IMPACT OF HOUSE DESIGN ON THE ENTRY AND EXIT OF INSECT DISEASE VECTORS IN THE GAMBIA

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IMPACT OF HOUSE DESIGN ON THE ENTRY AND EXIT OF INSECT DISEASE VECTORS IN THE GAMBIA

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List of Acronyms

ACT	Artemisinin-based Combination Therapy
BHC	Benzene Hexachloride
CDC	Centre for Disease Control
CI	Confidence Interval
CQ	Chloroquine
CSD	Central Statistics Department
DDT	Dichlorodiphenyltrichloroethane
DOSH	Department of State for Health
ELISA	Enzyme-Linked Immunosorbent Assay
GBC-CAMA	Global Business Coalition – Corporate Alliance on Malaria in Africa
GFATM	Global Fund to fight AIDS, Tuberculosis and Malaria
GEE	Generalised Estimating Equations
GLIM	Generalised Linear Model
GPS	Geographic Positioning System
HIV	Human Immunodeficiency Virus
IEC	Information, Education and Communication
IPT	Intermittent Preventive Therapy
IQR	Inter-Quartile Range
IRS	Indoor Residual Spray
ITN	Insecticide Treated Nets
IVM	Integrated Vector Management
LLIN	Long-Lasting Insecticide Treated Nets
MDG	Millennium Development Goals
MRC	Medical Research Council
NGO	Non Governmental Organisation
NMCP	National Malaria Control Program
PCR	Polymerase Chain Reaction
RBM	Roll Back Malaria
SP	Sulfadoxine-pyrimethamine
STOPMAL	Screening Homes to Prevent Malaria
UNICEF	United Nations International Children Emergency Fund
UTM	Universal Transverse Mercator
WHO	World Health Organisation

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Dedication

This work is dedicated to my father, Alieu Njie, who fought strongly to see me educated. He made himself poor by investing into my education. God bless you Dad and I would always be proud of you.

Executive Summary

The impact of house design on the entry and exit of insect vectors of disease in The Gambia

House design may affect the exposure of the residents to vector-borne diseases in rural areas in Africa. Improving a house by simply closing the eaves gap or by installing a ceiling could go a long way to preventing diseases such as malaria, which is killing millions of people every year. These two structural adjustments were the main focus of this thesis, which addresses three major questions:

1. What effect does eave closure have on mosquito house entry in houses that have screened doors and no other route of entry?
2. Are torn ceilings treated with insecticide as good as intact untreated ceilings at preventing mosquito house entry?
3. Is full screening and/or screened ceilings efficacious at preventing house entry by houseflies?

To determine the importance of eaves to mosquito house entry, a crossover study was conducted using 12 single-roomed houses with screened doors, in which the eaves were either open or closed for half of the study. Closing the eave gaps reduced the house entry of the malaria vector *Anopheles gambiae s.l.* by 65%, but no significant reduction was observed for culicine mosquitoes. To test the efficacy of insecticide-treated torn ceilings against mosquito house entry, three different insecticide treatments were compared with an intact untreated ceiling, an untreated torn ceiling, and a no ceiling control, using six experimental huts, with a man sleeping under an untreated bednet in each hut. Here treatments were rotated between different huts on different nights. The insecticide-treated ceilings failed to reduce the number of vectors entering the hut compared with the untreated torn ceilings. Finally, the number of houseflies, pests of public health importance, entering fully screened and screened-ceiling houses was estimated by sticky trap catches.

The findings indicate that anopheline mosquitoes largely enter houses through open eaves, whilst culicine mosquitoes enter through the doors. Failure to demonstrate enhanced protection with the insecticide-treated torn ceilings may have resulted from a failure of the insecticides to adhere well to the treated fabric. Fully screened houses reduced housefly entry by 24% whereas ceilings increased the houseflies by 440% compared to unscreened houses.

It is likely that the increase in houseflies in houses with screened ceilings was an artefact caused by sticky traps over-estimating the number of flies in a room. Whilst ceilings failed to reduce flies entering houses, fully screened doors and windows were protective.

These results demonstrate that simple changes in house design can reduce the risk of exposure to malaria and flies. However, further work is required to determine whether different insecticide formulations on screening can increase the efficacy of this intervention. These studies demonstrate that house screening can play an important role in the control of malaria, and perhaps other diseases as well.

Study Rationale

Mosquito-proofing homes was a fundamental technique of malaria control in the early 1900s (Takken et al., 1990, Lindsay et al., 2003) and it should protect against malaria in Africa since most people get the disease when they are bitten in their homes at night. House screening provides protection against malaria by reducing the exposure to malaria parasites, and has the added benefit of protecting everyone in the room, avoiding issues of inequity within the household (Lindsay et al., 2002). The research presented in this thesis was conducted with the support of The Screening Homes to Prevent Malaria (STOPMAL) project. STOPMAL was a randomized control trial of house screening in The Gambia (Kirby et al., 2008c). The rationale for this trial was to examine an alternative method to antimalarials and insecticides for controlling malaria, because these are unsustainable in the long term because of the rise of resistant parasite strains and vectors. The trial measured the number of *An. gambiae s.l* caught in light traps from unprotected control houses, houses with full screening and houses with screened ceilings. Full screening was where houses had untreated screening on the doors and windows, with the eaves, the gap between the top of the wall and the roof, closed with a mixture of mud and mortar. Screened ceilings allowed mosquitoes to enter the roof space through the open eaves, but they could not feed on people in the room. Children sleeping in these houses during the trial were screened for haemoglobin density and parasite prevalence and treatment provided at the end of each year's intervention.

Closing eaves has been suggested to reduce density of anophelines (Lindsay and Snow, 1988) and culicines (White, 1969) entering houses, but it is unclear how important blocking the eaves is at reducing mosquito entry for houses with no other route of entry. It was important to find out whether blocking the eaves, in addition to screening the doors and windows, is essential when constructing mosquito-proof homes in this West African setting. During the life of an untreated ceiling it is likely to get torn. For this reason I wanted to find out whether treating badly torn ceilings with insecticide could reduce entry by mosquitoes. Since screening protects against mosquitoes I also wanted to find out whether it was effective against house flies which are active during the day, instead of at night like mosquitoes. Since these issues have a potential impact on the operational efficacy of house screening they formed the basis of my research. The specific objectives of my thesis are detailed overleaf.

Study Objectives

- To determine the percentage reduction in mosquito house entry (anophelines and culicines) that can be achieved by closing the eave gap in houses with screened doors and no other routes of entry;

- To determine whether impregnating torn screened ceilings with insecticide provides a greater protection against mosquito house entry than untreated torn screened ceilings;

- To compare the protective efficacy of three insecticides on torn screened ceilings i.e. (1) Deltamethrin impregnated synthetic-netting ceiling (2) Chlorpyrifos impregnated synthetic-netting ceiling (3) Permethrin impregnated synthetic-netting ceiling;

- To find the optimal position of sticky traps in the house for sampling houseflies.

- To compare the efficacy of sticky traps (placed optimally) against knock down (insecticide spray) catches for sampling housefly populations;

- To establish the diel flight activity of houseflies;

- To re-evaluate the efficacy of full screening and screened ceilings against house fly entry using the most appropriate sampling technique.

CHAPTER 1

Malaria Control in Africa and in The Gambia

1.1 The global malaria burden

Despite the preventive and control methods available today, malaria still remains a major public health problem worldwide, especially in the world's poorest countries. Between 350-500 million clinical episodes of malaria and over 1 million deaths occur each year (Roll Back Malaria, 2005). Over 107 countries are affected by malaria worldwide, and the disease is endemic in 24 of these. Around 60% of cases and 80% of deaths occur in Africa, south of the Sahara. The parasite *Plasmodium falciparum* causes the vast majority of infections in this region and about 18% of deaths in children <5 years of age. The estimated *P. falciparum* annual parasite incidence is ≥ 0.1 per 1000 people in stable risk areas south of the Sahara (Guerra et al., 2008a). Malaria is also a major cause of anaemia in children (Menendez et al., 2000) and pregnant women (Huddle et al., 1999), low birth weight (Brabin and Piper, 1997), premature birth and infant mortality (Luxemburger et al., 2001). In endemic African countries, malaria accounts for 25–35% of all outpatient visits, 20–45% of hospital admissions and 15–35% of hospital deaths, imposing a great burden on already fragile health-care systems (GBC-CAMA, www.gbcimpact.org).

The cost of malaria prevention and treatment is huge, and there are great economic losses caused by malaria as a result of a decline in productivity. These losses are suffered by both people afflicted with the disease and those caring for them, and the loss is even higher as result of deaths due to malaria. Malaria will inevitably affect trade and movement of people and foreign investment, for example tourists will not travel to regions or countries with high malaria risk (Gallop and Sachs, 2001).

Parasite-resistance to anti-malarials and mosquito-resistance to insecticides have complicated the control of malaria worldwide, especially in Africa. One of the reasons for resurgence and increased burden of malaria is the development of resistance to traditional first line anti-malarial drugs for treatments such as Chloroquine (CQ) and Sulfadoxine-pyrimethamine (SP) by *P. falciparum*, the parasite that causes the severe form of malaria, and as such, this spread of drug resistance has raised the cost of treatment for the disease (White, 1998). Resistance to anti-malarials has been responsible for increases in morbidity and mortality in many sub-Saharan countries (Snow et al., 2001). A range of 17-30% resistance to CQ, has been reported (Wellems and Plowe, 2001), and a recent study reported 60%

resistance to CQ in Mali, West Africa (Sangho et al., 2004). The quality of anti-malarial products and patient adherence to dosage regimens are also important determinants of drug effectiveness (Amin et al., 2004). The use of substandard drugs could have serious clinical consequences for patients, prompting the need for continuous monitoring of the quality of marketed drugs, to ensure safety and efficacy of these products in the treatment of malaria in endemic areas (Minzi et al., 2003).

1.2 *An. gambiae* complex in Africa

There are about 400 species of *Anopheles*, but only about 60 are vectors of malaria under natural conditions, some 30 of which are of major public health importance (Wernsdorfer & McGregor, 1988). Not all species of anophelines are vectors of malaria and, even among those that are vectors; there are great differences in their ability to transmit the disease. Almost all *P. falciparum* parasite rates above 50% are reported in Africa, in a latitude band consistent with the distribution of *Anopheles gambiae* s.l. (Guerra et al., 2008b). This species complex include some of the most efficient vectors of malaria known from anywhere in the world. Formerly regarded as a single species with ecological salt-water variants, it has been shown to comprise of five fresh water and two salt-water species (Gillies, 1968, Bryan, 1983, Lindsay et al., 1993b).

Members of the complex are distinguished from other anophelines by the palps, which have three bands, one wide ring at the tip and two narrow towards the head, and by the wings which have a pale interruption at the third black band on vein one. No satisfactory morphological characters have been discovered to separate the adults of the species within the complex, and although meristic characters for separating the species at the population level have been demonstrated (Coluzzi, 1964), the identification of individual specimens by this technique is unreliable. More recently a PCR technique has been developed that can achieve this (Scott et al., 1993).

Among the complex members (Gillies and Coetzee, 1987, Hunt et al., 1998)1998), *An. gambiae s.s* and *An. arabiensis* are the most efficient malaria vectors because of their ability to feed readily on people. In the dry savannas of Africa, vector populations typically display strong seasonal fluctuations in abundance, being present in large numbers during the rainy season and dropping to very low levels when breeding sites dry up (Taylor et al., 1993, Charlwood et al., 1995, Lemasson et al., 1997). The main reason why Africa is severely affected by this deadly disease is because the continent is a tropical region and provides abundant breeding grounds and ambient temperatures that favour mosquitoes, especially *An.*

gambiae s.l. It is also the place where the most dangerous parasite, *P. falciparum*, is found, which causes the severe form of the disease.

1.3 Malaria control and prevention in Africa

Recognition of the unacceptable mortality and morbidity rates from malaria and malaria-related illness in World Health Organisation (WHO) Africa region, and of the availability of a number of evidence-based and cost-effective interventions, led to the formation of the Roll Back Malaria (RBM) Initiative (www.rbm.who.int/wmr 1998). Launched in 1998 by the WHO, the World Bank, the United Nations Children's Fund, the United Nations Development Program, and other partners, RBM aims to cut the malaria burden in half by 2010 by advocating and promoting treatment and preventive strategies. Insecticide-treated nets (ITNs) are one of the core vector interventions of the Global Malaria Action Plan of the RBM partnership, which aims to reach a universal coverage of one long-lasting insecticidal bednet for every two people in a household (Partnership, 2006). The RBM is not a financing mechanism; it works by encouraging others to dedicate resources to malaria control, to strengthen health systems, and to use a variety of tools through existing networks and partnerships. Progress is slow but substantial, particularly in surveillance, promotion of insecticide-treated bed nets (ITNs) and closer linkage of research to strategy.

The Initiative sets out clear goals and objectives to reduce the toll of malaria through public-private and sustainable actions towards strengthening the country health system through the following interventions and service delivery areas:

- Supporting, promoting and ensuring access to correct, affordable and appropriate malaria treatments, especially for young children within 24 hours of the onset of the disease;
- Prevention and control of malaria in pregnancy for pregnant women through the support and promotion of preventive measures such as intermittent preventive treatment (IPT), especially those in their first pregnancies;
- Supporting and promoting access to a suitable combination of personal and community protective measures such as ITNs;
- Prediction and containment of malaria epidemic.

This initiative was further strengthened by the Bill and Melinda Gates Foundation and the Global Fund. The purpose of the Global Fund is to attract, manage and disburse additional resources through a new public-private partnership that will make a sustainable and significant contribution to the reduction of infections, illness and death, thereby mitigating the impact caused by HIV/AIDS, tuberculosis and malaria in countries in need, and contributing to poverty reduction as part of the Millennium Development Goals (MDGs).

Malaria control in the tropics is largely based on case management and personal protection against malarial mosquitoes using ITNs or indoor residual spraying (IRS) (www.rbm.who.int). ITNs are currently the most efficacious tool against malaria (Lengeler, 2004). However with such an increase in usage of pyrethroid treatment, the selection for development of pyrethroid resistance in malaria vectors is inevitable (Hargreaves et al., 2000). There is already widespread pyrethroid resistance in many countries of West (Martinez-Torres et al., 1998), East (Greenwood and Mutabingwa, 2002, Ranson et al., 2000) and southern Africa (Hargreaves et al., 2000, Chandre et al., 1999). The deterioration of IRS programmes in some countries has also contributed to the resurgence of malaria and was responsible for the abandonment of the global campaign for eradication in the 1950s and 1960s. Eventually this failure sparked renewed interest in larval source management and personal protective measures for the reduction of malaria transmission (WHO, 2006). The current malaria control strategy calls for the selection of those control measures which are most appropriate to local circumstances.

1.4. Integrated Vector Management (IVM), Environmental Management & House Screening

This is a process for managing vector populations in such a way to reduce or interrupt transmission of disease (WHO, 2004). Characteristic features of Integrated Vector Management (IVM) include:

- Methods based on knowledge of factors influencing local vector biology, disease transmission and morbidity;
- Use of a range of interventions, in combination and synergistically;
- Collaboration within the health sector and with other public and private sectors that impact on health;
- Engagement with local communities and other stakeholders;
- A public health regulatory and legislative framework.

The most effective vector management and control strategy is an organized programme under the direction of an entomologist or other qualified expert. These programmes are based on sound biological, physical and chemical data and the integration of the best techniques and materials. The goal is the control of mosquitoes while preventing adverse effects on humans, wildlife or the environment by reducing the dependency on insecticides.

Of the many methods available for malaria control environmental management is considered the bedrock on which to launch other interventions. Environmental management is where the environment is modified or manipulated to reduce malaria transmission by attacking local vector mosquitoes, and requires an understanding of the ecology of these species. One of the oldest methods of environmental management was house screening, protecting people from mosquitoes that fed on people indoors.

Mosquito-proofing homes was common in the early 1900s (Celli, 1901, Takken et al., 1990, Lindsay et al., 2003). In the summer and autumn of 1899, Angelo Celli carried out the first intervention trial against malaria by protecting people against mosquito bites in their homes. He recorded the number of malaria cases in the workers and their families living near two malarious railway lines near Rome. Some of the families were left unprotected, whereas in the homes of others, the windows were covered with thin muslin and the doors were screened with a metal net to prevent mosquitoes getting indoors. Those employees who worked outdoors at night were also provided with hats with a veil of netting, similar to a bee-keeper's, and large leather gloves to prevent mosquito bites. The results were spectacular: nearly all those who were unprotected contracted malaria, compared with only four out of 24 people in the protected houses.

During the second year of Celli's studies, Patrick Manson also published a seminal paper proving that mosquitoes transmitted human malaria (Manson, 1900). Manson described how Sambon, Low, Terzi and their helpers spent the summer of 1900 living in a screened hut under untreated bednets in a malarious area near Rome. Not one of them contracted malaria, unlike most of their neighbours, who fell sick with the disease. Here was a demonstration that the simple practice of reducing exposure to mosquitoes by house-screening and sleeping under nets could protect people against malaria.

So it seems likely that house design affects an individual's exposure to malaria parasites and hence to the disease. Many of the main mosquito disease vectors bite man in his home, therefore improving the traditional dwelling may reduce the risk of disease transmission (Schofield and White, 1984). Open eaves have been shown to be important portals of entry into houses (Snow, 1987), consequently closed eaves significantly reduce the densities of culicines in houses (White, 1969). Houses with open eaves, or which lack ceilings, are associated with increased numbers of mosquitoes and higher levels of malaria compared with neighbouring houses with closed eaves or ceilings (Lindsay et al., 2003). Mosquito-proofing dwellings by covering windows, eaves, and doors with screening and repairing cracks and holes by which mosquitoes enter may reduce transmission both by protecting people from bites and by preventing the spread of the disease from infected human reservoirs (Walker, 2002). Screening and general housing improvements may reduce malaria transmission while raising overall living conditions. Improved house construction played a role in controlling malaria in the United States in the early 20th century (Boyd, 1926, Fullerton and Bishop, 1933a). Some researchers indicated that residents of poorly constructed houses were as much as 2.5 times more likely to contract malaria than neighbours in houses of good construction (Gamage-Mendis et al., 1991, Gunawardena et al., 1998).

1.5 The Gambia

The Gambia is a country in West Africa with an area of approximately 11,000 km^2, of which almost 10% is covered by the Gambia River and another 20% by swampy land and flood plains. It is bordered by Senegal on all sides except the west coast bordering the Atlantic Ocean. The river stretches about 400 km eastwards and a narrow strip of land extends 15-30 km north and south of its banks.

The climate consists of two seasons; a rainy season which lasts for about five months (June-October) and peaks in August, and a seven month long dry season. The average annual rainfall varies between 963-1202mm throughout the country. During the dry season, maximum temperatures are frequently as high as 37-40°C and relative humidity falls as low as 40-55%.

The country has five rural administrative regions headed by Governors and two urban councils headed by Mayors. Under the Regions are the Districts and Villages headed by Chiefs and Alkalos respectively. Each of the Regions has a Local Government Area Council that looks after the development of the area. These Councils are headed by Chairpersons supported by counsellors from Wards within their Districts. The Districts also have elected representatives at the National Assembly were legislative issues are addressed.

The projected population was over 1.4 million inhabitants in 2003 (CSD 2003 census) of which 15% were women of child bearing age and 20% children under five years of age. These two age groups are the most vulnerable to infection and most malaria interventions should be targeted at them.

1.6 Malaria in The Gambia

In The Gambia, malaria is one of the leading causes of morbidity and mortality, especially among children under 5 years. Twenty percent of antenatal consultations and 40% of under-five visits in Maternal and Child Health services are due to malaria (DOSH 2006desktop 2000). Accurate figures for admission are not available but the trend may be the same as elsewhere in West Africa. Each year, over 800 children are admitted in the Royal Victoria Teaching Hospital with severe malaria (DOSH, 2006). The situation is worst in the rural areas where a child experiences at least one to two episodes of malaria each year making malaria one of the most frequent cause of presentation at outpatients' clinics (Brewster and Greenwood, 1993). Although the economic burden of malaria has not been fully determined in The Gambia, there is no doubt that the disease accounts for considerable lost days of productivity among the adult population, absenteeism from schools and workplaces and increased household expenditure on health. Malaria is therefore not only a health problem but also a developmental one.

Malaria in The Gambia is transmitted by the *An. gambiae* complex, which includes *An. gambiae s.s.*, *An. arabiensis* and *An. melas* (Bryan, 1983). *An. gambiae s.s***.** and *An. arabiensis***,** the major vectors, are distributed throughout the country with the former comprising the majority. *Anopheles melas* is however restricted to the western half of the country and probably contributes less to the disease burden because of its habit of frequently feeding on animals. *Anopheles gambiae s.s.* which breeds in fresh water, is very efficient in transmitting malaria and is widely distributed throughout the country during the rainy season (June-October). This vector prefers to feed on man, feeds indoors and rest indoors (anthropophilic, endophagic and endophilic respectively). *Anopheles arabiensis*, a freshwater breeder, is found mainly in northern regions near Senegal and may not contribute significantly to malaria transmission in the country apart from in the dry season, though in some years *An. arabiensis* can also make up a large percentage of the complex caught in the rainy season (M. Kirby, pers. com.). *Anopheles melas* breeds in salt water and is less efficient at transmitting malaria, restricted mainly to the western half of the country and is found for several months after the rainy season. The annual entomological inoculation rate is in the range of 1–80 infective bites per person per year, and the average gonotrophic cycle of mosquitoes in The Gambia is two days (Quiñones et al., 1997).

Malaria occurs in The Gambia throughout the year but the majority of cases occur between September and December. Transmission of malaria during this period is very intense and the number of cases can be 20 times that of the dry season. Although the whole population is at risk of contracting malaria throughout the year, the prevalence also varies from area to area. The highest rates are recorded in rural areas where large numbers of people sleep in houses made of mud blocks, and where the eaves are open (Kirby et al., 2008b). There are differences between rural areas, with more intense transmission and more severe disease in the Upper River Division than in any other area. Gambian villages situated furthest from mosquito breeding sites suffer greatest morbidity (Clarke et al., 2002). Part of the explanation for this finding might be that, here, people are less willing to use bed nets because nuisance biting by mosquitoes is reduced (Lindsay et al., 2002).

1.7 Malaria control in The Gambia

To eliminate malaria in many areas of intense transmission is beyond the scope of methods which developing nations can currently afford. New cost-effective, practical tools are needed if malaria is ever to be eliminated from highly endemic areas (Killeen et al., 2000)**.** In the Gambia, the Department of State for Health (DOSH) is in charge of all health delivery systems. It consists of deferent Directorates, Units and Programmes that look into specific health issues. The National Malaria Control Programme (NMCP) is responsible for all malaria interventions in the country under the Directorate of Disease Control and Prevention. The activities of the NMCP are integrated into the health care system of DOSH.

The NMCP in 2002 developed a five-year strategic Plan of Action for the control of malaria in the country. The main strategic approaches include Case Management, Malaria in Pregnancy, Vector Control and Personal Protection, Management and Partnership, Information, Education and Communication (IEC) and Advocacy, Surveillance and Research.

1.7.1 Case management

The aim of case management in malaria control in The Gambia is to ensure early diagnosis and prompt treatment through improved access to effective anti-malarial drugs. Although resistance has been recorded in some parts of the country, CQ remained the first line drug for treatment of uncomplicated malaria and Sulphadoxine pyrimethamine as the second line drug with quinine reserved for the treatment of severe and complicated malaria. The Department of State for Health is anticipating to change first line management from CQ to artemisinin based combination therapy to incorporate Artemether-Lumefantrine (COARTEM®) as the first line treatment for uncomplicated malaria (DOSH., 2005). An effectiveness study testing artesunate/lumefantrine combined, started in Dec 2007 at the Armed Forces Provisional

Ruling Council (AFPRC) General Hospital in Farafenni with the technical support of Medical Research Council (MRC) in the Gambia. The quality of care in public and private health facilities needs to be improved and the capacity of health facilities and community based malaria control activities should also be strengthened in order to reduce the malaria burden of The Gambia.

1.7.2 Malaria in pregnancy

In pregnancy there is an immune suppression response that leads to an increase susceptibility to malaria. The effects of malaria on pregnancy are dependent on malaria epidemiology and the immunity of women. In The Gambia pregnant women are protected by the increased access and use of Insecticide Treated Nets (ITNs) and Long Lasting Insecticide Nets (LLINs), provision of the regular supply of anti-malaria drugs and other essential supplies at all levels, provision of prompt diagnosis and treatment for acute cases of malaria at all levels and provision and promotion of effective malaria chemoprophylaxis for all pregnant women through Intermittent Preventive Therapy (IPT).

1.7.3 Vector control and personal protection

The Vector Control Unit was established in the early 1950s and activities of the unit were confined only to Banjul, the capital city. These activities include larviciding, residual spraying, fogging and rodent control. At the inception of the unit, organochlorines such as DDT, BHC in combination with organophosphates such as Pynerzone EC25, Malathion, Dursban, Abate emulsion 500E, and carbamates such as Baygon, were used in The Gambia. Synthetic pyrethroids using permethrin and deltamethrin for mosquito treatment were piloted in The Gambia in 1985 by Ministry of Health and MRC.

In 1992, the National Impregnated Bed net Program (NIBP) was established jointly by Ministry of Health and MRC with support from WHO and UNICEF. During the first year of implementation when insecticides were supplied free of charge to communities, coverage was 85%. However, the introduction of user fees reduced coverage to 16%. The net user rate is around 60% as revealed from studies done in The Gambia (ITN evaluation 2001, Malaria Situational analysis 2002). The operational approaches of this component include: promoting the use of mosquito nets and ITNs, improving access to ITNs, strengthening partnership and collaboration with relevant institutions, establishing a functional entomological laboratory, piloting indoor residual spraying in selected areas and supporting research in biological control of larvae in rice fields and flood plains.

1.7.4 Programme Management, Coordination and Partnership

Effective malaria control requires the participation of multiple partners at various levels with varying responsibilities and interests. In the light of this, it is important that an effective framework for management, co-ordination and partnership is put in place at all levels. An effective system for management and co-ordination minimizes duplication of efforts and waste of resources. The importance of community participation in malaria control programmes cannot be over-emphasized. There are many types of structures and organized informal groups, with considerable potentials in Gambian communities, but NMCP does not seem to be taking full advantage of this great opportunity. Initiatives to involve communities are often *ad hoc* and sporadic. It is therefore important for the NMCP to involve and use the expertise of these local structures and individuals in the fight against malaria.

CHAPTER 2

Importance of eaves for house entry by mosquitoes

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M. Njie, E .Dilger, S.W. Lindsay & M.J. Kirby. 2009. The importance of eaves to house-entry by Anopheline, but not Culicine, mosquitoes. *Journal of Medical Entomology*, **46**, 505-510.

2.1 Summary

Background: Screening homes is an effective way of reducing house entry by mosquitoes, and closing eaves in particular has been suggested to reduce density of anophelines and culicines entering houses. It is uncertain how important blocking the eaves is at reducing mosquito entry in houses for which there are no other routes of entry. This study was designed to find out whether blocking the eaves, in addition to screening the doors, windows, and sealing cracks in the walls, is essential when constructing mosquito-proof homes in a West African setting.

Methods: Twelve traditional windowless houses in a rural Gambian village were selected, in which a single male adult slept. The doors of these houses were screened and any gaps in the walls were sealed. Six of the houses chosen randomly had their eaves blocked for four weeks then opened for another four weeks. The other six underwent the opposite treatment (a simple crossover design). Mosquitoes were sampled using CDC light traps from each house twice a week during the study period. Mosquito control activities and the number and type of domestic animals within the compound was recorded on each sampling occasion.

Findings: A total 2029 mosquitoes were caught from 187 light traps. *Anopheles gambiae s.s.* was the major vector caught (70.7%). With eaves closed a three-fold reduction in *An. gambiae s.l.* indoors was observed (geometric mean number *An. gambiae s.l,* /trap/night with opened eaves= 6.1, 95% Confidence Intervals, CIs 3.5-10.0; eaves closed = 2.1, 95% CIs 1.3-3.1, t_{11}=3.6, p = 0.004). However, there was no equivalent reduction in total culicine numbers observed (eaves open, geometric mean no. total culicines /trap/night = 2.1, 95% CIs 1.4-3.2; eaves closed = 2.1, 95% CIs 1.4-3.2, t_{11}= -0.07, p = 0.95). No significant difference between median room temperatures of open eave houses (28.8^{o}C, Inter Quartile Range, IQR, 27.7-29.5^{o}C) and that of closed eaves houses (28.7^{o}C, IQR 27.5-29.4^{o}C, t_{178}= -0.4, p = n.s.) was observed. Mean percentage relative humidity was also similar in open- (74.0%) and closed-eave houses (74.2%).

Interpretation: Eaves are a major route by which *An. gambiae* enters houses but in contrast culicine mosquitoes must enter largely through other routes. Sealing the eave gap is an important method for reducing malaria transmission in homes where all other routes of entry are screened or closed.

2.2 Background

Any disease control measure that aims to reduce human-vector contact must first identify, and then modify, the precise locations where this contact occurs. Household factors can account for about 28% of the total variability in malaria incidence (Mackinnon et al., 2005) and from this it can be inferred that the house is clearly a principal point of human-vector contact. This is reflected in the high degree of endophily and endophagy exhibited by the dominant malaria vectors worldwide: *Anopheles gambiae sensu stricto* and *An. funestus* in Africa (Gillies and DeMeillon, 1968), *An. minimus* in much of Asia (Van Bortel et al., 1999, Jana-Kara et al., 1995, Nutsathapana et al., 1986), *An. stephensi* in urban India (Sharma et al., 1993) and *An. darlingi* in parts of South America (Roberts et al., 1987). The ability to enter houses has evolved in some mosquito species and, more specifically, within some species populations but not others (Trpis and Hausermann, 1978). The African malaria vectors *An. gambiae s.s.* and *An. funestus* have evolved with humans and feed late at night indoors when hosts are asleep and less able to protect themselves from blood-feeding mosquitoes (Gillies and DeMeillon, 1968). The attractiveness and ease of entry into a house is affected by structural factors and social practices; a recent study in a semi-rural area of The Gambia showed that *An. gambiae s.l.* are more likely to enter houses with open eaves, mud brick walls and many occupants, whilst burning churai, a local incense, reduced house entry (Kirby et al., 2008a).

The main malaria vectors in The Gambia are members of the *An. gambiae s.l.* species complex, namely *An. gambiae s.s*., *An. arabiensis* and *An. melas* (Bryan, 1979, Lindsay et al., 1993a). These vectors are predominantly nocturnal and endophagic in their feeding behaviour (Lindsay et al., 1993a, Costantini et al., 1999) and, therefore, seek entry to dwellings occupied by humans at night. Much of the malaria transmission in this setting takes place at home, with around 80% occurring indoors (Lindsay et al., 1995) and so mosquito-proofing homes should be effective against malaria transmission here by reducing exposure to parasites. There are three major routes of entry into a typical West African house, that is, through the doors, windows or the eave gap, that is, the gap between the top of the wall and the roof. Closing eave gaps alone has been shown previously to reduce the density of anophelines (Lindsay and Snow, 1988) and culicines (White, 1969) entering houses, however the relative importance of closing eaves compared to closing the other routes of entry has not been fully explored. This study was designed to determine whether blocking the eaves, in addition to screening the doors, is essential to the design and construction of mosquito-proof homes in West Africa. It was carried out alongside a larger, randomized controlled trial

(ISRCTN51184253) assessing whether house screening can substantially reduce exposure to malaria vectors, and so reduce parasitaemia and anaemia in children sleeping in those houses.

2.3 Materials and Methods

2.3.1 Study area

The study village, Dibba Kunda Wollof, is situated approximately 170 km from the mouth of the River Gambia and 15km East of Farafenni town (UTM coordinates: 1500200N, 435500E) in the North Bank Division in The Gambia. The area is dominated by open Sudan savanna vegetation, and the climate consists of a single rainy season from June to October followed by a long dry season. Dibba Kunda Wollof comprises 1,226 people, predominantly farmers by trade. 98% belong to the Wollof ethnic group and there are roughly equal numbers of men (47%) and women.

2.3.2 Study houses and treatments

Houses in the village are typically arranged in familial compounds demarcated by a fence or wall, containing usually four to six houses, but sometimes as many as 20. Twelve similarly-sized, single-roomed houses with two doors, unplastered mud-brick walls, a thatched roof and open eaves, in which a single adult male slept, were selected for inclusion in the study. A full written consent agreement was obtained from the occupants of the houses, and each occupant provided with an untreated bed net.

All 12 houses had their doors screened (fig.2.1a and b). Doors were made of 15 x 30mm softwood, strengthened at the corners, with PVC-coated fibreglass netting (Vestergaard Frandsen group, Kolding, Denmark) stapled to the frame. A handle was fixed to both sides of the door and a catch on the inside helped to hold the door tight in the frame when shut. Elastic cord was attached to the outside of the door to keep the door closed when not in use.

Fig. 2.1a) screened door, b) eaves closed with rubble and mortar

For the first four weeks of the study six of the houses had their eaves completely closed with a mixture of sand, rubble and cement (fig 2.1b), whilst the eaves of the other six houses remained open. The list of houses and of possible treatments were randomised and then paired up using the list randomiser function available at www.random.org. These treatments were then crossed over; the eave filling was broken up and removed from the first group and the second group had the eaves closed in the same manner. The houses were then observed for another four weeks. Eaves were closed completely, rather than screened in the manner of the doors, for two reasons. Firstly the irregular surfaces of the thatched roof and the mud brick wall made it difficult to achieve a tight closure using the netting; secondly our design replicated the local method of closing eaves.

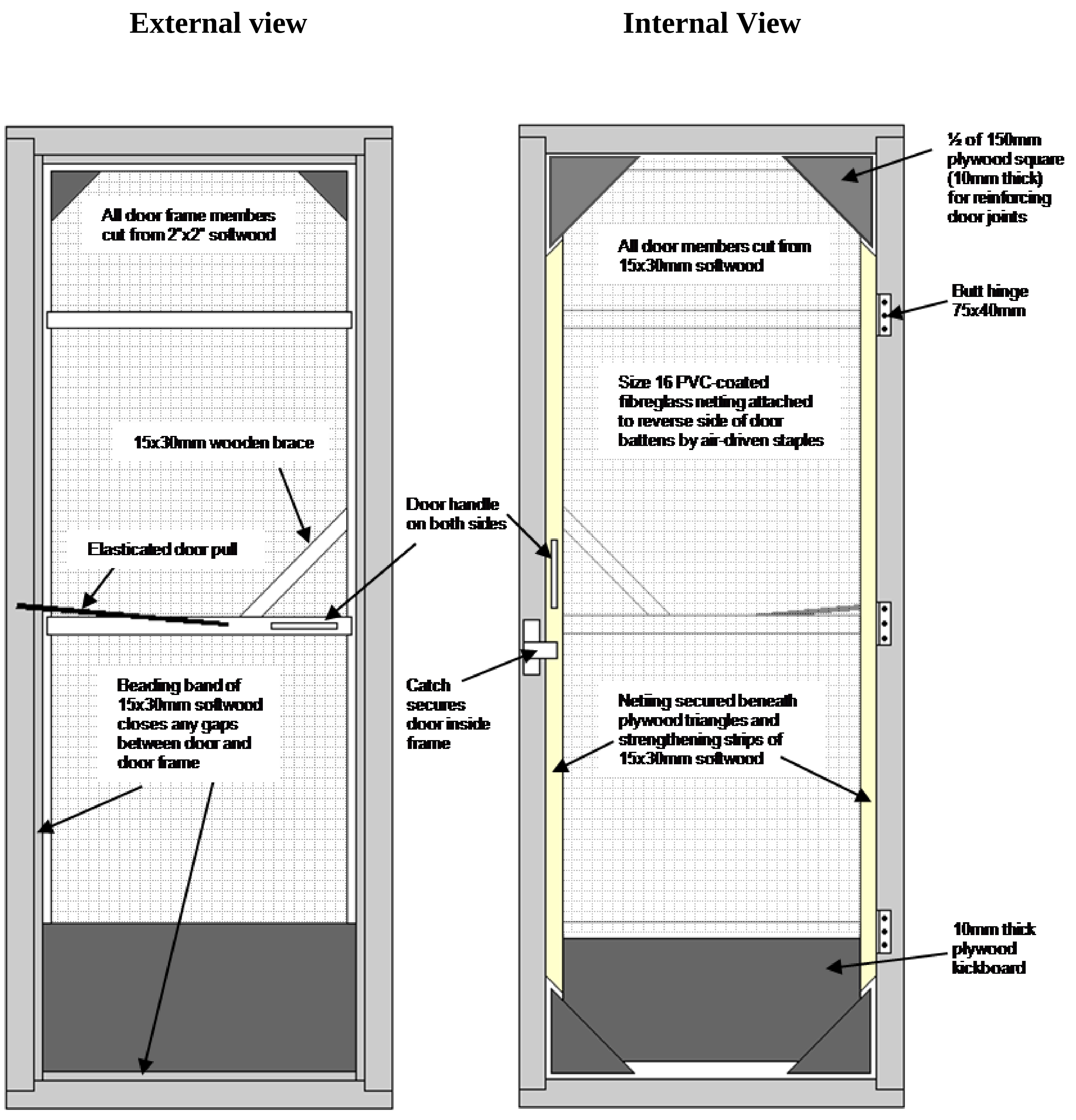

Fig. 2.2 Door design used during the study to screen doors (adapted from WHO Offset Publication no.66 Environmental Management for Mosquito Control).

2.3.3 Mosquito collections

The 12 houses were each sampled twice weekly between 3 September and 6 November 2007. A CDC miniature light trap (Model 512, John W. Hock Co., Gainesville, FL, fig. 2.3) was positioned 1m above the ground within 1-2m of the foot end of a bed. A village assistant was appointed to distribute the traps to the houses, put on the light trap at 19:00h and help the investigator to collect them the following morning at 07:00h. A screening questionnaire form was filled for each trapping house to record house data, risk factors and the environmental data (see appendix 3). Mosquitoes were killed by freezing at -25^{o}C for two hours and identified using morphological criteria. Where possible, 30 *An. gambiae s.l* from each house were identified to species by polymerase chain reaction (PCR) analysis (Scott et al., 1993).

Fig. 2.3 CDC light trap (photo source: www.johnwhock.com)

2.3.4 Putative risk factors and environmental factors.

The number of cows and horses in the compound between 19.00h and 07.00h on each night of trapping was recorded. Despite the request that only a single sleeper resided in each house, the number of occupants was recorded in case this changed during the course of the study. The use of mosquito coils, local incense (churai), and insecticide spray within the house on the night of trapping was also recorded, as participants were not discouraged from using anti-mosquito measures. In each house a single data logger (Model HOBO® U12 Temp/RH/Light External Data Logger, Onset Computer Corporation, Bourne, MA) was suspended next to the light trap to record temperature (^{o}C) and percentage relative humidity (%RH) every 30 minutes. The loggers were pre-set to turn on automatically at 19.00h. Loggers were collected each morning at 07.00h, switched off, and the data downloaded to Onset HOBOware™ version 2.0 software (Onset Computer Corporation, Bourne, MA).

2.3.5 Statistical analysis

Mosquito data within houses was described by arithmetic mean *An. gambiae s.l.* per trap per night. Variation between houses was positively skewed and therefore described by geometric mean. A paired-samples t test was used to compare the differences in mosquito catch between treatments. Room temperature within treatment group was negatively skewed and therefore this variable was reflected by subtracting every score from a constant that was one greater than the highest score, and then square-root transformed. A comparison of room temperature and relative humidity between the treatments was performed on normalized data using an independent-samples t test. A generalized estimating equation (GEE) was used to estimate treatment effects, allowing for repeated measures in the same houses, and including an adjustment for the covariates, week of trapping and crossover group. Mosquito data (counts) were fitted to a negative binomial distribution with a log link function. House ID was used as subject unit for repeated measure assuming an exchangeable correlation matrix. All analyses were done using SPSS version 15.0 (SPSS Inc, Chicago, IL).

2.3.6 Ethical approval

Approval for this study was given by the Gambia Government/Medical Research Council Joint Ethics Committee and Durham University Ethics Advisory Committee. Verbal and written consent was obtained from home owners prior to the start of the study.

2.4 Findings

2.4.1 Data exclusions

Four light traps failed during the trapping period. On one night only a house was occupied by more than one inhabitant. These data were excluded from the analysis.

2.4.2 Mosquito numbers.

2,029 mosquitoes were caught from 187 light trappings, of which 914 (45%) were *An. gambiae s.l.*, <1% were other anophelines and 54% were culicines. The other Anopheline species caught were *An. ziemanni* (11 specimens), *An. pharoensis* (4) and *An. squamosus* (1). The common culicines were *Culex thalassius* (906), *Cx. quinquefasciatus* (126), *Aedes aegypti* and *Ae. vittatus* (all *Aedes spp* combined = 43). A total of 499 *An. gambiae s.l.* specimens were identified to species by PCR; 70.7% were *An. gambiae sensu stricto*, 25.3% *An. melas* and 3.6% *An. arabiensis*. 0.4% failed repeat amplification.

2.4.3 Treatment effect.

Fig. 2.4 summarizes the variation in catch size between treatments during the study for *An. gambiae s.l.* and for all culicines. When houses had their eaves closed there was a 65% reduction in *An. gambiae s.l.* caught indoors (eaves open, geometric mean no. *An. gambiae s.l.* per trap per night = 6.1, 95% CIs 3.5-10.0; eaves closed = 2.1, 95% CIs 1.3-3.1, t_{11}=3.6, p = 0.004). However, there was no equivalent reduction in total culicine numbers observed (eaves open, geometric mean no. total culicines per trap per night = 2.1, 95% CIs 1.4-3.2; eaves closed = 2.1, 95% CIs 1.4-3.2, t_{11}= -0.07, p = 0.95).

No mosquito coils, local incense (churai), insecticide spray or insecticide-treated nets were used in any house during the study. These factors were therefore not incorporated in the GEE model. Incorporating the effects of trapping week and cross-over group, and adjusting for the numbers of horses and cows in the compound, *An. gambiae s.l.* were three times less likely to be found in houses with closed eaves compared to houses with open eaves (OR = 0.34; 95% CI = 0.20-0.56; $P < 0.001$). However the treatment had no impact on total culicine house entry (OR = 1.05; 95% CI = 0.65-1.70; *P* = n.s.). The presence of horses in the compound reduced the number of total culicines caught (OR = 0.46; 95% CI = 0.32-0.64; $P < 0.001$, fig 2.5) but not the number of *An. gambiae s.l.* (OR = 0.27; 95% CI = 0.01-6.21; p = n.s.). The presence of cows in the compound had no effect on culicine (p = 0.28) or *An. gambiae s.l.* numbers (p = 0.42).

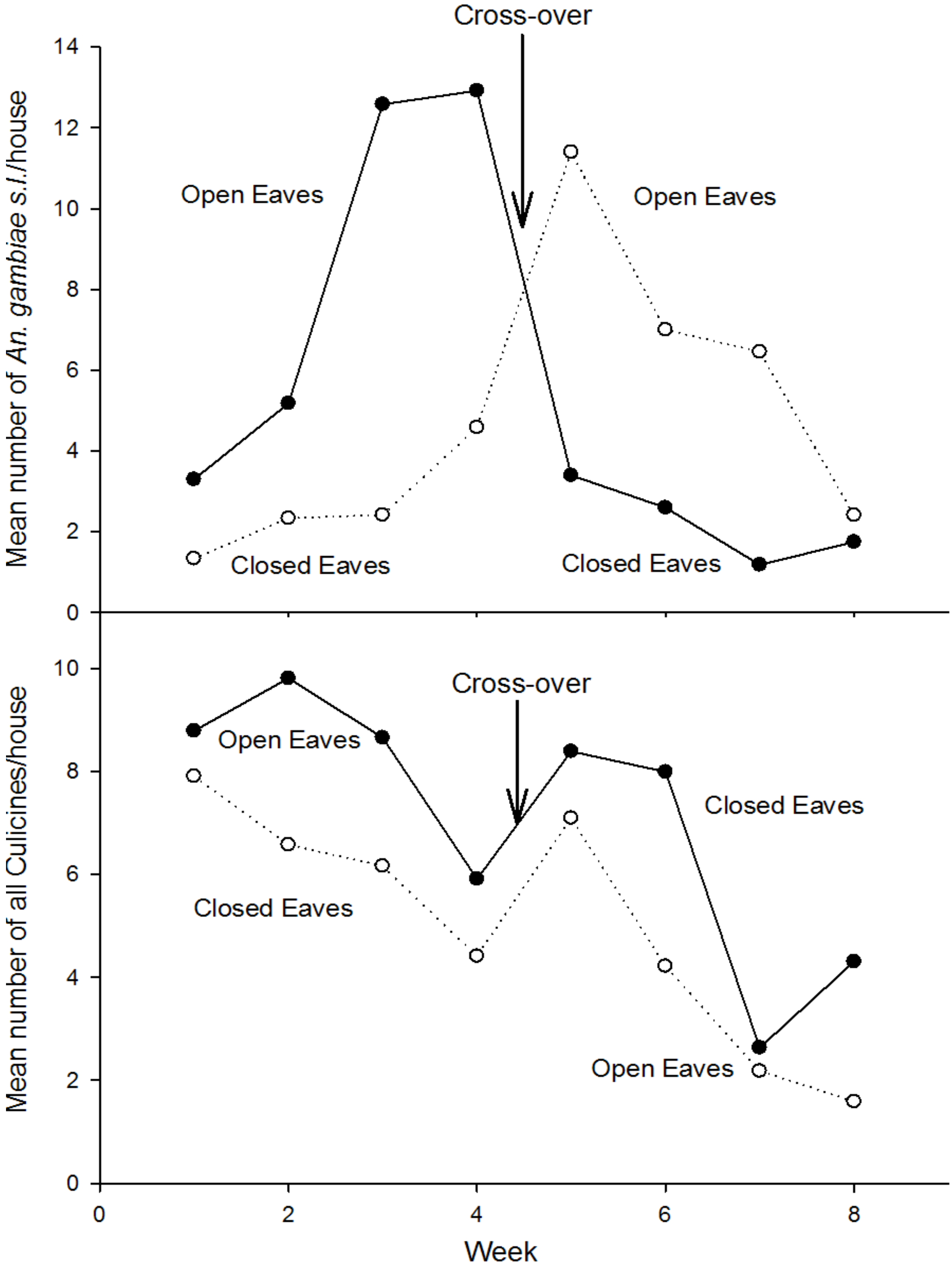

Fig. 2.4 Mean number of *An. gambiae s.l.* and all culicines caught/trap/night caught from two crossover groups of six houses with eaves open or closed.

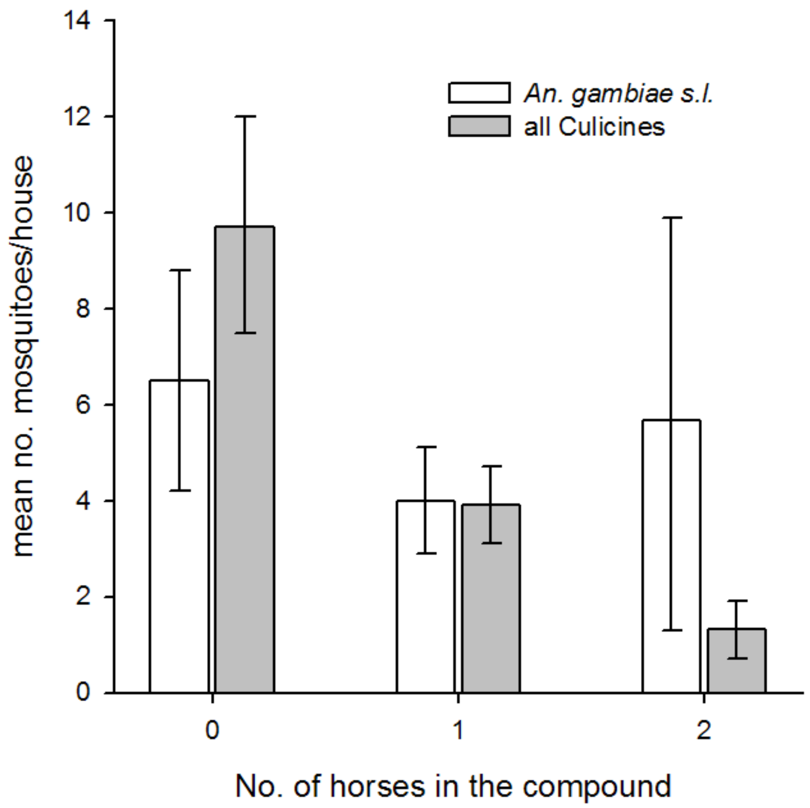

Fig. 2.5 Relationship of mean number of mosquitoes caught/trap/night with the number of horses in the compound

There was no difference in the median room temperatures of open eave houses (28.8^{o}C, Inter Quartile Range, IQR, 27.7-29.5^{o}C) and that of closed eaves houses (28.7^{o}C, IQR 27.5-29.4^{o}C, t_{178}= -0.4, p = n.s.). However it was significantly hotter during the second part of the study (29.3^{o}C, IQR 28.7-29.7^{o}C, t_{178}= 6.7^{o}C, p = <0.001) than the first (27.9^{o}C, IQR 27.1-28.9^{o}C). Similarly, though there was no difference in the mean percentage relative humidity between open eave houses (74.0%, 95% CIs 73.0-75.1%) and closed eave houses (74.2%, 95% CIs 73.2-75.3%, t_{178}= -0.3, p = n.s.), it was 8% more humid during the first period of the study (78.4%, 95% CIs 77.8-79.0%) than the second (70.4%, 95% CIs 69.6-71.1%, t_{178}= 17.0, p = <0.001).

2.5 Discussion

It has been demonstrated here that closing the eaves results in a 66% reduction in the number of *An. gambiae s.l.* entering houses in which the eave gap is the major route of entry for mosquitoes. This is a greater reduction than the 43% seen between open- and closed-eave houses in a previous study in The Gambia (Lindsay and Snow, 1988). Similarly closing the eaves, whilst leaving the windows and door slightly ajar, resulted in only a 39% reduction in *An. gambiae s.l.* house entry in an experimental hut study, again conducted in The Gambia (Lindsay et al., 2003). In both of these earlier studies the results probably reflect the fact that many mosquitoes will have entered through the doors and windows.

Installing a well-fitted ceiling has a similar effect to that of closing eaves on reducing mosquito house entry, acting as a barrier to mosquitoes attempting to enter the room space. Netting ceilings reduced exposure to *An. gambiae s.l.* by 80% and *Mansonia spp* by approximately 70% in experimental huts, even when the windows and door were left ajar (Lindsay et al., 2003). Comparable reductions in *An. gambiae s.l.* and *Mansonia uniformis* entrance were achieved by fitting Louvre traps to openings created one foot below the roof (Smith et al., 1972). These results all suggest *An. gambiae s.l.* seek initially to enter huts and houses predominantly through the open eaves. Routes of entry into houses are recognized by olfactory cues i.e. the carbon dioxide and body odours emanating from the hosts inside (Bertram and McGregor, 1956). *Anopheles gambiae s.l.* flies typically at a low (<1m) (Snow, 1979) or intermediate height (Gillies and Wilkes, 1976), and so it seems that it must fly upwards when encountering a vertical wall surface, following the cues emanating from the eaves and becoming channelled indoors through the open eaves by the overhanging roof. This concept is supported by the evidence that increasing wall height results in only a slight decrease in house entry for *An. gambiae s.l.*, despite their typical vertical distribution (Snow, 1987). This upward flight behaviour is probably a common trait of endophagic mosquitoes only. *Anopheles vestitipennis* lands low down on exterior walls before moving along walls in short flights either vertically or horizontally (Grieco et al., 2000). Thus doors (44%) are the primary route of entry for this species, though windows and eaves are also important (both 26%) and cracks in the walls are less so (4%) (Grieco et al., 2000). Similarly *An. pharoensis* will enter houses if presented only with a ground level opening but not if presented with an opening only at eave level (Snow, 1987). In the present study only 16 individuals of other anopheline species were caught, 6 from open eave houses and 10 from closed eave houses. Among these were the low-flying *An. pharoensis* (Snow, 1979). Though these numbers are too low to be statistically useful, they do hint at the possibility that this upward flight behaviour is unique to *An. gambiae s.l.* amongst the anophelines in this setting.

While the focus of this research was *An. gambiae s.l.*, the most important vectors of malaria in Africa, some of the culicines trapped are also important vectors of disease, and thus the effect of control measures against these should also be considered. Approximately

10% of the culicines caught were *Cx. quinquefasciatus*, vectors of lymphatic filariasis in East Africa, approximately 2% were *Ae. aegypti*, vectors of Yellow Fever and dengue, and 2% were *Mansonia africana* or *Mn. uniformis*, also vectors of lymphatic filariasis and of Rift Valley Fever and West Nile Virus in nearby Senegal (Diallo et al., 2005). The observation that closing the eaves has a much greater impact on *An. gambiae s.l.* house entry than on most culicines merits an explanation. Mosquito species with opportunistic feeding behavior may readily take human bloodmeals outdoors but fail to feed once humans retreat indoors. There are three potential explanations for this restriction. Firstly, repeated failure to enter through the narrow apertures of doors, windows or small holes in walls; secondly the inaccessibility of eaves to low-flying mosquitoes and the absence of the upward flight behaviour seen in *An. gambiae s.l.*, and lastly the inability to locate a host in a darkened or micro climatically altered environment (Gillies, 1988). As very few bloodfed mosquitoes were caught, probably because indoor light trap catches are biased towards catching unfed females (Service, 1976), it is not appropriate to comment on the last possibility here. We have at least in part controlled for the first explanation by choosing houses with no windows, screening their doors and filling small holes in the walls. Of course the eaves also represent a narrow aperture and as such may be impenetrable to the culicines rather than unencountered due to vertical distribution. If this were true it might be expected that culicine house entry success would vary with eave gap size. This has been shown for several culicine species (White, 1969). However in the present study the size of the eave gap was very similar for all houses, and in another study in the same location, eave gap size did not show a relationship with culicine house entry (M.J.K., unpublished data). That leaves the possibility that these culicine species are flying at a different height to *An. gambiae s.l.* and/or do not change their flight behaviour when coming into contact with house walls. *Mansonia uniformis*, *M. africana* and several *Aedes* species are consistently reported as a low flying species with a high percentage of the total catch taken in traps below 1m from the ground (Snow, 1979, Gillies and Wilkes, 1976, Snow, 1975). This might in part explain the unimportance of eave closure to the numbers of these species caught in the present study, where it was seen that similar proportions of *Aedes spp* and *Mansonia spp* were caught from houses with eaves closed and eaves open (1.5:1 and 0.7:1 respectively). However the evidence of other research suggests *Mansonia spp* do favour eaves as routes of entry (Snow, 1987, Lindsay et al., 2003), so the fact that this was not witnessed here may be an artefact of the small numbers (17) caught rather than their vertical distribution.

The vertical distribution of *Cx. thalassius*, the most common culicine caught in the present study, cannot explain why closing the eaves does not reduce house entry by this species. It has no obvious vertical flight limitation, being abundant at all sampling heights (Snow, 1979). Nevertheless, *Cx. thalassius* has been shown to be prevented from entering houses by increasing wall height; a 62% decrease in *Cx. thalassius* house entry was observed between a hut with a 60cm wall compared to one with a 172cm wall (Snow, 1987). This suggests that vertical distribution is only one of several factors that could explain the ineffectiveness of eave closure on culicine house entry. An alternative possibility is that the culicines are better adapted to enter through the doors than *An. gambiae s.l.* Despite the fact that the doors were screened, it was observed that often these doors were propped open during daylight hours, only being closed at 19:00-20:00h. Most of the culicines caught in this study are active during diurnal or crepuscular hours and are thus better placed to take advantage of the open doors than *An. gambiae s.l.*, which is active at night after the doors have been closed. In summary it can be said that the importance of entry route treatments on the indoor abundance of culicines is unclear in this study; other studies have found few or contradictory relationships between entry route sizes or treatments and culicine indoor catch (Howell and Chadee, 2007, Charlwood et al., 2003, Kohn, 1991)

Here we have shown that the presence of horses, but not cows, in the compound reduces house entry by culicine mosquitoes. Many culicines are opportunistic feeders and it is likely that horses, abundant in this area, are an important bloodmeal host. It has previously been demonstrated that the presence of horses in the compound also reduces house entry by *An. gambiae s.l.* (Kirby et al. 2008). That we failed to demonstrate that here is probably a result of the small sample size.

Most people in rural Gambia do not appreciate the significance of closing eaves in relation to mosquito house entry. They usually relate it to a reduction of house ventilation and an increase in indoor temperatures. However in this study no significant difference in temperature was observed between open and closed eave houses, contrary to a previous survey in The Gambia of open and closed eave houses, which found that closing the eaves increased indoor temperatures by $1^{o}C$ (Lindsay and Snow, 1988). It is suggested that in the present study the external environment contributed more to temperature within the houses than the eave treatment. The rains stopped close to the crossover date and it is probable that the external temperature after the crossover was higher compared with before; unfortunately no outdoor temperatures were recorded in the study village.

2.6 Conclusion

There is compelling evidence that house screening is associated with protection against malaria transmission, infection and morbidity (Lindsay et al., 2002). Our studies illustrate that blocking the eaves in houses with well-screened doors and windows is essential for reducing house entry by anophelines, although it is of no benefit against culicines. It has been shown here that if house screening against malaria is to be effective in The Gambia then blocking the route of entry through the eave gap to the room space, by closing eaves or installing ceilings, must be of primary importance.

CHAPTER 3

Experimental hut evaluation of insecticide treatment of damaged screened ceilings against house entry by malaria mosquitoes

3.1 Summary

Background: House design may affect the exposure of the house occupants to disease vectors. The installation of netting ceilings has been shown to be very effective at reducing mosquito house entry. However, these ceilings have a limited life-span and holes and tears will appear over time, compromising their effectiveness. It is therefore important to determine whether insecticide-treated torn netting ceilings are as efficacious as intact ceilings.

Methods: Six experimental huts were used in which a single adult male slept. A 12-night pilot study determined whether there were any residual insecticidal effects of the use of treated ceilings within the huts. In the main study three insecticide-treated damaged ceilings were tested against three controls. All damaged ceilings had 5 x $10cm^2$ holes, one at each corner and one at the centre. The insecticides were deltamethrin ($55mg/m^2$), permethrin ($500mg/m^2$) and chlorpyrifos ($500mg/m^2$). The controls were an intact untreated ceiling, a water-treated damaged ceiling, and a hut with no ceiling. The door and window of each hut was left ajar.

Results: The pilot study did not show any significant residual effect of treated ceilings on either the number of mosquitoes caught alive inside the huts or on the number of dead mosquitoes recovered (P=0.08 and 0.29 respectively), therefore the six treatments were rotated nightly between the huts for 48 consecutive nights. A total of 18,760 mosquitoes were caught in the main study of which 9,132 (48.7%) were *Anopheles gambiae* s.l. In comparison with the 'no ceiling' control (mean no. *An. gambiae* s.l./hut/night = 45.7, 95% CI 34.8-56.7), the intact untreated ceiling reduced *An. gambiae* s.l. house entry by 55%, the water-treated damaged ceiling by 29% (32.4; 23.4-41.3, p=0.06) and the insecticide-treated damaged ceilings by 29-36% (deltamethrin = 29.1, 21.8-36.5, p = 0.03; permethrin = 30.0, 23.3-36.8, p = 0.05; chlorpyrifos = 32.4, 22.4-42.4, p = 0.045). The highest number of dead mosquitoes was recovered from huts with chlorpyrifos-treated ceilings (4/hut/night) and huts with permethrin-treated ceilings had the least blood-fed mosquitoes (1.8/hut/night).

Interpretation: Intact ceilings reduced the number of mosquitoes entering houses by more than a half. However, with torn ceilings, treating them with insecticide did not result in a significant reduction in entry of malaria mosquitoes when compared to water-treated torn ceiling huts. Dipping PVC-coated nylon netting was an ineffective method of applying the insecticide, and alternative application techniques for this type of netting must be developed.

3.2 Background

Modifications to the house environment can take many forms. Reducing proximity to neighbours and mosquito breeding sites by careful site selection will reduce exposure to malaria vectors, but is not usually an option for most rural African house-owners restricted by the cost of available building plots. Such inhabitants will also find it hard to afford to construct their houses from quality long-lasting materials and to build houses large enough to avoid over-crowding, yet by these improvements malaria incidence was reduced in the USA (Hackett and Missirolli, 1932, Fullerton and Bishop, 1933b, Reiter et al., 2003).

But there is another option: house screening is a low-cost option that can be made from locally-available materials and quickly installed in existing house structures. Several studies (reviewed in (Lindsay et al., 2002)) have provided compelling evidence that house screening is associated with protection against malaria transmission, infection and morbidity. House screening was used to great effect to protect against malaria in Italy, Greece, Panama and the USA in the early 20th century.

A recent study in The Gambia using experimental huts demonstrated that netting ceilings reduced exposure to malaria vectors by 80% (Lindsay et al., 2003). Sleepers in the huts attracted mosquitoes emerging from the nearby irrigated rice fields, which then entered the huts through the open eaves but were prevented from entering the room space by a netting ceiling (fig. 3.1).

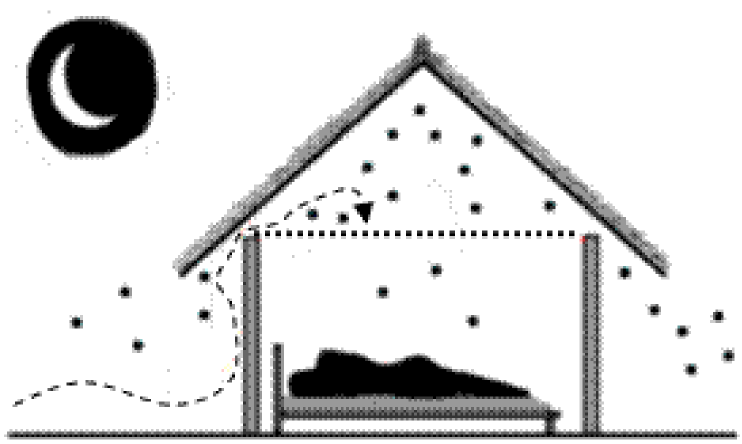

Fig. 3.1 Path taken by house-entering mosquitoes, attracted by host odour (dots). The netting ceiling keeps mosquitoes in the roof and prevents access to the room space (Source: Lindsay et al 2003).

In practice these screened ceilings will develop holes and tears. The Screening Homes to Prevent Malaria trial (STOPMAL) of house screening was run concurrently with this study in The Gambia, using the same netting to screen doors, windows and ceilings of houses. A STOPMAL durability study showed that some damage occurs to the netting within six months of installation. It was found that 26% of ceilings were intact i.e. no holes, 72% had some damage and 2% were badly damaged or removed altogether. The number and size of holes varied considerably between houses. There were on average 0.6 holes of 3-10cm diameter (max. 8 holes), 2.5 holes of 1.5-3cm diameter (max. 10 holes) and 1.7 holes of <1.5cm (max. 10 holes) (Kirby, unpublished).

Insecticides have been used in the past for the control of disease vectors and agricultural pests. Some have been proven to be effective in malaria control and have since been used in district and national malaria control programs. The use of bednets, highly accepted and widely used by local people, was proven to be significantly effective in malaria prevention when impregnated with insecticides (Lindsay et al., 1991, Miller et al., 1991). They found that impregnating bednets with pyrethroids like permethrin reduced number of human bloodfed mosquitoes by 90% and lambda-cyhalothrin killed a significant proportion of endophilic mosquitoes (91%) when compared to the untreated nets. Some researchers also considered impregnation of curtains draped inside the walls of houses, across doorways, windows and even under open eaves as appropriate mosquito control in small houses (Majori et al., 1987, Mutinga et al., 1992). To test these findings, this study was design to determine whether insecticide impregnation of netted ceilings will protect against mosquito house entry when the screens have holes and also test the efficacy of three different insecticides on torn ceilings.

3.3 Materials and Methods

3.3.1 Study Area

The study was carried out at MRC Wali Kunda (13° 34′N, 14° 55′W), a small field station in rural Gambia from 27th June to 7th August 2007. The area as described by Miller et al (1991) is situated in an area of Sudan savannah on the south bank of the River Gambia, approximately 290km from the coast. The station is mainly used for entomological research as it is perfectly located close to a large area of irrigated rice fields in the south which serve as the main mosquito breeding sites. The station is situated in the western part of the village of less than 100 inhabitants, who are mainly fishermen.

3.3.2. Huts

The six identical experimental huts (fig 3.2a), approximately 2m x 2m,were made with mud walls, thatched roof, open eaves, a veranda and window on each side and a door to the south and arranged in a straight line, 12m apart. The huts were raised 50cm off the ground on concrete legs surrounded by water-filled moats to prevent infestation of ants that might forage on dead mosquitoes. The east and west sides of the huts were fitted with screened verandas to capture mosquitoes exiting through the eaves and 30cm^3 exit traps to capture mosquitoes leaving through the windows (fig 3.2b).

Fig. 3.2 a) Experimental huts at Walikunda, b) Veranda trap with exit trap fixed over window

Mosquitoes could enter each hut through the eaves and windows on the north and south sides and the door. The door and windows were held ajar to simulate village conditions (30mm gap on the windows; 20mm gap between the edge of the door and frame). Mosquitoes could leave the room via the eaves, where open, or through the windows and door. Mosquitoes that left via the north and south sides were lost, but those leaving on the east and west sides were captured in the exit traps or enclosed verandas.

3.3.3 Ceilings

PVC-coated fibreglass netting (Vestergaard Frandsen group, Denmark) was used for the ceilings. The 'damaged' ceilings had 5 (10cm×10cm) holes cut into them (1 at each corner and 1 in the centre). Investigators and sleepers were blinded to the identity of the four ceilings with holes. An independent scientist was assigned to treat and number of the ceilings and only released their identity after the analysis. Each treatment was randomly allocated a number using the list randomiser function available at www.random.org.

Five different treatments were tested against a control of no ceiling: intact ceiling, water-impregnated torn ceiling, deltamethrin-impregnated torn ceiling 55mg/m^2, permethrin-impregnated torn ceiling 500mg/m^2 and chlorpyrifos-impregnated torn ceiling 500mg/m^2. Ceilings were installed below the open eaves and following an imperfect Latin square design (Design-Expert® Software) of six by six, ceilings were rotated every day between 16:00h-18:00h. During the pilot study, battens were attached to the ceilings and screwed to the hut walls. It was difficult and time consuming to release the battens from the walls when rotating ceilings. So, in the main study, a strip of Velcro was sewn to the ceilings leaving a gap of 10cm from the edges, and the second strip was stapled to battens permanently fixed to the walls. This made the rotation of the ceilings easier and quicker.

3.3.4 Human Subjects

Six Gambian adults aged between 18-60yrs were recruited as 'sleepers'. One sleeper was from Wali Kunda itself and the rest from Wellingara village, approximately 3km south of the study site. They were randomly allocated to one hut each for the duration of the trial. Every night they slept under an untreated bed net between 21:00-06:00h. The sleepers gave their consent to participate in the trial (see appendix 1) and each was paid 50 dalasis (USD2.5) per night, and provided with a bicycle and rain coat to facilitate their travel from the villages to the study site each day.

3.3.5 Temperature and Humidity

Room temperatures were recorded with a maximum-minimum thermometer (ALLA®, France) and evaporation measured (fig. 3.3) between 21:00hrs and 06:00hrs with a Piche evaporimeter (Casella CEL, Kempston, Bedfordshire, UK).

Fig. 3.3 Maximum and minimum thermometer and evaporimeter

3.3.6 Mosquito Collection and Identification

At 05:00h both the door and the north and south windows of each hut were closed to prevent mosquitoes leaving. At 06.00h sleepers left the huts and the window traps were plugged with a piece of cloth to ensure captured mosquitoes did not escape. All huts and the enclosed verandas were searched for mosquitoes between 06.45h and 10.30h for a total of 30 man-minutes per hut (15mins in the room and 7.5mins in each veranda). Exit traps from each hut were emptied after the 30min searching (fig. 3.4). Mosquitoes were held in paper cups, labelled with hut number, source, state (alive or dead), and taken to the laboratory for identification using a dissecting microscope. Live mosquitoes were killed by freezing at -25^{o}C for two hours and identified using morphological criteria.

All blood-fed anophelines and culicines were stored for bloodmeal ELISA described by (Burkot et al., 1981). Bloodfed *An. gambiae s.l* were also tested for sporozoites (Burkot et al., 1984). One unfed *An. gambiae s.l.* was collected from each hut everyday for Polymerase Chain Reaction (PCR) identification (Scott et al., 1993). Mosquitoes were stored singly in eppendorf tubes half filled with drierite (W.A. Hammond Drierite Co. Ltd, Xenia, USA); cotton wool and filter paper were used as stoppers to prevent direct contact with the chemical. All stored samples were transported to Farafenni Field Station for analysis.

Figure 3.4 Exit trap being emptied by the investigator

3.3.7 Statistical analysis

In order to determine whether insecticide-treated ceilings had a residual effect the pilot study, I compared the ratio of (1) number of mosquitoes and (2) number of dead mosquitoes caught the following day if a hut had a treated ceiling followed by an untreated ceiling the previous day and after. Total number of mosquitoes entering huts was obtained by doubling the catches from the veranda traps and adding it to the catches from the room and exit traps. Mosquito numbers per hut/intervention were described by an arithmetic mean. Comparison of the mosquito numbers between treatments was carried out using a multivariate analysis (general linear model). The dependent variable was log transformed and fitted to a negative binomial model. Maximum and minimum temperatures, plus evaporation over night were included as covariates. An independent samples t-test was used for the pilot study analysis. All statistical analysis was performed using SPSS version 15.0 (SPSS Inc. Chicago, USA).

3.4 Findings

3.4.1 Mosquito house entry

During the pilot study, a total of 2,210 mosquitoes were caught. To test the assumption that there was no residual effect of insecticide, I compared the geometric mean no. of *An. gambiae* from two groups of huts with an untreated ceiling; those that had an untreated ceiling the preceding day and those that had an insecticide-treated ceiling the preceding day. There was no significant effect on *An. gambiae s.l.* (Alive P =0.08 and Dead P=0.29). Most of the dead mosquitoes were found in the exit traps and one would assume that in any residual effects of insecticides, high numbers of dead mosquitoes would have been found in the room which was not the case here.

During a 48 night trial, a total number of 18,760 mosquitoes entered the six experimental huts. Out of this, 9,132 (48.7%) were *An. gambiae* s.l., 6,224 (33.2%) were *Mansonia* spp, 2,665 (14.2%) were other culicines mostly *Cx. thalassius* and *Cx. quinquefasciatus*, and 739 (3.9%) were other anophelines including *An. pharoensis*, *An. zeimanni* and *An. rufipes.*

A total of 18,760 mosquitoes were caught in the main study of which 9,132 (48.7%) were *An. gambiae s.l.*, 6,224 (33.2%) were *Mansonia* spp, 739 (3.9%) were other anophelines and 2,665 (14.2%) were other culicines.

Of the *An. gambiae s.l*, 625 were caught dead and 540 were caught bloodfed. As shown is table 3.1, most mosquitoes entered the hut without a ceiling and as expected, less entered the hut with an intact ceiling. All insecticide-treated ceilings had similar reduction to the water-treated torn ceiling in malaria mosquito entry.

Table 3.1 Means & odds ratios of *An. gambiae s.l.* caught/hut/night by treatment

Intervention	Williams mean (95% CI)	p	Odds Ratio (95% CI)
No ceiling	34.2 (27.2-42.8)	-	1.00
Intact ceiling	20.6 (15.7-25.6)	<0.001	0.46 (0.31-0.70)
Water-treated ceiling	32.4 (23.4-41.3)	0.062	0.67 (0.45-1.02)
Deltamethrin-treated ceiling	29.1 (21.8-36.5)	0.026	0.63 (0.41-0.94)
Permethrin-treated ceiling	30.0 (23.3-36.8)	0.05	0.66 (0.44-1.00)
Chlorpyrifos-treated ceiling	32.4 (22.4-42.4)	0.045	0.65 (0.43-0.99)

3.4.2 Blood feeding

Of all mosquitoes caught 652 (3.5%) were bloodfed, of which 553 were *An. gambiae s.l* and 99 were *Culex* spp. Out of the *An. gambiae s.l*, 139 (25%) were found to have fed on humans, and the rest fed on equines. 132 were caught alive and 7 dead. It was also found that only the intact ceiling (P=0.002, OR=0.45) and the permethrin treated ceiling (P=0.016, OR=0.55) showed significant reduction in blood feeding over the no-ceiling hut. The bloodfed *An. gambiae s.l* were also tested for presence of sporozoites. Out of the 553 tested, 7 (1.3%) were found to be positive.

Most bloodfed mosquitoes were collected in the room and were alive irrespective of treatment (fig. 3.5). The intact and permethrin-treated ceilings provided the best protection against bloodfeeding.

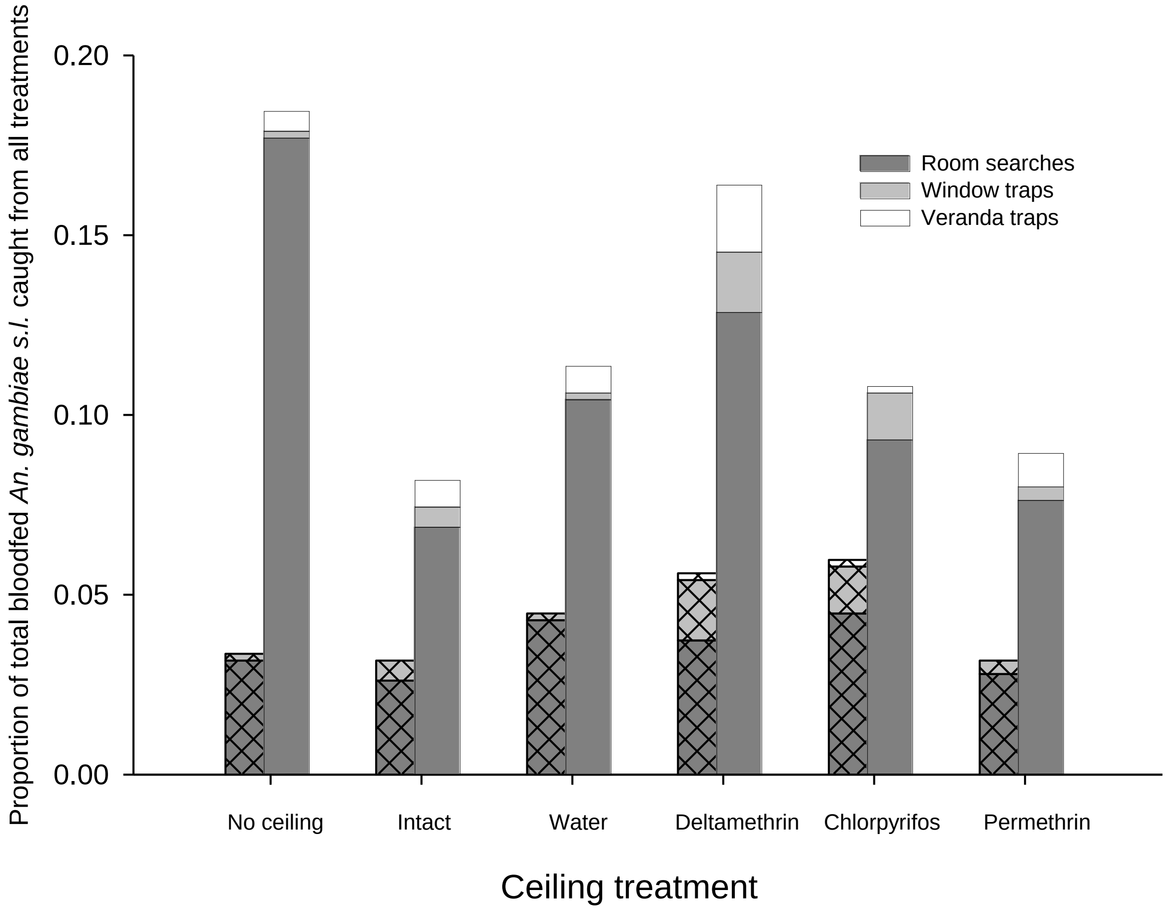

Fig. 3.5 Proportion of alive (unhatched bars) and dead (hatched bars) bloodfed *An. gambiae s.l.* females collected in different parts of the huts between treatment groups.

3.4.3 Mosquito mortality

Of mosquitoes caught in the experimental huts 7.5% (625/8307) were dead. Fewer culicines died than anophelines (fig. 3.7), but there was no difference in mortality rates between treatments.

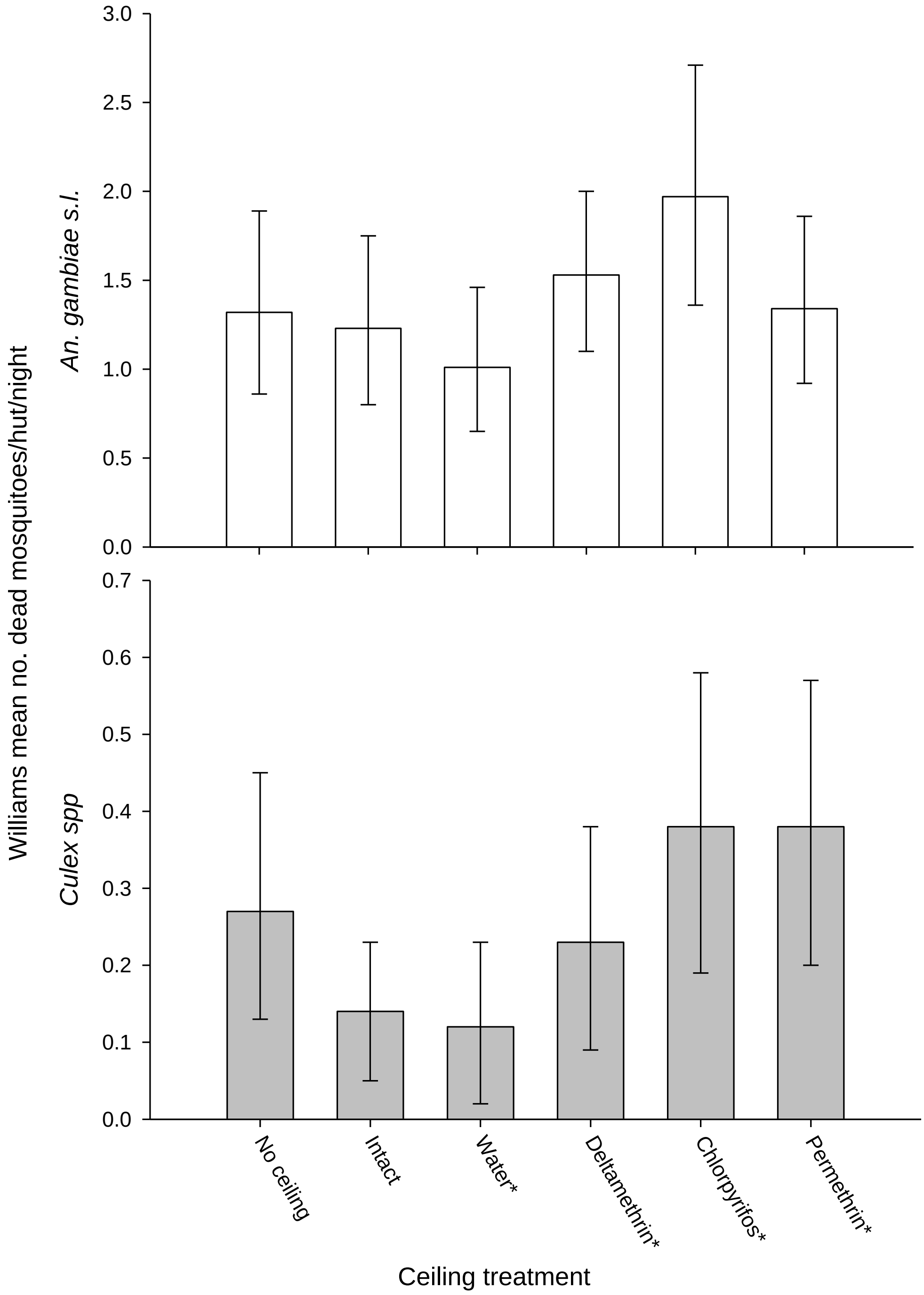

Fig. 3.6 Number of dead *An. gambiae s.l.* females (open bars) and *Culex* mosquitoes (closed bars) collected in different treatment groups.

3.4.5 PCR analysis

During the study period, one *An. gambiae s.l* was collected from each hut every day for PCR. A total of 289 samples were collected and out of which, 253 (87.5%) were *An. gambiae s.s*, 34 (11.8%) were *An. arabiensis*, 1 (0.3%) were *An. melas* and 1(0.3%) failed repeated amplification.

3.4.6 Environmental factors

Maximum and minimum temperatures between 21:00h-06:00h and evaporation rates were fitted as covariates in the GLM. The mean maximum and minimum temperatures showed no significant effect on mosquito entry, 30.7°C (IQR=25°C-30°C, P= 0.21) and 27°C (IQR=23.5°C-30°C, P=0.15) respectively. However evaporation was shown to be significant with a mean of 1.04cm (IQR=0.1cm-3.9cm, P<0.001). Slight differences in minimum and maximum temperatures about a degree varied between huts but this was not observed on evaporation over night.

3.5 Discussion

It has been demonstrated that intact ceilings significantly reduced *An. gambiae* entry by 55% compared to the control. Though with a smaller proportion, this concurs with previous findings by Lindsay (2003) where synthetic-netting ceilings reduced *An. gambiae* by 79%. The insecticide-treated damaged ceilings also reduced house entry by *An. gambiae* between 29%-36% but this was similar to water-treated torn ceiling with 29%. This means that treating netting with insecticide provided no additional protection when torn. This was a surprising finding since permethrin was shown to have a significant personal protection against malaria due to its repellent effect (Lindsay et al., 1991) as it reduced mosquito blood feeding by 91%. Even unwashed treated nets significantly reduced blood feeding (Pleass et al., 1993). This study also found a significant reduction of blood fed *An. gambiae s.l.* in huts with intact untreated ceiling and permethrin treated torn ceilings over the no-ceiling hut, but not over the water-treated torn ceiling. It was also found that 25% of blood fed *An. gambiae s.l.* and 29% of blood fed *Culex* spp fed on humans. It is likely that some mosquitoes inside the rooms fed on sleepers by probing through the untreated bednets during the night, or sleepers did not properly tuck their bednets under the mattress as some engorged mosquitoes were found resting inside the bednets, or mosquitoes might have fed somewhere else and sought for shelter in the huts as described by (Boreham and Port, 1982). The blood fed *An. gambiae* were tested for sporozoite rates and results showed a rate of 1.3%. This is similar to the findings of (Bogh et al., 2007) where they found sporozoite rate of 1.5% in their study area in The Gambia. The dominant species of *An. gambiae s.l* as confirmed by the PCR result was *An. gambiae s.s* which comprised of 87.5% of mosquitoes tested. This is the most common and efficient vector in The Gambia and most parts of Africa (Lindsay and Bayoh, 2004). This is simply because *An. gambiae s.s.* prefers more humid habitats and this area is highly suitable for their survival.

Higher numbers of *An. gambiae s.l* were found dead than *Culex* spp during this study. Chlorpyrifos, an organophosphate, was found to have the highest mortality rate of 23.5% compared to the deltamethrin (13.6%) and permethrin (17.8%), but these differences were not statistically significant. Miller (1991) found that pirimiphos-methyl and other pyrethroids (except permethrin) killed higher proportion of endophilic mosquitoes and concluded that this would give community protection when these insecticides are used on a large scale, whereas permethrin only enhances personal protection as it deters mosquitoes from house entry.

My findings show that the insecticide-treated torn ceilings neither deterred nor killed significant numbers of *An. gambiae* to enhance protection. This surprising finding might be an artefact since no chemical analysis was done to measure how much of the insecticides were absorbed by the netting ceilings. The netting used in the ceilings is poorly absorptive, thus it is possible that the ceilings were under-dosed. It is therefore important in future studies to make sure that the insecticide treatment adheres to the fibre.

Maximum and minimum temperatures measured during the study period did not show any significant effect on mosquito entry. Presumably this is because the study was conducted between June and August, and this period is part of the rainy season where temperatures are uniformly very high. However, increased humidity was seen to be significant in mosquito house entry. It has been demonstrated by some researchers that temperature and humidity plus carbon-dioxide are important olfactory cues that influence mosquito behaviour (Takken and Knols, 1999, Takken et al., 1997). It may be that high humidity resulted in more sweating by the human subjects sleeping in the huts, with greater concentrations of host odours being produced attracting more mosquitoes into the huts.

3.6 Conclusion

Although intact ceilings are effective against reducing mosquito house entry, no significant reduction was not observed when torn ceilings were treated with insecticides. Further studies are required to test other types of netting materials using long lasting insecticide treated nettings.

CHAPTER 4

Fly sampling using sticky traps and knockdown catches

Photograph courtesy of Paul Emerson

4.1 Summary

Background: During the 2006 rainy season the STOPMAL project found that 21% fewer houseflies were caught on sticky traps in fully-screened homes, but 72% more in houses with screened ceilings, compared to unscreened houses. However it was possible that the housefly numbers in screened ceiling houses were overestimated, because by preventing access to the roof space to flies that entered through doors and windows may have increased their activity in the room near the trap. The position of the sticky traps may also affect catching efficiency as they were positioned in one corner of the house. It was not known whether the sticky traps were placed in the optimal position to maximise the number of flies caught. It was also unknown at what time of day to set up the traps in order to maximise the number of flies caught. Ultimately the efficacy of screening to reduce house fly entry may have been significantly underestimated and therefore here I tried to determine the optimum position of sticky traps, the diel flight activity of houseflies and compare methods of estimating fly numbers indoors, in order to re-assess the efficacy of house screening against house flies.

Materials and methods: In 30 houses sticky traps were hung for 24h in three different locations (centre of room, an area of light:dark and the furthest corner from the door) to determine the optimal sticky trap position. House fly diel flight activity study was recorded using sticky traps positioned in the centre of 30 houses and replaced every hour from 07:00h-20:00h. A comparison of sticky trap efficacy versus non-residual insecticide knock-down catch was made in fully screened, screened ceiling and unscreened houses. Sticky traps were positioned in the centre of 71 houses and left for 24hrs. The following week, the same houses were sprayed using a non-residual insecticide.

Results: Some 235 flies were caught during the optimisation study, of which 74.9% were from the centre, 17.9% from light:dark area and 7.2% from the furthest corner. Of the 261 flies caught in the flight activity study, 59% were caught between 12:00h to 15:00h. Ceiling houses had more flies caught than other houses using both sampling methods. Sticky traps; ceilings 1.67/trap, 95% Confidence Interval, CI= 0-4.44/trap, fully screened 0.29, 95% CI= 0-0.61, controls 0.38, 95% CI= 0-0.78 and knock down catches; ceilings 2.38, 95% CI= 1.24-3.52, fully screened 1.29, 95% CI= 0-2.57, and controls 0.93, 95% CI= 0.40-1.46).

Interpretation: Maximum fly catches were obtained by positioning traps at the centre of houses and focus trapping activity in the early afternoon. Ceilings were not effective in preventing housefly entry as they entered through doors and windows, but fully screened houses prevented housefly entry provided the screened doors are not propped opened. Spray catches are 100% more effective at catching flies than sticky traps.

4.2 Background

House screening has been shown to be effective at reducing house entry by mosquitoes (Lindsay 2003), but to date no one has investigated whether screening reduces house entry by other flies. This is surprising since many Muscids, particularly *Musca domestica,* the housefly, are commonly found indoors. Preventing these flies from entering houses may be of major public health importance since they are vectors of diarrhoeal pathogens (Levine and Levine, 1991) and, in the case of *Musca sorbens*, trachoma (Emerson et al., 2004).

Houseflies are diurnal insects and their activity is favoured by high temperature, low humidity and shade. As the name implies, they are highly adapted to humans and usually complete their life cycle within human habitats (eusynanthrophic) and are endophagic. Their breeding sites are heaps of accumulated animal faeces, garbage and waste from food processing, heavily manured fields with organic manure, sewage sludge and solid organic wastes in open drains and accumulated plant materials. Flies will disperse from these breeding sites if it is less attractive than houses (Boase, 2007) and migrate to other areas which lead them to entering houses where they constitute potential disease vectors and household pests.

Musca domestica feeds on human foodstuff and waste products where they pick up and transport disease agents. *Musca sorbens,* a close relative of *M. domestica,* is also important and considered in the spread of eye infections because they are eye-seeking flies (Emerson et al., 2004). This species has been shown to breed preferentially in exposed human faeces (Hafez and Attia, 1958, Emerson et al., 2001). Houseflies are regarded as potential vectors of diarrhoeal diseases because they spend most of their life with humans, visiting and landing on faeces and faces of people.

It is thought that house screening to prevent mosquito entry could also prevent housefly entry. The STOPMAL project sampled flies in fully screened, screened ceiling and unscreened houses in 2006 using yellow sticky traps. The traps were suspended from the ceiling and positioned in one of the corners of houses. House flies house entry was reduced by 21% in fully screened homes but increased by 72% in houses with screened ceilings compared to unscreened houses. This prompted consideration of the catching efficiency of the sticky traps. This study was therefore designed to firstly determine the best positioning of sticky traps in a house when sampling flies, secondly to determine the flight activity of flies in houses, thirdly to estimate fly numbers indoors in three different types of houses and finally to compare the efficacy of sticky traps with knock down catches for sampling houseflies.

4.3 Materials and Methods

4.3.1 Study area

The studies were carried out in four villages: Duta Bulu (13° 34' 0N, 15° 37' 0W) 2km west of Farafenni, and Kunjo (13 34 0N, 15 34 60W), Yallal Ba (13 34 60N, 15 34 0W) and Dibba Kunda Fula (13 33 0N, 15 28 0W), 2km, 5km and 16km east of Farafenni respectively, in the North Bank Region of The Gambia. The area is dominated by Sudan savanna vegetation and the climate consists of one single rainy season from June to October followed by a long dry season. The villages have a population of 394, 393, 441 and 336 respectively with over 95% belonging to the Fulani tribe, with the exception of Kunjo, which is a mixture of Mandinka and Wollof with few Fulani. All the villages have roughly equal numbers of men and women, and the majority are farmers and herdsmen.

4.3.2 Study houses

Thirty unscreened houses were selected in Duta Bulu for the sticky trap positioning experiment. In each house three sticky traps were hung; one in the centre of the room, one at a light:dark intersection and one in the furthest corner from the door. Each trap was hung about 1.5m high and left for 24hrs. The experiment was repeated over three days in all houses. The diel flight activity experiment was conducted in Duta Bulu and Kunjo. Sticky traps were hung at the centre of 30 selected houses (15 from each village) and changed every hour over the period 07:00 to 20:00h. Validation of indoor fly sampling was conducted in 30 randomly selected houses from each arm of the STOPMAL trial of 2007 in Yallal Ba and Dibba Kunda Fula. All the studies were conducted between November 2007 and January 2008.

4.3.3 Fly Trapping

Flies were sampled using sticky traps and a non-residual spray. The sticky traps were yellow polythene targets 20 x 24.5cm coated on both sides with adhesive (AgriSense-BCS ltd, Pontypridd, UK) hung 1.5-2m above ground level and left for 24hrs except for the diel flight activity study where they were removed hourly. During the diel flight activity study, exit traps were also positioned in windows of houses between 17:00hrs and 20:00hrs to determine the time flies exit houses.

The non-residual insecticide “BOP” (McBride International, UK) used during the spray catches was bought locally and sprayed between 11:00hrs to 16:00hrs. The active ingredients were tetramethrin 0.3%w/w, d-allethrin 0.12% w/w and cypermethrin 0.07%w/w.

The doors and windows of the houses were closed, white cloth sheets were spread on the floors, and the aerosol sprayed for 30sec starting at the eaves. Doors and windows were opened after 10mins. Knocked-down flies were collected and taken to the laboratory for identification. Flies were identified to species and sexed using a dissecting microscope and relevant taxonomic keys described by Crosskey and Lane (1993) and Pont (1991).

4.3.4 Statistical Analysis

Fly numbers were described by arithmetic mean, a proportional description of the sum of *Musca* species per trap was calculated for the optimisation of sticky traps and an hourly sum of *Musca* species was calculated for the diel flight activity. A proportional description of fly numbers/house/trapping method was conducted for the validation of indoor fly sampling. Variation between house designs was described by arithmetic mean. A paired-samples t test was used to compare the differences in fly catches between methods of sampling. A Wilcoxon matched pairs test was used to analyze variables which were not normally distributed. All calculations were done using SPSS version 15.0.

4.3.5 Ethics

This study was approved by The Gambia Government/MRC Joint Ethics Committee and verbal consent was obtained from village alkalos and compound heads prior to the start of the study.

4.4 Findings

4.4.1 Optimising sticky traps

A total of 266 flies were caught from 30 houses during three 24h trapping periods. Traps hung in the centre of the room caught 71% of all *Musca* spp trapped (table 4.1), 90% more than the total caught from the corner of the room and 69% more than the total caught from the area of light:dark intersect (Friedman = 36.2, df = 2, $P<0.001$). Centrally hung traps also caught 67% (12/18) of all other Muscoidea, although this was not different from the trapping efficacy of the other locations (Friedman = 5.7, df = 2, $P<0.06$). Subsequently all sticky traps were hung centrally.

Table 4.1 The effect of sticky trap location on the total number of *Musca* spp caught in 30 houses.

	Trap location indoors		
Day	Centre	Light:Dark Intersection	Furthest Corner
1	33 (67%)	14 (29%)	2 (4%)
2	73 (69%)	27 (26%)	5 (5%)
3	70 (74%)	14 (15%)	10 (11)
TOTAL	**176 (71%)**	**55 (22%)**	**17 (7%)**

4.4.2 Diel flight activity

A total of 261 houseflies were caught from 16 houses, of which 70% (183/261) were caught between 11:00-15:00hrs (fig. 4.1). No flies were caught at any time from the other 14 houses, or from the window exit traps. Fly activity followed a normal distribution with maximum number of flies collected at 14:00hrs.

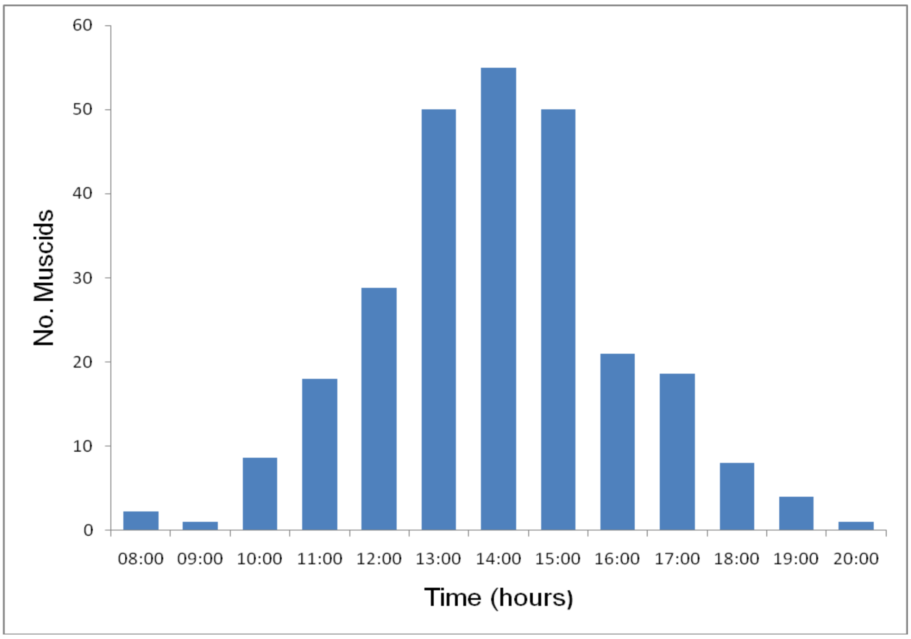

Fig. 4.1 Number of *Musca* spp caught hourly between 07:00-20:00h from 30 houses on three consecutive trapping days.

4.4.3 Validation of indoor fly sampling

A total of 156 *Musca* spp. were caught from both methods in 71 houses, 52 (33%) from sticky traps and 104 (67%) from spray catches. This represents an average of two flies per house but with a range of 0-28. When comparing the two collection methods, the mean number of *Musca* spp caught was 0.73 from sticky traps and 1.46 from non-residual spray catch. Thus twice as many flies were caught from the spray catch compared to the sticky traps ($Z=-4.66$ and $P<0.001$). Of those from sticky traps (table 4.2), 38% were males and 62% females; and from the spray catches, 45% were males and 55% females. *Musca domestica* dominated the catches from sticky traps and spray catches, 83% and 65% respectively.

Table 4.2 Sex distribution of *Musca* spp caught during the study

Collection method	*M. domestica*		*M. sorbens*	
	Male	Female	Male	Female
sticky traps	17	26	3	6
spray catches	33	35	14	22
Total	**50**	**61**	**17**	**28**

The mean number of *Musca* spp caught using sticky traps from fully screened rooms was 0.29 (95% CI= 0-0.61), ceiling rooms was 1.67 (95% CI= 0-4.44) and control house was 0.38 (95% CI= 0-0.78). This shows that more flies were caught from houses with ceilings compared with other houses and more flies caught from control houses than fully screened houses ($P<0.001$). When using a non-residual spray to sample flies, the mean number of flies caught from fully screened houses was 1.29 (95% CI= 0-2.57), ceiling houses was 2.38 (95% CI= 1.24-3.52) and control houses 0.93 (95% CI= 0.40-1.46).

4.5 Discussion

This series of studies demonstrates the dynamic nature of Muscid behavior. I found that the centre of houses is the best position to place sticky traps for sampling house flies, compared to light:dark areas and furthest corner from the door. The reason for this could be that the centre has more space in the house and when flies are agitated by movement of people in the house, they tend to fly upwards to the roof space and had the possibility of being caught by the sticky traps.

Sticky traps hung from the centre of houses was used to determine hourly flight activity of houseflies from 07:00h to 20:00h. The findings of the diel flight activity study showed that the flies were most active in the early afternoon with an average peak activity at 14:00h. This could be due to the fact that sampling was done in December and January which are the coldest months in The Gambia. During the morning and evening, the outdoor temperatures are as low as 20^{o}C which favours outdoor flight and they then enter houses in the afternoon when the temperatures start to increase. They are more active in shade than in sunlight and during this period, the sun is usually covered by patches of clouds which allows them to stay outdoors in the open air (Rozendaal, 1997). People in The Gambia usually have their lunch between 13:00-15:00hrs and the aroma from the food may attract more flies into the house. This is the time when they make contact with food if it is not properly covered and can act as vectors of food borne diseases.

Exit traps positioned in windows to record the time flies exited houses (17:00-20:00h) did not catch any houseflies, despite efforts to ensure all other routes of exit were blocked. Unfortunately the doors of houses could not be kept closed because women were busy going in and out to complete their domestic work, cook supper, bathe children and fetch water into houses and so the flies probably exited through the doors instead of the windows. The flies could have also preferred to rest indoors on hanging clothes, ceilings and roofing material as the outdoor evening temperature was very low during the sampling period.

Using three different types of houses, full screening, ceilings and control houses, flies were sampled to compare the efficiency of these interventions to housefly entry prevention. From the sticky traps, houses with ceilings had 440% more *Musca* spp than homes without ceilings, whilst fully screened houses had 24% fewer flies than control houses. These findings concurs with results in 2006 which showed 72% more flies in ceiling houses and 21% less in fully screened houses compared with control houses (Kirby, unpublished). This could simply be explained by the fact that flies usually get into houses through the doors and some exit through the eaves. In ceiling houses, they will not be able to exit through the eaves and would increase their activity in the centre or rest on ceilings which are their favourite resting places, increasing the likelihood of being caught by the suspended sticky traps. For the fully screened houses, the number of flies that get into the houses is limited because of the screened doors and closed eaves. The few flies that get in is a result of people propping the screened doors open during the day for quick access to the room and to prevent children damaging the screening material.

My findings were not the same when flies were sampled using non-residual sprays. Houses with ceilings again had the highest number of flies caught compared to homes without ceilings. However, surprisingly, fully screened houses had more flies than the control houses. This could be that during the spraying process, most of the flies escaped through the eaves in control houses, whereas in the other two groups of houses, they are trapped inside the rooms when the doors were closed and had the greater chances of being exposed to a lethal dose of the insecticide and knocked down. Thus spray catch collections are a biased method for collecting flies entering houses with different types of screening.

Musca domestica were more common than *M. sorbens* on both sticky traps and in knock-down catches. One possible explanation for this finding is that breeding sites for *M. domestica* were more abundant than those for *M. sorbens*. Refuse and organic waste are poorly managed and this provides ideal breeding ground for *M. domestica* within close range which can disperse into houses as adults. *Musca sorbens* prefers to lay its eggs on human faeces, yet only if these are situated in the open (Emerson et al. 2001). In the study area many people have pit latrines close to their houses, limiting the breeding media available for oviposition.

Spray catches were approximately twice as good at catching houseflies compared with sticky traps. More flies were recovered from houses with ceilings despite the likelihood that those in the roof space would have fallen on to the ceiling and not been collected. Spraying was tolerated because most people would like their houses to be sprayed. It provided a greater chance of all flies within the house being caught where as the sticky traps only caught flies that landed on them.

4.6 Conclusion

This study clearly demonstrates that the centre of houses provides the best position of sticky traps for sampling flies indoors and most flies are active between 12:00-15:00hrs. Ceilings are not effective in preventing houseflies into houses as they get in through the doors. Fully screened houses provide some prevention of housefly entry provided that the screened doors are not propped opened during periods of peak fly activity.

CHAPTER 5
Conclusions

5.1 General Conclusions

This series of studies were designed to investigate the impact of house design on the entry and exit of disease vectors in The Gambia. This study supported a randomised control trial of screening homes to prevent malaria (STOPMAL) in The Gambia. STOPMAL is a three-arm trial measuring the efficacy of screened ceilings and fully screened houses against malaria transmission and morbidity. This trial was designed to investigate in a 'real life' setting whether two different types of house-screening can halve the number of malaria mosquitoes entering houses.

During the running of the STOPMAL trial three questions about house screening arose that needed to be addressed:

1. ***Is it necessary to close eaves in houses with no other mosquito entry points?***

There is strong evidence that closing eaves reduces the number of anophelines entering houses (Lindsay and Snow 1988), but it was uncertain whether screening doors and windows would affect entry of mosquitoes through the eaves. This was important since in the STOPMAL trial, houses in one arm of the trial had their doors and windows screened and the eaves sealed with mud. I wanted to know whether closing the eaves was really necessary. A cross-over study was designed using 12 single-roomed houses with mud brick walls and a thatch roof where a single man slept, to determine the importance of eaves to mosquito entry into houses with no other entry points. All houses had their doors screened. Six houses had their eaves closed for four weeks, and then this treatment was crossed over, so that the houses with closed eaves were opened and those which were opened were closed for the second half of the trial. When houses had their eaves closed there was a 65% reduction in *An. gambiae* s.l. caught indoors, but no significant reduction in total culicine numbers. This was because *An. gambiae*, the main malaria vector in Africa, is well adapted for entering houses through the eaves because it flies upwards when encountering a vertical surface (Snow 1987).

Attracted to human odours pouring out of a house, many *An. gambiae s.l.* reach an outside wall and fly up, funneled indoors by the over-hanging roof, through the open eaves, whereas some culicines will fly sideways when they encounter a wall. The culicines were able to enter through the doors because people used to prop open their screened doors during the day and close them between 19:00-20:00h (Kirby unpublished). This study demonstrated that anophelines enter houses through the open eaves, whilst culicines largely enter through the doors and windows. It also emphasised the importance of sealing the eave gap if house screening is adopted as a method of reducing malaria vector-human contact in homes in The Gambia.

2. ***Are damaged ceilings protective?***

Netting ceilings have a limited life-span - they may wear out and get torn or holed, increasing accessibility for mosquitoes. This begs the question "do mosquitoes enter rooms in greater numbers when ceilings are torn? If so, could this be prevented by treating ceilings with insecticides as is the case with insecticide treated bed nets (Miller et al. 1991)? The field trial of insecticide-treated torn ceilings addressed this issue using six identical experimental huts in which five different ceilings were tested against a control with no ceiling. It was demonstrated that when torn, treating ceilings with insecticide does not prevent mosquito house entry when compared to untreated torn ceilings. However, one should be cautious since in the trial I do not know how much insecticide was on the netting.

3. ***Does screening stop houseflies entering houses?***

As house screening has been shown to be effective at reducing house entry by mosquitoes (Emerson et al. 2004). During the rainy season in 2006 the STOPMAL project sampled flies entering houses using sticky traps suspended from ceilings and positioned at one of the internal corners of a house. This study detected 24% fewer house flies in fully screened homes but 440% more in those with screened ceilings compared to unscreened houses. Thus implying that, house flies largely enter homes through open doors and windows.

There were also some uncertainties about fly sampling methods. We did not know whether the sticky traps were placed in the right position to maximise the number of flies caught. It was also unknown at what time of day to set up the catches in order to maximise the number of flies caught. Moreover there was concern that sticky traps in homes with ceilings were more efficient at catching flies than those in other homes. The hypothesis was that where there were ceilings flies had less room to move in and would contact the traps at a higher frequency than in other houses. I therefore carried out three separate studies to investigate these concerns:

a) Determining the optimum position for sticky traps in a house to sample flies;

b) Determining the daytime flight activity of flies in houses;

c) Estimating fly numbers indoors in 3 different types of houses (fully screened, screened ceiling, and control (no intervention) houses) using and comparing the efficacy of two techniques - sticky traps and knock down catches.

The first experiment found that the centre of a house is the best position for hanging sticky traps to sample house flies since these collected 90% more flies than those positioned in the furthest corner from the door and 76% more from light/dark intersection areas. Secondly I discovered that most houseflies are active between 12:00-15:00hrs; 57.8% of flies were caught between these times. Thirdly I confirmed that ceilings are not effective in preventing houseflies getting into houses as they probably still get in through the doors but then fail to find their way out again. Houses with ceilings caught 440% and 61% more flies than the control using sticky traps and knockdown catches respectively. Fully screened houses provide effective prevention of housefly entry provided that the screened doors are not left propped opened. Fully screened houses caught 31% less flies than the control houses using sticky traps but, surprisingly, 28% more in the knockdown catches. This could be because during the spraying process most of the flies escaped through the eaves in control houses, whereas in the other two groups of houses, they were trapped inside the rooms when the doors were closed and had the greater chances of being exposed to a lethal dose of the insecticide and knocked down. In comparing the two methods, knockdown catches were shown to be 100% more effective at catching flies than sticky traps, mean numbers caught were 1.46 and 0.73 respectively.

5.2 Further considerations

House design has been proven to be effective against disease vectors and today it is almost forgotten and rarely used in malaria prevention programs. Studies in the early 1900s by prominent scientists like Angelo Celli and Patrick Manson and of present century scientists like Professor Steven W. Lindsay and others show compelling evidence that a structural adjustment when constructing a house in a rural area, where malaria is endemic, could have a significant impact on its prevention. Lots of programmes, partnerships and initiatives like the World Malaria Eradication Programme in the 1960s, the RBM Initiative in 1998 and recently the GFATM by the Gates Foundation have called for or are calling for malaria eradication/elimination or control and yet still a solution has not been achieved. I think the necessary tools exist, but people neglect them. Integrated vector management (IVM) which includes use of LLINs, source reduction, IRS together with the use of effective chemotherapy like artemisinin-based combination therapy (ACT), are important weapons that need to be used. And where appropriate, coupled with a vigorous Information, Education and Communication (IEC) package based on achievements in malaria research and how they could be applied to the community for behavioural change and to have sound knowledge of mosquito behaviour and disease transmission. House screening could play an important role in this endeavour.

Many tropical countries report that malaria is a major killer and all efforts are being made to fight this disease. It is important for governments of these countries to unite and give strong political, social and most important, financial commitments to this disease and initiate intra-continental programmes to fight the disease. These are long term goals that require proper planning and organisation and when properly implemented could give significant results. Maintaining such programmes to achieve all their objectives has been a problem, for instance the campaign for polio eradication has stalled in a few especially tough countries where refusals are high and accessibility difficult and raising funds to complete the job is proving difficult. Real commitment and exceptional leadership are required when such programmes are near to the end.

5.3 Study Limitations

Despite the proper design and implementation of the studies described in this thesis, there is always room for improvement, as lessons are learnt in any activity. Lessons learnt in the past do not only help to improve implementation but also help during the planning stages of subsequent programmes.

The following are some observations made during the study and at least include some recommendations that I think could have helped in one way or other. The netting used in the field trial of insecticide-treated torn ceilings was PVC-coated fibreglass. The netting used for screening has excellent strength and flexibility, heat- and corrosion-resistance, but might have not been able to absorb the required concentration of the insecticides. A chemical bioassay from the treated nets would have helped to determine the amount of insecticide on the fibres. I would also recommend that in future, insecticides be incorporated into the fibres of the netting during the manufacturing process and then tested, as has been done with LLINs (Graham et al. 2005). If the required concentration of insecticides were absorbed by the netting to knock down or kill mosquitoes, I would have been tempted to spread a white sheet of cloth on the floor of the rooms and verandas to make sure that no dead mosquitoes were lost and also test the efficiency of the collectors by spraying rooms with a non-residual insecticide on the last day of the study after collection.

The fly sampling study was conducted after the rains had stopped and the most frequent remark from people in the villages was that 'more flies could have been trapped if it were in the rainy season'. Unfortunately this was not possible as some of the houses were being used by the STOPMAL project and hanging a sticky trap or spraying an insecticide into these rooms could affect their study by catching mosquitoes in the sticky traps or deterring them by the insecticide sprayed. I would suggest that if one is to conduct a similar study it should be during the rainy season where fly populations are greatest and to use separate houses from any other study.

5.4 Major Conclusions

The findings of this thesis concur with those of several previous studies of house design. Namely;

- Netting ceilings reduce mosquito house entry even if torn.
- Closing eave gaps of fully screened houses reduces *An. gambiae* entry by 65% and thus the risk of being bitten by mosquitoes indoors.
- Anopheline mosquitoes enter houses through the eaves, unlike most culicines which enter through the doors and windows
- Ceilings are not effective in prevention of housefly entry as they pass through the doors.
- Therefore, full screening of houses gives the advantage of both preventing mosquito and housefly entry provided that the screened doors are not propped opened during the day.

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Appendix

1.INFORMED CONSENT FORM FOR FIELD TRIAL OF INSECTICIDE-TREATED SCREENED CEILINGS

I, ……………………………………………………….(name) do hereby consent to participate in the research study entitled: **Field trial of Insecticide-treated Screened Ceilings**.
This study is part of a larger research project entitled **Screening Homes to Prevent Malaria (STOPMAL)**.

I have been given the opportunity to ask questions concerning this project. Any such questions have been answered to my full satisfaction. Should any further questions arise concerning this study I may contact Mbye Njie/Dr Matthew Kirby, MRC Farafenni.

I also understand that I may revoke this consent at any time without penalty or loss of benefits, if any.

Signature/thumb print of study subject…………………………….

Date……………………..

Sleeper No………………………

Address……………………………………………………………….

Compound Head……………………………………………………

Investigator's name, signature and date…………………………………………………….

2.INFORMED CONSENT FORM FOR THE IMPORTANCE OF EAVES FOR HOUSE ENTRY BY MOSQUITOES

I ……………………………………………………., (name) do hereby consent to participate in the research study entitled:

The impact of house design on the entry and exit of insect vectors. This study is part of a larger research project entitled **Screening Homes to Prevent Malaria (STOPMAL)**.

I have been given the opportunity to ask questions concerning this project. Any such questions have been answered to my full satisfaction. Should any further questions arise concerning this study I may contact Mbye Njie/Dr Matthew Kirby, MRC Farafenni.

I also understand that I may revoke this consent at any time without penalty or loss of benefits, if any.

Signature/thumb print of study subject…………………………….

Date……………………..

Address |__|__|__| |__|__|__| |__|__|

Compound Head……………………………………………………

Investigator's name, signature and date…………………………………………………….

3. SCREENING QUESTIONNAIRE (4)

SECTION 1 – TO BE COMPLETED IN THE FIELD BY MN

Before setting traps:

HOUSE

Q1. Date of Trapping |__|__| |__|__| |_2_|_0_|_0_|_7_|

Q2. Week No. |__|__|

Q3. House Address |__|__|__| |__|__|__| |__|__|

block compound house

Q4. Compound Head ……………………………………………………………

Q5. House Status Opened Eaves= 1,Closed Eaves = 2 ☐

EQUIPMENT

Q6. Data Logger Number (between 01-18, Not Used = 99) ☐☐

Q7. Thermometer Number (between 40-60, Not Used = 99) ☐☐

Q8. Light Trap Number (between 01-40) ☐☐

Q9. Pot Number (between 01-42) ☐☐

Q10. Battery Number (between 01-60) ☐☐

Q11. Time light trap turned on (24h clock): ………..……....…..…………....|__|__| : |__|__|

Q12. Time thermometer reset (24h clock, Not Used = 99:99): ...………....|__|__| : |__|__|

After turning off the trap the following morning:

Q13. Time light trap turned off (24h clock):……………………….…………..........|__|__| : |__|__|

Q14. Was light trap working before you turned it off? (Yes = 1, No = 2) ☐

Q15. Was the light weak i.e had it deemed since trap turned on? (Yes = 1, No =2) ☐

Q16. Light Trap Number (between 01-40) ☐☐

Q17. Pot Number (between 01-42) ☐☐

Q18. Battery Number (between 01-60) ☐☐

Q19. Data Logger Number (between 01-18, Not Used = 99) ☐☐

Q20. Thermometer Number (between 40-60, Not Used = 99) ☐☐

After removing trap **These questions relate specifically to the trapping night only:**

COMPOUND

Q21. No. of horses in the compound between 19.00 and 07.00h ☐

Q22. No. of cows in the compound between 19.00 and 07.00h ☐

TRAPPING ROOM

Q23. Number of children (6 months – 10 years old) that slept in the trapping room? ☐☐

Q24. Total no. of people that slept in the trapping room? ☐☐

Q25. Was Churai (local incense) burnt between 19.00 and 07.00h: (Yes = 1, No = 2) ☐

Q26. Was a mosquito coil burnt between 19.00 and 07.00h: (Yes =1, No = 2) ☐

Q27. Was insecticide spray used between 19.00 and 07.00h: (Yes = 1, No = 2) ☐

Q28. Were any insecticide-treated nets used between 19.00 and 07.00h: (Yes = 1, No = 2) ☐

SECTION 2 – TO BE COMPLETED IN THE FIELD BY MN

Q29. Maximum thermometer reading |__|__| ^oC
Q30. Minimum thermometer reading |__|__| ^oC

Initials & date…………………………………………………………………

SECTION 3 – TO BE COMPLETED IN THE OFFICE BY MN

Q31. Date of data logging |__|__| |__|__| |_2_|_0_|_0_|_7_|

Q32. Data logger no. |__|__|

Q33. Maximum room temperature |__|__|.|__| ^oC

Q34. Minimum room temperature |__|__|.|__| ^oC

Q35. Mean room temperature |__|__|.|__| ^oC

Q36. Maximum room relative humidity |__|__| %RH
Q37. Minimum room relative humidity |__|__| %RH
Q38. Mean room relative humidity |__|__| %RH

Initials & date…………………………………………………………………..

4. LIGHT TRAPPING DATA ENTRY FORM   Form: LTEF2

Date Trap Collected: |___|___| |___|___| |_2_|_0_|_0_|___| Lab Worker Initials:|__|__|

		Anophelines						**Culicines**				
	Pot no.	***An. gambiae s.l.***	***An. pharoensis***	***An. rufipes***	***An. squamosus***	***An. ziemanni***	**Unidentifiable**	***Cx. thalassius***	***Mansonia spp.***	***Aedes spp.***	**Other Culicines**	**Unidentifiable**
1												
2												
3												
4												
5												
6												
7												