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Importance of improved housing for reducing infectious diseases of children in sub-Saharan Africa

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Abstract

Since 2000, housing in sub-Saharan Africa (SSA) has markedly improved due to significant economic progress in the region. However, the majority of these improvements are concentrated in urban settings, leaving rural areas, primarily inhabited by impoverished communities, exposed to substandard housing and heightened risks of communicable diseases such as malaria, diarrhoea, and respiratory illnesses. Consequently, there is an imperative to devise healthier housing solutions to shield children in these communities from communicable diseases.

The screening of windows, open eaves and doors has been demonstrated to effectively reduce malaria transmission, parasitaemia, and anaemia in children. Additionally, homes equipped with constant access to clean water, proper latrines, improved flooring, and sewage systems exhibit a lower incidence of diarrheal diseases compared to those lacking these amenities. Well-ventilated houses, characterized by ample and strategically positioned windows, manifest a decreased risk of respiratory diseases such as tuberculosis.

In northern Tanzania, a novel two-storey house, featuring mosquito screens and designed to combat malaria, was piloted and demonstrated a significant reduction in the entry of malaria mosquitoes. The prototype, named the ‘Star home’, not only aims to diminish malaria but also seeks to lower the incidence of diarrheal and respiratory infections in young children compared to traditional house types. A household randomized-controlled trial assessing the impact of Star homes on the incidence of these diseases commenced in January 2022 in rural Mtwara in southern Tanzania.

My PhD evaluated the efficacy of Star homes in reducing mosquito and fly entry compared to traditional dwellings. The study comprises three parts: 1) pilot study to assess whether the light-permeable walls and the narrow eaves gaps found in Star homes increase mosquito entry in experimental huts, 2) the household RCT measuring mosquito and housefly densities, and 3) the potential risk factors contributing to the indoor abundances of malaria vectors was assessed using the traditional house type. This trial also evaluated indoor and outdoor temperature, relative humidity, and carbon dioxide levels in both control and intervention groups.

The pilot study demonstrated that transparent-walled huts, allowing light penetration from an indoor CDC light trap, increased indoor *An. arabiensis* mosquito abundance by 84% compared to opaque-walled huts with limited light visibility. Furthermore, well-ventilated huts reduced indoor *An. arabiensis* abundances by 99% compared to poorly ventilated traditional houses.

In the randomized controlled trial (RCT), Star homes exhibited a reduction in indoor mosquito density: 54% for *Anopheles gambiae s.l.*, 81% for *Anopheles funestus s.l.,* and 64% for *Culex species*. Moreover, Star homes decreased the risk of malaria transmission by 55% when contrasted with traditional houses. No discernible effects were observed on indoor environmental conditions measurements (temperature, relative humidity, and CO_2 concentrations) between Star homes and traditional houses. The main external doors of Star homes were open for 60% less time than those in traditional houses.

The Star homes also decreased domestic fly populations by over 40% in kitchens, with a 46% reduction specifically observed in *Chrysomyia putoria* populations in toilets compared to traditional houses.

When assessing potential risks factors for indoor malaria vectors (*gambiae* s.l. and *An. funestus* s.l.) hotspots for indoor malaria vectors were identified in the central and far eastern regions of the study area. Proximity to open water bodies within 15 m of a traditional house amplified indoor malaria vector abundances by 61%, while open windows doubled their presence. Conversely, increased built-up areas reduced indoor malaria vector abundance by 99%, each 100 m increase in altitude decreased it by 29%, and the presence of chickens in peri-domestic areas was associated with an 8% reduction in malaria vector abundance.

The pilot study findings informed the selection of a suitable mosquito trapping method and the design of Star homes used in the main trial. The main study emphasizes integrating various previously identified beneficial housing design elements, such as screened windows and doors, sealed eave gaps, and toilet designs with blocked main holes and smoothly cemented floors. These measures aim to minimize mosquito and non-hematophagous fly entry into dwellings, thereby reducing the transmission risk of multiple infections to children in rural areas of sub-Saharan Africa (SSA). Additionally, the results underscore the importance of improving drainage around nearby open water bodies or implementing larviciding measures to decrease malaria vector populations in the vicinity.

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Abbreviations

AA:	Double A battery
ArcGIS:	Geographical information system software
ARI:	Acute respiratory tract infection
CDC:	Centers for Disease Control and Prevention
CI:	Confidence intervals
CO_2:	Carbon dioxide
CO_2 Meter:	Carbon dioxide metre
COVID-19:	Coronavirus disease 2019
DALY:	Disability-adjusted life years
DC:	Direct current
DELIVER:	Door & Eave-gap, Lift house, Insecticidal nets, improved Ventilation, improved domestic Environment & sloping Roof
DEM:	Digital Elevation Model
DNA:	Deoxyribonucleic acid
EIR:	Entomological Inoculation Rates
ELISA:	Enzyme-Linked Immunosorbent Assays
ESA:	European Space Agency
GDP:	Gross domestic product
GIS:	Geographical information system
GLM:	Generalized linear model
GLMM:	Generalized Linear Mixed model
HAP:	Household air pollution
ID:	Identifier
IDW:	Inverse Distance Weighted
IQR:	Interquartile ranges
IRS:	Indoor residual spraying
ITNs:	Insecticide-treated bed nets
MoH:	Ministry of Health
NA:	Not applicable

NASA:	National Aeronautics and Space Administration
NBS:	National Bureau of Statistics
NDVI:	Normalized Difference Vegetation Index
NIMR:	National Institute of Medical Research
NMCP:	National Malaria Control Program
OCGS:	Office of the Chief Government Statistician
OR:	Odds ratios
PCR:	Polymerase Chain Reaction
PM:	Post Meridiem
PPM:	Parts per million
RCT:	Randomized controlled trial
RH:	Relative humidity
RR:	Rate ratio
SD:	Standard deviation
SDGs:	Sustainable Development Goals
SFS:	Semi-field system
SH:	Star homes
SPR:	Sporozoites rate
SSA:	sub-Saharan Africa
TB:	Tuberculosis infection
TH:	Traditional house
TMB:	Template model builder
UAV:	Unmanned aerial vehicle.
UK:	United Kingdom
UN:	United Nations
UNHCR:	United Nations High Commissioner for Refugees
URL:	Uniform Resource Locator
USA:	United States of America
USD:	United States Dollar
USGS:	United States Geological Survey

VICOBA: Village Community Banks

WASH: Water, sanitation, and hygiene

WHO: World Health Organization

Declaration

The content presented in this thesis has not been previously submitted for any other degree or qualification and represents the original work of the author unless explicitly stated otherwise.

Statement of Copyright

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Lastly, my gratitude goes to the rural Mtwara Village leaders and community members who generously volunteered their houses for data collection purposes.

Introduction

Previous studies have demonstrated a strong link between poor housing and ill health, particularly for communicable diseases like malaria, diarrhoea, and respiratory tract illnesses (WHO 2010, Hershey et al. 2011, Tusting et al. 2015). It has been shown that screening windows and doors and blocking or screening the eave-spaces of a house, reduces indoor mosquito biting rates, clinical malaria, and infection in children (Atieli et al. 2009, Tusting et al. 2015). Proper sanitation, a constant supply of water, appropriate waste management, and hygiene in houses reduce the incidence of diarrhoea in children (Yaya et al. 2018). Overcrowded houses with poor or no ventilation increase the risk of respiratory diseases, such as pneumonia and tuberculosis (Hill et al. 2006).

To date studies have primarily focused on assessing single housing features' efficacy against specific diseases. However, the Phase I pilot study of the Star homes project in 2015 in northeastern Tanzania introduced complete housing structures with various features, such as screened windows, sealed eave gaps, and self-closing solid doors. These features, reducing malaria vector entry by 70%-95%, garnered high community acceptance (von Seidlein et al., 2017). Prototype houses boasted 1) optimal shading, 2) durable roofs with closed eaves,3) raised concrete floors, 4) screened cooking areas, 5) lockable storage, 6) raised sleeping areas, 7) water harvesting systems, 8) fly-proof latrines, 9) shade net walls and windows, and 10) solar power. Compared to traditional houses, they were cooler, less prone to mosquito entry (70%-95% reduction), easier to clean, less smoky, offered security and privacy, water harvesting, and constant water and lighting supply. Preference for two-story buildings over single-story ones was common due to better mosquito prevention and sleeping arrangements (von Seidlein et al., 2019).

Despite the absence of an epidemiological assessment in the pilot study, it paved the way for a large-scale randomized controlled household trial (Phase II) in the rural Mtwara district, south-eastern Tanzania, to evaluate the health impact of these innovative housing designs. Consequently, our current study assesses the effectiveness of completed healthy housing structures, incorporating features like window screening, sealed eave gaps, and self-closing solid doors, in mitigating mosquito and domestic fly entry. This aims to reduce the transmission risk of multiple infectious diseases to children in rural Tanzania and other parts of sub-Saharan Africa (SSA).

The primary objective of Phase II was to assess the health impacts of the Star homes. Specifically, we compared the health outcomes of children residing in 110 Star homes with those residing in 110 traditional houses through a randomized controlled trial. The primary goals of the project were to evaluate the impact of Star homes on malaria, diarrhoea, and respiratory diseases in children compared to traditional African-style houses, which are not reported in this thesis. One of the secondary goals, and the main aim of this thesis, was to assess whether Star homes could reduce the entry of anopheline mosquitoes, which transmit malaria, and domestic flies, which transmit diarrhoeal diseases, using these as proxy measures for malaria and diarrhoea incidence in children under 13 years old. To accomplish these objectives, we measured mosquito and domestic fly abundance, as well as the entomological inoculation rate, in both Star homes and traditional houses. We also recorded indoor environmental factors, such as temperature, humidity, carbon dioxide (CO_2), and particulate matter ($PM_{2.5}$) particle pollution, in both study groups. Additionally, we recorded the opening and closing of windows and doors between the Star homes and traditional houses. Furthermore, we noted household characteristics in traditional houses, as these could vary from one house to another. These household characteristics included the number and size of windows, building materials, door types, eave-space status, and other features of the house. The assessment of indoor microclimatic conditions was closely associated with the health parameters affecting children living in Star homes compared to those living in traditional houses. Additionally, environmental conditions measurements were assessed to determine the comfort and adherence to the use of primary malaria vector control tools, such as insecticide-treated bed nets, between the two types of houses.

Aims and Objectives

Goal

To measure the impact of Star homes on the number of malaria mosquitoes and domestic flies as a proxy measure of malaria and diarrhoea transmission.

Aim

To assess the impact of Star homes on house entry by malaria mosquitoes and houseflies, vectors of diarrhoeal diseases.

Objectives

1. Reviewing existing literature on housing interventions to reduce communicable diseases among children in rural communities (**Chapter 1**).

2. Evaluating the influence of light and ventilation on indoor malaria vector abundance through a semi-field assessment (**Chapter 2**).

3. Contrasting indoor mosquito abundance and malaria transmission risks between Star homes and traditional houses (**Chapter 3**).

4. Comparing domestic fly densities between Star homes and traditional houses (**Chapter 4**).

5. Assessing potential risk factors contributing to indoor malaria vector abundance in traditional houses (**Chapter 5**).

Thesis Overview

Chapter 1: reviewed the existing literature on various house interventions to reduce transmission of malaria, diarrheal illnesses and acute respiratory tract (ARI) infections occurring in children living in sub–Saharan Africa.

Chapter 2: described the pilot studies conducted inside the semi-field environment to assess the impact of light and ventilation on indoor malaria vector abundances.

Chapter 3: assessed the impact of the Star homes in reducing the indoor mosquito biting risks and malaria transmission compared to the traditional houses, through a large-scale randomized household field trial conducted in rural Mtwara district, Tanzania.

Chapter 4: assessing the impact of the Star homes in reducing the domestic fly abundance in the kitchen and toilets compared to the traditional houses, in a randomized household trial conducted in rural Mtwara district, Tanzania.

Chapter 5: assessing the potential risks factors contributing to the indoor malaria vectors abundance in the traditional houses located in rural Mtwara district.

Finally, **Chapter 6** discusses the main findings of the thesis, study limitations, wider implications of the research, the way forward for house screening as a policy and future directions.

Contributions

Chapter 1 compiles data primarily from randomized control trials and cross-sectional surveys, focusing on the correlation between housing conditions and health outcomes, especially among children in sub-Saharan Africa. It emphasizes major communicable diseases impacting children, such as malaria, respiratory tract infections, and diarrhoeal illnesses, which collectively contribute to about 50% of child mortality in underserved communities. The chapter outlines the individual effects of housing on health, highlighting key interventions like window and door screening, sealing eave gaps, improving ventilation, and upgrading building materials (e.g., metal roofs, brick walls). Furthermore, it discusses water, sanitation, and hygiene (WASH) measures, underscoring the importance of providing clean and safe water in households to mitigate diarrheal illnesses. Chapter 1 also serves as a concise introduction to the study.

Chapter 2 was published as Mmbando et al. (2022) and describes a pilot study conducted in a semi-field system. Prof. Lorenz Von Seidlein and Dr. Jokob Knudsen secured the funds for this research. The study was conceived by Arnold Mmbando, Fredros Okumu, and Steve Lindsay. Arnold Mmbando conducted the semi-field tests and drafted the initial manuscript under the supervision of Dr. Fredros Okumu and Prof. Steve Lindsay. Prof. John Bradley oversaw data analysis, and Fredros Okumu, John Bradley, and Steve Lindsay contributed to the final paper.

Chapter 3 presents unpublished research, focused on assessing the impact of the Star homes in reducing indoor mosquito abundance compared to the traditional houses. The study was conceived by Lorenz Von Seidlein, Jakob Knudsen, and Steve Lindsay. Arnold Mmbando and Amos Ngonzi collected light trap survey data together with the field assistants (Mr Deogratius Kazimbaya, Salum Mohamed, Jazino Chonde and Benjamin Mdendemi). Mr. Said Abbas and Mr. Francis Tumbo processed and analysed field mosquito samples submitted to the Ifakara Health Institute Molecular Laboratory. Arnold Mmbando and Amos Ngonzi performed data entry, in collaboration with Steve Lindsay and Fredros Okumu. Salum Msham assisted in the enrolment of study houses and other logistics in the field. Arnold Mmbando and Halfan Ngowo conducted data cleaning and analysis under the supervision of Fredros Okumu, John Bradley, and Steve Lindsay. Arnold Mmbando, Fredros Okumu, and Steve Lindsay authored the chapter.

Chapter 4 also comprises unpublished work. Conceived by Fredros Okumu and Steve Lindsay, the study involved data collection on domestic flies abundances between the Star homes and traditional houses performed by Arnold Mmbando and Amos Ngonzi, under supervision of Steve Lindsay and Fredros Okumu. Arnold Mmbando and Halfan Ngowo conducted domestic fly data cleaning and analysis, supervised by Fredros Okumu and Steve Lindsay. Arnold Mmbando, Fredros Okumu, and Steve Lindsay contributed to writing the chapter.

Chapter 5 presents unpublished work, conceived by Fredros Okumu and Steve Lindsay, focused on the assessment of potential risk factors contributing to indoor entry of malaria vectors. Arnold Mmbando and Amos Ngonzi collected mosquito and house characterization data and performed data entry, in supervision of Steve Lindsay and Fredros Okumu. Dr. Luca Neili provided coarse scale spatial data and supervised the Geo-spatial analysis. Arnold Mmbando and Dr. Luca Neili analysed the datasets under the supervision of Fredros Okumu and Steve Lindsay. Arnold Mmbando, Fredros Okumu, Steve Lindsay, and Luca Neili authored the chapter.

Chapter 6, also an unpublished work, was authored by Arnold Mmbando, Fredros Okumu, and Steve Lindsay

Chapter 1 : Literature review and introduction to the research: Importance of improved housing for reducing infectious diseases of children in sub-Saharan Africa.

Abstract

Since 2000 there has been a rapid improvement of housing in sub-Saharan Africa (SSA), resulting from huge population growth and advancement of the economies of many countries in the region. In SSA most housing improvements are made in urban settings, leaving rural areas dominated with poor communities living in sub-standard housing at higher risks of encountering communicable diseases, such as malaria, diarrhoea and respiratory tract illness. Thus, there is need to develop healthier houses to protect children living in poor communities from these communicable diseases. Screening of windows, eave spaces and doors have been shown to reduce malaria transmission, parasitaemia and anaemia in children. Houses with constant clean water, proper latrines, improved floor and sewage systems, have a lower incidence of diarrhoeal diseases than those without. A well-ventilated house, with large and numerous windows have reduced risk of respiratory diseases such as tuberculosis.

A novel mosquito-screened two-storey house designed to protect people from malaria was piloted in northern Tanzania and shown to reduce the entry of malaria mosquitoes. It was also acceptable to the local community. A new prototype house, a Star home, has been designed to not only reduce malaria, but also decrease the incidence of diarrhoeal and respiratory infections in young children. A household randomised-controlled trial (RCT) to measure the impact of the Star home on the incidence of the three groups of diseases was conducted in Mtwara district, in southern Tanzania. Children living in 110 Star homes and 440 traditional village houses were followed for three years.

The goal of my PhD was to assess whether Star homes reduced the entry of mosquitoes and flies compared with traditional houses. My research was divided into three parts: 1) pilot study to assess whether the light-permeable walls and the narrow eaves gaps found in Star homes increase mosquito entry in experimental

huts, 2) the household RCT measuring mosquito and housefly densities, and 3) the potential risk factors contributing to the indoor abundances of malaria vectors was assessed using the traditional house type. In the trial, indoor temperature, relative humidity and carbon dioxide in both control and intervention groups and outdoors was assessed. Data generated in the pilot study will inform us whether or not we need to change the design of the Star homes before commencing the randomized household controlled trial (main study). Outcomes from the main study demonstrated the effectiveness of housing improvements in reducing the indoor densities of malaria and diarrhoea vectors and the risks of malaria and diarrhoea diseases in SSA children. The findings from this study will be of relevance to house designs in Tanzania and other parts of SSA.

Housing in sub-Saharan Africa

The population of SSA is growing rapidly, at a faster rate than anywhere in the world, accompanied by people migrating from rural to urban areas (Tusting et al. 2019). By 2050, the population of SSA countries is expected to be 4.2 billion, which is more than three times the population of 1.3 billion in 2015 (UN 2019). From 2000 to 2015, housing in the region improved significantly, with the proportion of modern structures (e.g., iron sheets and brick walls) increasing from 11% to 23%. This shift from traditional materials (e.g., thatched roofs and mud walls) was primarily driven by rapid economic growth and urbanization (El-hadj et al. 2018). The economy of Africa has grown rapidly since 2000, and the region had a collective gross domestic product (GDP) of USD 2.3 trillion by 2019 (WB 2019). From 2000, Africa’s economy has grown by approximately 4.3% GDP by 2022 and will further increase by 5.2% by 2024 (UN 2023), although the impact of COVID-19 is likely to drastically reduce this growth. In 2020 the pandemic reduced the GDP of SSA by 2.4% (WB 2020a).

Household surveys done in 31 SSA countries demonstrated an increase of housing improvements (with improved water and sanitation, sufficient living area and durable constructions) from 32% in 2000 to 51% in 2015 (Tusting et al. 2019). There is, however, an unequal distribution of housing improvements between and within countries, with 53% of urban dwellers and 82% of rural dwellers living in sub-standard accommodation (Tusting et al. 2019). Since housing is amongst the most basic of human needs, millions of new homes are required to accommodate the growing population of SSA. It is projected that by 2050 there will be 144 million new housing structures in Africa (Knols et al. 2016). Thus, rapid investment in housing and building materials is crucial in ensuring there is sufficient quality housing for the growing population, (Fig 1.1). The traditional housing stock of SSA is changing, with a transition from thatched roofs and mud-walled homes to corrugated iron-roofs and brick-walls (Rek et al. 2018).

In the past, poor housing has been associated with poor health in many parts of the world, particularly communicable diseases, like malaria, diarrhoeal illnesses and respiratory diseases (WHO 2010, Hershey et al. 2011, Kaindoa et al. 2018). As we observe a rapid improvement of housing structures and design it represents an extraordinary opportunity to improve human health and wellbeing through reducing the burden of communicable diseases in those living in unhealthy accommodation (Parby et al. 2015).

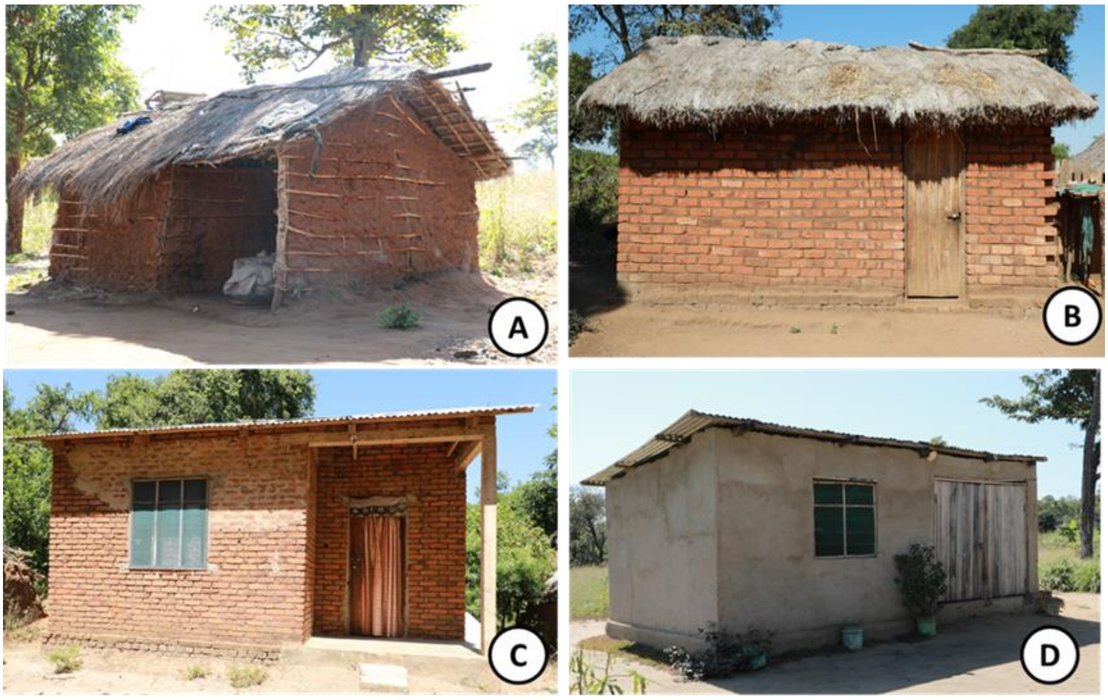

Figure 1.1: Transition of housing improvements in rural Tanzania villages; mud-walled with thatched roof, A), brick-walled with thatched roof, B), un-plastered brick-walled with corrugated iron roof, C) and plastered with corrugated iron-roof, D.

Major infectious diseases of children in sub-Saharan African

Between 2000 and 2021, communicable diseases like malaria, diarrhoea, and respiratory infections have remained leading causes of mortality among children under 5 in East and Southern Africa (WHO 2016a, UN 2022). Prematurity, lower respiratory tract infections (pneumonia), diarrhoea, and malaria are the primary causes of child mortality, (Kinney et al. 2009). A systematic review of global and regional under-5 mortality causes from 2000 to 2019 identified preterm birth complications (18%), lower respiratory tract infections (14%), diarrhoea (9%), and vaccine-related infectious diseases such as measles (22%) as major contributors to child deaths (Perin et al. 2022). These deaths are exacerbated by risk factors including malnutrition, low birth weight, and overcrowded living conditions for non-breastfed children (WHO 2016a). Prevention strategies such as reducing household air pollution, vaccination, exclusive breastfeeding, and proper nutrition can mitigate these mortality rates.

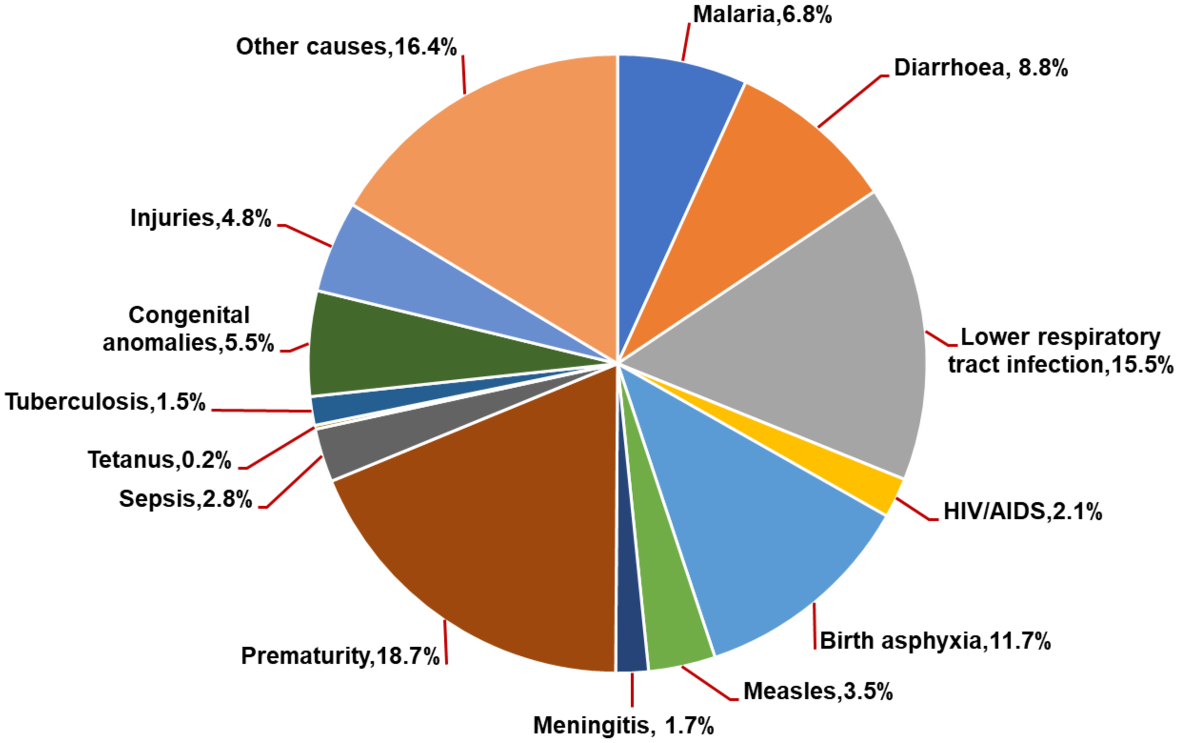

Figure 1.2: Causes of death in under five years old children in eastern and south African region.This figure was obtained from the United nation inter-agency group for child mortality estimations (UN 2022). Combination of mortality rates due to malaria, diarrhoea and respiratory tract infection account for almost 32% of children deaths in the region.

Malaria

Between 2000 and 2015, the prevalence of *Plasmodium falciparum* in SSA has reduced by half and incidence rates by 40% (Bhatt et al. 2015, Cibulskis et al. 2016). The unprecedented reduction of malaria cases and mortality has resulted from the wide-scale deployment of insecticide-treated nets (ITNs), indoor residual spraying

(IRS) and prompt and effective treatment with anti-malarial drugs. ITNs are the most effective intervention, accounting for 68% of the reduction of malaria cases between 2000 and 2015 (Bhatt et al. 2015) and have been deployed on a massive scale across the region. Between 2016 and 2018, 578 million nets have been distributed, with 50% directed to SSA countries (WHO 2019c). Net use in SSA has increased from 47% in 2010 to 83% in 2022 (WHO 2023).

Nonetheless malaria remains a major cause of ill health with 233 million cases globally and 580,000 deaths in 2022 (WHO 2023). Children under five years old account for about 67% of the global malaria deaths, with >90% of these in SSA. Unfortunately, the decline of malaria in many malaria endemic countries in SSA has stalled and we are seeing a rebound of malaria cases in some countries such as Nigeria, Uganda, Mozambique, and Democratic-Republic of Congo with heaviest burden of malaria in SSA (WHO 2023).

In SSA most of the malaria mortality and morbidity is due to infections with *P. falciparum*, which is a major cause of anaemia, low-birth weight and death of the under five years old and pregnant women (Walker et al. 2014, WHO 2019c). In moderate and high transmission settings in SSA, many infected individuals are asymptomatic which act as a reservoir of infection (Sturrock et al. 2013). In SSA region malaria transmission is dominated mainly by members of the *Anopheles gambiae* complex, including *An. gambiae s.s.*, *An. coluzzii* and *An. arabiensis* and members of the *An. funestus* complex which are distributed heterogenously in the region (Sinka et al. 2012).

In the past, malaria in Tanzania was transmitted largely by *An. gambiae s.s*. (Russell et al. 2011). These vectors have, however, disappeared and replaced by its sibling species, *An. arabiensis* (Russell et al. 2010). The disappearance of *An. gambiae s.s.* was mainly attributed to the widespread use of ITNs and IRS killing these highly anthropophilic and endophilic malaria vectors (Russell et al. 2010). Currently, in Tanzania, about 80% of malaria is transmitted by *An. funestus* and 20% by *An. arabiensis* (Charlwood et al. 2000, Lwetoijera et al. 2014, Kaindoa et al. 2017). In terms of numbers of mosquitoes, some of the malaria endemic regions in SSA such as in Tanzania are dominated by *An. arabiensis* mosquitoes with lesser number of *An. funestus* mosquitoes (Lwetoijera et al. 2014, Kaindoa et al. 2017). The reason for the change in species composition is related to the feeding and resting behaviours of the different species (Lwetoijera et al. 2014, Sougoufara et al. 2014). *Anopheles funestus* prefers mostly human blood and bites and rest inside the house, whilst *An. arabiensis* mosquitoes are more opportunistic feeders on people and domestic livestock, indoors and outdoors (Lwetoijera et al. 2014, Sougoufara et al. 2014). The biting, resting behaviours as well as ability to withstand the insecticides commonly used on our core vector control tools, allows *An. funestus* to persist and transmit disease even where vector control tools are widely used.

Diarrhoeal diseases

In SSA, diarrhoeal diseases are an important group of infectious diseases in childhood, responsible for 1.87 million deaths of children under five years old each year (Boschi-Pinto et al. 2008). Most diarrhoeal cases are attributed to five species of pathogens: (viruses) rotavirus, (bacterial) *Shigella*, enterotoxigenic *Escherichia coli, Campylobacter jejuni* and *Cryptosporidium parvum* (Mwenda et al. 2010). Globally, about 827,000 people in low-and middle-income countries die annually due to poor sanitation, inadequate water and hygiene, of which about 50% of these deaths are due to poor sanitation (WHO 2020). The systematic analysis of global burden of diseases done in 95 countries between 1990 and 2016, demonstrated that rotavirus was the leading cause of mortality in under five years old with estimated 128,515 deaths (Troeger et al. 2018). In SSA, there is limited data on diarrhoea illnesses, however, the surveillance of rotavirus conducted in eight African countries showed that about 40% of stools samples collected from children were positive for rotavirus infection (Mwenda et al. 2010). A systematic analysis of the global burden of diseases, demonstrated that Shigella was the second cause of mortality in under five years old children with estimated 63,713 deaths (Khalil et al. 2018). Enteropathogenic *E. coli* estimated to account about 4.2% of all diarrhoea deaths in under five years old children in 2016 (Khalil et al. 2018).

Safe water, sanitation and hygiene (WASH) all contribute to a reduction in diarrhoea incidence; (safe water) by 37% and (Sanitation and hygiene) by 25% (Fewtrell et al. 2005). Diarrhoea disease can be controlled by drinking safe and clean water as well as adequate sanitation and hygiene condition. A global burden of diarrhoea diseases conducted in 145 low-middle-income countries showed that about 502,000 diarrhoea deaths was estimated to be caused due to lack of drinking water and about 280,000 deaths was due to poor sanitation (Prüss-Ustün et al. 2014)

Globally, about 785 million people lack access to safe and clean water and 2.5 billion people lack improved sanitation, many in SSA (WHO 2019b). In Nigeria, a lack of WASH contributed to about 88% of the disease burden in the country (Pruss-Ustun et al. 2002, Gunther & Fink 2010). Diarrhoeal diseases are also a problem in refugee camps, accounting for about 17% of children mortality (Hershey et al. 2011). Immunocompromised people such as HIV-infected people and malnourished individuals are also at high risk of dying when encountering diarrhoea illnesses (WHO 2017a). In a cluster-randomized trial of school-based WASH program on student absence in schools done in Nyanza province in Kenya, from 2007 to 2008, schools which received water treatment and hygiene promotion had a 58% reduction in the odds of absence for girls compared to the control group (Freeman et al. 2012). Improved water, sanitation and hygiene combined, however, did not reduce the prevalence or duration of illnesses in pupils between the intervention and water-scarce (control) schools (Freeman et al. 2014).

Respiratory tract illnesses

Respiratory tract illnesses, including pneumonia, accounted for 920,136 deaths among children under 5 years old globally in 2015, representing 16% of all child deaths (WHO 2016a). It also led to 4 million increases of major of disability adjusted life (DALY's) in under five years old (Ferkol & Schraufnagel 2014). The four major pathogens causing respiratory tract illnesses in children are *Streptococcus pneumoniae, Haemophilus influenza type B* (Hib)*, influenza* and respiratory syncytia virus (Troeger et al. 2018). In SSA, about 16% of children die from respiratory tract illnesses, such as pneumonia, each year (WHO 2016a). Pneumonia is a form of acute respiratory infection which affects the lungs, and it is caused by fungi, bacteria and viruses. In 2015 pneumonia caused 16% of deaths of five years old globally and is common in SSA (WHO 2016a). Most of the deaths due to pneumonia infection are attributed to *Streptococcus pneumoniae*, *H. influenza* type b and respiratory syncytial virus (WHO 2016b). Death due to pneumonia can be prevented by immunizations such as pneumococcal and *H. influenza* type B, adequate nutrition and by improving ventilation, as well as reducing overcrowding and air pollution (WHO 2016b). Children who are immunocompromised and those with nutritional problem are at higher risk of developing pneumonia infection. Environmental factors such as indoor air pollution, living in crowded homes and parental cigarette smoking attribute to the risk of children getting pneumonia (WHO 2016b). A systematic review using 13 electronic databases showed significant association between biomass fuel and acute respiratory tract illnesses in rural population with an odds ratio of 3.53 (Po et al. 2011).

Association between poor housing and infectious diseases in children

Housing and malaria disease

There is a strong correlation between malaria and poverty, with the poorest of the poor twice as likely to have malaria as the less poor (Tusting et al. 2013). A multi-country analysis of survey data gathered in 21 SSA countries between 2008 to 2015 demonstrated significant reductions of odds of malaria by 9% (by microscopy) and 14% (by rapid diagnostic test) of the children living in modern houses compared to those in traditional houses (Tusting et al. 2017). A systematic review and meta-analysis of studies conducted from 1900 to 2013 found that individuals residing in modern houses had a 47% lower likelihood of contracting malaria compared to those in traditional houses (Tusting et al. 2015).

Most malaria transmission occurs indoors at night, with most *An. gambiae* s.l. and *An. funestus* entering the house via eave-spaces, and secondarily through open windows and doors (Huho et al. 2013, Kaindoa et al. 2017). Screening windows and doors, along with blocking eaves, leads to a decrease in human biting rate by 48% and parasitaemia by 57% in children (Rek et al. 2018). Since 2000, ITNs and IRS interventions have globally saved 663 million lives, with approximately 93% of the global malaria burden occurring in the SSA region (Knols et al. 2016). In some parts of SSA during the past 20 years, *An. gambiae* s.l. have transitioned from indoor biting and resting during midnight hours to outdoor activity in the early evening (Russel., et al., 2013). This shift has rendered bed nets and indoor residual spraying less effective in controlling them. Moreover, the rise of insecticide-resistant mosquito populations, coupled with inadequate compliance with bed nets and insecticide-treated nets (ITNs) prone to tearing and having poor residual activity, underscores the necessity for new vector control tools (Moiroux et al. 2012, Strode et al. 2014).

Mosquito-proof houses could be one of the additional vector control tools which could be developed over time (Lwetoijera et al. 2013, Knols et al. 2016, Rek et al. 2018). Thus, the improvements to the built environment provide a way for the communities to protect themselves against malaria and non-malaria vectors. Key features of a mosquito-proof house are captured in the mnemonic, DELIVER (Lindsay et al. 2021), which stands for: 1) D, is for door, which should be well fitted, self-closing and screened to prevent anopheline and culicine mosquitoes (Jawara et al. 2018), 2) E is for eave-spaces, which should be closed or screened and shown to reduce malaria vectors entry by 94% (Ogoma et al. 2010), 3) L, is for lifting the house off the ground (Carrasco et al. 2023), 4) I is for ITNs nets must be used when sleeping, 5) V is for ventilation should be ensured by increasing number and size of the windows, 6) E is for environmental management should also be well maintained to reduce potential mosquito habitats both inside and around the home and 7) R is for roof, which should be solid like corrugated iron-roof instead of thatched and slopping (Tusting et al. 2020). Blocking the eave-spaces, and screening windows and doors can reduce indoor mosquito entry and reduce malaria transmission risk by 59%, reducing anaemia in children by 47% (Knols et al. 2016, Tusting et al. 2016, Tusting et al. 2020). Raising sleeping rooms aided in the 96% reduction of mosquito densities in the sleeping room, as well as ensuring a cooler environment which increase compliance on bed net use (Pulford et al. 2011, von Seidlein et al. 2017, Carrasco et al. 2023). Environmental management, such as filling, draining and levelling aquatic habitats can, in certain situations, also reduce mosquito vector numbers around a house (Service & Authority 1947, Lindsay et al. 2017).

In rural Tanzania, as in many parts of SSA, there is a transition of housing from traditional houses with mud-walls, thatched-roof, unscreened window and doors to modern houses (with plastered wall, screen windows and doors) which reduce disease vector entry three-fold (Kaindoa et al. 2018).

Housing and diarrhoea illnesses

The 2013 World Bank report highlighted a strong correlation between improved housing conditions, including access to better water supply and advanced WASH facilities such as modern toilets, and reduced incidence of diarrheal illnesses (Gunther & Fink 2010). Cross-sectional surveys from 33 African countries (Tusting et al. 2020), found that children living in houses built with better construction materials, sanitation facility and drinking water source had an 8% lower incidence of diarrhoea than those living in their counterpart houses (Tusting et al. 2020). In Nigeria, housing with poor wastewater disposal systems, overcrowding, lack of clean water and poor latrines predispose individuals to diarrhoeal diseases by 14% (in urban) and 16% (in rural) (Yaya et al. 2018). Children under five years old living in a poor house without plumbed water had a 51% increased risk of encountering diarrhoeal pathogens (Yaya et al. 2018). This is also seen in adult population when about >40% of people who are living in houses with no quality water, sanitation and hygiene services are also at high risk of getting diarrhoea infection. Diarrhoea is more of a problem in rural communities, which can have double the incidence of diarrhoea than those living in urban areas, mainly due to lack of proper water supply and toilet facilities (Yaya et al. 2018). Houses are more likely to have access to piped water in urban settings than in rural settings (Regassa et al. 2008). In Ethiopia key environmental risk factors for childhood diarrhoea included large distances between the house and drinking water source, lack of ownership of latrine, poor refuse disposal, presence of faeces around the pit-hole, and absence of a pit-hole cover and presence of faeces in the compound (Regassa et al. 2008).

Domestic flies, *Musca domestica* and *Chrysomya putoria* are important mechanical vectors of diarrhoeal diseases, as they have a strong association with human faeces (Levine & Levine 1991, Lindsay et al. 2012). Eliminating breeding sites for domestic flies and prevention of contact between flies and diarrhoeal pathogens reduces the incidence of diarrhoea by 0.8 episodes per year (Das et al. 2018). Fly-proof houses, with screened windows and doors have been shown to reduce housefly densities inside the house which are the major contributor to the spread of diarrhoea pathogens (WHO 1991). In Brazil, an increase of sewerage coverage from 26% to 80% reduced the prevalence of diarrhoea by 22% (Barreto et al. 2007), demonstrating that proper waste management can reduce the incidence of diarrhoea illnesses (Barreto et al. 2007, Mara et al. 2010).

Housing and respiratory tract illnesses

Reducing crowding contributed to a reduction in respiratory tract illnesses and is strongly recommended by the WHO in their guidelines of housing and health (WHO 2018). For example, in Ethiopia (Tesema et al. 2015) and Guinea Bissau (Gustafson et al. 2004), houses with a floor area of 4 m^2 size and occupied by more than four individuals increased the risk of tuberculosis infection by 68% (Gustafson et al. 2004, Tesema et al. 2015). A case-control study done in The Gambia found an association between tuberculosis infection (TB) and increased household crowding, which mainly affected the Jola ethnic group and smokers (Hill et al. 2006). Individuals residing in smaller houses with limited or no windows face twice the risk of tuberculosis compared to those living in adequately ventilated houses, underscoring the significance of ventilation in reducing respiratory diseases (Hill et al. 2006). Children residing in well-ventilated homes with affordable clean indoor stoves, even in crowded conditions, experienced a reduction of over 90% in the risk of contracting pneumonia illnesses (Tiewsoh et al. 2009, Howie et al. 2016).

Fuel biomass for cooking or heating homes causes 1.6 million deaths from household air pollution (HAP) related diseases, due to pollutants from smoky, often open fires, with incomplete combustion (Boy et al. 2000). Global health agencies in (Cookstoves and fuels market) in Guatemala advocate that communities shift from using charcoal, firewood, coal to the cleaner fuels which have less pollutants (WHO 2019a). In The Gambia, and rural Tanzania a child being carried on mother's back during cooking, cigarettes smoking by the father and living in a polygamous family increased the risk and mortality of children by 60% (Armstrong & Campbell 1991, Armstrong Schellenberg et al. 2002).

When a child is suffering from more than one communicable disease

Children living in SSA countries repeatedly suffer from a barrage of infections during early life, encountering malaria, diarrhoea and respiratory tract illnesses, resulting in a higher risk of morbidity and mortality. In a study conducted in 90 African refugee camps, children who had both malaria and pneumonia accounted for 20% more child mortality compared to when a child had malaria or pneumonia alone (Hershey et al. 2011). In the Peruvian Amazon, children aged 0-72 months who experienced both malaria and a fever due to other infections, were more likely to be stunted than those that had only malaria (Lee et al. 2012).

The syndemics model of health demonstrates that the cause of ill health in individuals and population are not just caused by multiple health problems, but also that social inequalities and injustices contribute to disease burden (Singer et al.

2017). For example, malnutrition and poverty contribute to severity of several diseases in children like malaria, diarrhoea and respiratory tract illnesses (Tusting et al. 2016).

Scaling-up healthy housing

SSA is the region with the largest number of people living in extreme poverty, with more than 400 million surviving off less than $2/day, with 104 million children under five years old living in poor communities (WB 2020b). Unsurprisingly, poor communities live in poorly-constructed houses, and the health risks, and are constrained by poverty and competing interests for finance (Kaindoa et al. 2018). Low socio-economic status, low-income generation, and community priorities such as educating their children are major obstacles to housing improvements in rural communities. There are several ways in which healthy homes could be financed. Governments could subsidize building materials so that communities purchase building materials at subsidised prices to improve their houses (Kaindoa et al. 2018). This was done in Ethiopia when the government provided a micro-loan to people who could not afford to improve their homes (Ström & Petersson 2015). In this study, family members made the building blocks and the government hired local masons to build the foundation and erect the buildings (Ström & Petersson 2015). Apart from poverty, lack of inter-sectoral linkages between housing and human health is one of the major factors leading to poor housing (Lawrence 2004). There is no consensus on integrating housing into health policies. For example, malaria control authorities in SSA countries need to work closer with the building sector to facilitate building of the health homes. It is necessary to consider inter-sectoral collaborations to integrate housing into health policies (Lawrence 2004).

Poor quality housing is also common amongst people that move because of their occupation, such as forest workers, pastoralists, and migratory farmers (Taylor 1951, Makungu 2011). These migratory communities live in temporary houses which offer little protection against disease vectors and lack access to clean and safe water and sanitation (WHO 1999). Migratory communities faced significant risks of vector-borne diseases such as malaria due to poor housing as their makeshift houses often do not allow the use of ITNs and IRS (Fillinger et al. 2009, Swai et al. 2016). This is because most makeshift houses are constructed from timber and thatch, and do not provide wall surfaces suitable for producing a residual insecticidal effect. Poor ventilation is also common in these houses since they lack windows, making the houses hot and uncomfortable for people to use bed nets (Pulford et al. 2011).

Health and housing in relation to the SDGs

In 2017, 1.6 billion people lived in inadequate housing that are frequently concentrated in slums (UN 2017a). While, millions of people lack suitable homes, stocks of vacant houses are increasing (UN 2017a). Thus, the United Nation's (UN) sustainable development goals (SGDs) recognise the value of adequate housing policies as an important part of human basic needs. UN SDG 1 which aims to end poverty in all its forms, showed a substantial reduction in people leaving in extreme poverty situation, from 36% in 1990 to 10% in 2015 (UN 2023). People living in poverty have little access to basic needs like health, education, water and sanitation as well as shelter. Since, poor housing is mainly caused by the vicious poverty circle of the SSA communities, the UN SDG 1 target is to reduce poverty to men and women by at least by 50% in 2030 (UN 2023). Some of the SDG policies focused on the health such as UN SDG 3 which mainly focused on health lives and promoting wellbeing of all age of people by ensuring safe housing and promoting wellbeing of individuals (UN 2019). The UN SDG 3 aim to reduce the under five years old mortality to at least 25 deaths per 1000 children by 2030, by ensuring essential health-care services, reduction of pollutions and contamination are highly accessible to everyone. The UN SGD 6 focus on availability of clean water and sanitation for all, estimated that 2.4 billion people lack access to basic sanitation services, such as toilet or latrines (UN 2023). This is very crucial as unimproved houses are mostly characterized by lack of proper latrines and constant supply of clean water and sanitation (WASH) which are major contributor of diarrhoea illnesses. The UN SDG 6 target, to ensure the universal and equitable access to safe and affordable drinking water, sanitation as well as to end open defecation for all. To accelerate this UN SDG 11 much focus was put on making better cities and communities with human settlements which is sustainable, resilient and safe living environment within home (UN 2019). Rapid urbanization and cities expansion create pressure on fresh water supplies, sewage, the living environment, and public health. This will solve rapid growth of urbanization and human population especially in SSA countries so that they can live in a safe, health and resilient houses. The SDGs related to health and housing requires having proper, safe, and comfortable houses which prevent major diseases of childhood such as malaria, diarrhoea and respiratory illnesses (Figure 1.3).

Figure 1.3: The United Nation's Sustainable development goals relevant to housing and health.

The need for healthy houses, the Star homes project

To date, most studies on healthy homes have focused on protecting children from individual diseases, not multiple diseases. To address this issue, a pilot study of six prototype houses was conducted in north-eastern Tanzania between July 2014 and July 2015 (Figure 4). The features of these houses that were aimed at improving the health of the occupants is shown in Table 1.1.

In this study, most people preferred a two-storey house to single-storey ones, as the higher buildings reduced mosquito entry and provided a desirable place for sleeping (von Seidlein et al., 2019). During Phase I of this project, the health impacts of the health houses were not assessed, paving the way for a randomized controlled trial in Phase II of the project, which assessed the impact of a healthy home on malaria, diarrhoea, and respiratory tract illnesses in children living in rural communities.

Phase II of the healthy home project was primarily designed to assess the health impacts of a new prototype house, the 'Star home', on the incidence of malaria, diarrhoea, and respiratory tract illnesses in a randomized controlled trial. Children living in 110 Star homes were compared with those in 440 traditional houses. The features of the two-storey Star homes were designed to: (1) reduce malaria by closing the eave spaces, having the bedroom on the first floor, using windows, walls, and doors that provided a cooler environment to encourage the use of bed nets, (2) reduce diarrhoeal diseases by having an easy-to-clean toilet with a flap to prevent houseflies from coming into contact with faeces, and (3) reduce respiratory infections by ensuring maximum ventilation, airflow, and having a chimney in the kitchen to prevent wood smoke inhalation (Fig 1.5).

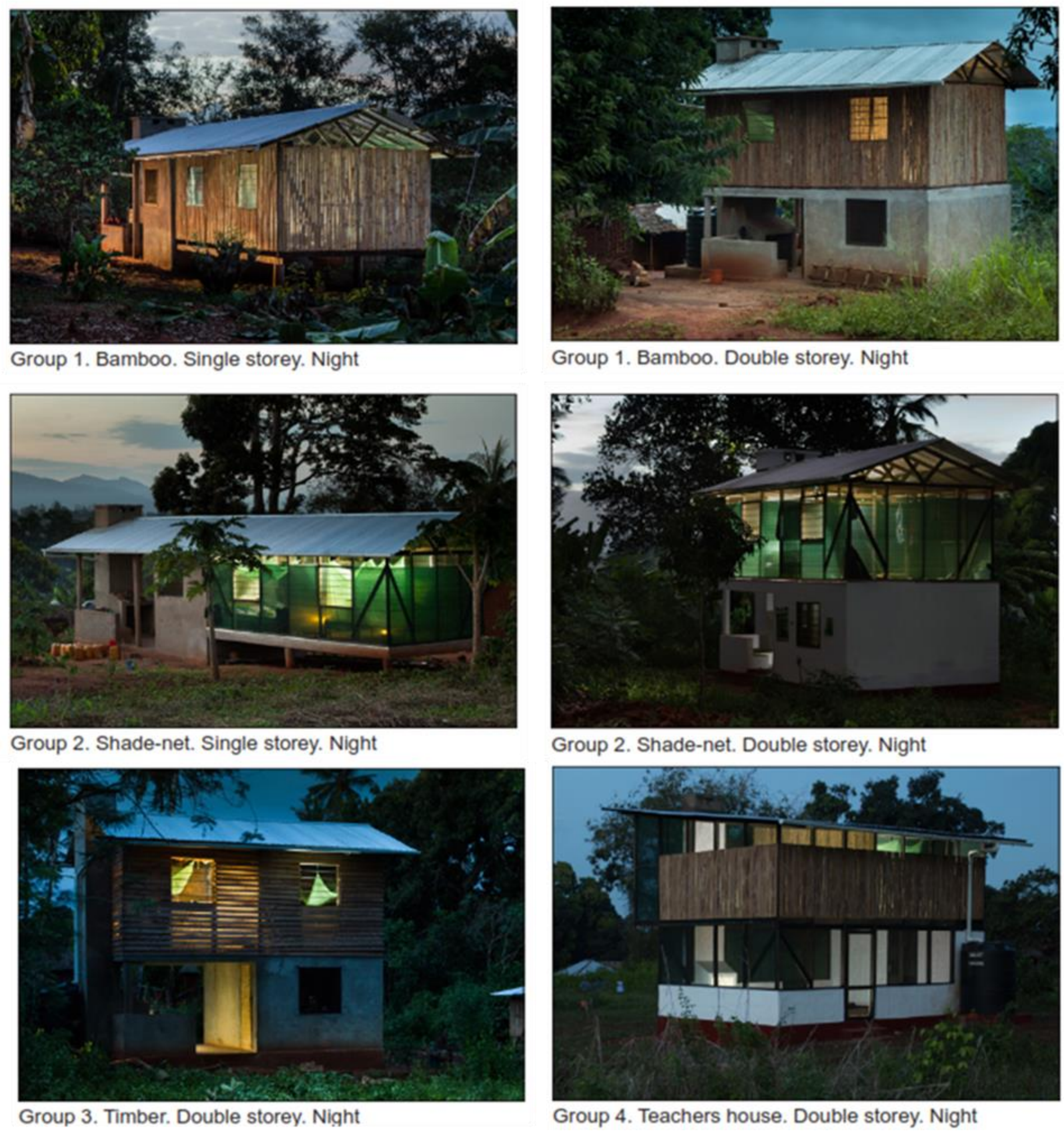

Figure 1.4: Different prototypes of healthy houses constructed during Phase I of the study in north-eastern Tanzania, (von Seidlein et al. 2017).

Table 1. 1: Features of Star homes (prototype used in the randomised controlled trial).

Feature	Effect	Impact
1) Building orientation with optimal shading	Ensuring optimal shading during the day	Keep the house cooler
2) Lightweight & durable roof with closed eave gaps	Reduced the entry of malaria vectors by 70% to 95% (von Seidlein et al. 2017)	Preventing indoor malaria transmission risks
3) Shade nets walls	Reduced indoor temperatures by 2.3^{o} C, and indoor mosquito entry (von Seidlein et al. 2017)	Would make it more comfortable and likely that people would sleep under an ITN and reducing indoor malaria transmission risks
4) Raised concrete ground floor	For easy cleaning and increased hygiene	Reduce the risk of enteric and soil-transmitted infections
5) Screened indoor cooking area	Remove smoke inside and household air pollutions and prevent domestic fly entry	Reduce risks of getting respiratory tract illnesses and diarrhoeal illnesses transmission risks
6) Protected lockable storage	Reduced rodent infestation and feeling of security	Ensuring household safety
7) Raised sleeping areas	Ensuring comfortability and airflow and increase use of bed net	Reducing mosquito densities indoor and well as good climatic condition
8) Water harvesting system	Ensuring constant availability of water	Reducing the risk of diarrhoea illnesses

9) Outdoor fly-proof latrine	Prevent housefly from contact with faecal materials	Reducing risks of diarrhoea illnesses
10) Solar power panel	For lighting the house during the night	Providing safety to the household

Figure 1.5: Components of the Star home. Where 1) building orientation, 2) lightweight roof, 3) facade openings, 4) raised concrete floor, 5) screened indoor cooking area, 6) lockable storage area, 7) raised sleeping area, 8) water harvesting system, 9) fly-proof latrine and 10) solar power, (Mshamu et al., 2022).

Star homes study goal

The main goal of the randomized household control trial (RCT) was to measure the efficacy of Star homes in reducing the incidence of malaria, diarrhoeal disease, and respiratory infections in children under five years old compared to those living in traditional houses.

Star homes objectives

The project objectives of the RCT were to:

1) Determine the relative risk of malaria, respiratory infections and diarrhoeal disease among children living in the Star homes compared

with those living in traditional houses.

2) Compare mosquito and fly abundance and the entomological inoculation rate inside the Star homes and traditional houses.
3) Compare the prevalence of malaria parasitaemia among children living in the Star homes and traditional houses.
4) Compare indoor temperature, humidity, CO_2 and $PM_{2.5}$ particle pollution in the Star homes and traditional houses.
5) Develop a process for scaling-up the Star homes outside the health sector.
6) Assess the acceptability of the Star homes to local people.

Primary outcomes

1) Incidence of malaria, respiratory tract infections and diarrhoeal disease in children <13 years of age.
2) Mean number of female *Anopheles* mosquitoes and houseflies between the Star homes and traditional rural African houses.
3) Prevalence of malaria parasitaemia, major respiratory pathogens and diarrhoeal among children living in the Star homes and traditional houses.
4) Mean indoor temperature, humidity, CO_2 and $PM_{2.5}$ particle pollution in the Star homes and traditional houses.
5) Qualitative and quantitative evaluations of knowledge, acceptance, and practice of the Star homes.

PhD study goal

To assess the impact of Star homes on house entry by malaria mosquitoes and houseflies, vectors of diarrhoeal diseases.

PhD study objectives

1. Compare number of mosquitoes entering light-permeable walled (clear plastic) experimental huts and the opaque-walled huts (opaque plastic).

2. Compare mosquito numbers caught inside the light-permeable walled huts (shade cloth) with narrow eave-gaps against closed eave-gap huts.
3. Compare indoor mosquito and domestic fly densities between Star homes and traditional houses.
4. Compare the indoor microclimatic conditions (temperature, relative humidity, CO_2 and particle pollution) between the Star homes and traditional houses.

Study hypotheses

1) The number of mosquitoes entering light-permeable walled (clear plastic) experimental huts is higher than the number entering opaque-walled (opaque plastic) huts.
2) The number of mosquitoes caught inside light-permeable walled huts (shade cloth) with narrow eave-gaps is greater than the number caught in huts with closed eaves.
3) Indoor mosquito and domestic fly densities are lower in Star homes compared to traditional houses.
4) The night-time temperature and CO_2 concentration are lower in Star homes than traditional houses.

Rationale

A pilot study in north-eastern Tanzania showed that a prototype two-storey building reduced the entry of mosquitoes by 95% and increased the comfort of the house by lowering indoor temperature by 2.3°C compared to unmodified traditional African houses (von Seidlein et al. 2017). Apart from reduced mosquito densities and lowered temperature, the community expressed their satisfaction, especially with the two-storey sleeping area due to safety and privacy concerns. This study led to a randomised controlled trial in Mtwara district, Tanzania, which assessed the health impacts of children living in Star homes compared to those in traditional houses.

My PhD, as a part of the Star homes Phase II project, included measuring the impact of Star homes on the numbers of malaria mosquitoes and flies, as a proxy measure of malaria and diarrhoea transmission. Before the main study commenced, however, we conducted two pilot studies to explore whether two features of the Star home would increase mosquito collections indoors. We assessed the effect of light, and later, the effect of the small gaps along the eave-space of the Star homes on mosquito densities indoors. Data generated from pilot studies provided insights into potential biases in the indoor trapping approach that may have arisen during the main study due to the structural differences between the Star homes and traditional houses.

Methods

Pilot study: Firstly, the aim was to determine whether light traps collected more mosquitoes in the light-permeable Star homes than in traditional houses with opaque walls. Secondly, the goal was to ascertain whether a small eaves gap (the space between the top of the wall and the roof) in Star homes led to a substantial increase in house-entering mosquitoes compared to whether this gap was closed. Both pilot studies were conducted inside large screen cages where laboratory-reared *An. arabiensis* mosquitoes released each night for 72 nights.

In the first pilot study, two transparent-walled and two opaque-walled huts were tested, leaving the eave gaps open for mosquito entry. Four consented male volunteers, one per hut, slept inside each hut under an insecticide-treated bed net. A CDC-light trap was hung beside each bed from 18.30 to 07.00h to collect mosquitoes inside the huts. Three hundred laboratory-reared *An. arabiensis* mosquitoes were released into each compartment each night. The primary outcome measure was the number of mosquitoes caught inside each type of hut.

The second objective of the pilot study followed similar procedures as the first objective, but all four huts were made using shade cloth, resembling the Star homes. Two had narrow eave gaps, while the other two huts had the eave gaps closed. Data generated from the pilot studies provided insights into potential biases in the indoor trapping approach that may arise during the main study due to structural differences between the Star homes and traditional houses.

Main study*:* The main study comprised a household randomized controlled trial involving 110 newly designed houses and 110 traditional houses. It aimed to achieve the following objectives: 1) measurement of entomological indices (Entomological

Inoculation Rates (EIR), mosquito densities, and housefly density) as proxies for malaria and diarrhoea transmission; 2) assessment of indoor microclimatic conditions (temperature, relative humidity, CO_2, and particle pollution) between the Star homes and traditional houses; and 3) evaluation of potential risk factors contributing to indoor malaria vector abundance in traditional houses.

Mosquito sampling

The study villages were divided into 7 clusters, each consisting of 16 Star homes along with 64 traditional houses, totalling 80 houses. Four consecutive nights of mosquito sampling per week were sufficient to cover all 80 houses in each cluster. Twenty CDC light traps (Service 1977) sampled indoor mosquitoes each night, equating to five traps per village. CDC light traps were deployed to collect mosquitoes indoors across all 220 houses participating in the study in a seven-week cycle. These traps were positioned 1 m above the ground at the foot end of a child's bed, with all individuals in the room sleeping under the ITNs. Trap operations occurred from 19.00 h to 07.00h the following morning. We sampled mosquitoes indoor from each house for two years, (Table 1.2). Each night, eight randomly selected houses were sampled within the same village, including four intervention houses (Star homes) and four traditional houses. Each week, the trap was relocated to another randomly selected cluster.

Mosquitoes collected from these houses were counted and sorted by species and sex using identification keys. Female malaria vectors were then packed and transported to the laboratory for further analysis. The circumsporozoite ELISA technique was employed to detect sporozoites in malaria vectors. Species identification among members of the sibling species of the *An. gambiae* complex and *An. funestus* group was achieved using Polymerase Chain Reaction (PCR) technique (Scott et al. 1993, Koekemoer et al. 2002).

Domestic flies sampling

Domestic flies were collected weekly from eight houses per cluster, which previously participated in mosquito collection. Sampling included four Star homes and four traditional houses, (Table 1.2). Optimal traps, specifically Lindsay Traps, were used and baited with 50 g of fish gills (Lindsay et al. 2012). Two baited domestic fly traps were positioned in each study house: one at the kitchen corner furthest from the main door and another at a corner away from the latrine entry door. The fly trap placed in the latrine was mounted on a metal frame with a cover on top to prevent rainwater from entering the traps as well as to prevent ants from accessing them. The flies were collected from the traps each day at 17.30 h before evening to prevent them from escaping.

Environmental measurements

Indoor temperature, relative humidity, and carbon dioxide concentrations were measured in the bedrooms of study children residing in four Star homes and four neighbouring traditional study homes every week. Readings were recorded at 30-minute intervals for 24 hours. GasLab® data loggers were positioned 1 m above the floor at the foot end of the study child's bed. Each logger was assigned a unique ID and designated to the corresponding house on the night of the mosquito and the day of the housefly collection. Additionally, four loggers were placed on a metal stand at a distance of 5 m from each house, with two loggers for the Star homes and another two for the traditional houses, to measure outdoor environmental conditions.

Door opening and closure

For a Star home to protect the occupants from mosquitoes it was important for house occupants to keep the external doors of the house closed at night. The duration of door opening and closing were recorded once in eight houses per week/cluster, comprising four Star homes and four traditional houses. For this study, two-door loggers were installed in each Star homes house, with one placed at the main entry door and the other at the stairway door. The data loggers recorded the opening and closing of the doors from 06.00 h until 06.00 h the following morning. This was done only during the second night of the week when the loggers were installed in four Star homes and four traditional houses. The data retrieved from the loggers were combined with other variables investigated in the study

Table 1. 2: Summary characteristic features of the common fly vectors found in the study area.

Group	Specie name	Behaviour	Disease/pathogens transmission
Hematophagous (blood sucking) flies	*Anopheles gambiae* s.l.	**Breeding habitats**: Breeds in a wide range of aquatic habitats both permanent and semi-permanent. More common in open sunlit pools but also found in shaded sites and polluted sites (Minakawa 2004) **Feeding and resting behaviour:** Prefers to feed on humans indoors at night, particularly *An. gambiae* s.s., and completes its gonotrophic cycle indoors, (Charlwood 2018) *An. arabiensis* are opportunistic feeders that can blood-feed on multiple hosts both indoors and outdoors. They complete their gonotrophic development outdoors, (Charlwood 2018)	Transmits malaria parasites
	Anopheles funestus s.l.	**Breeding habitats**: Prefers to breed in semi-permanent or permanent habitats characterized by clear water with emergent vegetation, located less than 100 m from human dwellings. These habitats include small spring-fed pools, natural ponds, and slow-moving river tributaries (Nambunga 2020).	

		Feeding and resting behaviours: Prefers to feed on humans and rest inside houses, especially *An. funestus* s.s. (Lwetoijera, 2014). Other sibling species, such as *An. rivulorum* and *An. parensis*, prefer to feed both indoors and outdoors on multiple hosts, particularly in areas where livestock are kept (Kamau, 2003)	
	Culex specie	**Breeding habitats**: Prefer a wide range of breeding habitats characterized by turbid water and emergent vegetation. *Cx. Quinquefasciatus* breeds in pit latrines. **Feeding and resting behaviours**: Demonstrated a wide range of host preference which cut across animals and birds, (Fall 2011)	Transmit viruses such as West Nile virus, Japanese encephalitis virus, St. Louis encephalitis virus, as well as Western and Eastern equine encephalitis viruses. It also transmits filarial worms causing lymphatic filariasis.
Non-hematophagous (non-blood feeding) flies	*Musca domestica* (housefly)	**Breeding habitats**: Prefers to lay eggs in decaying organic matter such as garbage and manure, providing warmth, food for the maggots, and the essential moisture required for their reproductive cycle (Malik, 2007). **Feeding behaviour**: They are opportunistic feeders and can consume a wide range of organic materials, making them common pests in both	Mechanically transports various pathogens from contaminated sources to food and water. Diarrhoeal pathogens carried by the common housefly include *Salmonella typhoid*, *Vibrio cholerae*, and *Escherichia coli*

		residential and agricultural settings	
	Chryosomya putoria (African Latrine fly)	**Breeding habitats:** Prefers to breed in human faeces, rotting meat or other decaying organic materials. **Feeding behaviours**: They prefers to feed on human faeces (Lindsay 2012).	Mechanically transmit *E. coli*, *Shigella spp*, *Salmonella spp*, *Campylobacter* and other diarhoea pathogens
	Sarcophaga species (flesh fly)	**Breeding habitats:** Their feeding and breeding biology is uncertain or even unknown, but it is suspected that they breed in the corpses of dead animals (Jordaens 2013). **Feeding behaviours**: Only *S. javanica* demonstrates a preference for feeding on carcasses of deceased animals; the food preferences of other *Sacrophaga* species remain unknown (Jordaens 2013).	Cause myiasis in animal flesh (Greenberg 1973).

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Chapter 2 : The effect of light and ventilation on house entry by *Anopheles arabiensis* sampled using light traps in Tanzania: an experimental hut study.

Abstract

Background: In sub-Saharan Africa, house design and ventilation affects the number of malaria mosquitoes entering houses. It was hypothesized that indoor light from a CDC light trap, visible from outside a hut, would increase entry of *Anopheles arabiensis*, an important malaria vector, and we examined whether ventilation modifies this effect.

Methods: Four inhabited experimental huts, each situated within a large cage, were used to assess how light and ventilation affect the number of hut-entering mosquitoes in Tanzania. Each night, two intervention and two control huts were tested. Three sets of experiments were conducted: 1) transparent vs. opaque-walled huts, 2) open vs. closed eave gaps, and 3) well-ventilated vs. poorly ventilated traditional houses. Each night, 300 female laboratory-reared *An. arabiensis* mosquitoes were released inside each chamber for 72 nights. Nightly mosquito collections were made using CDC-light traps. Temperature and carbon dioxide concentrations were measured using data loggers. Treatments and sleepers were rotated between huts using a randomized block design.

Results: In experiment 1, when indoor light was visible outside the huts, there was an 84% increase in the odds of collecting mosquitoes indoors (Odds ratio, OR=1.84, 95% confidence intervals, 95%CI=1.74-1.95, p<0.001) compared with when it was not. In experiment 2, although the odds of collecting mosquitoes in huts with closed eaves (OR=0.54, 95%CI=0.41–0.72, p<0.001) was less than those with open eaves, fewer mosquitoes entered either type of well-ventilated hut. In experiment 3, the odds of collecting mosquitoes was 99% less in well-ventilated huts, compared with poorly-ventilated traditional huts (OR= 0.01, 95%CI=0.01–0.03, p<0.001). In well-ventilated huts, indoor temperatures were 1.3°C (95%CI=0.9–1.7, p<0.001) cooler, with lower carbon dioxide (CO_2) levels (mean difference=97 ppm, 77.8–116.2, p<0.001) than poorly-ventilated huts

Conclusion: Although light visible from outside a hut increased mosquito house entry, good ventilation reduces indoor carbon dioxide concentrations, a major mosquito attractant, thereby reducing mosquito-hut entry.

Background

In sub-Saharan Africa, most malaria transmission occurs indoors at night (Huho et al. 2013, Sherrard-Smith et al. 2019). The design of a house (Jatta et al. 2018, Jatta et al. 2021), its height above ground (Carrasco-Tenezaca et al. 2021) and the degree of crowding in a building (Kaindoa et al. 2016) affect house entry by malaria mosquitoes. One reason for this is that the relative attractiveness of a building depends on how carbon dioxide produced by dwellers emanates from a house (Jatta et al. 2018). This gas is a major mosquito attractant (Gillies 1980), with large and concentrated plumes being more attractive than low concentrations diffusing out from numerous parts of the building (Jatta et al. 2018). Ultimately, it may be possible to design ‘stealth’ houses, where few mosquitoes find and enter a house.

Improved ventilation is critically important for reducing malaria transmission inside houses for multiple reasons: (1) it will dilute the carbon dioxide concentration indoors, reducing the odour plumes emanating from a building (Jatta et al. 2021) , making it less likely that a blood-seeking mosquito will locate a person to feed on and transmit malaria, (2) it will keep the bedroom cooler, cooling the body and reducing carbon dioxide production from those sleeping in the room (Jatta et al. 2021), and, (3) cooling the house makes it more likely that people will sleep under a bed net (Pulford et al. 2011). Based on the need to keep a house well-ventilated and cool, scientists have designed several prototype houses to reduce mosquito-house entry (von Seidlein et al. 2017). The houses were constructed with walls made of shade cloth, which is permeable to both air and light, with a low heat capacity, resulting in rapid cooling of the house at night. A pilot study of six prototype houses in Tanzania showed that there was a 95% reduction in mosquito-house entry in double-storey buildings and a 70% reduction in screened single-storey buildings elevated on stilts compared with unmodified reference houses. Both elevated single- and two-storey buildings were 2.3°C (95% CI 2.2–2.4) cooler than traditional housing. Thus, using materials to construct walls that increased ventilation and had a low thermal mass resulted in few mosquitoes indoors and cooler indoor temperatures. In addition, elevating a house also reduces mosquito entry, as shown by an experimental hut study in The Gambia, where individual huts were raised or lowered to different heights (Carrasco-Tenezaca et al. 2021).

Based on these encouraging findings, a randomised controlled trial (RCT) exploring the impact on health of a new healthy house, known as a Star home (Fig 2.1A), is in progress in rural south-eastern Tanzania (von Seidlein et al. 2017). Star homes are two-storey buildings, with the bedrooms on the upper storey and a kitchen and storeroom on the ground storey. The house is designed to be cool and is well ventilated largely because it is constructed using shade-net panels, which are air permeable, for most of the walls. Before starting the trial, however, we had two concerns about our novel house and study design, which lead to the series of experiments reported here. Firstly, we were concerned that light from the Centers for Disease Control and Prevention (CDC) light traps used to evaluate the protective efficacy of these houses, would be seen from outside the house and might attract more mosquitoes into the house compared with nights when the trap was not used – inflating mosquito collections in Star homes. Secondly, the Star home has small gaps under the corrugate-roofing sheeting that might be an important entry point for mosquitoes (Fig 2.1B), given that open eaves are the major route by which *Anopheles gambiae sensu lato* enters traditional houses (Lindsay & Snow 1988, Njie et al. 2014). The experiments described here, were designed to answer these questions, but simultaneously enabled us to assess how light emanating from the light-traps and ventilation affected the house-entering behaviour of *Anopheles arabiensis,* the most common vector of malaria in the Rift valley and drier parts of sub-Saharan Africa (Wiebe et al. 2017).

Figure 2.1: Star home. A, exterior view; B, interior view showing the air-permeable green shade-net wall and the bright light above the purlins and below the corrugate iron roof showing the openings under the roof.

Methods

Study area

The study was conducted at Mosquito City, Ifakara Health Institute's semi-field system, located near Kining'ina village (8.11417 S, 36.67484 E), approximately 5 km north of Ifakara town, Tanzania, in the dry season, on 72 nights (i.e. 3 experiments x 24 nights) between September 2020 to February 2021 (Ogoma et al. 2012, Lwetoijera et al. 2014) (Fig. 2.2). Briefly, the semi-field system is a large outdoor cage constructed with a metal-framed shell and mesh walls, supported on a concrete floor 4.53 m high and 553 m^2 in area (Fig. 2.2) (Ferguson et al. 2008). The building contains six identical chambers, each 9.6 m × 9.6 m in floor area, with side-walls 4.1 m high, each housing an experimental hut. In each chamber, the floor is covered with local soil to a depth of 400 mm, which allows vegetation to grow inside (Lwetoijera et al. 2014, Mmbando et al. 2019). Each night, we used four chambers: two with one typology of hut and two with a comparator hut.

Figure 2.2: Ifakara Health Institute Semi-field compartments located at the Mosquito City facility in Kining'ina village, with experimental huts in separate cages.

Study design

A randomized block design was used to allocate treatments to the four chambers in four-night blocks. This was a balanced design such that every possible combination of hut typologies had been tested after six blocks, with each hut typology being tested in each chamber for three blocks (Supplementary table S1). At the start of each experiment, one sleeper was randomly allocated to a hut, and then rotated between huts for the following three nights, such that at the end of a four-night block, each man had slept in each hut six times. This design allowed us to quantify the effect of the hut typology, adjusting for variation from night to night, sleeper and chamber. For each experiment, light traps were used to collect mosquitoes indoors for four nights each week for six weeks (n=24 nights).

Three experiments were carried out using four experimental huts each occupied by an adult man. There were two huts in each study group and each hut was situated within a large-screened cage (Fig. 2.2). Each night 300 *An. arabiensis* female mosquitoes were released in each cage outside the hut and collected indoors using CDC light traps. Experiment 1 compared huts with light-permeable walls with light-opaque walled huts and was designed to assess whether more mosquitoes entered huts with light-permeable walls compared to those with opaque walls. Experiment 2 compared shade-cloth walled huts with openings under the corrugate roof, which mimicked Star homes, with similar huts without holes under the roof. This was designed to determine whether the small-roof openings increased mosquito entry. Experiment 3 compared 'Star home' style huts with traditional mud-walled and thatched roof houses, replicating the typologies of housing found in the RCT study area. The experiments are summarised in (Fig. 2.3).

Experimental huts

Details of the experimental huts are shown in figure 3 and in the supplementary material. Each hut was constructed using 25.4 mm^2 iron-metal frames and measured 2.62 m x 1.86 m in floor area, with 2.0 m high walls with 150 mm high eave gaps immediately under the over-hanging roof. In each hut, there was one metal door, 1.75 m high and 75 cm wide, with a 20 mm high by 750 mm wide slit above and below the door, to simulate a badly fitting door common in villages. The roof was made of corrugated sheeting with a sloping flat design.

In experiment 1, huts with light-permeable walls were compared with light-opaque walled huts. Importantly, the walls consisted of two layers, the external layer was shade cloth and the internal layer clear or opaque plastic. This design allowed us to compare the effect of light alone, keeping the indoor temperature similar in both hut typologies. Light-permeable walls were constructed from panels consisting of 80% shade green nets (high density polyethylene net, Ultra Violet stabilized, Multiknit Ltd, South Africa), with net holes measuring 2 mm x 2 mm on the external face and an internal face of either black opaque high density polythene measuring 2.4 m x 0.69 m x 0.8 mm thick (JK Plastopack Pvt Ltd, Ahmedabad, India) or similar, but clear, transparent plastic sheeting of the same dimensions (Bronze, JK Plastopack Pvt Ltd Ahmedabad, India; figure 3). Internal sheeting panels were fixed in place using Velcro strips so that the panels could be moved easily between huts.

In experiment 2, the Star home-style huts were a similar design to those described in experiment 1, although in this experiment there was no internal panels of plastic sheeting, and the eaves gaps were either open or closed. In this experiment we compared huts with small gaps (24 mm wide and 18 mm radius) which form under the corrugate-roofing panels when lying on purlins (horizontal beams along the length of the roof supporting the rafters), with huts with these roofing holes plugged with sponges (Fig. 2.3, Appendix 1).

In experiment 3, we compared the Star home-style hut described in experiment 2, with a traditional-style hut common in Tanzania and other parts of sub-Saharan Africa, (Fig. 2.3). The traditional hut had a floor area of 3.1 m × 2.7 m, with walls 1.8 m high. These huts were constructed with burnt-brick walls, 90 mm thick, with gabled, thatched roofs, 50 mm thick, with 200 mm open eaves on all sides of the hut. The hut had one door at the front, 1.75 m high and 0.75 m wide. There were four windows 0.56 m × 0.56 m in size, one on each side of the hut. Although windows were closed during the experiments, they had 5 mm wide gaps on the vertical side of each window to simulate poorly fitting windows.

Study procedures

Male sleepers were recruited to the study after providing their signed consent and tested for malaria parasites using a rapid diagnostic test (paraHIT®f, Span Diagnostics Ltd, Sachin (Surat), India). All tested negative. The men did not smoke, drink alcohol or use perfume during the study.

Three hundred, unfed five-to-eight-day old female laboratory-reared *An. arabiensis* mosquitoes were released in each chamber each night, 3 m from the front door of each hut at 18.30 h. One man, between 18-35 years old, slept in each hut under an intact insecticide-treated net (Olyset nets, Sumitomo Chemical, Arusha, Tanzania), measuring 0.9 m wide x 1.8 m long x 1.8 m high. Sleepers entered the huts at 19.00 h and left at 07.00 h the next day. Mosquitoes were collected from each hut using a CDC light trap (incandescent light, Model 512, BioQuip product, California, USA), with the bulb 1 m above the floor at the foot end of the bed and operated from 19.00 h to 07.00 h. Mosquitoes from the light trap were collected and killed by exposure to chloroform. Any remaining mosquitoes were cleared from inside and outside the huts each morning using a mechanical aspirator (Prokopack®, model 1419, John W. Hock Co., Gainesville, USA). Mosquitoes from the light trap and aspirator collections were counted (App.4: Supplementary material). Resting mosquitoes were collected to ensure no mosquito remained inside the huts and chamber that could affect the next day experiment. Resting mosquitoes collected both inside and outside by using mechanical aspirator were counted and recorded.

Indoor temperature, carbon dioxide concentration and relative humidity were recorded using data loggers (CO_2Meter.com, model CM-0018-AA, GasLab, Florida, USA). The data loggers were positioned in the centre of each hut, 1 m above the ground. All data loggers recorded at 30 min intervals from 18.30 h to 07.00 h. Outdoor temperature, carbon dioxide concentration and relative humidity were measured only in experiment 3, at a height of 1 m high in the centre of each large cage 5 m from each hut.

Outcomes

The primary outcome was the proportion of host-seeking and resting *An. arabiensis* collected inside each hut each night using light traps and Prokopack aspirators, respectively. Secondary outcomes were mean indoor temperature and mean indoor carbon dioxide concentration recorded between 19.00 h and 07.00 h.

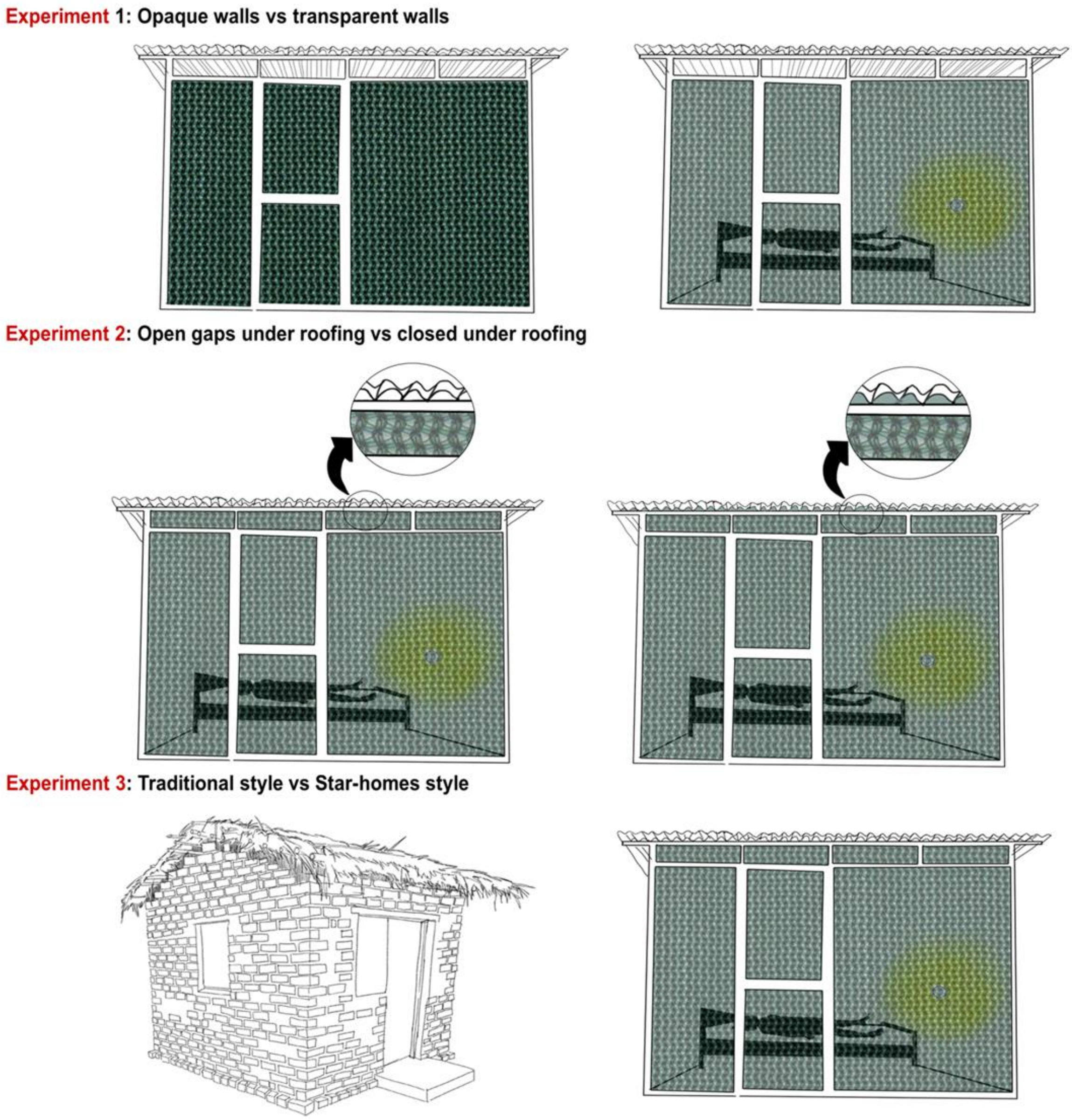

Figure 2.3: Summary of experiments. The reference hut in each experiment is shown in the first column of each row. In each experiment, local badly-fitting doors were mimicked by adding narrow gaps above and below each door.

Data analyses

Sample size was estimated based on a previous study (Mmbando et al. 2018) done at the study site where the mean number of *An. arabiensis* collected per trap per night was 10.4 (SD=21.5). The sample size simulation was based on a negative binomial distribution and designed to detect a 50% reduction in indoor mosquitoes at the 5% level of significance with 90% power, 24 nights (six weeks) of experimentation was sufficient.

Data analysis was done with R (version 3.3.2) (Team 2013), using *lme4* (Chaves & Chaves 2010, Bates et al. 2014), and *dplyr* (Wickham et al. 2015) packages*.* Mosquito count data were modelled using generalized linear mixed effect model (*glmer*) using a binomial distribution to account for a log-link function. The recaptured mosquito count numbers per SFS-chamber in a particular night were represented as a proportion of the released mosquitoes in the specific chamber. The response variable was the proportion of mosquitoes caught in light traps, while hut typology was included as fixed factors. The sleeper, chamber ID and nights were included as fixed effects. Model coefficients were exponentiated to obtain the odds ratios (OR) and 95% confidence intervals. Adjusted mean nightly difference of temperature, relative humidity and carbon dioxide concentrations together with its (95% CI) values per night/hut typology were calculated using linear mixed effect model (*lmer*) modelled using a normal distribution. Analysis of variance was used to assess the significance level (p-value) of mean difference of environmental conditions obtained from the huts typology/night. In experiment 3, matched-paired t tests were used to compare the mean indoor carbon dioxide concentrations in each hut type with the mean outdoor concentrations.

Results

Experiment 1. Light-opaque walls versus light-transparent walls

During the experiment, 69.5% (10,010/14,400) of the mosquitoes released were collected using CDC-light traps in the huts with transparent walls compared with 53.8% (7,747/14,400) in those with opaque walls. The average nightly percentage of mosquitoes collected in each hut was 69.9% (95% confidence intervals, CIs=67.4–72.3) in those with transparent walls and 55.8% (95% CIs= 52.9–58.6) in those with opaque walls. The adjusted analysis showed that the odds of finding mosquitoes in huts with transparent walls, where the light could be seen from outside, was 84% greater than huts with opaque walls, where little, if any light, was visible from outside (Odds ratio, OR=1.84, 95% CIs=1.74–1.95, $p<0.001$, Table 2.1). There was no difference in mean nightly indoor temperature or indoor carbon dioxide levels between the two types of hut (Table 2.3), suggesting that differences in mosquito entry were due to light alone, rather than temperature or carbon dioxide.

There was no difference in indoor resting *Anopheles arabiensis* collected in the different typologies of houses (Odds ratio=0.89, 95% CIs=0.74 – 1.05, p=0.17). Since we caught more mosquitoes in transparent huts, there were fewer outdoor resting *An. arabiensis* in transparent-walled houses compared to opaque-walled houses (OR=0.57, 95% CIs=0.54 – 0.64, p<0.001; Table 2.2).

Experiment 2. Open gaps under roofing versus closed gaps under roofing

During this experiment, just 1.0% (144/14,400) of the mosquitoes released were collected in huts with open gaps under the roofing compared with 0.6% (80/14,400) in those where the gaps were closed. The average percentage of mosquitoes collected in each hut was 0.03% (95% CIs = 0.01–0.12) in those with gaps and 0.02% (95% CIs = 0.0–0.1) in those without. In the adjusted analysis, 46% fewer mosquitoes were collected in huts with no gaps than those with open gaps (OR=0.54, 95% CIs=0.41–0.72, p<0.001;Table 2.1). There was no difference in temperature nor carbon dioxide between the two types of hut (Table 2.3).

Huts with closed-eaves were 81% lower indoor resting *An. arabiensis* than those with open-eaves (OR=0.19, 95% CIs= 0.08–0.46, p<0.001; Table 2.1). There was a corresponding 3% increase in outdoor-resting mosquitoes in cages with huts with closed eaves compared to cages with open eave huts (OR=1.03, 95% CIs= 0.97–1.09, p<0.05; Table 2.2).

Experiment 3. Poorly ventilated versus well ventilated

In this experiment, only 0.3% (46/14,400) of the mosquitoes released were collected in the well-ventilated Star home-style huts compared with 29.5% (4,246/14,400) in the poorly ventilated traditional-style huts. The average percentage of mosquitoes collected in each hut was 0.3% in the Star home style huts (95% CIs= 0.16–0.66) and 19.3% in the traditional-style huts (95% CIs= 17.0–21.9). The adjusted analysis showed that the odds of mosquito house entry was 99% less in well-ventilated huts than poorly-ventilated huts (OR=0.01, 95% CIs=0.01–0.03, p<0.001, Table 2.1).

The odds of collecting indoor-resting mosquitoes was 88% less in well-ventilated, Star home-style huts than traditional-style huts (OR=0.12, 95% CIs= 0.06 – 0.23, p<0.001; Table 2.1). Consequently, the cages of Star home style huts had an increased odds of collecting outdoor resting *An. arabiensis* mosquitoes than traditional-style huts (OR=3.04, 95% CIs= 2.90 – 3.20, p<0.001; Supplementary table 2)

The indoor temperature was 1.3° C (95% CIs= 0.9–1.7, p<0.001) cooler in the Star home-style huts (24.8°C, 95% CIs = 24.6–25.1) than traditional-style huts (26.1°C, 95% CIs =25.7–26.4). There were also lower concentrations of carbon dioxide indoors in Star home-style huts (mean concentration = 320 ppm, 95%, CI=314–327) than traditional-style huts (541 ppm, 95%CI=516.4–565.4, p<0.001). Importantly, carbon dioxide concentrations in Star home-style huts were similar to outdoor levels (mean difference = 11 ppm, 95% CIs=4-13, p=0.95), but were 232 ppm higher in the traditional-style huts than outdoors (95% CIs= 176-298, p=0.03; Table 2.2). The environment conditions between the hut typologies was similar. During this experiment, the mean nightly outdoor temperature (19.00-07.00 h) was 25.1°C (95% CIs= 24.3–27), and carbon dioxide concentration was 309 ppm (95% CIs= 290–320), (Table 2.2).

Discussion

This series of experiments assessed three aspects of the Star home-type huts; (1) transparency vs opacity walls, (2) presence vs absence of small eave gaps under the corrugated iron roofs and (3) ventilation achieved through the permeable walls of shade cloth huts. These experiments provide new insights into the effect of light and ventilation on house entry by one of sub-Saharan Africa's most important malaria vectors, *An. arabiensis*. In this experimental setting, when the light from the CDC-light trap was visible from outside the hut, the odds of catching mosquitoes indoors increased by 84% compared with when the light was not visible from outside. Clearly, in our experiment light and human odours were attracting mosquitoes from outside the inhabited hut. In the 1960s, in the first pioneering studies where light traps were used to collect African mosquitoes, Odetoyinbo showed that light was an essential element of the CDC light trap, since without light the trap collected 95% fewer *An. gambiae s.l.* (Odetoyinbo 1969). Similarly, when Costantini and co-workers used a light trap indoors without a light they collected 63% fewer *An. gambiae* s.l. than a trap with a light bulb (Costantini et al. 1998).

This finding is important for several reasons. Firstly, light traps are a standard sampling tool for collecting indoor mosquitoes during randomised trials of vector control interventions (Massebo & Lindtjørn 2013, Tiono et al. 2018). Whilst this is probably not a concern in most studies where the sampling units are traditional houses with opaque walls and doors, they may bias the sample where screened doors are used, or if used in houses with multiple small openings (e.g. a bamboo house) which allows the light to be viewed from outside a house. In a recent trial in The Gambia, where screened doors were installed in village houses, the number of mosquitoes collected indoors was higher than in the reference group, with solid doors (Pinder et al. 2021). It seems likely that the Gambian study may have over-estimated the mosquito densities in houses with screened doors because the light from the trap would have been visible from outside the house. Secondly, it also raises concerns about whether light traps should be used in the trial comparing mosquito-house entry in Star homes with traditional houses. In the randomised controlled study we decided it was better to use light traps even though they could inflate the numbers collected in Star homes, the prototype healthy house.

Thirdly, our findings beg the question: will domestic lighting increase malaria transmission? The conclusions are mixed, with most studies indicating increased malaria mosquito biting associated with electrification (Barghini & de Medeiros 2010, Yamamoto et al. 2010, Pellegrini & Tasciotti 2016), perhaps due to people staying outside longer in the night and getting bitten by malaria mosquitoes. In a study in Tanzania, however, houses with electricity had fewer indoor mosquitoes than those without electricity (Finda et al. 2019). Since electricity is associated with greater wealth, fewer mosquitoes may be due to better built homes with fewer mosquito entry points than poorer households or the use of mosquito coils (James 1920, Finda et al. 2019). Clearly, further research is needed to clarify whether electric light, including that generated from tungsten and light-emitting diode bulbs, are attractive to mosquitoes and at what light intensity.

Responses of mosquitoes to light are complex, since it varies according to the time of day, feeding status of the mosquito, as well as the intensity and wavelength of light. At dawn and dusk, under natural conditions, a substantial proportion of indoor-resting *An. gambiae* s.l., including those that are semi-gravid, gravid and bloodfed, are attracted to the faint light from the windows, whilst intense light experienced during the day prevents exiting (Thomson 1948, Marchand 1983). Host-seeking mosquitoes are also stimulated to fly by low light intensities at dusk, with this behaviour being under circadian control (Jones & Gubbins 1978). Interestingly, feeding can be interrupted for up to four hours when mosquitoes are exposed to bright white light for 10 minutes at the start of the night (Sheppard et al. 2017). In Brazil, there was a tenfold reduction in *An. gambiae* s.l. (now known to be *An. arabiensis*) inside brightly-lit houses compared to the darkest houses (Causey et al. 2002). In Canada, nocturnal blood-questing mosquitoes are attracted to low intensity light, like black, blue and red, rather than high intensity colours like white and yellow (Browne & Bennett 1981), suggesting that this behaviour could be related to the choice of darker day-resting sites. In The Gambia, host-seeking mosquitoes also appear to be attracted to large solid objects over distances of 15-20 m (Gillies & Wilkes 1982). Gillies and Wilkes suggested that the outline of a house or its degree of isolation from other houses or patches of tall vegetation could affect the attractiveness of one house over another. In conclusion, the evidence suggests that while light in the presence of human odours is attractive to host-seeking mosquitoes, the shape and position of a dwelling may also be important.

Small gaps formed where the corrugate-metal roof rested on a purlin and, as seen in experiment 2, resulted in more mosquitoes entering the hut compared with gap-free huts. This is expected since open eaves, the gap between the top of the wall and the roof, are the major route by which *An. gambiae* s.l. enters a house (Lindsay & Snow 1988, Njie et al. 2014, Mburu et al. 2018). In our experiment, only a few mosquitoes entered these huts, suggesting that the holes might not cause an appreciable rise in mosquitoes in similarly constructed houses, such as the Star homes. The most plausible explanation for this finding is that shade-cloth walled huts attracts fewer mosquitoes as it allows carbon dioxide to rapidly be dissipated from the huts, unlike those with solid-plastic walls used in experiment 1.

In experiment 3, we found a 99% reduction in mosquitoes entering the well-ventilated, Star home-type huts, compared to the poorly-ventilated huts, which resembled traditional houses. The principal explanation for this difference in attractiveness is related to the concentration gradient of carbon dioxide leaving the two typologies of hut. In the well-ventilated hut the carbon dioxide concentration was just 11 ppm higher than outdoor levels, illustrating how effectively the gas is removed from the hut through the permeable walls. Since mosquitoes can only detect differences in carbon dioxide concentrations greater than 40 ppm (O'Connell 1996), this suggests that they may not be able to readily detect people sleeping in Star home style huts. In marked contrast, the poorly-ventilated huts have carbon dioxide concentrations considerably higher, 232 ppm above background levels, providing steep concentration gradients of the gas which allows outdoor mosquitoes to locate a host indoors. Our findings are supported by a recent study in The Gambia, which showed that a well-ventilated house could reduce indoor mosquito densities by 80% compared with a poorly-ventilated house

(Jatta et al. 2021). The well-ventilated huts also reduced indoor temperature by 1.3°C compared to the poorly-ventilated hut, which is likely to increase human comfort and, hence, usage of bed nets (von Seidlein et al. 2017). Star homes with their well-ventilated walls are likely to act as 'stealth houses', especially as the bedrooms are situated on the second storey. Recent research shows that the number of *An. gambiae* s.l. entering an inhabited building declines with increasing height, with 84% fewer mosquitoes when houses are elevated 3 m from the ground (Carrasco-Tenezaca et al. 2021).

The present study has several limitations. First, the experimental huts were smaller than Star homes and village houses, so that our findings are unlikely to be directly comparable with the field. Second, only one man slept in each hut, whilst in the villages two to six people sleep in the same house (Maia et al. 2016). Third, the study was conducted in a semi-field system with laboratory-reared *An. arabiensis*, which may differ in their behaviour to wild mosquitoes since colonisation is likely to reduce the variation in behavioural traits seen in wild populations. Fourth, we did not vary the time when the sleepers went to bed nor allowed them to open and close the hut door as they chose, behaviours that would influence mosquito-house entry. Fifth, the present study was based on indoor mosquito collections using CDC light traps and it may be that the findings would differ if using sampling techniques that did not use light as an attractant, such as human landing catches.

Conclusion

Light from a CDC light trap when seen from outside a hut increases the number of host-seeking mosquitoes entering the building compared to a hut with opaque walls. Whilst small gaps under corrugate roofing increase indoor entry, in our huts with air-permeable walls, few mosquitoes entered the huts. Indeed, the well-ventilated huts had markedly fewer mosquitoes entering the huts compared with traditional dwellings which are hotter and poorly ventilated. Although light traps and holes under the roofing increases the number of mosquitoes entering the building, the presence of air-permeable walls, that increases ventilation, results in remarkably fewer mosquitoes entering the building compared with traditional buildings. Our findings suggest that increasing ventilation in buildings will substantially reduce mosquito entry in Tanzania and is supported by studies from The Gambia (Jatta et al. 2021) suggesting that this may be broadly applicable for malaria control in the region. Considering the absence of other simple sampling tools that are not subject to operator bias, it also suggests that light traps could be used for routine sampling in the Star homes, even though this may slightly over-estimate the true mosquito entry rate. In relation to the design of our new design for a healthy house, filling in the small holes under the roofing is likely to make little difference to overall mosquito numbers entering this type of house. Most importantly, our findings add to the literature suggesting that increasing ventilation in houses in sub-Saharan Africa may contribute to a reduction in malaria transmission and makes bedrooms cooler at night.

Table 2. 1: Comparison of indoor densities of malaria vectors between different hut typologies. Covariates in the model include sleeper, hut position and night. Where CI = confidence intervals, OR = odds ratio.

Category	**Description**	**Mean (%) of mosquitoes /night (95%CI)**	**Adjusted Odds ratio (95% CI)**	***p*-value**
Experiment 1: Light-opaque walls vs light-transparent walls				
Typology	Opaque-walled	55.8 (52.9–58.6)	1	
	Transparent-walled	69.9 (67.4–72.3)	1.84 (1.74–1.95)	<0.001
Experiment 2: Open gaps under roofing vs closed gaps under roofing				
Typology	Open gaps	0.03 (0.01–0.12)	1	
	Closed gaps	0.02 (0.00–0.10)	0.54 (0.41–0.72)	<0.001
Experiment 3: Poorly ventilated vs well-ventilated				
Typology	Poorly ventilated	19.3 (17–21.9)	1	
	Well ventilated	0.3 (0.16–0.66)	0.01 (0.01–0.03)	<0.001

Table 2. 2: Environmental measurements between the different hut typologies.Where CI = confidence intervals, ppm = parts per million

Variable	Type of hut	Mean (95% C.I)	Adjusted mean difference (95% CI)	*p*-value
Experiment 1: Light-opaque walls vs light-transparent walls				
Temperature (°C)	Opaque-walled	27.1 (26.1-28.1)	1	
	Transparent-walled	26.2 (24.8-27.6)	0.9 (0.1–2.4)	=0.84
Relative humidity (%)	Opaque-walled	59 (56-62)	1	
	Transparent-walled	63 (60-66)	4 (0.4–8)	=0.27
Carbon dioxide (ppm)	Opaque-walled	414 (394-434)	1	
	Transparent-walled	407 (383-430)	-7 (-21–34)	=0.80
Experiment 2: Open gaps under roofing vs closed gaps under roofing				
Temperature (°C)	Open gaps	28.3 (28–28.5)	1	
	Closed gaps	28.2 (28–28.5)	-0.1 (-0.8–0.1)	=0.84
Relative humidity (%)	Open gaps	64.0 (62.8–65.2)	1	
	Closed gaps	65.0 (63.7–66.3)	0.8 (-0–2)	=0.50
Carbon dioxide (ppm)	Open gaps	398 (387–408)	1	
	Closed gaps	388 (377–399)	-10 (-22–2)	=0.43
Experiment 3: Poorly ventilated vs well-ventilated				
Temperature (°C)	Traditional	26.1 (25.7–26.4)	1	

	Star homes types	24.8 (24.6–25.1)	-1.3 (-1.7–0.9)	<0.001
Relative humidity (%)	Traditional	74.6 (72.4–76.7)	1	
	Star homes types	82.2 (81.1–83.3)	7.8 (5.9–9.7)	<0.001
Carbon dioxide (ppm)	Traditional	541 (516–565)	1	
	Star homes types	320 (314–327)	-97 (-116–78)	<0.001

Chapter 3 : Effect of a novel house design (Star homes) on indoor mosquito densities in rural Tanzania: a household randomised controlled trial.

Abstract

Background: Screening traditional houses can reduce malaria transmission. A randomized controlled household study was conducted in Mtwara, Tanzania, to assess whether a novel type of screened house (Star home), with bedrooms on the second storey, reduced the children's exposure to night-time biting malaria mosquitoes compared to traditional houses.

Methods: Indoor malaria-vector abundance was assessed in 110 Star homes and 110 traditional houses with thatched roofs and mud walls in 59 villages from September 2021 to December 2023. CDC light traps measured indoor mosquito densities at night in study houses every seven weeks. Multiplex polymerase chain reaction (PCR) identified members of the *Anopheles gambiae and Anopheles funestus* species complexes, enzyme-linked immunosorbent assays detected *Plasmodium* sporozoites and data loggers measured nightly temperature, carbon dioxide (CO_2) concentrations in bedrooms and duration of door opening. Generalized Linear Mixed effect models were used to compare differences between study groups.

Finding: A total of 9,832 mosquitos were collected, of which 23% (2,235/9,832) were anophelines and 77% (7,597/9,832) culicines The *An. gambiae* complex consisted of 68% (1065/1872) *An. gambiae* s.s, 25% (402/1872) *An. arabiensis* and 5% (84/1872) *An. merus*, while the *An*. *funestus* group consisted of 98% (256/259) *An*. *funestus* s.s.. Star homes reduced indoor densities of *An. gambiae* s.l. by 52% (adjusted mean rate ratio (RR)=0.48 (95% Confidence intervals [CI], 0.34–0.69, p <0.001), *An. funestus* by 81% (RR=0.19 [0.01–0.39], p<0.001), and *Culex* species by 64% (RR=0.36 [0.30–0.45], p<001) compared with traditional houses. Star homes were associated with a 55% reduction in entomological inoculation rates compared to traditional houses. During the night, Star homes were 0.54°C cooler (95% CI: -0.85°C to -0.21°C, p=0.002) compared to traditional houses, but the concentration of CO_2 was similar in both house types. The external doors on the Star

homes were open for shorter durations (52% less [95% CI: 43% to 65%]) than those in traditional houses.

Conclusion: Star homes reduced the abundance of indoor night-time biting mosquitoes and reduced the risk of malaria transmission compared to traditional houses. During the night, Star homes were cooler but had similar CO_2 concentrations to traditional houses. These findings illustrate the protective efficacy of well-screened houses in rural Africa.

The trial was registered to ClinicalTrials.govNCT04529434.

Background

The population of sub-Saharan Africa is experiencing rapid growth at a rate faster than anywhere else in the world and is accompanied by significant rural-to-urban migration (UN 2023). A recent analysis of housing in sub-Saharan Africa showed that between 2000 and 2015, the prevalence of improved housing (with improved water and sanitation, sufficient living area and durable construction) in rural areas increased from 11% to 23% (Tusting et al. 2019). There is, therefore, an urgent need to build better-quality housing for the growing population.

Building houses screened against mosquitoes is important, since most malaria transmission occurs indoors at night (Huho et al. 2013, Sherrard-Smith et al. 2019, Mshamu et al. 2020). In traditional houses most *Anopheles gambiae* s.l. primarily enter homes through the open eaves (the gap between the top of the wall and the roof) and secondarily through gaps around windows and doors (Lindsay & Snow 1988, Njie et al. 2014). A systematic review and meta-analysis of studies conducted between 1900 and 2013 showed that residents living in modern houses had 47% lower odds of getting malaria infections compared to those in traditional houses (Tusting et al. 2015). Similarly, a multi-country analysis of survey data gathered between 2008 and 2015 in 21 sub-Saharan African countries showed significant reductions in malaria incidence of 9% (by microscopy) and 14% (by rapid diagnostic test) for children living in modern houses compared to those living in traditional houses (Tusting et al. 2017).

The available evidence emphasizes the need to add development interventions such as house screening to conventional biomedical tools, to fully leverage the 3rd Sustainable Development Goals (SDGs) which focusing on good health and wellbeing of the population (UN 2017b). The need for housing improvements as a core intervention against vector-borne diseases was also recommended by the World Health Organization (WHO) with a theme of building the vector out, by advocating for screening of windows, doors and eaves and improved ventilation to increase the use of insecticide-treated nets (ITNs) in hot climates (WHO 2017b).

In 2014 and 2015, a pilot study conducted in north-east Tanzania, tested six prototype houses constructed from different materials and were either single or double storey. Two-storey houses reduced mosquito house entry by 95% compared to traditional houses (von Seidlein et al. 2017). Although the prototypes were highly valued by the communities, their impact on health outcomes was not assessed during the pilot study. Therefore, a randomized household-controlled trial was conducted in rural Mtwara to evaluate the impact of the novel house design on three major childhood diseases (malaria, diarrhoea illnesses, and respiratory tract infections) in sub-Saharan Africa (Mshamu et al. 2022). The trial compared the abundance of mosquitoes collected indoors in a novel screened house (Star homes) and traditional houses in rural Mtwara, south-east Tanzania over two years. In addition, the study evaluated the impact of the Star homes on nightly temperature, carbon dioxide concentration, as well as duration of door opening compared to traditional houses. This is the first randomised controlled trial of a new type of house designed to reduce malaria transmission. Our study findings will be of relevance to those planning the large-scale building of rural homes in the SSA regions.

Methods

Study design

A detail description of this household randomized control study was provided by (Mshamu et al. 2022). Briefly, indoor mosquito densities were recorded in 110 intervention houses (Star homes) and 110 control houses (traditional houses) every seven weeks from September 2021 to September 2023. Additionally, the study assessed the night-time indoor temperatures, CO_2 concentration, and duration of door opening between the two-house types. The principal outcome of this study was to compare indoor mosquito densities caught in these two-house types.

Study area

This study was conducted in 59 villages in the rural part of Mtwara region (10.5181° S, 40.0633° E), south-east Tanzania from 2021 to 2023 (Fig. 3.1). The study area was a coastal strip of sandy low-lying land with undulating hills inland, with a maximum altitude of over 350 m above sea level, covered with forest and shrubland. There are two rainy seasons each year: the long rains between February and April and shorter rains between October to December (Fig. 3.2). The main ethnic group in the study area are the Makonde followed by Makua and Yao people. The main economic activities in the study area are the cultivation of cashew nuts, cassava, maize, and rice. Additionally, a minority of the residents of coastal villages are engaged in fishing activities (Mshamu et al. 2020).

Species abundance within the study area is dominated by *Culex* species, followed by *An. gambiae* s.l., and *An. funestus* s.l. (Lupenza et al. 2021). The study area had a malaria prevalence exceeding 20% among children aged 2-10 years old and pregnant women (Ministry of Health (MoH) [Tanzania Mainland] 2023). ITNs are the primary vector control tool. Each household owns at least one bednet, with an ownership rate of 74%. Additionally, at least two people sleep under one net per household, with a coverage rate of 51% (Ministry of Health (MoH) [Tanzania Mainland] 2023).

Star homes

All 110 Star homes were constructed between January and June 2021 (Fig. 3.2), representing the intervention group, with 440 traditional houses recruited to the control group (Mshamu et al. 2022). In each study village, there were two to three Star homes that were compared to the nearest two traditional houses (Mshamu et al. 2022). Star homes represented less than 10% of the houses in each village to minimize the possibility of mosquitoes that were prevented from entering an intervention house from entering a control house in larger numbers than usual (Maia et al. 2016). Previous studies suggest that the risk of such diversion was low and unlikely to increase exposure in unprotected homes (Clarke et al. 2001). From July 2021 to February 2023 (Fig. 3.2), two ITNs (Olyset, 2% Permethrin, Sumitomo Chemical, and A to Z Textile MillsTM, Arusha, Tanzania) were provided by the study team to each study house irrespective of the number of households per family, representing current best practice (WHO 2011).

The design of the Star homes has been described in detail in previous publications (von Seidlein et al. 2017, Mshamu et al. 2022). In brief, the Star homes are two-storey houses with two sleeping rooms on the second floor (Mshamu et al. 2022). Star homes had four features that could reduce the entry of mosquitoes indoors, including (1) walls made of screened netting, (2) self-closing external solid metallic doors with an additional internal screened door leading to the bedroom, (3) bedrooms on the second storey, and (4) a screened kitchen on the first storey, where people often stay in the early evening (Mshamu et al. 2022). The Star homes' walls are made of air-permeable shade nets to optimize airflow across the surface, helping to cool the building and reduce indoor carbon dioxide levels (CO_2), which are major attractants for mosquitoes (Gillies & Wilkes 1970). The self-closing external doors provide security, privacy, and prevent disease vectors from entering the house. The raised sleeping bedroom offers privacy and creates a cooling environment inside the house and may also help reduce mosquito entry (Carrasco-Tenezaca et al. 2021). The screened kitchen is designed to prevent domestic flies and mosquitoes from entering the house, as well as to deter rodents, while ensuring proper ventilation during cooking to reduce indoor air pollution (Figure 3.3a), (Mshamu et al. 2022).

Inclusion criteria for the study households

Houses were eligible for inclusion in the study if they met the following criteria: (1) traditional construction with mud walls, a thatched roof, and a dirt floor, (2) had a pit latrine, (3) absence of grid electricity, (4) no access to piped water, (5) sufficient land for Star home construction, (6) residence of at least two children under 13 years old, and (7) willingness of occupants to participate in three years of disease surveillance, (Fig 3.3b) (Mshamu et al. 2022).

These criteria led to 862 eligible households. Subsequently, eligible participants underwent a two-stage village-level lottery process. The first lottery selected 110 households, ensuring at least two families per village where Star homes were to be built. The second lottery designated 440 traditional houses. Within each study village, no more than two to three Star homes were constructed, and only one house was allocated per family (Mshamu et al. 2022). As a result, the study included 550 houses, 110 Star homes and 440 traditional houses, chosen from 59 study villages. For the entomological surveillance, however, all 110 intervention houses were included in the study together with the nearest traditional house from the study cohort, making a total of 220 study houses.

Randomization and masking

To reduce the distances travelled by field staff in this large study area, the 220 study houses were divided equally into seven geographical clusters. Each cluster consisted of four to five adjacent villages. Entomological surveillance was conducted in each cluster, with six clusters each containing 16 Star homes and 16 control houses, and one cluster comprising 14 Star homes and 14 traditional houses. Each cluster was further subdivided into four sub-clusters (geographical nearby villages), each containing eight houses, including four Star homes and four control houses selected as the nearest neighbours.

Figure 3.1: Study houses, a) Star homes and b) traditional house.

The order of cluster visits was randomly assigned and remained consistent throughout the trial to eliminate systematic biases related to cluster position. The designated order of cluster visits was 1, 6, 7, 3, 2, 5, and 4 (source: https://www.random.org/sequences/). The order of visiting each sub-cluster was also randomly determined and maintained throughout the study. This helped avoid systematic biases related to space and time (seasons). The random selection allowed each study village to have an equal chance to be visited at a specific period of time. Star homes were assigned numbers from 001 to 110, while traditional houses were assigned unique IDs ranging from 200 to 640 for clear distinction. The 110 traditional houses were randomly selected from a total of 440 houses, ensuring proximity to the Star homes.

Mosquitoes were collected from one sub-cluster of eight houses each night. Collections were performed in one cluster per week, with a different sub-cluster sampled over four consecutive nights, from Monday to Thursday, every week. Thus host-seeking mosquitoes were sampled once every seven weeks using CDC light traps for each house type (both Star homes and traditional houses). When traditional households declined or were unable to participate, the next closest traditional house within the same study village was selected as a replacement. Sampling took place in a room where at least one study child slept, and this room was consistently used throughout the study. In cases where study children occupied multiple rooms within the same house, the first child was randomly chosen from all the study children residing there.

As the study design was an open-label randomized household trial, complete masking of all study procedures was not feasible. However, bias in mosquito collection was minimized by using standard light traps that did not rely on the fieldworker's ability to collect specimens. Trap catches were examined and analysed by different technicians who were not involved in mosquito trapping on the respective night and in the village. Datasets were unblinded only after locking all critical data for primary and secondary endpoints.

Mosquito sampling

CDC light traps (incandescent light, Model 512, BioQuip product, California, USA) are a standard method of mosquito collection and not subject to operator bias and were placed inside the study child’s sleeping area or bedroom of the study children and operated by a field assistant, who was individually assigned to two study houses. Each trap was suspended 1m above the ground at the foot end of an ITN occupied by a study child bed.

In cases where the study child did not sleep under an ITN, the light traps were still operated, but it was noted that ITNs were not used. If a study child was not present in the room, a note was taken, and the light trap was suspended near the bed occupied by an adult. Traps operated from 19.00h to 07.00h the following morning. Each house was sampled every seven weeks, for 24 months. Each morning, mosquitoes were killed and sorted by taxa, using the mosquito identification keys (Coetzee 2020) and sexed. Mosquito data were recorded along with the household ID and other relevant experimental design information.

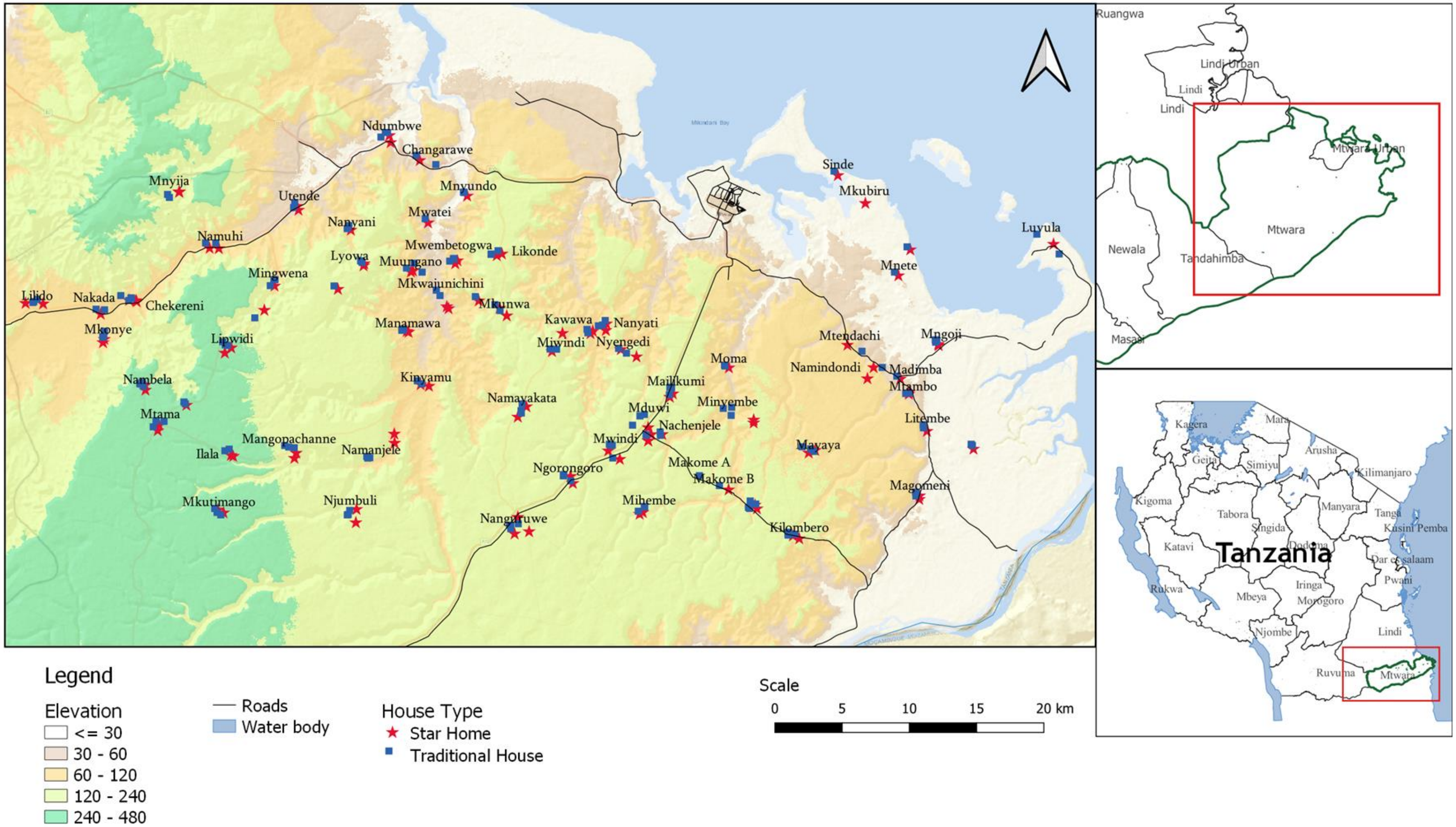

Figure 3.2: Location of study houses.

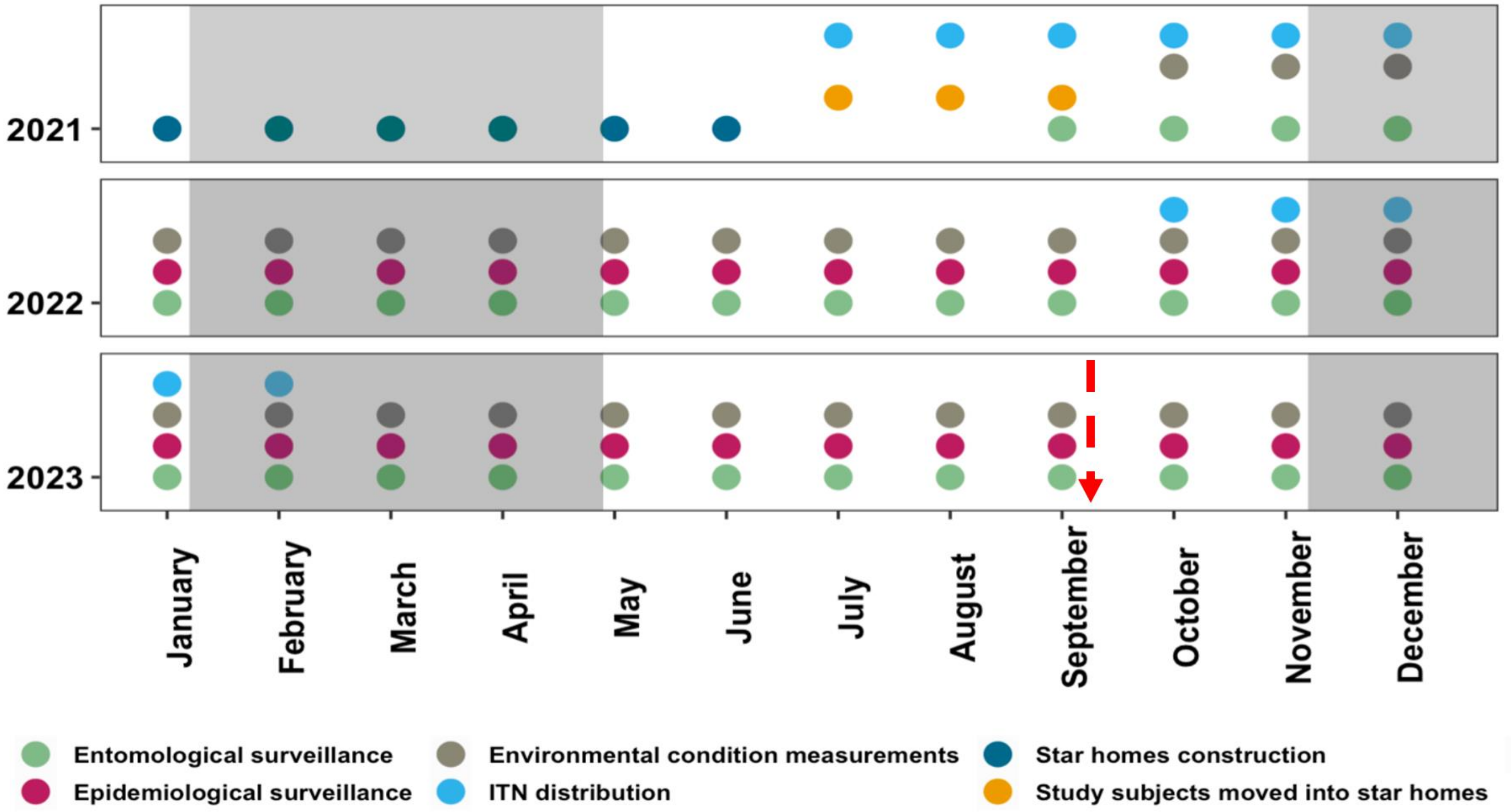

Figure 3.3: Trial design. Where grey rectangles represent rainy seasons, ITNs are distributed by the study project in three phases (July-December 2021), followed by (October-December 2022) and last phase in (January-February 2023). Entomological surveillance began in (September 2021 to September 2023, presented in red dotted arrow).

Validating light traps

The indoor mosquito densities collected using light traps were low and so we wanted to check that we were not missing large numbers of mosquitoes. To validate the efficiency of light traps, a subset of resting mosquito collections was conducted using a mechanical aspirator (Prokopack®, model 1419, John W. Hock Co., Gainesville, USA) from March to August 2023 (Fig. 3.2). One field worker performed these collections in two houses for 25 minutes each morning, immediately after retrieving the light traps between 06.20 h and 06.45 h (Vazquez-Prokopec et al. 2009). The number of mosquitoes collected with both methods in each study group was counted and compared. Specimens caught by the light traps and Prokopack aspirators were transferred to the field laboratory for sorting and data recording. Mosquito count data from the two methods were then compared.

Laboratory analysis

Sub-samples of primary malaria vectors were identified to species using multiplex Polymerase Chain Reactions (PCR) (using ribosomal DNA fragments) to distinguish between members of the *An. gambiae* complex (Scott et al. 1993) and the *An. funestus* group (Koekemoer et al. 2002). Enzyme-Linked Immunosorbent Assays (ELISA) detected the presence of circumsporozoite proteins in mosquito salivary glands (*Plasmodium* infections) (Durnez et al. 2011).

Environmental measurements

Every Tuesday night, 12 data loggers recorded indoor temperature, relative humidity, and CO_2 concentrations in the children's sleeping areas. Data collection was conducted in four Star homes and their corresponding four traditional houses used for mosquito collection on Monday night. To understand how these two-house types regulate environmental conditions, four additional loggers were deployed for outdoor control measurements.

Indoor and outdoor temperature and CO_2 were recorded using electronic data loggers (GasLab, CO2Meter.com, model CM-0018-AA, GasLab, Florida, USA), recording every 30-minutes from 19.00 h to 06.00 h the following morning. Indoor loggers were positioned at the foot end of the child's sleeping area, 1 m above the floor. Outdoor loggers were mounted on a metal stand, positioned 1 m above the ground, 5 m from the house. Each data logger was assigned a unique identifier (ID) corresponding to its respective location (indoor or outdoor) and the specific house number each night during mosquito collection, (Fig 3.4).

Figure 3.4: Environmental conditions loggers recording measurements; a) inside the study child bedroom and b) outside the house.

Duration of door opening

The duration of time a door was open was recorded in eight houses per week/cluster in four Star homes and four traditional houses. In the Star homes, two door loggers (Onset UX90-001-HOBO/state/pulse: HOBO®:On State data loggers) were installed, one on the main external door and the other placed on the bottom of the stairways leading to a study child's bedroom which the light trap was suspended in the previous night. For the traditional houses, a single data logger was installed only on the main external door, since control houses lacked bedroom doors. A total of 12 door loggers were deployed each night, with eight placed in Star homes and four in traditional houses. These loggers recorded door openings every 30 s from 19.00 h until 06.00 h the following morning. This data collection occurred on the second night of the week, specifically on Tuesday, involving four Star homes and four traditional houses used for the environmental condition measurements (Table 3.1, Fig.3.5). Households were informed about the purpose of the door loggers prior to installation to prevent any changes in behaviour that might affect the data.

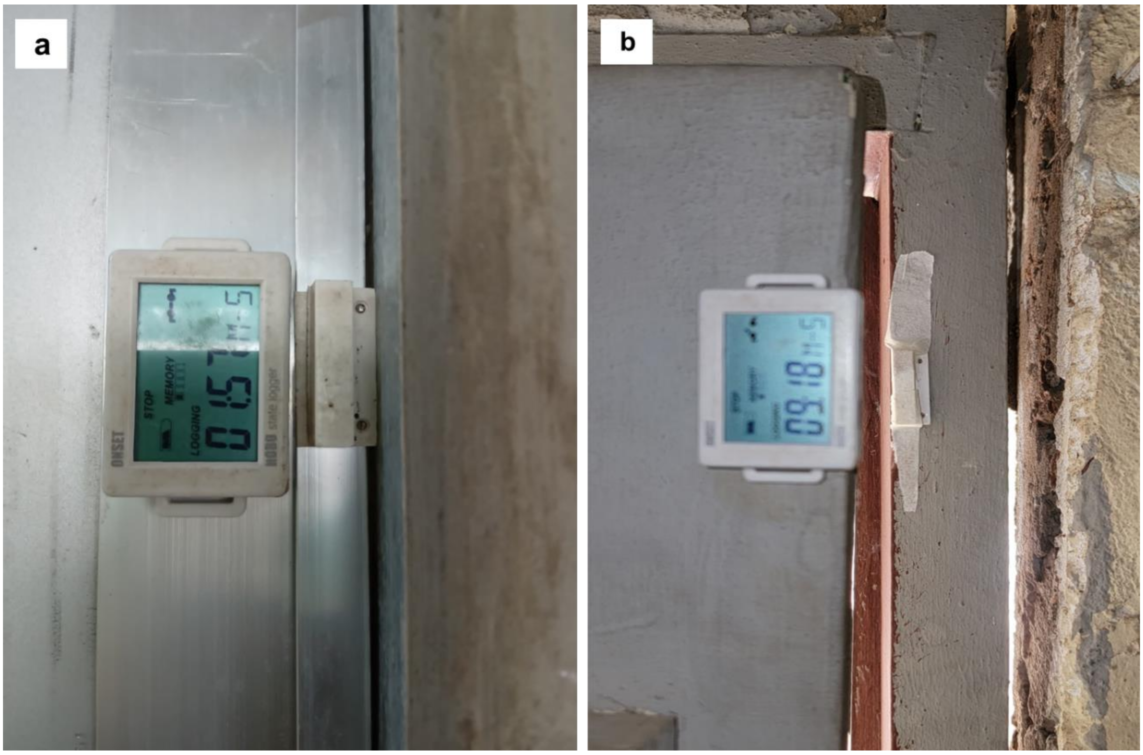

Figure 3.5: Door loggers fitted inside the doors of, a) the Star homes, a) and b) the traditional house.

Statistical analysis

Computer simulations based on a negative binomial distribution indicated that detecting a 50% reduction in indoor-entering mosquitoes (i.e., *An. gambiae* s.l.) required 110 Star homes and 110 control houses. This was based on a previous study (Mmbando et al. 2018) at the study site, where the mean number of *An. arabiensis* collected per trap per night was 10.4 (SD=21.5). This sample size would achieve 89% power at a 5% significance level.

Data were analysed using R language version 3.5.0, on an intention-to-treat basis. Mosquito count data were modelled using Generalized Linear Mixed model (GLMM) using template model builder (TMB) under *glmmTMB* package (Brooks et al. 2022), following a negative binomial distribution accounting for zero-inflation function with a random effect for house and pair. This analysis was adjusted for village and repeat measures (Lee et al. 2012, Bates et al. 2014). In this model, the response variable was the number of mosquitoes captured in each study house each night, while the main fixed variable was the intervention groups. To account for pseudo replicates and unexplained variation between days, villages, house pair ID, rounds and clusters, a series of nested random terms were included. Each mosquito species was analysed separately for each season. Finally, the model estimates were exponentiated to obtain the risk ratio along with its 95% confidence intervals. The differences in the proportions of malaria vector sibling species (*An. gambiae* s.l. and *An. funestus* s.l.) caught between the two-house types were compared using the chi-square test with Yates' correction at a 95% significance level.

Means and sum were used to assess the sporozoites rates (number of sporozoite positive mosquitoes per specie/total mosquito caught) between Star homes and the traditional houses. Entomological inoculation rate (EIR) in each study arm was calculated separately for *An. gambiae* and *An. funestus* using the following formula:

$$EIR = m \times SPR \times n$$

Where m = mean no. mosquitoes/light trap/night, SPR = sporozoite rate, the proportion of mosquitoes testing positive for malaria parasites divided by the total tested and n days in a year (365 days for 12 months). The annual EIR was computed independently for each species.

Data on nightly environmental conditions and door opening were summarized into hourly readings and linked with other variables investigated in the study. The adjusted mean nightly differences along with their 95% confidence intervals (CI) of temperature and carbon dioxide CO_2 concentration, with their 95% confidence intervals (CI), using a linear mixed-effects model (*lmer:Test*) with a normal distribution. Similarly, mean nightly differences in the duration of opening for the main door of the traditional house and the doors of the Star homes (main door and stairway door), were calculated per night/house type.

Descriptive statistics (mean, standard error) were used to calculate the mean nightly outdoor measurements. The outdoor measurements served as a standard measure for comparing the mean indoor temperature and CO_2 concentrations in each house type with the mean outdoor measurements.

Ethics declarations

The trial was registered to the ClinicalTrials.gov Identifier database with the registration number: NCT04529434. The study was approved by the National Research Ethics Committee of Tanzania with registration number reference

NIMR/HQ/R.8a/Vol.IX/3695) and the Department of Biosciences ethics committee, Durham University, United Kingdom (Approved 24th June 2020). Study participants provided with a written informed consent and those who consented were recruited into the study. The study was conducted in compliance with principles set out by the International Conference on Harmonization Good Clinical Practice, the Declaration of Helsinki and the ethical requirements of Tanzania.

Results

Indoor mosquito densities

A total of 9,832 mosquitoes were collected during 2,860 trapping nights of which 26% (2,518/9,832) were caught in the Star homes and 74% (7,314/9,832) in the traditional houses. Of these, 77% were *Culex* species (7,597/9,832), 19% were *An. gambiae* s.l. (1,872/9,832) and 4% *An. funestus* s.l. (363/9,832). The species composition was similar in both study groups. Overall, indoor mosquito densities were lower in the Star homes than traditional houses (Table 3.1). Only 7% of trapping occasions resulted in the collection of *An. gambiae* s.l. (98/1480) in Star homes and 13% (150/1480) in traditional houses. For *An. funestus*, at least one mosquito was captured per house per night on 1% (14/1480) of occasions in the Star homes and 2% (25/1480) in traditional houses.

After adjusting for villages, rounds, house ID, and seasons, there were 52% fewer *An. gambiae* s.l. (Rate ratio, RR=0.48 (95% Confidence Intervals, CI=0.34–0.69, $p<0.001$), 81% fewer *An. funestus* s.l., (RR=0.19 (0.01–0.39), $p< 0.001$) and 64% fewer *Culex* species mosquitoes (RR=0.36 (0.30–0.45), $p<0.001$; Table 3.1) in Star homes than traditional houses.

Validation of light traps

These collections were done to determine whether a substantial proportion of indoor mosquitoes were uncollected by light traps. Over 440 comparisons were made between collections with light traps and Prokopack® aspirators. Regardless of house type, light traps captured more than 96% of *An. gambiae* s.l. and 80% of *Culex* species. In Star homes, light traps collected 96% (172/178) of *An. gambiae* sl., and 85% (823/966) *Culex* mosquitoes, whilst in traditional houses, light traps caught

100% (272/272) of *An. gambiae* s.l., and 89% (1310/1478) of *Culex* mosquitoes (Table 3.2).

Sibling species in the An. gambiae complex and An. funestus group

Of the 1,872 *An. gambiae* complex mosquitoes collected, 39% (723) were from the Star homes, and 61% (1149) traditional houses. For *An. funestus* complex, 363 were collected, with 15% (55) from Star homes and 85% (308) traditional houses. Of the 1,631 *An. gambiae* complex identified to species level, 68% were (1065) *An. gambiae s.s*, 25% (402) *An. arabiensis*. and 5% (84) *An. merus.* Of the 264 *An. funestus* s.l. sampled, 98% (256) were *An. funestus* s.s, 0.8% (2/259) were *An. rivulorum* and 0.4% (1/259) *An. vanedeen*. Overall PCR amplification rate was 96% (1816/1895), (Table 3.3).

The abundance of *An. gambiae* s.s. was significantly higher in Star homes, with a mean of 0.40 mosquitoes per house, compared to 0.27 in traditional houses (χ^2 = 56.8, $p < 0.001$). Conversely, *An. arabiensis* was more abundant in traditional houses, with a mean of 0.19, compared to 0.01 in Star homes (χ^2 = 34.2, $p < 0.001$). There was no significant difference in the abundance of *An. funestus* s.s. between the two-house types, with means of 0.04 in Star homes and 0.07 in traditional houses (χ^2 = 0.1, $p > 0.05$), (Table 3.3).

Malaria transmission

Overall, upon adjusting for village, house ID, and date, the mean number of malaria vectors per night was 0.07 for *An. gambiae* s.l. in Star homes and 0.14 in traditional houses. Similarly, for *An. funestus*, the means were 0.00006 in Star homes and 0.0003 in traditional houses per mosquito per night (Table 3.1).

Unadjusted mean numbers were employed to calculate the Annual Entomological Inoculation Rate (EIR) for each year. For *An. gambiae* s.l. in Star homes per night, the first year recorded a mean of 0.29, increasing to 0.71 in the second year. Traditional houses showed means of 0.76 and 0.89 mosquitoes per night for the respective years. The *An. funestus* group exhibited a mean of 0.05 mosquitoes per Star home per night in the first year and 0.02 in the second year, while traditional houses recorded means of 0.42 and 0.02 mosquitoes per night for the same periods, (Table 3.4).

*Table 3. 1: Indoor mosquito densities in study houses at different times of the year. Where CI = 95% Confidence intervals, RR =Rate ratio, *= Model was not fitted for* Anopheles funestus *mosquito caught in the wet seasons due to the low number caught.*

Mosquito species	House type	Trap nights	Total caught	Predicted Mean (95% C.I)	Adjusted RR (95% C.I)	% Protection	p
Dry Season							
Anopheles gambiae s.l.	Traditional house	804	260	0.06 (0.03 – 0.10)	1		
	Star homes	804	66	0.02 (0.01 – 0.04)	0.29 (0.14 – 0.60)	71	<0.0001
Anopheles funestus s.l.	Traditional house	804	276	4.8 x e^{-04} (9.4 x e^{-05} – 0.002)	1		
	Star homes	804	53	1.2 x e^{-04} (2.0 x e^{-05} – 6.8 * e^{-05})	0.24 (0.12 – 0.47)	76	<0.0001
Culex species	Traditional house	804	2770	1.82 (1.40 – 2.37)	1		
	Star homes	804	429	0.38 (0.25 – 0.58)	0.21 (0.14 – 0.30)	79	<0.0001
Wet season							
Anopheles gambiae s.l.	Traditional house	626	889	0.47 (0.32 – 0.70)	1		
	Star homes	626	657	0.29 (0.18 – 0.45)	0.61 (0.43 – 0.86)	49	0.004
**Anopheles funestus* s.l.	Traditional house	626	32	N/a	N/a		N/a
	Star homes	626	2	N/a	N/a	N/a	N/a
Culex species	Traditional house	626	3087	2.87 (2.16 – 3.82)	1		
	Star homes	626	1311	1.40 (1 – 1.92)	0.48 (0.39 – 0.60)	52	<0.0001
Dry & Wet seasons combined							
Anopheles gambiae s.l.	Traditional house	1430	1149	0.14 (0.09 – 0.21)	1		
	Star homes	1430	723	0.07 (0.04 – 0.11)	0.48 (0.34 – 0.69)	52	<0.0001
Anopheles funestus s.l.	Traditional house	1430	308	3.0 x e^{-04} (6.0 x e^{-05} – 0.002)	1		
	Star homes	1430	55	5.7 x e^{-05} (9.2 x e^{-06} – 3.5 x e^{-04})	0.19 (0.01 – 0.39)	81	<0.0001
Culex species	Traditional house	1430	5857	2.17 (1.70 – 2.77)	1		
	Star homes	1430	1740	0.79 (0.59 – 1.05)	0.36 (0.30 – 0.45)	64	<0.0001

Seventeen *Anopheles* mosquitoes, 13 from the *An. gambiae* complex and four from the *An. funestus* group, tested positive for *P. falciparum* sporozoites. Of these, 13 were found in traditional houses and four in Star homes. There was no significant difference between the sporozoite rates for *An. gambiae s.l.* in both study groups (Chi-square = 0.955, p = 0.328). For this reason, the data were combined for both study groups yielding an overall sporozoite rate of 0.84% (13/1553) for *An. gambiae* s.l. and 1.62% (4/256) for *An. funestus* s.l. The overall EIR during the 24-month study period was 7.1 in traditional houses compared to 3.2 in Star homes, indicating an 55% (95% CI= 40–69%) reduction in malaria transmission risk in the intervention houses (Table 3.4).

Environmental conditions measurements

Temperature

Outdoor and indoor temperatures progressively decreased throughout the night across both house types (Fig. 3.6). Typically, the nightly temperature at dusk (19.00h) decreased from 27.8 °C (26.7 – 27.6) in Star homes and 28.4 °C (27.4 – 28.8) in traditional houses to 23.3°C (22.8 – 23.6) in Star homes and 24.2 °C (23.8 – 24.7) in traditional houses before dawn (05.00h). The Star homes were slightly cooler than traditional houses, with this temperature difference growing as the night advanced. By 05.00 h early morning, Star homes were over 1 ^{o}C cooler than traditional houses. In the early evening hours (from 19.00 to 21.00), the temperatures in both house types were generally similar. However, starting from 21.00 h when people began to sleep, a gradual temperature decline was observed between the two-house types, with Star homes consistently recording lower temperatures compared to traditional houses. Beyond 23.00h, Star homes consistently maintained cooler temperatures than traditional houses (Fig. 3.6).

The mean nightly temperature measurements indicated a difference in mean temperature between the two-house types (adjusted mean difference of -0.5 ^{o}C [-0.9, -0.2], p = 0.002). In the rainy seasons, it was -0.5 ^{o}C (-1, -0.01, p=0.05) cooler in Star homes than traditional houses and -0.6 ^{o}C (-1, -0.2, p=0.001) cooler in Star homes in the dry seasons (Table 3.5 and Fig. 3.6).

Table 3. 2: Comparisons between light traps and Prokopack aspirators per house type.Values were derived from a Generalized Linear Mixed Model (GLMM) with a negative binomial distribution, NA: Values could not be generated due to zero catches in one trap (Aspirator). The model was adjusted for the house types, village and trap nights.

Mosquito species	House type	n	No. caught by light traps	No. caught by Prokopack® aspirators	Proportion of mosquitoes caught by light trap (%)	p
An. gambiae s.l.	Traditional house	220	272	0	100 (272/272)	NA
	Star homes	220	172	6	96 (172/178)	<0.001
An. funestus s.l.	Traditional house	220	10	4	71 (10/14)	0.071
	Star homes	220	22	0	100 (22/22)	NA
Culex spp	Traditional house	220	1310	168	89 (1310/1478)	<0.001
	Star homes	220	823	143	85 (823/966)	<0.001

Table 3. 3: Composition of malaria vector species using Chi-square test with Yates correction, p= statistical probability.

Metric	**Study group**			
	Star homes	**Traditional houses**	**Chi-square with Yates correction**	**p**
***An. gambiae* complex**				
An. arabiensis	18% (126/684)	32% (276/873)	34.2	<0.00001
An. gambiae sensu stricto	79% (537/684)	60% (528/873)	56.8	<0.00001
An. merus	3% (21/684)	7% (63/873)	12	<0.0005
Non-amplified	2% (16/700)	6% (58/931)	-	-
***An. funestus* group**				
An. funestus sensu stricto	100% (62/62)	98% (193/197)	0.09	=0.7612
An. rivulorum	0% (0/62)	0.8% (2/197)	NA	NA
An. vanedeen	0% (0/62)	0.3% (1/197)	NA	NA
An. parensis	0% (0/62)	0.3% (1/197)	NA	NA
Non-amplified	2% (1/63)	8.2% (4/201)	-	-

NA= Mosquito species with zero proportions that did not yield Chi-square values

Table 3. 4: Assessment of malaria transmission intensity. Where EIR is the entomological inoculation rate.

Metric	**Study group**		
	Star homes	**Traditional houses**	**Both study groups**
Unadjusted mean (Year 1) – number of mosquitoes/trap/night			
An. gambiae s.l	0.29	0.76	-
An. funestus s.l	0.05	0.42	-
Unadjusted mean (Year 2) – number of mosquitoes/trap/night			
An. gambiae s.l	0.71	0.89	-
An. funestus s.l	0.02	0.02	-
Sporozoite rate (Year 1) - percentage of sporozoite positive mosquitoes			
An. gambiae s.l	0% (0/84)	0.9% (2/232)	0.6% (2/316)
An. funestus s.l	0% (0/60)	2.2% (4/183)	1.6% (4/243)
Sporozoite rate (Year 2) - percentage of sporozoite positive mosquitoes			
An. gambiae s.l	0.7% (4/602)	1.1% (7/635)	0.9% (11/1237)
An. funestus s.l.	0% (0/4)	0% (0/9)	0% (0/13)
Annual EIR (Year 1)			
EIR (*An. gambiae* s.l)	0.29 x 0.006 x 365 = 0.6	0.76 x 0.006 x 365 = 1.7	-
EIR (*An. funestus* s.l)	0.05 x 0.016 x 365 = 0.3	0.42 x 0.016 x 365 = 2.5	-
Overall EIR (*An. gambiae* & *An. funestus*)	0.6 + 0.3 = 0.9	1.7 + 2.5 = 4.2	
Annual EIR (Year 2)			
EIR (*An. gambiae* s.l)	0.71 x 0.009 x 365 = 2.3	0.89 x 0.009 x 365 = 2.9	-
EIR (*An. funestus* s.l)	0.02 x 0.0 x 365 = 0.0	0.02 x 0.0 x 365 = 0.0	-
Overall EIR (*An. gambiae* & *An. funestus*)	2.3 + 0.0 = 2.3	2.9 + 0.0 = 2.9	-
Overall EIR (Year 1&2)			
	0.9 + 2.3 = 3.2	4.2 + 2.9 = 7.1	-
% EIR reduction	-	-	((7.1-3.2) / 7.1) x 100 = 55%

Carbon dioxide

CO_2 concentrations increased progressively from early evening into the night (Figure 3.7). During the night, no differences in night-time CO_2 concentrations were observed between Star homes and traditional houses, with an adjusted mean difference of -4.15 PPM (-17.47, 9.49, p=0.543). Outdoors, a slight 2% (1.8% - 7%)

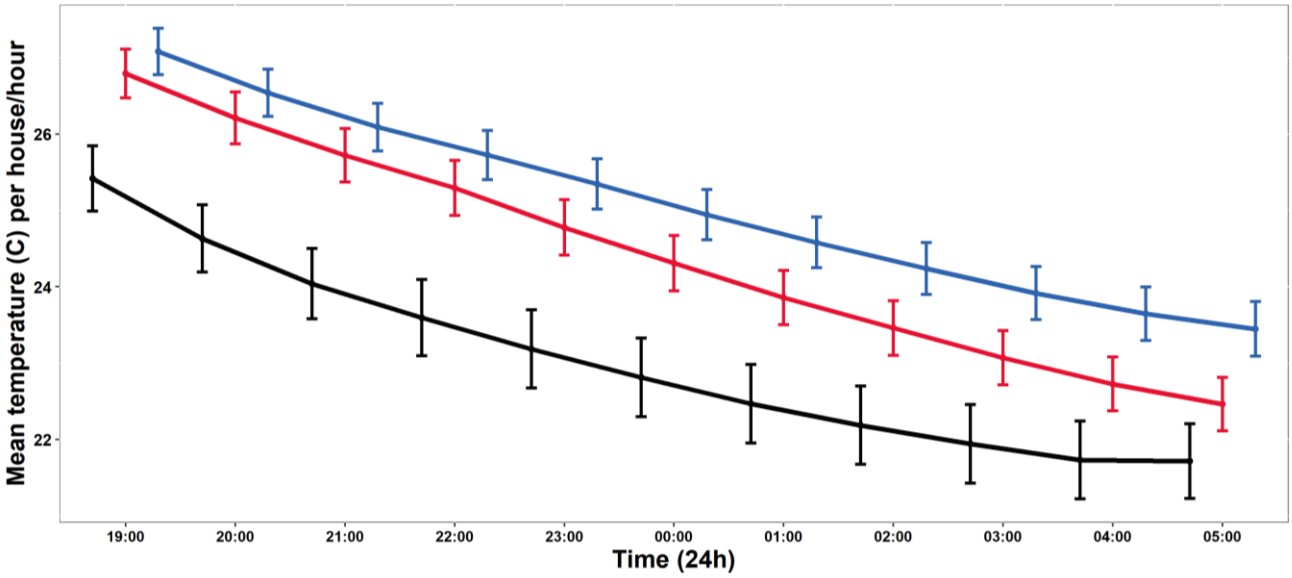

Figure 3.6: Mean hourly temperature in study houses and outdoors.Where red line represents Star homes, blue line traditional houses and black line is outdoors. Error bars are (95% CI's). Indoor data from 170 Star homes and 170 traditional houses. Outdoor data from 77 study houses. Error bars are 95% CIs.

increase in CO_2 concentration (ppm) was recorded during wet seasons, with a mean of 572 PPM (95% CI: 544.8 – 634), compared to the mean of 560.9 PPM (95% CI: 531.7 - 589.9) recorded in dry seasons.

A slight disparity in CO_2 concentration was noted in Star homes between seasons, with an adjusted mean difference of 10.3 ppm (95% CI: -1.9 – 23.3, p = 0.095) in dry seasons and -15.9 ppm (95% CI: -40.2 – 8.4, p = 0.202) in rainy seasons when compared to traditional houses (Table 3.5).

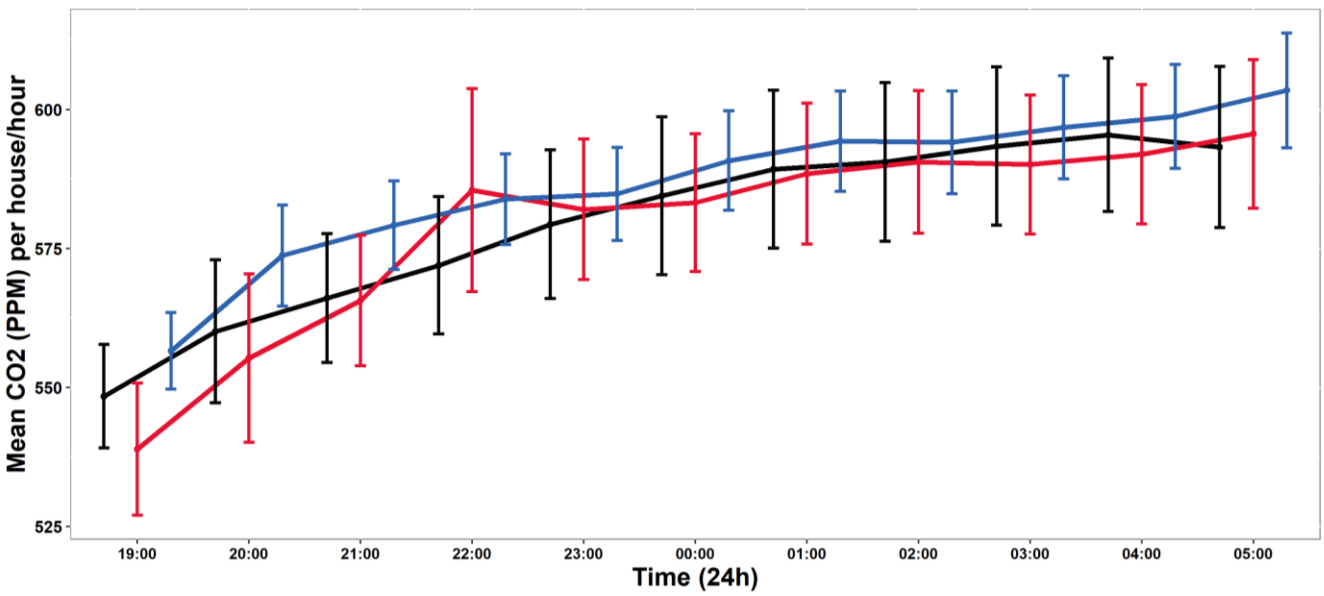

Figure 3.7: Mean hourly CO_2 concentrations (PPM) in study houses and outdoors collected from January 2022 to October 2023.Where red line represents Star homes, blue line traditional houses and black line is outdoors. Indoor data from 170 Star homes and 170 traditional houses. Outdoor data from 77 study houses. Error bars are 95% CI's.

Duration of door openings

During the night, most door openings (80%) occurred between 19.00 h and 21.59 h in both Star homes and traditional houses. In the Star homes, both the external and internal doors to the stairways were open for shorter durations than the main doorways of traditional houses. Notably, the shortest door opening duration was observed in the main doorways of Star homes, (Figure 3.8). The main doors of Star homes demonstrated the shortest door opening duration (minutes) (adjusted mean difference = -8.3, 95% CI: -12. to -4.6, p < 0.001), followed by -4.5 (95% confidence interval: -8.2 to -0.8, p = 0.024) of stairways doors when compared to the main doorways of traditional houses (Table 3.6).

Table 3. 5: Environmental measurements between the different house types during the night.Where CI = confidence intervals, ppm = parts per million.

Variable	Description	n	Mean (95% C.I)	Adjusted mean difference (95% CI)	p
Dry season					
Temperature (ºC)	Traditional houses	96	24.5 (23, 26)	1	
	Star homes	96	23.9 (22.4, 25.4)	-0.6 (-1.0, -0.2)	0.001
	Outdoors	44	22.1 (20.8, 23.3)	NA	NA
Relative humidity (%)	Traditional houses	96	76.3 (74.4, 78.1)	1	
	Star homes	96	76.95 (75.07, 78.77)	0.7 (-1.2, 2.4)	0.462
	Outdoors	44	84.6 (81.6, 87.7)	NA	NA
Carbon dioxide (ppm)	Traditional houses	96	565.7 (540.1, 591.5)	1	
	Star homes	96	575.9 (550.2, 601.7)	10.3 (-1.9, 23.3)	0.095
	Outdoors	44	560.9 (531.7, 589.9)	NA	NA
Wet season					
Temperature (ºC)	Traditional houses	74	26.6 (25.1, 28.2)	1	
	Star homes	74	26.1 (24.6, 27.7)	-0.5 (-1.0, -0.01)	0.050
	Outdoors	33	24.8 (23, 26.8)	NA	NA
Relative humidity (%)	Traditional houses	74	78.5 (72.8, 8)	1	
	Star homes	74	79.2 (73.4, 84.7)	0.68, (-1.32, 2.68)	0.503
	Outdoors	33	81.3 (69.1, 93)	N/A	NA
Carbon dioxide (ppm)	Traditional houses	74	584 (535.3, 630.4)	1	
	Star homes	74	584 (519.4, 614.5)	-15.89 (-40.16, 8.38)	0.202
	Outdoors	33	572 (544.8, 634)	N/A	N/A
Dry & Wet seasons					
Temperature (ºC)	Traditional houses	170	25.2 (23.9, 26.6)	1	
	Star homes	170	24.7 (23.3, 26.1)	-0.54 (-0.85, -0.21)	0.002
	Outdoors	77	23.5 (21.8, 25.3)	N/A	N/A
Relative humidity (%)	Traditional houses	170	78.1 (75, 81.2)	1	
	Star homes	170	78.4 (75.3, 81.5)	0.31 (-1.27, 1.82)	0.692
	Outdoors	77	82.9 (76.2, 89.1)	N/A	N/A
Carbon dioxide (ppm)	Traditional houses	170	577.8 (551.2, 604.1)	1	
	Star homes	170	573.7 (547, 599.2)	-4.15 (-17.47, 9.49)	0.543
	Outdoors	77	574.6 (538.6, 607.8)	N/A	N/A

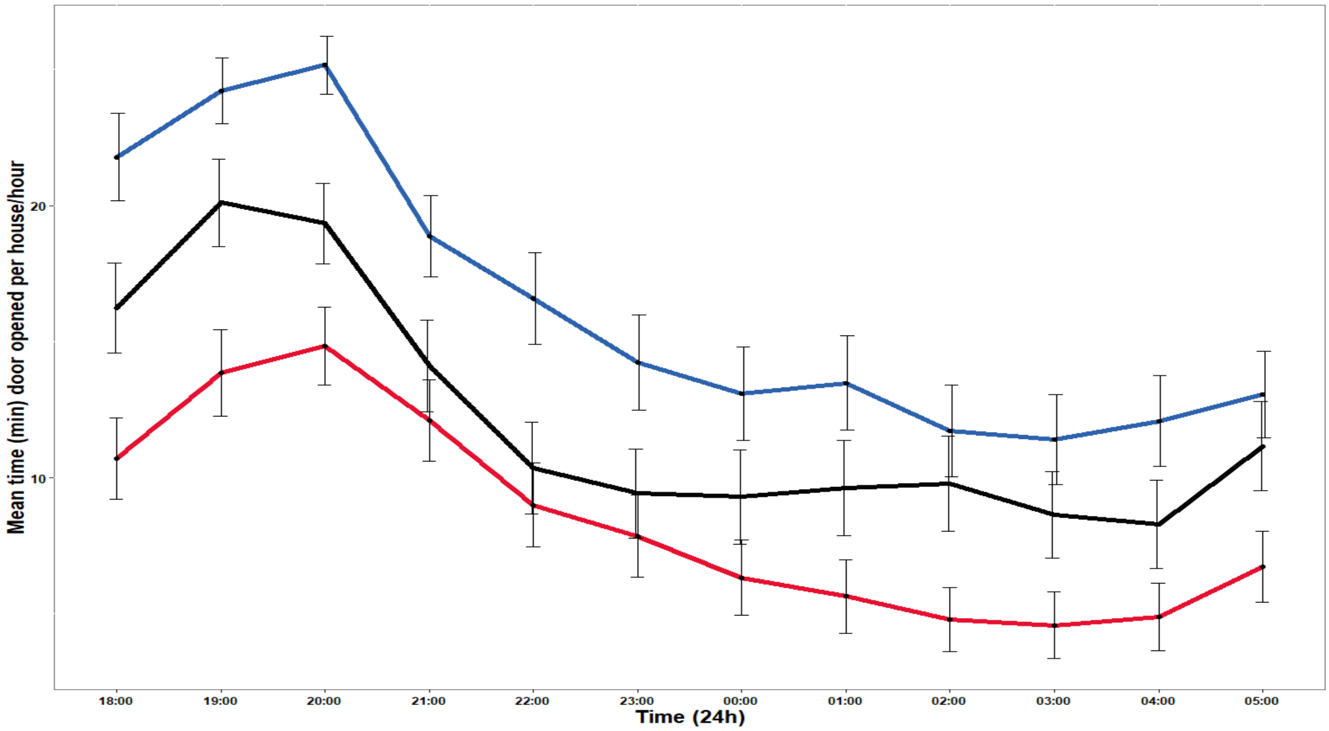

Figure 3.8: Duration of time the door spent open per hour/house type between 18.00h and 07.00h.Data from 132 Star homes and 132 traditional houses. Where red line represents Star homes (main door), blue line traditional houses (main door) and black line is Star homes (stairways door). Error bars are (95% CI's).

Table 3. 6: Nightly duration of door opening (minutes per hour) with a 95% confidence interval (CI). Mean values were adjusted for house type, rounds and month of collections.

Variable	Description	n	Mean (min) (95% C.I)	Adjusted mean difference (95% CI)	p
Dry season					
Traditional house	Main door	74	17.7 (14.5, 21)	1	
Star homes	Stairway door	74	11.4 (7.9, 14.9)	-6.4 (-10.9, -1.8)	0.008
Star homes	Main door	74	6 (2.5, 9.5)	-11.7 (-16.3, -7.2)	<0.00001
Wet season					
Traditional house	Main door	58	14.1 (9.9, 18.2)	1	
Star homes	Stairway door	58	11.4 (7.3, 15.5)	-2.6 (-7.2, 1.9)	0.256
Star homes	Main door	58	9.8 (5.8, 13.8)	-4.2 (-8.7, 0.2)	0.070
Dry & Wet seasons					
Traditional house	Main door	132	16 (13.1, 18.9)	1	Ref
Star homes	Stairway door	132	11.5 (8.5, 14.6)	-4.5 (-8.2, -0.8)	0.024
Star homes	Main door	132	7.7 (4.7, 10.8)	-8.3 (-12, -4.6)	<0.00001

Discussion

This study is the first randomised controlled trial to evaluate protective efficacy of newly built houses on malaria transmission relative to traditional rural housing in an African setting. The promising results from the comparison between Star homes and traditional houses in this area, Mtwara, Tanzania, highlight the important role that housing design can play in mitigating malaria transmission in endemic regions. A 52% reduction in the indoor abundances of *An. gambiae* s.l., an 81% reduction for *An. funestus*, and a 64% reduction for *Culex* species underscore the efficacy of Star homes in reducing exposure to mosquito bites during the night, when most malaria transmission occurs.

In this area of persistent malaria transmission and with 79% coverage of at least single ITNs per house (Stuck et al. 2017, Ministry of Health (MoH) [Tanzania Mainland] 2023), the novel screened house resulted in a 56-81% reduction in indoor malaria mosquitoes compared to traditional houses. There are likely to be a number of explanations for this finding. Firstly, the walls of Star homes were impermeable to mosquitoes and the new homes were fitted with solid doors, both providing a physical barrier against mosquito ingress. Secondly, self-closing metal doors provided added protection from mosquito entry. In our study we showed that this reduced the time doors were open in the Star homes. Properly fitted, screened, and self-closing doors have previously been shown to prevent the entry of mosquitoes into a house (Massebo & Lindtjørn 2013, Jawara et al. 2018). Thirdly, the elevated bedrooms are likely to reduce the entry of anopheline mosquitoes, partly because fewer mosquitoes fly at higher altitudes (Carrasco-Tenezaca et al. 2021). Fourthly, the eave gaps in Star homes are considerably smaller than in traditional houses.

Our findings are similar to those from a randomised controlled trial of house screening in The Gambia where indoor densities of malaria mosquitoes were reduced by 59% (Kirby et al. 2009). Additionally, a systematic review and meta-analysis revealed a 47% lower likelihood of malaria infection among children residing in modern houses compared to traditional ones (Tusting et al. 2015).

The evidence suggests variations in the effectiveness of Star homes in protecting against mosquito bites across different species and taxonomic groups. Given that *An. gambiae* s.s. comprised over 60% of the mosquito population within the *An.*

gambiae complex group, the reduction in abundance had a more pronounced impact on *An. gambiae* s.s. than *An. arabiensis*. The higher proportion of *An. gambiae s*.s. relative to *An. arabiensis* in Star homes was probably due to the more endophilic nature of *An. gambiae* s.s. compared with *An. arabiensis*. Star homes exhibited greater biting protection against *An. gambiae* s.l. and *Culex* species during dry seasons compared to wet seasons. The limited presence of *An. funestus* s.l. during the rainy season precluded a comparative analysis for this species. The diminished protection against *An. gambiae* s.l. in the rainy season may be attributed to the increased density of *An. gambiae s.s.* at this time of year.

Concern about the low number of malaria vectors caught in light traps made us check the catching efficiency of this sampling tool. Overall, light traps were highly effective at capturing indoor mosquitoes in comparison to resting collections, capturing over 80% of all mosquitoes entering the trapping room, while only a small number were caught using the mechanical aspirator. This phenomenon was observed in both arms of the study. It is likely that our collection efficiency varied according to the house design. Since in Star homes there are few exit points, most mosquitoes once indoors would shelter in the house. In marked contrast, in traditional houses with numerous openings in the walls and doors and large eave gaps, many would have left the room during the night. In an experimental hut study in East Africa, 51% of female *An. gambiae*, whether fed, unfed, or gravid, exited the hut each night (Smith 1965). Among these escaping mosquitoes, approximately 85% used windows as exit points, while the remaining 15% utilized eave gaps.

In addition to malaria vectors, Star homes have demonstrated a reduction in indoor abundances of *Culex species*, known for causing significant nuisance biting and transmitting other mosquito-borne pathogens such as arboviruses and worms. The reduction in indoor *Culex species* abundances in Star homes mirrored observations in anophelines, with greater reductions noted during dry seasons compared to rainy seasons.

Star homes reduced the EIR by 55% compared to traditional houses, which was entirely related to the similar reduction in indoor resting mosquitoes since there was no significant difference in the sporozoite rates of *An. gambiae* s.l. between study groups.

Star homes were slightly cooler at night compared to traditional houses, likely due to differences in building materials. Star homes were roofed with low-thermal mass metal sheets, while traditional houses had thatched roofs. Additionally, the shade nets on the walls of Star homes facilitated heat dissipation more effectively than the mud and stick walls of traditional houses. These factors contributed to the Star homes cooling down more rapidly after sunset than traditional houses. It is worth noting that during the study, occupants installed curtains in their bedrooms for privacy, unintentionally restricting air circulation and cooling. Cooler homes provide greater comfort to households (Jatta et al. 2021) and may encourage households to use bed nets compared to traditional houses (Pulford et al. 2011), thereby helping to reduce the risk of malaria transmission.

The gradual increase in CO_2 at night is primarily attributed to indoor human respiration and the nocturnal release of CO_2 by plants (Nobel & Hartsock 1983, Pedersen et al. 2008). The study identified no disparities in CO_2 concentrations between Star homes and traditional houses in both dry and wet seasons.CO_2 levels were lower in the dry seasons than the wet seasons illustrating the lower plant growth rates in the dry seasons. Importantly, there was no difference in CO_2 concentrations between house types, suggesting similar ventilation in both housing typologies. The similarity in CO_2 levels might be due to the use of curtains covering the shade net walls in Star homes, which enhanced privacy but hindered the free dissipation of CO_2 from inside to outside. For traditional houses, a study in The Gambia showed that open eave gaps and leaky doors readily dissipated CO_2 outdoors (Knudsen 2020). Thus, the protective effect found with Star homes is likely to be due to the physical barrier created preventing mosquito ingress.

Doors in Star homes were opened less frequently than those in traditional houses. We do not think that the presence of data loggers changed householder's behaviour since from our regular visits to study houses, we did not discern any difference in behaviour when data loggers were added to the doors.

Door opening patterns were consistent in both house types, with approximately 80% of door activity occurring during the early evening hours (19.00 to 21.00), gradually decreasing as people went to bed. This peak corresponds to times when individuals are actively moving in and out of the houses for activities like cooking, fetching water, and socializing, which also coincides with increased mosquito biting activity (Fornadel et al. 2010, Takken 2024). Open doors during these hours provide mosquitoes with opportunities to enter houses. Notably, internal stairway doors in Star homes remained open longer than external doors, potentially allowing mosquitoes to reach bedrooms on the second floor. These findings suggest that keeping doors open in both house types may facilitate mosquito entry. Despite having more open doors, traditional houses also allowed mosquito entry through eave gaps, unscreened windows, and ill-fitting doors.

Estimates of the protective efficacy of Star homes against indoor mosquitoes may have been underestimated due to two factors. First, the visibility of light traps from outdoors as seen with the ground floor Star homes may have artificially increased mosquito collections, potentially inflating the numbers recorded in Star homes (Mmbando et al. 2022). Double-net traps or Furvela tent traps could also be used for sampling mosquitoes inside houses but both methods are labour intensive and could not be readily scaled-up as easily as light traps. Second, the larger open eaves and other gaps typically found in traditional houses allowed a greater proportion of mosquitoes to exit the room during the night, reducing the catching efficiency of light traps in traditional houses.

Conclusion

Our study demonstrates that the novel design of screened houses, known as Star homes, can be effective strategy in combating malaria in rural communities by protecting individuals from major malaria-transmitting mosquitoes; *An. gambiae* and *An. funestus*. Moreover, Star homes mitigate indoor abundances of *Culex* species, known for nuisance biting and transmitting arboviruses and worm infections. Thus, the development of affordable, well-designed mosquito-proof housing should be prioritized in sub-Saharan Africa.

Chapter 4 : Effect of a novel house and latrine design (Star home) on domestic fly densities in rural Tanzania: a household randomised controlled trial.

Abstract

Background: Diarrhoeal diseases pose a significant health burden in sub-Saharan Africa, particularly among children in rural communities with poor hygiene practices and limited access to clean water, improved toilets, or fly-proof housing. A randomized controlled trial was carried out in rural Tanzania to assess the effectiveness of a novel house design, "Star homes", which has fly-proof kitchens and toilets with a self-closing flap under the poop hole, in reducing the abundance of domestic flies, the potential carriers of diarrhoeal pathogens.

Methods: The study recorded domestic fly populations in 28 randomly selected Star homes and 28 traditional houses, which featured thatched roofs and mud walls. Data collection was from November 2021 to September 2023. In all houses, domestic flies were sampled in kitchens and toilets using baited fly traps, every seven weeks from 07.00 to 17.30 h. Additional domestic fly traps were placed on top of the toilet holes to trap flies emerging from the toilets. Data loggers measured the duration of door openings in both Star homes and traditional houses. Generalized linear mixed-effect models were used to analyse differences between study groups.

Findings: A total of 24,754 domestic flies were captured on 706 occasions, 93% of these in the toilets and 7% in the kitchens of both house types. Of the flies collected, 75% were *Chrysomya putoria* (African latrine fly), 17% were *Musca domestica* (house fly), and 8% were *Sarcophaga* spp. (flesh fly). There were 48% fewer *C. putoria* flies (adjusted mean rate ratio [RR]=0.52, 95% confidence intervals [CI]: 0.35–0.78, $p = 0.001$), 47% fewer *M. domestica* (RR=0.53, CI: 0.32–0.89, $p = 0.02$), and 62% fewer *Sarcophaga* spp. (RR=0.38, CI: 0.23–0.62, $p = 0.001$) in the Star home kitchens compared to traditional houses. In Star home toilets, there was 46% fewer *C. putoria* (RR=0.54, CI: 0.44–0.67, $p < 0.001$), but no difference was observed for other domestic fly species. No flies emerged from Star homes toilets compared with a mean of (4.2, CI: 3.2 – 5.2) in traditional toilets. During the day, the

doorways on Star homes were open for an average of 14 fewer minutes/hour than the traditional house doors.

Conclusion: Star homes reduced the abundance of domestic flies in the kitchen, and there were fewer *C. putoria*, a putative vector of diarrhoeal diseases, in Star home toilets compared to those associated with traditional houses. Fly screening of kitchens and reducing the time doors are kept open probably contributed to a reduced fly entry, whilst the self-closing flap over the toilet holes prevents breeding of *C. putoria* in faeces collected in latrines. Changing the design and use of buildings can contribute to a decline in domestic flies and may lead to a reduction in diarrhoeal diseases.

The trial was registered to ClinicalTrials.govNCT04529434.

Background:

Non-biting synanthropic flies, such as *Musca domestica* (house flies), and *Chrysomya* putoria (African latrine flies), can be mechanical vectors of diarrhoeal diseases (Graczyk et al. 2001, Lindsay et al. 2012). They are particularly attracted to various organic materials, including decaying food, faeces, and garbage (Moon 2019), which contribute to their role as mechanical vectors (WHO 1997). Flies acquire pathogens through direct contact or ingestion of contaminated substances. and subsequently transfer them to human food, utensils, or surfaces, thus facilitating the transmission of the pathogens (Graczyk et al. 2001).

Domestic flies, such as *M. domestica*, can transmit pathogens like *Salmonella*, *Shigella*, and toxic *Escherichia coli*, leading to acute gastroenteritis and gastrointestinal infections (Graczyk et al. 2001). These pathogens, found in faecal matter, can be transferred by flies to food and surfaces that they come into contact with leading to infection (Lindsay et al. 2012, Khamesipour et al. 2018). *C. putoria*, with a strong preference for breeding and feeding on human faecal materials, can carry enteric bacteria, including toxic *E. coli*, *Shigella* and *Salmonella spp.* which causes diarrhoeal illnesses, particularly in children (Lindsay et al. 2012). Diarrhoeal diseases, are a major threat to childhood survival, are estimated to account for one in ten global child deaths (Leu 2021). In sub-Saharan Africa, these illnesses contribute to approximately 750,000 deaths annually among children under five years old, predominantly in low- and middle-income countries (Jamison 2006).

A systematic analysis conducted across 95 countries from 1990 to 2016 identified rotavirus as the primary cause of death among children under five years, leading to an estimated 128,515 fatalities (Troeger et al. 2018). A large-scale rotavirus surveillance program in eight African countries revealed that approximately 40% of stool samples from children tested positive for rotavirus infection (Mwenda et al. 2010). Shigella was the second leading cause of mortality in children under five, resulting in an estimated 63,713 deaths (Khalil et al. 2018). Additionally, enteropathogenic *Escherichia coli* was estimated to be responsible for approximately 4.2% of all deaths related to diarrhoea among children under five in 2016 (Khalil et al. 2018).

Various household-level measures have been proposed to reduce diarrhoeal illnesses, including ensuring access to safe water, sanitation, and hygiene (WASH) (Fewtrell et al. 2005, Mshamu et al. 2020). The importance of this approach was emphasized in a global study conducted in 145 low- and middle-income countries, estimating that approximately 502,000 diarrhoeal deaths were linked to inadequate safe drinking water, and around 280,000 deaths were associated with poor sanitation (Prüss-Ustün et al. 2014). Unfortunately, at least 785 million people still lack access to safe water, and 2.5 billion people lack improved sanitation, with a significant proportion residing in sub-Saharan Africa (WHO 2019b). A systematic review and meta-analysis of data from 1970 to 2016 (Wolf et al. 2018), demonstrated that point-of-use filter interventions reduced diarrhoea risks by 61%, piped water at the premises by 75%, and continuous availability of piped water by 36% (Wolf et al. 2018).

Previous studies demonstrate that controlling adult flies can reduce the transmission of diarrhoeal pathogens. In a prospective crossover intervention in Israeli army camps the use of baited traps resulted in a 65% reduction in housefly counts, a 42% decrease in clinical diarrhoea visits, and an 85% reduction in cases of shigellosis in the intervention bases, as compared to the control ones (Cohen et al. 1991). A pilot study of indoor residual spraying with deltamethrin in The Gambia resulted in a 75% reduction in muscid flies and a 75% decrease in trachoma incidence compared to the control village (Emerson et al. 1999). Additionally, in the sprayed village, there was a 22% reduction in childhood diarrhoea cases observed during the wet seasons and a 26% reduction during the dry seasons when compared to the control group (Emerson et al. 1999).

While physically killing adult flies can help reduce infestation, effective management requires the elimination of breeding areas (Sanchez-Arroyo & Capinera 2013). Proper waste management practices, such as using tightly fitting lids on garbage cans and placing trash in sealed plastic bags away from building entrances, are crucial in limiting the attractiveness and availability of breeding sites for flies (Sanchez-Arroyo & Capinera 2013, Iqbal et al. 2014). Housing modifications, like screening or covering windows, and air doors, have proven effective in preventing domestic flies from entering houses (Sanchez-Arroyo & Capinera 2013).

To expedite efforts in housing modifications aimed at preventing the entry of domestic flies, an open-labelled randomized household trial was conducted to evaluate the impact of novel-designed health houses (Star homes) on domestic fly abundance in rural Mtwara district, Tanzania. The study also compared the duration of the kitchen doors remained open in comparison to traditional houses. Finally, a small-scale study nested within the larger investigation compared the emergence of domestic flies in the toilets of the two-house types.

Methods

Study design

A detailed description of the study design is provided by (Mshamu et al. 2022). In brief, this is a cohort, open-label household randomized control study. Domestic fly abundance was measured in 28 intervention houses and 28 control houses every seven weeks from November 2021 to September 2023. Collections were made using baited traps situated in the kitchen and toilet. Additionally, the study assessed the duration of door opening at the kitchen area between the two study groups.

Study area

The study was conducted in rural Mtwara district (Fig. 4.1) south-eastern Tanzania from 2021 to 2023. The study area consists of a coastal strip of sandy low-lying land and undulating hills inland, with elevation <400 m above sea level. The region is mainly covered by dense forests and shrublands. It experiences two distinct rainy seasons: a longer one from February to April and a shorter one from October to December. Only 10% of households have a toilet, with 85% of these toilets being pit latrines with earth floors (Mshamu et al. 2020). Most families in the study area relied on firewood for cooking, with their kitchen situated outdoors, approximately 2 m from the house. These kitchens were mostly open, with some having only thatched roofs while leaving the walls open. During the rainy season, families cooked indoors, typically to the living room, until the rainy season ended. Like much of rural mainland Tanzania, there is a high prevalence (71%) of unimproved pit latrines (NBS & ICF Macro 2010). Between 11% and 20% of the residents practice open defecation (Sara & Graham 2014). In common with other rural regions of mainland Tanzania, there are various Diptera families, including Psychodidae, Culicidae, Calliphoridae, Syrphidae, Stratiomyidae, and Sarcophagidae (Irish et al. 2013). The prevalence of diarrhoea in children was 48% in 2009 and 37% in 2010 (Kulwa et al. 2014).

Inclusion criteria

Inclusion criteria of study participants were described previously (Mshamu et al. 2022). Briefly, to be included in the study the house should: (1) have a child aged six months to 12 years old (male or female), who resided in the study area for a period of not less than three years, (2) parents were willing and able to consent to their child participating in the study and (3) they lived in a house made with mud walls, thatched roof with open eave-spaces, unscreened windows, badly fitting doors, unfinished floor and unplastered walls.

Participants who met the eligibility criteria were provided with informed consent forms and invited to participate in a randomization process in the form of a village lottery. The names of household heads who met the inclusion criteria were put on cards and inserted in identical envelopes. Those who were provided with a Star home were selected at a public meeting by a child picking envelopes from a transparent bucket (Mshamu et al. 2022).

Star homes

A total of 110 Star homes were constructed between January and June 2021 (Fig. 4.3), while 440 traditional houses were included in the study (Mshamu et al. 2022). In this present study a sub-sample of the houses randomly selected for mosquito collection on the first day of the week was used for the domestic fly collections. In each study village, there were at least two Star homes that were compared to the nearest two traditional houses (Mshamu et al. 2022).

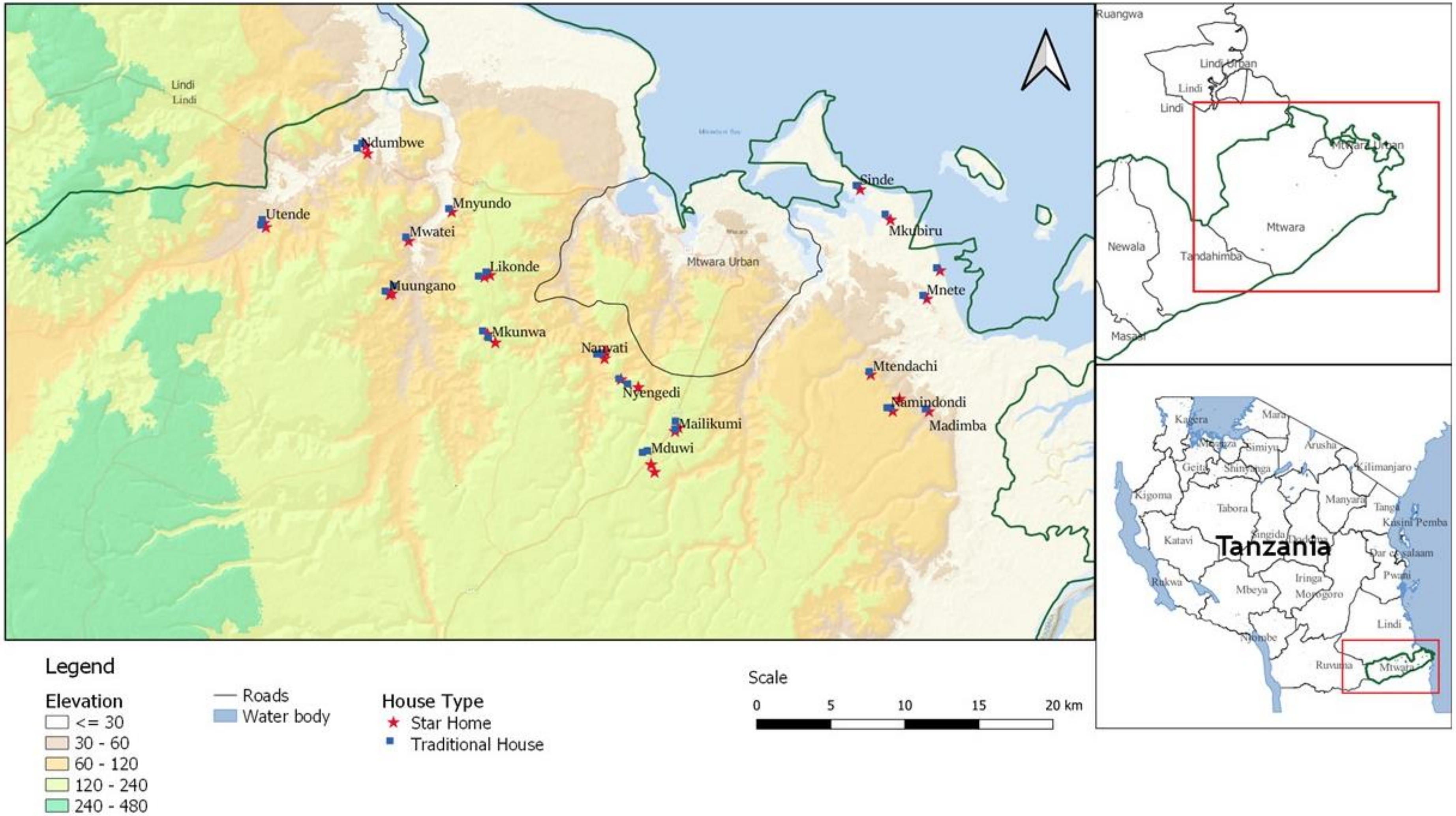

Figure 4.1: Location of study houses.

The design of the Star homes has been described in previous publications (von Seidlein et al. 2017, Mshamu et al. 2022). Star homes were equipped with specific features aimed at reducing domestic fly abundances, serving as proxy measures for the potential transmission of diarrhoeal illnesses in children. Star homes had four features that could reduce the entry of domestic flies at the kitchen and toilets, including (1) walls made of screened netting, (2) self-closing and well-fitting external doors, (3) a screened kitchen on the first storey, for cooking and storing cooking utensils and 4) a cement-floor toilet with a flap under the poop hole (Mshamu et al. 2022). The Star homes' screened kitchen wall was designed to prevent domestic flies from entering the house, while ensuring proper ventilation during cooking. The self-closing external doors should help reduce the entry of domestic flies into the house. The cemented toilet with the flap prevented the directed contact between the domestic flies and human faecal materials which preferred by *Chrysomya* species as a main breeding habitat and source of contaminants (Lindsay et al. 2012) (Fig. 4.2a).

Figure 4.2: Study houses. a) Star home and b) traditional house.

Randomization and blinding

Of the 110 Star homes and 440 control houses, a sub-sample of 56 houses were included in the study upon consent: 28 Star homes and 28 traditional houses, selected from 17 study villages. For each Star home the nearest traditional house enrolled in the study was selected. In domestic fly collections the sampled houses were purposively selected following the list of four randomly selected Star homes and four nearby traditional houses which were involved in mosquito collections on the Monday night each week. The domestic fly traps were introduced to those eight houses each Tuesday morning just after the light trap retrieval (07.00 h). This was because of the logistical difficulties of collecting flies over a large geographical area.

The order in which clusters were visited was randomly assigned and remained consistent throughout the trial. The order of visiting each sub-cluster was also randomly determined and maintained throughout the study.

Not all study procedures were fully masked, but bias was reduced using fly traps independent of fieldworker skill. Different technicians, aware of trap locations, analysed catches. Datasets were unblinded after locking primary and secondary endpoint data.

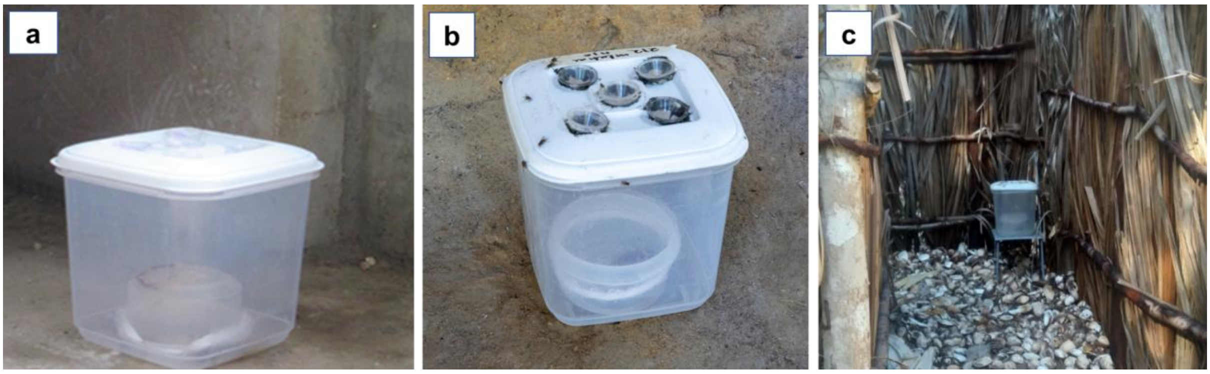

Figure 4.3: Odour-baited fly trap,a) side view, b) the top/lid view showing the holes for domestic fly entry and c) the traps placed on the metal stand in the toilet.

Study procedures

Domestic fly trapping:

Odour-baited fly traps as previously described (Lindsay et al. 2012) were used for collecting domestic flies. Each trap consisted of a 3 L rectangular-shaped polypropylene box (Whitefurze, Coventry United Kingdom) with a white opaque lid containing five circular entrance holes (6-8 mm in diameter). In the box 50 g of members of saltwater fish was placed in a 9 cm plastic diameter and 6 cm height) bowl (W. K. Thomas, Chessington UK) covered with untreated cotton netting (Figure 4.2), (Lindsay et al. 2012). Many of the traditional toilets had no roof, so for traps placed in latrines, the traps were raised 20 cm above the ground on a metal frame and a small roof constructed over the trap to prevent flooding during heavy rains (Figure 4.7). Each study house had two traps: one in the kitchen at the furthest corner from the main door, and another in the latrine at the furthest corner from the door (Figure 4.4 & 4.5). Each week, 16 odour-baited fly traps were positioned in the kitchens and toilets of both house types. After seven weeks, a total of 56 houses (8 houses x 7 weeks) houses were sampled. Sampling was conducted between 07.00 h and 17.30 h. To evaluate the reduction of domestic flies in the kitchen, we conducted an observational survey to assess the proportion of families who actively used their kitchens versus those who did not. Factors assessed included the presence of ashes, remaining firewood, and enquiries about typical cooking locations. For families conducting outdoor cooking, traps were placed inside the house near cooking utensils and leftover food during domestic fly collection in the kitchen. We compared the number of flies in both house types for families with an active kitchen and those without. We hypothesised that houses with active kitchens would attract more flies due to the presence of food materials or dirty cooking utensils. Therefore, to assess the actual impact of Star homes on reducing domestic fly abundance in the kitchen, a comparison was made between households actively using their kitchens and those that were not.

Collection of flies emerging from latrines

Since latrines can produce prodigious numbers of *C. putoria*, (Lindsay et al. 2012) we assessed the productivity of latrines in our study. Weekly sampling was carried out to capture flies.

Figure 4.4: Kitchen spaces in study houses.a) Star homes and b) traditional house.

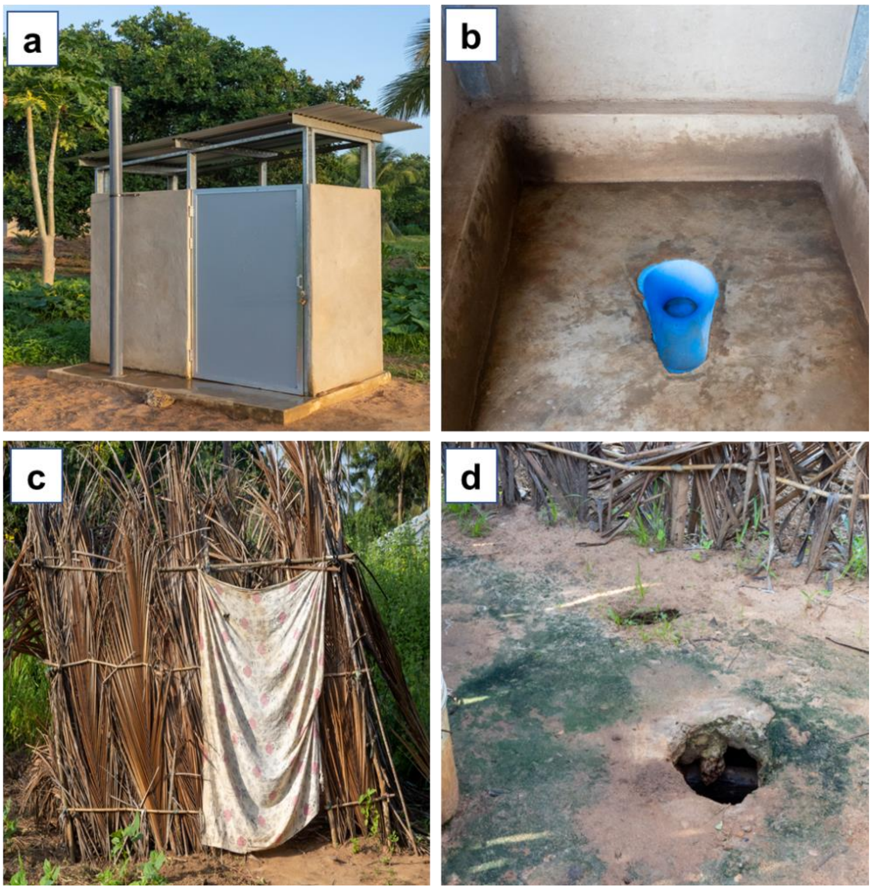

Figure 4.5: Toilet types.a) exterior of a Star home toilet, b) interior of a Star home toilet showing the main hole with a flap, c) exterior of a traditional toilet and d) interior of a traditional pit latrine showing open hole.

using a funnel trap (Fig 4.6). A 50 cm long, 25 cm wide, and 60 cm high metal cage, fitted with untreated bed nets, was positioned with the open mouth of the trap immediately above the main toilet hole. These traps were placed in the same toilets where odour-baited traps had been previously deployed. Flies were collected for two hours from 10.00-12.00 h. This period was chosen to coincide with reduced household presence and infrequent toilet usage. mechanical aspirators (Prokopack®, model 1419, John W. Hock Co., Gainesville, USA) (Vazquez-Prokopec et al. 2009) were used to aspirate all trapped flies. The collected flies were then carefully packed into labelled nets and transported to the field laboratory for sorting and data recording.

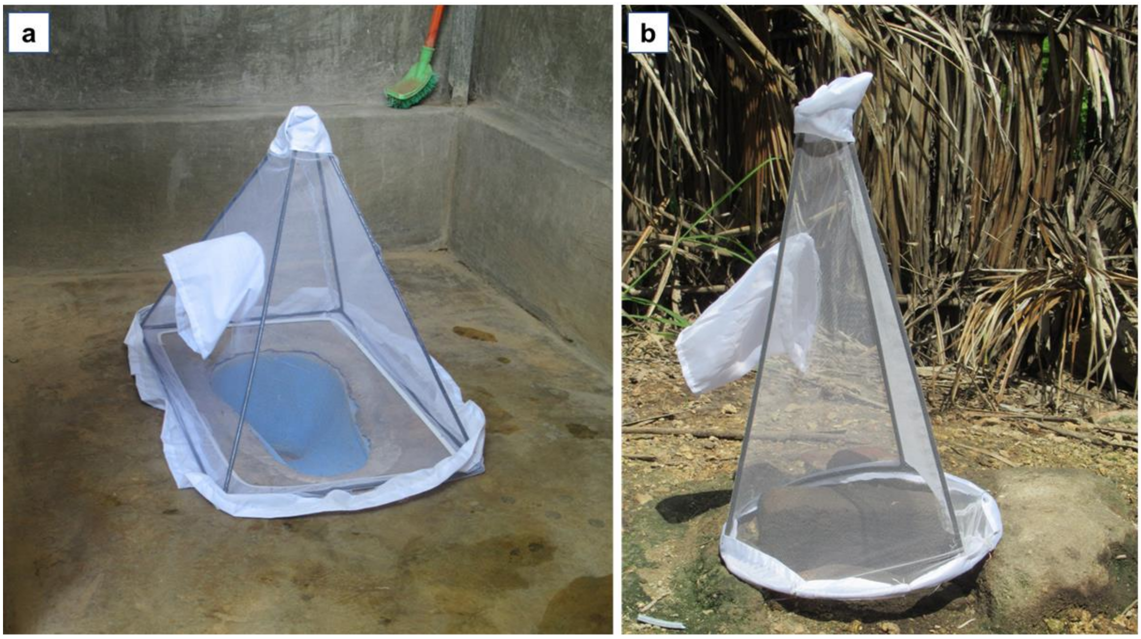

Figure 4.6: Emergence domestic flies traps placed at the main hole toilets.a) Star homes and b) traditional house.

Figure 4.7: Lindsay domestic fly traps were affixed to metal stands for fly sampling at the toilets.

Door opening and closing

The duration of door opening was recorded in eight houses per week/cluster, comprising four Star homes and four traditional houses. For each house type, one door logger (Onset UX90-001-HOBO/state/pulse) was installed on the inner side of the main door. The data loggers recorded the duration of the door opening every 30 seconds from 06.00h to 18.00h, every Tuesday morning of the week (Fig 4.8)

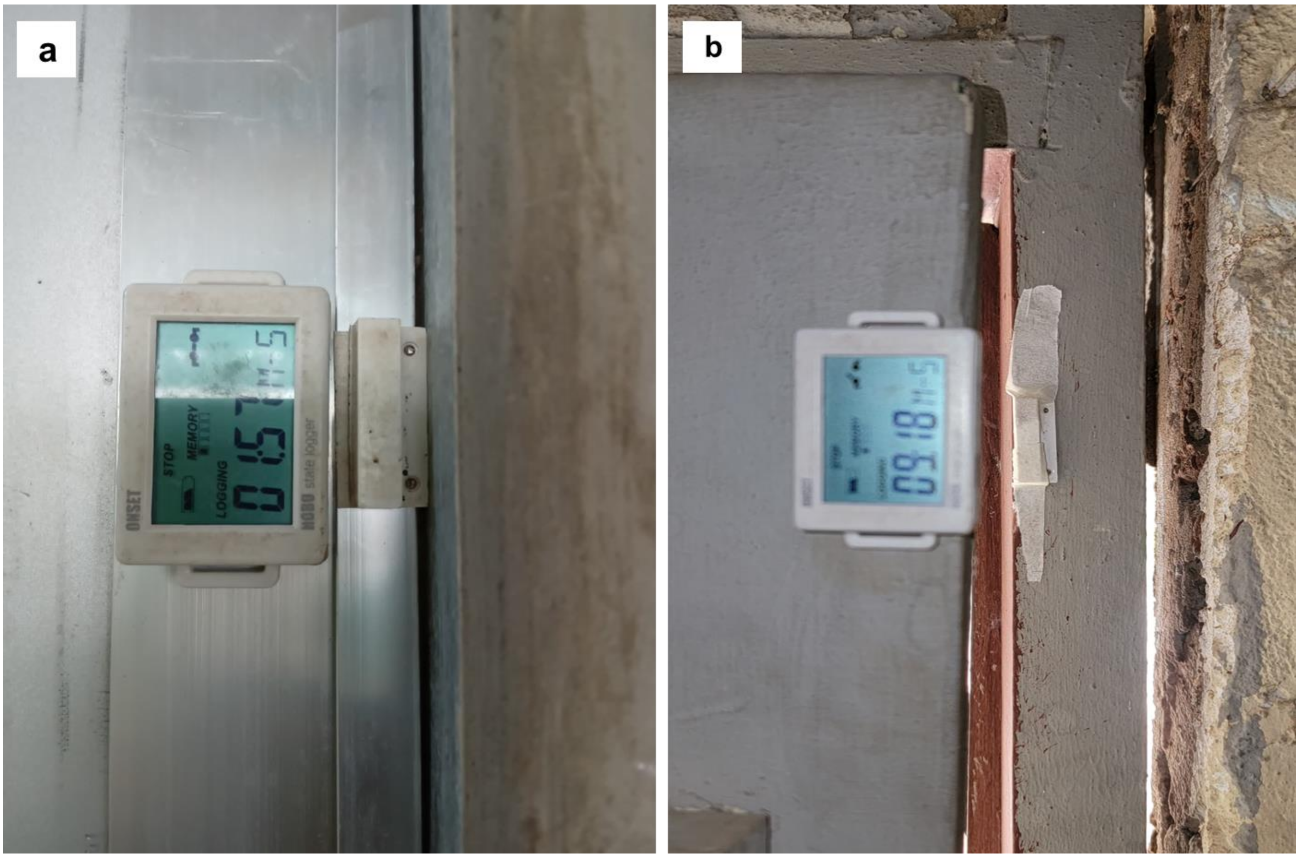

Figure 4.8: Door loggers fitted inside the doors of, a) the Star homes, and b) the traditional house.

Statistical analysis

Data were analysed using R language version 3.5.0 and followed an analysis plan written before study completion. The primary outcome measure was the number of domestic fly species collected at the Star homes kitchen compared to the traditional houses. Also, the count of domestic fly species caught at the Star homes toilets compared to the traditional houses. All analyses were conducted on an intention-to-treat basis i.e. regardless of whether families actively used the kitchen or not. Additionally, the analysis accounted for potential overestimation of the number of flies caught in completely open-structured kitchens. An additional study was conducted to assess any difference in domestic fly abundance between families who actively used the kitchen and those who did not. Domestic fly counts were modelled using a Generalized Linear Mixed Model (GLMM) with the *glmmTMB* package (Brooks et al. 2022), employing a negative binomial distribution to account for over-dispersion The response variable was daily fly counts per house, and the main fixed variable was the interventions applied. Nested random terms were added to account for variations across days, villages, house pair IDs, rounds, and clusters. Each domestic fly species was analysed separately for each season. An independent Student's t-test compared fly counts in Star homes' kitchens between users and non-users. Door opening duration analyses included data from 06.00h to 18.00h, excluding night-time readings. These data were segmented into hourly intervals and merged with other study variables. A linear mixed-effects model (*lmer*) with a normal distribution determined the adjusted daily mean differences in door opening durations across traditional and Star homes, including their 95% CIs.

Ethics declarations

The study was registered on ClinicalTrials.gov (NCT04529434) and approved by Tanzania's National Research Ethics Committee (NIMR/HQ/R.8a/Vol.IX/3695) and Durham University's Department of Biosciences ethics committee (Approved 24th June 2020). Participants gave informed consent before joining. The study was conducted in compliance with principles set out by the International Conference on Harmonization Good Clinical Practice, the Declaration of Helsinki and the ethical requirements of Tanzania.

Results

Fly abundance in kitchens

During the study, 1,727 domestic flies were collected over 712 trapping occasions from kitchens, 32% (556/1727) in the Star homes and 68% (1171/1727) in the traditional houses. Of these 51% were *C. putoria* (880/1727), 30% *M. domestica* (514/1727) and 19% *Sarcophaga* spp. (333/1727).

Overall, Star homes were associated with approximately half the number of flies found in traditional houses (Table 4.1). There were, however, different effects in the dry and wet seasons. Whilst significant reductions were found in all fly species in the dry seasons, this only occurred for *Sarcophaga* spp. during the wet seasons, when fly numbers were highest.

During the study we found that often people living in Star homes were not using the indoor kitchen for cooking (53%, 187/356 occasions). In the dry seasons, there were more households cooking outdoors (40%, 144/356 occasions) than during the rainy seasons (12%, 43/356 occasions). The households pointed out several factors hindered them from using the Star homes kitchen such as the smaller diameter of the cooking perimeters, smoke, and the preferred type of fire. We therefore carried out an analysis to see whether fly catch sizes differed between those occupants of Star homes who used the kitchens and those who did not. In all comparisons there were no difference in fly abundance between those who used Star home kitchens and those who did not, (Table 4.2).

Conversely, approximately 26% (92/356 occasions) of households residing in traditional dwellings engaged in outdoor cooking within proximity to their residences. During dry seasons, the prevalence of outdoor cooking rose to 17% (62/356 occasions), surpassing the 8% (30/356 occasions) observed during rainy seasons. Notably, no discernible variation in domestic fly abundance was observed between traditional houses practising outdoor and indoor cooking, except for the *M. domestica* fly species, which exhibited greater abundances at indoors than outdoors (Table 4.3).

Fly abundance in toilets

Among the 28 traditional houses examined in the study, 82% (23/28) were equipped with pit latrines, leading to the exclusion of the remaining houses practising open defecation from the analysis. Of those houses with a latrine, 17% (4/23) had a roof. The pit latrines in all 23 traditional houses were characterized by earth floors and walls constructed from sticks and grass. Approximately 79% (16/23) of these pit latrines had an entry door covered by curtains, 17% (4/23) were fully open, and 13% (3/23) featured doors made of sticks and grass.

A total of 23,027 domestic flies were collected over 698 trapping occasions, 40% of them in Star home toilets and 60% in traditional toilets. Of these, 77% were *C. putoria* (17,636/23,027), 16% *M. domestica* (3,713/23,027) and 7% *Sarcophaga* spp. (1,678/23,027).

Overall, Star home toilets exhibited a 50% reduction in the presence of *C. putoria* fly species compared to traditional houses, a trend not observed for other domestic fly species. The influence on *C. putoria* was significant in both dry and wet seasons, with no discernible impact on *Sarcophaga spp* and *M domestica*. No difference in a number of domestic fly abundances was observed between the dry and wet seasons (Table 4.3).

Exit fly collections from toilets

During this sub-study we collected flies from directly over the poop hole of 52 Star homes and 52 traditional toilets. No flies were caught in exit traps in Star home toilets compared to 219 flies traditional toilets. Of the flies collected, 72% (158/219) were *C. putoria*, 24% (52/219) *M. domestica* and 4% (9/219).

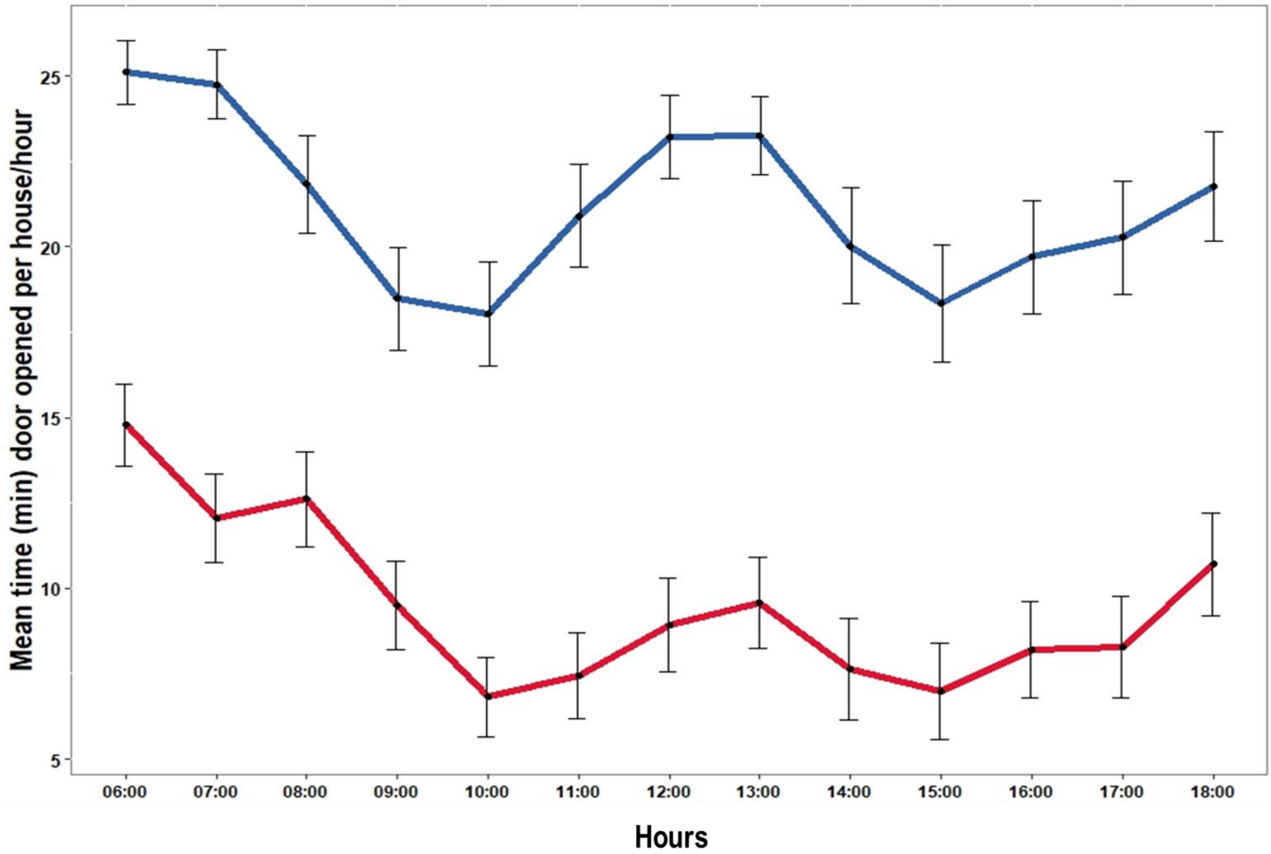

Figure 4.9: Proportion of time (minutes) spent with door open between 07.00h and 17.30h.Data from 120 Star homes and 120 traditional houses. Where red line represents Star homes (main door) and blue line traditional houses (main door). Error bars are (95% CI's).

Duration of main door openings

In Star homes the main door leads directly into the kitchen, was compared the main door leading to both indoor and outdoor traditional house kitchen areas. The daily (07.00 to 1730h) trendlines for door opening were similar for both house typologies. The duration of door opening was greatest in the early morning at 06.00 which declined until 10.00h. There was a second peak at midday from 12.00 to 13.00 h followed by a further decline until a gradual rise from 15.00 to 18.00 h (Fig 4.5). Control houses had a higher mean door opening duration (mean = 20.9 minutes, 95% CI: 17.8 –24) than Star homes (mean = 7.4 minutes, 95% CI: 4.2–10.7). Star homes' kitchen doors remained open for 65% less time (equivalent to 14 minutes/hour) compared to traditional houses, with an adjusted mean difference of -13.5 minutes per hour, 95% CI: -16.9, -10.1, $p < 0.0001$ (Table 4.4, Fig 4.8).

Table 4. 1: Domestic fly abundance in study house kitchens. Where, RR=Risk ratio, CI= 95% Confidence intervals.

Domestic fly species	House type	Trap days	Total caught	Unadjusted mean (95% CI)	Adjusted mean (95% CI)	Adjusted RR (95% CI)	% reduction	p
Dry season (June – November)								
Chrysomya putoria	Traditional house	208	227	1.1 (0.5 – 1.5)	0.72 (0.33 – 1.60)	1		
	Star homes	208	106	0.5 (0.3 – 0.8)	0.32 (0.14 – 0.73)	0.45 (0.26 – 0.77)	55	0.004
Musca domestica	Traditional house	208	163	0.8 (0.2 – 1.4)	0.31 (0.10 – 0.96)	1		
	Star homes	208	66	0.3 (0.1 – 0.5)	0.12 (0.04 – 0.38)	0.38 (0.18 – 0.83)	62	0.013
Sarcophaga species	Traditional house	208	112	0.5 (0.2 – 0.7)	0.17 (0.05 – 0.54)	1		
	Star homes	208	51	0.3 (0.2 – 0.4)	0.08 (0.02 – 0.28)	0.48 (0.25 – 0.92)	52	0.027
Wet season (December – May)								
Chrysomya putoria	Traditional house	148	357	2.5 (1.6 – 3.4)	1.50 (0.67 – 3.33)	1		
	Star homes	148	190	1.3 (0.9 – 1.7)	1.03 (0.47 – 2.23)	0.69 (0.38 – 1.25)	31	0.217
Musca domestica	Traditional house	148	174	1.2 (0.5 – 1.9)	0.60 (0.23 – 1.57)	1		
	Star homes	148	111	0.8 (0.4 – 1.2)	0.47 (0.18 – 1.22)	0.77 (0.38 – 1.57)	23	0.476
Sarcophaga species	Traditional house	148	138	0.9 (0.5 – 1.3)	0.27 (0.06 – 1.21)	1		
	Star homes	148	32	0.2 (0.1 – 0.3)	0.08 (0.02 – 0.36)	0.27 (0.14 – 0.52)	73	0.0001
Dry and Wet seasons (October 2021 – October 2023)								
Chrysomya putoria	Traditional house	356	584	1.7 (1.2 – 2.1)	0.99 (0.52 – 1.88)	1		
	Star homes	356	296	0.8 (0.6 – 1)	0.52 (0.27 – 0.99)	0.52 (0.35 – 0.78)	48	0.001
Musca domestica	Traditional house	356	337	1 (0.5 – 1.5)	0.45 (0.19 – 1.07)	1		
	Star homes	356	177	0.5 (0.3 – 0.8)	0.24 (0.10 – 0.58)	0.53 (0.32 – 0.89)	47	0.017
Sarcophaga species	Traditional house	356	250	0.7 (0.5 – 0.9)	0.24 (0.09 – 0.62)	1		
	Star homes	356	83	0.2 (0.1 – 0.3)	0.09 (0.03 – 0.25)	0.38 (0.23 – 0.62)	62	0.0001

Table 4. 2: Domestic fly abundance in the Star homes families who utilize the kitchen versus non-users. Where, CI= 95% Confidence intervals and p= 0.05 significant level.

Domestic fly species	Kitchen use status	Total number of houses	Actual domestic fly counts	Mean (95% CI)	p
Dry seasons (June – November)					
Chrysomya putoria	Non-user	144	69	0.5 (0.2 – 0.8)	0.67
	User	64	37	0.6 (0.2 – 1)	
Sarcophaga species	Non-user	144	42	0.3 (0.1 – 0.5)	0.25
	User	64	9	0.1 (-0.1 – 0.3)	
Musca domestica	Non-user	144	31	0.1 (-0.1 – 0.3)	0.27
	User	64	35	0.6 (0.1 – 1.1)	
Rainy seasons (December – May)					
Chrysomya putoria	Non-user	43	74	1.7 (0.8 – 2.6)	0.23
	User	105	116	1.1 (0.6 – 1.6)	
Sarcophaga species	Non-user	43	12	0.3 (0.1 – 0.5)	0.50
	User	105	20	0.2 (0.1 – 0.3)	
Musca domestica	Non-user	43	39	0.9 (0.2 – 1.6)	0.59
	User	105	74	0.7 (0.2 – 1.2)	
Dry & Wet seasons (October 2021 – October 2023)					
Chrysomya putoria	Non-user	187	143	0.8 (0.5 – 1.1)	0.13
	User	169	153	0.9 (0.6 – 1.2)	
Sarcophaga species	Non-user	116	54	0.3 (0.2 – 0.4)	0.07
	User	154	29	0.2 (0.1 – 0.3)	
Musca domestica	Non-user	116	70	0.4 (0.1 – 0.7)	0.06
	User	154	107	0.6 (0.3 – 0.9)	

Table 4. 3: Domestic fly abundance in indoor and outdoor kitchen in traditional houses.Where, CI= 95% Confidence intervals and p= 0.05 significant level.

Domestic fly species	Kitchen location	Total number of houses	Actual domestic fly counts	Mean (95% CI)	p
Dry season (June – November)					
Chrysomya putoria	Indoor	146	165	1.1 (0.6 – 1.6)	0.76
	Outdoor	62	62	1 (0.3 – 1.7)	
Sarcophaga species	Indoor	146	82	0.6 (0.4 – 0.8)	0.82
	Outdoor	62	30	0.5 (-0.1 – 1.1)	
Musca domestica	Indoor	146	146	1 (0.2 – 1.8)	0.09
	Outdoor	62	17	0.3 (0 – 0.6)	
Rainy season (December – May)					
Chrysomya putoria	Indoor	118	285	2.4 (1.4 – 3.4)	0.99
	Outdoor	30	72	2.4 (0 – 4.8)	
Sarcophaga species	Indoor	118	119	1 (0.6 – 1.4)	0.42
	Outdoor	30	19	0.6 (-0.2 – 1.3)	
Musca domestica	Indoor	118	151	1.3 (0.3 – 2.2)	0.43
	Outdoor	30	23	0.8 (0 – 1.6)	
Dry & Wet seasons (October 2021 – October 2023)					
Chrysomya putoria	Indoor	264	450	1.7 (1.2 – 2.2)	0.64
	Outdoor	92	134	1.5 (0.6 – 2.4)	
Sarcophaga species	Indoor	264	201	0.8 (0.6 – 1)	0.42
	Outdoor	92	49	0.5 (0 – 1)	
Musca domestica	Indoor	264	297	1.1 (0.5 – 1.7)	0.05
	Outdoor	92	40	0.4 (0.1 – 07)	

Table 4. 4: Domestic fly abundance in toilets. Where, RR=Risk ratio, CI= 95% Confidence intervals.

Domestic fly species	House type	Trap days	Total caught	Unadjusted mean (95% CI	Predicted (Mean [95% C.I]	Adjusted (RR (95% C.I)	% reduction	p
Dry seasons (June – November)								
Chrysomya putoria	Traditional house	200	4458	22.5 (17.8 – 27.2)	13.82 (6.70 – 28.48)	1		
	Star homes	200	2366	12 (8.7 – 15.2)	6.89 (3.32 – 14.30)	0.50 (0.37 – 0.67)	50	<0.0001
Musca domestica	Traditional house	200	368	1.7 (1.1 – 2.3)	1.39 (0.74 – 2.60)	1		
	Star homes	200	275	1.4 (1 – 1.8)	1.06 (0.56 – 1.99)	0.76 (0.50 – 1.17)	24	0.218
Sarcophaga species	Traditional house	200	345	1.7 (1.4 – 2.1)	1.57 (0.97 – 2.53)	1		
	Star homes	200	314	1.6 (1.4 – 1.8)	1.32 (0.82 – 2.13)	0.84 (0.62 – 1.15)	16	0.271
Wet seasons (December – May)								
Chrysomya putoria	Traditional house	149	6596	45.5 (35.5 – 55.5)	35.31 (14.46 – 86.22)	1		
	Star homes	149	4212	28.3 (22.3 – 34.4)	18.82 (7.75 – 45.74)	0.53 (0.38 – 0.74)	47	0.00022
Musca domestica	Traditional house	149	1543	10.6 (7.4 – 13.5)	3.79 (1.41 – 10.24)	1		
	Star homes	149	1527	10.2 (6.6 – 13.8)	3.07 (1.14 – 8.26)	0.81 (0.55 – 1.18)	19	0.271
Sarcophaga species	Traditional house	149	540	3.7 (2.6 – 4.8)	2.37 (1.06 – 5.32)	1		
	Star homes	149	479	3.2 (2.4 – 4)	2.23 (1.00 – 4.97)	0.94 (0.67 – 1.32)	6	0.720
Dry and wet seasons (October 2021 – October 2023)								
Chrysomya putoria	Traditional house	349	11058	32.2 (26.9 – 37.5)	19.03 (9.67 – 37.35)	1		
	Star homes	349	6578	19 (15.7 – 21.3)	10.28 (5.23 – 20.21)	0.54 (0.44 – 0.67)	46	<0.0001
Musca domestica	Traditional house	349	1911	5.6 (4.1 – 7.1)	2.34 (1.11 – 4.93)	1		
	Star homes	349	1802	5.2 (3.5 – 6.9)	1.83 (0.87 – 3.89)	0.79 (0.58 – 1.06)	21	0.112
Sarcophaga species	Traditional house	349	885	2.6 (2.1 – 3.1)	1.83 (1.07 – 3.11)	1		
	Star homes	349	793	2.3 (1.9 – 2.7)	1.58 (0.93 – 2.69)	0.87 (0.69 – 1.09)	13	0.215

Table 4. 5: Duration of door opening in study houses.

House typology	No. houses	Mean (min) (95% C.I)	Adjusted mean difference, (min) (95% CI)	p
Dry seasons (June – November)				
Traditional house	64	19.8 (16.3, 23.4)	1	
Star homes	64	5.2 (1.4, 9)	-14.6 (-18.3, -11)	<0.00001
Wet seasons (December – May)				
Traditional house	56	21.3 (16.9, 25.9)	1	
Star homes	56	10.8 (6.5, 15)	-10.6 (-15.5, -5.7)	0.0002
Dry and wet seasons (February 2022 – October 2023)				
Traditional house	120	20.9 (17.8, 24.0)	1	
Star homes	120	7.4 (4.2, 10.7)	-13.5 (-16.9, -10.1)	<0.00001

Discussion

Preventing the interaction between humans and domestic flies, which serve as mechanical vectors for diarrhoeal pathogens, is amongst of the approaches to reduce the transmission of these pathogens (Emerson et al. 1999). This study demonstrated the effectiveness of Star homes in reducing domestic fly populations in the kitchen and toilets compared to traditional houses. The innovative design features of the Star homes roughly halved the entry of domestic flies into the kitchen and toilets which could lower the risks of transmitting diarrhoeal pathogens.

The study observed a 50-57% reduction in domestic fly abundance in the kitchen of the Star homes compared to the traditional houses. The reduction varied depending on the fly species and seasons. In dry seasons, the Star homes kitchen reduced domestic fly abundances by 50-63% compared to traditional houses. This reduction is especially important for *C. putoria* and *M. domestica*, both of which can transmit diarrhoeal pathogens (Emerson et al. 1999). These fly species can transmit pathogens such as *Salmonella*, *Shigella*, and *Escherichia coli*, leading to acute gastroenteritis and gastrointestinal infections (Graczyk, Knight et al., 2001). These pathogens, commonly found in faecal matter, can be transferred by flies to food and surfaces they come into contact with, leading to potential infection (Lindsay, Lindsay et al., 2012; Khamesipour et al., 2018). *C. putoria*, which exhibits a strong preference for breeding and feeding on human faecal materials, can carry enteric bacteria, including *E. coli*, known to cause diarrheal illnesses, particularly in children (Lindsay, Lindsay et al., 2012). By minimizing fly contact with cooking utensils and food preparation areas in the kitchens, the risk of diarrhoeal illnesses may be mitigated. While the *Sarcophaga* species, commonly known as flesh flies, do not serve as vectors for diarrheal pathogens, it is imperative to underscore the significance of Star homes kitchens in mitigating the prevalence of this fly species due to their association with myiasis (Sukontason et al. 2014).

Although Star homes reduced the entry of domestic flies during the dry season, this was not the case during the rainy season. During the rainy seasons, people tend to stay indoors to seek shelter from the rains, especially after returning from farms or when children come back from school, prolonging the duration that doors remain open and providing opportunities for flies to enter. The shift from outdoor to indoor cooking during rainy seasons also increases the chances of flies entering through open doors.

The design features of the intervention kitchens which included fly-proof shade net walls, self-closing solid doors, and cemented floors may have contributed to the observed reduction in domestic fly abundances. The shade netting on the walls acted as physical barriers, preventing domestic flies from entering the kitchen, as observed in previous studies (Hald et al. 2007). The self-closing solid doors in the intervention house kitchens also acted as physical barriers against the flies, and ensured the doors stayed closed for longer than in traditional houses. Using data loggers recording door opening in the study showed that on average Star home doors were on average open for 14 min less each hour. Lastly, the smooth cemented floors in the intervention houses facilitated easier cleaning in contrast to the frequently encountered porous and earthen floors in traditional houses. This ensured efficient removal of all food materials dropped during cooking, thereby reducing the presence of decaying food odours that could attract flies to the kitchen. While, in contrast, for the traditional houses kitchen presence of earth floor allows food spilled on the floor to be absorbed into the ground and smells to persist.

During the study, a large percentage of occupants in Star homes cooked outside their houses during dry seasons (40%, 144/356 occasions) than during the rainy seasons (12%, 43/356 occasions). The reasons cited by households for not using the Star homes kitchen included factors such as the smaller diameter of the cooking stove, smoke, and the preferred type of fire. However, we observed no disparity in domestic fly abundance between kitchen users and non-users in Star homes. Nonetheless, those cooking outdoors are at greater risk of encountering diarrhoeal pathogens, as their food materials and cooking utensils are exposed to domestic flies, which can easily contaminate them compared to families cooking in the Star homes' kitchens.

During the day, the main door to Star homes were open for 14 min/h less time than the main door of traditional houses. The general patterns seen reflect how people use the house. High activity is observed post 06.00 h, coinciding with people waking up, gradually diminishing during the period around 10.00 h when both men and women are engaged in farm work, children are heading to school, and household cleaning is underway. A subsequent surge in activity occurs, reaching its peak at 13.00 h, aligning with households preparing and consuming their lunches. Following a subsequent decline, there is a gradual increase in door openings after 15.00 h, as individuals return home from the farms and children return from school. During this timeframe, households are involved in domestic activities such as fetching water, washing dishes, and commencing the preparation of their evening meal. During this timeframe, there is alignment with the active period of outdoor domestic flies, such as *Musca domestica*, which is recognised for its activity during the late morning (09.00 h) to early afternoon (12.00 h) (Klong-klaew et al. 2020), providing an opportunity for domestic flies to enter the houses when doors were left open (Keiding 1986).

While house screening proved to be an effective approach in preventing fly entry, the presence of shade net walls in Star homes' kitchens served as a physical barrier, making doors the primary entry point for flies. While the study could not directly assess the correlation between domestic fly abundances and door opening duration, as fly trapping and door logging occurred on different days, it is probable that doors served as a significant entry route for flies in Star home kitchens. In addition to doors, the number of flies in Star homes' kitchens might also be influenced by entry through holes in the shade net walls, predominantly located on the ground floor of the house. For the traditional houses, doors were open for longer than in Star homes and, in addition, could enter the house through open windows and holes in the walls.

Star home toilets had roughly half the number of *C. putoria* compared with traditional toilets. This is an important finding since this species represented 77% of the total flies trapped in the toilets and is a putative vector of diarrhoea pathogens (Lindsay et al. 2012) since it breeds in faeces contained within the toilet. This reduction can be attributed to the flap under the poop hole that prevents flies entering or exiting the faecal waste chamber. These findings suggest that *C. putoria* flies collected at the toilets originated from the surrounding environment, particularly in villages, especially those located in coastal areas where open defecation practices prevail, facilitating the breeding of these species (Sara & Graham 2014). This conclusion was drawn from a sub-study where no flies exited the defecation hole, in contrast to 219 flies exiting traditional toilets lacking a protective cover.

The study had two main limitations. Firstly, it is probable that baited traps in the Star homes captured a higher proportion of house-entering flies compared to traps in traditional houses, given that Star homes had significantly fewer exit points than traditional houses. Secondly, a similar source of bias is expected when sampling flies within the Star home toilets. Both limitations would underestimate the true efficacy of our interventions in capturing flies.

Conclusion

Star homes reduced the abundance of two vectors of diarrhoeal pathogens in Star home kitchens compared to those in traditional houses. Similar reductions were seen in Star home toilets compared with traditional toilets. These results show how well-screened houses and toilets which prevent flies entering the faecal collecting pit can reduce the abundance of important vectors of diarrhoeal diseases. It is likely that even greater reductions would occur if the quality of housing and toilets were improved on a larger scale. Our findings are of relevance to those designing and constructing new homes in sub-Saharan Africa.

Chapter 5 : Environmental risk factors associated with the abundance of malaria vectors in traditional houses in rural south-eastern Tanzania.

Abstract

Background: Malaria transmission in sub-Saharan Africa is primarily mediated by indoor-biting members of the *Anopheles gambiae* complex and *Anopheles funestus* group. Understanding the factors influencing indoor mosquito densities is vital for developing effective interventions to reduce malaria transmission risk. Entomological surveillance data -obtained from a randomized controlled trial evaluating a new house design in rural south-eastern Tanzania, was used to identify risk factors associated with malaria vector abundance inside traditional homes in the area.

Methods: Mosquitoes were sampled in 110 traditional houses in 59 villages, each housing at least one child under 13 years old. CDC light traps were utilised to sample indoor malaria vectors in each house once every seven weeks for two years. Remotely sensed imagery was employed to measure coarse-scale environmental factors, while questionnaires were used to describe finer-scale house characteristics that could influence indoor malaria vector abundance. Generalized linear mixed-effects models were applied to assess the association between mosquito count data and the putative risk factors.

Results: Of the 7,314 mosquitoes captured, 14% (1,149) were *An. gambiae s.l*., and 4% (308) *An. funestus s.l.* Among the identified *An. gambiae s.l*., 60% (528) were *An. gambiae ss*, 32% (276) were *An. arabiensis*, and 7% (63) *An. merus*. In the case of *An. funestus s.l*., 98% were *An. funestus ss*, with the remaining 2% comprising other sibling species. Higher indoor densities of malaria vectors were localized in the central and eastern regions, leaving the western and southern parts of the study area with low numbers. Factors associated with increased indoor malaria vector abundance (*An. gambiae* s.l. and *An. funestus* s.l. combined) included the presence of open water bodies within 15 m of the house (Risk Ratio RR = 1.61, 95% CI: 1.04–2.50, p = 0.03) and unscreened windows (RR = 2.47, 95% CI: 0.73–8.39, p = 0.01). Conversely, the proportion of the area covered with human-made features reduced malaria vectors (RR = 0.01, 95% CI: 0–0.15, p = 0.001), higher altitudes (RR = 0.71, 95% CI: 0.55–0.90, p = 0.01), and presence of chickens (RR = 0.92, 95% CI: 0.87–0.97, p = 0.002).

Conclusion: Higher indoor abundance of malaria vectors occurred in the central and eastern part of the study area. The close proximity of open-water bodies, potential aquatic habitats, and unscreened windows increased indoor malaria vector densities, whilst built areas, high altitude and the presence of chickens were associated with reduced abundance. These findings suggest that drainage of open water bodies near villages or larviciding, with screened windows, doors and eaves may help reduce the entry of malaria mosquitoes indoors.

Background

African regions remain at the forefront of global malaria cases and mortality, accounting for approximately 94% of cases and 95% of deaths by 2022. This represents a decline of 146.7 million cases and 87.1 million deaths from 2000 to 2022 (WHO 2023), primarily attributed to the widespread adoption of an integrated approach (Bhatt et al. 2015), encompassing vector control tools and effective treatment strategies (Mboera et al. 2007, Bhatt et al. 2015). However, since 2015, progress in reducing malaria cases and deaths has stalled, particularly in sub-Saharan Africa (SSA), due to the COVID-19 pandemic and other humanitarian emergencies (WHO 2023). This has led to a significant increase in malaria cases in countries such as Nigeria, Ethiopia, Madagascar, Uganda, the United Republic of Tanzania, Mali, and Mozambique (WHO 2023).

Despite considerable progress in malaria control since 2000, SSA still faces numerous challenges, including insufficient financing for malaria prevention and treatment services, hindering access to prevention measures and treatment for at-risk populations (WHO 2022). Additionally, changing climatic conditions favour the breeding and distribution of malaria vectors (Karypidou et al. 2020), while pervasive poverty hampers community engagement in prioritization for malaria prevention measures and adequate housing (Tusting et al. 2016). Vector control tools, such as insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS), have emerged as the most widely used and cost-effective approaches for mitigating malaria transmission risks in the region (malERA Consultative Group on Vector Control 2011, Bhatt et al. 2015). A cluster-randomized open-label trial conducted in Mozambique between 2016 and 2018, evaluating the combined benefits of ITNs and IRS, demonstrated a significant additional protection of 46% against malaria prevalence in children under five years old (Chaccour et al. 2021).

Sub-Saharan Africa is home to a diverse array of *Anopheles* mosquito species (Sinka et al 2012, Kyalo et al 2017), with the prominent species responsible for transmitting malaria parasites including *Anopheles gambiae s.l*., (Mason 2003), *Anopheles funestus s.l*. (Kahamba et al. 2022) and a newly invasive species, *Anopheles stephensi* (Sinka et al. 2020). The role of these mosquitoes in malaria transmission can be attributed to their biting and resting behaviours, life span (survival longevity) as well as ecological factors that favour their distribution (Kyalo et al 2017).

Given that approximately 80% of malaria transmission occur indoors (Huho et al. 2013, Sherrard-Smith et al. 2019), it is important to gain a deeper understanding of the risk factors favouring indoor mosquito densities and, hence, high transmission. Key factors contributing to the risk of indoor mosquito densities include house structure (Njie et al. 2014), building materials (Jatta et al. 2018)), the presence of domestic animals near a house (Animut et al. 2013), the number of human occupants, weather conditions and the characteristics of the environment around a house (Minakawa et al. 2002). Environmental factors, such as the presence of potential breeding habitats like marshland, irrigated farming areas, crop cultivation (Ijumba & Lindsay, 2001) and water wells located near houses, have been linked to an elevated risk of malaria vector biting indoors (Minakawa et al. 2002, Sogoba et al. 2007). Weather conditions also play an important role, as rainy seasons create numerous aquatic habitats, thereby increasing mosquito population densities compared to dry seasons (Ngowo et al. 2017). Previous studies have shown that keeping large domestic animals, such as cattle, close to the house increases indoor mosquito abundance, as these animals attract mosquitoes from nearby breeding habitats into the house (Minakawa et al., 2002; Animut et al., 2013). Conversely, other studies have demonstrated a reduction in mosquito abundance, as these animals provide an alternative blood meal source outdoors, particularly in areas where bednets are widely used (Kirby et al., 2008).

Housing features that increase mosquito numbers include open eaves, the major route of *Anopheles gambiae* s.l. entry (Njie et al. 2014), unscreened windows, and poorly-fitting doors (Lindsay et al. 2002). Mud-walled and thatched-roof houses have been associated with higher indoor mosquito densities compared to brick-walled and metal-roofed houses (Jatta et al. 2018). Increased household occupancy has been correlated with higher indoor entry of malaria vectors (Kaindoa et al., 2016). Additionally, people spending time outdoors in the early evening for activities such as cooking, dishwashing, and storytelling are more exposed to mosquito bites (Tirados et al. 2006) and can facilitate vector entry into the house through open doors, windows and eaves (Fig 5.1).

Within the framework of a household randomized controlled trial assessing the impact of a novel type of home on childhood malaria (Mshamu et al. 2022), routine entomological data was collected from traditional houses. This cohort of houses were routinely sampled for the presence of malaria mosquitoes over two years and allowed us to undertake a secondary analysis of risk factors for the presence of indoor mosquitoes in traditional houses. Findings from this study may identify potential ways to reduce the risk of indoor biting by malaria mosquitoes.

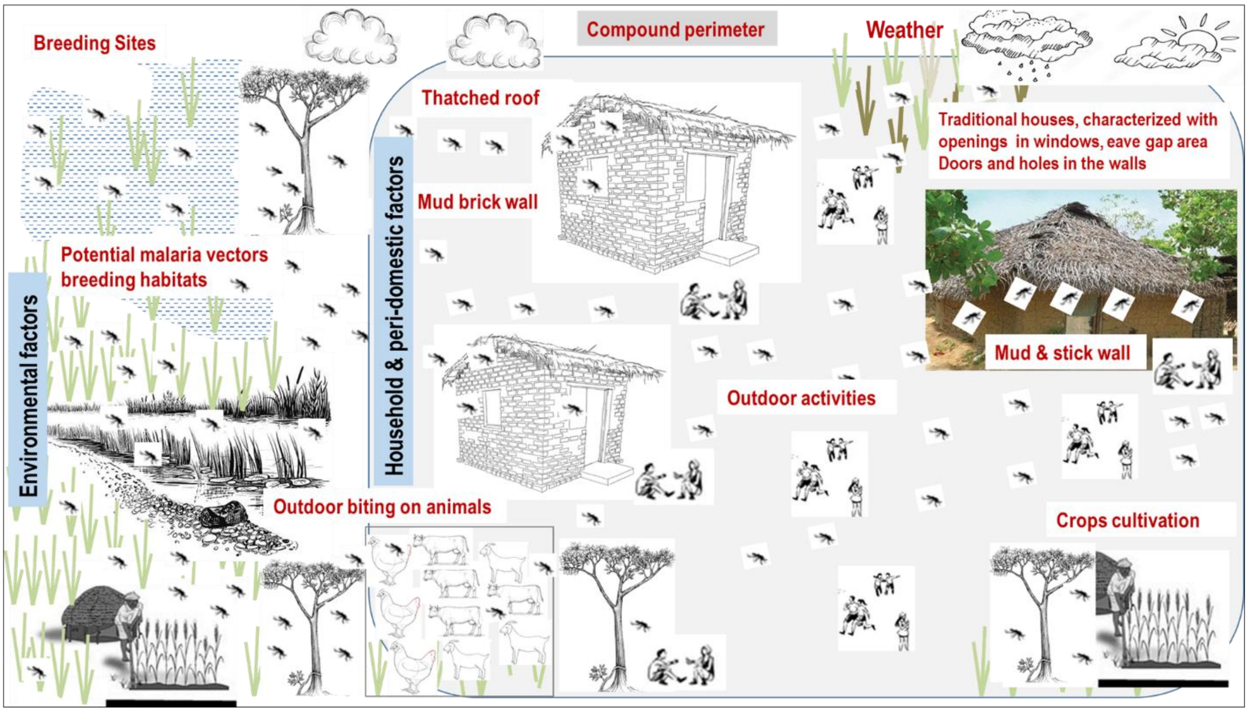

Figure 5.1: Potential environmental and household risk factors influencing the indoor abundance of malaria vectors, adapted from Yaro et al. 2021. These factors include the proximity of the house to larval habitats, the presence of domestic animals and

birds that may attract or divert mosquitoes away from the dwelling, and outdoor activities such as cooking, washing dishes, and storytelling, which increase the frequency and duration of open doors, thereby providing entry points for indoor mosquitoes. Additionally, favourable conditions for mosquito breeding are influenced by weather conditions such as temperature and rainfall.

Methods

Study area

This study was conducted in Mtwara region (10.5181° S, 40.0633° E), south-east Tanzania from September 2021 to October 2023 (Fig. 5.2). The study area consists of a coastal strip of sandy low-lying land with undulating hills inland, <400 m above sea level, covered with agricultural fields, forest and scrubland. There are two rainy seasons; the long rains between February and April and shorter rains between October to December. The major crops grown in the study area are cashew, cassava, maize and rice. About 90% of residents are farmers, with a smaller population engaged in fishing activities (Mshamu et al. 2020), (Fig 5.2).

Earlier studies have shown that *An. gambiae* s.l., and *An. funestus* s.l. were the major malaria vectors (Lwetoijera et al. 2014, Lupenza et al. 2021). In 2022, 20% of children aged 2 to 10 years old had *Plasmodium falciparum* infections (Ministry of Health (MoH) [Tanzania Mainland] 2023). Starting in 2010, the Tanzania National Malaria Control Program (NMCP) distributed ITNs to the most vulnerable population, including school-aged children and pregnant mothers residing in rural areas of the country (Ministry of Health (MoH) [Tanzania Mainland] 2023). The school bed nets program distributed bed nets semi-annually, coinciding with the commencement of each academic term for students. Pregnant women received bednets upon initiation of antenatal clinics. The project team implemented three phases of bednet distribution, providing one pair of Olyset nets (Olyset, 2% Permethrin, Sumitomo Chemical, and A to Z Textile MillsTM, Arusha, Tanzania) per household. The initial distribution phase occurred in August 2021, followed by the subsequent phases in October 2022 and January 2023.

Study design

This study investigated the risk factors associated with indoor malaria vector abundance within traditional houses. Mosquito surveillance data were systematically

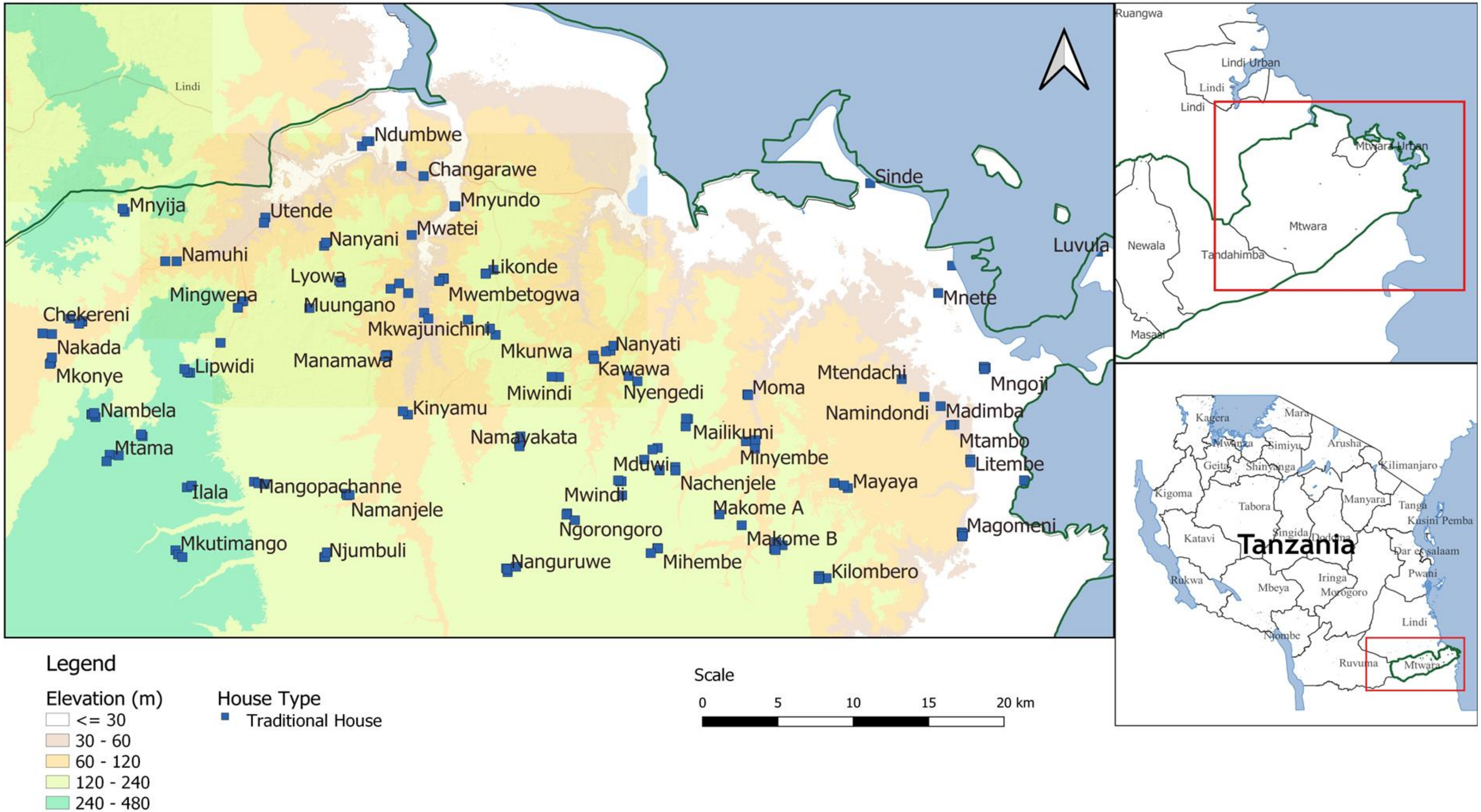

Figure 5.2: Study area.

Table 5. 1: Selection of potential risk factors associated with indoor abundance of malaria vectors(combined An. gambiae s.l and An. funestus s.l.).

Co-variates	**Source of measurements**	**Outcome definition**	**Rationale for risk factor**
Remotely sensed data			
Altitude (*at logarithmic scale, to get rid of collinearity*)	Satellite imagery (Sentinel-2) collected in rural Mtwara district from September 2021 to October 2023. .	Height (m) above sea level.	Water pooling is less likely at higher elevations due to run off than at lower altitudes. The more surface stagnant, the more likely it is to find aquatic habitats for mosquitoes.
Slope		Percentage of the ratio between (change in height/horizontal change) * 100	Steep slopes impede water accumulation, unlike gentle slopes that facilitate slow water runoff, creating opportunities for water to accumulate along the stream's periphery. These areas subsequently serve as breeding habitats for malaria vectors.
Normalized Difference Vegetation Index (NDVI), measures (greenness or photosynthetic		An NDVI value close to -1 indicates an area covered with water, close to 0 represents an area without green leaves (urbanised area) and values	Highly green vegetation indicates a high moisture content, which may correlate with wetness. Wet areas are likely to support the aquatic habitats of *Anopheles* mosquitoes.

activity), within 2km of each house		close to +1 indicate a dense green leaf area.	
Flow accumulation (where water accumulates) within 2km of each house	Flow accumulation was computed using the D8 algorithm, which is contingent upon the topographical features of the terrain. In GIS, these data were derived from the Digital Elevation Model (DEM) of the study area obtained via satellite imagery, providing an elevation depiction of the terrain. Flow direction was determined for each cell by employing this DEM data, contributing to the overall understanding of water flow, particularly at a specified threshold.	Cumulative number of cells contributing flow at a specified location on the landscape, OR Cubic meters per second (m^3 /s) mostly in the hydrological models which mostly consider the drainage area, the cell size and the flow accumulation values.	Water accumulates as run-off in low-elevated areas. Aquatic habitats are likely to be more plentiful in areas where water accumulates.

Built up environment	The quantity of human-made structures and infrastructure in a study area from the data retrieved from the satellite imagery.	Percentage or proportion of the building structures and infrastructures found in the study area	Increased urbanisation can reduce the availability of aquatic habitats, for both anophelines and culicine species.
Temperature within 2km of the house	Satellite imagery taken in each study village extracted from NASA satellite, (Sentinel-2) obtained from the study area, between September 2021 to October 2023.	Environmental temperature (°C)	Temperature affects mosquito development and survival, i.e., Higher temperature of 30°C to 35°C reduce lifespan of adult mosquitoes. Moderate temperature conditions (20°C – 30°C promote higher survival and faster development of adult mosquitoes
Rainfall within 2km of the house		Amount of rainfall (mm)	Increased rainfall is associated with more surface pooling providing aquatic habitats for malaria vectors.
Land cover within 2km of the house	Satellite imagery data on all physical and biological cover on Earth's surface. factors such as crops land, tree densities, built up environment, flooded vegetation. Additionally, satellite imagery data on ways in	Proportion or percentage of each landcover feature found in the study area.	Landcover factors such as seasonal swamps, and grassland favoured mosquito breeding and resting.
Land use within 2km of the house		Proportion or percentage of the total land area within a specific region.	Land use factors, such as the presence of agricultural fields and densely populated areas, contribute to the facilitation of increased mosquito populations.

	which land was utilized by humans such as agricultural, building and estate and populated areas		
Peri-domestic environment			
Presence of cattle near the house	Median number of cattle collected in house survey number two collected between May and June 2022	Number of cattle within 30 m of the child's bedroom.	Cattle provide an alternative blood meal source for mosquitoes especially *Anopheles arabiensis* and may attract them closer to houses, increasing the likelihood of house entry. Alternatively, the presence of cattle near a house may divert mosquitoes such as *An. arabiensis* away from humans.
Presence of chicken near the house	Median number of chicken collected in house survey number two collected between May and June 2022	Number of chickens within 30 m of the child's bedroom.	Chickens serve as an alternative blood meal source for *An. arabiensis* (Ngom et al. 2013, Mmbando et al. 2021), drawing them closer to houses and thereby increasing the likelihood of their entry.
Presence of stagnant water near the house 30m from the house	Stagnant water status selected from house survey number two collected	Number of stagnant water sources within 30 m of the child's bedroom.	Stagnant water bodies serve as aquatic habitats for mosquitoes.

	between May and June 2022		
House architecture			
Eave gap area (m^2) (max height * max width) (cm) of open eaves in child's bedroom	Mean open eave gap area (m^2) collected in three house surveys. The mean was used to account for potential modifications of eave gap areas between surveys for each house and to address any measurement errors that might occur during the surveys.	Open eave gaps area (m^2)	The main entry point for *An. gambiae* into a dwelling is through open eaves, which denote the gap between the top of the wall and the roof.
Window area (m^2) (length * width)	Mean window area (m^2) collected in three house surveys. The mean was used to account for potential modifications of window sizes between surveys for each house and to address any measurement errors that might occur during the surveys.	Window area (m^2)	Large window areas, if unscreened provide potential entry points for mosquitoes, thereby contributing to the overall porosity of the house with regard to mosquito entry.

Number of windows in child's bedroom	Median number window collected in three house surveys. The median was used to account for potential modifications of number of windows in the study between surveys.	Median number of windows present in the study child's bedroom.	Mosquito entry may increase in houses with unscreened windows, thereby contributing to the overall porosity of the house with regard to mosquito entry
Window covering status in child's bedroom	Window covering status gathered in survey number two.	The window covering status was documented, classifying it as curtains, completely open, partially open, or shutters	Completely open and partially open windows allow mosquitoes to enter and exit the child' bedroom
Area of door gap (cm^2) is measured either at the external door (in the case of houses with a single bedroom) or the internal door for houses with an inner sleeping bedroom, which is occupied by the study child.	Mean door gap area (cm^2) collected in three surveys.	The measurement pertains to the gap area above and below the child's bedroom door, with assessments conducted at either internal or external doors, contingent upon the structural characteristics of the house.	Gaps located at the top and bottom of doors can function as entry points for mosquitoes, thereby contributing to the overall porosity of the house with regard to mosquito entry.

Door condition include internal and external bedroom doors	Door covering status obtained during survey number two.	Presence of different door opening types in the study child's bedroom, including those with curtains, iron sheets, solid, and those with no doors.	An entrance to a child's bedroom that is not closed is likely to increase mosquito entry.
Area of holes in walls (cm^2)	Area (cm^2) of holes both small and large in the study child wall	The total area of holes in the study child's bedroom walls (cm^2).	Holes in the walls of the child's bedroom are likely serve as pathways for mosquitoes to enter the room.
Number of people sleeping in the child's bedroom	Median count of individuals (both children and adults) who occupied the study child sleeping place collected during each retrieval of the light trap.	Number of children (<=16 years) and number of adults =>16 years old in the study child's bedroom.	More people sleeping in the child's bedroom will increase the human biomass, increasing the quantity of attractive volatiles leaving the room and increasing mosquito entry (Kaindoa, 2019).
Number and type of sleeping places inside the study child sleeping place	Proportions of sleeping arrangements observed in the study of children's sleeping places.	Proportion numbers of different sleeping place type found within study child sleeping place, traditional bed, normal bed, mat e.t.c.	More and different sleeping place types increase the number of people within the study child room hence attracting more mosquitoes (Kaindoa, 2019).
Personal protection method			

A child sleeping under an ITN	Bed net use status gathered during each light trap setting and retrieval time was recorded.	In each house visit, prior to setting the light traps, and in the morning when retrieving the light traps, the bed net use status was recorded	Sleeping under ITNs is protective

collected over a seven-week cycle from September 2021 to October 2023 in 110 houses from 59 villages in rural Mtwara, south-eastern Tanzania. Indoor mosquito collections were performed using CDC-light traps within the sleeping areas of study children, conducted from 18.30h to 06.00h the following morning. Where a study child relocated or parents declined participation, the original house was substituted with another nearby traditional house.

The study used remotely sensed data to evaluate potential influences on indoor mosquito abundances. Landscape, climatic, and land use data were acquired from satellite imagery captured by the European Space Agency (ESA) Sentinel-2 satellite in October 2023, encompassing eight distinct land cover classes at a spatial resolution of 200 m (Potapov et al. 2022). In addition, data pertaining to the structural aspects of each study child's bedroom were collected. These data were linked to house characterization surveys conducted within the study child's bedroom during survey two, carried out between May 2022 and June 2022. House characteristic data were obtained through a structured questionnaire administered to households, augmented by observational data gathered during the setting and retrieval of light traps. The evaluated features in traditional housing structures encompassed architectural characteristics, environmental attributes, and seasonal variations, (distinguishing between rainy and dry months). Additionally, anthropogenic factors within the study house and child sleeping places were collected, including bednet usage, type of bednet, and the practice of keeping animals.

Recruitment of participants

Enrolment of study participants was described elsewhere (Mshamu et al. 2020). Briefly, traditional houses were selected if they had: 1) mud-walls, thatched roof and dirt floor, 2) without electric power supply from the grid, 3) no water supply, and 4) with at least two children under 13 years old willing to participate in the trial for three years (Mshamu et al. 2020). This study aimed to assess factors influencing the entry of malaria vectors into traditional houses by systematically examining house characteristics known or suspected to affect vector entry (Fig 5.3).

Participants who met the eligibility criteria were provided with informed consent forms and invited to participate in a randomization process in the form of a village lottery. The participants underwent two open lotteries to randomly select the participants, firstly, to select the owners of the new Star homes and, secondly, to select those in the control arm. There was a maximum of three control houses in each village (Mshamu et al. 2022).

Randomization and masking

Light trap collections were made in 16 randomly selected traditional houses surveyed over four consecutive days (from Monday to Thursday) each week. After six weeks, a total of 96 traditional houses (16 houses * 6 weeks) were sampled within 52 study villages located nearby the field laboratory in Mtwara town. The remaining cluster, consisting of seven study villages, included 14 houses located in the furthest villages from the field laboratory, which were sampled in the seventh week. Each day within the same week, four traditional houses were surveyed, ensuring the entire cluster was sampled within one week. The order in which clusters were visited was randomly assigned and remained consistent throughout the trial. The order of cluster visits was as follows: 1, 6, 7, 3, 2, 5, and 4 (source: https://www.random.org/sequences/). The order of visiting each sub-cluster was also randomly determined and maintained throughout the study. The 110 traditional houses included in this study were randomly selected from a list of 440 traditional houses with unique ID numbers ranging from 200 to 640. The house identification (ID) used in the clinical study was the same for linking the clinical case with the house characterization datasets in a specific house. If a study house had multiple children participating, randomization was employed to select one study child bedroom for the survey.

Where there were study children in multiple rooms within the same house, the first child was chosen randomly from all the study children residing in that particular house. This random selection was generated by Random Allocation Software computer program (https://mahmoodsaghaei.tripod.com/softwares/dnld /RA.zip). If the first child was not present in the designated room, the second child bedroom was selected for the observational data collection. If the study child's room was closed or the child had moved away from the house, the study child bedroom/sleeping spaces was characterized. These circumstances were documented in both field monitoring and data recording sheets.

Figure 5.3: Traditional house.

Mosquito collection and laboratory analysis

CDC light traps (incandescent light, Model 512, BioQuip product, California, USA) were placed inside the study child's sleeping area or bedroom (Yaro et al. 2021) which was operated by field assistants, each one assigned to two study houses. Each trap was hung 1m above the ground at the foot end of the bed covered by an ITN occupied by the study child. Traps operated from 19.00h to 07.00h the following morning. Each house was sampled every seven weeks, for 28 months.

In cases where the study child did not sleep under an ITN, the light traps were still operated, but it was noted that ITNs were not used. The same bedrooms were sampled for each collection. If a study child was not present in the room, a note was taken, and the light trap was suspended near the bed occupied by an adult. Each morning, the mosquitoes were killed and sorted by taxa, using the mosquito identification keys (Coetzee 2020), sex, blood-feeding status and gravidity.

Sub-samples of primary malaria vectors were subjected to species identification using multiplex Polymerase Chain Reactions (PCR) (using ribosomal DNA fragments) to distinguish between members of the *An. gambiae* complex (Scott et al. 1993) and the *An. funestus* group (Koekemoer et al. 2002). Enzyme-Linked Immunosorbent Assays (ELISA) techniques for the detection of the presence of circumsporozoite proteins in mosquito salivary glands (*Plasmodium* infections), as previously described (Durnez et al. 2011). The mosquito data, household ID and other relevant experimental design information were recorded.

Putative risk factors

Landscape and climate data

A digital elevation model (DEM) featuring a resolution of a surface area of 200 m was obtained from a free online website the United States Geological Survey (USGS) website via URL: http://earthexplorer.usgs.gov., served as the basis for extracting data related to elevation, slope, terrain, and aspect at the designated traditional house sampling locations (NASA , Mwangungulu et al. 2023). Land cover data, derived from imagery captured by the European Space Agency (ESA) Sentinel-2 satellite in October 2023, encompassed eight distinct land cover classes with a spatial resolution of 200 m, demonstrating an overall accuracy of 75% (ESRI 2020). The ESA imagery facilitated comprehensive analyses of both land-cover and land-use characteristics, including features such as forested areas, bare lands, and small-scale landscape attributes (Brown et al. 2022). Additionally, temperature (°C) and precipitation (mm) data were extracted from climate coverages within a 200 m radius of each traditional house included in this study, and compared with indoor malaria vector abundance. Lastly, the distance from each sampled house to the nearest feature of each land-cover class was measured and linked with malaria vector abundance (Table 5.1).

Peri-domestic and household data

Data from a cross-sectional survey conducted in May-June 2022, at the transition from the rainy to the dry seasons, was utilized. This dataset, derived from traditional houses, was integrated into the model, establishing a link with indoor mosquito abundances.

The administration of an electronic open-structured questionnaire, programmed using the open-source KOBO toolkit, took place before light trap installation, between 16.00h and 17.30h. Android personal digital system assistants were employed for questionnaire administration, with responses recorded on electronic tablets. The collected house characterization features were categorized into two primary sections. The initial section focused on the general description of the entire house, specifically addressing the type of walls and roof. The second section concentrated on the sleeping area of the study child, assessing pertinent risk factors within that space. Assessed risk factors during the visit included: 1) the number of individuals sleeping in the same area as the study child, 2) the use of ITNs and the type of ITNs used, 3) the construction of the child's sleeping space (roof, wall, and floor), and the status of the eaves (whether open or closed), 4) the size of holes within the study child's room, 5) the distance from the study child's room to the water source, and 6) the number and types of larger domestic animals (cattle, goats, pigs) tethered within 30 m of the study houses. The sleeping locations were geolocated using a handheld global positioning system (GARMIN eTrex) during the eligibility survey conducted before the large trial commenced.

Additional observational data were gathered before trap setting and retrieval periods at the designated study house. This survey was conducted each morning before 07.00 h during the retrieval of light traps and identified the number of children and adults who slept in the trapping room and the type and quantity of bed nets used the preceding night. These data were subsequently integrated with the house characteristic survey (Table 5.1).

A newly variable, house porosity, consolidates all openings identified within the sleeping areas of study children in traditional houses, including the presence of holes in the wall, open eave gaps, door gaps area above and below, and window opening areas.

Hotspots analysis for the indoor malaria vectors densities

A hotspot analysis for high mosquito abundance was conducted to identify areas with varying concentrations of malaria vectors in the study region. Indoor malaria vector abundances collected from 110 traditional houses across 59 study villages over a period of two years were included in the hotspot analysis. Data from a Microsoft Office Excel sheet, encompassing the total number of indoor malaria vector catches for each location, was imported into ArcGIS for analysis. Initially, spatial autocorrelation (Moran's I) was employed to assess the statistical significance of data values, determining their spatial distribution whether random, clustered, or exhibiting a regular pattern, irrespective of the seasons (Bousema et al. 2010, Noé et al. 2018, Tewara et al. 2018), (Fig 5.5). Subsequently, a count of events within a 200 m radius was conducted using utility tools in the spatial statistical package. This count, termed icount, was utilized for hotspot analysis, distinguishing between areas with heightened and reduced vector concentrations (Bousema et al. 2010, Noé et al. 2018). A rasterized hotspot analysis followed, utilizing the spatial analyst package and (Inverse Distance Weighted (IDW) interpolation tools with hotspot and cold spot as input features and GiZscore for the z-value field (Bousema et al. 2010, Noé et al. 2018). To enhance interpretability, the resulting rasterized map was reclassified into five classes, categorizing malaria vector levels as very high, high, moderate, low, and very low, respectively.

Data management and statistical analysis

Data were gathered using KOBO-collect software on Samsung Galaxy Tab A7 Lite, model SM-T220, made in Vietnam, incorporating drop-down boxes and consistency checks to mitigate data entry errors. Subsequently, the data underwent retrieval from the tablets and cleaning through the utilization of the R statistical program version 4.2.1 (R Core Team 2019) before the analysis. The principal metric of interest is the count of indoor malaria vectors captured per night in each study child sleeping place.

The generation of hotspot maps for malaria vectors (combined *An. gambiae* s.l and An. *funestus* s.l.) was done using the ArcGIS system Geographical Information System version 10 *(License (EFL96036612 ArcGIS for Desktop Advanced)* (Scott & Janikas 2009). Hotspot classes were defined based on actual counts of malaria vectors obtained during routine indoor trapping in specific areas. This analysis aimed to identify locations with concentrated malaria vectors for potential future interventions, utilizing spatial autocorrelation statistical techniques. Five hotspot classes were identified starting from very low, low, medium, high, and very high categories. These analyses were crucial for elucidating localized relationships with indoor malaria vector abundance, (Fig 5.5). To visually represent predictions as continuous surfaces and highlight mosquito abundance hotspots, ordinary Kriging estimation was employed (Isaaks & Srivastava 1989), utilizing study house.

Landscape and climatic features within a 200 m buffer around the study houses, derived from satellite image data, were analysed to assess their influence on indoor malaria vector abundances using a generalized linear model (GLM) with a negative binomial distribution to address overdispersion. Landscape data encompassed proportions of areas covered by trees, crops, water bodies, altitude (m), bare lands, and grasslands. Climatic data, including rainfall (mm), temperature (°C), and relative humidity, were also summarized into means (95% CI) per house. All landscape and climatic data proportions were summarized into means (95% CI) per house before model analysis.

House characteristics survey data categorized, with numeric variables summarized using means (95% CI) or medians (interquartile ranges (IQR)), while categorical variables obtained during survey two (May-June 2022) were included. Field monitoring data collected during trap set and retrieval were initially linked with house characteristic data before being merged with spatial covariates, climatic data, and mosquito counts. Subsequently, after merging the house characteristic data, field monitoring data, spatial covariates, mosquito counts, and climatic data, GLM models with a negative binomial distribution were employed for analysis. Potential house characteristic features influencing indoor malaria vectors abundance, (combined *An. gambiae* s.l and *An. funestus* s.l.) were selected a priori based on their relevance to malaria vector entry. These features included roof and wall types, eave gap, window and door statuses, bed net use and type, number of study children and adults in the trapping room, presence of domesticated animals within 15 m of the house, and potential anopheline breeding habitats within 15 m of the study houses.

Initially, a comprehensive model incorporating all factors was estimated using backward stepwise selection, employing likelihood ratio tests to identify significant variables for inclusion in the final model. Model fitness was assessed by visually examining residual versus fitted plots to confirm homogeneity (Zuur et al., 2010). Subsequently, a multivariate model was applied to all biologically and mathematically significant variables identified after conducting backward model selection. Variables from the multivariate analysis underwent univariate analysis, including every risk factor regardless of significance in the multivariate model. Interactions among a subset of variables, considered biologically relevant for malaria vector house entry, were evaluated. The model was adjusted for the random factors such as village names, house IDs and rounds of data collections. Lastly, the model estimated means of the final selected models were exponentiated to obtain Relative risk ratios and means, along with their 95% confidence intervals, were calculated using R statistical program version 4.2.1, utilizing packages such as lme4, vsreg, MASS, and glmm (R Core Team 2019).

Results

Characteristics of study houses

A description of measurements made for study houses is shown in Table 5.2.

Entomology

A total of 7,314 mosquitoes were collected during 1,430 trapping nights of which 80% (5,857/7,314) were *Culex spp*, 14% (1,149/7,314) *An. gambiae* complex and 4% (308/7,314) *An. funestus* group.

Of the 76% (873/1,149) *An. gambiae* complex identified to species, 60% (528/873) were *An. gambiae ss*, 32% (276/873) *An. arabiensis*, 7% (63/873) *An. merus* and 2% (58/931) were unknown. For the *An. funestus* group, out of 197 mosquitoes, 98% (193/197) were *An. funestus s.s.*, 0.8% (2/197) *An. rivulorum*, 0.3% (1/197) *An. parensis*, 0.3% (1/197) *An. vanedeen* and 8.2% (4/201) were non-amplified. Malaria vector abundance peaked in rainy seasons (January to May).

Overall, an adjusted mean number of *An. gambiae* complex was 0.14 per house per night, and for *An. funestus* group were 0.0003 per mosquito per house per night. A total of 13 circumsporozoites positive malaria vectors were caught, nine were *An. gambiae* complex and four were from *An. funestus* group which account for the overall sporozoites rates of 1% (9/867) and 2% (4/193), respectively.

Table 5. 2: Characteristics of study children's sleeping spaces.Where a = mean (for continues variables (95% CI), b = n (%), c = median (for discrete data (inter-quartile range).

Characteristic	Status	Measure
Potential landscape characteristics		
Proportion of land covered with water within 200 m radius		0.02 (0 – 0.64 m^2) [a]
Proportion of land covered with vegetation within 200 m radius		0.32 (0.1 – 0.90 m^2) [a]
Proportion of land covered with flooded and vegetation within 200 m radius		0.01 (0 – 0.24 m^2) [a]
Proportion of land covered with crops within 200 m radius		0.01 (0 – 0.1 m^2) [a]
Proportion of land covered by trees within 200m radius		0.55 (0 – 0.93 m^2) [a]
Proportion of land represented as bare land within 200m radius		0.02 (0 – 0.12 m^2) [a]
Proportion of land covered with buildings at 200m radius		0.1 (0 – 0.37 m^2) [a]
Peri-domestic characteristics		
Mean number of chickens within 15 m of the child's sleeping space		2.4 (1.7 – 3.6)[a]
Toilet within 15m of child sleeping places	Present	166 (92%) [b]
	Absent	15 (8%) [b]
Open water bodies within 15m of the child's sleeping space	Present	11 (6%)[b]
	Absent	170 (94%)[b]
Construction of child's sleeping space		
Roof material	Thatched	139 (77%)[b]
	Metal roof	42 (23%)[b]
Wall material	Mud & stick	180 (99%)[b]

	Brick	1 (1%)[b]
Average eave gap area (m^2)		0.72 (0.1 – 2.4 m^2) [a]
Bedroom window	Present	77 (43%) [b]
	Absent	104 (57%) [b]
Average window area (m^2)		0.3 (0 – 1.5 m^2) [a]
Window cover	Open	14 (18%) [b]
	Partially open	38 (49%) [b]
	Curtain	18 (23%) [b]
External door on child's bedroom	Absent	156 (86%) [b]
	Present	25 (14%) [b]
Internal door on child's bedroom	Absent	25 (14%) [b]
	Present	156 (86%) [b]
Internal door cover status	Open doorway	37 (24%) [b]
	Curtains	112 (72%) [b]
	Solid door	6 (4%) [b]
Bed type	Normal bed	110 (61%) [b]
	Traditional bed (Dogi dogi)	26 (14%) [b]
	Mattress & Mat	20 (17%) [b]
House porosity (m^2)		1.2 (0.3 – 4.5 m^2) [a]

Hotspots for indoor malaria vectors

The global Moran's I value, denoting spatial autocorrelation, was positively observed for malaria vectors, registering a Moran's index of 0.126 (p < 0.001). The significantly elevated Z-score (24.31) and lower P-value indicate the existence of a hotspot for both *An. gambiae* s.l. and *An. funestus* s.l. within the study area (Figure 5.4).

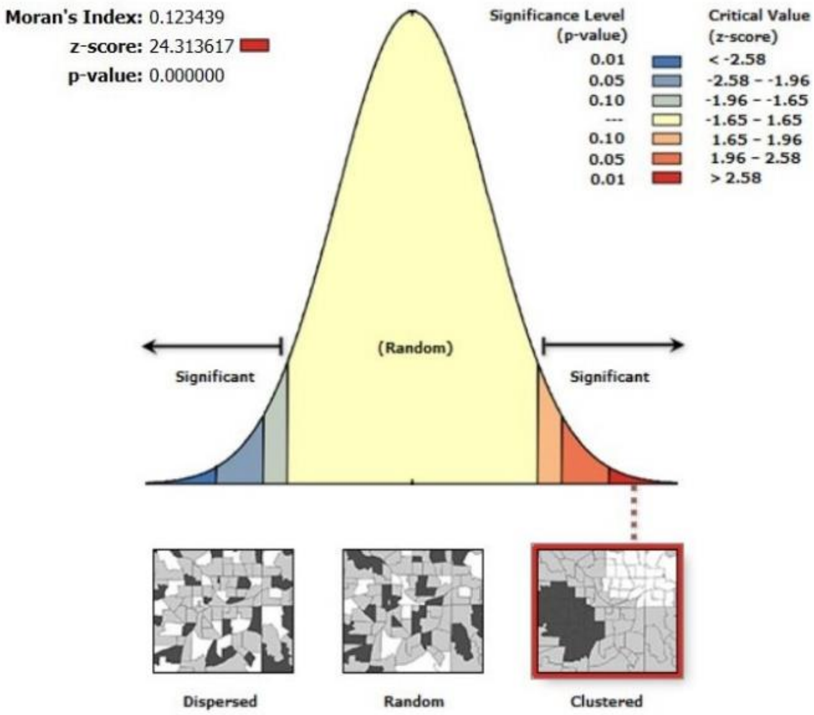

Figure 5.4: Spatial autocorrelation pattern for the total number of indoor malaria vector catches at each location in the study area, (Geremew et al. 2021).

In contrast, malaria vector cold spots (99% and 95% confidence) were predominantly located in the south of the study area. Conversely, statistically significant malaria vector hotspots, with confidence levels of 99% and 95%, were primarily identified in the central and eastern parts of the district, as depicted in geolocations and predicted mosquito counts (Fig. 5.5).

The hotspot villages were characterized by direct observations of the physical attributes in areas delineated on the malaria vectors hotspot map, systematically organized into distinct levels as indicators for the prevalence of malaria vectors in specified locations. The environmental characteristics of the high, medium and low risk areas are described in table 5.3.

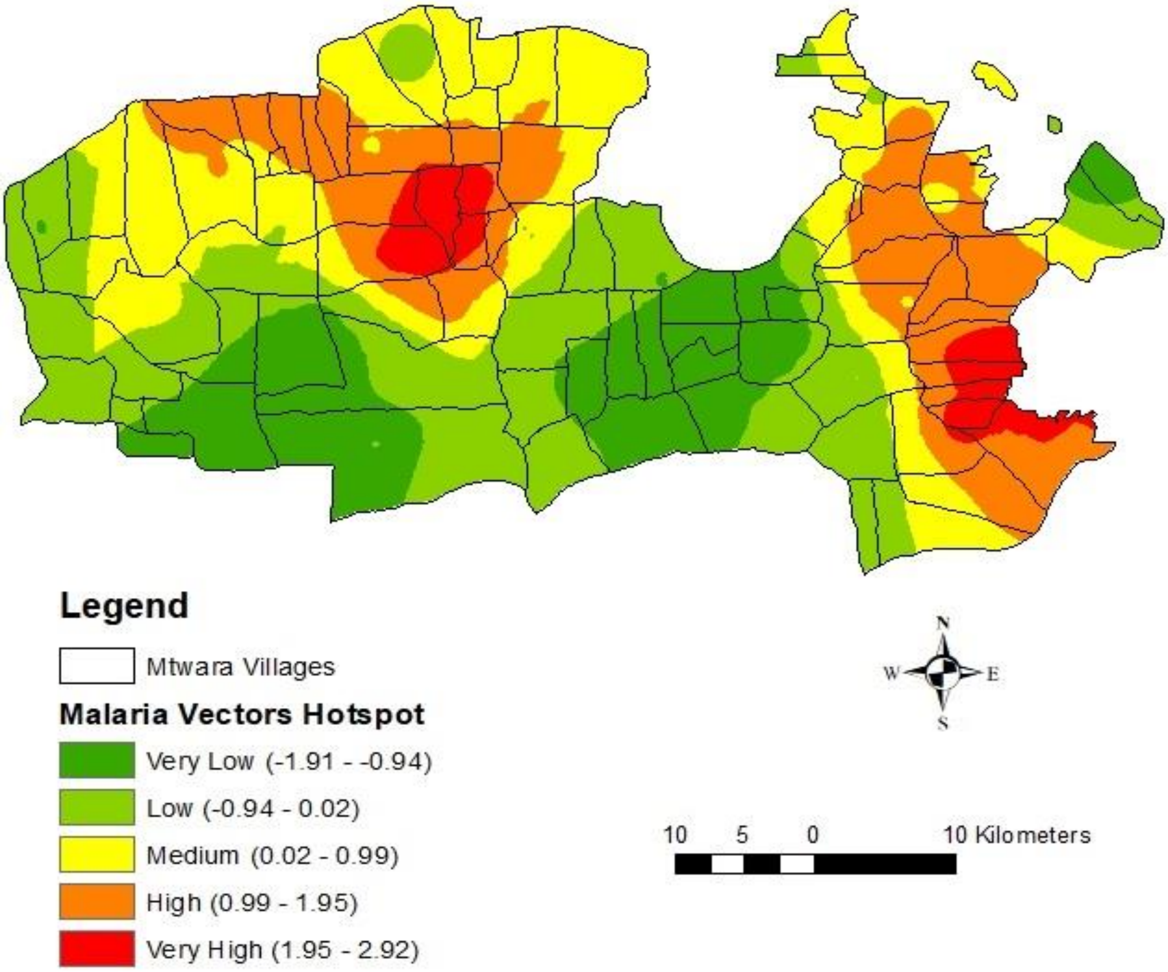

Figure 5.5: Global Moran's Index values (GMI) showing indoor malaria vector (An. gambiae s.l. and An. funestus s.l.) hotspots in rural Mtwara district. Indoor mosquito data were collected from 110 traditional houses across 59 study villages over a two-year period.

Table 5. 3: Physical characteristics of indoor malaria vector hotspots

Hotspot category	Characteristics
Very high and higher hotspots villages. With Global Moran's Index (GMI) values; *GMI (0.99 – 2.92)*	Villages with very high and high hotspots are located in the central part of the study area, marked by low altitude (ranging from 30m to 120m), the practice of irrigation rice farming, and a high concentration of houses nearby. In contrast, the eastern (coastal) villages are characterized by much lower altitudes (30m to 60m),

	featuring marshlands and a prevalence of irrigation farming.
Medium hotspots *GMI (0.02 – 0.99)*	Villages identified as medium hotspots are marked by altitudes ranging from 60m to 180m, featuring sparse stagnant water pools and sparse distribution of houses is notable.
Very low and low hotspots villages *GMI (-1.91 – 0.02)*	These villages are located in highland areas with hills, characterized by altitudes ranging from 200m to 300m. The residential houses are situated in very remote areas and are involved in low-scale cultivation of cassava, sesame, and maize, primarily dependent on rainfall.

Risk factors

Peri-domestic characteristic

Chickens were the most prevalent domesticated bird or animal found close to the houses, followed by cattle with the median =2.4 (IQR= 0.7 – 13) chickens which revealed an association with a reduction in indoor malaria vectors (RR= 0.92, 95% CI 0.87 – 0.97, p<0.01) as their numbers increased. Conversely, there was a minimal number of open water source which acted as a potential breeding habitat for anopheline close to the of the study house was also associated with an increase in indoor malaria vector abundances (RR=1.61, 95% CI 1.04 – 2.50, p<0.05), (Table 5.3).

Bedroom characteristics

The mean size of a study child's bedroom was 2.4 m wide (95% CI = 0.1 – 4.6), shortest side) by 2.8m long (95% CI = 1.2 – 20), longest side) and 2.2 m (95% CI = 1.1 – 4.4, high. Most houses had a thatched roof (77%, 139/181), mud walls (99%, 180/181), no windows 57% (104/181) and one internal door (86%, (156/181). Of the bedrooms with windows, approximately 96% had one window (74/77), 4% had two windows (5/77). Some 48% of these windows were partially open (38/77), with 18% completely open (14/77) and 23% (18/77) covered by the curtains. The average surface area of the window at the study child sleeping place was (0.3 m^2, 95% CI 0-1.5). About 72% of the internal doors at the child sleeping places (11/156) were covered by the curtains and 24% (37/156) were completely open and only 4% (6/156) were solid doors. About 85% of the study child sleeping place do have open eave gap area (154/181), with 62% (95/154) with all four-sided open eave gaps area and 34% (52/154) with three-sided open eave gaps area. The average surface area open eave gap in each study child sleeping place was (0.72 m^2, 95% CI 0.1 – 2.4).

A median of 2 people (Interquartile range, IQR 2-3) in 2 beds slept in each room (IQR = 1 – 2.2). Most bedrooms had two or three sleeping spaces, with most sleeping with other children without an adult in the room (64%, 115/181), and about 17% (31/181) adult slept with younger children in the room. Normal bed was the mostly preferred type of sleep in the room with 61% (110/181) followed by 14% (26/181) of traditional bed (Dogi dogi) and about 11% (20/181) slept on the mats. Approximately, 93% of the children were observed utilizing bed nets (169/181) during the night of the survey, with Olyset net type the most common net 75% (127/169) followed by Parmanet 23% (39/169) (Table 5.2).

Statistical modelling

In the final multivariate model, a number of risk factors were associated with increasing mosquito abundance including: 1) open water sources such as open-water wells, swamp spring which are potential breeding habitats for anophelines, close to study houses (RR=1.61, 95% CI 1.04 – 2.50, p<0.05) and 2) presence of unscreened windows at the study child sleeping rooms (RR=2.47, 95% CI 0.73 – 8.39, p<.05) (Table 5.4). Whilst the following risk factors were associated with a decline in mosquito abundance: 1) an increase in built-up areas (RR=0.01, 95% CI 0.001 – 0.15, p<0.001) increasing altitude (RR=0.71, 95% CI 0.55 – 0.90, p<0.05) and 2) the number of chickens (RR= 0.92, 95% CI 0.87 – 0.97, p<0.01).

Table 5. 4: Risk factors for primary malaria vectors (combined An. gambiae s.l and An. funestus s.l.) abundance in the study children's sleeping place. RR=Rate ratio and its 98% Confidence intervals, p=significance level

Variable	Total house visits	Mean mosquito abundance/trap/ night (95% CI)	Univariate analysis		Multivariate analysis	
			RR (95% CI)	**p**	**RR (95% CI)**	**p**
Remote sensing factors						
Proportion of land covered with water (m^2) within 200m radius	12	0.53 (0.07 – 4.38)	0.27 (0.03 – 2.49)	>0.05	0.11 (0.01 – 1.31)	>0.05
Proportion of land covered with man-made buildings (m^2) within 200m radius	12	0.59 (0.16 – 2.14)	0.01 (0.0 – 0.08)	<0.001	0.01 (0.001 – 0.15)	<0.001
Altitude in (100m) within 200m radius	12	1.2 (0.1 – 2.9)	0.62 (0.51 – 0.76)	<0.001	0.71 (0.56 – 0.90)	<0.05
Slope (m) within 200m radius	12	1.09 (1.0 – 1.13)	1.07 (0.99 – 1.16)	>0.05	1.02 (0.93 – 1.13)	>0.05
Peri-domestic factors						
Open water within 15m of the house						
Absence	3	1.18 (1.03 – 1.35)	1		1	
Presence	3	2.33 (1.59 – 3.44)	1.98 (1.31 – 2.99)	<0.001	1.61 (1.04 – 2.50)	<0.05
Number of chickens within 15m of the house	3	0.95 (0.90 – 0.99)	0.90 (0.85 – 0.95)	<0.001	0.92 (0.87 – 0.97)	<0.05

Seasons						
Dry seasons (June – November)	3	1.12 (0.94 – 1.33)	1		1	
Rainy seasons (December – May)	3	1.38 (1.06 – 1.81)	1.24 (0.90 – 1.70)	=0.192	1.20 (0.87 – 1.67)	>0.05
Study child sleeping place factors						
Type of bedroom entrance						
External door	3	0.74 (0.47 – 1.17)	1		1	
Internal door	3	1.33 (1.16 – 1.52)	1.80 (1.12 – 2.89)	=0.016	1.42 (0.83 – 2.44)	>0.05
Number of people sleeping in the child's sleeping place	3	1.07 (1.01 – 1.14)	1.05 (0.89 – 1.24)	=0.592	1.13 (0.95 – 1.34)	>0.05
Window in study child's sleeping place						
Absent	3	1.4 (0.7 – 2.6)	1		1	
Present	3	1 (0.9 – 1.2)	0.73 (0.37 – 1.47)	= 0.39	2.47 (0.73 – 8.39)	<0.05
House porosity (m^2)	3	1 (1 – 1)	1 (1 – 1)	=0.065	1 (1 – 1)	>0.05

(House porosity= (all open areas (m^2) such as opening eave gap areas, window, door gaps, and holes in the walls found at the child's sleeping place)

Discussion

The present study identified potential risk factors linked to indoor malaria vector abundance in traditional rural dwellings in southeastern Tanzania. Hotspots of indoor malaria vector abundance were pinpointed in specific central and eastern parts of the study area, characterized by distinct landscape and land use features. These hotspots commonly consist of lowland areas situated between <30 m to 120 m above sea level, susceptible to water pooling from highland runoff, notably during rainy periods. These lowland study villages are characterized by diverse water features including seasonal swamps, river streams, and irrigated rice fields, which offer conducive habitats for the aquatic phases of malaria vectors. Communities engage in irrigation agriculture, utilizing these water bodies for cultivating rice and bananas, thus serving as potential breeding sites for malaria vectors (Ijumba & Lindsay, 2001). These water bodies provide breeding habitats for malaria vectors, with larvae and eggs thriving in stagnant water at low altitudes (Bødker et al. 2003). The accumulation of larvae and eggs in slow-moving water at low altitudes leads to increased malaria vector densities, facilitating their entry into houses (Atieli et al. 2011, Mwangungulu et al. 2023).

The study findings revealed an inverse relationship between altitude and indoor malaria vector abundance. Specifically, an average increase of 100 m in altitude reduced indoor malaria vector abundance by 29%. This reduction is attributed to the steep slopes of highland landscapes, which prevent water accumulation and reduce mosquito breeding sites, consequently lowering mosquito density. Highland areas feature permanent breeding habitats, such as boreholes and springs, preferred by *An. funestus* mosquitoes (Nambunga et al., 2020). Conversely, lower altitude regions harbour numerous breeding sites, such as marshlands and floodplains, especially during rainy seasons, favoured by *An. arabiensis* mosquitoes. However, the impact of altitude on indoor malaria vector abundance may be influenced by lower *An. funestus* densities in the study area, potentially diminishing altitude's effect in highland areas despite the presence of permanent breeding habitats (Nambunga et al., 2020). Additionally, *An. arabiensis*, known for its broad range of aquatic habitats, including human-made and natural locations like rock pools and marshlands, is commonly found in irrigated agricultural zones at lower altitudes (Gouagna et al., 2011). Our findings show an increase in malaria vector densities during the rainy seasons. Interestingly, the densities of these vectors peaked at the onset of the rainy season (December-January) and during the transition between the end of the rainy season and the beginning of the dry season (May-June), when rainfall is low.

The study also uncovered a negative correlation between the increase in built-up environments, mainly comprising human settlements and roads, and reduced indoor malaria vector abundance. However, in different settings, the built-up environment positively influenced malaria vector densities by creating potential human-made aquatic habitats, including broken pipes, roadside ditches, potholes, and unpaved roads and paths within and around house compounds, which can support anopheline mosquito aquatic stages (Keating et al. 2003).

The proximity of open water bodies within 15 m of the house demonstrated a positive correlation with indoor malaria vector abundance. These water bodies, including water wells and irrigated agricultural fields in the study area, served as potential breeding habitats for malaria vectors, leading to increased mosquito density in the environment and subsequently inside houses (Ijumba & Lindsay 2001), (App.6: Fig.5.5c). Conversely, keeping chickens in peri-domestic areas was found to reduce indoor malaria vector abundances. Chickens in peri-domestic areas act as alternative hosts, diverting opportunistic feeders like *An. arabiensis* away from humans, particularly in areas where bed nets are widely used (Mwangangi et al. 2013). Additionally, these findings align with another study suggesting that chicken volatiles repel mosquitoes in peri-domestic areas, potentially reducing indoor mosquito entry into houses (Jaleta et al. 2016), (App.6:Fig.5.5d).

The study revealed a positive correlation between the presence of unscreened windows in the bedrooms of the study children and indoor mosquito abundances. Given that the houses selected for this study belonged to impoverished families with multiple openings such as wall holes, poorly fitting doors, and open eave gaps, the presence of windows was found to contribute to increased indoor mosquito abundances, (App.6: Fig.5.5e).

This study encountered several limitations. Firstly, integrating field environmental data with remote sensing land cover data posed challenges due to the resolution limitations of the land cover data, which failed to capture fine details such as isolated residences and small water bodies, affecting habitat suitability mapping. This could be addressed by utilizing unmanned aerial vehicle (UAV) imagery to capture finer details in specific areas. Secondly, the model selection process may have overlooked important factors such as ecology, human behaviours, landscape characteristics, and seasonal variations, which are critical determinants of indoor malaria vector abundances. Thirdly, the spatial statistical model used does not directly assess the relationship between all potential risk factors (including environmental, anthropological, and peri-domestic factors) and risk of malaria transmission. Thirdly, co-linearity may exist between altitude and some environmental variables. In future analyses, this possibility will be explored by removing potentially correlated variables from the model.

Further research, including field studies and comprehensive data collection on malaria cases and transmission risks, is necessary to strengthen the evidence and establish a direct link between putative risk factors and malaria transmission hazards. Identified hotspot areas for indoor malaria vector abundance revealed two localized regions with significantly higher indoor malaria vector counts. These hotspots should be further investigated in correlation with actual malaria prevalence in those specific regions. Moreover, further studies are needed to quantify how outdoor behaviour may affect malaria transmission. For example. whether people are cooking or resting outdoors, the relative number of people outdoors compared to the number indoors and the level of activity of people outdoors may all influence the number and movement of mosquitoes.

Consequently, the current environmental variables associated with indoor malaria vector abundances provide a foundation for assessing control progress and identifying geographic areas requiring prioritization.

Conclusions

The study identified two malaria vector hotspots, one centrally located and the other in the east, both at low elevations prone to water accumulation. Proximity of open water bodies within 15 m of the house and unscreened windows in children's sleeping rooms correlated with increased indoor malaria vector abundance. Conversely, increased built-up areas, higher altitude, and presence of chickens at 15 m from the house were associated with reduced indoor malaria vector abundances.

Based on these findings, we recommend draining or larviciding hotspot areas and implementing housing interventions such as screening windows, doors, and eave gaps as supplementary measures alongside insecticide-treated net use

Chapter 6 : Discussion

Overview and summary of findings

The primary aim of this thesis was to produce high-quality evidence to support improved housing as an intervention against malaria and diarrhoea diseases.

This study differs from previous ones by evaluating entire houses featuring multiple interventions such as window screening, self-closing solid doors, raised sleeping bedrooms, closed eave gap areas, domestic fly-proof kitchens, and toilets, rather than assessing these interventions individually. Additionally, it simultaneously assesses the impact of housing on multiple communicable diseases of children, including malaria, diarrhoea infections, and respiratory tract infections, instead of studying each disease separately. The main focus was on evaluating the effectiveness of a newly designed healthy house, known as a Star home, in reducing indoor densities of mosquitoes and domestic flies. Indoor mosquito and fly measurements served as indicators for assessing the risk of malaria and diarrhoeal diseases among children aged 13 years and younger living in rural Tanzanian villages with inadequate housing. Comparisons were made in entomological indicators between Star homes and traditional houses to determine the protective efficacy of the novel healthy home.

Chapter 1 provided a concise overview of the relationship between housing and communicable diseases, with a focus on malaria, diarrhoeal illnesses, and acute respiratory tract infections (ARI), affecting children in rural areas of the tropics. It summarizes various studies utilizing housing interventions to prevent these diseases, primarily in sub-Saharan Africa.

Chapter 2 summarised the pilot experiments conducted in a controlled semi-field system in Ifakara, Tanzania, to investigate the impact of light and ventilation on indoor malaria vector abundance (Mmbando et al. 2022). This pilot study was aimed at informing the type of mosquito trapping method to use indoors in our household randomised controlled field trial in rural Mtwara district, Tanzania. Transparent-walled huts, allowing light from a CDC light trap placed indoors, caught 84% more *Anopheles arabiensis* mosquitoes compared to opaque-walled huts, where the light could not be seen. It was also shown that well-ventilated huts resulted in a 99% reduction in indoor densities of *An. arabiensis*, emphasizing the critical role of ventilation in preventing mosquito entry, probably by reducing the concentration of carbon dioxide, a major mosquito attractant, emanating from a building.

The study findings suggest that light traps tend to overestimate indoor mosquito captures in Star homes because their construction features impermeable walls, allowing light to be visible from outside. This phenomenon attracts more mosquitoes, while the easy dissipation of CO_2 from inside to outside the house reduces indoor mosquito entry compared to traditional houses with impermeable walls. Having understood the likely interactions between CDC light traps and the design of the Star homes superstructure, a decision was made to use this trapping method since it was reproducible, and its catching efficiency did not depend on field staff unlike other techniques used for collecting indoor mosquitoes such as aspirator collections, pyrethrum spray collections and human-landing catches.

Chapter 3: This chapter provides an overview of the findings from large-scale randomized household field trials conducted in rural Mtwara district, Tanzania, from September 2021 to October 2023. The trial aimed to assess the efficacy of Star homes in reducing indoor mosquito abundance and malaria transmission risks compared to traditional African-style houses.

Compared to the traditional houses, Star homes resulted in a reduction in indoor mosquito abundance: 54% for *Anopheles gambiae s.l*., 81% for *Anopheles funestus s.l*., and 64% for *Culex species*. This level of reduction is important since approximately 80% of malaria transmission occurs indoors (Huho et al. 2013, Sherrard-Smith et al. 2019).

The protective efficacy of Star homes likely stems from a combination of design factors. Firstly, in Star homes, bedrooms are located on the second story, elevated 3 m above the ground, in contrast to traditional houses, which are typically situated on the ground floor. Prior research suggests that elevating sleeping quarters by 3 m can decrease indoor mosquito densities by up to 84% if the ground floor remains open

(Charlwood et al. 2003, Carrasco-Tenezaca et al. 2021). Even when the area beneath the house is enclosed with walls, the protective effect persists but diminishes to 77% (Carrasco-Tenezaca 2023). Secondly, the ventilation effect, particularly notable in Star homes with shade-net walls facilitating efficient carbon dioxide dissipation (Knudsen et al. 2020, Jatta et al. 2021), differs from the mud and stick walls of traditional houses. While indoor CO_2 concentrations did not differ between the Star homes and traditional houses, Star homes were slightly cooler at night. Ventilation in Star home bedrooms may have been compromised by residents covering the walls with cloth for privacy, whereas traditional houses with open eaves and windows would naturally be well-ventilated, as suggested by experimental studies in The Gambia (Knudsen et al. 2020). On the other hand, the metal roofs in Star homes, with low thermal mass, dissipate heat more rapidly than thatch roofs with higher thermal mass, contributing to night-time cooling of Star home bedrooms. Thirdly, the full-screened shade net walls of Star homes act as a physical barrier against mosquito intrusion (von Seidlein et al. 2017), unlike the mud and stick walls of traditional houses which have many entry points. These shade-net walls cover all openings, including eave gaps and windows, important entry points for malaria vectors, as indicated by prior studies examining window and door screening (Massebo & Lindtjørn 2013), and eave gap closure (Njie 2010). In contrast, traditional houses with open eave gaps, partially or fully open windows and doors, and wall perforations facilitate malaria vector entry. Fourthly, the installation of well-fitted self-closing doors in Star homes, including on the main outdoor wall and indoor stairway entrances to the sleeping quarters, resulted in shorter door opening times compared to traditional houses. These findings are consistent with previous studies focusing on proper door fittings and door screening as strategies to reduce malaria vector entry into homes (Lindsay et al. 2021).

There was a lower proportion of *An. gambiae s.s.,* the most efficient vector of malaria in sub-Saharan Africa, than in Star homes. The higher proportion of *An. gambiae s.s*. in Star homes, probably represent their greater inclination for indoor biting and resting, compared with *An. arabiensis*, known for its opportunistic feeding habits on both indoor and outdoor hosts (Tirados et al. 2006). In other words, *An. arabiensis* is more easily deterred from entering Star homes than *An. gambiae* s.s.

Ninety-nine per cent of all *Anopheles funestus* groups mosquitoes captured in both house types were identified as *An. funestus s.s*., a species recognized for its inclination towards human blood meals and indoor resting (Lwetoijera et al. 2014). Although an 84% reduction in indoor biting of *An. funestus s.l.* was observed in Star homes compared to traditional houses, no observable difference in the relative proportion of sibling species was shown between the two-house types.

The findings of this study indicate that constructing houses with design features such as screened window, eave gaps areas and self-closing solid doors can effectively reduce indoor mosquito entry. Star homes reduced the risk of malaria transmission by 55% compared to traditional houses. Furthermore, the 64% reduction in indoor *Culex* species populations attributable to Star homes is important, given their role as vectors of nuisance biting and the transmission of mosquito-borne diseases such as filariasis, Rift Valley fever and West Nile Virus. Employing locally available well screened housing, constructed from low thermal mass materials, promoting community education on the importance of closing doors during the early evening hours, repairing screen windows and doors, and utilizing ITNs are crucial strategies for enhancing malaria control in endemic regions.

Chapter 4 describes the effectiveness of Star homes in reducing domestic fly species, mechanical vectors of diarrhoeal pathogens in children, in kitchens and toilets compared to traditional houses. Baited-fly traps were used to sample flies in both the kitchen and toilet areas of both house types (Lindsay et al. 2012). The predominant species comprised 75% *Chrysomya putoria* (African latrine fly), 17% *Musca domestica* (house fly), and 8% *Sarcophaga* species (flesh fly). *Chrysomya putoria* and *M. domestica* are vectors of diarrhoeal pathogens such as enteropathogenic *Escherichia coli*, *Klebsiella species*, *Shigella species*, and *Rotaviruses*. *Sarcophaga* species, known for feeding on animal flesh, are linked with myiasis (Azarmi et al. 2024).

Star homes had 40% fewer domestic flies (vector of diarrhoea illnesses) in kitchens compared to traditional houses. This decline in fly abundance in Star home kitchens can be attributed to several design factors. Firstly, the presence of 60% shade-net walling in the kitchens of Star homes served as a physical barrier against domestic fly entry, unlike the perforated walls commonly found in traditional house kitchens. This finding aligns with previous studies that emphasized the efficacy of door (double doorways) and window screening as interventions to prevent domestic fly entry into households (Moon 2019). Secondly, the well-fitted self-closing main doors of Star homes also acted as a barrier against domestic fly entry into the kitchen, unlike the poorly fitting and completely open doorways typically found in traditional house kitchens. The shorter duration of main doorway openings in Star homes significantly deterred fly entry compared to traditional houses, as also seen in the previous study which applied the positive-pressured doors to reduce the domestic flies entry (Moon 2019). Thirdly, the smoothed cemented floors in Star home kitchens facilitated easy cleaning and prevented food spillage, thereby minimizing the attraction of flies. This contrasts with the earth floors commonly found in traditional house kitchens, which are less easy to clean and may attract flies due to food residue.

Star home toilets had 40% fewer *C. putoria* than traditional toilets. The main reason for this reduction was due to the flap under the main hole preventing flies from entering the pit to lay eggs. *C. putoria*, preferentially breeds in human faecal materials (Lindsay et al. 2012) and can produce prodigious numbers of flies leaving the pit (Emerson et al. 2005). In marked contrast, traditional house toilets are open allowing easy access for flies. This conclusion is supported by pilot studies that showed that no flies exited Star homes' main holes compared to over 200 flies from traditional house latrines. Cemented flooring, and enclosed sewage systems in Star home toilets may also have contributed to fewer flies, particularly *C. putoria* as it prevents the direct contact *C. putoria* to have a direct contact with human faeces which allows them to feed and breed.

The protective effect of Star homes against the principal mechanical vector, *C. putoria*, in both kitchens and toilets is important, given its ability to transmit various diarrhoeal pathogens. These findings highlight the need for kitchen and toilet modifications to prevent fly entry, alongside maintaining hygienic practices and access to safe water. Community sensitization on the hazards of open defaecation, door closing, and proper waste disposal is essential for reducing fly density near homes. Additionally, proper diagnosis and treatment of diarrhoeal illnesses are vital for mitigating their impact on children living in rural communities.

Chapter 5: summarizes environmental risk factors contributing to indoor malaria vector entry in the traditional African houses, to propose appropriate measures to mitigate malaria transmission risks for children living in rural communities. These potential risks are categorised at different spatial scales from coarse scale data obtained from remotely sensed imagery to fine scale data in and around the home derived from questionnaire surveys. A total of 1,457 malaria vectors were captured indoors over 1,430 trapping occasions, with 79% (1,149) identified as *Anopheles gambiae s.l*. and 21% (308) as *Anopheles funestus s.l*. Within the *An. gambiae* complex group, 60% (528) were identified as *An. gambiae s.s*., 32% (276) as *An. arabiensis*, and 2% as *An. merus*. Among the *An. funestus* group, 98% were *An. funestus s.s*., with the remaining 2% comprising other sibling species within the group.

Hotspots of indoor malaria vector abundance were identified in the central and far eastern regions of the study area, characterized primarily by low altitudes (30-90m above sea level) (Hast et al. 2019), the presence of marshlands, irrigation rice farming, and banana plantations, in agreement with an earlier study (Mwangungulu et al. 2023). The presence of open water bodies, such as water wells, marshlands, and irrigated farming, within 15 m of the house were associated with increased numbers of malaria vectors. These water bodies serve as potential breeding habitats for malaria vectors (Hast et al. 2019). The presence of at least one window in the study child's sleeping area was found to double the number of malaria vectors caught indoors. Open windows at the study child's sleeping area provide a pathway for malaria vectors to enter the house, as evidenced by previous studies advocating for window screening as a means of preventing mosquito entry (Jatta et al. 2021).

A reduction in indoor malaria vector abundance was associated with more built-up areas, lower altitudes and the presence of chickens near study houses. The predominant human-made features in the study area were houses and schools, reflecting the rural character of the villages, characterized by low population density and dispersed settlement patterns. This attribute has also been linked to decreased indoor malaria vector abundances in prior research (Kaindoa 2019). Furthermore, a decline in indoor malaria vector abundance was observed with an average increase in altitude by 100 m above sea level. Higher altitude areas were marked by steep slopes with fast-moving water, which prevents egg laying by *An. gambiae* s.l and *An.*

funestus s.l., unlike lower altitude regions that favoured water accumulation, providing the still water ideal for mosquitoes (Hast et al. 2019). The practice of keeping domestic animals such as chickens near the house has been found to reduce indoor malaria vector abundance. The presence of chickens near the house has been found to reduce indoor malaria vector abundance. Chickens in peri-domestic areas serve as alternative hosts, diverting opportunistic feeders like *An. arabiensis* away from humans. Additionally, another study suggested that the preference of malaria vectors for feeding on chickens may be influenced by widespread bed net use, as observed in our study area, where approximately 95% of children use bed nets. This high bed net usage could potentially drive mosquitoes to seek alternative hosts for blood meals (Mwangangi et al. 2013). Moreover, a separate study suggests that chicken volatiles repel mosquitoes, potentially lowering indoor mosquito entry when chickens are present in peri-domestic areas (Jaleta et al. 2016).

The study will be of interest to malaria control programmes which can target malaria control at areas of high mosquito abundance, by carrying out larval control through the application of microbial larvicides or draining marshland. They can also encourage householders to install mosquito screening in windows and close opening in their houses to reduce mosquito ingress.

The spatial analysis models used in this study were suitable for assessing correlations between indoor malaria vector abundance and a wide range of spatial, environmental, human behavioural and climatic factors. However, these models have two disadvantages: 1) since many factors were included in the model, some correlations were more likely to occur by chance alone, 2) scale dependency was not evaluated since different environmental factors are likely to operate at different scales.

Study limitations

The study had several limitations. Firstly, the selection criteria for traditional houses was purposive, selecting only houses with thatched roofs, mud and stick walls, open eave gaps, and poorly fitted doors. This approach thus targeted the poorest households in the community and does not fully represent the housing structures in the study areas. Some families not included in the study were improving their houses by replacing thatched roofs with corrugated iron sheets, installing window screens, and sealing eave gaps. These improvements could impact our findings, as traditional houses with multiple openings allow mosquitoes and domestic flies to enter and exit more freely compared to modern houses in the study villages. Poorly fitting doors, wall holes, and open eave gaps in traditional houses also allow CO_2 to dissipate easily, resulting in similar CO_2 concentrations between the two-house types. Differences in CO_2 concentrations might be more apparent between Star homes and brick-walled houses with corrugated iron roofs, ceilings, and closed windows. Random selection of traditional houses for comparison could have accounted for these changes in housing structures within the study area.

Secondly, the effectiveness of Star homes in reducing indoor disease vectors was underestimated since a higher proportion of mosquitoes and flies entering a Star home would be trapped than in traditional houses where there are more exit holes. Thirdly, the study did not measure outdoor exposure to disease vectors which is likely to contribute to around 20% of infective bites. Fourthly, mostly during the holidays season children in study homes occasionally slept in other traditional houses, compromising the accuracy of estimating protection against disease vectors, especially for those sleeping in Star homes. Fifthly, approximately 70% of families in Star homes preferred cooking outdoors, which does not effectively prevent domestic fly contact with food during cooking. This outdoor cooking behaviour not only puts families at risk of encountering diarrhoeal pathogens but also increases their exposure to malaria, as they are exposed to mosquito bites in the evening and early night. To protect them, I propose constructing screened verandas where they can cook while being protected from both mosquitoes and domestic flies. Alternatively, spatial repellents could be added around their cooking areas or on the mats they use outdoors to protect them from mosquito bites. Sixthly, the use of curtains to provide warmth and privacy in Star home bedrooms reduced ventilation, impeding carbon dioxide dissipation and resulting in no discernible difference in carbon dioxide concentration between Star homes and traditional houses. Seventhly, since correlation does not equal causation, the risk factor survey only identifies putative risk factors for indoor malaria vectors. Moreover, since we measured so many risk factors it is likely that some correlations simply arose by chance alone. Further experimental studies are required to show causation.

Further direction and wider applicability of this research

The study findings underscore the potential of house screening, a significant feature of Star homes, as an additional intervention for controlling vector-borne diseases. This is particularly noteworthy given that approximately 80% of vector-borne disease transmission, such as malaria, occurs indoors (Carnevale & Manguin, 2021). In areas where outdoor malaria transmission results in significant levels of transmission, housing interventions could be supplemented with environmental modifications, spatial repellents, and larviciding to reduce the densities of malaria vectors in the surrounding environment. Complementary to ITNs, untreated house screening is recommended by the World Health Organization (WHO) to prevent vectors like mosquitoes from entering homes (WHO 2017b). The reductions of over 50% in indoor mosquito abundance and over 40% in domestic flies in Star homes, indicate promising avenues for future research on house screening as a strategy for preventing vector-borne disease transmission. Readily available and easy to implement housing improvement approach such as window and door screening and sealing the eave gaps area is applicable even in rural settings. In contrast, other methods like using cement or mud-brick sealing would require significant expertise, time, and compromise ventilation. House screening can be applied to impoverished housing structures, including makeshift shelters used by fishermen, pastoralists, forest workers, and farmers (Swai et al. 2016)

The main challenge of house screening technology lies in accessibility and affordability, particularly for low-income communities in remote areas. Engaging communities and policymakers, seeking funding partnerships, and advocating for government subsidies for screening materials can address these challenges. Future research should also address human behavioural aspects, such as the tendency of residents in poor communities to leave doors open, facilitating vector entry. Additional sensitization efforts are needed to promote the maintenance of shade net walls to reduce entry points for disease vectors, especially at the first storey. Moreover, assisting coastal communities in constructing toilets and increasing awareness about the hazards of open defecation can aid in diminishing fly populations in the environment and their subsequent infiltration into homes. This approach can be drawn from the Indian government's initiative, which involved the construction of eight million toilets and could similarly be applied to residents in the coastal areas of the Mtwara region (Banerjee et al. 2016)

In order to expand house screening and other housing interventions many approaches can be taken through the involvement of multiple stakeholders:

1) Community involvement: Engaging the community is crucial as they are the primary beneficiaries of the technology. A well-engaged community can accelerate the adoption of technology and identify alternative funding sources to support the implementation of screening and other housing interventions.
2) Policy makers: Policy makers, including parliamentary, executive, and district commissioners, can facilitate the advancement of these technologies and housing modifications by advocating for supportive policies. They can also help establish connections between the housing and health sectors, leading to better health-oriented house designs.
3) Capacity building: Collaboration between health and housing experts is essential for capacity building. Training architects to consider health aspects in their designs can lead to healthier housing modifications. This interdisciplinary connection enhances the effective application of health-conscious housing approaches.

Scaling up house screening and other housing modifications should aim to cover a broader population for maximum impact. This involves extending coverage to more communities and regions and fostering partnerships across various sectors, including commercial, health, housing design, and government. Government policies can support this integration by subsidizing building materials such as screening, thus improving accessibility and facilitating home improvement.

The economic implications of house screening and other housing interventions can be categorized into direct and indirect costs. Direct costs involve initial investments such as material purchases and labour charges. Indirect costs include community benefits from successful house screening, such as fewer hospital visits, reduced treatment costs, and increased productivity due to healthier populations. Additionally, broader economic benefits encompass improved health and reduced disease transmission, contributing to a better quality of life, which can positively impact economic development.

Recommendations for moving house screening into policy and practice.

Based on the findings of this study, I make the following recommendations:

1) *Expansion of research*: Further research is required to evaluate the effectiveness of house screening in diverse geographic and socio-economic contexts. This involves examining its impact on indoor mosquito biting and malaria transmission risks in areas beyond rural Mtwara, including different geographical, ecological, and malaria intensity settings. Moreover, the efficacy of house screening should be assessed against other vector-borne diseases such as dengue fever, Zika virus, and Chagas disease, rather than solely focusing on malaria and diarrhoeal illnesses as discussed in Chapters 3 and 4. Additionally, it is imperative to conduct microbiological assessments on domestic flies collected in Chapter 4 to determine presence of diarrhoeal illness pathogens carried by the flies and to compare the associated risks between the two types of houses. Further research is also needed to understand why households tend to leave their doors open, facilitating the entry of disease vectors. This behaviour was observed during the measurement of door opening and closing in both study groups and its impact on vector entry. Despite having modern, self-closing, solid doors, residents of Star homes often use stones to block these doors from closing. This practice allows mosquitoes and flies to enter, reducing the protective efficacy of the intervention housing.

2) *Community engagement*: Community involvement in implementing house screening programs can boost acceptance and sustainability. This entails raising awareness about the importance of screening and engaging community members in decision-making processes. Initiatives like Village Community Banks (VICOBA) in Tanzania can facilitate fundraising efforts (Mponzi et al., 2023). In this study, various community engagement activities

were conducted to raise awareness about the need for improved housing structures, including screening, to protect against vector-borne diseases. These campaigns involved bonanzas, where the research team interacted with local communities through sports and disseminated health and housing knowledge.

3) *Policy advocacy*: Advocating for supportive policies for house screening interventions is essential. This involves lobbying for funding and resources, integrating screening requirements into building codes, and promoting housing regulations that facilitate screening. Policymakers can also promote the integration of housing improvements with health measures to enhance living conditions, particularly for impoverished communities. In this study, we collaborated closely with the District Commissioner's office and village chairpersons to establish connections with government officials responsible for housing and building material policies.
4) *Capacity building*: Boosting the capacity of local communities and health workers to carry out and maintain house screening interventions is crucial. This involves training in screening techniques, maintenance protocols, and evaluation methods. It is also essential to educate local house constructors about incorporating health aspects into construction practices, such as ensuring proper ventilation through appropriate window sizes, fitting doors, suitable house size relative to family members, improved latrine facilities with adequate sewage systems, and consistent water availability. Additionally, village health workers should receive training and assistance, including access to enhanced disease diagnostic tools for use at the household level.
5) *Integration with other interventions* Integrating house screening with other vector control measures like ITNs, indoor residual spraying, and larval control can enhance overall effectiveness and sustainability. This study's recommendations, based on findings in Chapter 3, emphasize the importance of combining these interventions, as mosquitoes can still enter homes through doors, especially if individuals left the doors open. Beyond vector control, proper diagnosis and treatment of vector-borne illnesses are essential to reduce disease transmission risks in the community. Additionally, promoting safe water, sanitation, and hygiene (WASH) practices is crucial for reducing

the risk of diarrhoeal illnesses, as observed by the entry of domestic flies into Star homes' kitchens in Chapter 4.

6) *Evaluation and monitoring*: Continuous evaluation and monitoring of house screening interventions are vital for assessing effectiveness, identifying challenges, and making necessary adjustments to improve outcomes. Additionally, assessing the cost effectiveness of screening is essential to demonstrate its added protection against vector-borne disease transmission compared to other interventions such as larviciding. Moreover, evaluating the supplementary benefits of screening in kitchens, in addition to existing WASH practices and other diarrhoeal illness control measures such as covering of food and cooking utensils, is imperative.
7) *Scaling up*: Expanding house screening interventions to a wider population is essential for maximum impact. This involves extending coverage to more communities and regions and fostering partnerships across various sectors: commercial, health, housing design, and government. Government policies can support this integration by subsidizing the cost of building materials such as screening, thus enhancing accessibility and enabling home improvement.

Future research addressing human behaviour is essential. In this study, we faced the challenge of individuals leaving their doors open, allowing disease vectors such as mosquitoes and domestic flies to enter, thereby increasing the risk of pathogen transmission. To address this issue, a mixed-method research approach involving both qualitative and quantitative methods could be carried out to better understand the factors why people leave their doors open and to develop ways of reducing the length of time doors are open. There is also a need to study why people cook outdoors and to develop improved cooking stoves that people will use indoors.

Conclusion

The study findings suggest that constructing houses with features like screened windows and walls, small eave gaps, raised sleeping areas, and self-closing solid doors, effectively prevent indoor mosquito entry. Utilizing locally available low thermal mass building materials and promoting community education on door closure, screen repair, and ITN use are vital for malaria control in endemic regions.

Identification of hotspots with higher indoor malaria vector abundance is crucial for targeted mosquito bite control measures, including window screening and sealing house openings like eave gaps and wall holes. Conducting larvae surveys, applying larvicides in aquatic habitats, and covering water bodies close to homes are necessary strategies, along with bed net use, for controlling indoor malaria transmission.

Star homes also provide protection against the principal mechanical vector of diarrhoeal illnesses in children, *C. putoria*, in kitchens and toilets, thereby helping to reduce the transmission of diarrhoeal pathogens. This highlights the necessity for kitchen and toilet modifications, improved hygiene, and ensuring access to safe water. Community awareness to reduce open defaecation and improve door closure at night and before dawn is essential, along with diagnosing and treating diarrhoeal illnesses to mitigate their impact on rural children.

Housing improvement technologies, such as house screening and self-closing solid doors, have been demonstrated to protect against indoor entry of disease vectors while also enhancing household comfort through improved ventilation.

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Appendix

Appendix 1: Huts used in the study; opaque-walled, (A), transparent-walled houses, (B), partial open eave-gaps, (C), completely closed eave-gaps, (D), Star home-style house (E), and traditional-style house (F).

Appendix 2: Treatment rotations between the semi-field chambers experiment 1black fill represents opaque-walled and no fill represents transparent walled, experiment 2 black fill represent open gaps and no fill represent closed gap, and experiment 3 black fill represent poorly ventilated huts and no fill represent well ventilated huts (Star home style). Each experiment was conducted over 24 nights and the entire project over 72 nights.

Nights	Round	Chamber 1	Chamber 2	Chamber 3	Chamber 4
1 – 4	1	■		■	
5 – 8	2		■	■	
9 - 12	3		■		■
13 - 16	4	■	■		
17 - 20	5			■	■
21 - 24	6	■			■

Appendix 3: Volunteer rotations between semi-field chambers

Day of the week	Sleeper			
	Chamber 1	Chamber 2	Chamber 3	Chamber 4
Monday	1	2	3	4
Tuesday	2	3	4	1
Wednesday	3	4	1	2
Thursday	4	1	2	3

Appendix 4: Collection of resting mosquitoes indoors and outdoor

Resting mosquitoes were collected after the light traps switched off at 07:00 h. All mosquitoes remaining inside the huts and outside were cleared using a mechanical aspirator (Prokopack®, model 1419, John W. Hock Co., Gainesville, USA), each morning from 07:15 to 07:45 h. Collection of resting mosquitoes started inside the huts, followed by outdoor collections. Resting mosquitoes were collected to ensure no mosquito remained inside the huts and chamber that could affect the next day experiment. Resting mosquitoes collected both inside and outside by using mechanical aspirator were counted and recorded.

Appendix 5: Supplementary results

Experiment 1. Indoor light intensity and resting-mosquitoes

There was no difference in indoor resting *Anopheles arabiensis* collected in the different typologies of houses (Odds ratio=0.89, 95% CIs=0.74 – 1.05, p=0.17). There were fewer outdoor resting *An. arabiensis* in transparent-walled houses compared to opaque-walled houses (OR=0.57, 95% CIs=0.54 – 0.64, p<0.001; Supplementary table 1).

Experiment 2. Open gaps under roofing vs closed gaps under roofing and resting mosquitoes

Huts with closed-eaves were less likely to have indoor resting *An. arabiensis* than those with open-eaves (OR=0.19, 95% CIs= 0.08–0.46, p<0.001). There was a corresponding increase in outdoor-resting mosquitoes in cages with huts with closed eaves compared to cages with open eave huts (OR=1.03, 95% CIs= 0.97–1.09, p<0.05; Supplementary table 1).

Experiment 3. Traditional style vs Star homes style and resting mosquitoes

The odds of collecting indoor-resting mosquitoes was 88% less in well-ventilated, Star home-style huts than traditional-style huts (OR=0.12, 95% CIs= 0.06 – 0.23, p<0.001). Consequently, the cages of Star home style huts had an increased odds of collecting outdoor resting *An. arabiensis* mosquitoes than traditional-style huts (OR=3.04, 95% CIs= 2.90 – 3.20, p<0.001; Supplementary table 1). The environment conditions between the hut typologies was similar. The outdoor relative humidity was 83.1% (95% CIs= 79.4 – 88.3) (Table 2.2).

Supplementary Table 1: Comparisons of indoor and outdoor resting of malaria vectors between two house types.

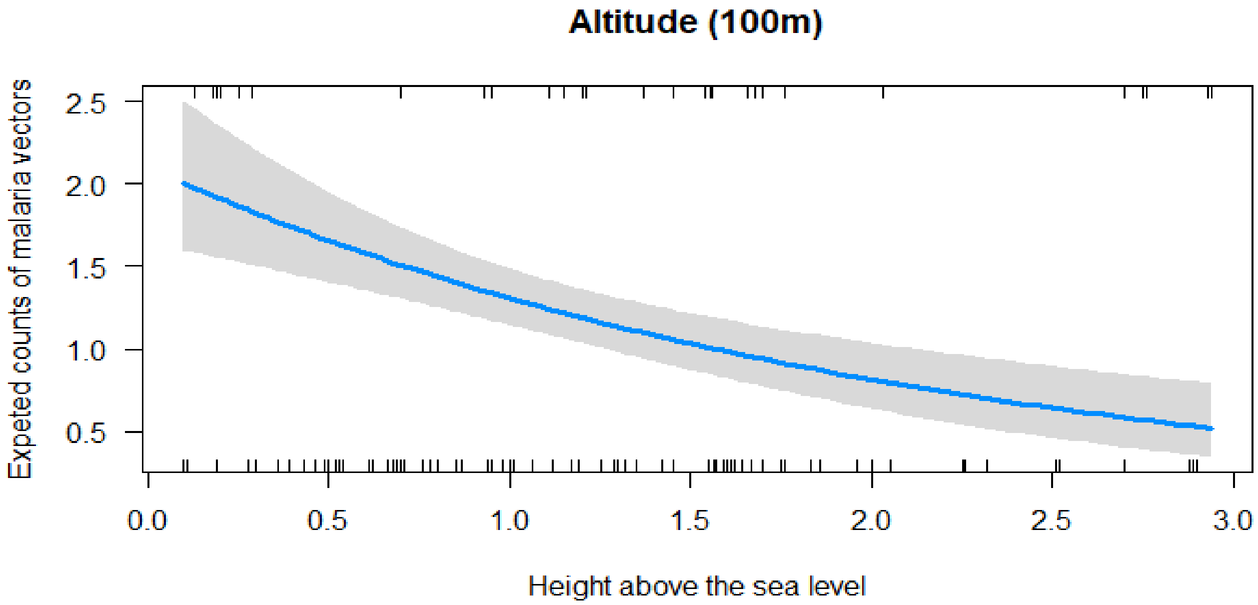
Experiments	Description	Indoor resting mosquitoes caught by Prokopack® aspirators			Outdoor resting mosquitoes caught by Prokopack® aspirators		
		Mean no. mosquitoes/night (95% CI)	**Odds ratio (95%CI)**	***p*-value**	**Mean no. mosquitoes/night (95% CI)**	**Odds ratio (95%CI**	***p*-value**
Experiment 1: Light-opaque walls vs light-transparent walls							
	Opaque-walled	1.3 (0.8–2.1)	1		8.1 (6.9–9.5)	1	
	Transparent-walled	1.1 (0.7–1.9)	0.89 (0.74–1.05)	=0.17	4.9 (4.1–5.8)	0.57 (0.54–0.64)	<0.001
Experiment 2: Open gaps under roofing vs closed gaps under roofing							
	Open eave-gaps	9.4*e-4 (0.0–0.1)	1		59.1 (56–62)	1	
	Closed eave-gaps	1.7*e-4 (0.0–0.01)	0.19 (0.08–0.44)	<0.001	60.6 (57.6–63.5)	1.07 (1.02–1.12)	=0.008
Experiment 3: Poorly ventilated vs well-ventilated							
	Traditional	0.5 (0.3–0.8)	1		50.1 (46.4–53.8)	1	
	Star homes type	0.1 (0–0.1)	0.12 (0.06–0.23)	<0.001	75.3 (72.5–78.0)	3.04 (2.90–3.20)	<0.001

24 nights of experimentations done in each experiment; each house type test was replicated inside two chambers. 300- host-seeking laboratory reared *An. arabiensis* released in each SFS-chamber. Prokopack® aspirators used to collect resting mosquitoes inside and outside the huts.

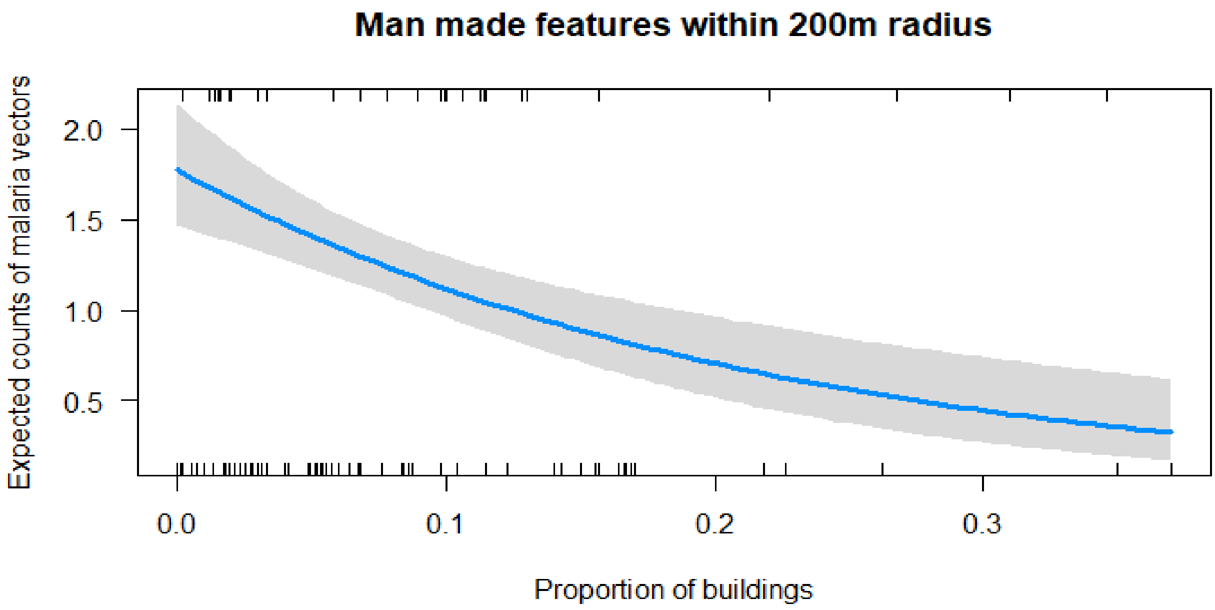
Supplementary Table 3. 7: Workplan per round. Where SH = Star homes and TH = traditional houses.

Activity	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Total
Light traps collection	16 SH vs16TH	16 SH vs 16TH	16 SH vs 16TH	16 SH vs 16TH	16 SH vs 16TH	16 SH vs 16TH	14 SH vs 14TH	110 SH vs 110TH
Indoor temperature measurements	4SH vs 4TH	4SH vs 4TH	4SH vs 4TH	4SH vs 4TH	4SH vs 4TH	4SH vs 4TH	4SH vs 4TH	28SH vs 28TH
Outdoor temperature measurements	2SH vs 2TH	2SH vs 2TH	2SH vs 2TH	2SH vs 2TH	2SH vs 2TH	2SH vs 2TH	2SH vs 2TH	14SH vs 14TH
Duration of door opening (main door)	4SH vs 4TH	4SH vs 4TH	4SH vs 4TH	4SH vs 4TH	4SH vs 4TH	4SH vs 4TH	4SH vs 4TH	28SH vs 28TH
Duration of door opening (Stairways door)	4SH	4SH	4SH	4SH	4SH	4SH	4SH	28SH

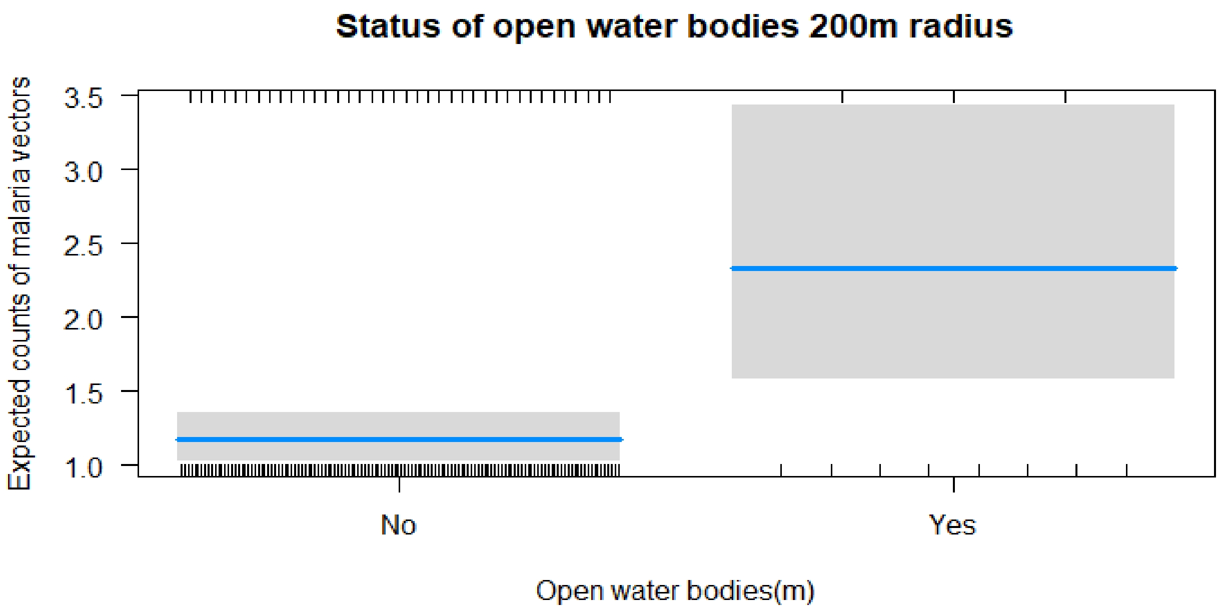
Appendix 6: Spatial model prediction plots depicting indoor densities of primary malaria vector abundances.



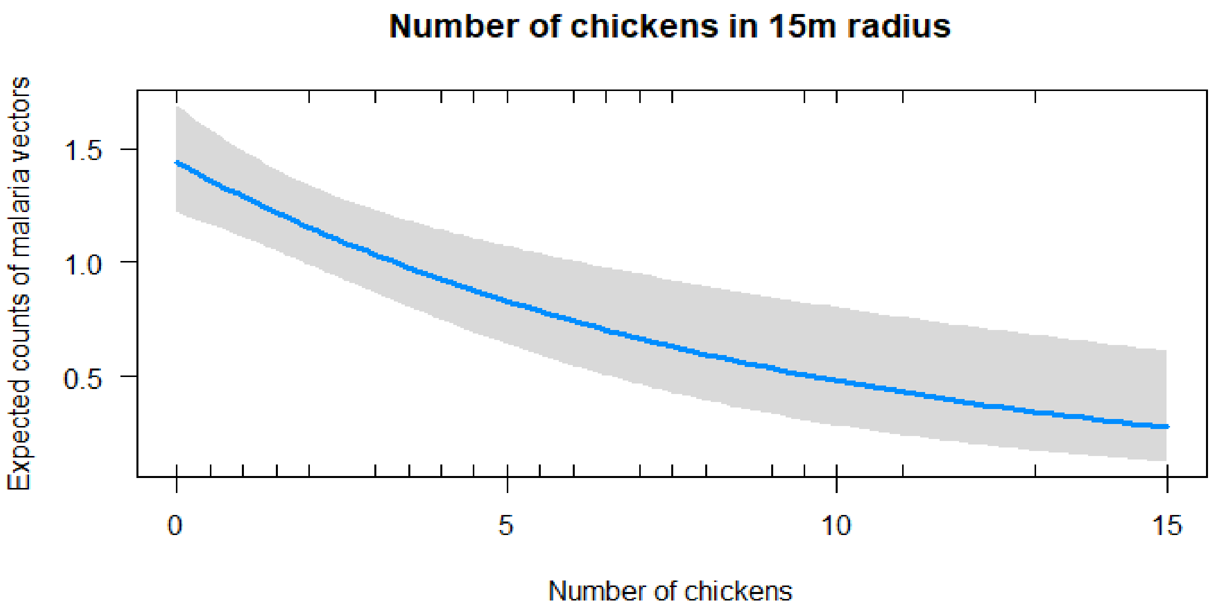
Supp: Fig.5.4a: Expected indoor malaria vector counts in relation to an average increase of altitude by 100m above sea level.



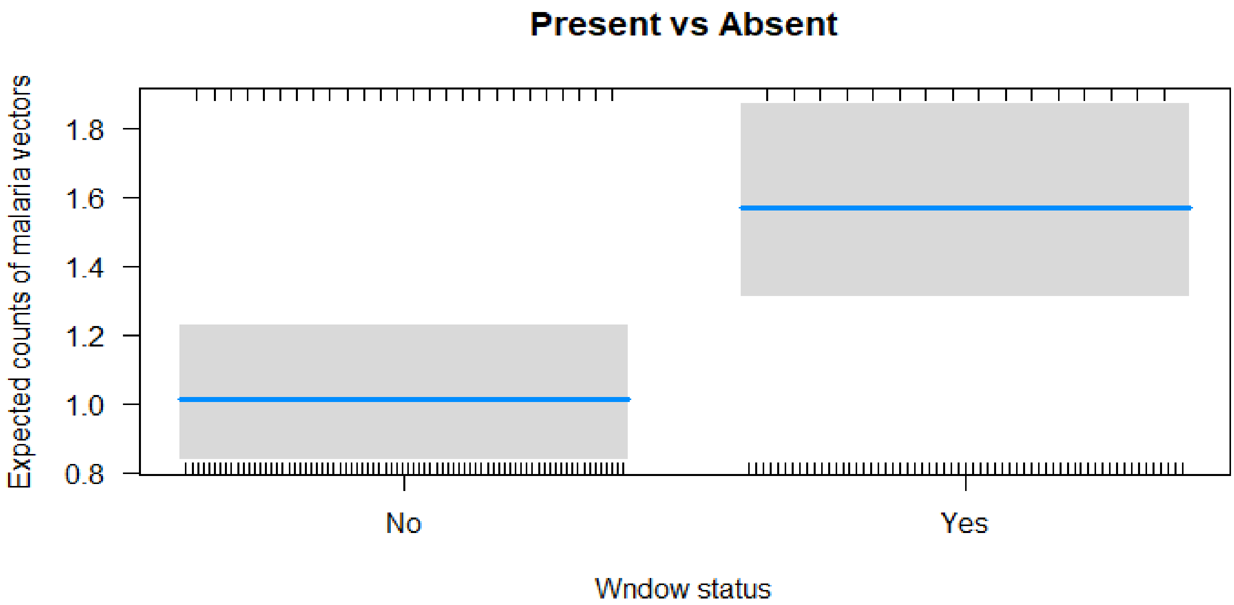
Supp: Fig.5.4b: Expected indoor malaria vector counts on an average increase in the proportion of man-made features within 200m.



Supp: Fig.5.4c: Expected indoor malaria vector counts with open water status within 15m around the house.



Supp: Fig.5.4d: Expected indoor malaria vector counts with an average increase of chicken within 15m from the house.



Supp: Fig.5.4e: Expected indoor malaria vector counts with window status at the child's sleeping place.