11 − 5.81) (antilog of absolute value of -0.830) times more mosquitoes (t = 10.37, p <0.0001) than the SHK-wè trap (Figure 20, right side). The agreement analysis also suggested that the level of bias tended to reduce significantly to nearly zero when the density of *A. gambiae s.l* was significantly higher, e.g. at neighbourhoods 2 (the main rice field, denoted for simplicity as B2RF) and, also, at neighbourhoods 5 (B5) and neighbourhoods 6 (B6) (R^2_{adj} = 0.2235 p = 0.00243), as evidenced by the variation of the regression line slope (Fig 20). This suggests that the Shock-wè trap may have under-sampled *A. gambiae s.l* in those regions where *A. gambiae s.l* were present in lower numbers, such as in the neighbourhood 2 near the irrigation channel (denoted as B2CH) and at neighbourhoods 3 (B3) and 4 (B4).

In the case of *A. pharoensis*, the log-transformed HLC and SHK-WÈ catches showed no obvious linear correlation (R^2_{adj} = - 0.0069, p = 0.387). Visual inspections of the regression line suggest that the SHK-wè efficiency might have been influenced by some exceptionally high catches, such as those from neighbourhood B6 (Fig. 20). The global estimated bias (i.e., mean sampling efficiency) of SHK-wè over HLC and its back-transformed lower and upper agreement interval limits was -1.07 (-0.41 − 2.80) and paired *t* test analysis on the log-transformed catches from the two methods indicated that they were collecting approximately the same proportion of *A. pharoensis* (t = 0.83069, p-value = 0.4119).

For *Anopheles tenebrosus* and *A. ziemanni*, the estimated global amount of bias was similar, at -1.19 (-1.91 – 1.35) and -1.21(-2.30 – 1.15), respectively. However, the

agreement analysis indicated that SHK-wè trap only performed similar to HLC in regions with lower mosquito density such as neighbourhood 2 near the irrigation channel (B2CH). The performance decreased linearly with increasing of density (Figure 20) in the remaining neighbourhoods with the highest *A. tenebrosus* and *A. ziemanni* density.

Analysis of variance also indicated that the relative sampling efficiency of SHK-wè varied significantly according to species ($F = 58.33$; $p < 0.001$) and village regions ($F = 3.98$; $p = 0.0044$). The overall bias (antilog of the mean difference) between *A. gambiae s.l* catches from SHK-wè and HLC was relatively high compared to the bias observed with *A. paroensis* (difference was 2.15 times higher), *A. tenebrosus* (2.50 times higher) and *A. ziemanni* (2.52 times higher). Comparing regions, the significant difference in bias in the sampling efficiency was only observed between the overall catches from regions B4 and B6 (Tab. 3). Hence, these findings suggest that the efficiency of the two methods in collecting *Anopheles* indoors was more biased toward both the more abundant species and regions with high mosquito density.

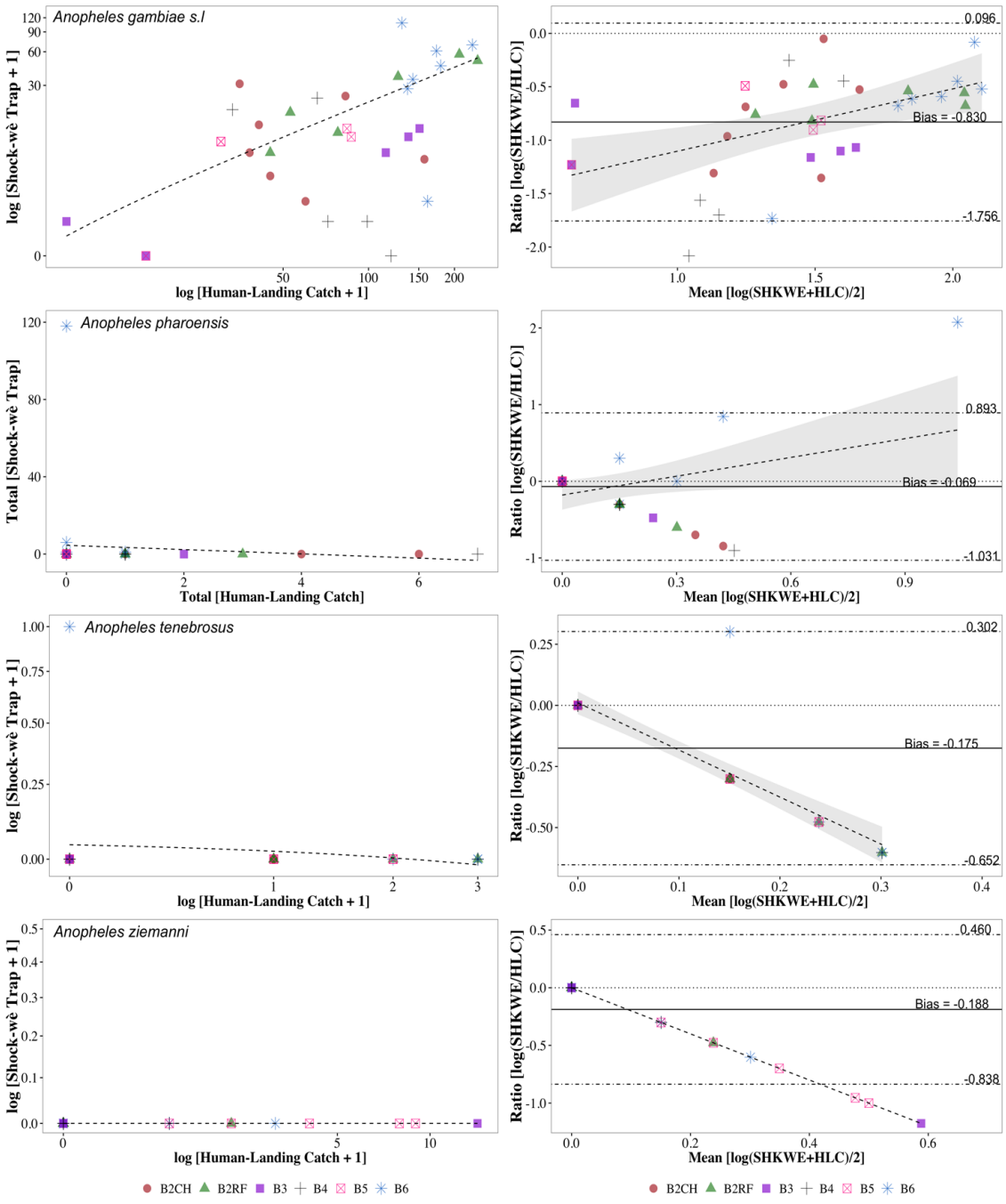


Figure 20. Bland and Altman plots showing the mean difference of bias (solid line), interval of agreement (twodash lines) and the proportional variation of the catch efficiency between HLC and SHK-wè trap catches in sampling the most common *Anopheles* species of Massavasse village indoors. The shaded area between the regression line represent the standard error (± se) of the mean efficiency expressed as the ratio between the logarithm of the catch from HLC logarithm of the catch from SHK. The legend symbols indicate Massavasse village regions where the experiments were undertaken, namely: neigbourhood 2 near irrigation channel (B2CH) and rice field

(B2RF), neigbourhoods 3 (B3), neigbourhoods 4 (B4), neigbourhoods 5 (B5) and neigbourhoods 6 (B6).

Table 3. Multiple comparisons analysis using TukeyHSD showing the significance of the difference between HLC and SHK sampling efficiency in collecting the most common *Anopheles* species indoors according to species and region. Sandwich estimator (Sn) of covariance matrix (312) was used to correct the effect of any possible variance heteroscedasticity between groups on the estimates of the sampling efficiency.

Comparison	**Efficiency**	**Std. Error**	***t* value**	***P*-value**
Species				
A. pharoensis vs. *A. gambiae*	-0.761	0.082	-9.336	**< 0.001**
A. tenebrosus vs. *A. gambiae*	-0.918	0.082	-11.254	**< 0.001**
A. ziemanni vs. *A. gambiae*	-0.924	0.082	-11.336	**< 0.001**
A. tenebrosus vs. *A. pharoensis*	-0.156	0.082	-1.918	0.461
A. ziemanni vs. *A. pharoensis*	-0.163	0.082	-2.000	0.407
A. ziemanni vs. *A. tenebrosus*	-0.007	0.082	-0.082	1.000
Neighbourhood				
Neighbourhood B3 - Neighbourhood B2	0.049	0.090	0.548	0.999
Neighbourhood B4 - Neighbourhood B2	0.164	0.090	1.826	0.526
Neighbourhood B5 - Neighbourhood B2	-0.134	0.090	-1.495	0.753
Neighbourhood B6 - Neighbourhood B2	-0.190	0.080	-2.374	0.202
Neighbourhood B4 - Neighbourhood B3	0.115	0.108	1.063	0.948
Neighbourhood B5 - Neighbourhood B3	-0.183	0.108	-1.699	0.616
Neighbourhood B6 - Neighbourhood B3	-0.239	0.100	-2.393	0.194
Neighbourhood B5 - Neighbourhood B4	-0.298	0.108	-2.763	0.080
Neighbourhood B6 - Neighbourhood B4	-0.354	0.100	-3.541	**0.008**
Neighbourhood B6 - Neighbourhood B5	-0.056	0.100	-0.557	0.999

5.4.2. Efficiency of Shock-wè on sampling malaria vectors outdoors

Evaluation of the SHK-wè trap's sampling efficiency for *Anopheles gambiae s.l* and *Anopheles pharoensis* indicated similar performance (Figure 21). There was significant linear association between the logarithms of *A. gambiae s.l* daily catches from SHK-wè trap and HLC (R^2_{adj} = 0.1; p = 0.037); and, as well as, the logarithms of daily catches of *A. pharoensis* (R^2_{adj} = 0.147; p = 0.013). However, the results of average sampling bias (± 95% agreement interval) indicate that SHK-wè trap sampled -4.46 (-10.61– -1.87; t = 20.03, p <0.001) and -2.74 (- 8.33 – -1.11; t = 10.52, p<0,001) times less *Anopheles gambiae s.l.* and *Anopheles pharoensis* respectively, than HLC. Linear regression analysis of trends of sampling bias indicates that the catch efficiency of SHK-wè trap was not significantly dependent on the variation of mosquito density both for *A. gambiae s.l* (R^2_{adj} = 0.0629, p = 0.0791) and *A. pharoensis* (R^2_{adj} = 0.0117, p = 0.245). For *Anopheles tenebrosus* (R^2_{adj} = 0.124; p = 0.02145) and *Anopheles ziemanni* (R^2_{adj} = 0.448; p <0.0001), there was also significant correlation between the daily catches of those species obtained by SHK-wè and HLC (Figure 21, left-side). However, the mean sampling bias indicated that SHK-wè trap collected -3.01 (-7.33 – -1.24) and -2.03 (-7.03 – 1.71), respectively, less *A. tenebrosus* and *A. ziemanni* than HLC and; linear regression analysis indicated that catch efficiency depended significantly on the density of the two mosquito species (Figure 21), that is, *A. tenebrosus* (R^2 = 0.1726; p = 0.0096), *A. ziemanni* (R^2 = 0.537; p <0.001).

Analysis of variance indicated that the relative sampling efficiency of the two types of catches varied significantly according to species (F = 15.488; p <0.001) and regions (F = 6.986; p <0.001). The most significant difference in sampling efficiency was observed between the catch of *Anopheles gambiae* and the other *Anopheles* species (Table 4). Moreover, a significant bias in sampling efficiency was observed between the overall catches from regions B2 and those of B4 and B5 (Tab. 4). As with the indoor data, this suggests that the efficiency of the two methods in collecting *Anopheles* outdoors was more biased toward both the most abundant species and regions with high mosquito density.

Table 4. Multiple comparisons analysis using TukeyHSD showing the significance of the difference between HLC and SHK sampling efficiency in collecting the most common *Anopheles* species outdoors according to species and region. Sandwich estimator (Sn) of covariance (312) was used to correct the effect of any possible variance heteroscedasticity between groups on the estimates of the sampling efficiency.

Comparisons **Species**	**Efficiency**	**Std. Error**	***t* value**	***P*-value**
A. pharoensis vs. *A. gambiae*	-0.487	0.117	-4.166	**< 0.001**
A. tenebrosus vs. *A. gambiae*	-0.392	0.117	-3.352	**0.015**
A. ziemanni vs. *A. gambiae*	-0.789	0.117	-6.745	**< 0.001**
A. tenebrosus vs. *A. pharoensis*	0.095	0.117	0.814	0.988
A. ziemanni vs. *A. pharoensis*	-0.302	0.117	-2.579	0.127
A. ziemanni vs. *A. tenebrosus*	-0.397	0.117	-3.393	**0.013**
Neighbourhood				
Neighbourhood B3 - Neighbourhood B2	0.550	0.129	4.277	**< 0.001**
Neighbourhood B4 - Neighbourhood B2	0.526	0.129	4.091	**0.001**
Neighbourhood B5 - Neighbourhood B2	0.198	0.129	1.538	0.726
Neighbourhood B6 - Neighbourhood B2	0.296	0.115	2.580	0.126
Neighbourhood B4 - Neighbourhood B3	-0.024	0.155	-0.155	1.000
Neighbourhood B5 - Neighbourhood B3	-0.352	0.155	-2.278	0.246
Neighbourhood B6 - Neighbourhood B3	-0.255	0.143	-1.778	0.560
Neighbourhood B5 - Neighbourhood B4	-0.328	0.155	-2.123	0.330
Neighbourhood B6 - Neighbourhood B4	-0.231	0.143	-1.610	0.677
Neighbourhood B6 - Neighbourhood B5	0.098	0.143	0.683	0.996

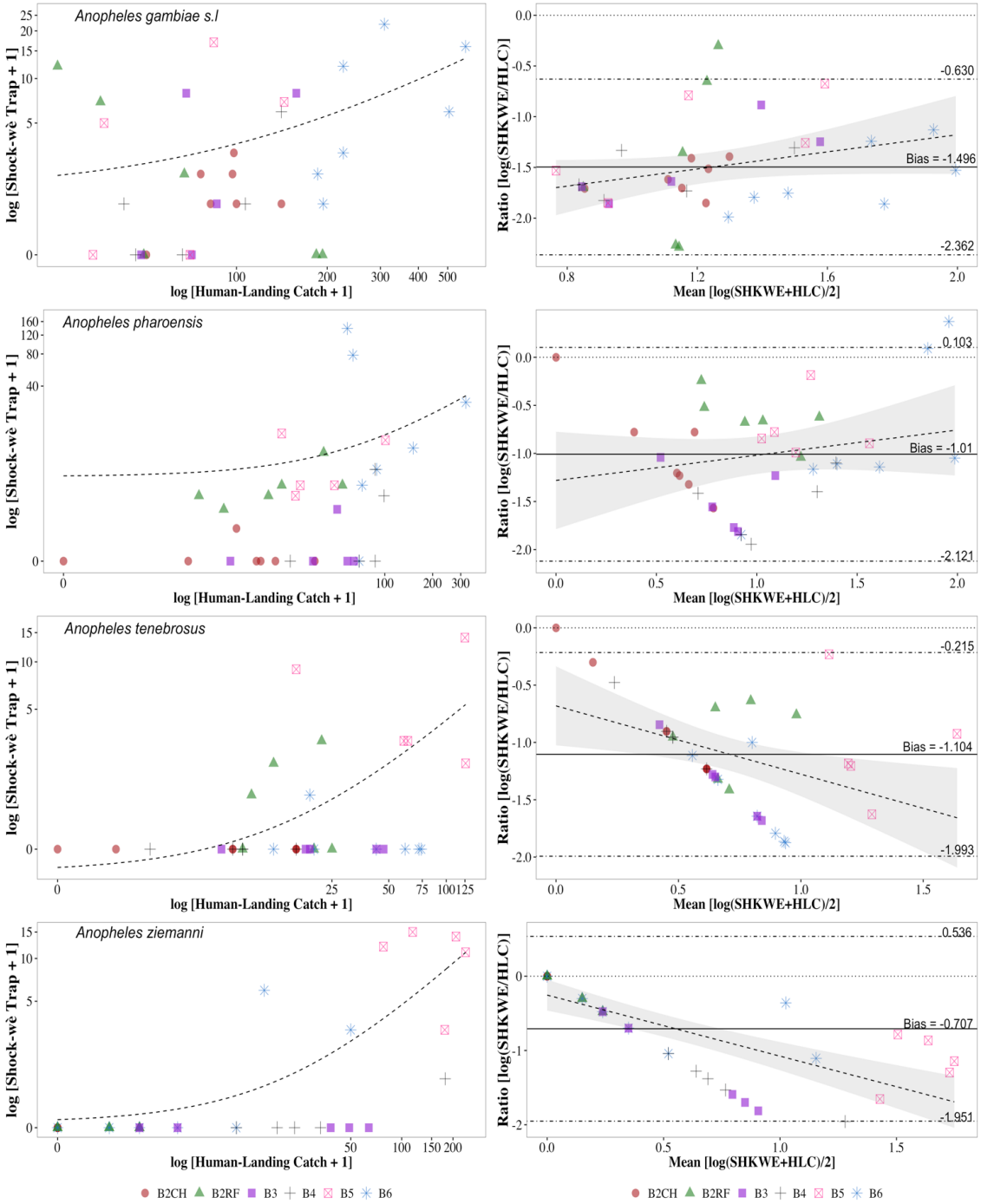


Figure 21. Bland and Altman plots showing the bias (solid line grey), interval of agreement (twodash line) and the proportional variation of the catch efficiency between Human landing catches and Shock-wè trap catches in sampling the most common *Anopheles* species of Massavasse village outdoors. The shaded area between the regression line represent the standard error (±se) of the mean efficiency expressed as the ratio between the logarithm of the catch from HLC logarithm of the catch from SHK. See figure above for the meanings of legend symbols.

5.5. Response of malaria vectors to houses structural components

5.5.1. Species composition and mosquito density

The total numbers of mosquitoes collected from all indoor and outdoor control points are reported in the table 5. A total of 23, 249 mosquitoes, comprising 17 species in 5 genera, were collected over 50 nights of sampling. *Mansonia sp.* was the most common genus (n = 20,884) followed by *Culex* (n = 1,828), *Anopheles* (n = 527) *Ochlerotatus* (n = 8) and *Coquilletidia* (n = 2). *A. gambiae s.l* was by far the most common *Anopheles* species collected. Similarly, *Cx tritaeniorhynchus*, *Cx. quinquefasciatus*, *Cx. poicilipes* and *Cx. antennatus* were the most frequently sampled *Culex* species, whilst both species of *Mansonia*, *M. uniformis* and *M. africana* were abundant (18,999 and 1885 respectively).

There was significant difference of relative density of mosquitoes between the two neigbourhoods studied. Great number of mosquito was collected in neighbourhood 6 than neighbourhood 5 ($t = -16.83$; $p<0.0001$).

Table 5. Richness and total combined number mosquitoes collected at manipulation and indoor vs. outdoor controls collection points.

Species	Total collected	Relative density (%)
Anopheles funestus	51	0.22
Anopheles gambiae	398	1.71
Anopheles pharoensis	41	0.18
Anopheles tenebrosus	10	0.04
Anopheles ziemanni	27	0.12
Ochlerotatus sp	8	0.03
Coquilletidea aurites	2	0.01
Culex antennatus	284	1.22
Culex sinaiticus	9	0.04
Culex bitaeniorhynchus	8	0.03
Culex pipiens sp	29	0.12

Culex poicilipes	291	1.25
Culex quinquefasciatus	306	1.32
Culex sitiens	14	0.06
Culex tritaeniorhynchus	887	3.82
Mansonia africana	1885	8.11
Mansonia uniformis	18999	81.72

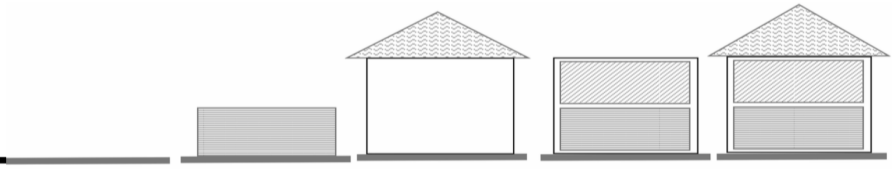
5.6. Effect of house structure on mosquito catches

5.6.1. Effect on *Anopheles gambiae s.l.* population

Table 6 shows the results of the response of *Anopheles gambiae s.l.* to manipulation of hut structures. Of the 398 mosquitoes collected, 76.88% (306/398) and 7.54% (30/398) were sampled inside the indoor control hut and outdoor control sentinel point, respectively. The remaining 62 mosquitoes were collected at the treatment assignment sentinel point, that is, 8.04% (32/398) in the complete hut; 2.26% (9/398) with the lower or both lower and upper walls, without roof, respectively, 1.76 (7/398) with roof only and 1.26% (5/398) with no treatment (Table 6).

Results of mosquito incidence rate ratio (IRR) estimates, obtained by fitting Generalized estimating equations (GEE) to the data, indicate that, compared with no treatment, there was a 4.83 greater chance [(2.03 – 11.54), p <0.001)] of *A. gambiae s.l.* being caught in the experimental hut when the lower and upper walls and the roof were present. In contrast, the presence of roof without walls may have prevented 87% [IRR = - 0.13 (-1.12 – 0.99), p = 0.826] of mosquitoes from entering the experimental hut, whilst, walls without roof and lower walls only, also without roof, prevented, respectively, 72% [IRR = - 0.28 (-0.76 – 1.31), p = 0.826] and 52% [IRR = 0.48 (-0.53 – 1.48), p = 0.349]. However, the non-significant difference of *A. gambiae s.l.* suppression by the presence of aforementioned three types treatment compared with no treatment levels (Tab. 6), suggests that the density of mosquitoes collected was, at some point, similar among the three type of treatment levels (Fig. 22).

Table 6. Marginal mean and Incidence rate ratio (IRR ± 95% Wald CI) of *A. gambiae s.l.* collected at different treatment levels.



Estimates	No treatment	Lower walls only	Roof	Walls only	Complete hut
Number collected	5	9	7	9	32
Mean (± 95% Wald CI)	0.73 (0.27 - 1.98)	1.11 (0.78 - 1.58)	0.68 (0.36 - 1.27)	0.81 (0.47 - 1.39)	3.36 (1.65 - 6.84)
IRR (± 95% Wald CI)	-	0.48 (-0.53 - 1.48)	-0.13 (-1.24 - 0.99)	-0.28 (-0.76 - 1.31)	1.50 (0.63 - 2.37)
P values	-	0.349	0.826	0.826	**0.001**

Pair-wise comparisons also showed that the mean number of mosquitoes collected when the hut was fully built was significantly higher compared to all other treatments, including the outdoor control catches. However, no statistically significant differences were observed between the mosquito catches from indoor control and the completely built experimental hut also that, the mean number of mosquito collected when the treatment was walls, roof and lower walls only, respectively, did not differ with the number collected when there was any type of treatment (Fig. 22). Moreover, the number of mosquito collected with each one of the previously mentioned four types of treatments did not differ significantly compared to outdoor control but differed compared to indoor control.

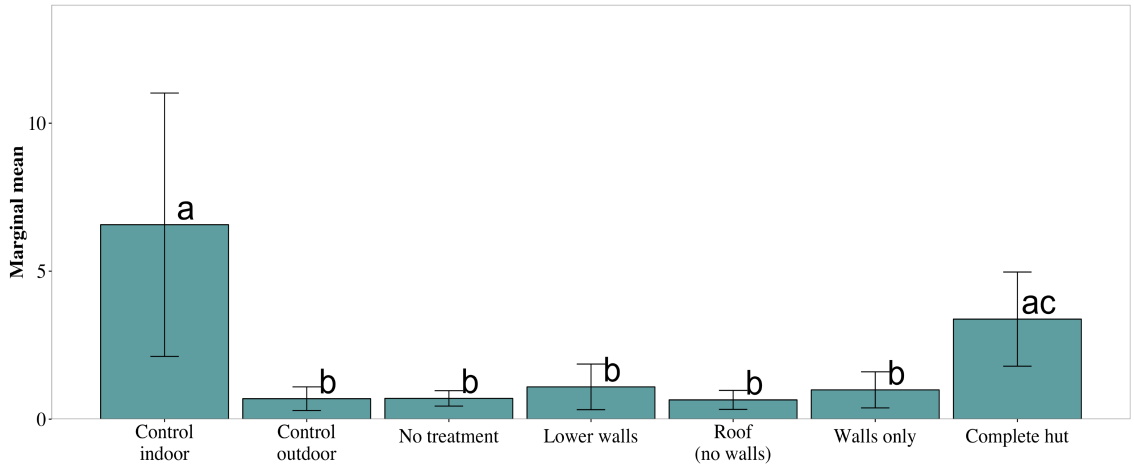


Figure 22. Results of pairwise comparisons of the differences between the mean catch of *Anopheles gambiae s.l.* obtained in the presence of all five types of manipulation

tested. Error bars represent Wald´s confidence interval. Bar followed by same letters are not significantly different at 5% significance level.

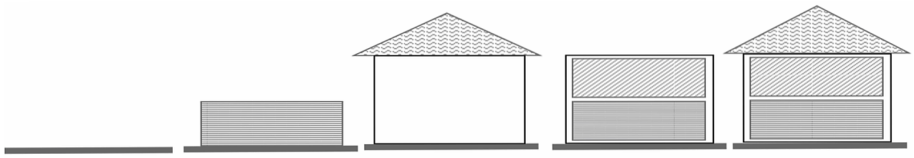
5.6.2. Effect on other vector species of medical importance

Result of the effect of treatments on the collection and entry rate of the three most common *Culex* mosquitoes with distinct feeding habits and, that were frequently collected with *A. gambiae s.l.* is showed in table 7. The probability ratio, calculated by the antilog of Incidence Rate Ratio (IRR), of *Cx. quinquefasciatus* occurrence indoors increased significantly with the increase of walls height (Tab. 7), that is, from 4.14 [IRR = 1.42 (0.33 – 2.05), p = 0.01] with lower walls to 7.54 [IRR = 2.02 (0.57 – 3.49), p = 0.006] and 8.93 [IRR = 2.19 (0.89 – 3.49), p = 0.001] when the experimental hut was fully walled but without a roof, and when it was completely built, respectively. However, there was no significant difference between the mean numbers of mosquitoes collected in the aforementioned three types of treatments (Tab. 7).

Significantly higher numbers of *Cx. tritaeniorhynchus* occurred either when the walls were at lower height [IRR = 2.07 (1.71 – 2.42), p <0.0001] or when the roof was in place without walls [IRR = 1.26 (0.96 – 1.56), p <0.0001] (Tab. 7). The presence of high height walls with roof might have significantly prevented the entry of 5% [IRR = 0.95 (0.12 – 1.78), p = 0.025] of *Cx. tritaeniorhynchus*.

There was significant reduction of *Culex poicilipes* number associated with presence of walls and roof. The probability of *Cx. poicilipes* being caught in the complete hut was 3.63 [IRR = -126 (-1.90 – 0.68), p <0.0001)] lower compared with no treatment (Tab. 7).

Table 7. The influence of different type of experimental hut structural manipulations on the number of *Culex quinquefasciatus*, *Cx. tritaeniorhynchus* and *Cx. poicilipes* collected in neighbourhood 5 and 6 of Massavasse village from April to June 2016.



	No treatment	Lower walls only	Roof	Walls only	Complete hut
Estimates					
			Culex quinquefasciatus		
Total collected	1	4	1	22	38
Mean (± 95% Wald CI)	0.58 (0.19 - 1.75)	2.42 (1.30 - 4.50)	0.30 (0.11 - 0.77)	3.02 (1.07 - 8.58)	3.27 (0.82 - 12.98)
IRR (± 95% Wald CI)	-	1.42 (0.33 - 2.51)	-0.33 (-1.16 - 0.51)	2.02 (0.57 - 3.49)	2.19 (0.89 - 3.49)
***P* values**	-	**0.011**	0.446	**0.006**	**0.001**
			Culex tritaeniorhynchus		
Total collected	13	180	119	50	78
Mean (± 95% Wald CI)	2.19 (0.79 - 6.07)	20.19 (8.11 - 50.27)	11.11 (5.08 - 24.30)	5.59 (1.78 - 17.55)	7.57 (2.45 - 23.41)
IRR (± 95% Wald CI)	-	2.07 (1.71 - 2.42)	1.26 (0.96 - 1.56)	-0.59 (-0.14 - 1.31)	0.95 (0.12 - 1.78)
***P* values**	-	**<0.0001**	**<0.0001**	0.113	0.025
			Culex poicilipes		
Total collected	32	25	37	18	8
Mean (± 95% Wald CI)	5.60 (3.35 - 8.84)	4.29 (2.65 - 6.94)	4.08 (2.18 - 7.63)	2.73 (0.90 - 8.26)	1.70 (0.85 - 3.41)
IRR (± 95% Wald CI)	-	-0.42 (-0.77 - 0.057)	-0.24 (-0.85 - 0.37)	-0.73 (-1.9 - 0.68)	-1.29 (-1.90 - 0.68)
***P* values**	-	**0.023**	0.445	0.218	**<0.0001**

5.7. Influence of environmental and climate factors

5.7.1. Descriptive summary of environmental and climate data

The variations of daily air temperature, rainfall wind speed and direction patterns during the study period (15th February to 31st May 2016) are shown in the figures 23 and 24, respectively. The mean air temperature (± standard deviation) was 25.08 ºC (± 6.13) and mean rainfall (± SD) was 1.21mm (± 4.26).

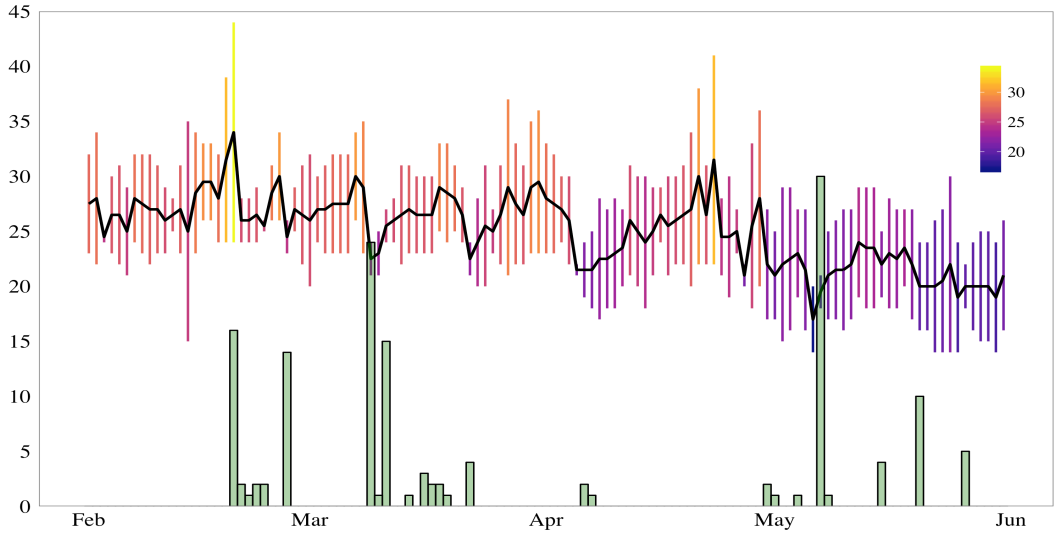


Figure 23. Mean daily air temperature (ºC**,** black line) and precipitation (mm, green bars) in Lionde administrative region, from 15th February to 1st June 2016. Colour key indicates fluctuations between the low and high temperatures (ºC**)**.

The wind direction was most frequently from East-South-East, corresponding to an angle in degrees Celsius (± circular Standard Deviation) of 109.98 ± 25.57ºC. The average wind speed (± SD) and wind gust was 9.29 ± 5.54 kmh^{-1} and 22.19 ± 7.10 kmh^{-1}, respectively.

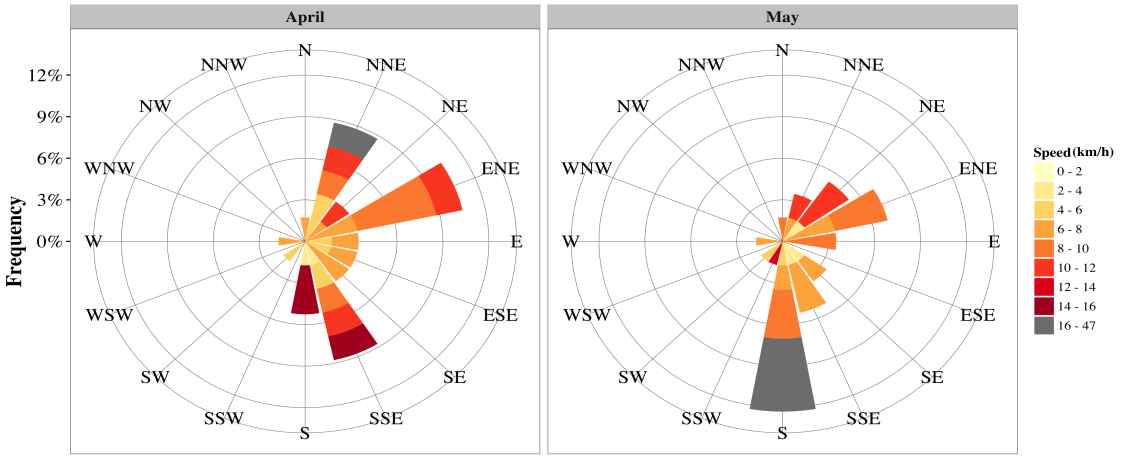


Figure 24. Patterns of wind direction (in degrees Celsius) and wind speed (in km/h) in Massavasse village from April to May 2016.

5.7.2. Effect of environmental and climate factors on the sampling efficiency of Shock-wè trap.

The effect of moon phases, daily variations of air temperature on sampling efficiency of both SHK-wè trap and HLC was analysed. There was no significant effect of either moon phases (F = 1.20; p= 0.142) or air temperature (F = 0.53; p = 0.472) variations on the performance of either sampling method in collecting *A. gambiae s.l* indoors, where the effect of moon phase and air temperature combined explained only 17.6% of variation in *A. gambiae s.l* sampling efficiency (p = 0.199). Analysis of the influence of moonlight on the sampling efficiency of outdoor host-seeking *Anopheles* is reported on table 8. Results suggest that the Shock-wè trap caught significantly fewer (3.97 times fewer) *A. pharoensis* on nights when the moon was full (F = 7.90; p = 0.0034).

Table 8. Efficiency of Shock-wè trap on sampling host-seeking anophelines outdoors at Massavasse village, from 16th February to 09th April 2016.

Species	Sampling efficiency				*P values*
	New moon	**First quarter**	**Full moon**	**Last quarter**	
Anopheles gambiae s.l	1.58 (1.05 - 2.11)	1.58 (1.37 - 1.79)	1.61 (1.42 - 1.80)	1.16 (0.60 - 1.74)	0.356
Anophles pharoensis	0.92 (0.56 - 1.26)	0.91 (0.56 - 1.26)	1.38 (1.10 - 1.66)	0.64 (0.39 - 0.89)	**0.0034**
Anopheles tenebrosus	1.41 (0.75 - 2.06)	1.10 (0.71 - 1-50)	1.13 (0.94 - 1.32)	0.91 (0.55 - 1.27)	0.542
Anopheles ziemanni	0.61 (0.03 - 1.21)	0.40 (0.02 - 0.78)	0.95 (0.47 - 1.42)	0.81 (0.36 - 1.27)	0.802

Variations in air temperature were associated with approximately 75% of the variation in sampling efficiency of both methods, indicating that sampling bias of the two methods varied proportionally, reducing nonlinearly when the air temperature was higher than 25 ºC (F = 2.10, p = 0.00934) (Figure 25).

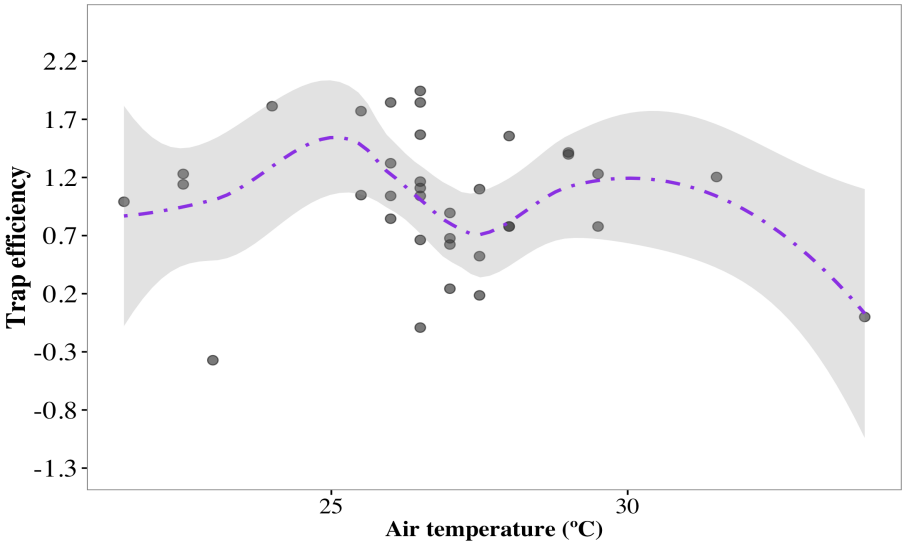


Figure 25. Effect of fluctuation of air temperature on the sampling efficiency of *Anopheles pharoensis* in Massavasse village, from 16 February to 09 April 2016. The shaded area represents the 95% confidence interval of the ratio of log(HLC catches) to log(Shock-wè trap catches).

There was no evidence of any effect of moonlight on the sampling efficiency of the other mosquito species (Tab. 8). However, results indicated a non-linear association between variation of air temperature and the deviance of 50% of sampling efficiency of *A. tenebrosus* by the two methods (figure 26). The sampling bias between the two methods reduced significantly with increase of air temperature ($F = 10.37$; $p = 0.0065$).

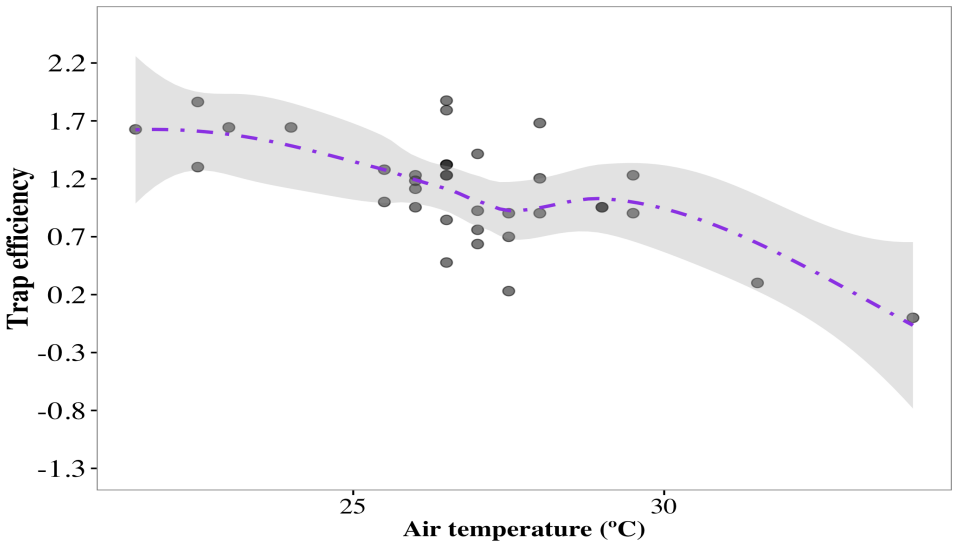


Figure 26. Effect of variation of air temperature on the sampling efficiency of the Shock-wè trap for *Anopheles tenebrosus* in Massavasse village, from 16 February to 09

April 2016. Shaded areas represent 95% confidence interval of the ration of log(HLC catches) to log(Shock-wè trap catches).

5.7.3. Influence of moonlight, environmental factors and level of manipulations on mosquito density.

Analysis using a generalized additive model (GAM) with Poisson distribution error indicated a significant interaction between some levels of treatment and moon phase and the number of *A. gambiae s.l.* collected (χ^2_{wald} = 38.75, p = 0.0137). There was significant reduction of mosquito from new moon to crescent phase (first quarter) and then an increase toward full moon but in general, the number collected on moonlit nights was less than that collected at non-moonlit nights (t = -3.38; p = 0.00286) (Figure 27).

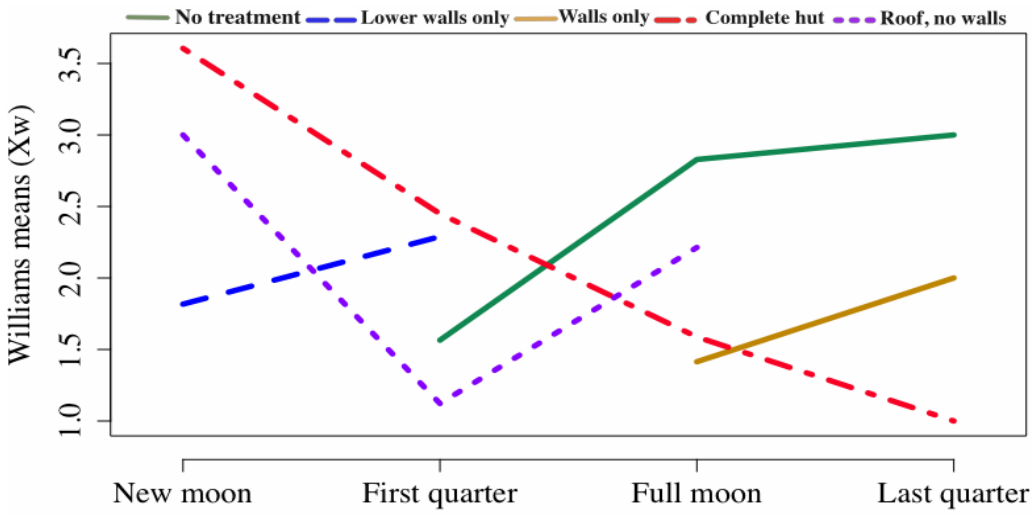


Figure 27. Interactions between moonlight and type of structural manipulation on the number of *A. gambiae s.l.* collected.

There was also significant non-linear correlation between the changing of wind direction and mosquito catches (χ^2wald = 11.8; p = 0.0377). Changing of wind direction significantly explained 67.2% (R^2 = 0.724) of the deviances of mosquito catches during the experiment (Fig. 28). Results indicated that more mosquitoes arrived at the sentinel post when the wind was blowing from East and southeast quadrants, that is, when the wind speed did not exceeded 4 km/h. However, no significant association was observed between variations of either wind speed (p = 0.255) or gust (p = 0.297) and mosquito numbers collected during the experiment.

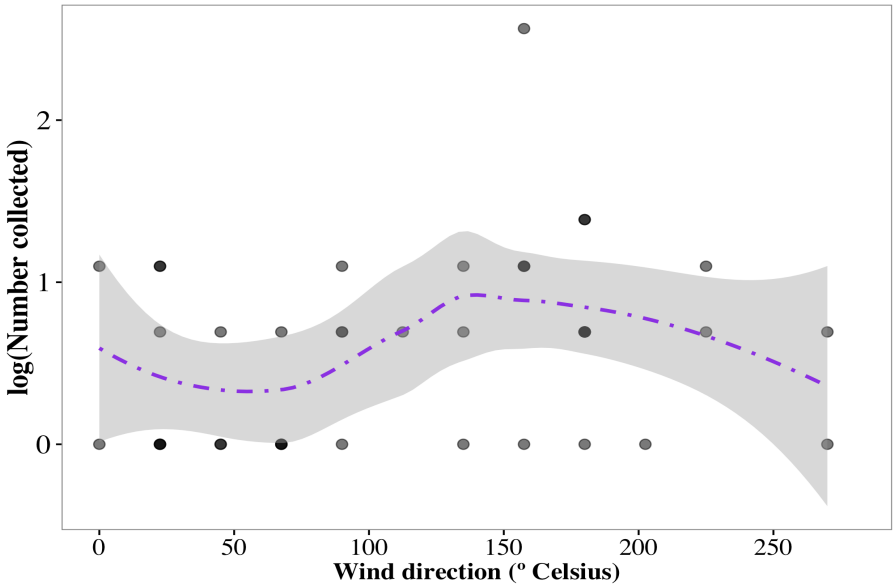


Figure 28. Relationship between variation of wind direction and the number of mosquito collected during the experiment period (11th April to 31st May 2016) in Massavasse village.

6. General discussion

6.1 Shock-wè trap as an exposure-free alternative to landing catches

Results of the evaluation of the performance of Shock-wè trap (SHK-wè) in comparison with human landing catches (HLC) indicated that the sampling bias between the two methods in collecting *Anopheles gambiae s.l* was significantly different from zero (*i.e.* 0.83) suggesting that, on average, SHK-wè collected relatively 2.29 times fewer *A. gambiae s.l* than HLC. The efficiency of SHK-wè was density-dependent indicating that the trap may have under-estimated endophagic members of the *A. gambiae* complex in neighbourhoods with relatively lower mosquito abundance. In contrast, such a density dependent effect was not observed with outdoors catches of *A. gambiae s.l*, although the average sampling bias outdoors was relatively higher than that obtained indoor with the same vector. A possible explanation for this conflicting result may have been due to site and species-specific differences in the responses of different members of the *Anopheles gambiae* complex from Massavasse village to electrocution traps. This difference was previously observed with *A. gambiae s.s.* and *A. arabiensis* in Tanzania in a study using basic insect electrocution devices (313). The authors collected more mosquitoes with electrocution trap outdoors than indoors.

Previous surveys performed in Massavasse suggested that *A. arabiensis*, a malaria vector with a wide range of feeding habits, was the most common member of *Anopheles gambiae* complex occurring in the village (283). In that study, a series of paired spatial collections reported that *A. arabiensis* was less common in the CDC light-trap than the tent trap, suggesting that the vector might exhibit within-species differential response to traps. Further field evidences suggest that there may be age-specific responses of malaria vectors to collection methods. Paired collections and field comparisons of the performance of CDC light traps and HLC in sampling Asian (*A. culifacies*, *A. farauti s.l*) and African (*A. gambiae s.l*) malaria vectors, reported that HLC consistently collected more young (nulliparous) females than light traps (254, 255, 314, 315). In this study dissections to determine the age structure of *A. gambiae s.l* were not carried out so, whether age-specific differential response to both methods might explain the variability and bias in mosquito catches is not known.

For *Anopheles pharoensis*, results showed that SHK-wè and HLC sampled proportionally the same quantity of mosquito, irrespective of site (i.e. indoor vs outdoor). The sampling bias, particularly from indoor catches, was nearly zero and there was no evidence of any density dependent sampling by SHK. This may be the first evidence of field satisfactory result of collections of this possibly important exophilic vector using an alternative to the HLC method. However, close inspections of the results suggest the efficiency of SHK-wè in sampling *A. pharoensis*, particularly indoors (Fig 20) may have been influenced by exceptionally higher mosquito density values such as those found at neighbourhood 6, whilst the efficiency outdoor may have been influenced by variations of moonlights. Field reports about the negative influence of moonlight on the efficiency of mosquito traps are not unusual (250, 251, 316). In fact, moonlight has been found to exert influences on both abundance, biting cycle and reproductive cycles of important malaria vectors such as *A. gambiae s.l* (317), *A. farauti* (52, 318) and *A. funestus* (53).

SHK-wè performed poorly relative to HLC irrespective of location for *Anopheles constani* group, *A. tenebrosus* and *A. ziemanni*. Though rarely mentioned in the literature, both species are vectors, whose role in malaria transmission has been confirmed elsewhere (319, 320). Here, the trap performance showed highest bias and strong density dependence (Fig. 20 and Fig. 21). Published reports evaluating the sampling efficiency of HLC and other alternative method for collection of *A. tenebrosus* and *A. ziemann* are scant. In Senegal, Dia *et al.* (321) collected these two species in landing catches but not in an odour-baited entry trap. On this basis, it can be hypothesised that SHK-wè trap might perform better than OBET in sampling *A. tenebrosus* and *A. ziemann* as well.

In general, HLC collected more anophelines than SHK-wè trap but the two methods detected effectively the same range of species (Tab. 2, Fig. 17). Similar findings have been reported in other comparisons between HLC and CDC light traps (253-255). Unfortunately, no field or laboratory studies have determined the reason why differences occur between absolute HLC catches and numerous proposed alternative methods. Such information would help to maximize the sampling performance of subsequent candidate methods. However, differences in stimuli presented to vectors and

the effectiveness of these stimuli is likely to be one of the main causes of the discrepancies found. HLC combines a complete broad range of stimuli whilst light traps, even when deployed close to an occupied bed net (and therefore exploiting the host attractants nearby), is dependent on visual stimuli whose role on attracting night-biting mosquito is poorly understood. In fact, some of the light trap's inherent properties may even be repellent to host seeking mosquitoes. Moreover, light traps collect females of nearly all gonotrophic conditions (unfed, fed and gravid females), rendering catches unrepresentative of the host seeking population and unreliable for estimation of practical malaria transmission indexes (239, 240, 255).

Limitations also exist on the performance of synthetic odour-baited traps, such as carbon dioxide-baited trap; OBET; Mbita trap; Magnet® trap; mosquito landing box; Mosquito electrocution trap; Suna trap, etc (270, 272, 322-325). Increasing evidence suggests that odour blends are less effective at close-range, where warmth and humidity, rather than odours, play an important and possible primary role in orientation and stimulating landing (177, 217, 326). Additionally, the use of chemical compounds derived from human odour or synthetic mimics of human odours may attract less efficiently than the complete host or cause the trap to under-estimate the importance of predominantly zoophilic vectors that will also feed on humans. It has been recently demonstrated that the same odour concentration can be attractive and repulsive depending on the physiological status of the targeted insect (327).

The SHK-wè is a fully human-baited trap and in this first evaluation, all catches were comprised entirely of unfed females attesting to its suitability as a replacement for HLC. It provided an accurate sample of the relative abundance of the entire range of species in the study site and was satisfactory indoors and outdoors. Hence SHK-wè catches can be considered as reliable as those obtained from HLC for the estimation of malaria transmission risks. Furthermore, the trap offers an alternative to explore in real time the key behavioural components concerning host-finding behaviour by disease vectors using recently developed image-capturing technology such as those reported by Parker *et al*., (285). However, it is also worth noting that, although both SHK-wè and HLC employ the same source of stimuli, it is very unlike that the two methods could, thereby, collect absolutely the same quantity of mosquitoes since both are subjected to

imprecisions and analytical errors that can generate significant levels of variability (302-304). Moreover, as with all sampling devices, both baited and non-baited, SHK-wè is subject to the influence of variation in environmental and climatic conditions, particularly wind and moonlight (212, 250, 251, 328, 329). However, as Hii *et al*., (255) remarked, the lower numbers of mosquitoes caught by an alternative method do not *per se* invalidate the use of the method for estimation of relevant entomological indexes of malaria vectors populations; the crucial criterion is whether the new method is collecting mosquitoes that are in proportion to the gold standard. This condition is fulfilled by SHK-wè for *A. gambiae s.l* and *A. pharoensis* but not for *A. tenebrosus* and *A. ziemanni*. Additional field studies need to be conducted in other locations and with other populations and species of mosquitoes, particularly *Anopheles sp.* to evaluate its reliability thoroughly.

6.1.2. Observations on limitations of the Shock-wè trap

While performing the experiments in the field some operational constraints concerning the usage of SHK-wè trap were recorded. It was noted that the high voltage would sometimes inflict significant damage on mosquitoes, particularly on small-sized mosquitoes such as *Anopheles funestus*, rendering them difficult or even impossible to identify morphologically. Further laboratory experiments need to be carried out to reduce the voltage to an optimal level that will avoid such severe damage while not compromising the performance of the trap in sampling larger mosquitoes such as *Mansonia sp*.

Secondly, adapting the trap to allow it to be powered by DC battery source rather than a portable AC generator as at present would also be a distinct advantage.

6.2. What does a mosquito perceive as 'indoors'?

Using the manipulated experimental hut, the results of experiments to determine the influence of the main structural components of human houses on entry rates by mosquitoes indicated that different genera/species were influenced by or responding to different elements of the structure. The probability of sampling *Anopheles gambiae s.l* inside the hut increased by a magnitude of approximately 5 times when all the walls and

roof were assembled together (*i.e.* the complete hut structure) compared to other levels of treatments (Tab. 6). Moreover, there was no significant differences between the mean numbers of mosquitoes collected indoors compared to others remaining levels of treatments tested (Fig. 22). These findings suggest that the presence of roof might be a key structural component perceived by the endophagic/endophilic fraction of *A. gambiae s.l* complex populations, as an indicator of a structure, or the point of entry into that structure, associated with the presence of humans or associated animal hosts. The roof may also help direct or focus an odour plume coming from inside houses toward an insect approaching upstream.

Eaves may be one of the most important routes for *A. gambiae s.l* entry into houses (282, 330-332). In the present study, the results showed that the likelihood of *A. gambiae s.l* finding such a route reduced significantly – by nearly 72% (1 - 0.28) - when the roof was absent. An earlier and very similar study by Snow (282) reported an increasing rate of *A. gambiae s.l* entry as direct consequence of increasing walls height. Unfortunately, the differences in the experimental hut designs between the present study and that of Snow (282) prevent direct comparisons. During Snow´s experiments, the roofing of the experimental hut was kept intact so he was unable to capture the importance of that structural component on house entry habit.

Endophily may have evolved independently from anthropophagy and the behaviour that influences location and house entry in several vector populations may differ from that of host seeking (53). Results for *Culex quinquefasciatus*, another species also highly associated with endophilic environments (Tab. 7), suggested that the perception of indoors by that vector species differed dramatically from *A. gambiae s.l.* Here, the probability ratio of *Cx. quinquefasciatus* occurrence indoors increased significantly with the increase of walls height from 1.42 (lower walls) to 2.02 and 2.19, when the experimental hut was fully walled but without a roof, and when it was completely built, respectively (Tab. 7). Since, there was no significant difference between the mean numbers of mosquitoes collected in the aforementioned three type of treatments, these results suggest that the presence of walls, not roof, may be the most important structural component for house entry by *Cx. quinquefasciatus*. These findings also suggest that *Cx. quinqusfasciatus* might exploit different entry routes other than eaves which would

also explain why eave-screening did not impact the indoor population of *Cx. quinquefasciatus* in a study in Tanzania (333). In general, the evidence from the present study suggest that, rather than the earlier claim by Lindsay & Snow (330), the trouble may not be with eaves but rather with the roof.

Analysis of results from two other exophagic and zoophilic culicines, *Culex tritaeniorhynchus* and *Cx. poicilipes*, that are frequently caught together with *A. gambiae s.l.,* suggest great differences in the response to the hut components of those species. Significantly higher numbers of *Cx. tritaeniorhynchus* occurred either when the walls were at lower height or when the roof was in place without walls (Tab. 7). The numbers of *Cx. tritaeniorhynchus* increased by a magnitude of 4.40 when treatment was changed from roof only to lower walls. Hence, a complete or fully walled hut without a roof inhibited *C. tritaeniorhynchus*, suggesting that this species may largely avoid closed spaces. *Culex poicilipes* was greatly suppressed by the presence of walls and roof, or a complete hut. Highest numbers of both *Cx tritaeniorhynchus* and *Cx. poicilipes* occurred when the treatment levels were semi-outdoor (lower walls, no roof or roof without walls) or entirely outdoors (no treatment). These scenarios resemble the corrals or stables where these two predominantly exophagic vectors of Japanese Encephalitis and Rift valley fever virus would very likely to find their preferred source of blood meals, namely: swine (feral and domestic pigs) and wading birds (334-337). Clearly, keeping the reservoir hosts inside enclosed environments would help tackle the transmission of these important arboviruses.

6.2.1. Limitations of the study

The behavioural responses and patterns discussed here were obtained from relatively low quantities of mosquitoes. The experiments were undertaken at the beginning of winter season when mosquito populations naturally decreased. Nonetheless, the rigorous statistical approaches applied to the data indicated significant differences and the behaviour patterns are very likely to be indicative of those actually occurring in above described vector populations with regard perception of environs. Naturally, further studies need to be extended to other regions to determine whether the patterns are consistent over time and space.

7. Conclusion

Based on the results from paired comparisons between Shock-wè trap (SHK-wè) and Human landing collections (HLC) it can be concluded that SHK-wè was as effective as HLC at sampling malaria vectors and allied species, in Massavasse village. Though additional validation is required it seems likely that the trap could eventually be recommended as a reliable exposure-free alternative to HLC for sampling and monitoring the exophagic malaria vectors for *A. gambiae s.l.,* and possibly other vector populations. Further evaluation needs to be done in order to determine its accuracy when collecting indoor active vector populations and vectors of possible secondary importance such as *A. tenebrosus* and *A ziemanni*.

Malaria vectors and associated species that bite and transmit disease to humans exhibit different responses to structural component of houses or enclosures. The presence or absence of certain components will influence vector perception of environs where they are likely to get blood meals. The presence of a roof may be the key component that helps *A. gambiae s.l* recognizes a [human] house. In the absence of a roof the likelihood of *A. gambiae s.l* either being willing or able to enter houses is greatly disrupted. For *Cx. quinquefasciatus* the height of walls was the main determinant for indoor recognition.

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9. Annexes

Annex I. Copy of informed consent in Portuguese

Instituto Nacional de Saúde (INS), Maputo, Moçambique

&

Liverpool School of Tropical Medicine (LSTM)

Estudo sobre a localização dos hospedeiros pelos vectores da malária, dentro e fora das casas

Consentimento informado para participar no estudo

O Instituto Nacional de Saúde de Moçambique (INS) e a Liverpool School of Tropical Medicine (LSTM) da Inglaterra, tem estado a trabalhar em conjunto num projeto que visa determinar o comportamento de procura e localização dos seus hospedeiros pelos vectores da malária, dentro e fora das habitações humanas. Escolhemos realizar este estudo aqui na aldeia de Massavasse porque acreditamos que, os resultados que possam surgir do mesmo irão ajudar-nos na formulação de novos e melhores métodos destinados a proteger as pessoas contra as picadas de mosquito e contra a transmissão da malária.

Alguns mosquitos preferem picar as pessoas quando estas encontram-se a socializarem-se dentro das suas casas, enquanto que os outros preferem picar tanto dentro assim como fora das casas. Assim, pretendemos identificar quais os factores que permitem os vectores da malária localizarem os seus locais preferenciais de picada. Para tal, iremos usar armadilhas elétrica para a captura dos mosquitos que vem para picar um voluntário posicionado. A armadilha é segura e previne que os mosquitos entrem em contacto com o voluntário. Durante o uso da armadilha, o voluntário terá que dormir, no máximo de sete dias durante dentro de uma rede mosquiteira, e a armadilha eléctrica será montada por fora da rede, imediatamente acima do cabeça e do tronco do voluntário. A armadilha elétrica irá neutralizar e colher todos os mosquitos que tentarão alcançar o voluntário. Os mosquitos colhidos serão posteriormente identificados e processados no nosso laboratório em Maputo.

Deste modo, vimos através deste consentimento solicitar a sua participação como voluntário para este estudo. Como voluntário, deverás dormir no interior da armadilha durante pelo menos 6 horas. Durante a experiência, poderás escutar o rádio ou usar o seu celular. Contudo, não deverás usar qualquer tipo de repelente contra as picadas de insectos ou mosquitos, cremes, perfumes ou outro tipo de cosmético.

Aos voluntário, serão dispensados medidas de proteção contra malária, tais como redes mosquiteiras, Fasidar® (Sulfadoxine-Pyrimethamine) ou Malarone® (Atovaquone/proguanil), recomendado pela Organização Mundial da Saúde (OMS) para tratamento profilático contra a malária e; redes mosquiteiras para protegerem-se contra a picada de mosquitos. As redes mosquiteiras não serão tratadas com inseticidas para prevenir qualquer influencia dos insecticidas na captura dos mosquitos Adicionalmente, durante as experiências, um membro sénior da nossa equipa de trabalho presente para prestar apoio ou esclarecimento, sempre que necessitares.

Confidencialidade

Nenhuma amostra de sangue, nem mesmo os seus dados pessoais, exceptuando o seu nome e idade, serão recolhidos durante o estudo. O seu nome não irá constar em qualquer relatório ou outro tipo de documento que poderá resultar desse estudo.

Fim de participação no estudo

Sendo voluntário, não serás pressionado ou obrigado a participar em nenhuma fase do estudo. Sendo assim, quando manifestarem o desejo em deixar de participar do estudo, a sua decisão será aceite incondicionalmente, sempre que desejares fazê-lo. Recusa em participar ou abandonar o estudo não afectará o seu direito de beneficiar de qualquer tratamento médico em qualquer momento ou qualquer lugar.

Obrigado pela sua cooperação

ASSINATURAS

Voluntário alfabetizado

Se aceita em fazer parte deste estudo como voluntário por favor assine abaixo

Eu,……

…… concordo em participar deste estudo; estou ciente de que posso parar de voluntariar assim que eu desejar

Assinatura…….

Data……..

Voluntário não alfabetizado

Se o voluntário for não alfabetizado, uma testemunha alfabetizada será escolhida pelo próprio voluntário para confirmando a aceitação do mesmo em fazer parte do estudo. A testemunha escolhida pelo voluntário irá assinar o formulário de consentimento informado em nome do voluntário, conforme indicado abaixo

Assinatura

(testemunha)…….Data……

……

Nome do voluntário……Impressão digital 

Em caso de dúvida ou mais detalhes por favor contacte:

Ayubo Kampango (Pesquisador e Coordenador de Campo, Laboratório de Entomologia): +258 820087990

Ana Paula Abílio (Pesquisadora e chefe do Laboratório de Entomologia-INS): +258 821508079

Comité Nacional de Bioética para Saúde (CNBS): Tel: (+258) 824066350

Obrigado pela atenção e participação

Assinatura do investigador

Eu confirmo que testemunhei a leitura deste consentimento informado na presença do voluntário e/ou da testemunha. Sendo assim, Eu confirmo que o voluntário foi administrado este consentimento livremente

Assinatura……

Date……..

Uma cópia deste formulário deve ser retida pelo voluntário e/ou sua ou seu testemunha.

Annex II. Ethical approval granted by the "Comitè Nacional de Bioética para a Sáude" to undertake the study

REPÚBLICA DE MOÇAMBIQUE

MINISTÉRIO DA SAÚDE
COMITÉ NACIONAL DE BIOÉTICA PARA A SAÚDE
IRB00002657

Exmo Senhor
Dr. Ayubo Kampango
INS

Ref: 208/CNBS/15 **Data 22 de Julho de 2015**

Assunto: Parecer do Comité Nacional de Bioética para saúde (CNBS) sobre o estudo: "*Host Location by Exophagic African Malaria Vectors",*

O Comité Nacional de Bioética para Saúde (CNBS) analisou as correcções efectuadas no protocolo intitulado: ***"Host Location by Exophagic African Malaria Vectors"",*** Registado no CNBS com o número 61/CNBS/2015, conforme os requisitos da Declaração de Helsínquia,
Não havendo nenhum inconveniente de ordem ética que impeça a realização do estudo, o CNBS dá a sua devida aprovação aos seguintes documentos:

- Protocolo de estudo versão 3.0
- Consentimento informado versão 3.0
- Instrumento de recolha de dados versão 3.0

Todavia, o CNBS informa que:

1- A presente aprovação não substitui a autorização administrativa.
2- Não houve declaração de conflitos de interesse por nenhum dos membros do CNBS.
3- A aprovação terá a validade de um ano, terminando esta a 20 de Julho de 2016. Os investigadores deverão submeter o pedido de renovação da aprovação um mês antes de terminar o prazo.
4- Recomenda-se aos investigadores que mantenham o CNBS informado do decurso do estudo.
5- A lista actualizada dos membros do CNBS esta disponível na secretaria do Comité.

O Presidente

Dr. João Fernando Lima Schwalbach

C/c Comité Institucional de Bioética para Saúde do Instituto Nacional de Saúde

ENDEREÇO:
MINISTÉRIO DA SAÚDE
C. POSTAL 264
Av. Eduardo Mondlane/Salvador Allende
MAPUTO – MOÇAMBIQUE

Telefones: 430814/427131(4)
Telex: 6-239 MISAU MO
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258 (1) 33320

Annex III. Ethical approval granted by the Liverpool School of Tropical Medicine Bioethics Committee

LSTM
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Dr Phil McCall
Liverpool School of Tropical Medicine
Pembroke Place
Liverpool
L3 5QA

Wednesday, 28 January 2015

Dear Dr McCall,

Research Protocol (14.055) Host Location by Exophagic African Malaria Vectors

Thank you for your letter of 16/01/2015 responding to the action points requested by the Research Ethics Committee. The protocol now has in principle ethical approval from the Chair of LSTM Research Ethics Committee.

Full approval will only be issued once the necessary in-country ethical approval documents have been submitted to the Research Office. Please send the documents to the LSTM Ethics Secretariat via lstmrec@liv.ac.uk.

Failure to provide these documents will result in withdrawal of in principle approval. This will constitute a breach of the LSTM Research Code of Conduct and may result in disciplinary proceedings. Please note that no research activities can be carried out for this project until full ethical approval has been given.

Please remember to quote your ethics reference number with all related correspondence,

Yours sincerely

Dr Angela Obasi,
Chair,
LSTM Research Ethics Committee