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Assessing Relationship Between Human Settlement Patterns and Malaria Risk in a Residual Transmission Setting in South-Eastern Tanzania



Thesis submitted to the Faculty of Science, University of the Witwatersrand, in fulfilment of the requirement for the degree of Doctor of Philosophy

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Candidate declaration

I, **Emmanuel W. Kaindoa**, declare that this thesis is my own work and has not been submitted for any other degree or at any other university. All materials adopted from previously published work have been appropriately cited in this thesis.



22nd October 2019

_____ _____

Emmanuel Willson Kaindoa **Date**

Abstract

Background: Spatial targeting of interventions is increasingly recognized as essential for malaria control, particularly in areas aiming for elimination. The associations between house characteristics and malaria transmission is known, but gaps remain on whether transmission is also influenced by factors such as distances between households or the degree to which houses are clustered. It is also important to identify new interventions that can be implemented by targeting critical points in the biology of major malaria vectors and common characteristics of human settlements. Such efforts will be essential to complement current major tools.

Aims: This study had the following aims; (i) understand how households densities and their distances influence malaria transmission the study area , (ii) identify housing-related factors affecting residual transmission risks in the area (iii) assess opinions of residents on how house characteristics, settlement patterns and other environmental factors influencing malaria transmission, and (iv) identify effective complementary approaches that could be used to improve malaria control in these residual transmission area with high coverage of basic interventions such as long lasting treated nets.

Methodology: A mixed methods approach was used, which included: (i) indoor and outdoor mosquito collections from fixed as well as randomly selected households over 12 months, (ii) characterization of physical and microclimatic conditions of the houses and surrounding environmental variables, (iii) assessing effects of spatial clustering of households on malaria transmission risks, and (iv) assessing community concerns regarding household and environmental factors influencing transmission. During these studies, swarms of *Anopheles* mosquitoes were discovered for the first time in this area. Therefore, additional quantitative and qualitative observations were made to characterize the swarms and assess how they could be targeted to complement malaria control efforts.

Key findings: Despite the expansive use of LLINs in the area, factors associated with housing characteristics and settlement patterns as well as people's perceptions contribute to persistent malaria transmission, and will need to be addressed for eventual elimination to be reached. The main findings of this study were as follows: (i) household occupancy influenced indoor mosquito density (ii) high house densities

increased *Anopheles* biting risk but mosquito density declined as distances between houses increased beyond 50m; (iii) abundance of the vector *Anopheles arabiensis* peaked during high rainfall months (February-May), but *An. funestus* densities remained stable into the dry season (May-August); (iv) there were higher indoor densities of *An. arabiensis* and *An. funestus* in houses with mud walls compared to plastered or brick walls, open eaves compared to closed eaves and unscreened windows compared to screened windows; (v) most respondents were aware of associations between their house characteristics and malaria risks but carrying out improvements was constrained by financial costs and other household priorities; (vi) in all our surveys, *An. funestus* mosquitoes contributed approximately 85% of ongoing malaria transmission in the areas, even when occurring in far smaller densities than *An. arabiensis*; and (vii) with regards to complementary interventions, small scale studies demonstrated that mating swarms of both *An. arabiensis* and *An. funestus* could be readily identified and characterized (in this case by volunteer community members), and that targeting the swarms using aerosol spraying could possibly reduce overall biting risk in the communities.

Conclusions: This study yielded evidence that malaria transmission risks are significantly lower when the distances between houses are high. Variation in malaria transmission risk within a village might be important for planning and implementing spatially targeted interventions. With limited resources, malaria control efforts must be prioritized in locations with significantly higher risk in order to obtain maximum benefit. Thus, the distances between houses should be considered when planning malaria vector control strategies. Furthermore, the overall clustering of indoor densities of major disease-transmitting mosquitoes is significantly associated with household occupancy and its spatial distribution within the villages. Further research is needed to assess the potential of using this information for predicting, identifying and targeting the most intense foci of mosquito-borne disease transmission based on household occupancy.

Understanding the factors influencing malaria transmission at a small scale is crucial for planning malaria control interventions. Malaria transmission heterogeneity does not only exist on a large scale, but also at small scales, which may be influenced by small variations of environmental features, such as seasonality. These variations should be considered when improving malaria control interventions. While *An. funestus* showed

variation in density during the year, numbers of mosquitoes remained high throughout the dry season, whereas *An. arabiensis* showed a definite peak in the rainy season with a subsequent drop during the dry season. Further studies are required to investigate the survival strategies of major malaria vectors during the dry season. Understanding this particular feature of *An. funestus* may lead to the design of new control strategies or improve existing interventions so as to reduce the malaria burden in such rural areas.

This study also documented high mosquito densities in mud-walled houses compared to houses with brick or plastered walls. These findings further indicate the necessity of considering house improvement as a malaria control strategy. While the community members had fairly high awareness that improved houses were associated with reduced mosquito nuisance, many families in rural areas are living in poorly constructed houses. It is therefore necessary to consider inter-sectoral collaborations to integrate housing into health policies in Tanzania. Further studies are also needed to provide epidemiological evidence on how housing design affects malaria transmission. Furthermore, community-based house improvement programs should be promoted, including simple, scalable and affordable house improvements for the populations living in high malaria endemic areas in rural communities. Generally, this study has generated information which sets the basis for further studies on the relationship between the exact house location and malaria transmission risk in rural malaria endemic countries.

Key words: Malaria transmission, house design, house distances, settlement patterns, environmental factors, community participation, swarm spraying, complementary tools.

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2. **Kaindoa EW**, Finda M, Kiplagat J, Mkandawile G, Nyoni A, Coetzee M, et al. Housing gaps, mosquitoes and public viewpoints: A mixed methods assessment of relationships between house characteristics, malaria vector biting risk and community perspectives in rural Tanzania. Malar J. 2018;17. https://doi.org/10.1186/s12936-018-2450-y
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1. **Emmanuel Kaindoa**, Halfan Ngowo, Alex Limwagu, Gustav Mkandawile, Japhet Kihonda, John Paliga, Hamis Bwanary, Abdoulaye Diabate & Fredros Okumu. Targeting *Anopheles* swarms and nocturnal behaviors to control outdoor malaria transmission. **May 2018**. The 8th Indian Alliance, New Delhi, India. Poster presentation.

2. **Emmanuel Kaindoa**, Halfan Ngowo, Alex Limwagu, Gustav Mkandawile, Japhet Kihonda, John Paliga, Hamis Bwanary, Abdoulaye Diabate & Fredros Okumu. New evidence of mating swarms of *anopheles arabiensis* in rural Tanzania. **November 2017**. The 66th Annual Meeting of the American Society of Tropical Medicine & Hygiene Baltimore USA. Oral presentation.

3. **Emmanuel Kaindoa**, Nancy Matowo, Halfan Ngowo, Gustav Mkandawile, Arnold Mmbando, Marcelina Finda & Fredros Okumu. Interventions that effectively target *Anopheles funestus* mosquitoes could significantly improve control of persistent malaria transmission in south–eastern Tanzania. **September 2016**. Pan Africa Mosquito Control Association (PAMCA) - Lagos Nigeria. Oral presentation.

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Table of contents

Candidate declaration iii

Abstract iv

Acknowledgments vii

Original papers x

Conference proceedings xii

Awards xiii

List of abbreviation xxvi

Chapter I: Introduction and Literature Review 1

1.1 Malaria transmission 1

1.2 Malaria burden 2

1.3 Heterogeneity of biting risk and malaria transmission 3

1.4 Existing gaps, challenges and contribution of this study 5

1.5 Literature Review 6

1.5.1 Residual malaria transmission 6

1.5.2 Factors affecting malaria transmission 8

1.5.2.1 Climatic and environmental factors 8

1.5.2.2 Human behaviours and activities 8

1.5.2.3 House characteristics, human settlements and malaria transmission 9

1.5.2.3 Mosquito behaviour 10

1.5.2.4 Vector densities and competence 10

1.5.2.5 Biting times and locations 11

1.5.2.6 Sugar-feeding and blood-feeding 11

1.5.2.7 Swarming and mating 11

1.5.2.8 Mosquito flight patterns in the environment 12

1.6 Community participation in malaria control 12

1.7 Study justification 13

1.8 Overall aim of this study 13
1.8.1 Specific objectives 13
References 14
Chapter II: Materials and methods 26
2.1 Study area 26
2.2 Study design and data collection 26
2.3 Study procedures 27
2.3.1 Objective-1: To assess how densities of households and distances between them affect the risk of malaria transmission 27
2.3.2 Objective-2: To identify environmental features associated with residual malaria transmission in the Kilombero valley 28
2.3.3 Objective-3: To assess opinions of residents on how settlement patterns and the other environmental factors affect transmission in their study area 28
2.3.4 Objective-4: To identify effective complementary approaches that could be used to target malaria transmission in the Kilombero valley in south-eastern Tanzania 28
2.3.4.1 Identification of *Anopheles* swarms and assessing the potential of targeting them as a complementary approach. 29
2.3.4.2 Assessment of insecticide resistance profiles of dominant malaria vectors 29
2.4 Data analysis 29
2.4.1 Quantitative data 29
2.4.2 Analysis of housing pattern and distances 29
2.4.3 Analysis of quantitative survey data 30
2.4.4 Processing and analysis of qualitative data 30
2.4.5 Laboratory analysis of sub samples of *Anopheles* mosquitoes 31
2.5 Ethical approval 31
References 32
Chapter III: Correlations between household occupancy and malaria vector biting risk in rural Tanzanian villages: implications for high-resolution spatial targeting of control interventions 34
3.1 Abstract 34
3.2 Background 36

3.3 Methods 37
3.3.1 Study area 37
3.3.2 Study procedures 38
3.3.3 Controlled experimental verification of correlations between household occupancy and mosquito house-entry 39
3.3.4 Longitudinal vector surveys to assess empirical relationships between indoor mosquito densities and household occupancy 39
3.3.5 Data analysis 41
3.3.6 Ethical consideration 42
3.4 Results 43
3.4.1 Relationship between household occupancy and mosquito house-entry in experimental huts 43
3.4.2 Relationships between indoor mosquito densities and occupancies of the households of trapping rooms 46
3.4.3 Spatial clustering and correlations between house occupancy and indoor vector densities 46
3.4.4 Species composition of malaria vectors and Plasmodium infection rates 49
3.5 Discussion 49
3.6 Conclusion 54
References 56
Chapter IV: Housing gaps, mosquitoes and public viewpoints: a mixed methods assessment of relationships between house characteristics, malaria vector biting risk and community perspectives in rural Tanzania 61
4.1 Abstract 61
4.2 Background 63
4.3 Methods 65
4.3.1 Study area 65
4.3.2 Study procedures 67
4.3.3 Longitudinal surveys of indoor mosquito densities 68
4.3.4 Characterization of the houses and their environments 69

4.3.5 Survey questionnaires: quantitative assessment of community knowledge, opinions and concerns regarding house designs and environmental characteristics, and how these factors influence malaria transmission 70

4.3.6 Focus group discussions: qualitative assessment of community knowledge, opinions and concerns regarding house designs and environmental characteristics, and how these factors influence malaria transmission 72

4.3.7 Data analysis 73

4.3.7.1 Analysis of quantitative data 73

4.3.7.2 Processing and analysis of qualitative data: 73

4.4 Results 74

4.4.1 Indoor densities of malaria and non-malaria mosquito vectors 74

4.4.2 Physical characteristics and microclimate of sampled houses 75

4.4.3 Household risk factors associated with indoor mosquito densities 76

4.4.4 Socio-demographic characteristics of survey participants 80

4.4.5 Results of the questionnaire survey: participants' knowledge regarding indoor mosquito density and how this relates to housing characteristics and environmental variables 81

4.4.6 Focus group discussions: participants' knowledge regarding indoor mosquito density and how this relates to housing characteristics and environmental variables 82

4.5 Discussion 86

4.6 Conclusion 89

References 92

Chapter V: Effects of household densities and their distances on malaria transmission risk in rural Tanzania 98

5.1 Abstract 98

5.2 Background 99

5.3 Methods 100

5.3.1 Analysis of housing pattern and distances 100

5.4 Results 101

5.4.1 Effects of distances and housing density on malaria vectors 101

5.5 Discussion 102
5.6 Conclusion 103
References 105
Chapter VI: Interventions that effectively target Anopheles funestus mosquitoes could significantly improve control of persistent malaria transmission in south–eastern Tanzania 108
6.1 Abstract 108
6.2 Background 110
6.3 Methods 111
6.3.1 Study area 111
6.3.2 Study procedures 112
6.3.2.1 Monitoring indoor densities of host-seeking and resting disease-transmitting mosquitoes 113
6.3.2.2 Identification of sibling species and detection of *Plasmodium* infection in malaria vectors 114
6.3.2.3 Identification of mosquito blood meals 114
6.3.2.4 Assessment of insecticide susceptibility status 114
6.3.2.5 Assessment of parity status 115
6.3.3 Data analysis 115
6.4 Results 116
6.4.1 Indoor densities of *Anopheles* mosquitoes 116
6.4.2 Sibling species of malaria vectors and *Plasmodium* infection rates 117
6.4.3 Indoor densities of culicine mosquitoes 119
6.4.4 Mosquito blood meal sources 122
6.4.5 Spatial distribution of sporozoite infected *An. funestus* and *An. arabiensis* 122
6.4.6 Insecticide susceptibility test results 123
6.4.7 Proportion of *An. arabiensis* and *An. funestus* that are parous 124
6.5 Discussion 126
6.6 Conclusion 132
References 135

Chapter VII: Evidence of mating swarms of the malaria vector Anopheles arabiensis in Tanzania 143
7.1 Abstract 143
7.2 Background 145
7.3 Methods 146
7.3.1 Study area 146
7.3.2 Training on swarm surveys and characterization 147
7.3.3 Initial exploratory surveys of mosquito swarms 149
7.3.4 Detailed mapping and characterization of *Anopheles* mosquito swarms, and swarm markers in three selected villages 149
7.3.5 Laboratory processing of mosquitoes collected in the swarms 151
7.3.6 Assessment of female insemination status and sexual maturity in males 151
7.3.7 Data analysis 151
7.4 Results 152
7.4.1 Results of exploratory surveys: initial evidence of *Anopheles* swarms 152
7.4.2 *Anopheles* mosquito swarms in the three selected villages 152
7.4.3 Start times, end times, duration and height of *Anopheles* swarms 153
7.4.4 Estimated swarm sizes: data collected through direct visualization by the community volunteers compared to data collected using sweep nets 154
7.4.5 Potential swarm markers survey 156
7.4.6 Copulation events, mating status, sexual maturity and parity rates in mosquitoes collected from the swarms 157
7.5 Discussion 158
7.6 Conclusion 160
References 163
Chapter VIII: Swarms of the malaria vector, Anopheles funestus in Tanzania 167
8.1 Abstract 167
8.2 Background 169
8.3 Methods 170
8.3.1 Study area 170

8.3.2 Observation and characterization of swarms 171
8.3.3 Morphological and molecular identification of *Anopheles* mosquitoes collected in swarms 173
8.3.4 Assessing sexual maturity and wing sizes of sampled mosquitoes 173
8.3.5 Data analysis 173
8.4 Results 174
8.4.1 Mean density of *An. funestus* and copulation events in swarms 174
8.4.2 Correlations between visual and sweep net estimates of swarm sizes 176
8.4.3 Location, time and height at which the swarms occurred 177
8.4.4 Environmental features associated with swarm occurrence 177
8.4.5 Molecular identification, wing lengths and sexual maturity of sampled *An. funestus* mosquitoes 178
8.5 Discussion 179
8.6 Conclusions 182
References 184
Chapter IX: Discussion and Conclusions 190
9.1 Household occupancy and mosquito biting risks 191
9.2 House distances and mosquito biting risks 192
9.3 Housing characteristics and malaria transmission risks 193
9.4 Community knowledge and involvement in malaria control efforts 194
9.5 Targeting swarms as complementary approaches for controlling malaria transmission 196
9.6 Role of different *Anopheles* species in malaria transmission 198
9.7 Limitations of the study 199
9.8 Conclusions 200
References 202
Appendices 205
Appendix 1: Original paper I 206
Appendix 2: Original paper II 218

Appendix 3: Original paper III 234
Appendix 4: Original paper IV 253
Appendix 5: Original paper V 272
Appendix 6: Ethical Clearance certificate from Wits 282
Appendix 7: Ethical Clearance certificate from IHI 283
Appendix 8: Household observation sheet 284
Appendix 9: Questionnaire 286
Appendix 10: Plagiarism report 288

List of figures

Figure 1.1: Lifecycle of the human malaria *Plasmodium* parasite spp. 2

Figure 1.2: Malaria prevalence by regions in Tanzania (percentage of children aged 6-59 months with a positive Rapid Diagnostic Test (RDT). Adopted from Tanzania Malaria Indicators Survey. 4

Figure 1.3: Diagrammatic representation of progress made against malaria over the past decade. Approximate values are based on observations in the Kilombero Valley, south eastern Tanzania 7

Figure 1.4: Diagrammatic representation of processes and behaviours of mosquitoes 10

Figure 2.1: Maps of the study villages in Ulanga and Kilombero districts in the Kilombero Valley in south-eastern Tanzania. 26

Figure 3.1: Map of the study area, showing the villages in Ulanga district where the study was conducted. 38

Figure 3.2: Effects of host biomass on indoor densities of malaria vectors: comparison of the number of *Anopheles arabiensis* and *Anopheles funestus* mosquitoes caught in experimental huts occupied by varying numbers of adult male volunteers. 44

Figure 3.3: Effects of host biomass on indoor densities of non-malaria vectors: Comparison of the number of *Culex* and *Mansonia* mosquitoes caught in experimental huts occupied by varying numbers of adult male volunteers. 45

Figure 3.4: Maps showing statistically significant clusters of households with the high occupancy (A) and statistically significant clusters of households with high

densities of *Anopheles arabiensis* in the same study area (B). The grey circles represent the rest of the households in the study area……..47

Figure 3.5: Maps showing statistically significant clusters of households with the high occupancy (A) and statistically significant clusters of households with high densities of *Anopheles funestus* in the same study area (B). The grey circles represent the rest of the households in the study area..... 48

Figure 3.6. Maps showing statistically significant clusters of households with high occupancy (A) and statistically significant clusters of households with high densities of *Culex* in the same study area (B). The grey circles represent the rest of the households in the study area. 48

Figure 4.1: Illustration of the study villages. The study was conducted in households across four villages in Ulanga district, south of the Kilombero river in south-eastern Tanzania. 66

Figure 4.2: Pictorial representation of typical local house types in the study area ... 67

Figure 4.3: Diagrammatic illustration of the mixed methods study design used in this study..... 68

Figure 4.4: Monthly trends of mean number mosquitoes of different species collected per house per night 75

Figure 5.1. Correlations of distances between houses and mosquito biting risks in rural Tanzania 101

Figure 5.2. Relationship between house densities and mosquito biting risks in rural Tanzania. 102

Figure 6.1: Map of the study area, showing the villages and households in Ulanga district where the study was conducted..... 112

Figure 6.2: Mean number of *Anopheles funestus s.l.* and *An. arabiensis* mosquitoes caught per house per night. Y error bars represent the 2-standard errors of the mean 117

Figure 6.3: Monthly variations in the composition of sibling species of *Anopheles funestus* group in the study area 118

Figure 6.4: Distribution of Plasmodium infected An. funestus and An. arabiensis.. 122

Figure 7.1: Map of the study area, showing villages in south-eastern Tanzania, where swarm surveys were conducted 147

Figure 7.2: Pictures of volunteers and researchers learning how to identify and sample mosquito swarms in one of the study villages..... 148

Figure 7.3: Comparison of swarm distribution between the three study villages 153

Figure 7.4: Observed start and end times of *Anopheles arabiensis* swarms in the study villages 154

Figure 7.5: Estimated swarm sizes and correlation between estimates done by visualization and estimates done using sweep nets 156

Figure 8.1: Map of the study area, showing villages in south-eastern Tanzania, where swarm surveys were conducted 171

Figure 8.2: Median number of *An. funestus* caught per swarm per evening in the two study sites. 175

Figure 8.3: Correlations between visual estimates of the swarm sizes and the swarm densities per sweep net collection per evening. 176

Figure 8.4: Pictorial illustration of commonest locations where swarms of *Anopheles funestus* mosquitoes were observed 177

Figure 8.5: Distribution of *Anopheles funestus* swarms observed in study sites. ... 178

Figure 8.6: Comparison of wing sizes between swarming and resting *An. funestus* male mosquitoes. 179

List of tables

Table 4.1: Description of the main themes addressed in the survey to assess community knowledge, opinions and concerns regarding house designs and environmental characteristics, and how these factors influence malaria transmission 71

Table 4.2: Physical characteristics and microclimatic conditions in sampled houses..... 76

Table 4.3: Summary statistics for mosquitoes caught in houses with different characteristics, showing the mean nightly catches of *An. arabiensis* and *An. funestus*, relative rates and the associated significance levels calculated from the generalized linear mixed models (GLMMs) at 95% confidence interval 78

Table 4.4: Summary statistics for mosquitoes caught in houses with different characteristics, showing the mean nightly catches of *Culex* and *Mansonia* species, relative rates and the associated significance levels calculated from the generalized linear mixed models (GLMMs) at 95% confidence interval..... 79

Table 4.5: Socio-demographic characteristics of participants who responded to the survey questionnaire to assess people's awareness of how house characteristics affect malaria transmission..... 80

Table 4.6. Summary of community members' knowledge about indoor mosquito density and how these relate to certain housing and environmental characteristics.. 81

Table 6.1: Infectious status of malaria vectors and dominant contribution of *Anopheles funestus* mosquitoes to ongoing malaria transmission, despite their low abundance relative to *Anopheles arabiensis*. 120

Table 6.2. Monthly sporozoite rates and entomological inoculation rates (EIR) for *Anopheles funestus* and *Anopheles arabiensis* from January to December 2016 121

Table 6.3. Host blood antigens detected in blood-meals obtained from *Anopheles* mosquitoes..... 122

Table 6.4: Summary of data on susceptibility of wild-caught adult female Anopheles funestus mosquitoes collected in the study sites..... 125

Table 6.5: Examples of localities where *Anopheles funestus* mosquitoes have been demonstrated to mediate most of the residual malaria transmission, and where control programmes targeting *An. funestus* with effective interventions, could drastically reduce local transmission 125

Table 7.1: Swarm sizes estimated by either visual observations by trained volunteers, or sweep net sampling in the three study villages. 155

Table 7.2: Common types of swarm markers observed in the three study villages (and number of markers that actually had *Anopheles swarms* over them)..... 157

Table 8.1: Descriptive variables assessed during field surveys of *Anopheles funestus* swarms. 172

Table 8.2: Environmental features of locations where *Anopheles funestus* swarms were observed in the study villages. 178

List of abbreviation

ACT	Artemisinin Combination Therapy
CARTA	Consortium for Advanced Research Training in Africa
CDC	Centres for Disease Control
CI	Confidence Interval
DDT	Dichloro-diphenyl-trichloroethane
DNA	Deoxyribonucleic Acid
DSS	Demographic Surveillance System
ELISA	Enzyme-linked Immunosorbent Assay
EHES	Environmental Health and Ecological Science Department
EIR	Entomological Inoculation Rate
FGD	Focus Group Discussion
GAMMs	Generalized Additive Mixed Models
GIS	Geographical Information System
GLM	Generalized Linear Model
GLMM	Generalized Linear Mixed Model
GLMMER	Generalized Linear Mixed Effect Model
GPS	Global Positioning System
HDSS	Human Demographic Surveillance System
HLC	Human Landing Catch
IHI	Ifakara Health Institute
IPTp	Intermittent Preventative Treatment in Pregnancy
IRB	Ifakara Review Board
IRSS	Indoor Residual Spraying
IVCC	Innovative Vector Control Consortium
ITNs	Insecticide Treated Nets

JAS	Joint Advance Seminar
MaIERA	Malaria Eradication Research Agenda
LLINs	Long Lasting Insecticide Treated Nets
NIMR	National Institute for Medical Research
OMC	Outdoor Mosquito Control
kdr	Knockdown Resistance
pf	*Plasmodium falciparum*
NA	Not applicable
NMCP	National Malaria Control Program
PAMCA	Pan-African Mosquito Control Association
PCR	Polymerase Chain Reaction
RBM	Roll Back Malaria
RR	Relative Rates
SDGs	Sustainable Development Goals
SNP	Single Nucleotide Polymorphism
TACAIDS	Tanzania Commission for Aids (Acquired Immune Deficiency Syndrome)
TMIS	Tanzania Malaria Indicators Survey
WHO	World Health Organization
WITS	University of the Witwatersrand

Chapter I: Introduction and Literature Review

1.1 Malaria transmission

Malaria is transmitted to humans by five species of *Plasmodium* parasites. The species include *P. falciparum*, *P. ovale*, *P. malariae* and *P. vivax* [1], while a fifth species (*P. knowlesi*) is known to transmit malaria between Macaque monkeys and humans in south-east Asia [2,3]. The most dangerous species is *P. falciparum*, as it causes the most severe form of malaria with the highest morbidity and mortality rates [4]. The disease is transmitted by parasite-infected female *Anopheles* mosquitoes biting uninfected humans. During a blood meal, *Anopheles* mosquitoes inject parasites in their infective stage (sporozoites) from the salivary glands into the human blood circulation. Sporozoites then travel by blood to the liver, where they develop into merozoites, another life-cycle form which then invades red blood cells once they are released again into the blood circulation. Once merozoites have entered these cells, they replicate and rupture the red blood cell and invade even more cells. However, a few young merozoites undergo another developmental pathway. These parasites develop into male and female gametocytes within the red blood cells and remain inside these cells. This cycle in the humans takes approximately 10 days.

The gametocytes are then taken up again by female *Anopheles* mosquitoes during a blood meal. Once the gametocytes reach the mid-gut of the mosquito they mature into male and female gametes and fertilization takes place. A motile zygote (ookinete) is formed and penetrates the mosquito's gut wall where it develops into an oocyst. Sporozoites develop within the oocyst and, when mature, rupture the oocyst and migrate to the mosquito's salivary glands. The mosquito is then infective for life and will pass on the parasites every time she takes a blood meal. The maturation process of the parasite within the mosquito, starting from ingestion of gametocytes, is referred to as the sporogonic period, which varies in duration between eight to twelve days, depending on environmental conditions (Figure 1.1).

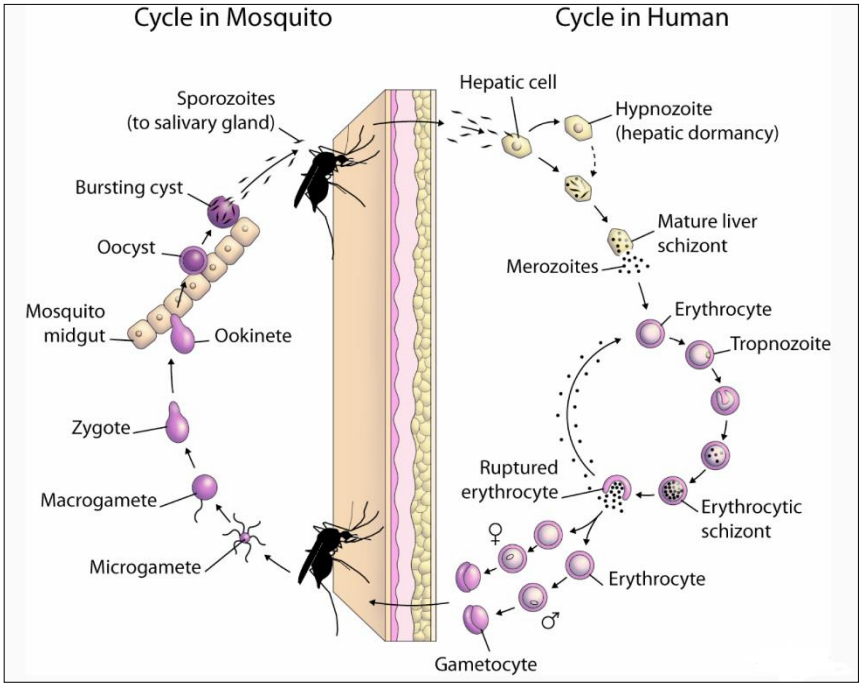


Figure 1.1: Lifecycle of the human malaria *Plasmodium* parasite spp. (Adopted from Wirth 2002 [5]).

1.2 Malaria burden

Malaria is an important parasitic disease transmitted by *Anopheles* mosquitoes, killing 435,000 people and infecting more than 200 million annually worldwide [6]. The most affected group are children under 5 years old. In 2017, under-five deaths accounted for 61% of all malaria deaths globally. The desease also adversely affects socio-ecodomic development in sub-Sharan African countries [7]. About 93% of all malaria deaths now occur in Africa [6]. However, the current WHO estimates show a significant reduction of the morbidity and mortality globally by nearly 50% in the period between 2000-2015 [6], although this progress has since plateaued [6,8].

Tanzania is making significant efforts to reduce malaria transmission. Evidence shows that malaria prevalence among children was significantly reduced by more than 50% between 2000 and 2017 [9]. According to the Tanzania Malaria Indicator Survey, prevalence was estimated to be 18.0% in children under five in 2008 [10], but has been reduced to 7.3% in 2017 [11], due to mass use of long-lasting insecticide-treated bed nets (LLINs) [9,12] and other control tools such as application of indoor residual-sprays (IRS) [13], advanced diagnostic tools and access to health

care, proper medication and overall economic growth and urbanization [14]. For example, the Kilombero floodplains is a perennially meso-endemic area in Morogoro region, south-eastern Tanzania, where residents experienced >1,000 infectious bites/person/year in the 1990s and early 2000s [15], but this has recently gone down to below 20 infectious bites/person/year. The persistence of low-level transmission (also called residual transmission) in the community remains a major challenge [16], being no longer as responsive to the forefront malaria prevention measures as before. It means that some additional new tools will be required to bridge the gaps and drive transmission to zero [16], as Tanzania is aiming to eliminate malaria by 2030 [17]. Despite the fact that the Ministry of health in Tanzania highlighted the need for cost-effective use of limited resources, there was no clear pathway of how this would be achieved.

1.3 Heterogeneity of biting risk and malaria transmission

The latest Tanzania malaria indicator survey [11] shows that malaria prevalence varies between regions, ranging from 0 to 24% [11] (Figure 1.2). Most people now live in areas with less than 5% parasite prevalence, the greatest burden being in the north and southern areas with the central belt being mostly low-prevalence. However, even within the high transmission districts, some households are always at higher risks of malaria transmission than other households in the same villages [11]. Intervention strategies should be designed to also capture these variations [18,19]. Researchers have gathered useful information on housing designs and malaria transmission, yet the contribution of settlement patterns to small scale variations of malaria transmission is still unknown.

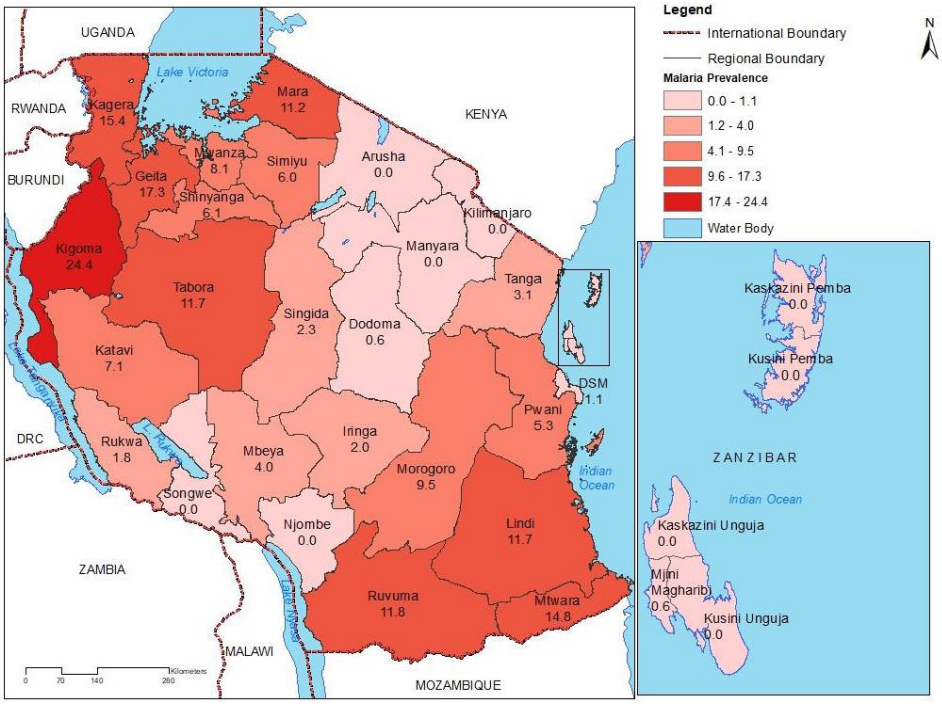


Figure 1.2: Malaria prevalence by regions in Tanzania (percentage of children aged 6-59 months with a positive Rapid Diagnostic Test (RDT). Adopted from Tanzania Malaria Indicators Survey [11].

Earlier research determined that distribution of the human host influences the distribution of adult mosquitoes inside houses [20]. This study also showed the relationship between mosquito distribution and number of people in the households (household occupancy). This means that researchers can use district-level population census data to map malaria transmission risk at fine scales. However, this study did not assess the distances between these households and the degree of clustering. Another study suggested that targeting the few households at higher risk of malaria transmission could significantly reduce the overall transmission in the villages [21]. The same trend was also observed in a semi-urban area where 60% of mosquitoes collected over a period of one year were collected from 0.6% of all light trap collections [22]. This kind of heterogeneity may affect the efficacy of control strategies specifically when the transmission is low. It also provides opportunity for malaria-targeted interventions on pockets of transmission that could be highly effective if identified.

Geographical Information systems (GIS) offer special capabilities in helping to identify malaria risk areas [23]. They can be used to detect how the interventions are

distributed, assess abundances and distributions of the malaria vectors and their associated breeding sites, distribution of insecticide resistance incidence, as well as mapping malaria transmission risks in general [23,24]. Environmental data are widely analyzed and presented using the GIS technology. However, there are also important factors such as distribution of hosts which can be integrated in the technology for mapping the likelihood of mosquito-borne disease transmission patterns [25]. Integrating this information will eventually lead to informed decision-making and provide guidance for malaria interventions.

1.4 Existing gaps, challenges and contribution of this study

Despite the effective use of malaria interventions such as long lasting insecticide-treated nets (LLINs), there are still major household and environmental characteristics contributing to persistent malaria transmission. Spatially targeting vector control tools is recognized as one of the best approaches for controlling malaria transmission in countries aiming for elimination [26]. However, there are limited studies on the relationship between household densities, distances between households and malaria transmission risk. Yet, such information is crucial for determining how and where to prioritize and target ongoing transmission at village level.

This thesis improves our understanding of the relationships between key environmental factors, distribution of human hosts and malaria transmission risks, influence of housing designs and human settlement patterns, community knowledge and persistent malaria transmission. Furthermore, it improves our understanding of the influence of environmental features as well as anthropological determinants of transmission and control and how elimination efforts can benefit from the information provided in this study. As malaria efforts move towards elimination, it is necessary to begin focusing our limited resources on hot spots (areas with high risk of malaria transmission), identifiable in small and large scale landscape levels. Furthermore, this research also yielded data on factors affecting mosquito distribution as well as infectiousness in rural Tanzania. This information will contribute to the goal of malaria elimination by enabling spatially targeted interventions.

In Tanzania, malaria has significantly declined due to scale-up of the use of long-lasting insecticidal nets (LLINs) [27]. However, more efforts should be put in place to control the persistent low-level malaria transmission, and targeting high risk areas would be an ideal way to do so in order to achieve zero malaria transmission, which is the target for the current national malaria strategy [17]. The role of settlement patterns in sustaining malaria transmission should not be ignored. Indeed, elimination will require that effective strategies are developed that enable detection of high risk areas in individual villages [28–30], thus enabling more effective control by current and future tools.

The geographical heterogeneity between villages and households significantly influences the distribution and variation of malaria transmission in a village [31]. Some households maybe at lower risk, while nearby households may be characterized by high risks due to the small scale variation of environmental features and variations in household designs [18,19,32]. While housing characteristics are historically known to influence the risk of mosquito borne diseases, information on the impact of actual location of the houses in a given area is minimal [33]. Such information would be immensely useful in determining how best to prioritize and target ongoing transmission at village and household level. This research focused on identifying key factors influencing fine-scale variation of ongoing malaria transmission inclusive of settlement patterns and distances between households. Additionally, locally-appropriate interventions are required to address any such challenges and effectively complement LLINs.

1.5 Literature Review

1.5.1 Residual malaria transmission

Residual transmission refers to the persistence of malaria transmission that continues to affect people after the implementation of recommended effective malaria control interventions over time and space [16,34]. WHO defines residual transmission as “Persistence of malaria transmission following the implementation in time and space of a widely effective malaria programme” [34]. In other words, residual transmission simply means the complete set of transmission events that continue to occur in communities where primary interventions such as LLINs, IRS, case management and larviciding have already been widely implemented at high

coverage, but where new *Plasmodium* infections still occur locally. It refers to all new local malaria transmission events in non-naive communities. The malaria eradication research agenda initiative (maLERA) indicated that while there were multiple possible reasons for the extensive reductions in malaria, numerous communities in settings where large-scale control operations have been implemented are experiencing a reduced but seemingly more difficult-to-control level of transmission [35]. It is also now widely believed that a significant proportion of this residual transmission may be transmitted by mosquitoes that bite humans when they are outside their dwellings or away from protection of indoor interventions such as LLINs and IRS [36]. Mosquitoes that are either resilient or physiologically resistant to the interventions, and can survive on non-human hosts, would require improved surveillance and control approaches to complement existing methods (Figure 1.3). Residual transmission has negative impacts on the ongoing efforts for malaria elimination [16]. The evidence suggests that current interventions are challenged by the vectors [9] and low-level residual transmission persists even in settings with high coverage of LLINs and IRS [37].

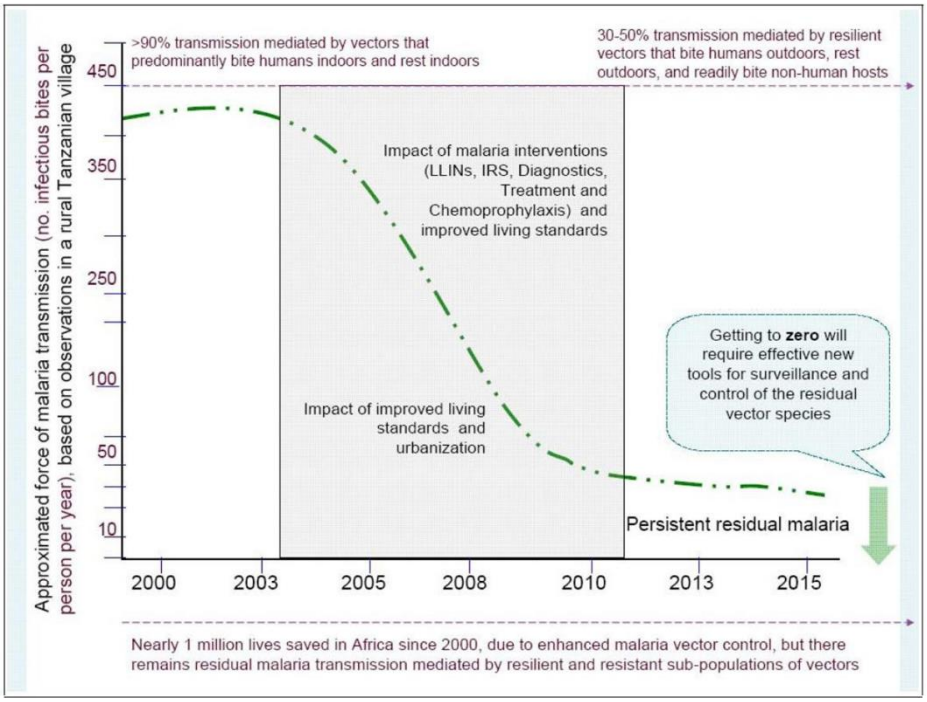


Figure 1.3: Diagrammatic representation of progress made against malaria over the past decade. Approximate values are based on observations in the Kilombero

Valley, south eastern Tanzania, adopted from Okumu *et al.* [38].

1.5.2 Factors affecting malaria transmission

There are several factors that influence malaria transmission ranging from suitable climates to human activities, mosquito behaviour and other non-human factors. These factors are described below with examples from different settings.

1.5.2.1 Climatic and environmental factors

Climatic factors are very important determinants of malaria transmission [39,40]. Rainfall, humidity and temperature influence mosquito population sizes and hence malaria transmission in a given setting [41]. Depending on the amount of rainfall, it can either increase aquatic habitats and thus mosquito numbers, or flush out some habitats, which interrupts the development of eggs and larva [42–44]. Rainfall patterns have been used to predict epidemics. Evidence from Sudan [45], Uganda [46] and Zimbabwe [47] found a correlation between rainfall increase and increased malaria incidence.

Temperature is another important factors which influences parasite development and mosquito survivorship [48]. Within a certain range, high temperatures shorten the gonotrophic cycle and thus the interval in which mosquitoes take subsequent blood meals. This increases the biting rate of a mosquito, hence increasing the risk of malaria transmission [49]. On the other hand, studies have demonstrated that very high temperatures above 40°C cause mortality in both mosquitoes and parasites [48]. Lastly, relative humidity also affects the life span of mosquitoes. The optimum relative humidity for *Anopheles* mosquito survival is between 60 and 80%, while below 60% or above 80% the adult mosquito life is shortened [48].

1.5.2.2 Human behaviours and activities

Human behaviours and activities are known to also influence malaria transmission. Socio-economic activities [50–52] and environmental changes [53] have direct effects on malaria transmission since they often cause modifications in human habitats and associated agricultural activities [54–56]. Human movement also is attributed to the increase in malaria transmission [57], thus malaria control interventions can be compromised by imported malaria [58].

1.5.2.3 House characteristics, human settlements and malaria transmission

Poor housing is associated with high incidences of numerous infectious diseases, notably insect-borne diseases like malaria, filariasis and arboviruses, which are major public health and economic concerns in Africa [54–56,59]. In Tanzania, approximately >90% of the population lives in areas at risk of insect-borne diseases [17,60], which could be preventable by proper housing. Most people in rural areas live in poorly finished houses with gaps in the eaves, windows and doors, allowing mosquitoes to enter freely, bite people and transmit the pathogens. Evidence suggests that house design, geographical location of the house within settlements and the number of people occupying the house, all influence malaria transmission [21,54,61,62]. Several studies carried out elsewhere show that house design is one of the factors influencing the abundance of indoor malaria vector densities and malaria transmission [54,61,63].

A systematic review of studies done in Asia, Latin America and sub-Saharan Africa showed that, compared to people living in traditional houses, people living in modern houses had 47% lower odds of getting malaria and up to 65% lower odds of getting clinical malaria [55]. A more recent study carried out in 10 countries in sub-Saharan Africa showed that children living in modern houses are up to 14% less likely to have malaria compared to the children living in traditional houses [64]. Better housing should be incorporated in the health policy as one of the approaches to reduce malaria transmission and other vector borne diseases [65]. Tanzania, like many other African countries, is aiming to achieve several Sustainable Development Goals by 2030: SDG No. 3 on good health and wellbeing; SGD No. 8 on sustainable economic growth; and SGD No. 11 on sustainable cities and communities. With regards to housing locations in a given area, there is evidence showing that the exact location of the house has an impact on malaria transmission risks, for example, a study by Wim *et al*, in Sri Lanka found a strong association of malaria transmission with houses which were located close to the breeding sites [66]. Furthermore, locations with clustered households can be considered to be at high risk of malaria transmission since mosquitoes are highly attracted toward these housing clusters [21].

1.5.2.3 Mosquito behaviour

Evidence suggests that vector behaviour determines the malaria transmission intensity in a given area [67–69]. From the control point of view, each behaviour provides an opportunity for designing specific complementary interventions. These behaviours are illustrated in Figure 1.4 below.

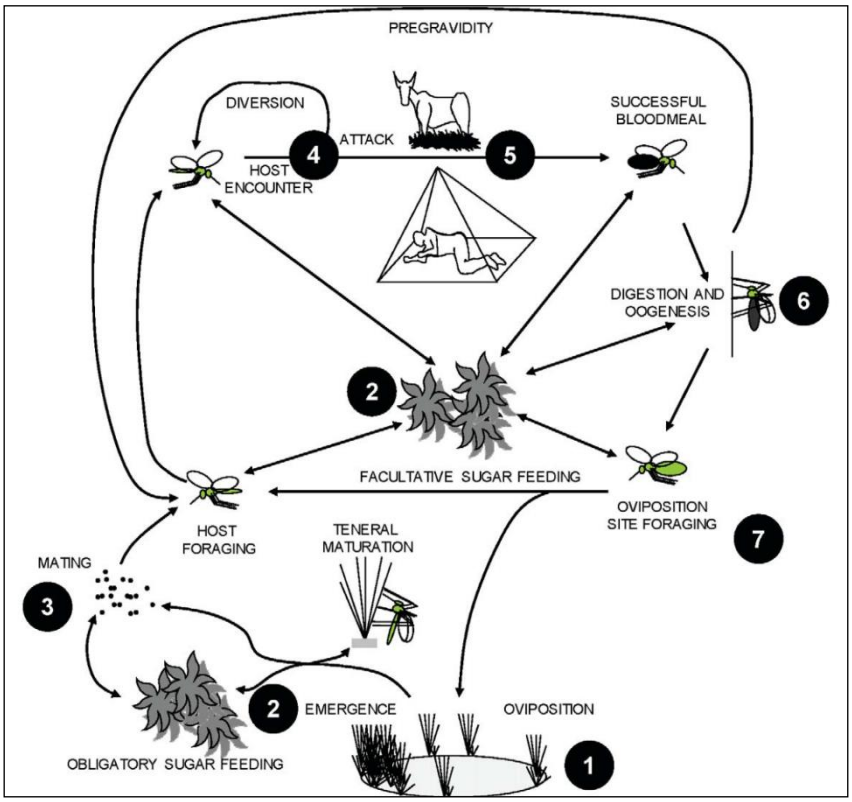


Figure 1.4: Diagrammatic representation of processes and behaviours of mosquitoes (Adopted from Ferguson *et al*. [70]).

1.5.2.4 Vector densities and competence

There have been different arguments on how mosquito abundance relates to malaria transmission in a given area. Some researchers have documented that high numbers of malaria vectors in an area translates into high malaria transmission [71], while others have argued that high transmission can also occur in areas with low vector densities [72]. Nonetheless, vector competency, which is a function of their preferences for humans over other vertebrates, their survival in nature, and sporogonic development of *Plasmodium* parasites inside the different *Anopheles* species, is likely a more important driver of malaria transmission in any given area.

1.5.2.5 Biting times and locations

Widespread use of effective interventions like LLINs and IRS, which target mostly mosquitoes which bite and rest inside human houses [73], have led to changes in the biting time of malaria vector populations [74,75]. As a result, mosquitoes have changed their biting time and location significantly and now outdoor biting is common with several studies reporting the increase of outdoor mosquito biting in many African countries [75–77] and also in south-east Asia [78,79]. This shift in biting time and place needs to be targeted by interventions that protect people from mosquito bites when they are not under the bed nets. Such interventions may include odour-baited traps [80,81], eave ribbons [82] and spatial repellents [83–85].

1.5.2.6 Sugar-feeding and blood-feeding

Anopheles mosquitoes are also known to depend on sugar meals [86,87]. This behaviour gives an opportunity to design an intervention that can control these vectors during sugar feeding [87–90]. However, with regards to blood-meals, mosquitoes also feed on other hosts apart from human beings. For example, *An. arabiensis* readily blood-feeds on cattle and several other animals when humans are unavailable [91,92], while other species such as *An. funestus* predominantly bites humans [93]. There are several studies that have shown that targeting these behaviours, e.g. using zooprophylaxis against *An. arabiensis*, can reduce malaria transmission [91].

1.5.2.7 Swarming and mating

Another important mosquito behaviour that can be exploited for control is swarming and mating. It is a major aspect in reproduction biology in mosquitoes, and *Anopheles* mosquitoes, like most arthropods are known to mate in swarms in nature [94,95]. Intervention should be designed to target this aspect of mosquito behaviour so as to reduce vector densities and malaria transmission. The current evidence of the efficacy of targeting swarms has been demonstrated in West African countries [96]. Information on mating swarms is extremely rare in East African countries, and there was previously no evidence of *Anopheles* swarms in Tanzania except for one study published in 1983 [97]. Recently, two studies have reported swarming, *An. funestus* in 2019 [98] and *An. arabiensis* in 2017 [99]. After the initial discovery of *An. arabiensis* swarms, this PhD study was extended to examine this as an option for complementing LLINs. We demonstrated that targeting swarms can significantly

reduce mosquito density (Kaindoa *et al.* unpublished) as was shown earlier in West Africa [96].

1.5.2.8 Mosquito flight patterns in the environment

Mosquito movement is also one of the important factors that affects malaria transmission in a given setting. The odour-mediated principle of host-seeking was first demonstrated by Rudolf Willem in 1922 [100] and led to the understanding that mosquitoes locate human hosts by following the odour trail [101–103] and therefore, host distribution has an influence on the flight patterns of mosquitoes in the villages [18]. This information is essential in determining how settlement patterns may be driving the spatial distribution of mosquitoes. Once confirmed that settlement patterns indeed influnce the variations in malaria transmission risks, interventions could be designed to incorporate this information so as to better target important areas with minimal resrources.

1.6 Community participation in malaria control

Engaging local communities in any intervention is known to influence the outcome [104–108]. For example, in Tigray, Ethiopia, mothers were taught to manage the treatment of malaria at home and were able to significantly reduce child mortality in the community [109]. Furthermore, studies have shown that knowledge about malaria and its control strategies can have a positive effect when working with community health workers [110]. Community participation is essential in the fight against malaria transmission and unless the community recognizes the importance of preventing diseases, the best designed interventions might not achieve the intended goals. It is however, not clear whether individuals understand how their surrounding environment and their activities impact on malaria transmission. It is likely that malaria vector densities can be disrupted if the communities understand local environmental factors influencing the distributions of malaria vectors.

A study done by Nganda *et al.* to assess how knowledge influences the uptake of malaria interventions, yielded evidence that LLIN uptake was higher compared to intermittent preventive treatment (IPTp) among pregnant women [111]. Despite the fact that this study was conducted in an urban area, the awareness for IPTp was very low. Similar evidence was obtained in a study conducted in rural Nigeria [112]. Both studies highlight the importance of increasing awareness of malaria

interventions. Another study by Levins *et al.,* provides evidence that community members can accurately distinguish mosquito species and breeding sites if well trained [113]. One aspect of the present study was to explore how communities understand relationships between environment and malaria risks.

1.7 Study justification

Spatial targeting of interventions is increasingly recognized as essential for malaria control, particularly in areas aiming for elimination [26]. Several studies are already published on associations between house characteristics and malaria transmission [54,56,61], but gaps remain on whether transmission is also influenced by factors such as distances between households or the degree to which houses are clustered. It is also important to identify new interventions that can be implemented by targeting critical points in the biology of major malaria vectors and common characteristics of human settlements. Such efforts will be essential to complement the current major malaria interventions. Therefore, this thesis is focusing on generating evidence to support spatially targeted interventions which would integrate housing characteristics and exploiting biology of malaria vectors to improve control.

1.8 Overall aim of this study

To investigate relationships between settlement patterns and malaria transmission in rural Ulanga district, southeastern Tanzania and identify new opportunities for control.

1.8.1 Specific objectives

1. To assess how densities of households and their distances from each other affect the risk of malaria transmission.
2. To identify environmental factors that may be affecting the ongoing residual malaria transmission risk in the study area.
3. To assess opinions of residents on how settlement patterns and other environmental factors affect transmission in the study area.
4. To identify complementary approaches that could be used to target and significantly improve malaria control in these residual transmission settings where LLINs are already widely used.

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Chapter II: Materials and methods

2.1 Study area

This study was conducted between 2016 and 2018 in seven villages in Ulanga and Kilombero districts in the Kilombero Valley in south-eastern Tanzania. These included Kivukoni (8.2135°S, 36.6879°E), Minepa (8.2710°S, 36.6771°E), Mavimba (8.3124°S, 36.6771°E), Milola (8.2135°S, 36.6878°E), Tuliza Moyo (8.354497°S, 36.705468°E), Lipangalala (8.1539°S, 36.6970°E) and Ikwambi (7.98244°S, 36.82167°E) (Figure 2.1). Current annual entomological inoculation rates (EIR) are estimated to average 4.2 infectious bites per person per year (ib/p/y) for *An. arabiensis* and 11.7 ib/p/yr for *An. funestus* [1]. The town of Ifakara, however, experiences EIRs below 1 ib/p/yr [2]. Most recent parasite surveys showed that malaria prevalence ranged from <1% in the northernmost villages to >35% in the southernmost villages (Minja *et al*., unpublished data).

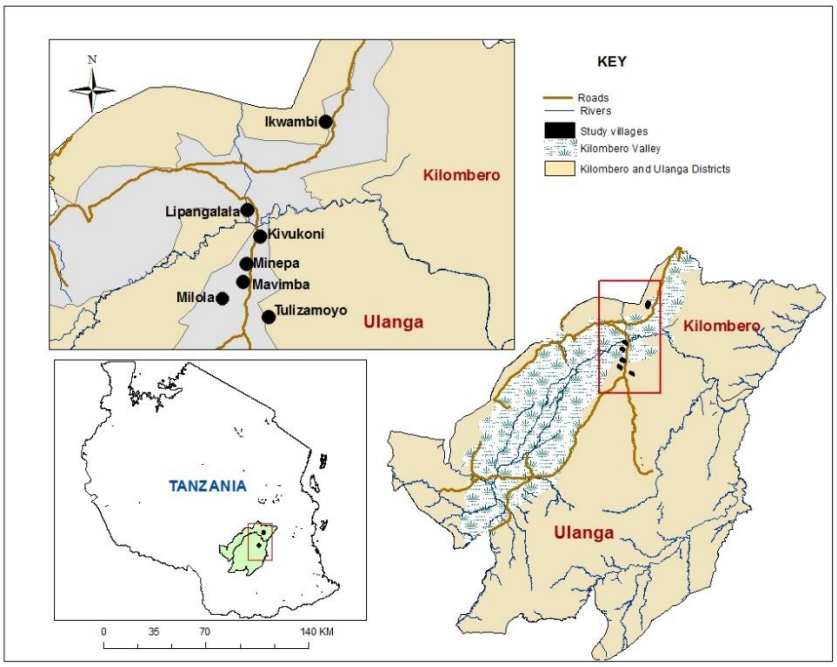


Figure 2.1: Map of the study villages in Ulanga and Kilombero districts in the Kilombero Valley in south-eastern Tanzania.

2.2 Study design and data collection

A mixed methods approach was used with the following components: (i) assessing how geographical foci of mosquito biting risk can be identified based on spatial

distributions of household occupancies in villages (Chapter III); (ii) longitudinal surveys of indoor mosquito densities (Chapter III); (iii) characterization of the houses and surrounding environmental variables including spatial densities and the distance between the houses (Chapter III); (iv) questionnaire and focus group discussions (FGDs) to assess and explore knowledge and perceptions of community members regarding household and environmental factors (Chapter III); (v) training of community members on *Anopheles* swarm surveys and characterization (Chapter III); (vi) laboratory analysis of sub-samples of *Anopheles* mosquitoes (Chapter III); and (vii) insecticide resistance tests (Chapter III).

For each research objective and activity, the specific study procedures are summarized below, but also the full details are provided in the attached appendices.

2.3 Study procedures

2.3.1 Objective-1: To assess how densities of households and distances between them affect the risk of malaria transmission**.**

Study households were selected from the study villages in the Kilombero Valley. Initially, a set of 48 households were randomly selected and recruited to participate in the study. These were sentinel (fixed) households, and mosquito collections were conducted once every month for a period of 12 months in 2017.

Then every month a total of 48 households were randomly selected (without replacement) from the pool of the remaining houses, and mosquitoes were sampled once. Altogether, mosquitoes were sampled from a total of 624 households in the Kilombero valley over 12 months.

Selection of the 48 households was done as follows: first, 1,600 households were randomly selected from the pool of 2,433 households in the seven villages in the valley. These households were then assigned to 16 geographical clusters, each containing 100 households. Every month mosquitoes were sampled from 6 houses in each cluster, three were sentinel households and three were randomly selected every month. Thus mosquitoes were sampled from 96 households every month. The full details are described in Kaindoa *et al.,* [3] (Chapter 4).

Centers for Disease Control and Prevention (CDC) light traps were used to sample mosquitoes for assessing indoor mosquito densities. The traps were set near an

occupied treated bed net. The collections were done from 18:00Hrs to 06:00Hrs. Resting mosquitoes were sampled each subsequent morning using backpack aspirators. The collected mosquitoes were killed and then morphologically identified using the taxonomic key developed by Gillies and Coetzee [4]. Molecular analysis done also done as explained in section 2.4.5.

2.3.2 Objective-2: To identify environmental features associated with residual malaria transmission in the Kilombero valley.

Characteristics of all the households surveyed were recorded. These included the geo-locations and whether the houses were located at the edge or centre of the villages. The conditions of windows, doors and walls, and whether the houses had closed or open eaves, were recorded. The surroundings of the households were also observed and nearby water bodies and animal sheds were recorded.

2.3.3 Objective-3: To assess opinions of residents on how settlement patterns and the other environmental factors affect transmission in their study area.

A structured questionnaire was used to assess community members' knowledge and perceptions regarding malaria transmission, treatment and prevention, and their perceptions on the association between various household and environmental characteristics and malaria transmission. The study also assessed and whether the community members consider malaria prevention when constructing houses. This survey was done with a total of 200 participants from the Kilombero Valley.

Following the questionnaire, Focus Group Discussions (FGDs) were conducted to further explore opinions and concerns of the community members regarding house and environmental characteristics and their association with malaria transmission. Eight FGDs were conducted with 12 participants, equally divided between youths (18-35 years) and adults (above 36 years). These discussions were held between January and March 2017. Full details are described in Chapter IV.

2.3.4 Objective-4: To identify effective complementary approaches that could be used to target malaria transmission in the Kilombero valley in south-eastern Tanzania. This work was focused on targeting of male mating swarms.

2.3.4.1 Identification of *Anopheles* swarms and assessing the potential of targeting them as a complementary approach.

Ten community members were selected with help from their respective leaders. They were provided with extensive training on how to identify and target-spray *Anopheles* mosquito swarms. All participants were male adults between 18 and 35 years of age. The trained community members were then asked to identify and characterize the swarms, and target-spray the confirmed *Anopheles* swarms. Full details are described in Chapter VIII.

2.3.4.2 Assessment of insecticide resistance profiles of dominant malaria vectors

The WHO guidelines were used to conduct insecticide susceptibility tests [5]. The Ifakara insectary-reared strain of *Anopheles gambiae s.s*. was used as a reference susceptible population. Wild female *An. funestus* mosquitoes collected from all the surveyed households were exposed to pyrethroids (0.75% permethrin, 0.05% deltamethrin, 0.05% lambda-cyhalothrin), organochlorine (4% dieldrin, 4% DDT), carbamate (0.1% bendiocarb, 0.1% propoxur) and organophosphate (0.25% pirimiphos-methyl, 1% malathion) classes of insecticides that are approved for use in malaria control by the WHO. The assay procedures were conducted as described in the WHO guidelines [5].

2.4 Data analysis

2.4.1 Quantitative data

Open source software, R was used to analyze all quantitative data [6]. Associations between household features and mosquito densities as well as monthly and nightly biting patterns were examined using generalized linear mixed effects models (*glmer).* Mosquito density was modelled as a function of household and environmental characteristics.

2.4.2 Analysis of housing pattern and distances

Distances between houses and possible influence on malaria transmission risks in the study area was assessed. Two approaches were used. Firstly, the point distance tool in ArcGIS 10 (ESRI, USA), was used to assign average distances of each house

within a radius of 150m, 200m and 400m. In each radius, the average distances of houses from each other were computed and the number of houses within the specific radius were counted. R version 3.5.0 [6] was used to perform this analysis, which focused on the association between house density and density of malaria vectors. Generalized additive mixed model (GAMMs) was used to model the number of mosquitoes caught per person per night [7].

The first sets of models were structured using number of houses within a hypothetical radius (200m, 300m, 400m and 500m). Only the number of houses within the 200m and 400m radius was included in the final model. The second sets of models were structured with distance between houses using the same radius as in the first set of models. In these sets of GAMMs, random terms were included to capture unexplained variations prevailing within households and to accommodate pseudo-replication within households and villages. Akaike Information Criterion (AIC) was used, and was used to generate the spline figures (Figures 5.1 and 5.2) in chapter V, showing the trends for each of the variables of interest.

2.4.3 Analysis of quantitative survey data

The data were summarized and presented descriptively in tables. Information was presented on the community members' knowledge about malaria transmission and risk factors, and on whether they consider these factors when constructing their houses.

2.4.4 Processing and analysis of qualitative data

All FGD audio files were transcribed in the local language that was used to conduct the discussions, and were then translated into English. The translated scripts were imported into NVivo software version 13 for further organization and analysis. Both deductive and inductive coding was used to arrange the themes. Discussion guides, as well as the specific objectives, were used to generate deductive coding, and inductive coding was used based on thorough review of the scripts. Some of the themes explored included: a) whether people associated household and environmental characteristics with malaria transmission; b) whether people consider these characteristics when constructing their houses; c) whether people associate seasonality and mosquito abundance with malaria transmission; d) whether people associate settlement patterns and human population numbers with malaria

transmission; and e) people's knowledge and perceptions of *Anopheles* mating swarms. Direct quotes from the participants were used to support the themes.

2.4.5 Laboratory analysis of sub samples of *Anopheles* mosquitoes

All mosquito samples collected from the village were sorted by sex, taxa and physiological status. Sub-samples of *An. arabiensis* and *An. funestus* mosquitoes were then further subjected to molecular identification by multiplex polymerase chain reaction (PCR), to identify sibling species as described by Scott *et al.* [8], Cohuet *et al.* [9] and Koekemoer *et al.* [10].

2.5 Ethical approval

Meetings were held with local leaders and their community members to describe the nature of the study. Written informed consent was obtained from all participants involved in each activity of the study. No identifying information was collected from the participants. All participants were assured anonymity. Ethical approval for conducting the study was obtained from institutional review board at the Ifakara Health Institute (IHI/IRB/No: 029-2016), as well as from the Medical Research Coordinating Committee at the National Institutes of Medical Research (Ref: NIMR/HQ/R.8a/Vol.IX/2428). Approval was also obtained from the Human Research Ethics Committee at University of the Witwatersrand (Approval Certificate No: M160806). Permission for publication was also obtained from the National Institutes of Medical Research. Approval numbers are provided in each publication and approval certificates are attached as appendices 7 & 8.

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Chapter III

Correlations between household occupancy and malaria vector biting risk in rural Tanzanian villages: implications for high-resolution spatial targeting of control interventions

3.1 Abstract

Fine-scale targeting of interventions is increasingly important where epidemiological disease profiles depict high geographical stratifications. This study verified correlations between household biomass and mosquito house-entry using experimental hut studies, and then demonstrated how geographical foci of mosquito biting risk can be readily identified based on spatial distributions of household occupancies in villages.

A controlled 4 x 4 Latin square experiment was conducted in rural Tanzania, in which zero, one, three or six adult male volunteers slept under intact bed nets, in experimental huts. Mosquitoes entering the huts were caught using exit interception traps on eaves and windows. Separately, monthly mosquito collections were conducted in 96 randomly selected households in three villages using CDC light traps between March 2012 and November 2013. The number of people sleeping in the houses and other household and environmental characteristics were recorded. ArcGIS 10 (ESRI-USA) spatial analyst tool, Gi* Ord Statistic was used to analyse clustering of vector densities and household occupancy.

The densities of all mosquito genera increased in huts with one, three or six volunteers, relative to huts with no volunteers, and direct linear correlations within tested ranges ($P<0.001$). Significant geographical clustering of indoor densities of malaria vectors, *Anopheles arabiensis* and *Anopheles funestus,* but not *Culex* or *Mansonia* species, occurred in locations where households with highest occupancy were also most clustered (Gi* P-value ≤ 0.05, and Gi* Z-score ≥ 1.96).

This study demonstrated strong correlations between household occupancy and malaria vector densities in households, but also spatial correlations of these variables within and between villages in rural southeastern Tanzania. Fine-scale clustering of indoor densities of vectors within and between villages occurs in locations where houses with highest occupancy are also clustered. The study indicates potential for using household census data to preliminarily identify households with greatest *Anopheles* mosquito biting risk.

Adapted from Kaindoa EW, Mkandawile G, Ligamba G, Kelly-Hope LA, Okumu FO. Correlations between household occupancy and malaria vector biting risk in rural Tanzanian villages: Implications for high-resolution spatial targeting of control interventions. Malar J. 2016;15:199. https://doi.org/10.1186/s12936-016-1268-8.

See Appendix 1.

3.2 Background

Significant efforts have been made to scale up appropriate interventions against malaria, an infectious tropical disease that still affects about 214 million people and kills 438,000 people annually [1]. The World Health Organization estimates that there has been a decline of malaria burden, and that morbidity worldwide reduced by 37% and mortality by 60% between 2000 and 2015, but sub-Saharan Africa accounts for approximately 90% of all malaria deaths and cases [1].

In Tanzania, country-wide malaria prevalence was last estimated at 9% among children under five years old, by rapid diagnostic tests (RDTs) [2]. Parasite prevalence has declined by between 50 and 60% in most of the country since 2000, although the southeastern and northwestern parts of the country have witnessed slower gains than the rest of the country [3]. These successes are mainly attributable to scale-up of long-lasting insecticidal nets (LLINs) [4,5] and indoor residual spraying (IRS) [6], but also improved diagnosis and treatment with effective drugs [7,8] . It is also possible that these successes were associated with overall improved health care, improved living standards, urbanization and overall economic transformation in the country [9]. The Tanzanian National Malaria Control Programme (NMCP) Strategy 2014-2020 was aimed to cut the prevalence to 5% by 2016 and to 1% by 2020 [10].

The current Global Technical Strategy for Malaria [11] recognizes that in order to achieve malaria elimination in today's endemic countries, it is imperative to develop and implement not only new complementary control methods, but also improved surveillance-response strategies to support resource allocation and implementation. More emphasis is needed to develop targeted approaches in intervention campaigns focusing on residual transmission foci. The need for fine-scale targeting of interventions is growing, particularly in countries where epidemiological malaria profiles increasingly depict high geographical stratification of risk [12–14]. In many cases, as transmission levels reduce, there remains geographically distinct pockets of transmission or demographically defined sub-populations, which must be identified and targeted to achieve zero transmission [12,15,16].

Based on the understanding of how disease-transmitting mosquitoes identify and follow cues from vertebrate hosts [17]. This study hypothesized that their dispersal within villages could be used as an indicator of areas where high biting risk occurs. Disease-transmitting mosquitoes are known to preferentially bite people with large body sizes [18], and households with high occupancy have also been shown to correspondingly have high *Anopheles* densities [19]. It is therefore likely that overall directional movement of mosquitoes within villages, and subsequently disease transmission risk, could be greatly influenced by spatial distribution of household biomass. In an earlier study, Russel *et al.* demonstrated the coincidence of increased malaria transmission hazard and vulnerability occurring at the periphery of two Tanzania villages [20]. The study postulated that the occurrence of *An. gambiae* was associated with the number of occupants. The study further suggests that most vector control could be effective by targeting few households at the periphery of two villages in rural Tanzania. These observations, though widely accepted, have not previously been developed into practical, actionable methodologies for disease surveillance, prevention or control. Yet this close association between human aggregations and mosquito biting risk may have significant influence on malaria parasite prevalence [21,22] and infectiousness [23].

This study used controlled experimental hut studies and high resolution household-level sampling of indoor mosquito-biting densities to demonstrate strong spatial correlations between household occupancy and indoor malaria vector densities in three contiguous villages in south-eastern Tanzania. The study also assessed whether regular census data could be used to identify households with the greatest *Anopheles* mosquito biting risk in rural Tanzania.

3.3 Methods

3.3.1 Study area

The study was conducted in three villages in rural Ulanga District, south-eastern Tanzania (Figure 1). This is an area with moderate to high malaria transmission, where prevalence was last estimated at 38% by polymerase chain reaction (PCR),

16% by RDTs and 6% by light microscopy [24]. Annual minimum and maximum rainfall ranges from 1,200 to 1,800 mm respectively, while the mean maximum and minimum temperatures are 20°C and 32.6°C respectively. Malaria vectors in the area include primarily the *Anopheles gambiae* complex, which comprises >99% *Anopheles arabiensis*, and the *Anopheles funestus* group. Houses are mainly mud and brick walled, with thatched or iron-sheet roofs. Most people rely on subsistence farming for their livelihood, cultivating rice and maize in the Kilombero river valley.

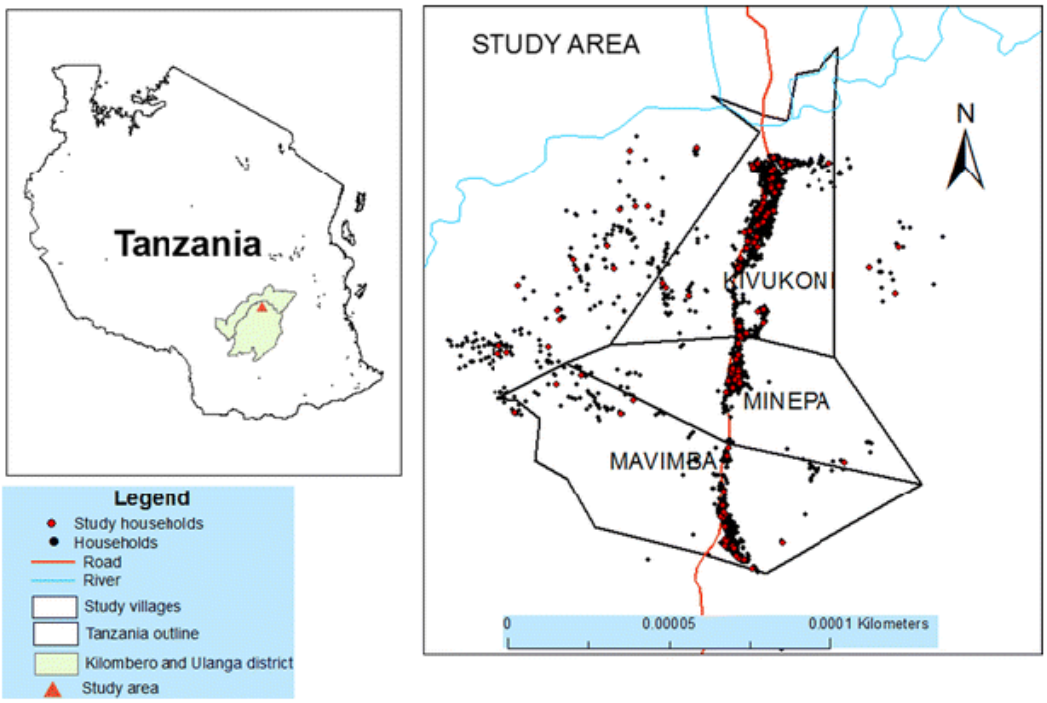


Figure 3.1: Map of the study area, showing the villages in Ulanga district where the study was conducted.

3.3.2 Study procedures

The study consisted of three parts: first, a controlled experimental assessment of the effects of host biomass on mosquito house entry, using specially designed experimental huts fitted with interception exit traps on eaves and windows to collect mosquitoes that enter the huts [25]. Second, longitudinal surveys of indoor mosquito densities were conducted from March 2012 to November 2013 in randomly selected households within the Ifakara Health and Demographic Surveillance System (HDSS) area [26]. Lastly, statistical assessments and

visualization of coincidental clustering between indoor malaria vector densities and household occupancy was done using ArcGIS 10 software (ESRI, USA).

3.3.3 Controlled experimental verification of correlations between household occupancy and mosquito house-entry

The Ifakara experimental huts, which have previously been demonstrated as effective for studying behaviours of disease-transmitting mosquitoes, including major malaria vectors in East Africa, were used [25]. The experiment was conducted in a 4 × 4 Latin square design replicated four times over 16 nights. Four experimental huts, designated A, B, C, and D were used. Each night, each of the huts was either left unoccupied, or was occupied by one, three or six volunteers. The number of volunteers for each hut was randomly assigned nightly, and was rotated across the four experimental huts over a four-night working week. A group of 10 male volunteers aged between 18 and 35 years participated in the experiment throughout the 16 nights.

Each night, the hut designated to have no volunteers was considered the control hut, and remained unoccupied. To eliminate any potential biases from differential attractiveness of individual volunteers to mosquitoes, the volunteers themselves randomly selected the huts in which they would sleep each night. Each day, just before the experiments began, each volunteer was asked to randomly select one piece of folded paper from a bowl containing several such folded papers, each with a specific hut label, which had been assigned by the researcher such that the specified number of volunteers per hut was always achieved. This way, there was always one hut with no volunteer (i.e. control hut), a hut with one volunteer, a hut with three volunteers and another with six volunteers, in all cases randomly assigned. Mosquitoes were collected in interception exit traps fitted on eave spaces of the experimental huts.

3.3.4 Longitudinal vector surveys to assess empirical relationships between indoor mosquito densities and household occupancy

A total of 96 households were randomly selected from an original HDSS household listing consisting of 2,433 households in three villages of Kivukoni,

Minepa and Mavimba, in Ulanga district, south-eastern Tanzania (Figure 3.1). The selection was conducted in two stages, where 1,600 households were first selected (randomly), and spatially assigned to 16 geographical clusters each consisting of 100 households. The sampling clusters were assigned based on household latitudes so that clusters 1 to 16 were obtained on a north-southerly direction. From each geographical cluster, six households were selected randomly, and the household heads were requested to volunteer in the study. Whenever a household heads did not consent, the next household in the random listing was selected, so that there were always six households per cluster.

The geo-positions (latitudes and longitudes) of all the households were recorded. In the same households, the total number of people in the household, and total number of people who slept in the specific trapping room were recorded on the night of mosquito sampling. The study also observed: a) type of roofing material (i.e. grass or iron sheets), b) material used on walls (i.e. brick or mud), c) whether the windows were screened or unscreened, d) whether the eave spaces were closed or open, and e) distance from nearest water body. All the 96 selected households were provided with a new intact long-lasting permethrin-impregnated bed net similar to what had been provided by the government during the universal LLIN coverage campaign, which covered the villages between November 2010 and January 2011 [5].

Mosquito sampling was conducted monthly in each of the study households, but the order in which the households and clusters were visited was randomized. Each week, mosquitoes were sampled in four of the 16 geographical clusters, by visiting all six households per cluster per night, working for four nights per week. In each household, one room with at least one person sleeping in it was selected for assessing indoor mosquito densities. CDC light traps set near occupied bed nets were used for the sampling [27], and were operated from 18:00 to 06:00hours. Each morning, the collected mosquitoes were killed in a closed container using petroleum fumes, then sorted by sex, taxa and physiological status as blood-fed, non-blood-fed or gravid.

A sub-sample of the female malaria vectors, *An. gambiae* complex, was examined

by multiplex (PCR), which amplifies the 28S intergenic spacer region of the ribosomal DNA to distinguish between sibling species in the complex [28]. Sub-samples of *An. funestus* were also examined by PCR, using techniques developed by Koekemoer *et al*. [29] and Cohuet *et al*. [30], which are based on species specific single nucleotide polymorphisms (SNP) in the internal transcribed spacer region 2 (ITS 2). The *Anopheles* samples were also examined by enzyme-linked immunosorbent assay (ELISA), to detect *Plasmodium* sporozoites in their salivary glands [31]. To avoid false positives, all the ELISA lysates were boiled for 10 minutes at 100˚C, so that any detected protozoan antigens were only the heat stable *Plasmodium* species [32,33].

3.3.5 Data analysis

Data were analysed by open source software, R version 3.1.0, using the lme4 package [34]. The total number of female mosquitoes of each taxon was compared between huts having one, three or six volunteer sleepers and the control hut with no volunteers. The data were fitted to a generalized linear mixed effects model (GLMM), with log-linked Poisson error distribution [34]. Total mosquito catches were modelled as a function of number of volunteers and hut, while day of collection and experimental block (i.e. a set of four study nights) were used as random variables in the model, taking into account variations associated with nightly and weekly randomization in the experiment. Mean number of mosquitoes of each species collected per hut per night, and relative rates (RR) of collecting mosquitoes in the huts, and the associated 95% Confidence Intervals (CI), were estimated by exponentiation the coefficients generated from GLMMs.

For the longitudinal mosquito survey, the total number of mosquitoes collected from each house was obtained by first summing all female mosquitoes per hut per night. Relationships between household or trap room occupancy and mosquito densities were examined also by GLMMs using the *lme4* package [34], and log-linked Poisson error distributions as above. The indoor densities of mosquitoes of different species were modelled as a function of: a) number of occupants in the mosquito trapping room, b) total household occupancy, c) month of mosquito collection, d) village of collection, e) whether the eave spaces on the houses were

closed or open and f) distance from nearest water bodies. Date of mosquito collection and house identification codes were incorporated as random variables in the GLMMs. Estimated mean indoor mosquito densities per hut per night and RR of these mosquitoes catches, and associated 95% confidence intervals (CI), were computed from exponentials of the coefficients generated from the GLMMs.

Identification of spatial patterns of indoor mosquito catches was done using ArcGIS 10 spatial analyst tool (ESRI, USA). The Getis-Ord Gi* statistic [35] in ArcGIS was used to identify locations of households with significant clustering of high indoor densities of disease-transmitting mosquitoes, including the malaria vectors, *An. arabiensis* and *An. funestus*, but also *Culex* species and *Mansonia* species. Clusters depicting both the high vector density foci (i.e. areas with households where the highest densities are most spatially concentrated) and low-vector density foci (i.e. areas with households where lowest densities are most spatially concentrated) were identified. Statistically significant clusters were then determined at a level of Gi* P value ≤ 0.05, and Gi* Z score ≥ 1.96. In this analysis, the conceptual relationship between households was assumed to be inversely related (so that houses far apart were considered more likely to be have different indoor vector densities than households near one another), and Euclidean distances between neighbouring features were considered [35,36].

3.3.6 Ethical consideration

All human volunteer participants were fully informed of the study objectives, benefits and risks involved in the experiment. Participation was only after the volunteer provided written informed consent. All households participating in this study and all volunteers sleeping in the experimental huts were protected by intact LLINs (Olyset® nets), to ensure basic minimum protection. Ethical approval for the study was obtained from Ifakara Health Institute's Institutional Review Board (IHI/IRB/No:10-2013), Liverpool School of Tropical Medicine (Approval No. 01, issued on 10thMarch, 2014), and the Medical Research Coordination Committee of the National Institute of Medical Research (Certificate No. NIMR/HQ/R.8a/Vol.IX/1816). Permission to publish this manuscript was obtained from National Institute of Medical Research (Ref: NIMR/HQ/P.12 VOL XVII/16).

3.4 Results

3.4.1 Relationship between household occupancy and mosquito house-entry in experimental huts

There were significant increases in numbers of all mosquitoes of different taxa, whenever the number of volunteer sleepers (proxy of human biomass) increased in the experimental huts. The increase was observed in catches of *An. arabiensis*, *An. funestus* and *Culex* mosquitoes. As shown in Figures 2 and 3, the results indicate that in all huts with one, three, or six volunteers, there were more mosquitoes than in the unoccupied control huts ($P<0.001$).

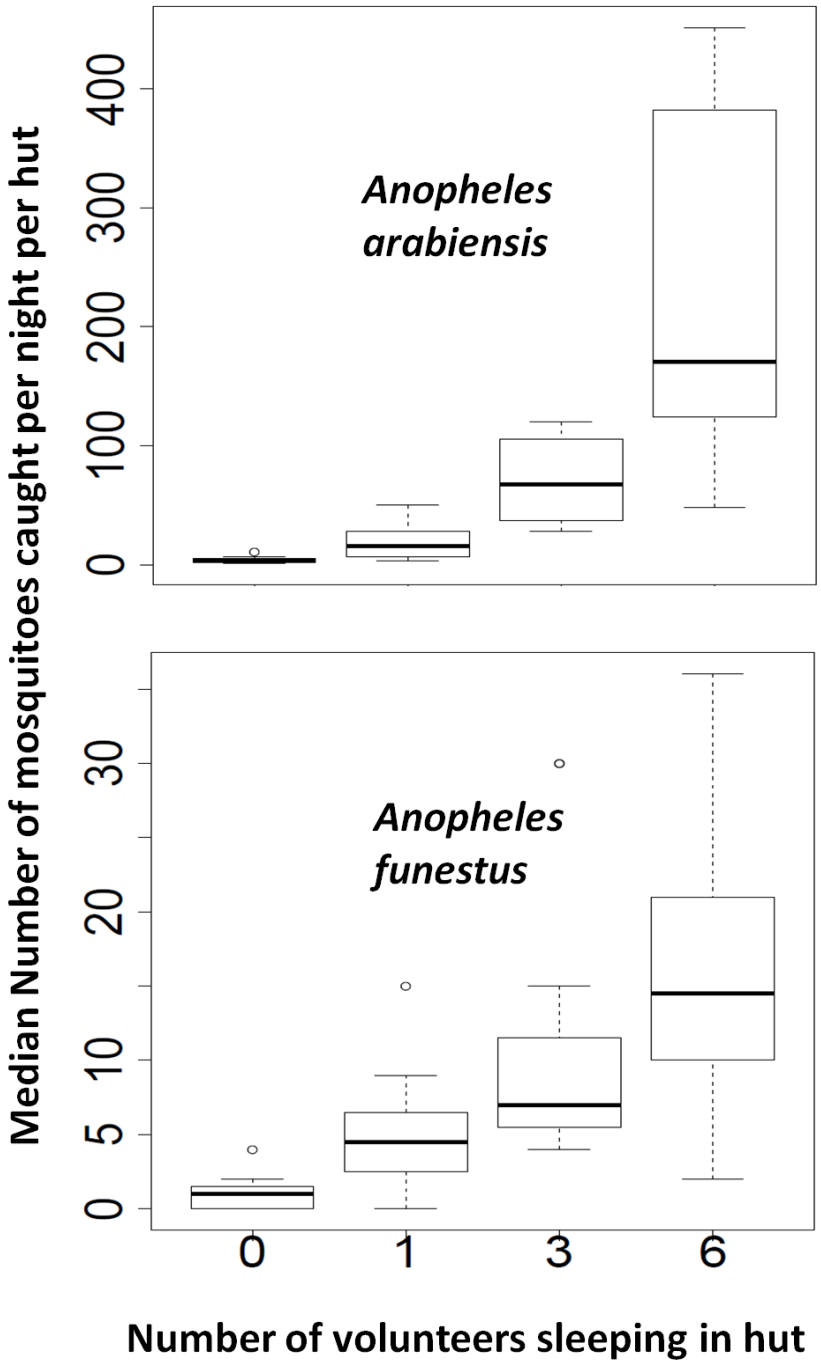


Figure 3.2: Effects of host biomass on indoor densities of malaria vectors: comparison of the number of *Anopheles arabiensis* and *Anopheles funestus* mosquitoes caught in experimental huts occupied by varying numbers of adult male volunteers. The y-error bars represent the inter-quartile ranges around the median.

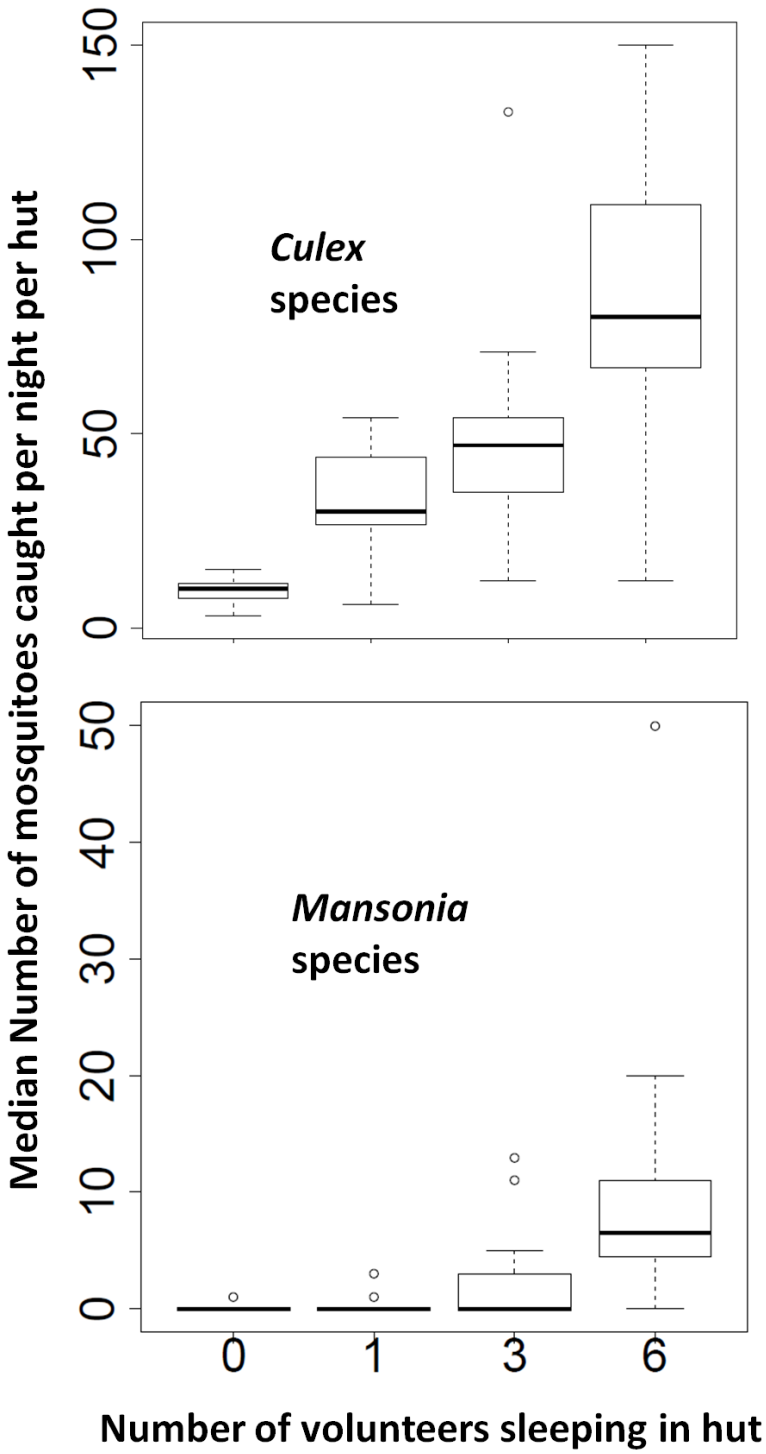


Figure 3.3: Effects of host biomass on indoor densities of non-malaria vectors: Comparison of the number of *Culex* and *Mansonia* mosquitoes caught in experimental huts occupied by varying numbers of adult male volunteers. The y-error bars represent the inter-quartile ranges around the median.

3.4.2 Relationships between indoor mosquito densities and occupancies of the households of trapping rooms

The number of malaria vectors caught in houses increased with number of people occupying the house. To match the field observations, where trapping rooms generally had at least one person and households generally had at least two members, the study assessed effects of trap-room occupancy and household occupancies relative to base-line levels of one person versus two persons respectively. The relative rate (RR) of catching *An. arabiensis* mosquitoes in trapping rooms with more than one occupant compared to rooms with one occupant was 1.6 (95% C.I 1.2 - 2.3), P<0.001. Similarly, for *An. funestus*, trapping rooms with more than one occupant had higher catches than rooms with just one person (RR = 1.1 (1.0 -1.4), P < 0.001). A similar trend was found in densities of non-malaria mosquitoes. With regard to overall household level occupancy, as opposed to just trapping room occupancy, there were also significantly more *An. arabiensis* (RR = 1.8 (1.3 - 2.7), P<0.001), more *An. funestus* (RR = 1.0 (0.9-1.2), P<0.001) and more *Culex* mosquitoes (RR = 1.3 (1.1-1.7), P<0.001) in houses with more than two occupants, compared to houses with two or fewer occupants.

Having open eave spaces on houses was also significantly associated with indoor vector densities. The number of *An. arabiensis* caught was significantly higher in houses with open eaves, compared to houses with closed eaves (RR=0.8 (0.4.1-1.4), P<0.001) and the number of *An. funestus* was consistently higher in huts with open eaves compared to huts with closed eaves (RR= 1.2 (1.0-1.5)) P<0.001). Similar trends were observed for *Culex* mosquito species (RR = 0.9 (0.7-1.1), P<0.005), and *Mansonia* species (RR= 3.2 (2.0-5.2), P<0.001). In this study, the CDC light traps used for sampling mosquitoes were set near human-occupied bed-nets in the selected households.

3.4.3 Spatial clustering and correlations between house occupancy and indoor vector densities

There were clearly identifiable and statistically significant clusters of households with high densities of the two main malaria vectors, *An. arabiensis* and *An. funestus*

in the central part of the study area (GI* Z ≥1.96, GI* P≤0.005), but also significant clusters of *Culex* mosquitoes in the northern part of the study area (Figures 4 - 6). Clusters of households with the highest occupancy occurred in these same geographical locations in the study area (GI* Z≥1.96, GI* P≤ 0.05). Since the study obtained the household occupancy data from all the houses where mosquito collections were also conducted, the analysis reveals that geographical clusters of households with the highest occupancy were individually the same clusters of households with highest densities of malaria vector species, but not *Culex* mosquitoes. Clustering of *An. arabiensis* (Figure 4) and *An. funestus* (Figure 5) were both geographically coincidental with clustering of household occupancy in the study area. No such geo-coincidence was observed for *Culex* species (Figure 6). The study a l s o found no statistically significant clusters of houses with high densities of *Mansonia* species mosquitoes in the study area.

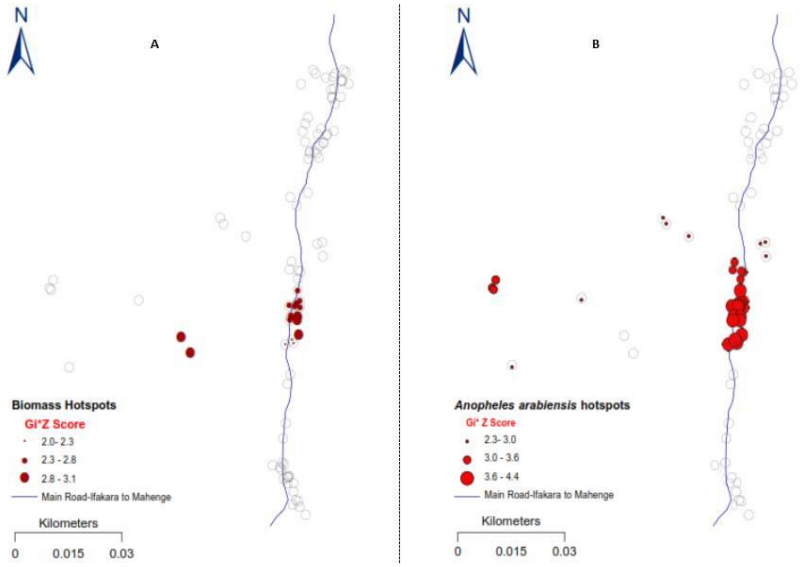


Figure 3.4: Maps showing statistically significant clusters of households with the high occupancy (A) and statistically significant clusters of households with high densities of *Anopheles arabiensis* in the same study area (B). The grey circles represent the rest of the households in the study area.

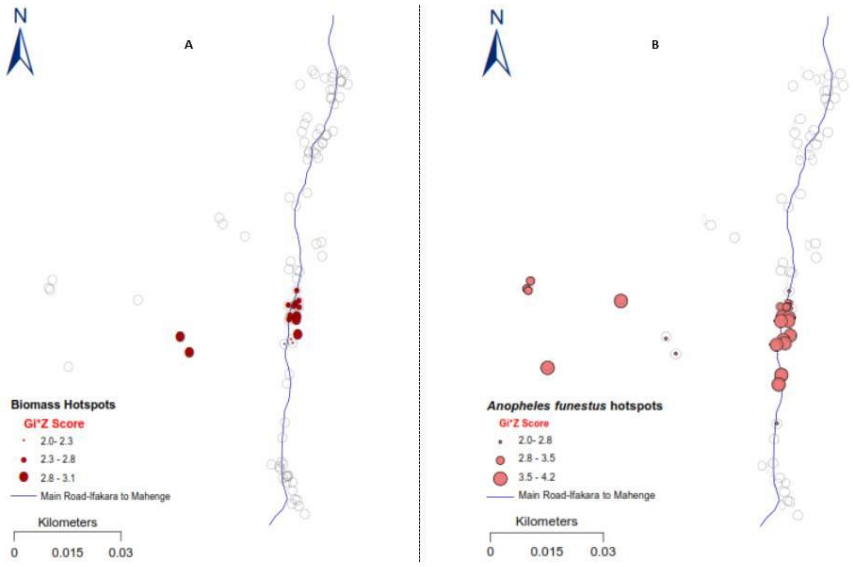


Figure 3.5: Maps showing statistically significant clusters of households with the high occupancy (A) and statistically significant clusters of households with high densities of *Anopheles funestus* in the same study area (B). The grey circles represent the rest of the households in the study area.

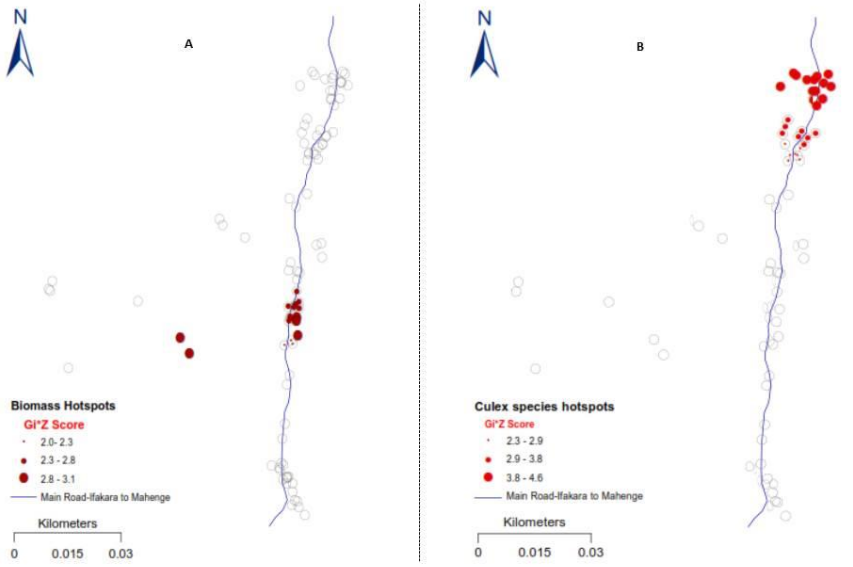


Figure 3.6. Maps showing statistically significant clusters of households with high occupancy (A) and statistically significant clusters of households with high densities of *Culex* in the same study area (B). The grey circles represent the rest of the households in the study area.

3.4.4 Species composition of malaria vectors and Plasmodium infection rates

A total of 39,754 *An*. *gambiae complex* and 14,817 *An. funestus* group mosquitoes were assayed in the laboratory. All of the *An. gambiae s.l.* mosquitoes assayed were confirmed to be *An. arabiensis* (100%), while 94% of the mosquitoes from the *An. funestus* group assayed were *An. funestus s.s* Giles. The rest of the An. funestus mosquitoes were *Anopheles rivulorum* (6%). Overall, the sporozoite infection rates were 4.04% in the *An. funestus* mosquitoes and 0.47% in *An. arabiensis* during the study period.

3.5 Discussion

Identification and targeting of high transmission foci particularly at fine scale levels within villages is essential for successful malaria control and eventual elimination [12,14]. Transmission of malaria pathogens, like many other infectious agents, is heterogeneous over host populations but also over geographical space [21,37], and this stratification increases significantly in settings with reduced transmission [12,14,15]. Understanding these dynamics and how they are influenced by the various biotic and abiotic factors is essential to improving planning for interventions of ongoing malaria prevention strategies.

The study hypothesized that household occupancy (being proxy to household-level biomass), would influence not only indoor vector densities as shown in several previous studies [18,19], but that it also influences mosquito dispersal within communities, and the resulting geographical distribution of human biting risk and pathogen transmission risks across these communities. By extension, it was assumed that overall dispersal of mosquitoes within villages is influenced by spatial distribution and demographic composition of households in these villages. As a result, locations where households with high biomass or occupancy are clustered would naturally form pockets of high transmission of mosquito-borne diseases, unless there are specific interventions or environmental variables, which significantly modulate such patterns.

Female mosquitoes need vertebrate host blood for reproduction and understanding this host-seeking behaviour is essential for estimating the transmission of mosquito

borne diseases, including malaria [17]. The host-seeking behaviour is influenced by many factors, including host odour cues, host density, dispersal ability of the mosquitoes and host distribution availability [17,38]. Indeed, where distribution of human populations is heterogeneous, the distribution of adult mosquitoes also tends to be heterogeneous even if the breeding sites are uniformly distributed in the environment [21].

The controlled experimental hut studies verified earlier observations of correlations between vector densities and human biomass [18], but also provided a clear pattern of the seemingly linear relationships between these variables. The design of the experiment, using exit interception traps, enabled mosquitoes to freely fly into huts, and quantifies the densities by trapping them upon exit. The human volunteers participating in the study were fully randomly assigned to the huts on a nightly basis, thereby excluding confounding effects of differential host attractiveness to mosquitoes [38]. Moreover, since the study restricted the age of volunteers to between 18 and 35 years, and relied on a fixed group of ten volunteers for this study, the observed associations between vector densities and volunteer numbers can be considered to represent correlations with human biomass, and not any other factor. The experiment therefore provides the first of such datasets obtained under controlled environments in a malaria-endemic community, and lends itself to future use for fitting models that simulate mosquito host seeking and pathogen transmission.

Similarly, the field surveys also showed that houses with higher occupancy tended to have more mosquitoes as compared to houses with low occupancy, even though the indoor vector densities were also modulated by factors such as whether the eave spaces were open or not. In this study, the effects of trap-room and household occupancy were assessed by considering observed base-lines of at least one person per trap room versus at least two persons per household. This was because individual trapping rooms generally had at least one person while households generally had at least two members. Although the study observed several other household characteristics other than biomass and eave spaces, the analyses revealed that these were the two most influential variables on indoor vector densities in the study area. A study by Al-Eryani *et al.* in Yemen has also yielded similar evidence that the number of *An. arabiensis* was positively and spatially

correlated with the number of occupants in the house [22].

There was a clearly observable geographical overlap in the spatial clustering of houses with high occupancy, and the clustering of houses with high densities of malaria vectors. The study analysed the data for household biomass separately from the data for vector densities, yet in both cases, there were significant clustering. The analysis thus provides a set of possible simple rules, which could be relied upon to predict at fine-scale, the parts of villages where the highest biting risk occurs and where intense, highly focalized vector control efforts would achieve greatest community-level impact. This study was conducted in an area which has historically had very high malaria transmission rates [3,39], but where LLIN coverage is now evenly very high. Even then, this study suggests that by simply mapping household occupancy and their spatial distribution in the area, one would be able to rapidly identify places with the highest and lowest indoor vector densities, even without any vector trapping. This information is of major significance for spatial targeted interventions, particularly at fine-scale [20,22], even within small administrative boundaries such as wards and villages.

Visual inspection of Figures 4-6 suggests that both intra- and inter-village variation in indoor mosquito-biting risk would be spatially correlated to household occupancy patterns, readily identifiable by using census and other demographic data in many other malaria-endemic countries. One point of caution is that whereas the assertions could hold true over geographically homogenous areas, and in the absence of any focalized vector control operations that disrupt mosquito-host seeking and density distributions such as IRS [40,41] or larval source management [42], there are several other features with potential to change or eliminate these spatial correlations. Features such as topography [43,44], ground water and surface water flows [45], growth of urban centres and increased settlement densities [46], as well as agricultural cultivation [47], are examples that could disrupt the geographical coincidences observed here. Indeed Thomas *et al.* recently concluded, after analyses of data from The Gambia, that mosquito dispersal would likely be landscape specific [44], necessitating that a reasonable level of characterization is conducted in the target communities. Despite these potential sources of discrepancies, the observations and experimental verifications have clearly

determined that vector control operations at local district level could rely heavily on readily available household census data to predict basic risk patterns across villages, but that use of other data layers would improve the outcomes and overall predictions.

Since household-level analyses revealed increasing mosquito numbers with increasing number of occupants, the results of these geo-spatial analyses must be interpreted with caution. The increased community-level biting risk implied by these analyses is primarily because of the increase in hazard levels, even if the individual level-exposure remained unchanged. Caution should be taken in the interpretation of these results so as not to imply that biting-risk per person was also increased inside the household in the areas where host biomass was highest, even if the bite-related hazard was higher. Interpretations of the results should therefore be limited to the understanding that increased concentration of potentially infectious mosquitoes in these areas would enable more effective targeted control, with lower amounts of resources, and also that in such locations, even a low-level exposure, would result in significant risk of malaria infections. For example, it is likely to be more dangerous to sleep without a bed net in these locations with high concentrations of large households, than it is to sleep without a bed net in the rest of the villages. The results should therefore be examined from the perspective of community level protection from the increased biting risk. Since potentially infectious mosquitoes disperse towards, and eventually end up being most abundant in areas with highest household biomass concentrations, creating opportunities for targeted control of these vectors community-wide mass effect. Moreover, locations with clusters of large households can therefore be considered as providing a significant level of protection to the smaller households elsewhere in the village [20], because mosquitoes are drawn mostly towards these locations, and away from the other areas. The greatest epidemiological value of the results is therefore more on their potential as a way to target community-wide vector control and achieve mass effect on potentially infectious vectors, rather than as a way to predict individual risk.

Other than the experimental studies and community-wide vector surveys, this study also showed that the proportion of *Plasmodium*-infected *An. funestus* was far higher than proportions of infected *An. arabiensis*. The former species thus plays

a much greater role in malaria transmission, contributing up to 87.9% of potential new infections in the study area, compared to only 12.1% from *An. arabiensis*. No other infected *Anopheles* species was found during this study. The concern over the increasing role of local *An. funestus* populations remains an important one, given its greater competence as a vector of malaria. Although *An. arabiensis* is still the most prevalent of the vector species in the area, determining that ongoing residual malaria transmission is mostly mediated by *An. funestus* suggests that highly effective household-level interventions that target the indoor-feeding and indoor-resting behaviours of these vector species could still be highly applicable to bring down transmission levels. Such interventions would be greatly enhanced if spatially targeted to the parts of villages where host biomass is most concentrated. Studies by Lwetoijera *et al.* in southeastern Tanzania [48] and MacCann *et al.* in western Kenya [49] have also yielded similar evidence of the increased role of *An. funestus*. The seemingly growing challenge would be further complicated in areas where the vector species is also increasingly resistant to insecticides commonly used for malaria prevention and control.

Considering both the experimental assays and the entomological survey data, this study indicates that household-level effects of host occupancy or biomass on the host-seeking and indoor vector densities are indeed transferable to community-level patterns. As a result, areas with concentrations of large households tend to have more mosquitoes than areas with sparsely distributed small households. Unfortunately, despite availability of vast quantities of household census and other demographic data regularly collected from large populations in many countries including Tanzania, no efforts have previously been made to triangulate such datasets with the knowledge of how vectors identify, locate and attack humans, so as to map the likelihood of mosquito-borne disease transmission within and between villages. One would propose that such triangulations should be considered as an initial step in the assessments of disease risk. The knowledge is essential for creating baseline estimates of transmission risk and disease burden, which enables actual transmission foci to be easily mapped on fine scales, using simply estimates of human biomass or household occupancy, from regular demographic surveys. In countries where HDSSs have been running for many years, such datasets could also be utilized to provide baseline spatial estimates for risk

prediction and prioritization of interventions. Future studies may therefore include modeling of malaria risk from existing datasets such as malaria indicator surveys (MIS) and Demographic Health Surveys (DHS), with the aim of confirming the results observed in this study. An obvious advantage here is that MIS and DHS datasets regularly record numbers and age of people in households and would provide reliable estimates of household-level biomass distribution across communities.

3.6 Conclusion

Directly observable household level effects of household biomass on mosquito house entry are manifest at community-level spatial scales. These relationships result in a situation where areas with clusters of large households tend to have most biting mosquitoes, while areas with sparsely distributed small households have least biting risk. Overall, fine-scale and within-village clustering of indoor densities of major malaria vectors in this study area occurred in the same locations where houses with the greatest number of occupants were also clustered. It is hypothesized that in similar communities; the most intense foci of *Anopheles* biting risk could therefore be preliminarily predicted directly from household-level population census data. Where regular census data are available, with records of ages and numbers of people per household, it may be possible to rely on these maps to generate spatially defined high-resolution malaria risk maps, to support disease control programmes. However, since mosquito dispersal over space is often landscape-specific, it may also be necessary to identify what other factors significantly modulate these spatial relationships, and how these observations could be used to improve vector-borne disease mapping and control.

Competing interests

The authors declare that they have no competing interests.

Author's' contribution

EK and FO conceived the study and developed study protocols. EK, GM and FO conducted the experiments and supervised data collection. EK, LKH and FO conducted data analysis and wrote the manuscript. All authors approved the

manuscript prior to submission.

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Chapter IV

Housing gaps, mosquitoes and public viewpoints: a mixed methods assessment of relationships between house characteristics, malaria vector biting risk and community perspectives in rural Tanzania

4.1 Abstract

House improvement and environmental management can significantly improve malaria transmission control in endemic communities. This study assessed the influence of physical characteristics of houses and surrounding environments on mosquito biting risk in rural Tanzanian villages, and examined knowledge and perceptions of residents on relationships between these factors and malaria transmission. The study further assessed whether people were concerned about these risks and how they coped. Entomological surveys of indoor mosquito densities were conducted across four villages in Ulanga district, south-eastern Tanzania.

The survey involved 48 sentinel houses sampled monthly and other sets of 48 houses randomly recruited each month for one-off sampling over 12 months. Physical characteristics of the houses and surrounding environments were recorded. Questionnaire surveys were administered to 200 household heads to assess their knowledge and concerns regarding the observed housing and environmental features, and whether they considered these features when constructing houses. Focus group discussions, were conducted to clarify emergent themes on people's perceptions on relationships between housing or environmental factors and malaria transmission.

The entomological surveys showed statistically higher indoor densities of the malaria vectors (*Anopheles arabiensis* and *Anopheles funestus*) in houses with mud walls compared to plastered or brick walls, open eaves compared to closed eaves and unscreened windows compared to screened windows. Most respondents reported that their houses allowed mosquito entry, at least partially. Participants were aware that house structure and environmental characteristics influenced indoor mosquito

densities and consequently malaria transmission. They were concerned about living in poorly-constructed houses with gaps in eaves, walls, windows and doors but were constrained by low income and competing priorities. In rural south-eastern Tanzania, significant proportions of people still live in houses with open eaves, unscreened windows and gaps in door frames. In conclusion, we propose that community-based house improvement initiatives combined with targeted subsidies could lower the financial barriers, improve access to essential construction materials or designs, and significantly accelerate malaria transmission control in these communities.

Adapted from Kaindoa EW, Finda M, Kiplagat J, Mkandawile G, Nyoni A, Coetzee M, et al. Housing gaps, mosquitoes and public viewpoints: A mixed methods assessment of relationships between house characteristics, malaria vector biting risk and community perspectives in rural Tanzania. Malar J. 2018; 17(1):298. https://doi.org/10.1186/s12936-018-2450-y

See Appendix 2.

4.2 Background

Community involvement in vector control programmes is essential for successful malaria transmission reduction [1–4]. Existing evidence suggests that acceptance and optimal use of interventions such as indoor residual spraying (IRS) and long-lasting insecticide treated nets (LLINs) depends on the level of education and awareness in target communities [5], as well as grass-root organization of such communities to support the health initiatives [4]. Similar successes have been observed with other vector control approaches such as larval source management. For example, in Tanzania, a large-scale community-based larvicide application programme in the city of Dar es Salaam, significantly reduced malaria infection prevalence among city residents [6], despite significant logistical and coverage challenges. In that programme, community-owned resource persons (CORPs) supported specific efforts such as mapping, larvicide application and vector surveillance [7,8]. Elsewhere, in an interesting integration of farmers' experiences, van den Berg and Knols [9] highlighted the importance and potential of using farmer field schools as a method for improving community participation in malaria control. They suggested that malaria control can benefit from complementarities between rural development, farming and pest management and argued for a form of education that uses experiential learning methods to build farmers experiences, thereby achieving improvements in both health and development [9].

Unfortunately, despite substantial evidence on benefits of community engagement in malaria prevention and various innovations on how to achieve this goal, community participation is often relegated, as malaria-endemic countries adopt vertical campaigns such as universal LLIN distributions and mass drug administration in some settings. Instead, the knowledge and experiences of recipient communities is rarely sought even where such additional knowledge would significantly improve outcomes. In rural Ulanga district, south-eastern Tanzania, recent studies have demonstrated that community members were either equally or more knowledgeable than experts in identifying locations where mosquitoes are most abundant [10] and locations where male mosquitoes regularly swarm [11]. Both examples emphasize the potential benefits of relying on communities for improved vector control.

Another important question is whether individuals understand how malaria transmission risk varies across their communities and how it is affected by factors such as housing characteristics and the environment. Evidence suggests that transmission intensities are hardly uniform between districts and villages or even between households in the same village. Instead some households are always disproportionally more exposed to biting risk and malaria infections than community averages [12]. A recent study demonstrated strong correlations between household occupancy and indoor densities of common malaria vectors (*Anopheles arabiensis* and *Anopheles funestus*), as well as spatial correlations of the two variables within and between villages in rural Tanzania [13]. That study also showed that high indoor vector densities cluster in locations where houses with highest occupancy are also clustered, suggesting possibility of relying on regular census data to predict malaria transmission patterns. Similarly, Smith *et al*. [14] illustrated that even though biting risk from malaria vectors may be highest at the edge of the villages near breeding habitats, infectious proportions of these mosquito populations (tending to be the older ones) are far more likely to be clustered in the middle of the villages, with peak populations of the infectious ones occurring when overall vector densities start declining [14].

Despite the above observations, and other evidence suggesting that house design, geographical location of the house within settlements, and characteristics of surrounding environments all influence malaria transmission [16–19], several questions remain unanswered, particularly with regard to community awareness of the same factors. For example, it is unclear whether community members fully understand the importance of such factors, whether they have any concerns about them, and what their counter-efforts are during site selection and house construction. Yet, as malaria transmission declines, control efforts aiming at elimination should target locations and possibly households with greatest risk [18], while also actively engaging members of those communities and households. Such measures should also tackle any competing priorities

The aims of this study were therefore: a) to assess how densities of households and their distances from each other affect the risk of malaria transmission, b) to identify environmental factors that may be affecting the ongoing residual malaria transmission risk in the study area, c) to assess opinions of residents on how

settlement patterns and other environmental factors affect transmission in the study area and d) to identify complementary approaches that could be used to target and significantly improve malaria control in these residual transmission settings where LLINs are already widely used. The study further identified common house characteristics that may be associated with increased vector biting risk and assessed community knowledge and experiences regarding housing factors that influence malaria transmission and how these experiences can be incorporated in ongoing and future intervention options.

4.3 Methods

4.3.1 Study area

The study was conducted in four villages of Kivukoni (8.2135°S, 36.6879°E), Minepa (8.2710°S, 36.6771°E), Mavimba (8.3124°S, 36.6771°E) and Milola (8.2135°S, 36.6878°E) in Ulanga district, south-eastern Tanzania (Figure 4.1). The local houses have either mud or brick walls. The roofs are either grass-thatched or covered with corrugated iron-sheets, and most houses have open eaves (Figure 4. 2). Mean household size is 4.2 [19]. This area is perennially meso-endemic for malaria, and has high mosquito densities throughout the year, peaking between March and May. Annual rainfall and mean daily temperatures range from 1,200 to 1,800 mm and 20°C to 32.6°C respectively.

The primary malaria vectors were *An. funestus* and *An. arabiensis,* with minor contributions from *An. rivulorum* [20]. Malaria transmission intensities, i.e. entomological inoculation rates (EIR) were last estimated at 4.2 and 11.7 infectious bites/person/year (ib/p/yr) by *An. arabiensis* and *An. funestus* respectively, totaling 15.9ib/p/yr [20]. The main vector control method is LLINs, usually distributed through mass campaigns done every 3-4 years, and keep-up campaigns done through reproductive health clinics. The main malaria vectors are resistant to the pyrethroids used in the LLINs, but still susceptible to organophosphates [20].

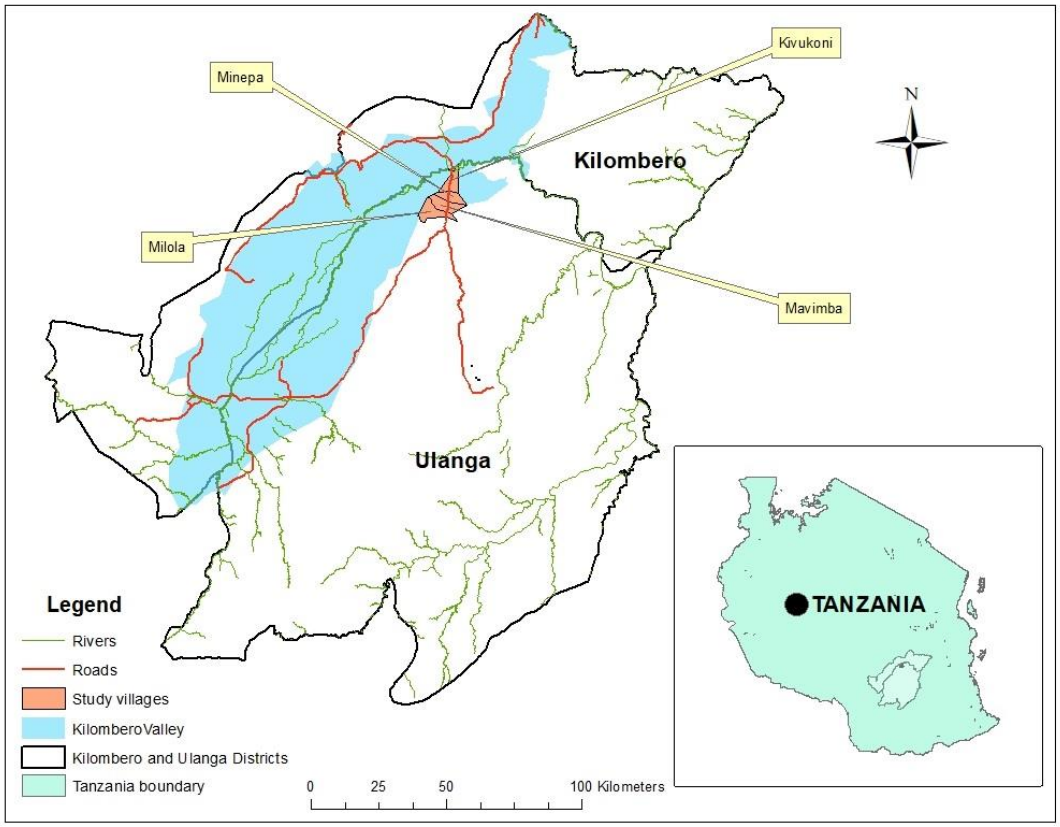


Figure 4.1: Illustration of the study villages. The study was conducted in households across four villages in Ulanga district, south of the Kilombero river in south-eastern Tanzania.



Figure 4.2: Pictorial representation of typical local house types in the study area. Top left - a house with grass thatch roofing with mud walls; top right - a house with corrugated iron roof and brick walls not plastered on the outside, and sometime also not plastered on the inside, bottom left - a grass thatch roof with brick walls not plastered on the outside, and sometime also not plastered on the inside, and bottom right - a house with iron sheet roofing and plastered brick walls. A variety of window and door designs are evident.

4.3.2 Study procedures

The study used a mixed methods approach with four critical components (Figure 4.3) including: (i) longitudinal surveys of indoor mosquito densities in sentinel and randomly selected households in the study area [19] from January 2015 to January 2016; (ii) characterization of the houses and surrounding environmental variables; (iii) a questionnaire survey administered to community members to assess their concerns about household and environmental factors and whether they consider such variables when they are constructing their houses; and (iv) focus group

discussions to assess people's knowledge and perceptions on relationships between house characteristics and environmental factors associated with transmission. Detailed explanations of each component are provided below.

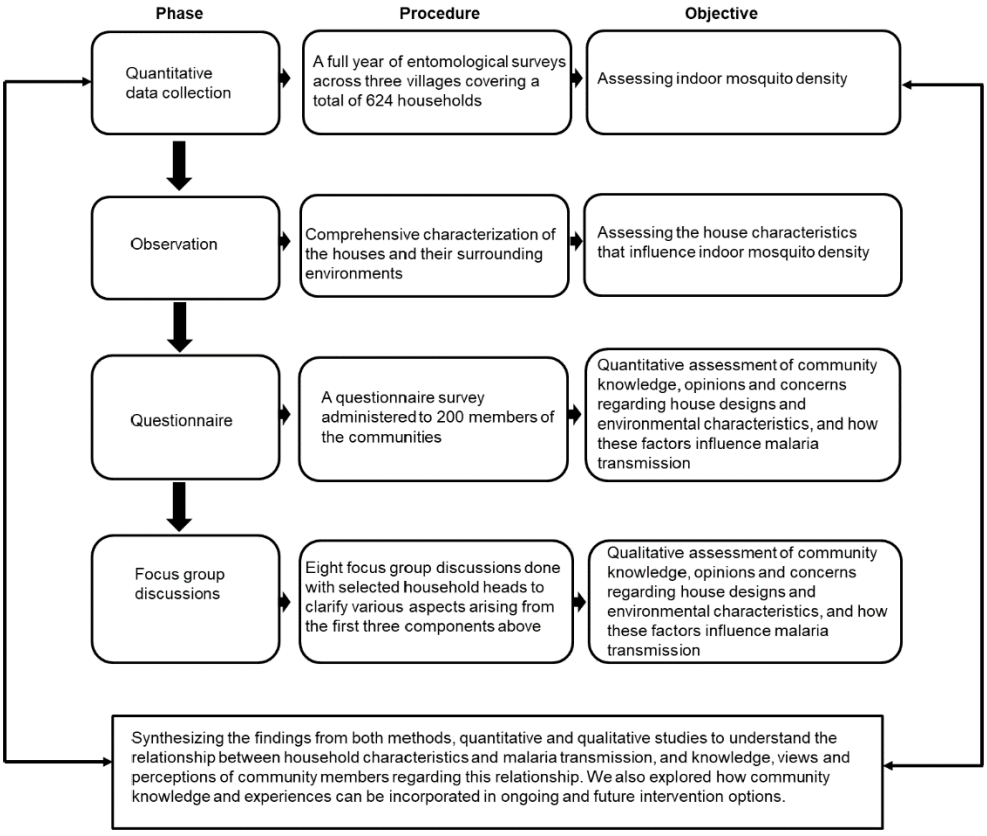


Figure 4.3: Diagrammatic illustration of the mixed methods study design used in this study.

4.3.3 Longitudinal surveys of indoor mosquito densities

Sampling was done using household listings originally obtained from the Ifakara Health & Demographic Surveillance Systems unit (2,433 houses) covering the study area. An initial set of 48 houses were selected and recruited upon consent of household heads, and were set as fixed sentinel houses where vector surveillance was conducted once every month for 12 months. Each month, another set of 48 houses was selected randomly from the same villages (with approximately similar spatial distribution as sentinel houses) for a one off mosquito collection that month. The following month a new set of 48 houses were selected without repeating those that had previously been sampled. In the end, a total 624 households were studied over the 12-month period, with the 48 sentinels sampled 12 times each.

Since the four study villages were different in size and number of households, actual selection and distribution of the sampling houses was done in such a way as to cover full geographical extents of the entire study area. First, 1,600 households were randomly identified and spatially assigned to 16 geographical clusters, each having 100 households. The sampling clusters were defined based on household latitudes so that clusters 1 to 16 were on a north-south direction. On the first month of study, the geographical clusters were visited and six households selected randomly per cluster. This way, there were 48 fixed sentinels throughout the study period and another 48 new houses freshly selected each month across the clusters.

Thereafter, mosquito collections were conducted monthly in each of the study households, each time selecting a new set of three random households from each cluster. A weekly schedule was followed, working for four weeks per month. Each week, mosquitoes were sampled in four of the 16 geographical clusters, by visiting all six households per cluster each night, working for four nights per week. This way, all the 16 clusters were covered once every month. In each household, one occupied room was selected for assessing indoor mosquito densities. CDC light traps set near occupied bed nets were used [21], and were operated from 18:00Hrs to 06:00Hrs. Backpack aspirators were also used to sample resting mosquitoes each subsequent morning. The collected mosquitoes were killed in a closed container using petroleum fumes, then morphologically identified using taxonomic keys for *Anopheles* Africa, south of Sahara, developed by Gillies and Coetzee [22]. All samples were then sorted by sex, taxa and physiological status. Sub-samples of *An. gambiae* complex were further identified by multiplex polymerase chain reaction (PCR), to identify sibling species using protocols developed by Scott *et al*. [23]. Also a sub samples of *An. funestus* were identified by using PCR, following the techniques developed by cohuet *et al.* [24] and Koekemoer *et al.* [25].

4.3.4 Characterization of the houses and their environments

At each initial visit, all the 624 human houses were characterized from where mosquito collections had been done over the one-year period. Geo-positions (latitudes and longitudes) of all the households were captured using hand-held GPS receivers (Magellan eXplorist 110) and recorded. In each house, the condition of windows, doors and walls, and whether the houses had closed or open eaves, was recorded. The surroundings of the households were examined and any nearby water

bodies, cattle sheds or other animal sheds recorded. Other environmental variables included whether the houses were located at the edge or in the middle of the villages and whether people kept animals, such as cattle, pigs or chickens.

4.3.5 Survey questionnaires: quantitative assessment of community knowledge, opinions and concerns regarding house designs and environmental characteristics, and how these factors influence malaria transmission

A structured questionnaire was developed, pretested and used for this survey. The questionnaire had three components. The first component captured socio-demographic characteristics of participants including variables such as age, gender, education, marital status, household size, household income and expenditure. The second component examined the general knowledge of the participants on malaria, malaria transmission, its symptoms, preventive measures and care-seeking behaviours. The third component of the questionnaire assessed participants' knowledge on how various house characteristics and environmental variables might influence malaria transmission, how the participants currently deal with those associations and whether the participants usually put in place any measures to address specific malaria risk factors associated with housing and the environment.

The questionnaires were translated and administered in Kiswahili language, which is the common language spoken by communities across the study area. After pre-testing and correcting all issues arising, the questionnaire was administered by trained research officers to consenting household heads across the three study villages where the entomological surveys had been done and in one village where there was no entomological surveillance. A total of 200 participants responded to the survey.

Table 4.1: Description of the main themes addressed in the survey to assess community knowledge, opinions and concerns regarding house designs and environmental characteristics, and how these factors influence malaria transmission.

	Concepts investigated	**Specific questions asked by the interviewer**	**Relevance of the concepts**
1	Knowledge and perception about house characteristics, mosquito entry and malaria transmission risks	Do you know if the house design can influence mosquito entry?	Assessment of knowledge and perception of malaria transmission risks in relation to house characteristics
		Does your house allow mosquito entry?	
		If your house does not allow mosquito entry, how do you prevent mosquito entry?	
		Why does your house allow mosquito entry?	
		How do mosquitoes enter your house?	
		When was your house constructed?	
		Why did you decide to construct this kind of house?	
		What did you consider during construction?	
2	Knowledge and perception about environmental variables influencing mosquito density	Do you know if the environments surrounding your house influence mosquito density?	Assessment of knowledge and perception of malaria transmission risks in relation to environmental characteristics
		How does the environments surrounding your house influence mosquito density?	
		Mention the common mosquito breeding sites in your area	
		What do you do to prevent mosquito bites?	

3	Knowledge and perception about settlements, mosquito density and malaria transmission risks	Do you think the number of houses in an area can influence mosquitoes and malaria transmission?	Assessment of knowledge and perception of malaria transmission risks in relation to settlement patterns
		Do you think constructing houses near other houses or far from other houses is an important factor in regard to mosquito biting risk and malaria transmission?	
		Why do you think close house have many mosquitoes?	
		What can be done to control mosquitoes in such kind of environment?	

4.3.6 Focus group discussions: qualitative assessment of community knowledge, opinions and concerns regarding house designs and environmental characteristics, and how these factors influence malaria transmission

Study participants were selected from among the households participating in the entomological survey and recruited upon consent with help from the community leaders. Eight focus group discussions (FGD) were conducted across the four study villages, each FGD comprised 12 participants recruited from the same village. Overall the study had a total of 96 participants, 48 being youths (18-35 years) and 48 being adults (above 36 years). There was equal representation of men and women in each FGD group (6 men and 6 women per group). The discussions were held between January and March 2017 and were conducted at local primary schools within the respective villages.

A study guide for the FGD was developed and pretested. The FGD guide followed the themes described in Table 4.1 for the questionnaire surveys. Adults and youths were separated during the interviews to avoid any chance of intimidation during the discussions. The purpose of the discussions was explained and verbal and written informed consents were obtained from the participants. The discussions were semi-structured: the facilitator asked a question and the participants discussed their knowledge, views and comments. The discussions were facilitated by two social

scientists: a note taker and an interviewer, and were tape recorded to ensure that all information was captured. Discussions lasted between 40 and 60 minutes each session.

4.3.7 Data analysis

4.3.7.1 Analysis of quantitative data: data were analysed using open source software, R version 3.1.0, using the *lme4* package [26]. Associations between house characteristics and indoor mosquito densities were examined by generalized linear mixed effects models (*glmer)* [27], by fitting the data in log-linked Poisson error distributions. The indoor densities of *An. arabiensis*, *An. funestus, Culex* and *Mansonia* species were modelled as a function of different house and environmental characteristics. The effects of a) wall type, b) roof type, c) window type, d) whether chickens were kept inside or outside the house, and e) whether the eave spaces were closed or open, were assessed. Date of mosquito collection and house identification numbers were incorporated as random variables in the *glmer* models. Estimated mean indoor mosquito densities per house per night, the relative rates (RR) of observing these mosquito catches, and associated 95% confidence intervals (CI), were computed from exponentials of coefficients generated from the *glmer* models.

The monthly temporal patterns of biting risk by the two malaria vector species and also the Culicine genera, i.e. *Culex* spp and *Mansonia* spp mosquitoes were presented graphically. Data generated through the questionnaire surveys were summarized and presented descriptively in tables to capture:

4.3.7.2 Processing and analysis of qualitative data: after the FGD session, all recordings were retrieved from the tape recorder and archived on a password-protected computer. A trained behavioural scientist then transcribed and translated the data from Kiswahili to English for further analysis. The scripts were then imported into NVivo software version 13.0 for organizing and indexing the data. Emergent themes were organized to assess: a) whether people worry about the characteristics of the house and if they consider such variables when constructing their houses, b) whether people care about environmental variables and if they consider such variables when constructing their houses, c) whether people know effects of

seasonality on malaria transmission, d) whether people know the relationship of settlement patterns and human population on malaria transmission, and e) how the people cope with these risk factors if they are actually aware of relationships with malaria transmission. Direct quotes from the participants are presented to support their responses on the themes.

4.4 Results

4.4.1 Indoor densities of malaria and non-malaria mosquito vectors

A total of 129,052 mosquitoes were collected by CDC light traps and backpack aspirators indoors. Of these, *Anopheles* mosquitoes were 19.9 % (n=25,670) and Culicines were 80.1% (103,382).The 25,670 *Anopheles* mosquitoes included 20,254 *An. arabiensis* (78.9%), 4,800 *An. funestus* (18.7%), 205 *An. coustani* (0.8%), 128 *An. pharoensis* (0.5%), 103 *An. squamosus* (0.4%), 77 *An. ziemanni* (0.3%) and 103 *An. wellcomei* mosquitoes (0.4%). of 103,382 culicine mosquitoes collected, there were 100,047 *Culex* spp (96.7%), 319 *Mansonia* spp (3.1%) and 138 *Aedes* spp (0.1%). The highest densities of host seeking mosquitoes were observed in Minepa village, where about 54% and 65% of all *An. arabiensis* and *An. funestus* mosquitoes were collected. The mean density (±se) of *An. gambiae* caught per village per night were, as follows: Minepa, 65 ± 20, Kivukoni, 19± 6 and Mavimba, 8±7. Mean monthly densities of the indoor mosquito catches are shown in Figure 4.4. Densities of *An. arabiensis* and *An. funestus* peaked between April and June in all study villages and were generally low between September and November (Figure 4.4).

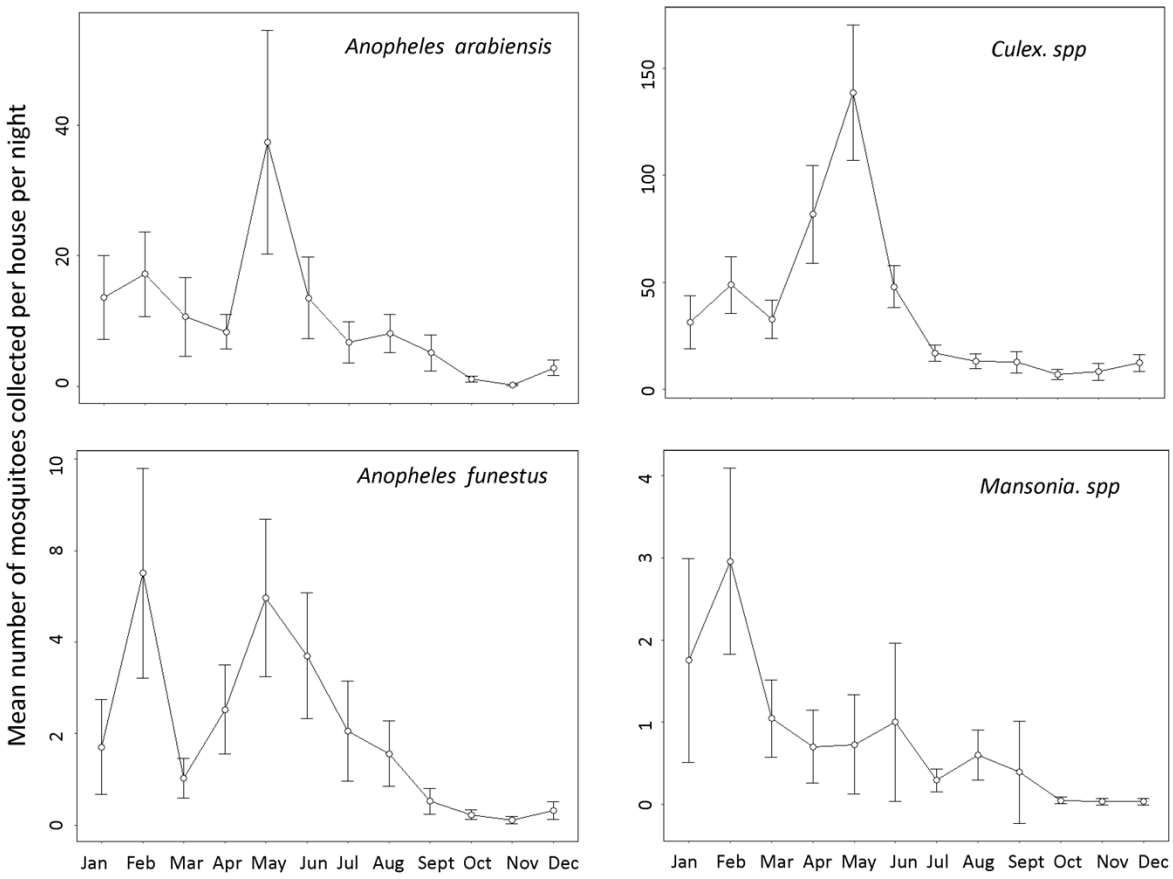


Figure 4.4: Monthly trends of mean number mosquitoes of different species collected per house per night. The Y-error bars represent 95% CI. All the species generally followed same trend peaking between April and June, except *Mansonia* spp, whose densities peaked between January and March.

4.4.2 Physical characteristics and microclimate of sampled houses

A variety of construction material was observed in the local houses (Table 4.2). Most houses had walls made of mud (50.5 %) and unplastered brick (48.6%), rarely with brick and concrete plastering (1.0%). With regard to roofs, a significant proportion had grass-thatched roofing (60.6%), while the rest had corrugated iron sheet roofing (39.4%). At the time when mosquito traps were being set in the evenings, we observed that 64% of the houses had open doors at that time, possibly letting in mosquitoes.

Table 4.2: Physical characteristics and microclimatic conditions in sampled houses.

Variables assessed	**Category**	**Percentage (N)**
Wall type	Plastered brick walls	1.0% (6)
	Mud walls	50.5% (315)
	Un-plastered brick walls	48.6% (303)
Eave space	Closed	25.3% (158)
	Open	74.7% (466)
Roof type	Corrugated iron sheet	39.4% (246)
	Grass-thatched	60.6% (378)
Window covers	With netting screen	25.3% (158)
	Without netting screen	74.7% (466)
Door (observed from 6pm to 7pm)	Open	63.9% (399)
	Tightly closed	29.5% (184)
	Partially closed	6.6% (41)

4.4.3 Household risk factors associated with indoor mosquito densities

Mud walled houses were associated with higher vector densities compared to brick walls. *Anopheles arabiensis* [RR=3.9 (2.3-5.6), P<0.005], *An. funestus* [RR=4.0 (2.5-5.3.) P<0.005], *Culex* [RR=2.3 (2.1– 2.6), P<0.001] and *Mansonia* species [RR=2.5 (1.7-3.8), P<0.005], were all more abundant in houses with mud walls than brick walls. Similarly, open eaves were significantly associated with higher indoor vector densities: *An. arabiensis* [RR=1.9 (1.8-2.0), P<0.001], *An. funestus* [RR=1.2 (0.9-1.6) P<0.005], *Culex* [RR=1.9 (0.8-1.0), P<0.001] and *Mansonia* species [RR=1.5 (1.4.1.8), P<0.001]. Tables 4.3 &4. 4 provide parameter estimates for different house characteristics and their significance in relation to mean catches of mosquitoes in the selected households. The number of *An. funestus* was significantly higher in houses with chickens indoors [RR=2.0 (1.8-2.1), P<0.001] compared to those without chickens, but no such association was observed with *An. arabiensis* [RR=1.8 (1.8-1.9), 0.343]. Similarly, there were higher densities of *Culex* [RR=1.2 (1.1-1.2),

P<0.001] and *Mansonia* mosquitoes [RR=2.6 (2.4-2.7), P<0.001] in houses with chickens indoors.

Table 4.3: Summary statistics for mosquitoes caught in houses with different characteristics, showing the mean nightly catches of *An. arabiensis* and *An. funestus*, relative rates and the associated significance levels calculated from the generalized linear mixed models (GLMMs) at 95% confidence interval.

Variables	**Category**	***Anopheles arabiensis***			***Anopheles funestus***		
		Mean [CI]	**RR [95% CI]**	**P value**	**Mean**	**RR [95% CI]**	**P value**
Wall type	Plastered brick	2.6 [0.0 – 6.8]	Ref		1.6 [0.0 – 5.0]	Ref	
	Mud	17.7 [10.1 – 25.2]	3.9 [2.7– 5.6]	0.00	5.0 [2.6 – 7.4]	4.0 [2.4 – 5.3]	0.01
	Bricks	16.0 [9.5 – 22.5]	1.2 [0.9 – 1.7]	0.00	3.8 [2.4 – 5.2]	1.6 [1.3 – 2.0]	0.00
Eave space	Closed	9.3 [4.2 – 14.4]	Ref		2.8 [1.3 – 4.2]	Ref	
	Open	19.2 [12.9 – 25.6]	1.9 [1.8– 2.0]	0.00	4.9 [3.2 – 6.7]	1.2 [0.9 – 1.6]	0.00
Roof type	Iron sheets	14.4 [6.6 – 22.2]	Ref		3.3 [1.9 – 4.8]	Ref	
	Grass	18.2 [11.8 – 24.6]	1.5 [1.4 – 1.7]	0.00	5.1 [3.0 – 7.1]	2.4 [2.0 – 3.0]	0.31
Window	Screened	15.8 [5.4 – 23.3]	Ref		4.2 [2.7 – 5.7]	Ref	
	Unscreened	17.1 [11.5 – 26.6]	1.9 [1.7 – 1.9]	0.00	4.9 [1.7 – 8.0]	2.9 [2.3 – 2.7]	0.13
Chicken indoors	No	15.3 [9.5 – 21.0]	Ref		4.2 [2.6 – 5.9]	Ref	
	Yes	20.4 [10.7 – 29.9]	1.8 [1.8 – 1.9]	0.34	4.8 [2.4 – 7.1]	2.0 [1.8 –2.1]	0.00

Table 4.4: Summary statistics for mosquitoes caught in houses with different characteristics, showing the mean nightly catches of *Culex* and *Mansonia* species, relative rates and the associated significance levels calculated from the generalized linear mixed models (GLMMs) at 95% confidence interval.

Variables	**Category**	***Culex* species**			***Mansonia species***		
		Mean [CI]	**RR [95% CI]**	**P value**	**Mean**	**RR [95% CI]**	**P value**
Wall type	Plastered bricks	39.5 [0.0 – 79.9]	Ref		0.2 [0.0 – 0.9]	Ref	
	Bricks	64.0 [41.2 – 86.9]	1.4 [1.2 – 1.9]	0.00	0.9 [0.3 – 1.5]	1.4 [0.1 – 2.4]	0.80
	Mud	66.2 [43.9 – 88.5]	2.3 [2.1– 2.6]	0.00	1.5 [0.4 – 2.5]	2.5 [1.7 – 3.8]	0.00
Eave space	Closed	57.9 [27.7 – 88.1]	Ref		0.3 [0.1 – 0.6]	Ref	
	Open	67.2 [48.7 – 85.8]	1.9 [1.8 – 2.0]	0.00	1.5 [0.7 – 2.3]	1.5 1.4 –1.8]	0.00
Roof type	Iron sheet	56.7 [33.9 – 79.4]	Ref		0.7 [0.1 – 1.4]	Ref	
	Grass	70.2 [48.7 – 91.7]	2.6 [2.4 – 2.7]	0.00	1.5 [0.6 – 2.3]	2.5 [1.8 – 2.4]	0.70
Window	Screened	55.4 [23.2 – 86.6]	Ref		0.9 [0.0 – 2.0]	Ref	
	Unscreened	68.4 [50.3 – 86.5]	2.5 [2.4 – 2.6]	0.00	1.3 [0.6 – 2.0]	1.8 [1.7 – 1.9]	0.00
Chicken indoors	No	63.8 [46.2 – 81.5]	Ref		1.1 [0.5 –1.8]	Ref	
	Yes	67.4 [34.6 – 100.0]	1.2 [1.1– 1.2]	0.00	1.3 [0.0 – 2.7]	2.6 [2.4 – 2.7]	0.00

4.4.4 Socio-demographic characteristics of survey participants

In the questionnaire survey, the study participants were 40.5% males and 59.5% females, with overall age range between 18 and 95 years (mean age = 36.9 years). The majority had acquired primary education and were farmers. All the demographic characteristics are shown in Table 4.5.

Table 4.5: Socio-demographic characteristics of participants who responded to the survey questionnaire to assess people's awareness of how house characteristics affect malaria transmission.

Variables assessed	Category	Percentage (N)
Gender	Males	40.5 (81)
	Females	59.5 (119)
Age	18- 35	48.7 (97)
	36 -50	26.5 (53)
	51 - 65	15 (30)
	> 65	20 (10)
Marital status	Married	66 (132)
	Unmarried	20 (40)
	Widow/widower	4.5 (9)
	Divorced	9.5 (19)
Level of education	No formal education	11.5 (23)
	Primary school	67.5 (135)
	Secondary school	18 (36)
	College/university	2 (4)
	Other trainings	1 (2)
Occupation	Peasant (self-employed in agriculture)	68.5 (137)
	Small scale business	4 (8)
	Formal employment	0.5 (1)
	Unemployed	0.5 (1)

	Other	26.5 (53)

4.4.5 Results of the questionnaire survey: participants' knowledge regarding indoor mosquito density and how this relates to housing characteristics and environmental variables

Generally, most respondents associated malaria infections with poor house design features and physical conditions (Table 4.6). More than 90% of participants were aware of different environmental characteristics influencing mosquito density in their area. Similarly, 98.5% of respondents reported that their houses allowed mosquito entry. Houses with open eave space, unscreened windows and mud walls were generally associated with higher mosquito densities in the houses. The age of houses observed was fairly even, with 37% of the houses less than 4 years old, 37% more than 9 years old and the remainder being between 5 and 8 years old.

Table 4.6. Summary of community members' knowledge about indoor mosquito density and how these relate to certain housing and environmental characteristics.

Variables assessed	Response category	Percentage (N)
Whether participants believe their house let in mosquitoes	Yes	98.5% (197)
	No	1.5% (3)
How participants believed mosquitoes entered their houses	Through open doors	28.5% (57)
	Through windows	32.5% (65)
	Through holes in the wall	3.0% (6)
	Through the eave space	19.5% (39)
	Through both open windows and doors	13.5% (27)
	Through open window doors, holes in walls and eaves	2% (4)
	Others	1% (2)
Age of house (date of construction)	< 4 years ago	36.5% (73)
	5- 8 years ago	26.5% (53)

	> 9 years ago	37.0% (74)
Main reasons for constructing the kind of house	Because it is permanent	36.5% (73)
	Because it prevents animals from entering the house	26.5% (53)
	Because it prevents insects	17,0% (34)
	Others	20.0% (40)
Whether people consider mosquito prevention as a key factor when constructing houses	Yes	58.5% (117)
	No	40.0% (80)
	Does not know	1.5% (3)
Specific practices considered by participants (during construction of their houses) for preventing mosquitoes	Netting on the window	38.0% (76)
	Blocking the eaves	8.0% (16)
	Using bricks on the wall	2.0% (4)
	Using cement on the wall	1 (2)
	Does not know	38.5% (77)
	Others	12.5% (25)
Whether participants knew open eave spaces let in mosquitoes	Yes	96.0% (192)
	No	4.0% (8)
Whether participants knew that surrounding environments influence vector densities in their houses	Yes	97.0% (194)
	No	3.0% (6)

4.4.6 Focus group discussions: participants' knowledge regarding indoor mosquito density and how this relates to housing characteristics and environmental variables

In the FGDs, participants stated that house structure had an effect on number of mosquitoes entering their houses, and consequently on malaria transmission. The

participants were concerned about living in poorly constructed, small houses with poor lighting and gaps in the eaves, walls, windows and doors. They mentioned that these gaps allow mosquito entry into their houses and expressed concern that they are frequently bitten by mosquitoes while indoors when they are not under bed nets. Most participants associated this situation with low household income, expressing concerns that they did not have enough money to construct proper houses.

> *"Many mosquitoes are found in those houses with smaller windows, in which there is less light because mosquitoes like dark places. Then mosquitoes enter the house and won't leave the area."* (46 years old male, Milola village).

Other house characteristics implicated in mosquito biting risk were nearness to mosquito breeding sites and also height of the house. The participants suggested that it was necessary to construct houses with greater heights and far from water bodies to prevent this menace. Here are two examples of quotes from participants on this subject

> "*It is important to make sure that houses are built as far away from the mosquito breeding sites as possible. It is good to know the distance that mosquitoes can fly from their breeding sites so that we can know where to build houses." (*37 years old male, Minepa village).

> "*It is necessary to build a house that makes it hard for mosquitoes to get inside. I know that houses that are built lower let mosquitoes in than the houses that have larger height."* (26 years old male Milola village).

There was also a strong linkage of environmental conditions with mosquito densities in houses. It was commonly mentioned that the proximity of the mosquito breeding sites and the state of the environment surrounding the houses had a lot of influence on the mosquito density, and consequently with higher number of malaria cases. Participants stated that mosquitoes liked to breed in dark, damp, grassy and bushy areas. Others said that mosquitoes liked to breed in dirty environments since the dirt provided mosquitoes with good hiding places. There were general references to "dirty water", by which participants mostly meant stagnant water pools outdoors ("dirty water" = water not fit for drinking). This type of water, found near dwellings was commonly mentioned as

being related to mosquito breeding. There was however no evidence that the community members knew the specific characteristics of breeding habitats used by malaria mosquitoes as opposed to other mosquitoes. For example, two of the participants said:

> "*If the environment is dirty mosquitoes increase in number, because they are able to breed in trash. That means if we clean our surroundings then there will be very few mosquitoes. But if our environment is dirty mosquitoes reproduce and increase in numbers."* (58 years old male, Minepa village).

> "*Mosquitoes are found in dirty water around the house, like in water that is in broken containers or cups. They are also found in the ponds, shrubs and in dark places. I believe that is where they breed."* (34 years old female, Mavimba village).

An interesting theme that arose during the FGDs was how behaviours of neighbours was thought to influence vector densities and malaria transmission in houses that are in close proximity. Some participants thought that if the behaviours and practices of their neighbours were encouraging mosquito breeding and survival, then those mosquitoes would also go to the other surrounding houses as well.

> "*When there are many houses together, it means that there is a lot of trash and other things in which mosquitoes can breed. For example, I may keep my surroundings clean, but if my neighbours do not clean their surroundings, and they live close to me, then mosquitoes can easily come from their houses to mine, it will make it easy for mosquitoes to bite me." (*37 years old female, Milola village)

Participants perceived that nucleated settlements encouraged malaria transmission more than dispersed settlements. Most participants expressed that when households were close to each other, mosquitoes could easily move from one house to another, easily transmitting malaria. According to the participants, when households are far apart then it would take time for mosquitoes to travel between the households. Here are comments from some of the participants:

"*If there are many houses close together then mosquitoes can move from one house to another. But if houses are few and far apart then the mosquitoes cannot really get from one house to another easily, because there can be many obstacles for them on the way, like the wind and rain." (*40 years old female, Milola village).

"*When there are a lot of houses together then it is a problem for us because this way many mosquitoes come, because they know that they can get a lot of people in close proximity. It is easy for the mosquitoes to transmit malaria."* (23 years old male, Kivukoni village).

"*If houses are scattered, then the wind will blow mosquitoes to other places where they cannot easily get people."* (51 years old male, Milola village).

LLINs were the main form of protection used against mosquito bites and malaria transmission. All participants reported that they used bed nets regardless of the season. Other means of protection mentioned were cleaning the environment, drying up ponds when possible, covering up with clothing when people are outdoors, using insecticide repellent lotions and indoor sprays and going indoors early. There were however concerns about the integrity of the available nets and how this influences their efficacy. Examples of relevant quotes included:

"*One thing that most of the people in the village can afford is to use bed nets, because everyone has bed nets here. But in order for these nets to be useful they have to be in a good shape. Sadly not everyone has new nets. I sleep with my children in the same bed because we have one net, but our net is not in a very good state, it has a lot of holes that sometime let mosquitoes in. But it still helps; it is not the same as if we did not use anything."* (31 years old female, Kivukoni village).

"We make sure that children are protected from mosquito bites by making sure they go to bed early when it gets dark, and then we make sure they are covered by bed nets. Sometimes when they are sitting outside then we use a piece of cloth to chase mosquitoes away." (54 years old male, Milola village).

Lastly, there was generally a high level of understanding regarding seasonality and malaria transmission. Participants mentioned that though mosquito numbers were generally high in the area, most biting and malaria transmission occurred in the rainy season, especially during the months of February through May, during the long rains. Here are some of the responses from the participants:

> "*Mosquitoes are found here throughout the year. However, their population is the highest during the rainy season because there is a lot of water around, hence many breeding sites. During the dry season there are fewer places where mosquitoes breed, so their population is lower."* (28 years old male, Milola village).

> "*There are a lot of mosquitoes from February to April because this is the time where there is a lot of water everywhere. There are many pools, ponds and bushes where mosquitoes can breed. The mosquito population starts to go down from May and June when the weather starts to get dry and cold."* (24 years old male, Minepa village).

4.5 Discussion

The WHO 2017 World Malaria Report showed significant successes in malaria control in various countries, but with an overall slight increase in malaria cases and deaths relative to the previous year [28]. Over the past decades, long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) have been widely used alongside improved diagnosis and treatment, primarily with artemisinin combination therapy (ACT). These tools have contributed to most of the gains accrued against malaria in the past decades [28,29], but evidence suggests that new interventions will be required to finally move to zero transmission [30,31]. Unfortunately, novel strategies such as improved housing [32] and environmental management for malaria control have only been scantily considered [33,34].

Tanzania experienced more than 50% reduction in malaria prevalence in children aged 2-10 years across the country between 2000 and 2015 [35]. Less than 2% of people in

mainland Tanzania now live in places considered as having intense year-round transmission, while more than 60% of Tanzanians live in areas considered as having low transmission [35]. The 2015-2016 malaria indicator surveys reported an increase in average malaria prevalence to 14.8% in children under 5 years [36], up from 9.5% in 2012 [37], but the most recent survey has shown much reduced prevalence of 7.3% [38]. Despite these gains, there are still numerous challenges, notably the rise of insecticide resistance [39] and sub-optimal net use each night [36]. Greater vigilance and additional interventions are therefore required to consolidate past gains and accelerate efforts towards the current national malaria control targets and eventual elimination.

Housing design has been shown in different studies to significantly influence malaria transmission [30–32]. Most people in rural and peri-urban areas live in poorly finished houses with open eaves, doors and windows, hence exposure to malaria transmission is very high. Mosquitoes enter the houses easily through eaves and other openings, thus increasing likelihood of biting and pathogen transmission [29, 30]. Unfortunately, these communities cannot afford essential house construction and mosquito-proofing materials due to competing priorities. Greater sensitization and enabling financing initiatives to promote better housing should therefore be considered in vector control initiatives to accelerate malaria elimination efforts. This study found statistically higher indoor densities of the main malaria vectors (*An. arabiensis* and *An. funestus*) in houses with mud-walls as opposed to plastered or brick walls, open as opposed to closed eaves, and also in unscreened as opposed to screened windows. Besides, keeping chickens inside the house was similarly associated with higher indoor densities.

There was already high levels of awareness about how housing characteristics and the surrounding environment influence mosquito densities, and consequently malaria transmission. Another factor identified was the small size of houses (area and height). People were concerned about living in poorly-constructed houses with gaps in the eaves, walls, windows and doors, and in small houses. However, they were constrained by low income and could not readily afford construction of properly ventilated and mosquito-proof houses. Nearly all respondents reported that their houses allowed

mosquito entry, but they could not afford modern building materials. They expressed that they are also being bitten by mosquitoes while indoors when they are not under bed nets.

The relationship between poor housing and low-income is expected in such settings. However, although many households in rural areas are unable to afford modern housing, there are cheaper and simpler ways of modifying or screening houses which could readily prevent mosquito entry, especially if the community members are adequately sensitized and financial barriers removed, e.g. through subsidies. Unfortunately, malaria control authorities in sub-Saharan Africa have not invested in house improvement programmes despite the significant available evidence [40,41].

Knowledge of mosquito breeding habitats is another important factor to consider for successful community-based vector control. In this study, participants in the FGD identified various sources of mosquitoes in their surroundings, such as stagnant water pools, dense vegetation and rice fields. Studies elsewhere have yielded similar findings [42,43], and such high levels of community awareness can be harnessed to improve disease prevention and control initiatives. In rural south-eastern Tanzania, the nucleated settlement patterns, combined with the previously described spatial distribution of vector densities across the villages, indicate that targeted interventions, if supported by local community champions, could indeed be highly effective. An earlier study by Mwangungulu *et al.* in the same villages yielded evidence that community members are capable of identifying places with low, medium and high mosquito densities [10]. Mosquitoes breed in shallow pools of water and puddles [44,45], which are numerous in rural areas especially during the rainy season. Draining pools of water, levelling land, construction of drains and proper waste management could eliminate mosquito breeding sites around homes [46,47], and subsequently reduce malaria transmission [48]. Given the existing high-levels of awareness, further control initiatives involving environmental management could be improved community members should be considered the main stake-holders. Such community-based approaches could also result in scale-up of better housing designs and access to commodities necessary for screening windows, eaves and doors, even in rural, low-income areas.

Community knowledge, perception and practices are important for designing or improving disease control programmes and identifying factors for effectiveness of the interventions [49]. Promoting community based programmes can help to achieve population level change in risk behaviours which in turn results in positive changes [50]. Unless communities recognize the importance of changing behaviours which influence disease transmission, the best designed interventions might not achieve the intended goals.

Another important point that emerged from this study was participants' impression that if the houses are close to each other, malaria transmission is likely to be higher because mosquitoes can easily move between houses. Though participants were not able to describe settlement patterns, they perceived that closer housing and congested households are at higher risk of malaria transmission, and were concerned with the distances that a mosquito can move from one house to another. Previous studies suggested strong correlations between household occupancy and malaria vector densities [13], but no specific analysis has been done on effects of distance between households and malaria transmission. By combining the new findings from this current study with the observations previously described by Mwangungulu *et al*. [10] and Kaindoa *et al*. [13], a community driven intervention could be designed, which relies on trained locals to support environmental management and house improvement initiatives for mosquito control.

4.6 Conclusion

To improve malaria control and elimination efforts, it is important to develop an understanding of community perspectives on persistent malaria transmission within and around their villages, then engage these communities in identifying and addressing the key factors contributing to the persistent transmission. This study has shown that in rural south-eastern Tanzania, significant proportions of people still live in houses with open eaves, unscreened windows and gaps in doors. Though the people are fully aware of associated mosquito biting and pathogen transmission risks, they are constrained by low-income levels and cannot readily afford better housing or house improvement (e.g.

through screening of eaves and windows), alongside other competing priorities. In conclusion, community-based housing improvement initiatives combined with targeted subsidies could lower the financial barriers, improve access to essential construction materials or designs, and significantly accelerate malaria transmission control in these communities.

Declarations

Author's contribution

EK and FO conceived the study and developed study protocols and supervised data collection. EK, MF, JK, conducted data analysis, EK, MF, JK, AN, MC and FO wrote the manuscript. All authors approved the manuscript prior to submission.

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Competing interests

The authors declare that they have no competing interest.

Availability of data and materials

The dataset generated by this study is available from the corresponding author upon reasonable request.

Ethical considerations

Meetings with local leaders were held in the study areas and the main aims of the study were explained by the research team. A written and signed informed consent was obtained from each study participant. All information was given in Kiswahili, the local language. The six household heads were requested to volunteer in the study, and half

of them requested for permission so their houses could be enrolled as fixed sentinel sampling stations for repeated monthly mosquito collections. Whenever a household head did not consent, the next household in the random listing was selected, so that there were always six households per cluster (three of them being fixed sentinels and another three being random households for one-off sampling).

Ethical approval for the study was obtained from Ifakara Health Institute Institutional Review Board (IHI/IRB/No: 029-2016), and from the Medical Research Coordinating Committee (MRCC) at the National Institutes of Medical Research (NIMR) (Ref: NIMR/HQ/R.8a/Vol.IX/2428). Approval was also obtained from the Human Research Ethics Committee (Medical) at University of the Witwatersrand (Approval Certificate No: M160806). All personal information that could identify the study participants was omitted from the final report so as to protect identification of respondents. The participants were assured anonymity in the presentation and publishing of the data. Permission to publish this manuscript was obtained from the National Institutes of Medical Research (NIMR) (Ref: NIMR/HQ/P.12/VOLXXV/).

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Chapter V

Effects of household densities and their distances on malaria transmission risk in rural Tanzania

5.1 Abstract

Associations between house characteristics and malaria transmission are known, but gaps remain on whether transmission is also influenced by factors such as distances between households or the degree to which houses are clustered. This study examined these factors to determine whether there was any influence on malaria transmission in a set of rural Tanzanian communities. We performed indoor and outdoor mosquito collections from fixed as well as randomly selected households over 12 months, and assessed effects of spatial clustering of household on malaria transmission risk. The study found that high house densities increased *Anopheles* biting risk but mosquito density declined as distances between houses increased beyond 50m. Despite the recent scale-up of effective vector control interventions, such as LLINs, there are still major household and environmental characteristics contributing to persistent malaria transmission.

Additional efforts such as house improvement programs targeted at village-level, could address the challenges by prioritizing high-risk household clusters. Such complementary strategies could be further improved by involving communities in the control programs, and removing financial and access barriers associated with poor-housing conditions

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5.2 Background

Vector control tools such as Long Lasting Insecticide-treated Nets (LLINs) and Indoor Residual Sprays (IRS) have resulted in significant successes in reducing malaria transmission in recent year [1–3]. However, between 2015 and 2018 malaria control has stalled. This is due, in part, to emerging insecticide resistance, behavioural avoidance of insecticides by mosquitoes, and inherent behavioural plasticity amongst some species that keep them out of contact with the mostly indoor-based methods of mosquito control [4–6].

Contact between humans and vectors is a key factor for the transmission of many vector-borne diseases [7,8]. Understanding how the distribution of humans influences distribution of mosquitoes will facilitate identification of key individuals and locations with risks for malaria transmission, which may provide useful information for targeted intervention. Malaria transmission is not uniformly distributed between districts (Figure 1.2, Chapter I), and between villages or households from the same village - some households are always more exposed to malaria than others [9]. It has also been documented that the overall distribution of mosquitoes in the village is influenced by the number and distribution of household occupancy, thus number of people in the household can be used to map malaria transmission risk at fine scale [10,11].

Despite the evidence, there is still a challenge of proper development and application of targeted interventions. Yet, to achieve malaria elimination, it is essential to develop effective strategies that will enable identification and targeting of transmission at fine scale levels within villages [12–14], thus enabling more effective control by current and future tools.

The role of settlement patterns on malaria transmission is currently not known. Thus this study was built on the hypothesis that that settlement patterns, as defined by degree of clustering, the distances between individual households, or the locations of individual households within villages, affect malaria transmission rates, and current and new interventions could be significantly improved by understanding these factors. This study tested the hypothesis that malaria transmission risk is higher in highly clustered

settlements than dispersed settlements, and that transmission is less likely to occur between households that are far apart than households that are close together.

5.3 Methods

The description of the methods, including the study area, study procedures and data collection has been fully described in the method sections of Chapters II and III.

5.3.1 Analysis of housing pattern and distances

Distances between houses was assessed to determine if this influences malaria transmission risks in the study area. Two approaches were used as follows. First, the point distance tool in ArcGIS 10 (ESRI, USA) was used to assign average distance between each house within different radius of 150m, 200m and 400m. In each radius, the average distances of houses from each other were computed and the number of houses within the specific radius were counted. R version 3.5.0 was also used to perform this analysis, which focused on the association between house density and density of malaria vectors density. Generalized additive mixed model (GAMMs) was used to model the number of mosquitoes caught per person per night. The first sets of models were structured using number of houses within a hypothetical radius (200m, 300m, 400m and 500m). This cumulative hypothetical radius shared common information, hence strong cross-correlation. Only the number of houses within the 200m and 400m radius was included in the final model. The second sets of models were structured with distance between houses using the same radius as in the first set of models. In these sets of GAMMs, random terms were included to capture the unexplained variation that is prevailing within households and to take care of pseudo-replication within households and villages. Akaike Information Criterion (AIC) was used to generate the spline figures (Figures 5.1 and 5.2) showing the trends for each of the variables of interest.

5.4 Results

5.4.1 Effects of distances and housing density on malaria vectors

Mosquito densities were affected by the distances between houses, but within a limited radius of 400m. The GAMM model showed higher numbers of mosquitoes in an area characterized by closer households, i.e. less than 50m. As the distance between houses increased, the number of mosquitoes decreased (Figure 5.1).

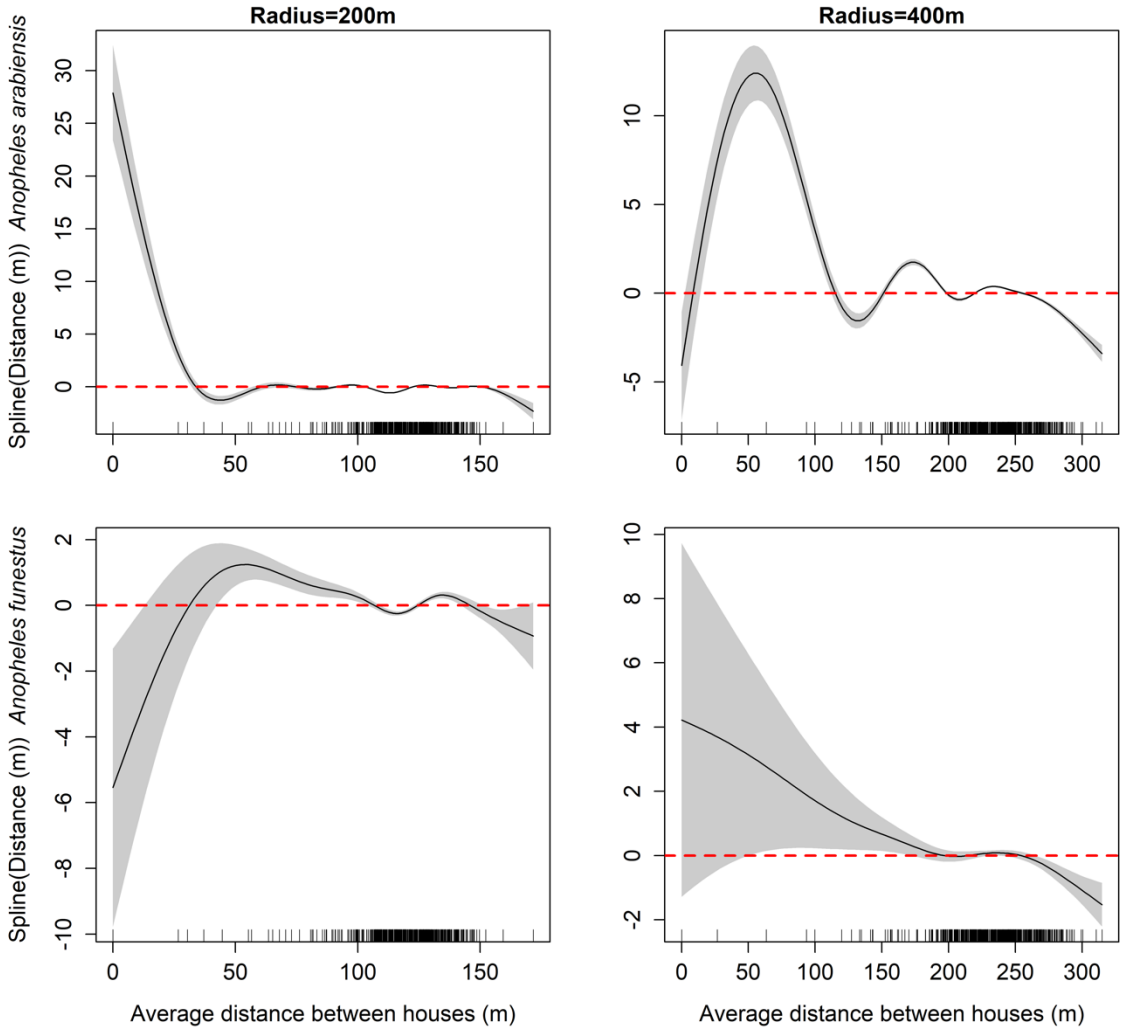


Figure 5.1. Correlations of distances between houses and mosquito biting risks in rural Tanzania.

Unlike the house distances, the house density in a given area was not associated with

mosquito biting risks (Figure 5.2).

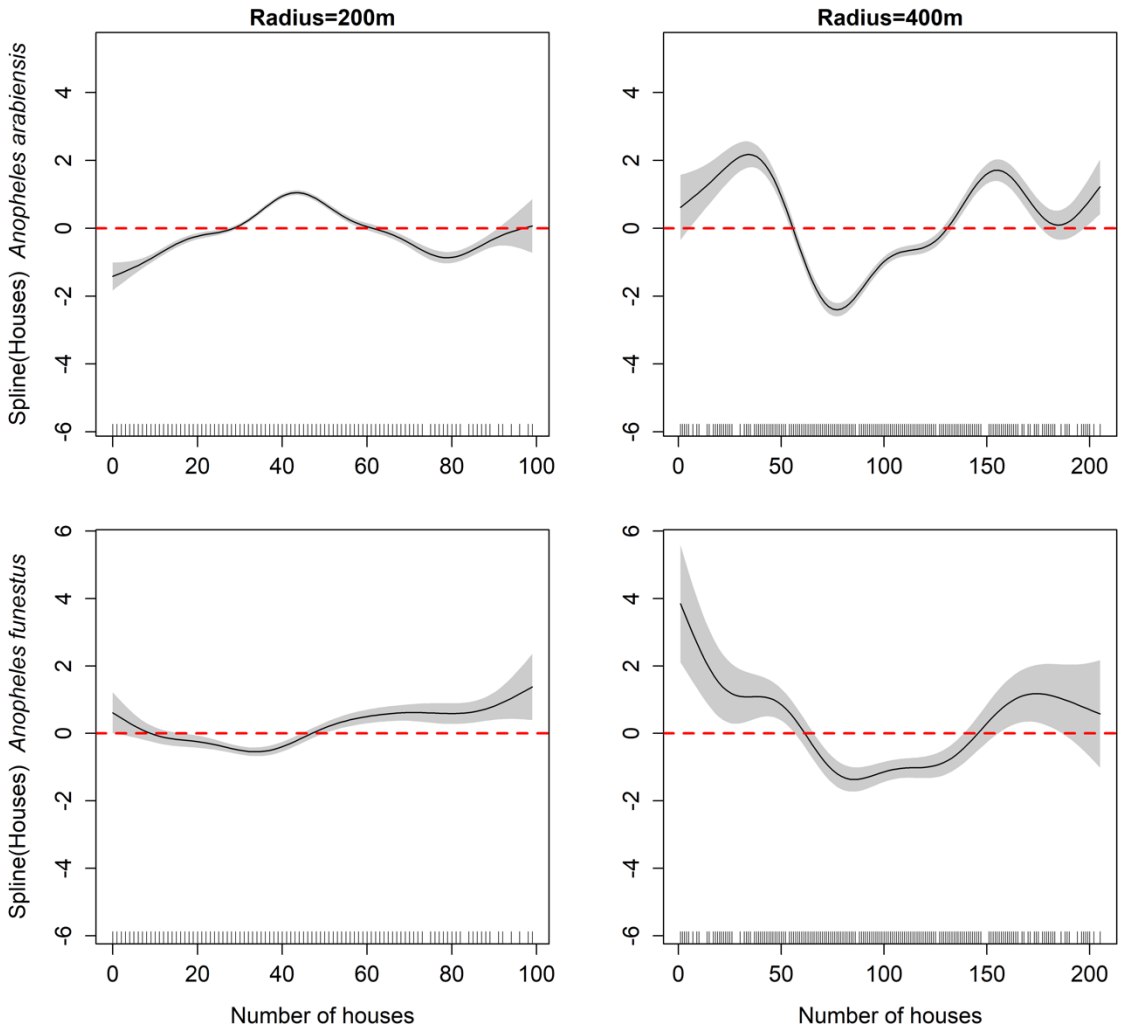


Figure 5.2. Relationship between house densities and mosquito biting risks in rural Tanzania.

5.5 Discussion

Malaria transmission is significantly declining in African countries [15] and many of these countries are aiming to achieve malaria elimination targets [16]. Thus, there is a necessity for increasing efforts and designing appropriate interventions and policies which would target interventions to fine scale and most importantly, target the areas

which are at higher risks of malaria transmission. Such approaches need evidence to guide the choice of areas for targeted control efforts. In this work, the relationship between human settlement patterns and malaria transmission risk was assessed, and yielded evidence that the risk is highest in locations where houses are most densely clustered, with less than 50 m distances from one house to another. The evidence shows that distances between houses not only influences malaria transmission, but also other mosquito borne diseases such as dengue [17]. A study by Bowman *et al*., indicated that dengue cases are usually aggregated within a short distance ranging between 100 and 800m [18]. This may be attributed to short flight distances of *Aedes* mosquitoes. Results from the present study confirmed that malaria vector density was also higher in houses which were very close hence the biting risk was very high. It is, however, not known whether infected malaria vectors also aggregate within short distance, thus further studies are need to determine whether infectiousness of mosquitoes would be influenced by distances of households.

Understanding the relationships between human settlement patterns and persistent malaria transmission could help malaria control efforts to use available resources in a more cost-effective manner. It is suggested that exact house location is an important and independent factor for malaria transmission and should be considered in designing the interventions [9]. Further evidence shows that only 20% of the population accounts for 80% of the transmission of infectious diseases [19,20].

Since this study focused only on mosquito density as an indicator of malaria transmission risks in the village, further studies should be carried out using parasitological surveys. This information could explain the variations of exposure to malaria transmission at fine scale levels. It is important to design interventions to target areas that are at high risk of malaria transmission and the approach used in this study could help to identify these areas.

5.6 Conclusion

The clustering of households in a given area influenced *Anopheles* biting risk with mosquito density declining as distances between houses increased beyond 50m. This

information would be necessary for designing village-level interventions against malaria transmission.

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Chapter VI

Interventions that effectively target *Anopheles funestus* mosquitoes could significantly improve control of persistent malaria transmission in south–eastern Tanzania

6.1 Abstract

Malaria is transmitted by many *Anopheles* species whose proportionate contributions vary across settings. The roles of *Anopheles arabiensis* and *Anopheles funestus* were reassessed and potential benefits of species-specific interventions examined in an area in south-eastern Tanzania, where malaria transmission persists four years after mass distribution of long-lasting insecticide-treated nets (LLINs). Monthly mosquito sampling was done in randomly selected households in three villages using CDC light traps and back-pack aspirators, between January-2015 and January-2016, four years after the last mass distribution of LLINs in 2011. Multiplex polymerase chain reaction (PCR) was used to identify members of the *An. funestus* group and *An. gambiae* complex. Enzyme-linked immunosorbent assay (ELISA) was used to detect *Plasmodium* sporozoites in mosquito salivary glands, and to identify sources of mosquito blood meals. WHO susceptibility assays were carried out on wild caught female *An. funestus s.l.* and physiological ages approximated by examining mosquito ovaries for parity. A total of 20,135 *An. arabiensis* and 4,759 *An. funestus* were collected. The *An. funestus* group consisted of 76.6% *An. funestus s.s.,* 2.9% *An. rivulorum*, 7.1% *An. leesoni,* and 13.4% unamplified samples. Of all mosquitoes positive for *Plasmodium*, 82.6% were *An. funestus*, 14.0% were *An. arabiensis* and 3.4% were *An. rivulorum*. *Anopheles funestus* and *An. arabiensis* contributed 86.21% and 13.79% respectively, to the annual entomological inoculation rate (EIR). *Anopheles arabiensis* fed on humans (73.4%), cattle (22.0%), dogs (3.1%) and chickens (1.5%), but *An. funestus* fed exclusively on humans. The *An. funestus* populations were 100% susceptible to organophosphates pirimiphos-methyl and malathion, but resistant to permethrin (10.5% mortality), deltamethrin (18.7%), lambda-

cyhalothrin (18.7%) and DDT (26.2%), and had reduced susceptibility to bendiocarb (95% mortality) and propoxur (90.1%). Parity rate was higher in *An. funestus* (65.8%) than *An. arabiensis* (44.1%). Though *An. arabiensis* is still the most abundant vector species here, the malaria transmission is primarily mediated by *An. funestus,* most likely due to its high vectorial capacity, high insecticide resistance and high survival probabilities. Interventions that effectively target *An. funestus* mosquitoes could therefore significantly improve control of persistent malaria transmission in south–eastern Tanzania.

Adapted from Kaindoa EW, Matowo NS, Ngowo HS, Mkandawile G, Mmbando A, Finda M, et al. Interventions that effectively target *Anopheles funestus* mosquitoes could significantly improve control of persistent malaria transmission in south-eastern Tanzania. PLoS One. 2017; 12(5):e0177807. https://doi.org/10.1371/journal.pone.0177807

See Appendix 3.

6.2 Background

Malaria continues to be one of the most significant mosquito-borne parasitic diseases, affecting about 212 million people, causing 429,000 deaths annually [1], and adversely affecting socio-economic development in sub-Saharan African countries [2]. The World Health Organization (WHO) estimates that there has been a decline in malaria burden, and that morbidity worldwide reduced by 21% and mortality by 29% between 2010 and 2015, but sub-Saharan Africa still accounts for approximately 92% of all malaria deaths and cases [1].

Tanzania has experienced a decline in malaria transmission following the introduction of insecticide-treated bed nets (ITNs) and the scale-up of long-lasting insecticide-treated nets (LLINs) [3]. By 2010, the country had made significant progress, and most areas that were experiencing prevalence above 50% in 2000, now had below 10% prevalence [4]. However, the most recent malaria indicator survey has returned an average prevalence of 14.8% prevalence in children under 5 years [5]. Getting to zero transmission, therefore, remains a major challenge, mainly because the persistent malaria transmission is mediated by mosquitoes that are not adequately responsive to control by existing indoor insecticidal interventions, such as LLINs and indoor residual spraying (IRS). The Tanzanian National Malaria Control Programme is aimed at cutting down malaria transmission to 1% by 2020 [6], thus the need for new complementary approaches is even more urgent.

Despite the high coverage of LLINs, high indoor densities of potentially infectious mosquitoes remain common in some places [7,8]. Evidence suggests that indoor densities of major malaria vectors such as *Anopheles funestus* and *An. arabiensis* depend on house design features, environmental conditions and demographic composition [8,9]. Previous studies have reported high levels of resistance in *An. funestus* to pyrethroids in Tanzania [10] and elsewhere in East Africa [11–13]. This is one of the major malaria vectors in Africa and is widely distributed across the continent [14,15]. It is highly anthropophilic and endophilic, with higher vectorial capacity than most other vector species [14,16]. The *An. funestus* group includes several sibling species, which are identical morphologically but can be separated by polymerase

chain reaction (PCR) using a cocktail of species-specific primers [17]. Previous studies in Tanzania have reported the presence of four member species in the *An. funestus* group, namely *An. funestus s.s.*, *An. rivulorum*, *An. leesoni* and *An. parensis* [18]. All these species have been found positive for *Plasmodium falciparum* though the infection rates are higher in the predominantly anthropophagic *An. funestus* than the rest. The importance of this vector is increasingly associated with persistent malaria transmission [8,10]. Indeed, it has been suggested that it could be responsible for resurgence of malaria transmission in rural Tanzania [10].

This study assessed the contributions of *An. arabiensis* and *An. funestus* to malaria transmission in endemic villages in rural south-eastern Tanzania, where the last mass distribution of LLINs had been completed in 2011 [19]. The study assessed prevalence of *Plasmodium* sporozoites in the vector species, survival rates of these vectors, and their resistance to WHO-approved insecticides for vector control. Inferences are made with regard to what would happen if appropriate species-specific interventions were deployed in this and similar settings. More specifically, interventions that effectively target mosquito species mediating most of the remnant malaria transmission should result in extensive declines in the transmission. Where one vector species dominates the transmission system, approaches against such vectors may offer opportunities to sufficiently disrupt local malaria transmission.

6.3 Methods

6.3.1 Study area: The study was conducted in three villages in the Kilombero floodplains in Ulanga district, south-eastern Tanzania (Figure 6.1). This area is perennially meso-endemic for malaria and has high mosquito densities throughout the year, peaking between January and May. Annual rainfall and temperatures range from 1,200 to 1,800 mm and 20°C to 32.6°C respectively. Malaria vector species in the area are comprised primarily of members of the *An. gambiae* complex (almost exclusively *An. arabiensis)* and the *An. funestus group*. Several other *Anopheles* mosquitoes, such as *An. coustani*, *An. pharoensis*, *An. squamosus, An. ziemanni* and *An. wellcomei,* are also found, as well as several culicine species belonging to the genera *Mansonia*,

Aedes and *Culex*. The major vector control intervention in this area is LLINs [19], and the last mass-distribution of nets, prior to this study, had been conducted between 2010 and 2011 [19].

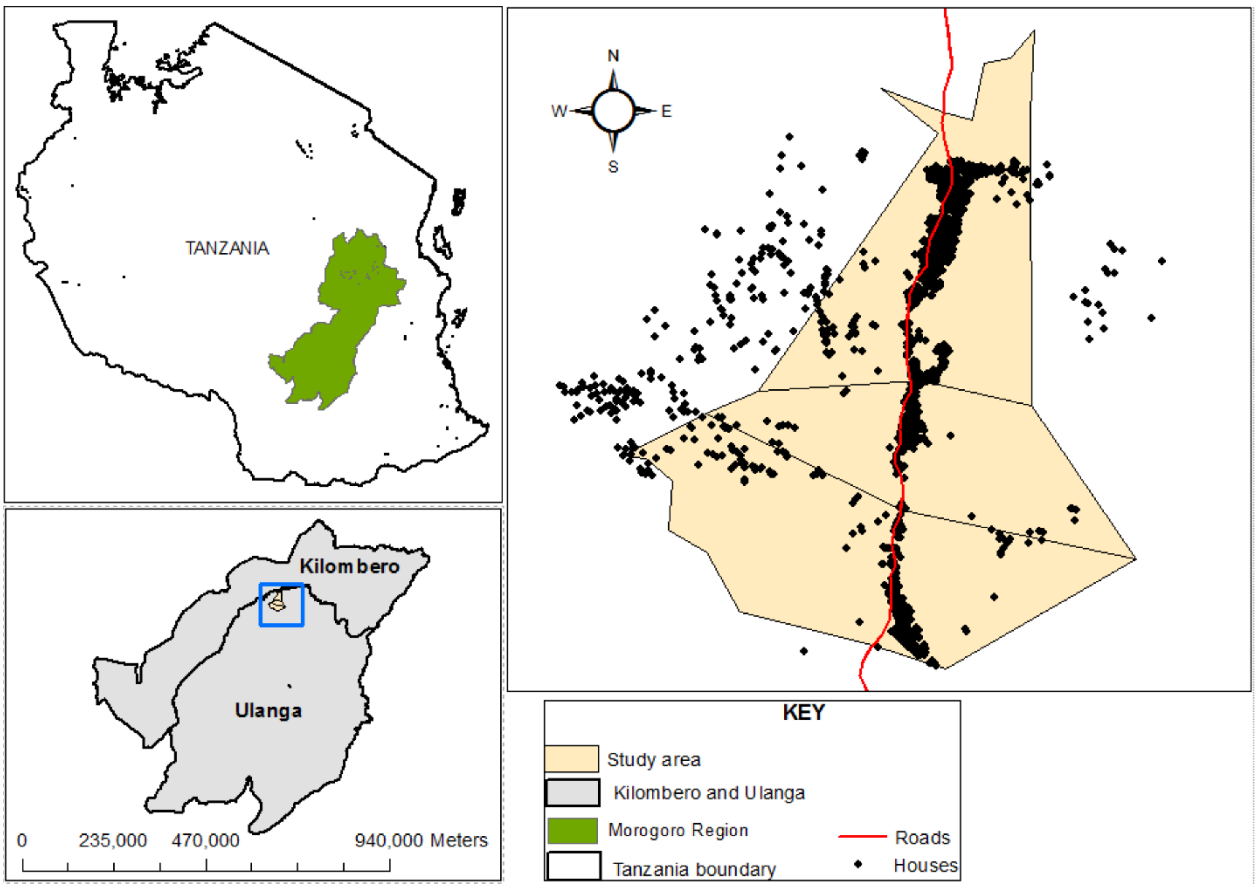


Figure 6.1: Map of the study area, showing the villages and households in Ulanga district where the study was conducted.

6.3.2 Study procedures

This study was part of a high-resolution longitudinal mosquito surveillance programme, where host-seeking and indoor-resting mosquitoes were collected between January 2015 and January 2016 in randomly selected households, and also selected sentinel households within the Health and Demographic Surveillance System (HDSS) maintained by the Ifakara Health Institute in the study area [20].

6.3.2.1 Monitoring indoor densities of host-seeking and resting disease-transmitting mosquitoes

Firstly, 1,600 households were randomly selected from the HDSS households database in three study villages of Kivukoni, Minepa and Mavimba, and assigned to 16 geographical clusters each consisting of 100 households. The sampling clusters were assigned based on household latitudes so that clusters 1-16 were arranged in a north-south direction in the study area. From each geographical cluster, six households were selected for sampling. These included three households randomly selected on a monthly basis, and another three households that were fixed as sentinel households and visited repeatedly every month. This way, 96 households were studied per month, each time consisting of 48 sentinel households across the study area, and 48 new households randomly selected each month. Geo-positions of all the households were recorded using hand-held GPS receivers (Magellan eXplorist 110). Each household was visited only once each month, and mosquitoes were sampled from one room with occupant(s) sleeping under mosquito nets, using miniature Centre for Disease Control (CDC) light traps set near occupied bed nets at the foot end, at approximately 1.5 m from the ground [21,22]. The traps were operated from18:00Hrs to 06:00Hrs each night.

Indoor resting mosquitoes were collected using a backpack aspirator [23] from the walls, bed nets and floors in the same households where the CDC light trap catches were conducted. Aspiration of resting mosquitoes was conducted for approximately 15 minutes in each household. Each morning, the collected mosquitoes were killed in a closed container using alcohol. The mosquitoes were identified morphologically and sorted by taxa and sex before they were identified by PCR. The identification was done following the taxonomic key developed by Gillies and Coetzee [24].

6.3.2.2 Identification of sibling species and detection of *Plasmodium* infection in malaria vectors

All female *An. gambiae* complex mosquitoes, were examined by multiplex polymerase chain reaction (PCR) technique as described by Scott *et al* [18] to distinguish sibling species. Similarly, all samples of *An. funestus* were examined by PCR, using techniques developed by Koekemoer *et al.* [17] and Cohuet *et al*. [16] to distinguish sibling species. All *Anopheles* mosquitoes caught were segregated by species and examined in pools by enzyme-linked immunosorbent assays (ELISA), to detect *Plasmodium* sporozoites in their salivary glands [25]. To avoid false positives, the ELISA lysates were boiled for 10 minutes at 100˚C, effectively eliminating heat-labile non *Plasmodium falciparum* protozoan antigens [26]. We assessed all the collected *An. gambiae s.l.* and *An. funestus s.l.* mosquitoes by ELISA, using heads and thoraces.

6.3.2.3 Identification of mosquito blood meals

Blood meal analysis was also done on a sub-sample, working only with the mosquitoes that were blood-fed. Each mosquito abdomen was separated from the thorax and homogenized in phosphate buffer saline (PBS) following the method described by Beier *et al.* [27]. This way, all the blood-fed mosquitoes were screened for human, bovine, chicken, goat and dog blood using ELISA.

6.3.2.4 Assessment of insecticide susceptibility status

The insecticide susceptibility tests were conducted using standard WHO guidelines [28]. Insectary-reared *An. gambiae s.s.* (Ifakara strain) was used as a reference susceptible population. Because of the difficulties in finding *An. funestus* larvae in the study area, adult female mosquitoes were collected indoors from April to July 2016, by CDC light traps from the same households in three villages. Instead of preserving in alcohol, the mosquitoes were transferred into net cages and maintained on 10% glucose solution. *Anopheles funestus* mosquitoes were then separated from other mosquito species by gently sucking them from the cages into a small-netting cage using mouth aspirators and all other mosquito species were killed.

Non blood-fed adult *An. funestus s.l.* females were exposed to 0.75% permethrin, 0.05% deltamethrin, 0.05% lambda-cyhalothrin, 4% dieldrin, 4% DDT, 0.1% bendiocarb, 0.1% propoxur, 0.25 % pirimiphos-methyl, and 1% malathion, as recommended by the WHO. Four replicates, each of 25 wild females of unknown age, were exposed to each insecticide during the experiment. In each test, mosquitoes collected from the same villages at the same time were exposed to oil impregnated papers, constituting additional controls. The number of knocked-down mosquitoes in each tube was counted and recorded after 10, 15, 20, 30, 40, 50 and 60 minutes. After one hour of exposure, mosquitoes were transferred to the holding tubes and maintained on 10% sugar solution then monitored for survival and mortality after 24 hours. The post exposure observation was conducted after 80 minutes only if the knockdown was less than 80% [28].

6.3.2.5 Assessment of parity status

Physiological age of the wild-caught female malaria vectors was approximated based on the status of their ovaries, i.e. whether they had previously laid eggs or not [29]. Abdomens of a randomly selected sub-sample of unfed female *An. funestus* and *An. arabiensis* collected from the selected households were dissected for parity [30]. Each unfed female mosquito was first anesthetized in a refrigerator. A drop of distilled water was added to a slide and each specimen kept still on the slide, then the seventh and eighth abdominal segment was pulled using a fine needle under a stereo dissecting microscope. The ovarial tracheoles were observed under a compound microscope at 10X magnification, to determine whether they were parous or nulliparous [29].

6.3.3 Data analysis

Statistical analyses were carried out using R statistical software version 3.3.1 [31]. Mosquito abundance was over-dispersed so we modeled it following a negative binomial distribution using the generalized linear mixed model automatic differentiation model builder (*glmmADMB*) package [32] with logarithmic function. Here, mosquito abundance (nightly total count) was treated as a response variable while locality

(villages) were treated as fixed effects.

Plasmodium infection rates and blood meal sources of malaria vectors were estimated as percentages of total mosquitoes assayed. Similarly, observed mean mortality after 24 hours of exposure to each insecticide was calculated as a percentage of total mosquitoes exposed in all four exposure replicates [28]. The insecticide susceptibility test findings were interpreted according to WHO guidelines [28].

The EIR was calculated as a function of biting rates and proportion of tested mosquitoes infected with sporozoites. Annual EIR was calculated by multiplying the daily EIR values by 365 nights (Annual EIR = Nightly biting rate x Sporozoite rate x 365). Earlier trials done in the same study area, where CDC light traps had been directly compared to human landing catches inside households [33], had indicated that CDC light traps consistently caught 0.3 times as many *An. gambiae s.l.* and 0.68 times as many *An. funestus* mosquitoes as human landing catches [33]. In this current study, the CDC light trap results were adjusted using these coefficients, but the data are also presented as unadjusted results.

6.4 Results

6.4.1 Indoor densities of *Anopheles* mosquitoes

A total of 25,670 *Anopheles* mosquitoes were collected by CDC light trap and back pack aspirator, comprising *An. funestus s.l.* (18.8%), *An. arabiensis* (79.0%), *An. pharoensis* (0.5%), *An. squamosus* (0.4%), *An. ziemanni* (0.5%) and *An. wellcomei* (0.8%). The highest densities, i.e. 54% and 65% of all *An. arabiensis* and 65% of all *An. funestus* were observed in the middle of study area, i.e. Minepa village. Densities of *An. arabiensis* peaked in May, while the density of *An. funestus* peaked between February and May, one month after onset of the long rainy season, which is the main malaria transmission season. The lowest vector densities were observed between September and November (Figure 6.2).

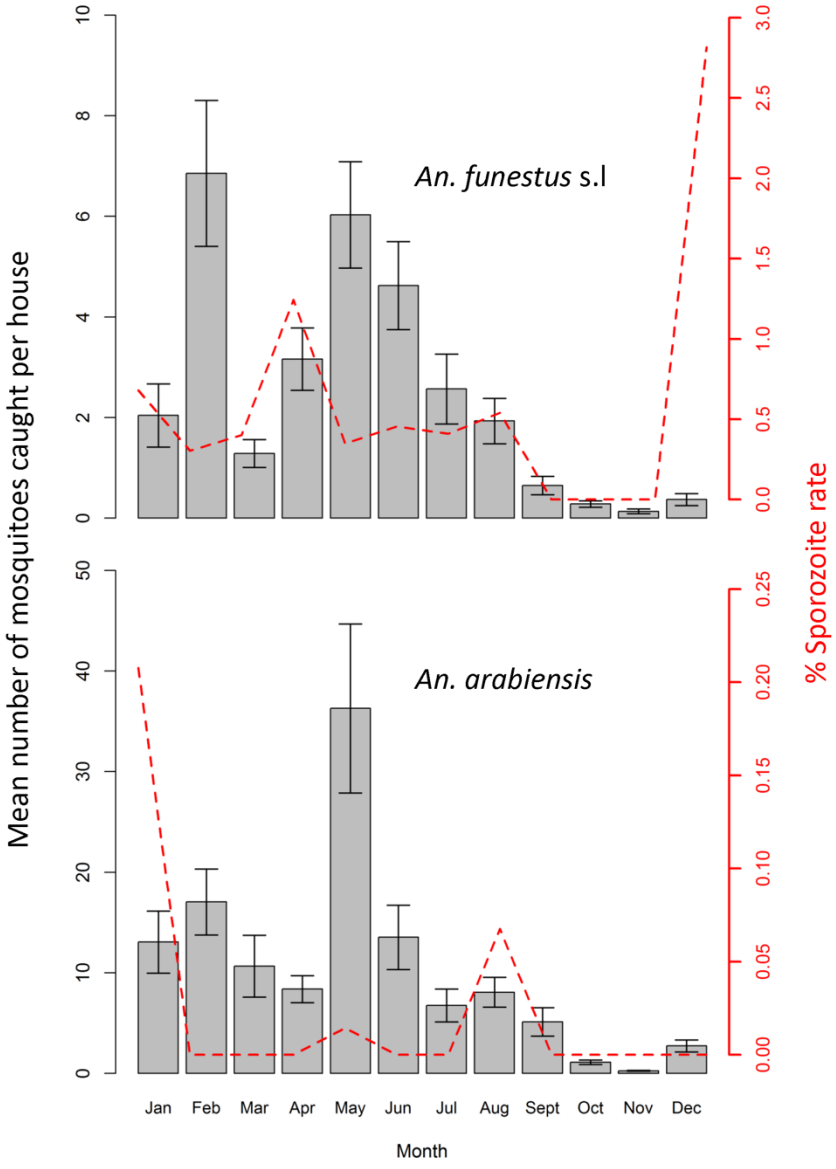


Figure 6.2: Mean number of *Anopheles funestus s.l. and An. arabiensis* mosquitoes caught per house per night. Y error bars represent the 2-standard errors of the mean.

6.4.2 Sibling species of malaria vectors and *Plasmodium* infection rates

A total of 20,135 *An. gambiae s.l.* and 4,759 *An. funestus s.l.* mosquitoes were assayed in the laboratory. All the *An. gambiae s.l.* mosquitoes tested by PCR were confirmed to be *An. arabiensis* (100%). On the other hand, the *An. funestus* group consisted of

76.6% *An. funestus,* 2.9% *An. rivulorum*, 7.1% *An. leesoni,* and 13.4% unamplified samples. Monthly variations of proportions of the *An. funestus* sibling species is shown in Figure 6.3.



Figure 6.3: Monthly variations in the composition of sibling species of *Anopheles funestus* group in the study area.

Infection rates were higher in *An. funestus* than *An. arabiensis*. Using unadjusted estimates, the overall EIR (bites per person per year) was 9.19, of which *An. funestus* contributed 86.21%, while *An. arabiensis* contributed 13.79% (Table 6.1). However, when considering estimates adjusted for relative efficiencies of CDC light traps compared to human landing catches, the overall EIR (bites per person per year) was 15.87, of which *An. funestus* contributed 73.39%, while *An. arabiensis* contributed

26.61% (Table 6.1). The highest sporozoite rate in *An. funestus* was in April, when 13.79% were found infected. Nightly biting rates of *An. funestus* was highest in May, i.e. 344 bites/month, while the highest biting rate of *An. arabiensis* was 2090 bites/month in the same month (Table 6.2). Of all *An. funestus s.l.* mosquitoes that tested positive for malaria parasites, 96% were *An. funestus s.s.*, the remaining 4% being *An. rivulorum*. None of the *An. leesoni* tested positive for *Plasmodium* sporozoites.

6.4.3 Indoor densities of culicine mosquitoes

A total of 103,382 culicine mosquitoes, comprising of 100,047 *Culex* (96.7%), 3,197 *Mansonia* (3.1%) and 138 *Aedes* (0.1%) were collected. Of all the *Culex* mosquitoes collected, males contributed 76.3%, while females contributed 23.7%. For *Mansonia*, males contributed 58.6% while females contributed 41.4%. Lastly, *Aedes* males contributed 26.1% while females were 73.9%.

Table 6.1: Infectious status of malaria vectors and dominant contribution of *Anopheles funestus* mosquitoes to ongoing malaria transmission, despite their low abundance relative to *Anopheles arabiensis*.

	Anopheles arabiensis	***Anopheles funestus s.l.****
Total number of mosquitoes collected by CDC Light Trap (January 2015 to January 2016)	20135	4759
Total number of trap nights	1152	1152
Biting rate per night	17.48	4.13
Relative efficiency (CDC-LT) relative to HLC (Derived from Okumu et al 2008 [38])	0.3	0.68
Corrected biting rate	58.26	6.08
Total number of mosquitoes analysed for *Plasmodium falciparum* circumsporozoite protein	20135	4759
Total number of sporozoite positive mosquitoes	4	25*
Sporozoite rate	0.0002	0.0053
Annual EIR (Adjusted)**	**4.22**	**11.65**
% EIR Contribution (Adjusted)**	**26.61%**	**73.39%**
Annual EIR (not adjusted)	**1.27**	**7.92**
% EIR Contribution (not adjusted)	**13.79%**	**86.21%**

- Annual EIR (Corrected biting rate x Sporozoite rate x 365)
- Overall EIR (EIR for *An. arabiensis* + EIR for *An. funestus)* = 18.45
- *86.3% of the mosquitoes from the *An. funestus* group tested were *An. funestus s.s.,* 8.4% were *An. leesoni* and 5.3% were *An. rivulorum*. Of the 25 *An. funestus s.l.* mosquitoes that tested positive for malaria parasites, 24 were *An. funestus s.s.* and one was *An. rivulorum.*
- **Where the estimates were adjusted, these were done using coefficients computed as the ratio of mosquito catches by CDC-Light Traps to Human Landing Catch methods. These coefficients were 0.3 for *An. arabiensis* and 0.68 for *An. funestus* as determined by Okumu *et al.* 2008 [33].

Table 6.2. Monthly sporozoite rates and entomological inoculation rates (EIR) for *Anopheles funestus* and *Anopheles arabiensis* from January to December 2016

	Anopheles funestus				***Anopheles arabiensis***			
Month	Number of Mosquitoes Tested	Monthly biting rates (adjusted**)	Sporozoite Infection Rate	Mean Monthly EIR	Number of Mosquitoes Tested	Monthly biting rates (adjusted **)	Sporozoite Infection Rate	Mean Monthly EIR
January	147	44.10	0.0219	0.97	963	288.90	0.0068	1.96
February	658	197.40	0.0107	2.12	1640	492.00	0.0000	0.00
March	249	74.70	0.0129	0.96	2111	633.30	0.0000	0.00
April	563	168.90	0.0413	6.98	1502	450.60	0.0000	0.00
May	1148	344.40	0.0113	3.89	6968	2090.40	0.0005	0.94
June	879	263.70	0.0153	4.04	2576	772.80	0.0000	0.00
July	489	146.70	0.0132	1.94	1283	384.90	0.0000	0.00
August	371	111.30	0.0174	1.94	1478	443.40	0.0023	1.00
September	109	32.70	0.0000	0.00	859	257.70	0.0000	0.00
October	49	14.70	0.0000	0.00	244	73.20	0.0000	0.00
November	26	7.80	0.0000	0.00	51	15.30	0.0000	0.00
December	71	21.30	0.0910	1.94	460	138.00	0.0000	0.00

** Values adjusted based on Okumu *et al.* 2008 [33] as in Table 6.1 above.

6.4.4 Mosquito blood meal sources

A total of 82 mosquitoes that were identified by PCR also had blood meals in their abdomen, and were assessed by blood-meal ELISA [29]. These included 64 *An. arabiensis*, 17 *An. funestus* and 1 *An. leesoni*. The study identified a broad spectrum of host blood antigens, mostly humans (79.2%), followed by cattle (17.1%), dogs (2.4%) and chickens (1.2%). The analysis did not detect any mixed blood meals. The results for mosquito blood meals are summarized in Table 6.3.

Table 6.3. Host blood antigens detected in blood-meals obtained from *Anopheles* mosquitoes

Blood meal source	*Anopheles arabiensis*		*Anopheles funestus*		*Anopheles leesoni*	
	N	%	N	%	N	%
Human	47	73.4	17	100	1	100
Bovine	14	22	0	0	0	0
Dog	2	3.1	0	0	0	0
Chicken	1	1.5	0	0	0	0

6.4.5 Spatial distribution of sporozoite infected *An. funestus* and *An. arabiensis*

Sporozoite infected *An. funestus* were widely distributed across all the study villages. It was also observed that the *An. arabiensis* were mostly confined to an area in the middle of the study area (Figure 6.4). The total number of sporozoite infected mosquitoes during the year was, however, too small to enable detailed analysis of spatial relations.

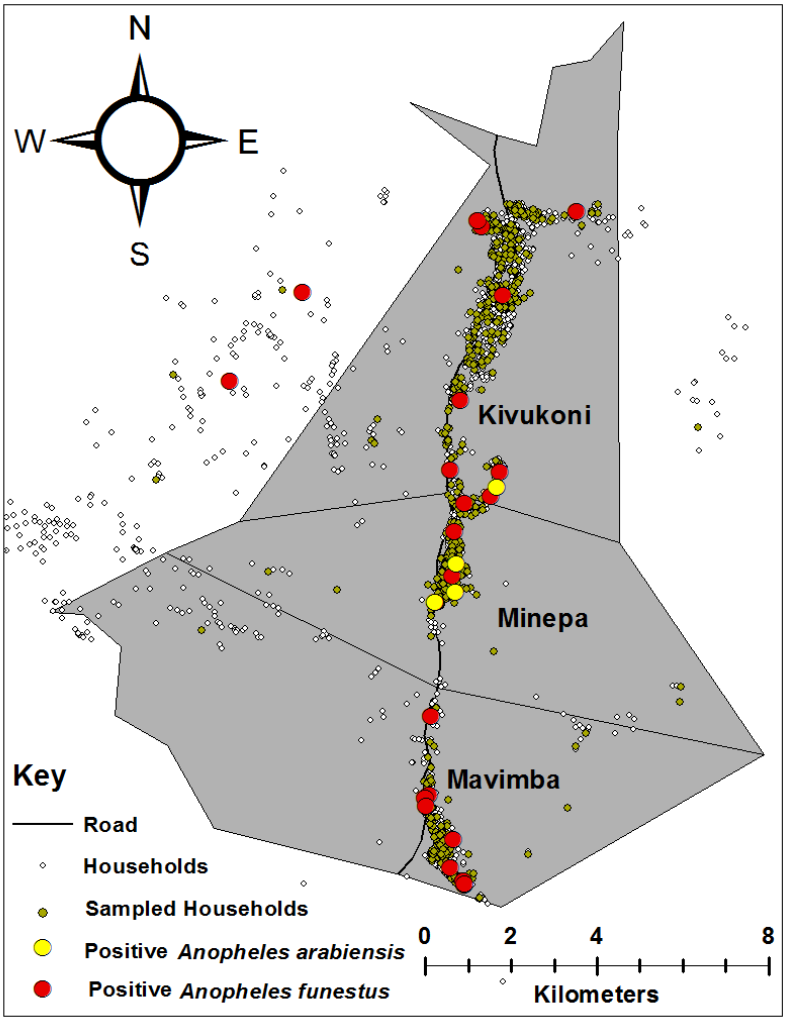


Figure 6.4: Distribution of *Plasmodium* infected *An. funestus* and *An. arabiensis.*

6.4.6 Insecticide susceptibility test results

The insecticide susceptibility test results are summarized in Table 6.4. There was 100% mortality in the susceptible reference *An. gambiae s.s.* colony when exposed to all the candidate insecticides tested. The wild-caught *An. funestus* were also fully susceptible to pirimiphos-methyl (100% mortality), malathion (100%), and dieldrin (100%), but they were resistant to permethrin (10.5% mortality), deltamethrin (18.7%), lambda-cyhalothrin (18.7%) and DDT (26.2%). In these tests, the *An. funestus* also showed reduced susceptibility to bendiocarb (95% mortality) and propoxur (90.1% mortality). The overall mortality of the *An. funestus* mosquitoes exposed to control papers was

5.4%. Susceptibility tests on *An. arabiensis* did not form part of this project as they were being carried out by colleagues at the Ifakara Health Institute.

6.4.7 Proportion of *An. arabiensis* and *An. funestus* that are parous

Of the 302 *An. funestus* dissected, 65.8% were parous, 28.8% were nulliparous, and 5.2% were pre-gravid. Of the 162 *An. arabiensis* dissected, 49.3% were parous, 37% were nulliparous and 13.5% were pre-gravid.

Table 6.4: Summary of data on susceptibility of wild-caught adult female *Anopheles funestus* mosquitoes collected in the study sites.

Insecticide tested	Total. No. Exposed	Number of mosquitoes knocked- down (KD)								Mortality		
		10 min	15 min	20 min	30 min	40 min	50 min	60 min	% KD 60 min	Total No. Dead	% Dead	Status*
Control	129	0	0	0	0	0	0	0	0	7	5.4	-
0.75% Permethrin	95	0	0	0	0	1	1	1	1.1	10	10.5	RR
0.05% Deltamethrin	80	2	2	1	2	3	3	6	7.5	15	18.7	RR
0.05% Lambda-cyhalothrin	80	0	0	1	1	1	2	4	5.0	15	18.7	RR
0.1% Bendiocarb	80	0	2	2	6	27	31	35	43.7	76	95.0	RI
0.1% Propoxur	81	21	36	46	67	58	75	77	95.0	73	90.1	RI
0.25 % Pirimiphos-methyl	84	6	3	8	46	63	80	80	95.2	84	100.0	SS
5% Malathion	80	7	13	32	55	74	78	79	98.7	80	100.0	SS
4% DDT	80	0	0	4	7	9	8	13	16.2	21	26.2	RR
0.4% Dieldrin	80	0	0	0	0	2	6	14	17.5	80	100.0	SS

*Susceptibility status:

- SS: Susceptible.
- RR: Confirmation of resistance in the tested mosquito population.
- RI: Suggests the existence of resistance and further investigation is needed.

6.5 Discussion

Malaria prevalence is declining across most African countries [1], due to improved vector control and case management, but also urbanization, improved health care and better living standards [1]. Long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS) contributed 78% of all gains accrued since 2000 [34]. In rural south-eastern Tanzania, where malaria prevalence has reduced by >60% since 2000, low-level transmission still persists despite persistently high LLIN coverage [35]. The present study re-assessed the residual malaria transmission dynamics in rural south-eastern Tanzania, in an area where the last mass distribution of bed nets was conducted between 2010 and 2011, four years before the study commenced [19]. This study therefore provides an essential update of the transmission situation and demonstrates the now dominant role of *An. funestus*. Like in most residual transmission settings in East Africa, this study confirms that populations of formerly notorious *An. gambiae s.s.* have significantly diminished, or completely vanished [10,36]. *Anopheles arabiensis* now dominates in numbers, but the far more competent vector *An. funestus* carries most of the residual malaria parasites, despite being highly outnumbered by *An. arabiensis*. This study provides evidence to test the hypothesis that interventions targeting *An. funestus* could dramatically reduce malaria transmission in areas where it is the dominant vector.

This study has also determined that *An. funestus* is resistant to pyrethroids used on LLINs, and survives unexpectedly longer than *An. arabiensis*, thus requiring new control approaches. Given the extensive resistance in the species population against a wide range of insecticides currently approved for public health use, it appears that we now have only a limited set of options, including organophosphates such as pirimiphos-methyl. Thus, one of the main control methods that could be used against this species is focal IRS with long-lasting formulations of organophosphates (e.g. Actellic® CS-300), against which *An. funestus* is still highly susceptible. Other potential interventions may include house improvement and house screening, as well as use of eave baffles treated with organophosphates, which would take far less insecticide, but deliver equal impact on the mosquito populations as IRS [37]. Going forward, it may be desirable to conduct new evidence-based studies in well-characterized areas such as these study sites, to

demonstrate possible vector elimination strategies leading to malaria transmission control. Approaches may include combinations of organophosphate-based IRS alongside other interventions like LLINs, improved housing and larval source management, especially where rice growing is commonly practiced.

To achieve malaria elimination, we must identify and target all pockets of transmission, including geographically distinct areas of high transmission and demographically high-risk sub-populations [38,39]. In this study setting in rural south-eastern Tanzania, low-level transmission persists, despite over 80% LLIN coverage [35]. The persistent residual transmission is most definitely mediated predominantly by *An. funestus s.s*., suggesting possibly that targeted, high impact, species-specific interventions may improve control and accelerate efforts towards eventual malaria elimination. Recent findings suggest that the overall distribution of malaria mosquitoes, including *An. funestus,* is strongly influenced by and can be predicted based on numbers and spatial variability of household occupancy [8]. The densities of *Anopheles* mosquitoes were highest in the rainy season even though transmission was evidently sustained at low densities even in the dry season, mostly by *An. funestus*. Since aquatic breeding habitats for *An. funestus* are not easily identified, it may be necessary to identify other environmental characteristics that affect natural abundance of *An. funestus* within villages and between households. This approach can be feasible by involving the local community and volunteers. The evidence from Mwangungulu *et al.* show that scientists can rely on community knowledge and experiences to identify areas where mosquitoes are most abundant [40].

The data presented here would be essential in developing control measures that target primarily the most important residual vector species, in this case *An. funestus*. In these specific study settings in Tanzania, the nucleated settlements, and spatial correlations between human biomass and vector densities [8], indicates this proposed study area renders itself well to spatially-targeted interventions. The study has rigorously demonstrated that *An. funestus* is now the primary vector of malaria in this area in rural-south eastern Tanzania, despite concurrently high densities of other

species such as *An. arabiensis*. The area historically had very high malaria transmission, averaged 400 infectious bites/person/year (ib/p/y) until early 2000s [41], with prevalence as high as 70% in the 1990s [42], but this study indicates that transmission has now dropped to around 20 ib/p/y. Moreover, this study has also demonstrated that *An. funestus* co-exists with *An. rivulorum* and *An. leesoni,* but malaria transmission was mainly mediated by *An. funestus.* It is not possible to determine conclusively if the greater contribution of *An. funestus* to malaria transmission is mostly due to its relatively higher abundance than that of the other sibling species, but it is clear that this species mediates most of the residual transmission.

Of the 25 *An. funestus* group mosquitoes that tested positive for malaria parasites, one was *An. rivulorum.* Given that the ELISA lysates were all boiled to eliminate any false positives [26], occasionally associated with non-*Plasmodium* protozoans transmitted by zoophilic mosquitoes [43], we can assume, based on the 4% sporozoite rate, that *An. rivulorum* is indeed an important vector in this setting, and that its role is limited mostly by its low densities (2.9% relative to the other species in the group). Other studies in East Africa have previously demonstrated the importance of this species in residual transmission settings [44], its role increasing after the decline of *An. gambiae s.s*. populations [45,46]. Some previous studies have reported sporozoite infections in *An. coustani* [47], *An. squamosus* [48] and *An. ziemanni* [49], but no such evidence was observed in this current study. Table 6.5 shows some of the other localities where *An. funestus* mosquitoes significantly contribute to residual malaria transmission.

Table 6.5: Examples of localities where *Anopheles funestus* mosquitoes have been demonstrated to mediate most of the residual malaria transmission, and where control programmes targeting *An. funestus* with effective interventions, could drastically reduce local transmission.

SN	Country	Intervention	Dominant Vectors	Other Vectors	Resistance Status	Sporozoite Rates	Implications for malaria transmission control	Reference
1	Kenya	ITNs and LLINs	*An. funestus*	*An. arabiensis, An. gambiae s.s.*	Pyrethroid resistance confirmed	*An. funestus* 4.5%, *An. arabiensis* 0.9%, *An. gambiae s.s*. 8.6%	Reduced effectiveness of the current interventions	[50]
2	Burkina Faso		*An. gambiae s.l.*	*An. funestus s.l. An. nili*	No pyrethroid resistance detected. *An. funestus* was highly resistant to dieldrin	*An. funestus* (2.6% - 9.7%)	*An. funestus* had higher biting rates and high sporozoite rate compared to *An. gambiae* complex	[51]
3	Tanzania	LLINs	*An. gambiae s.l.*	*An. funestus s.l.*	Pyrethroid resistance confirmed	*An. funestus* (0.16% - 1.47%)	Resurgence of malaria transmission in Kilombero valley, Tanzania	[10]
4	Senegal	LLINs	*An. funestus*	-	-	*An. funestus* 1.28%	*An. funestus* changed their host seeking behaviour following the introduction of LLINs, thus compromising effectiveness of LLINS.	[52]
5	Cameroon		*An. funestus s.s.*	*An. gambiae s.s*.	-	*An. funestus* 6.8%, *An. gambiae s.s.* 0.6%-4.1%	*An. funestus* accounts for 88% of the transmission in this setting.	[53]

6	Benin	LLINs IRS	*An. gambiae* s.l and *An. funestus*	*An. coluzzii*	-	*An. funestus* 26.08% *An. gambiae* 11.16%	Sporozoite prevalence was three times higher with *An. funestus* than *An. gambiae.*	[54]
7	Madagascar	LLINs IRS	*An. funestus* *An. gambiae s.l*	-	Pyrethroids resistance confirmed Bendiocarb resistance confirmed	*An. funestus* 1.8% *An. gambiae s.l* 1.2%	*An. funestus* is suspected to have cross-resistance between pyrethroids and carbamate.	[55]
8	Ghana	LLINs	*An. funestus* *An. gambiae s.l*	*An. rufipes*	-	*An. funestus* 7.2%	*An. funestus* contributes to higher sporozoite rate compared to *An. gambiae s.l*	[56]
8	Malawi		*An. funestus* *An. gambiae s.l*	*An. parensis*	Pyrethroids resistance confirmed Carbamate resistance confirmed	*An. funestus* 4.8% *An. rivulorum-like* 1.07%	Observation of multiple insecticide resistance in Malawian *An. funestus.*	[57]

The extremely high proportion of *Plasmodium* infections currently mediated by *An. funestus* mosquitoes in this area is compounded by, and is likely also a result of the very high insecticide resistance in this species. This study found that *An. funestus* mosquitoes were not only carrying most of the residual malaria parasites, but they were also very highly resistant to common pyrethroids used for vector control, i.e. deltamethrin, permethrin and lambda-cyhalothrin, as well as to the organochlorine, DDT. Moreover, there was also reduced susceptibility to the carbamate insecticides bendiocarb and propoxur, which would normally be candidates for replacing the pyrethroids. An earlier study by Lwetoijera *et al.* in the neighbouring Kilombero district, also found increasing importance of *An. funestus* in malaria transmission associated with high resistance [10]. Even though the insecticide susceptibility status for *An. arabiensis* was not evaluated in this study, a separate study from these villages by Matowo *et al.* [58] found *An. arabiensis* to be also highly resistant to pyrethroid chemicals.

The high human blood index observed in the *An. funestus* females clearly suggests that the species could still be best targeted by household-based interventions that protect humans. Observations of increasing outdoor biting by the different *An. funestus* sibling species has been reported in this study area [59,60], which warrants consideration of complementary interventions that also target outdoor-biting vectors. Also, given the high resistance to the common insecticides for vector control, it appears that use of organophosphates, against which the vector is still susceptible, notably pirimiphos-methyl, could provide a temporary solution against residual malaria transmission in this area. Long-lasting formulations of pirimiphos-methyl already exist [61], which could be used for IRS in this area to complement LLINs, as it has already been implemented in Zanzibar, northern region and lake zones in Tanzania [62].

Other possible high value interventions could be insecticidal eave baffles [37] especially in areas where even outdoor-biting populations may forage indoors during their adult life [63].

This study did not assess the role of *An. funestus* on outdoor malaria transmission, but a separate study conducted in the same villages by Ngowo *et*

al. [64] yielded evidence that some *An. funestus* collected outdoors were found to be sporozoite positive. Furthermore, new tools should be included, such as chemicals with new active ingredients available for IRS that are being evaluated by the WHO (including non-pyrethroid active ingredients like neonicotinoids), as well as bed nets that combine synergists with pyrethroids, or those that combine different insecticide classes, to tackle insecticide resistance and lower vector survival rates.

One limitation of this study is that tests for insecticide susceptibility were conducted on wild caught *An. funestus* females of variable age, since we could not find adequate larvae for the assays. WHO recommends where possible to use mosquitoes of the same age cohort (F1 progeny). Using wild mosquitoes that are older than the standard 3-5 days may lead to under-estimation of resistance levels. Secondly, blood meal analyses were carried out on visually blooded mosquitoes and so may have missed those with small amounts of blood in their abdomens, such as those that had interrupted feeding or had partially digested the blood. Another potential limitation is that parity assessments, such as the ones conduced here are greatly impacted by season - this study conducted parity assessment for mosquitoes collected for two months only and further studies should be done to assess parity status across seasons. Lastly, there was also a significant non-amplification of mosquito samples during the PCR analysis to determine the identity of sibling species. About 13.4% of all samples analyzed in the laboratory did not amplify. An analysis of the sample handling processes, the analysis process, and the control samples, suggests that the laboratory processes were unlikely to be the reason for the non-amplification. Instead, this situation might have arisen due to sub-optimal storage conditions of some samples, or discrepancies in the morphological identification. These specific processes therefore deserve greater attention in future studies.

6.6 Conclusion

This study provides an update on the residual malaria transmission situation in rural south-eastern Tanzania, where LLINs were already widely distributed, confirming the previous findings. Although *An. arabiensis* is still the most abundant vector species here, ongoing residual transmission is predominantly mediated by *An. funestus,*

possibly due to high resistance and high survival probabilities. Interventions that effectively target *An. funestus* could therefore significantly improve control of residual malaria transmission in the area.

Author's contribution

EWK and FOO conceived the study and developed study protocols. EWK, GM, MF NSM, AM and FOO conducted experiments and supervised data collection. EWK, HSN, and FOO did data analysis and wrote the manuscript. All authors approved the manuscript prior to submission.

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Ethical considerations

Meetings with local leaders were held in the study areas and the main aims of the study were explained by the scientists. Permission to conduct studies in the separate villages was obtained from respective leaders of each of the villages; Mr. Ayubu Salila, Mr. Kiluka Mdumba and Mr. Baalesa Ligola for Kivukoni, Minepa and Mavimba villages respectively. I confirm that the field studies did not involve endangered or protected species. A written and signed informed consent was obtained from each household head participating in the study. All information was given in Kiswahili, the local language. All the households were provided and protected by intact LLINs (Olyset®nets) as previously distributed by the universal bednets coverage campaign [65]. Ethical approval for the study was obtained from Ifakara Health Institute Institutional Review Board (IHI/IRB/No: 34-2014), and the

Medical Research Coordination Committee of the National Institute of Medical Research (Certificate No.NIMR/HQ/R.8a/Vol.IX1903).

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Chapter VII

Evidence of mating swarms of the malaria vector *Anopheles arabiensis* in Tanzania

7.1 Abstract

Malaria mosquitoes form mating swarms around sunset, often at the same locations for months or years. Unfortunately, studies of *Anopheles* swarms are rare in East Africa, the last recorded field observations in Tanzania having been in 1983. Mosquito swarms were surveyed by trained volunteers between August 2016 and June 2017, in Ulanga district, Tanzania. Identified *Anopheles* swarms were sampled using sweep nets, and collected mosquitoes killed by refrigeration then identified by sex and taxa. Sub-samples were further identified by PCR, and spermatheca of females examined for mating status. Mosquito ages were estimated by observing female ovarian tracheoles, and rotation of male genitalia. GPS locations, types of swarm markers, start/end times of swarming, heights above ground, mosquito counts/swarm, and copulation events were recorded. A total of 216 *Anopheles* swarms were identified, characterized and mapped, from which 7,142 *Anopheles gambiae s.l.* and 13 *Anopheles funestus* were sampled. The *An. gambiae s.l*. were 99.6% males and 0.4% females, while the *An. funestus* were all males. Of all *An. gambiae s.l.* analyzed by PCR, 86.7% were *An. arabiensis*, while 13.3% returned non-amplified DNA. Mean height (±SD) of swarms was 2.74±0.64m, and median duration was 20 (IQR; 15-25) minutes. Physical features found in locations where swarms occured included ant hills (22%), garbage heaps (21.7%), demolished houses (10%), pit latrines (10%), brick piles (7.9%) and woodpiles (3.4%). Visual estimates of swarm sizes by the volunteers was closely correlated to actual sizes by sweep nets (R=0.94; P=<0.001). All females examined were nulliparous and 95.6% [N=6787] of males had rotated genitalia, indicating sexual maturity. This is the first report of *Anopheles* swarms in Tanzania in more than three decades. The study demonstrates that the swarms can be identified and characterized by trained

community-based volunteers, and highlights potential new interventions, for example targeted aerial spraying of the swarms to improve malaria control.

Adapted from Kaindoa EW, Ngowo HS, Limwagu A, Mkandawile G, Kihonda J, Masalu JP, et al. New evidence of mating swarms of the malaria vector, *Anopheles arabiensis* in Tanzania. Wellcome Open Res. 2017;2:88. (doi: 10.12688/wellcomeopenres.12458.1)

7.2 Background

Recent successes in malaria control have been mostly attributed to vector control measures, in particular, long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) [1]. These tools have contributed to ~78% of all gains achieved since 2000 [1,2]. However, effectiveness of these interventions is compromised, partly due to spread of insecticide resistance [3] and increased outdoor exposure to mosquito bites, amongst other challenges [4–6]. Consequently, malaria transmission persists in many places, even where high and sustained coverage of LLINs has been achieved.

To develop new options for malaria vector control, it is important to re-examine the overall ecology of the malaria vectors [7], including not only the blood-feeding and resting habits commonly targeted by LLINs and IRS, but also other mosquito behaviours indoors and outdoors. Mating is one of the most important aspects in the maintenance of species [8], yet it is a widely under-investigated aspect of the mosquito biology. Understanding mosquito mating systems can provide new opportunities for expanding vector control options. Most insects mate in swarms, whereby dispersed populations aggregate at specific times and places [9,10]. In mosquitoes, including the malaria vectors, swarming flights are influenced by presence of visual markers on the ground [11,12]. Targeting swarms to deplete mosquito densities indeed offers unrivalled opportunities to drastically reduce mosquito-borne pathogen transmission [13]. Such an approach has proven effective against some *Anopheles* mosquitoes on a limited scale in Burkina Faso [14], but needs to be validated for other vector species in other areas. Besides, mosquito swarms are known to occur perpetually in the same locations at approximately the same time each day [10,15], thus targeting them could be easily achieved by trained community volunteers. The concentrations of males in swarms, predictability and accessibility of the swarming sites and the fact that swarms can be artificially manipulated [16], makes the male mosquitoes vulnerable and an easy control target.

While swarms have been commonly identified in west and central Africa [17–19], observations have rarely been reported in East and Southern Africa. Prior to this current report, there was only a handful of reports, including the seminal work by

R.P. Marchand in Tanzania in the early 1980s [20], the work by J.D. Charlwood in Mozambique in early 2000s [21], some observations in Kenya in 1965 [22] and unpublished observations of *An. arabiensis* swarms in the Kilombero valley in south eastern Tanzania (Dr. Kija Nghabi and Japhet Kihonda, Personal Communication).

This current study was designed to explore the *Anopheles* mating systems in rural south-eastern Tanzania, by assessing occurrence of natural swarming and outdoor nocturnal behaviours of *Anopheles* mosquitoes. The assessment involved accurate identification, mapping and characterizing of mosquito swarms in three study villages in Ulanga district, Tanzania. A combination of crowd-sourced community knowledge [23], intensive field surveys and expert advice was used. The initial objective was, therefore, to demonstrate natural occurrence of swarms of malaria vectors in rural, south eastern Tanzania and characterize these swarms.

7.3 Methods

7.3.1 Study area

The study was conducted in the three villages of Kivukoni (8.2135°S, 36.6879°E), Minepa (8.2710°S, 36.6771°E) and Mavimba (8.3124°S, 36.6771°E) in Ulanga district, on the Kilombero river floodplain in south-eastern Tanzania (Figure 7.1). The main malaria vectors in the area included *Anopheles funestus* and *An. arabiensis* with minor contributions from *An. rivulorum* [24]. Overall EIR was last estimated at 4.2 and 11.7 infectious bites/person/year by *An. arabiensis* and *An. funestus* respectively [24]. The main vector control approach used in the area is LLINs, and the villages benefited from universal LLIN coverage campaigns both in 2011 and in 2016. The main malaria vectors are resistant to pyrethroids used in the LLINs, but still susceptible to organophosphates [24,25]. The mean household size is 4.2 [26]. Most of the houses are mainly mud and brick walled, with thatched or iron-sheet roofs. The communities rely mainly on substance farming, cultivating rice and maize but also fishing.

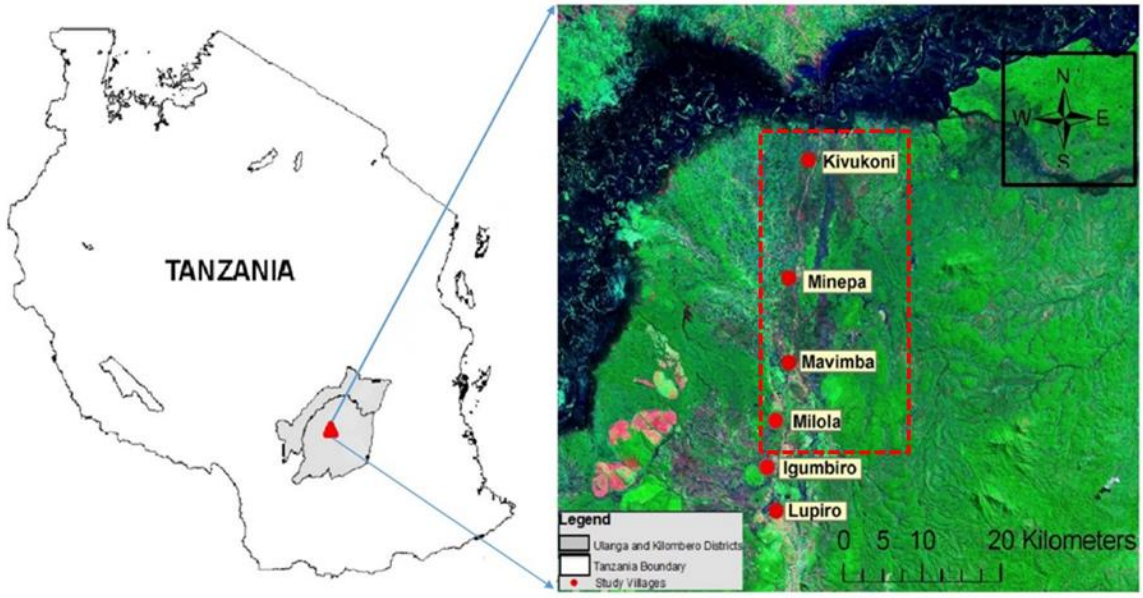


Figure 7.1: Map of the study area, showing villages in south-eastern Tanzania, where swarm surveys were conducted.

7.3.2 Training on swarm surveys and characterization

Initially, the project leaders and research assistants were trained on how to identify mosquito swarms and swarm markers by experienced entomologists from Burkina Faso. The first brief training was conducted in Ifakara, Tanzania, in December 2014 by Diabate, at which stage this project had just been conceived. The second training was done for project leaders during a visit to Burkina Faso in September 2016. Then ten participants were selected in the three study villages, with the help of community leaders were also trained. All participants were male adults aged 18 to 35 years, who provided written informed consent prior to being enrolled in the study.

In April 2016, five months before the second training of project leaders, exploratory swarm surveys were started in the study area. During and after these exploratory surveys, the project leaders trained the local community-based swarm searching volunteers on how swarms are identified and characterized, focusing on major features of both the swarms and their associated markers (Figure 7.2). This training was conducted over several weeks, and included: i) swarm searching techniques; ii) swarm sampling techniques; iii) swarm characterization, and iv) entomological procedures essential for examining male and female *Anopheles* mosquitoes collected from swarms. All training for local community volunteers was conducted in

Kiswahili language, native in the study area. These volunteers were then relied upon to identify swarms, which the researchers would then follow up for species confirmation.

Further advanced training on swarm surveys and characterization was conducted in Tanzania by a team of experts from the Institut de Recherche en Sciences de la Santé (IRSS), Burkina Faso. This advanced training covered: i) swarm searching techniques in dry and wet seasons; ii) swarm visualization techniques; iii) GIS-based analysis of swarms; iv) swarm markers and associated environmental features; v) swarm sampling techniques; vi) photographic survey of swarms; vii) swarm characterization (including key features to consider); and viii) identification of mating couples in swarms and examination of male and female mosquitoes to identify mated and non-mated individuals.



Figure 7.2: Pictures of volunteers and researchers learning how to identify and sample mosquito swarms in one of the study villages.

7.3.3 Initial exploratory surveys of mosquito swarms

To aid logistical assignment of the swarm searching activities, each volunteer was assigned one sub-village to search and report the occurrence of swarms. The trained community volunteers were dispatched to search and report any types of insect swarms in their areas, and report these to the researchers. Every time a swarm was reported by the volunteers, the researchers visited the site immediately (if nearby) or the next day (if in far locations), first, to verify whether the swarms were actually present, second, to determine whether they were swarms of mosquitoes as opposed to other insects, and third, to assess whether they were actually *Anopheles* swarms. The first confirmed *Anopheles* mosquito swarm was identified by a community-based swarm searcher in mid-August 2016 in Minepa Village. This provided our initial verified evidence of presence of *Anopheles* swarms in this area.

As a result of this initial discovery, the team was reorganized for more intensive swarm searching expeditions. From this point forward, the key objectives now included demonstrating that local community members could be trained to readily identify and characterize *Anopheles* swarms, and conduct comprehensive mapping and characterization of *Anopheles* swarms in selected villages. Subsequent efforts were restricted to just three villages (Mavimba, Kivukoni and Minepa) in Ulanga district, to enable detailed swarm studies.

7.3.4 Detailed mapping and characterization of *Anopheles* mosquito swarms, and swarm markers in three selected villages

After the initial swarm searching, a comprehensive mapping and characterization of the *Anopheles* swarms was conducted in three selected villages, Minepa, Mavimba and Kivukoni. Each volunteer was assigned a specific area (sub-villages) to search for mosquito swarms, by first identifying potential swarm markers in these areas during the day, and then actively searching for mosquito swarms across the area every evening from 6:00 pm. During the swarm surveys, the volunteers continued to receive supportive supervision and training on how to identify, locate and sample mosquitoes using sweep nets. This continuous training was important since this is the first time *Anopheles* swarms were being recorded in this area.

To facilitate the swarm surveys, we also identified, characterized and recorded all potential swarm markers in the study villages. The swarm markers were classified

based on the criteria developed by Diabate *et al*. [27], into four categories as follows: a) flat-contrast markers, such as bare land and footpaths, b) flat no-contrast markers, such as grasses and rice fields, c) elevated contrast markers, such as anthills, woodpiles, bricks and garbage bins, and d) elevated no-contrast markers such as banana trees. These markers were subsequently monitored in the evenings to identify and record those with swarms.

Following the initial identification and mapping, each verified swarm was comprehensively characterized by the expert team, repeatedly visiting the swarms at least three times. In addition to recording date, month, and name of village, a systematic process was followed based on various pre-determined characteristics: a) heights at which the swarms occurred, (measured as a distance between the base of the swarm and the ground level), b) time of day when the swarming started appearing, recorded to the nearest minute, c) time of evening/night when the swarms were completely dispersed, also measured to the nearest minute, d) physiological status of a sample of mosquitoes collected in the swarm, e.g. mating status and parity, e) any other mosquito species and insects occurring in the same swarm, f) exact geo-location of the swarm marker, measured using handheld GPS receivers (Magellan eXplorist GC, USA), g) ratio of males to females collected in the sample, h) number of copulation events observed in the swarm, i) number and proportion of females caught that had evidence of having been inseminated, j) type of swarm marker, k) parity status of female mosquitoes collected in the swarms, including number and proportion of females that were parous or nulliparous, l) morphological and molecular identification of the species of *Anopheles* mosquitoes collected in the swarm, m) any other unique observations made at the swarm site or on the swarms, and n) approximate number of mosquitoes collected in each swarm.

Swarm sizes were estimated in two ways: by sampling with a large sweep net (190 cm diameter), approximately 10 minutes after the start of the swarm, and visually, by the trained volunteers counting the flying mosquitoes and approximating the counts. Lastly, we observed other features which may affect the occurrence of mosquito swarms, such as time of sunset, human movements and other insects present.

7.3.5 Laboratory processing of mosquitoes collected in the swarms

Collected mosquitoes (dead or alive) were aspirated from the sweep nets, kept in separate paper cups and maintained on 10% glucose solution so that those still alive could survive till the next morning. The following morning, all mosquitoes were killed in a closed container by freezing, then identified morphologically by taxa and sex following keys developed by Gillies and Coetzee [28]. A sub-sample from each collection was further identified by multiplex polymerase chain reaction (PCR) to identify sibling species that are morphologically indistinguishable. The PCR assays were conducted using protocols developed by Scott *et al*. [29].

Physiological age of the wild-caught female malaria vectors was approximated based on the status of their ovaries, i.e. whether they had previously laid eggs or not, by observing the coiling or uncoiling of the ovarian tracheoles [30]. Each unfed female mosquito was first anesthetized in a refrigerator. A drop of distilled water was added to a slide and each specimen kept still on the slide, then the seventh and eighth abdominal segment was pulled using fine needles under a stereo dissecting microscope. The ovarian tracheoles were then observed under a compound microscope at 10X magnification, to determine whether the mosquitoes were parous (tracheoles uncoiled and stretched) or nulliparous (with tracheolar skeins, i.e. coiled) [30].

7.3.6 Assessment of female insemination status and sexual maturity in males

To determine the insemination rate, female *An. arabiensis* were dissected under a dissecting microscope and their spermathecae examined for the presence of spermatozoon under a compound microscope using a X10 objective lens [31,32]. To assess whether males had matured sexually, genitalia of *An. arabiensis* males were also observed for signs of rotation.

7.3.7 Data analysis

Data analysis was done by using R software [33]. Number of mosquitoes per swarms, duration of swarms, proportion inseminated, proportion parous/nulliparous, and proportion of males with rotated genitalia, were modeled as a function of different variables: village, swarm marker type, month, height of swarms, time of

swarming and volunteer ID. Geolocations of the swarms relative to households and other landmarks were visualized using ArcGIS 10.0 (ESRI, USA). Percentages of parity status, insemination rate, mean heights of swarms above ground, median duration of swarms, and time of day when swarms started or ended at the different months of surveys, were also calculated. Swarm markers were classified following the classification category developed by Diabate *et al*. [27]. Visualized volunteer estimates of swarm sizes, and sweep net swarm size estimates were compared and their linear correlation coefficients estimated.

7.4 Results

7.4.1 Results of exploratory surveys: initial evidence of *Anopheles* swarms

The initial exploration, conducted in multiple villages revealed that *Anopheles* swarms do indeed occur in villages along the Kilombero river valley in rural south-eastern Tanzania. The surveys also revealed that community members can be relied upon to identify these swarms and that by combining their local knowledge with expert knowledge, the *Anopheles* swarm surveys could be effectively characterized. The first *Anopheles arabiensis* swarm was observed on 15th August 2016, in the village of Minepa, by one of the local volunteers. To the best of our knowledge, this was the first recorded *Anopheles* swarm in this specific part of Tanzania, and the first in the country since the last report from the north-eastern part of the country in 1983 [20]. In the initial exploration, several non-mosquito swarms and also culicine mosquito swarms were incorrectly reported by the volunteers as *Anopheles* swarms, but were dispelled by the expert verification teams. However, one of the swarms was later identified as that of *An. funestus.* The reliability of the volunteers, however, increased during the course of the surveys.

7.4.2 *Anopheles* mosquito swarms in the three selected villages

After the initial exploratory surveys, and confirmation of occurrence of *Anopheles* swarms, subsequent surveys were concentrated in the three selected villages of Minepa, Mavimba and Kivukoni (Figure 7.1). A total of 216 swarms were observed from August 2016 to June 2017. The distribution of mosquito swarms in the study villages is shown in Figure 7.3. Swarm distribution varied across the three study

villages as follows: 58.3% of the swarms (n=126) were found in Minepa, 11.1% (n=24) in Mavimba and 30.5% (n=66) in Kivukoni. There were more swarms distributed in areas close to the rice fields and at the edges of the villages, compared to areas near human settlements (Figure 7.3). All the swarms observed consisted exclusively of *An. gambiae s.l.* PCR identification of a sub-sample of 97 *An. gambiae s.l.* mosquitoes showed them to be 100% *An. arabiensis*. In one instance in the early days of the surveys, we also collected 13 male *An. funestus* mosquitoes, though these were not separate, but were instead very near the *An. arabiensis*, swarms. Since no repeat observation was made of *An. funestus*, we are unable to verify presence of swarms of this species here until further investigations are completed.

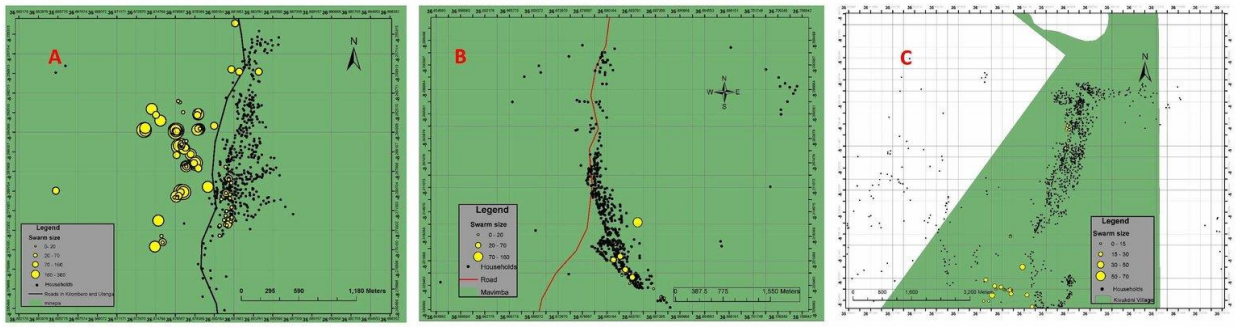


Figure 7.3: Comparison of swarm distribution between the three study villages

7.4.3 Start times, end times, duration and height of *Anopheles* swarms

It was observed that swarms began with two to three mosquitoes congregating after sunset, moving above a swarm marker. Then the numbers increased over the next 10 to 15 minutes, and slowly decreased in size then disappeared after 20 (IQR;15-25) minutes. Flying mosquitoes were observed by viewing them against the sunset. Mosquitoes tended to congregate at a mean height (±SD) of 2.74±0.64m from the ground. The swarm size increased and became compact above the marker but they disappeared when it became dark, which was also when swarm observations became impossible. Furthermore, the start and end time of swarming periods varied across months - swarms were appearing earlier between August 2016 and January 2017 while appearing later between February and June. (Figure 7.4) Overall, the average start and finish time was 18:25Hrs and 19:45Hrs respectively. According to weather information archived online by dateandtime.com, the latest time of sunset in the study area varied in a similar pattern as the start and end times

of the observed swarm sessions (https://www.timeanddate.com/sun/@157407) (retrieved August 2017). According to timeanddate.com, between August and November 2016, the latest sunset time ranged between 18:32Hrs and 18:42Hrs. However, between December 2016 and March 2017, the latest sunset times occurred between 18:56Hrs and 19:03Hrs, but was once again early between April 2017 and August 2017, when the latest sunset time over the Kilombero valley ranged from 18:26Hrs and 18:35Hrs (https://www.timeanddate.com/sun/@157407).

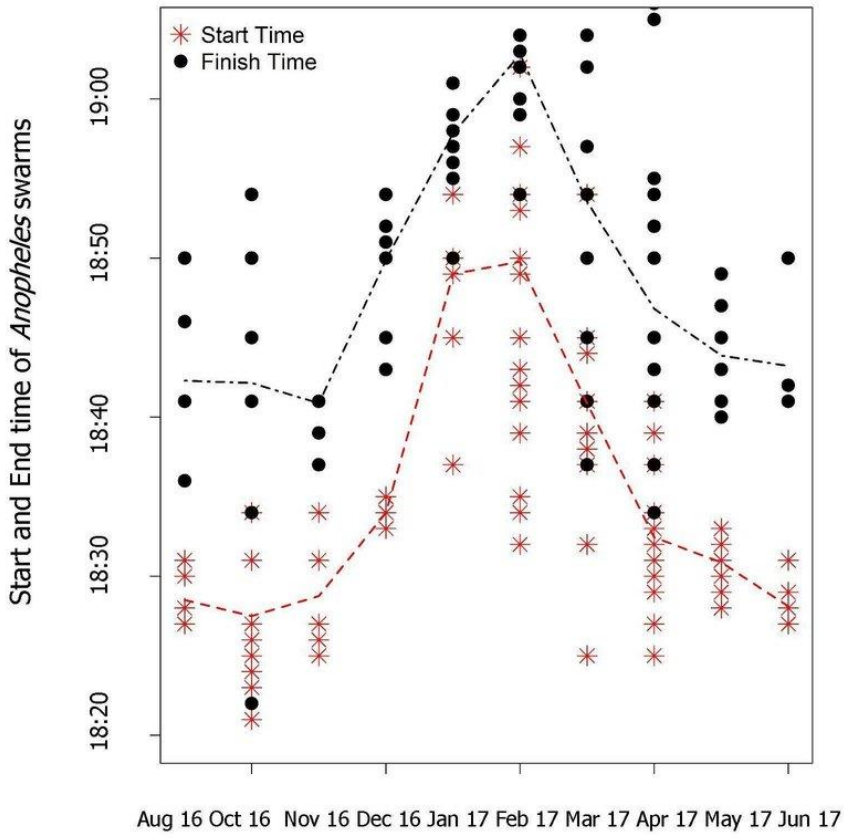


Figure 7.4: Observed start and end times of *Anopheles* arabiensis swarms in the study villages.

7.4.4 Estimated swarm sizes: data collected through direct visualization by the community volunteers compared to data collected using sweep nets

Swarm sizes were estimated from two data sources. First, were data provided from visual assessments by the volunteers looking at swarms and estimating how many mosquitoes were flying in the swarm. Second, were data from collections done using large standardized sweep nets, with which trained volunteers collected the

mosquitoes approximately 10 minutes after the start of swarming. Though the values provided from the two data sources were not equal (i.e. visual estimates being consistently higher than sweep net counts), there was a significant linear correlation (Table 7.1 & Figure 7.5) between the visual estimates provided by the community volunteers and estimates obtained from sweep net counts ($R^2 = 0.94$; $P < 0.001$). There were also variations of swarm sizes observed in different villages, with Minepa village having the largest swarms followed by Mavimba and Kivukoni villages (Table 7.1).

Table 7.1: Swarm sizes estimated by either visual observations by trained volunteers, or sweep net sampling in the three study villages.

	Visual estimates by volunteers		**Sweep net sampling estimates**	
	Mean	C.I	Mean	C.I
Kivukoni	34.0	31.4 - 36.6	13.1	11.5 -14.9
Mavimba	38.7	36.3 - 41. 3	20.6	19.9 - 22.5
Minepa	95.5	93.8 – 97.3	56.1	54.8 - 57.4

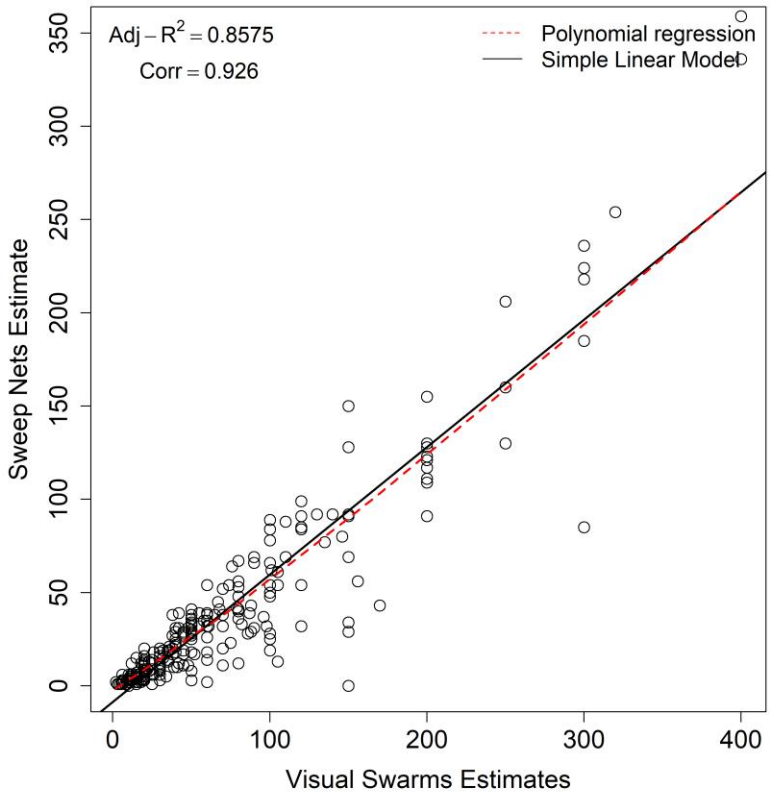


Figure 7.5: Estimated swarm sizes and correlation between estimates done by visualization and estimates done using sweep nets.

7.4.5 Potential swarm markers survey

The commonest swarm markers were ant hills (22%), garbage heaps (21.7%), demolished houses (10%), pit latrines (10%), brick piles (7.9%) and woodpiles (3.4%). Of the four marker categories classified by Diabate *et al*. [27], *An. arabiensis* preferred to swarm over bare land (category B), garbage heaps and bricks (category C) and rice fields (category D). Table 7.2 shows the commonest swarm markers and the frequency of swarms observed at each marker. Most of the swarm locations remained the same, several months after the swarms were first identified and verified.

Table 7.2: Common types of swarm markers observed in the three study villages (and number of markers that actually had *Anopheles* swarms over them).

	A. Flat contrast swarm markers	**B. Flat no-contrast markers**	**C. Elevated contrast markers**	**D. Elevated no-contrast markers**
Minepa Village	Burned ground (17) Well (0) Cowshed (0) Trench (0)	Rice fields (75) Footpath (1) Grass (8)	Bricks (17) Garbage heaps (10) Woodpile (1) Demolished house (2) Ant hill (7) Toilets (3)	Banana trees (24)
Mavimba Village	Burned ground (4) Well (0) Cowshed (0) Trench (2)	Rice fields (1) Footpath (1) Grass (5)	Bricks (0) Garbage heaps (10) Woodpile (1) Demolished house (0) Ant hill (14) Toilets (0)	Banana trees (0)
Kivukoni Village	Burned ground (0) Well (0) Cowshed (1) Trench (1)	Rice fields (1) Footpath (0) Grass (4)	Bricks (2) Garbage heaps (3) Woodpile (0) Demolished house (3) Ant hill (0) Toilets (0)	Banana trees (4)

7.4.6 Copulation events, mating status, sexual maturity and parity rates in mosquitoes collected from the swarms

Throughout the study period, a total of 22 copulation events in the swarms were observed and collected. Of these, 15 female *An. arabiensis* mosquitoes were successfully dissected and all were determined as nulliparous. The 7,114 male *An. arabiensis* collected from the swarms were assessed for sexual maturity and 95.4% (N=6,787) had fully rotated male genitalia, indicating sexual maturity. We also assessed 28 females recovered from the swarms, to assess whether their spermatheca were already filled with male sperms or not. Of all females dissected, 42.9% (N=12) had evidence of having been inseminated, while the remaining 57.1% (N=16) were not inseminated.

7.5 Discussion

Malaria vector control remains constrained by lack of comprehensive understanding of mosquito ecology, yet such ecological studies are considered a prerequisite for improved control and eventual disease elimination [7]. Though not widely studied, the mating behaviour of malaria mosquitoes may present some unique opportunities for improved vector control [13]. In a recent trial in Burkina Faso, it has been demonstrated that targeting *Anopheles* mosquito swarms with effective aerial insecticide spraying could rapidly crash vector populations in local communities [14]. However, this approach could be challenged by development of insecticide resistance, hence non chemical based interventions could be explored.

Though mating and swarming behaviours of *Anopheles* mosquitoes are among the most important components of the vector reproduction biology [8], there have been limited field studies of mosquito swarming in Tanzania, the last published field observations having been in 1983 [20]. This neglect is mainly because most scientist have not considered swarming behaviour as having any potential opportunities for disease prevention and vector control. On the contrary, studies on mating and swarming behaviours of *Anopheles* mosquitoes in West Africa have already demonstrated that it could possibly be relied upon to control mosquito densities and associated pathogen transmission, by killing mosquito swarms with effective insecticides [13,14].

This study yielded evidence of the occurrence of swarms of *An. arabiensis* mosquitoes in rural south-eastern Tanzania. Furthermore, the study proves that trained volunteers are able to successfully identify and locate swarms in their villages. A map of all swarm locations so far identified has been built-up with the help of the locally recruited personnel (Figure 7.3). Interestingly, most of the swarm locations remained the same, several months after they were first identified. This phenomenon of swarms occurring in the same location is widely reported by other scientists, particularly in west Africa [27,34].

The commonest swarm markers are shown in Table 7.2, and were found on landscapes that were not blocked by either trees or houses. Several other studies have also reported the roles of such markers on mosquito swarming [15]. Mosquitoes preferred to swarm in open areas such as over bare land, woodpiles

and bricks and it may be possible, therefore, to predict the distributions of *Anopheles* swarms based on the spatial distribution of the common swarm markers in the village. In this study, once the accurate representation of the main swarm markers was determined, it became easier to conduct trainings for the volunteers, who relied heavily on these swarm markers as a guide to search for mosquito swarms in their assigned areas. Secondly, with a clear definition of swarm markers, it could be possible, as has been demonstrated in some studies, to manipulate swarm markers, or create artificial swarm markers, to attract *Anopheles* males and enhance the chances of effective vector control targeting male swarms [16]. Following this initial step, further work was initiated to find swarms of *An. funestus*, the dominant vector in the area as presented in Chapter VIII.

This study also demonstrated that locally recruited and trained non-entomologist volunteers from the study villages were capable of not only identifying the swarm and associated markers, but also estimating the swarm size. This idea was derived from an earlier study conducted in the villages where community members were able to identify locations where mosquito densities were high [23]. An added advantage of this approach was that it also increased local community knowledge on mosquitoes, and the level of community engagement in research increased. By using such a participatory approach, we expect that by the time targeting *Anopheles* swarms for vector control is utilized, e.g. by aerial spraying, high levels of community acceptability could be achieved, and the volunteers could be relied upon to lower implementation costs.

This study did not observe any *An. funestus* swarms, except for the 13 *An. funestus* mosquitoes collected near one *An. arabiensis* swarm. Unfortunately, this was only one instance and could not be verified during this study. It may be possible that this was a separate distinct *An. funestus* swarm that was formed close to *An. arabiensis*. Since they were all male *An. funestus*, we speculate that *An. funestus* swarms also occur in the area, and that extended surveys could reveal greater details of their swarming behaviours. It is therefore recommended that more detailed surveys should be conducted to understand the swarming behaviours of *An. funestus* in the area. Evidence from other studies in Africa suggest that swarms of *An. funestus* appear in a similar way to those of *An.*

gambiae s.l. [21]. Since it is known that *An. funestus* contributes to a significant amount of the remaining malaria transmission in this area [24], it is therefore suggested that any control measures targeting *An. gambiae s.l.* without affecting *An. funestus* will have little impact on the ongoing malaria transmission in this area.

Coincidently, presence of dragonflies, though not initially targeted for observation, was an indication of swarm presence in many locations, though in this case it was not considered as a physical swarm marker. Similar observations were also reported by Sawadogo *et al*. [15] in their studies in Burkina Faso. Furthermore, human movements, sounds, strong wind and rainfall were found to affect mosquito swarming behaviours. For instance, in some cases when swarm sampling was conducted when it was raining, we rarely observed swarms even in the previously confirmed swarm sites. Dragonflies and other predators could thus be considered good indicators of presence of mosquito swarms in certain areas. Lastly, in this study, most of the swarms were found at the edge of the village and in the rice fields, possibly because the males prefer to swarm in locations that are not disturbed by human activities or movements.

One potential limitation of this study is that observation of genitalia rotation was done the following. This could have overestimated the maturity status of males since maturation could have occurred in the paper cups where samples were kept. It is recommended that observation of maturation should be done immediately after collection.

7.6 Conclusion

This is the first report of *Anopheles* swarms in Tanzania in more than three decades. The study provides evidence that the swarms can be identified, characterized and quantified by trained community-based volunteers, and opens potential new opportunities for targeting male malaria mosquitoes to improve disease control. Further investigations are recommended to further characterize the swarms in the area and to verify whether other residual malaria vector populations, such as *An. funestus,* also form similar mating swarms. More importantly, future studies should seek to demonstrate whether new interventions exploiting this phenomenon, such

as targeted aerial spraying of swarms, could significantly disrupt malaria transmission.

Author's contribution

EK, AD and FO conceived the study and developed study protocols. EK, GM, HB and FO conducted experiments and supervised data collection. EK, JK, JP, HN, AL, and FO did data analysis and wrote the manuscript. All authors approved the manuscript prior to submission.

Competing interests

The authors declare that they have no competing interests.

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Data availability

The file containing supplementary data used in this manuscript is available.

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Ethical considerations

Meetings with local leaders were held in the study areas and the main aims of the study were explained by the research team. Written and signed informed consent were obtained from all volunteers participating in the study. All information was given in Kiswahili, the local language. Ethical approval for the study was obtained from Ifakara Health Institute Institutional Review Board (IHI/IRB/No: 38-2016), and from the Medical Research Coordinating Committee (MRCC) at the National Institutes of Medical Research (NIMR), Ref: NIMR/HQ/R.8a/Vol.IX/2428. Approval was also obtained from the Human Research Ethics Committee (Medical) clearance at the University of the Witwatersrand with an Approval Certificate No: M160806, where EK is registered for PhD.

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Chapter VIII

Swarms of the malaria vector, *Anopheles funestus* in Tanzania

8.1 Abstract

Anopheles funestus currently contributes more than 85% of malaria transmission in south-eastern Tanzania, even where it occurs in lower densities than other vectors, such as *Anopheles arabiensis*. Unfortunately, its ecology is minimally understood, partly because of difficulties in laboratory colonization. This study describes the first-ever observations of *An. funestus* swarms in Tanzania, possibly heralding new opportunities for control. Using systematic searches by community-based volunteers and expert entomologists, *An. funestus* swarms were identified in two villages in the districts of Ulanga (Tuliza Moyo village) and Kilombero (Ikwambi village) in south-eastern Tanzania, starting June 2018. A total of 81 swarms were characterized by size, height, start- and end-times, presence of copulation and associated environmental features.

Samples of male mosquitoes from the swarms were examined for maturity (genitalia rotation), species identity (Polymerase Chain Reaction) and wing sizes. A total of 581 *An. funestus* (570 males and 11 females) and 13 *An. gambiae s.l.* (all males) were sampled using sweep nets from the 81 confirmed swarms in the two villages. Six copulation events were observed in the swarms. Mean density of *An. funestus* per swarm/village/evening was 6.6 (5.9–7.2) in Tuliza Moyo and 10.8 (5.8-15.8) in Ikwambi with 87.7% (n=71) of the swarms found in Tuliza Moyo and 12.3% (n=10) in Ikwambi. Mean height of swarms was 1.7m (0.9m–2.5m), while mean duration was 12.9 (7.9–17.9) minutes. PCR analysis confirmed that all were *An. funestus s.s*. Mean wing length of the males was 2.47mm (2.0mm-2.8mm), with no difference between swarming males and indoor-resting males. Most swarms (95.0%) occurred above bare ground, sometimes on front lawns of human dwellings, and repeatedly in the same locations.

This study has demonstrated occurrence of *An. funestus* swarms for the first time in Tanzania. Further investigations could highlight new opportunities for improved control of this dominant malaria vector, possibly by targeting the swarms.

Adapted from Kaindoa EW, Ngowo HS, Limwagu AJ, Tchouakui M, Hape E, Abbasi S, et al. Swarms of the malaria vector *Anopheles funestus* in Tanzania. Malar J. 2019;18:29. https://doi.org/10.1186/s12936-019-2660-y

8.2 Background

Global statistics indicate that malaria morbidity and mortality have declined mostly as a result of scaling up vector control interventions [1,2], but that the gains are stagnating in some countries [3]. This decline has also been observed in Tanzania with more than 50% reduction of malaria burden recorded since the year 2000 [4]. The 2016-2017 Tanzania malaria indicator survey demonstrated reduction of prevalence in children under five, from 18.0% in 2008 to 7.3% in 2017 [5–7] on the mainland. These successes may be attributed to scaling up of vector control tools such as long-lasting insecticide-treated bed nets (LLINs) [8] and indoor residual sprays (IRS) [9], as well as socio-economic developments and urbanization [10] and treatment with artemisinin combination therapy (ACT) [11].

In a recent study, it was demonstrated that just one of the main malaria vector species, *Anopheles funestus* contributes more than 85% of malaria transmission in south-eastern Tanzania, even in locations where iit occurs at far lower densities than *Anopheles arabiensis* [12]. Moreover, the ubiquity and high vectorial capacity of this vector species suggests it is potentially dominant elsewhere across the country. Unfortunately, the *An. funestus* populations are resistant to pyrethroid insecticides commonly-used on bed nets [12,13], survive longer than *An. arabiensis,* and feed almost exclusively on humans [12,14]. Other studies have also documented *An. funestus* resistance to pyrethroids: Malawi [15,16], Mozambique [17,18], South Africa [19], Zambia [20,21], Zimbabwe [21], Cameroon [22,23] and Senegal [24], a situation that compromises effectiveness of current vector control options [25], and perpetuates residual transmission even in communities where bed net coverage is more than 90% [26]. Given its dominance in Tanzania, it has been suggested that interventions that effectively target *An. funestus* mosquitoes could have a high impact on residual transmission in Tanzania [12]. One potentially effective approach involves suppressing mosquito populations by identifying and directly targeting *Anopheles* swarms with highly effective insecticides [27].

Swarming behaviours have previously been intensively studied, mostly in Western and Central Africa [28–30]. However in East and southern Africa, there have only been a few studies in Zambia, Mozambique and Kenya [20,31,32] in addition to an old set of observations in northern Tanzania in 1983 [33]. *Anopheles* swarms are naturally difficult to find since they occur at dusk when visibility is poor and last only

for a few minutes. In fact, the rarity of *Anopheles* swarms in East Africa has led some to hypothesise that they mate primarily indoors, such as previously observed in experiments by Dao *et al*. [34]. Nevertheless, a recent study that relied primarily on community volunteers rather than experts, demonstrated natural occurrence of swarms of *An. arabiensis* in villages across south-eastern Tanzania [35], more than three decades after the last records of this species swarming anywhere in the region [33]. In addition to providing detailed characterization of over 200 *An. arabiensis* swarms, the study also demonstrated that trained volunteers were able to locate and identify mosquito swarms in their villages [35]. Follow-up studies have since demonstrated potential for localized control by targeting the swarms using aerosol spraying (Kaindoa *et al.,* unpublished data).

An interesting revelation in the study on *An. arabiensis* swarms was a single incidence where 13 *An. funestus* males were caught in a sweep net [35], providing earliest indications that this species too formed swarms in the valley, but that these swarms were certainly more elusive than those of *An. arabiensis*. The aim of this current study was therefore to identify and characterize swarms of *An. funestu*s, which is now the dominant malaria vector species in rural south-eastern Tanzania.

8.3 Methods

8.3.1 Study area

The study was carried out in the two districts of Ulanga (Tuliza Moyo village, 8.354497°S, 36.705468°E) and Kilombero (Ikwambi village, 7.98244°S, 36.82167°E) in south-eastern Tanzania (Figure 8.1). The villages were selected because previous adult mosquito surveys had yielded high densities of *An. funestus* mosquitoes. The area is characterized by perennial meso-endemic malaria transmission, with mosquito densities peaking between February and May [36]. Malaria vectors comprise primarily *An. arabiensis* and *An. funestus*, the latter being the most important vector.

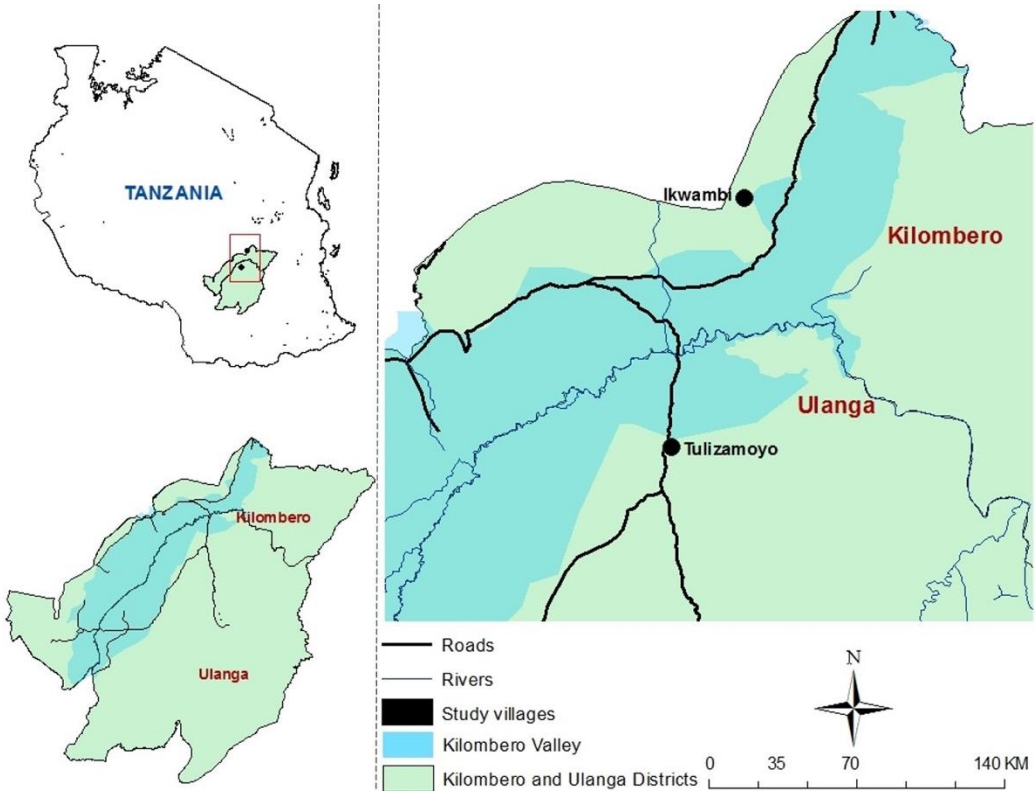


Figure 8.1: Map of the study area, showing villages in south-eastern Tanzania, where swarm surveys were conducted.

8.3.2 Observation and characterization of swarms

Following the successful training of village volunteers during the *An. arabiensis* swarm identifications in 2016 and 2017, refresher training was provided in early 2018 to the same volunteers, using the same procedures for swarm searching [35]. In this approach, the study villages were divided into sub-sections where volunteers had to first identify potential swarm markers, then search for swarms later in the evening. Whenever a swarm was seen, volunteers reported to the supervisors, after which physical expert verification, swarm sampling and characterization was performed the following day. Sampling was done 10-15 minutes after the start of swarming using a sweep net. Using a standard observation sheet, all the identified swarms were characterized as shown in Table 8.1.

Table 8.1: Descriptive variables assessed during field surveys of *Anopheles funestus* swarms.

	Variables	Methods used and indicators measured
1	Swarm size and copulation events	Visual estimates of swarm sizes: approximate number of mosquitoes in swarm, as estimated visually to the nearest 5 mosquitoes (observations were made between 10 and 15 mins after start of the swarm)
		Sweep net estimates of swarm sizes: approximate number of mosquitoes in the swarm, as estimated using standardized sweep nets, collected once by experienced collectors. Swarm size was a measure of density of mosquitoes per collection by sweep net per instance
		Copulation: number of copulation events observed in the swarm after ten minutes of observation
2	Location, time and height of swarms	Geo-location of the swarm measured using handheld GPS receivers.
		Unique ID of compound owner (each swarm was uniquely identified on this parameter)
		Time of day when the swarm begins appearing, recorded to the nearest minute
		Time of day when the swarms completely disperse, also measured to the nearest minute
		Height measured as distance between the base of the swarm and the ground level in meters
3	Molecular identification and characteristics of sampled mosquitoes	Morphological and molecular identification of the species of *Anopheles* mosquitoes collected in the swarm
		Proportion of males caught that have evidence of being capable of mating (based on the rotation of male genitalia)
		Measurements of wing size (mm)
4	Important landmarks and potential swarm markers	A record of important landmarks, at or near which swarms occur such as vegetation, house, mosques, markets, schools, water pumps, houses, cowshed, banana tree and cemeteries

8.3.3 Morphological and molecular identification of *Anopheles* mosquitoes collected in swarms

All collected mosquitoes were aspirated from the sweep nets, placed in paper cups and maintained on 10% glucose solution. The following morning, the mosquitoes were killed in a closed container by freezing, then identified morphologically by taxa and sex following keys by Gillies & Coetzee [37]. All samples from each collection were further identified by multiplex polymerase chain reaction (PCR) to sibling species [38].

8.3.4 Assessing sexual maturity and wing sizes of sampled mosquitoes

The following day, all collected *An. funestus* males were assessed for maturity through observations of genitalia rotation [39]. Wing lengths of male mosquitoes sampled from swarms were compared to those collected resting on indoor surfaces. One wing of each specimen was cut and placed onto a slide, then its length measured from the alula notch to wing tip under a dissecting microscope, following the procedures of Lyimo *et al.* [40] and Charlwood *et al.* [41].

8.3.5 Data analysis

Data analysis was done by using R software version 3.3.2 [42]. Swarm sizes were estimated as median number of mosquitoes sampled in each swarm (using a sweep net when the swarming was at its peak), and compared between the two study villages. The swarm size was therefore an estimate of mosquito densities in each sweep net collection. Swarm duration was obtained based on the difference between start and end times of each swarm in minutes. Mean height of swarms above ground, and mean duration of swarms were also calculated. Wing size of swarming and resting *An. funestus* males were compared using Student's T-test. Geo-locations of the swarms were visualized in ArcGIS 10.4 (ESRI, USA). Swarm sizes estimated visually were compared to those estimated by using sweep nets and their correlation coefficients computed.

8.4 Results

8.4.1 Mean density of *An. funestus* and copulation events in swarms

A total of 570 *An. funestus* males and 11 females were sampled using sweep nets, from 81 swarms observed in the two villages. A total of 6 copulation events were observed in the swarms, following a standardized observation period of ten minutes per swarm (Table 8.1). Mean density of *An. funestus* caught per swarm per village per evening was 6.6 (5.7 – 7.5) in Tuliza Moyo and 10.8 (5.8 – 15.8) in Ikwambi (Figure 8.2). Overall, 87.7% [n=71] of the swarms were found in Tuliza Moyo, while only 12.3% [n=10] were in Ikwambi. A single swarm was identified in Tuliza Moyo village where 13 *An. gambiae s.l.* males were found together with four *An. funestus* males, suggesting either mixed swarms or swarms of different species but occurring in close proximity.

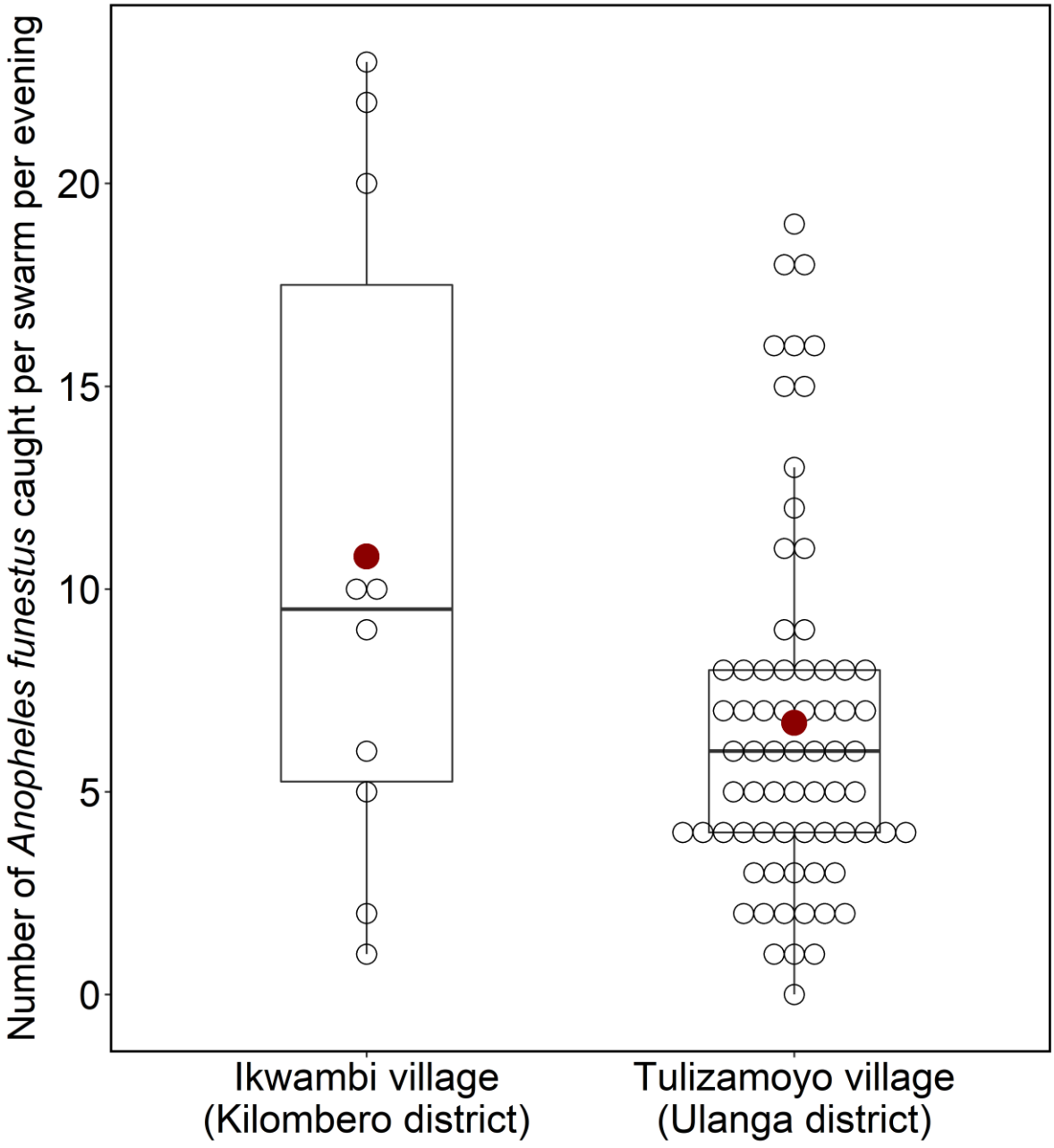


Figure 8.2: Median number of *An. funestus* caught per swarm per evening in the two study sites. Both, the actual mosquito counts per evening (hollow dots) and the model estimated mean catches (red dots) are shown.

8.4.2 Correlations between visual and sweep net estimates of swarm sizes

Anopheles funestus swarm size was estimated by visual assessments of approximate numbers of mosquitoes forming the swarm prior to sampling with sweep nets (as described in Table 8.1) to estimate mosquito densities per sweep. Regression models revealed significant but weak correlation between visual estimates and sweep net estimates (Figure 8.3) (Linear regression analysis: R = 0.4518, P-value <0.001; Polynomial regression: Adjusted R^2 = 0.2041, P-value <0.001).

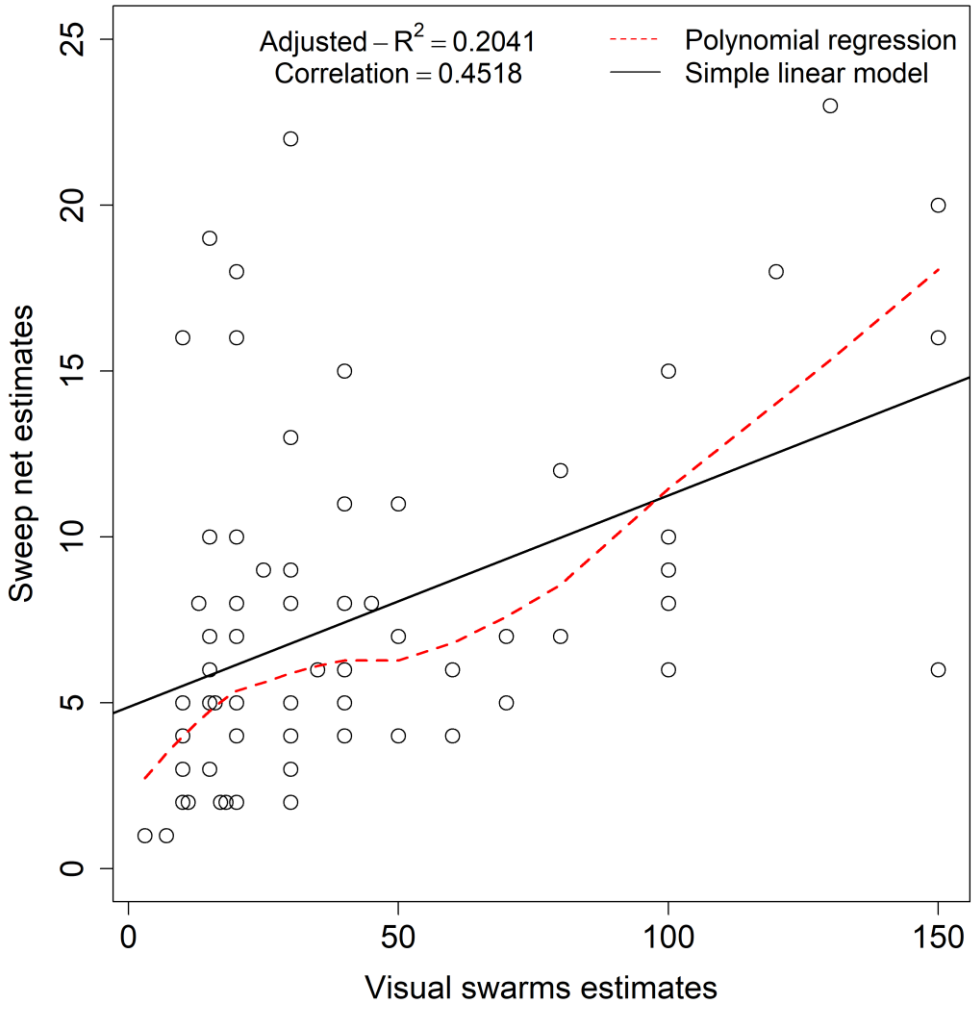


Figure 8.3: Correlations between visual estimates of the swarm sizes and the swarm densities per sweep net collection per evening.

8.4.3 Location, time and height at which the swarms occurred

The identified swarms were all characterized in the months of June and July 2018 and are shown in a georeferenced map in Figure 8.4. Most swarms started at approximately 6:40pm. Mean duration of the swarm was 12 (±5) minutes. The mean height of swarms above ground was 1.7 (±0.8) meters. It was observed that most *An. funestus* swarms occurred close to human houses.

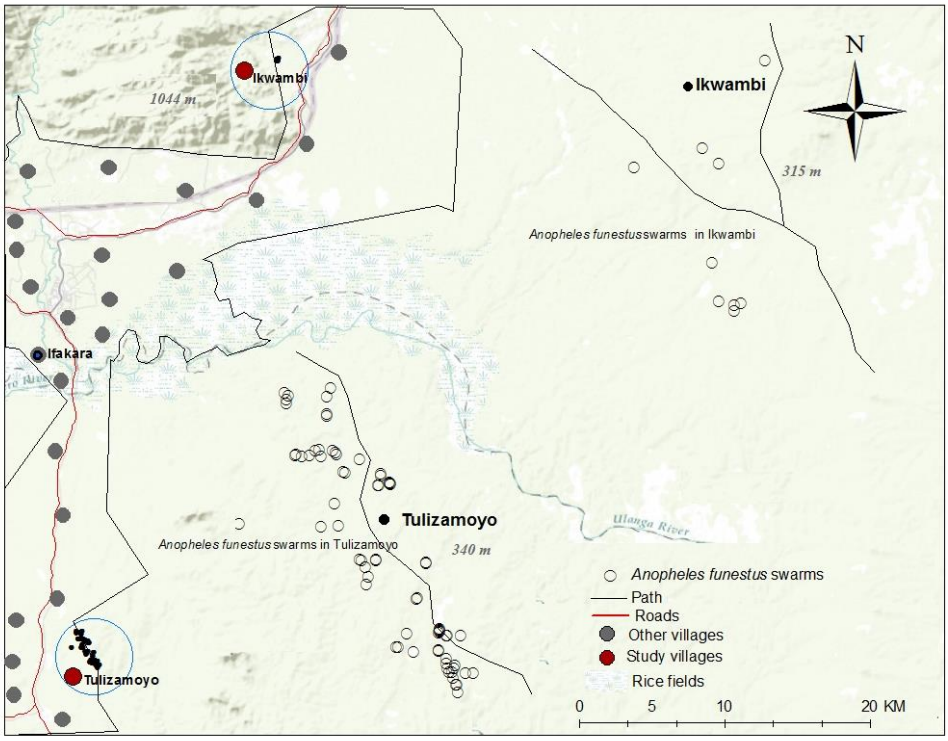


Figure 8.4: Distribution of *Anopheles funestus* swarms observed in the study sites.

8.4.4 Environmental features associated with swarm occurrence

There were no obvious physical features depictable as markers previously described for *An. arabiensis*. However, assessment of all the swarm locations resulted in four different categories of places where swarming by *An. funestus* occurred (bare ground near dwellings, demolished house, cleared farms and trees). The most common feature was bare ground near the houses (Figure 8.5), usually dusty but no obvious textural discontinuation and sometimes in the front lawns of human dwellings (91.4% (n=74) of the swarms occurred in such sites). Two swarms were observed on small cleared farms (2.5% of the swarms), one swarm under a teak tree and one over a demolished house (Table 8.2).



Figure 8.5: Pictorial illustration of commonest locations where swarms of *Anopheles funestus* mosquitoes were observed.

Table 8.2: Environmental features of locations where *Anopheles funestus* swarms were observed in the study villages

	Environmental features	Total marker (%)	No. mosquitoes caught
1	Bare ground near dwellings	77 (95.0)	535
2	Demolished house	1 (1.2)	18
3	Cleared farms	2 (2.5)	10
4	Trees	1 (1.3)	18
	Total	81 (100)	581

8.4.5 Molecular identification, wing lengths and sexual maturity of sampled *An. funestus* mosquitoes

A total of 413 *An. funestus* males were analysed for species identification in the laboratory, 93.9% (n=388) of which yielded successful DNA amplifications. All amplified samples were confirmed as *An. funestus s.s.* A total of 109 and 70 swarming and resting *An. funestus* males respectively, were examined for size using wing length as a proxy. The wing lengths ranged between 2.00 and 2.80mm with a mean of 2.47mm as shown in Figure 8.6. There was a very small difference in wing

size between the swarming and resting male *An. funestus* mosquitoes (t=2.301, df= 214.38, P=0.022). Nearly all (97.3%) of the male *An. funestus* had fully rotated genitalia, indicating sexual maturity.

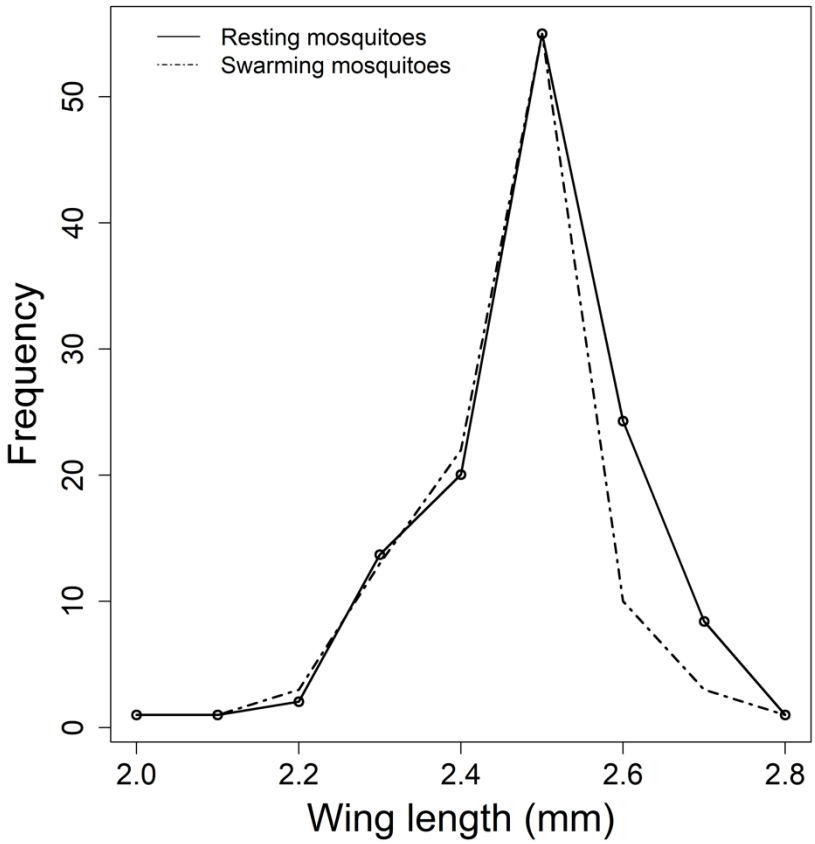


Figure 8.6: Comparison of wing sizes between swarming and resting *An. funestus* male mosquitoes.

8.5 Discussion

Improved understanding of malaria vector ecology and behaviour is crucial for achieving the ambition of malaria elimination through vector control tools [43]. The swarming and mating behaviours in particular, are components of mosquito behaviour that has been neglected for a long time [30,35,41]. Given that *An. funestus* contributes to more than 80% of the ongoing malaria transmission in the area, it is equally important to identify and characterize swarms of *An. funestus* so as to explore complimentary tools, possibly targeting swarming behaviour. Furthermore, it is important to understand mating behaviour for implementation of certain control methods, e.g. use of sterile insect techniques and release of genetically modified

males. To the best of our knowledge, this is the first verified report of *An. funestus* swarms in Tanzania. Together with the earlier study on *An. arabiensis* swarms in the same area [35], these reports confirm widespread occurrence of swarms by the main malaria vectors, even though they are generally elusive and characterization requires dedicated teams with local knowledge.

An attempt to characterize the swarm sites did not yield any obviously distinctive physical markers of the swarm stations. Instead, most of the swarms were observed to occur above bare ground, sometimes on the front lawns of human houses (Figure 8.5). This is very similar to observations by Charlwood *et al*., who suggested that *An. funestus* mosquitoes in Mozambique formed swarms close to the houses used for resting and that the swarming sites could be used as indicators of houses to be targeted for vector control interventions [41]. Proximity to houses, therefore, appears to be an essential condition for *An. funestus* mating swarms, and the possibility that some degree of mating happens indoors cannot be excluded.

Relative to previous observations in the same area [35], this current study has also demonstrated that *An. funestus* swarms differ from those of *An. arabiensis* in terms of height, swarm size and location. For example, while *An. arabiensis* males were observed swarming at mean heights of 2.5m, swarms of *An. funestus* occurred at much lower heights, averaging just 1.7m above ground, with several swarms lower than one metre. In terms of location and markers, *An. funestus* preferred to swarm very close to human houses, unlike *An. arabiensis* swarms, which were mostly at the edges of the villages [35]. The *An. funestus* swarms were small and generally consisted of less than 15 mosquitoes, as collected by sweep nets, while *An. arabiensis* swarm sizes ranged from approximately 10 to 60 mosquitoes [35]. It is possible that these differences in vector densities may be associated with season (the *An. funestus* swarm study was conducted between June and July 2018 while the *An. arabiensis* study was conducted between August 2016 and June 2017). Nonetheless, the natural differences in population sizes of the two species, as previously observed in the valley [12], could account for the difference in swarm sizes.

While the work with community volunteers certainly increased the ability to identify the *An. funestus* swarms, visual estimates of the swarm sizes by the volunteers did

not strongly correlate with the sweep net estimates (R = 0.4518). Though the volunteers were able to locate *An. funestus* swarms, either they could not accurately estimate the number of mosquitoes in the swarm, or the flying males were able to avoid the sweep nets. In our previous study, however, there was a strong correlation between sweep nets estimates and visual estimates of the number of *An. arabiensis* in the swarms, indicating that visual estimates could possibly be relied upon to estimate swarm sizes for this species [35]. Given that this current study was not done across multiple seasons, the discordance between estimates may indicate density dependence of such relationships, or that the correlations are non-linear (Figure 8.3).

The molecular analysis confirmed that 100% of all amplified mosquitoes were *An. funestus s.s.* Although *An. rivulorum* and *An. leesoni* have also been recorded from this area [12], the present study did not find any other sibling species in the swarms. However, in a recent study in Zambia, mixed swarm of *An. funestus* and *An. leesoni* was found [20]. Though rare, *An. arabiensis* and *An. funestus* mosquitoes may also swarm either in very close proximity or together. Indeed, in the previous study of *An. arabiensis* swarms, one instance of 13 male *An. funestus* mosquitoes was observed in a sweep net targeting the former species [35], and in this current work, one instance of 11 *An. arabiensis* males were caught in a sweep net targeting *An. funestus*. Previously, mixed swarms of *An. coluzzii*, *An. gambiae* and *An. funestus* have also been reported from other areas in Africa [20,44]. But such mixed swarms are very rare, and instead mating occurs predominantly assortatively.

Genitalia rotation is a physiological change that occurs a few hours after male mosquitoes emerge from the pupal case and become sexually mature. Dahan and Koekemoer (2014) indicated that full rotation is visible a few hours after emergence, but the rotation rate can increase with an increase in temperature [45]. During this process, the genitalia turn clockwise or anti-clockwise until sexual maturity is reached at 180 degrees full rotation. This current study showed that 100% of all sampled males had complete genitalia rotation.

It was also observed that there was no special selection based on size of mosquitoes entering the swarms. The wing lengths of *An. funestus* males ranged between 2.0 and 2.8mm, with no statistically significant differences observed in

mean sizes between the mosquitoes caught in the swarms and those caught resting indoors. The results are comparable to those of Charlwood *et al.* [31] on *An. funestus* in Mozambique.

The elusive nature of *Anopheles* swarms in East Africa is confirmed by the failure of several expert entomologists to identify them in the past. However, this study adopted the approach of working with trained community members to search for swarms, as previously described by Kaindoa *et al.* [35], and also in Burkina Faso [27]. Similar approaches have also been used by community members to accurately identify places with low, medium and high mosquito densities [46]. This has implications for vector control strategies using community participation in targeting mosquito swarms. Indeed, there are several examples where community participation in vector control programmes have been successfully relied upon for disease control [47–49].

The challenge of identifying *An. funestus* swarms in the study area was associated with the unexpected low height of the swarms, given that we had used our knowledge of *An. arabiensis* swarms when searching for *An. funestus* swarms. *Anopheles funestus* swarms occurred in close proximity to the houses, places that we could not predict or associate with mosquito swarming. Additional research and adoption of technologies such as the use of unmanned aerial vehicles fitted with high-resolution infrared cameras could help to locate swarms in areas that are inaccessible. There is also a need to develop methods for prediction and estimation of *An. funestus* swarms, which could help to improve the control of malaria in rural areas**.**

8.6 Conclusions

This study has demonstrated the occurrence of *An. funestus* swarms for the first time in Tanzania. More intensive studies should be conducted so as to map and characterise *An. funestus* swarms and assess the factors which influence the swarming and mating behaviours of this species. Targeting *An. funestus* swarms could be one of the complementary tools used alongside existing interventions such as long-lasting insecticide-treated nets (LLINs) and IRS to control malaria transmission. Since male mosquitoes use their wing beat beats frequency to attract

females, it offers an opportunity to design sound traps which could be used to attract and kill female mosquitoes.

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Chapter IX: Discussion and Conclusions

This study was built on the hypothesis is that settlement patterns, as defined by degree of clustering, the distances between individual households, or the locations of individual households within villages, affect malaria transmission rates, and that current and new interventions could be significantly improved by understanding these factors. It was hypothesized also that malaria transmission risk is higher in highly clustered settlements than dispersed settlements, and less likely to occur between households that are far apart than households that are close together. This study aimed to investigate relationships between human settlement patterns and malaria risk in a residual transmission setting in rural Ulanga district, south-eastern Tanzania, and how these relationships can be utilized to improve malaria control. The study generated the following key findings:

- Household occupancy influences the indoor vector density (Chapter III).
- High house densities increased *Anopheles* biting risk but mosquito density declined as distances between houses increased beyond 50m (Chapter V).
- Abundance of *An. arabiensis* peaked during high rainfall months (February-May), but *An. funestus* densities remained stable into dry season (May-August) (Chapter VI).
- There was higher indoor densities of *An. arabiensis* and *An. funestus* in houses with mud walls compared to plastered or brick walls, open eaves compared to closed eaves and unscreened windows compared to screened windows (Chapter IV).
- Most respondents were aware of associations between their house characteristics and malaria risks but were constrained by financial costs and other household priorities (Chapter IV).
- *An. funestus* mosquitoes contributed more than 85% of ongoing malaria transmission in the areas, even when occurring in far smaller densities than *An. arabiensis* (Chapter VI).
- With regards to complementary interventions, small scale studies demonstrated that mating swarms of both *An. arabiensis* and *An. funestus* could be readily identified and characterised by volunteer community

members, and that targeting the swarms using aerosol spray could reduce overall biting risk in the communities (Chapter VII and VIII).

Generally, this study is comprised of five published articles and one additional unpublished finding that are discussed below.

9.1 Household occupancy and mosquito biting risks

To achieve the current goal of malaria elimination, there is a need to understand the directional movements of malaria vectors within villages so as to identify and target the most intense foci of transmission for mosquito-borne diseases. Additionally, while either IRS or LLINs are considered the basic minimum intervention that all community members should receive under universal coverage programmes [1–3], it remains important that any supplementary new tools must be properly targeted to achieve maximum and sustainable impact, and eliminate the disease even from residual ‘hotspots’ [4,5].

Human-vector contact is an important driver for malaria transmission in any given setting. Those mosquitoes that prefer human hosts tend to live near humans and their dwellings, and heterogeneity of human hosts leads to heterogeneity of mosquito distribution in the environment [6]. Moreover, disease-transmitting mosquitoes prefer to enter houses with many occupants rather than houses with few occupants [7]. They make these choices by first detecting and then following odours emanating from the humans in the houses [8]. The present study has shown that the directional movement of mosquitoes within villages is influenced by spatial distribution and demographic composition of village households. As a result, households with many occupants attract more mosquitoes compared to houses with fewer occupants. This information is essential as it can be used to predict, identify and target the most intense foci of mosquito-borne disease transmission, based simply on household census data and models of mosquito host selection.

Current vector control strategies can be greatly enhanced by using a targeted approach that relies simply on household demographics (age and number of people per household) as a proxy of biomass, to predict the spatial distribution of disease transmission. This new methodology, if proven, would be applicable for large-scale operations, since the demographic data that are required are often already available

or can be readily collected prospectively through nation-wide or district-wide surveys, using well-established and cost-effective methodologies. However, this technique cannot yet stand alone and would need to be complemented with other approaches to verify actual occurrence of disease.

Key findings

The study found that household occupancy is associated with high indoor mosquito density.

Implication for intervention

- This information could greatly improve the planning of malaria vector control activities in many endemic areas using census data already available in malaria endemic countries.

Future studies

- Records of number of occupants in different geo-referenced households could be used to map out the fine-scale distribution of mosquito densities in rural areas. Studies should be done to assess whether this approach could be applicable in the whole district and possibly entire country, to map transmission foci using regularly collected census and demographic data.

9.2 House distances and mosquito biting risks

The exact location of houses in the villages is important when designing malaria vector control interventions. It was found in this study that houses separated by less than 50m had higher numbers of mosquitoes compared to the houses which were far from one another. The implication of this finding is that it may be possible to design a spatially targeted intervention focusing on groups of houses rather than blanket coverage of every individual household.

Key findings

Mosquito densities were affected by the distances between houses, but within a limited radius of 400m.

Implication for intervention

- Malaria risk would differ between villages with different levels of clustering and variations in distances between households.

Future studies

- Studies should be done to also assess the role human movements between these settlements on malaria transmission at local village level.

9.3 Housing characteristics and malaria transmission risks

It has been documented previously that house design strongly influences indoor malaria transmission risks in rural Tanzania [9–12]. Several studies elsewhere have also shown that house design affects the abundance of mosquitoes found indoors [13–15]. Simple house modification could significantly reduce malaria transmission [9,16,17], but the present study found that communities in rural Tanzania were unable to modify their houses due to cost constraints and competing household priorities. Better housing should be incorporated into the national health policy so as to significantly lower vector borne diseases. Initiatives should be taken to improve houses through community-based approaches and the government should provide subsidies for building materials so as to reduce the financial barriers described by the communities.

There is evidence that socio-economic development has a strong influence in reducing malaria transmission [18]. As countries in Africa progress towards achieving the Sustainable Development Goals around health, economic growth and cities by 2030 [19], malaria endemic countries should see associated reduction of malaria transmission in their communities. The evidence clearly shows that in developed countries, malaria was eliminated as a result of improved housing and

standard of living. One may argue that, if we are to achieve malaria elimination in African countries, the focus should not be entirely on vector control, instead more investments should be made into improving people's living conditions.

This study yielded evidence that people are aware of the challenges related to house designs and malaria transmission risks, but they have competing priorities. The government through its two ministries, the ministry of lands, housing and human settlements development and the ministry of health, community development, gender, elderly and children should work together and develop a program of work which would include housing improvement as one of vector control strategies.

Key findings

The study found that housing design is associated with high mosquito density.

Implication for intervention

- In order to achieve malaria elimination in Tanzania, house improvement is essential to complement vector control tools.

Future studies

- Development of simple, scalable, and sufficiently efficient house improvement technologies should be promoted. Such technology should be practical and affordable, suitable for all poor households, regardless of design, including houses in rural and remote settings, without requiring any major house modifications, electricity or sophisticated skills.

9.4 Community knowledge and involvement in malaria control efforts

Strategies to control malaria in Tanzania could benefit from community involvement in control efforts. To successfully achieve effective mosquito control, community engagement and participation is essential.

This study benefited from community engagement approach, for example, the community-based approaches enabled identification of *Anopheles* swarms of malaria

vectors [20,21]. The available data suggest that no one had previously observed these mosquitoes mating in the wild in Tanzania until 2018 when, with the help of local community volunteers, the study discovered *An. funestus* mating swarms. A study done by Mwangungulu *et al*., also found that community members are know the location with high and low vector abundancies in their environment [22]. Thus is abundantly clear that for longer term programmes, such as those necessary for malaria elimination, community engagement is very important.

Furthermore, it was found that members are aware of the relationship between housing designs, environmental factors and malaria transmission risks. It would be important to sensitize community members to consider housing and environmental modification one of the approach for vector control. Below are more examples of community engagement activities that have significantly reduced malaria transmission in different settings.

Ten years ago, a large larviciding programme was implemented in Dar es Salaam, the capital city of Tanzania. Community-owned resources and personnel supported the programme during identification of aquatic habitats, mapping and application of the microbial larvicide over the one-year study period. The approach achieved 32% reduction of malaria infections in the study area [23]. Although this study was done in urban areas, the same approach could still be used in rural areas. Where aquatic habitats are numerous, the communities can still be relied upon to locate and target them. In a previous study in the Kilombero valley, evidence was provided which showed that community members knew the locations with low, medium and high densities of mosquitoes [22].

Working with community health nurses and community health workers to deliver primary health care, is another example of community engagement in malaria control programmes. A study conducted in The Gambia resulted in the reduction of child mortality by approximately 50%, i.e. from 42/1000 to 28/1000 between 1992 and 1994, due to the involvement of trained community health workers [24]. Training of shopkeepers to recognize malaria so as to dispense appropriate malaria drugs has been shown to improve compliance with antimalarial treatments in Kenya [25]. This is important because many remote villages face challenges to obtain health services

and self-medication is common [26], with people tending to seek treatment at home or from traditional healers.

Community engagement should be integrated into malaria control programmes, however, guidelines should be formulated on how this engagement should be supervised and guided so that quality assurance in control programmes is guaranteed.

Key findings

- The communities are aware of the relationship between house design and mosquito-borne disease but financial barriers were identified as key challenges facing these communities.

Implication for intervention

- Community knowledge and experience of malaria transmission are essential and should be integrated into current malaria control efforts.

Future studies

- Further experiments should be designed to evaluate the specific knowledge required from the community in order to achieve malaria elimination in Africa.

9.5 Targeting swarms as complementary approaches for controlling malaria transmission

It is suggested that the malaria elimination agenda will hardly be achieved unless additional approaches are designed to be used alongside the current interventions. Current vector control tools have significantly reduced malaria mortality and morbidity in Africa, but they are challenged by development of insecticide resistance in the vector mosquitoes. Strong emphasis must be placed on targeting mosquito behaviours that are not currently targeted by existing interventions. In this regard, the

present study has yielded important information on the occurrence and potential of targeting *Anopheles* swarms in rural Tanzania.

Targeting swarming and mating behaviour provides a unique opportunity to achieve high impact and drastically reduce vector density, and hence malaria transmission in areas where LLINs have achieved significant reduction but low-level transmission persists. For example, studies in Burkina Faso have provided evidence that targeting swarms can reduce vector populations by 80% [27]. The present study demonstrated, for the first time in nearly four decades, the presence of *An. arabiensis* mosquito swarms, as described in Kaindoa *et al.* [20] and for the first time, the occurrence of *An. funestus* swarming in the same study area [21]. Swarms of *An. funestus* were found mainly close to human houses while those of *An. arabiensis* were mainly in the rice fields. Designing new interventions would need to take this variation into account.

Key findings

This was the first study in Tanzania to report swarms of *Anopheles funestus* mosquitoes, and to provide important information on swarming of *An. arabiensis* in the same area.

Implication for intervention

Targeting *Anopheles* swarms has potential of controlling vector densities and reducing malaria transmission. These findings open up new opportunities for improved malaria transmission control in Tanzania and beyond.

Future studies

More studies should be done to design simple, affordable and scalable tools that will help in detecting and targeting the swarming locations.

9.6 Role of different *Anopheles* species in malaria transmission

Another complimentary approach would be one designed to target specific mosquito species. For example, this study found that *An. funestus* accounted for about 80% of the infected females collected in the study area, despite occurring in low numbers compared to other mosquito species. When tested against different chemicals used for vector control, *An. funestus* was resistant to all pyrethroids used for treating bed nets. The blood meal analysis also found that *An. funestus* were highly anthropophilic, hence high vectorial capacity. This calls for additional interventions that will effectively target this species if malaria transmission in this area of Tanzania is to be reduced.

Moreover, it was found that sibling species of the *An. funestus* group, e.g. *An. rivulorum*, may also be important malaria vectors and strategies should be put in place to control these species. As an outcome of this study, the Ifakara Health Institute has initiated a study focusing on understanding the role of *An. funestus* sibling species in malaria transmission in rural Tanzania.

Key findings

Anopheles funestus mosquitoes persist, and are reasonable for higher infections in rural Tanzania, despite their low densities.

Implication for intervention

- We need to design better approaches which will specifically target this species so as to improve malaria control.

Future studies

- More studies should be done to demonstrate local disruption of residual malaria transmission by targeting and eliminating *An. funestus* mosquitoes, by combining innovative techniques that

maximize behavioral and physiological susceptibility of these vectors.

- Further studies are needed to investigate the role of *An. funestus* sibling species in residual malaria transmission settings in rural areas.

9.7 Limitations of the study

One limitation of the study is that it focused on entomological outcomes only and therefore limited inferences could be made about the effects on malaria transmission. Furthermore, improvements can be made on assessing the community knowledge on housing and malaria transmission by structuring questions in an open manner such that participants can express a wide range of opinions. The questions in the current format appear to be suggestive, which might have had an influence on the responses.

Another limitation was that sampling of indoor mosquitoes was done using CDC light traps placed only in a single, occupied room in the selected houses. It might be possible that the selected rooms had highly attractive or less attractive persons and the number of mosquitoes caught might not adequately be representative of all mosquitoes that entered the house. Another challenge is that CDC light traps are not capable of collecting all mosquitoes that enter a house, unlike exit traps. This may explain why the number of mosquitoes caught was more strongly correlated to number of people in the experimental hut study (where collections were done using exit interception traps fitted on house eaves and windows) than in the longitudinal surveys, where CDC light traps were used.

With regards to *An. arabiensis* mating behaviour, one potential limitation of this study was sampling mosquitoes in the swarm using sweep nets. Sweep netting does not remove all of the insects from a swarm and the use of alternative methods for estimating the size of swarms, such as photography, should be investigated. Other limitations, such as the use of wild mosquitoes for assessing insecticide resistance have been discussed in the specific chapters.

9.8 Conclusions

The study yielded evidence that malaria transmission risk is significantly lower when the distances between houses is high. Malaria transmission risk variations within a village might be important for planning and implementing spatially targeted interventions. With limited resources, malaria control efforts must be prioritized in locations with significantly higher risk in order to get the best results from these interventions. Thus, the distances between houses should be considered when planning for malaria vector control strategies.

Understanding the factors influencing malaria transmission at a small scale is crucial for planning control interventions. Malaria transmission heterogeneity does not only exist on a large scale, but also at small scales, which may be influenced by small variations of environmental features. These variations should be considered when improving malaria control interventions. To this end, the seasonal variation of malaria transmission has been established in the study area. While *An. funestus* showed variation in density throughout the year, numbers of mosquitoes remained high throughout the dry season, whereas *An. arabiensis* showed a definite peak in the rainy season with a subsequent drop during the dry season. Further studies are required to investigate the survival strategies of major malaria vectors during the dry season. Understanding this particular feature of *An. funestus* may lead to the design of new control strategies or improve existing interventions so as to reduce the malaria burden in such rural areas.

This study also documented high mosquito densities in mud-walled houses compared to houses with brick or plastered walls. These findings further indicate the necessity of considering house improvement as a malaria control strategy. While the community members had fairly high awareness that improved houses were associated with reduced mosquito nuisance, many families in rural areas are living in poorly constructed houses because of financial constraints. It is therefore necessary to consider inter-sectoral collaborations to integrate housing into health policies in Tanzania. Further studies are also needed to provide epidemiological evidence on how housing design affects malaria transmission. Furthermore, community-based house improvement programmes should be promoted, including simple, scalable

and affordable house improvements for the populations living in high malaria endemic areas in rural communities.

In summary, despite the recent high usage of LLINs, major household and environmental characteristics still contribute to persistent malaria transmission. Additional efforts such as house improvement programmes implemented at village-level, could address the challenges by prioritizing high-risk household clusters. Such efforts could be significantly improved by involving communities as well as removing financial barriers associated with poor housing, thus making this control strategy more accessible. The observation that *An. funestus* is now the major malaria vector suggests that effective and deliberate targeting of this species could accelerate elimination efforts. Generally, this study has generated information which sets the baseline for further studies on the relationship between the exact house location and malaria transmission risk in rural malaria endemic countries.

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Appendices

Appendix 1: Original paper I

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Malaria Journal

RESEARCH Open Access

Correlations between household occupancy and malaria vector biting risk in rural Tanzanian villages: implications for high-resolution spatial targeting of control interventions

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Abstract

Background: Fine-scale targeting of interventions is increasingly important where epidemiological disease profiles depict high geographical stratifications. This study verified correlations between household biomass and mosquito house-entry using experimental hut studies, and then demonstrated how geographical foci of mosquito biting risk can be readily identified based on spatial distributions of household occupancies in villages.

Methods: A controlled 4 × 4 Latin square experiment was conducted in rural Tanzania, in which no, one, three or six adult male volunteers slept under intact bed nets, in experimental huts. Mosquitoes entering the huts were caught using exit interception traps on eaves and windows. Separately, monthly mosquito collections were conducted in 96 randomly selected households in three villages using CDC light traps between March-2012 and November-2013. The number of people sleeping in the houses and other household and environmental characteristics were recorded. ArcGIS 10 (ESRI-USA) spatial analyst tool, Gi* Ord Statistic was used to analyse clustering of vector densities and household occupancy.

Results: The densities of all mosquito genera increased in huts with one, three or six volunteers, relative to huts with no volunteers, and direct linear correlations within tested ranges ($P < 0.001$). Significant geographical clustering of indoor densities of malaria vectors, *Anopheles arabiensis* and *Anopheles funestus,* but not *Culex* or *Mansonia* species occurred in locations where households with highest occupancy were also most clustered (Gi* $P \leq 0.05$, and Gi* Z-score ≥ 1.96).

Conclusions: This study demonstrates strong correlations between household occupancy and malaria vector densities in households, but also spatial correlations of these variables within and between villages in rural southeastern Tanzania. Fine-scale clustering of indoor densities of vectors within and between villages occurs in locations where houses with highest occupancy are also clustered. The study indicates potential for using household census data to preliminarily identify households with greatest *Anopheles* mosquito biting risk.

Keywords: Malaria, Household occupancy, Targeting interventions, Hot spots, Mosquitoes

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Background

Significant efforts have been made to scale up appropriate interventions against malaria, an infectious tropical disease that still affects about 214 million people and kills 438,000 people annually [1]. Most of these victims are African children below 5 years old. The World Health Organization estimates that there has been a decline of malaria burden, and that morbidity worldwide reduced by 37 % and mortality by 60 % between 2000 and 2015, but sub-Saharan Africa accounts for approximately 90 % of all malaria deaths and cases [1].

In Tanzania, country-wide malaria prevalence was last estimated at 9 % among children under 5 years old, by rapid diagnostic tests (RDTs) [2]. Parasite prevalence has declined by between 50 and 60 % in most of the country since 2000, although the southeastern and northwestern parts of the country have witnessed slower gains than the rest of the country [3]. These successes are mainly attributable to scale-up of long-lasting insecticidal nets (LLINs) [4, 5] and indoor residual spraying (IRS) [6], but also improved diagnosis and treatment with effective drugs [7, 8]. It is also possible that these successes were associated with overall improved health care, improved living standards, urbanization and overall economic transformation in the country [9]. Currently, there are new efforts in the Tanzanian National Malaria Control Programme (NMCP) Strategy 2014–2020 to cut the prevalence to 5 % by 2016 and to 1 % by 2020 [10].

The current Global Technical Strategy for Malaria [11] recognizes that in order to achieve malaria elimination in today's endemic countries, it is imperative to develop and implement not only new complementary control methods, but also improved surveillance-response strategies to support resource allocation and implementation. More emphasis is needed to develop targeted approaches in intervention campaigns focusing on residual transmission foci. The need for fine-scale targeting of interventions is growing, particularly in countries where epidemiological malaria profiles increasingly depict high geographical stratification of risk [12–14]. In many cases, as transmission levels reduce, there remains a geographically distinct pocket of transmission or demographically defined sub-populations, which must be identified and targeted to achieve zero transmission [12, 15, 16].

Based on the understanding of how disease-transmitting mosquitoes identify and follow cues from vertebrate hosts [17]. This study hypothesized that their dispersal within villages could be used as an indicator of areas where high biting risk occurs. Disease-transmitting mosquitoes are known to preferentially bite people with large body sizes [18], and households with high occupancy have also been shown to correspondingly have high *Anopheles* densities [19]. It is therefore likely that overall directional movement of mosquitoes within villages, and subsequently disease transmission risk, could be greatly influenced by spatial distribution of household biomass. In a recent study, Russel et al. demonstrate the coincidence of increased malaria transmission hazard and vulnerability occurring at the periphery of two Tanzania villages [20]. The study postulates that the occurrence of *An. gambiae* was associated with the number of occupants. The study further suggests that most vector control could be effective by targeting few households at the periphery of two villages in rural Tanzania. These observations, though widely accepted, have not previously been developed into practical actionable methodologies for disease surveillance, prevention or control. Yet this close association between human aggregations and mosquito biting risk may have significant influence on malaria parasite prevalence [21, 22] and infectiousness [23].

This study used controlled experimental hut studies and high resolution household-level sampling of indoor mosquito-biting densities to demonstrate strong spatial correlations between household occupancy and indoor malaria vector densities in three contiguous villages in south eastern Tanzania. The study also assessed whether regular household census data could be used to identify households with the greatest *Anopheles* mosquito biting risk in rural Tanzania.

Methods

Study area

The study was conducted in three villages in rural Ulanga District, southeastern Tanzania (Fig. 1). This is an area with moderate to high malaria transmission, where prevalence was last estimated at 38 % by polymerase chain reaction (PCR), 16 % by RDTs and 6 % by light microscopy [24]. Annual minimum and maximum rainfall ranges from 1200 to 1800 mm, respectively, while the mean maximum and minimum temperature are 20 and 32.6 °C, respectively. Malaria vectors in the area includes primarily *Anopheles gambiae* complex, which comprises >99 % *Anopheles arabiensis* sibling species, and *Anopheles funestus* group. Houses are mainly mud and brick walled, with thatched or iron-sheet roofs. Most people rely on subsistence farming for their livelihood, cultivating rice and maize in the Kilombero river valley.

Study procedures

The study consisted of three parts: first, a controlled experimental assessment of effects of host biomass on mosquito house entry, using specially designed experimental huts fitted with interception exit traps on eaves and windows to collect mosquitoes that enter the huts [25]. Second, longitudinal surveys of indoor mosquito

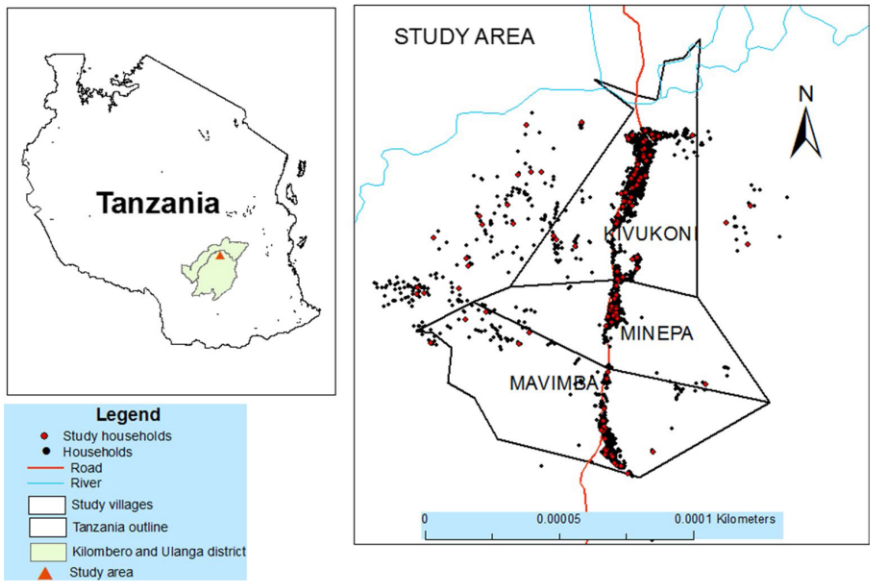


Fig. 1 Map of the study area, showing the villages in Ulanga district where the study was conducted

densities were conducted from March 2012 to November 2013 in randomly selected households within the Ifakara Health and Demographic Surveillance System (HDSS) area [26]. Lastly, statistical assessments and visualization of coincidental clustering between indoor malaria vector densities and household occupancy was done using ArcGIS 10 software (ESRI, USA).

Controlled experimental verification of correlations between household occupancy and mosquito house-entry

The Ifakara experimental huts, which have previously been demonstrated as effective for studying behaviours of disease-transmitting mosquitoes, including major malaria vectors in East Africa were used [25]. The experiment was conducted in a 4 × 4 Latin square design replicated four times over 16 nights. Four experimental huts, designated A, B, C, and D were used. Each night, each of the huts was either left unoccupied, or was occupied by one, three or six volunteers. The number of volunteers for each hut was randomly assigned nightly, and was rotated across the four experimental huts over a four-night working week. A group of ten male volunteers aged between 18 and 35 years participated in the experiment throughout the 16 nights. Each night, the hut designated to have no volunteers was considered the control hut, and remained unoccupied. To eliminate any potential biases from differential attractiveness of individual volunteers to mosquitoes, the volunteers themselves randomly selected the huts in which they would sleep each night. Each day, just before the experiments began, each volunteer was asked to randomly select one piece of folded paper from a bowl containing several such folded papers, each with a specific hut label, which had been assigned by the researcher such that the specified number of volunteers per hut was always achieved. This way, there was always one hut with no volunteers (i.e. control hut); a hut with one volunteer; a hut with three volunteers; and another with six volunteers, in all cases randomly assigned. Mosquitoes were collected in interception exit traps fitted on eave spaces of the experimental huts.

Longitudinal vector surveys to assess empirical relationships between indoor mosquito densities and household occupancy

A total of 96 households were randomly selected from an original HDSS household listing consisting of 2433 households in three villages of Kivukoni, Minepa and Mavimba, in Ulanga district, south-eastern Tanzania. The selection was conducted in two stages, where 1600 households were first selected (randomly), and spatially assigned to 16 geographical clusters each consisting of 100 households. The sampling clusters were assigned based on household latitudes so that clusters 1–16 were obtained on a north-southerly direction. From each geographical cluster, six households were selected randomly, and the household heads were requested to volunteer in

the study. Whenever a household heads did not consent, the next household in the random listing was selected, so that there were always six households per cluster.

The geo-positions (latitudes and longitudes) of all the households were recorded. In the same households, the total number of people in the household, and total number of people who slept in the specific trapping room were recorded on the night of mosquito sampling. The study also observed: (a) type of roofing material (i.e. grass or iron sheets), (b) material used on walls (i.e. brick or mud), (c) whether the windows were screened or unscreened, (d) whether the eave spaces were closed or open, and (e) distance from nearest water body. All the 96 selected households were provided with a new intact long-lasting permethrin-impregnated bed net similar to what had been provided by the government during the universal LLIN coverage campaign, which covered the villages between November 2010 and January 2011 [5].

Mosquito sampling was conducted monthly in each of the study households, but the order in which the households and clusters were visited was randomized. Each week, mosquitoes were sampled in four of the 16 geographical clusters, by visiting all six households per cluster per night, working for four nights per week. In each household, one room with at least one person sleeping in it was selected for assessing indoor mosquito densities. CDC light traps set near occupied bed nets were used for the sampling [27], and were operated from 18:00 to 06:00 h. Each morning, the collected mosquitoes were killed in a closed container using petroleum fumes, then sorted by sex, taxa and physiological status as blood-fed, non-blood-fed or gravid.

A sub-sample of the female malaria vectors, *An. gambiae* complex, was examined by multiplex (PCR), which amplifies the 28S intergenic spacer region of the ribosomal DNA to distinguish between sibling species in the complex [28]. Sub-samples of *An. funestus* were also examined by PCR, using techniques developed by Koekemoer et al. [29] and Cohuet et al. [30], which are based on species specific single nucleotide polymorphisms (SNP) in the internal transcribed spacer region 2 (ITS 2). The *Anopheles* samples were also examined by enzyme-linked immunosorbent assay (ELISA), to detect *Plasmodium* sporozoites in their salivary glands [31]. To avoid false positives, all the ELISA lysates were boiled for 10 min at 100 °C, so that any detected protozoan antigens were only the heat stable *Plasmodium* species [32, 33].

Data analysis

Data were analysed by open source software, R version 3.1.0, using the lme4 package [34]. The total number of female mosquitoes of each taxon was compared between huts having one, three or six volunteer sleepers and the control hut, being the hut with the no volunteers. The data were fitted to a generalized linear mixed effects model (GLMM), with log-linked Poisson error distribution [34]. Total mosquito catches were modelled as a function of number of volunteers and hut, while day of collection and experimental block (i.e. a set of four study nights) were used as random variables in the model, taking into account variations associated with nightly and weekly randomization in the experiment. Mean number of mosquitoes of each species collected per hut per night, and relative rates (RR) of collecting mosquitoes in the huts, and the associated 95 % Confidence intervals (CI), were estimated by exponentiating the coefficients generated from GLMMs.

For the longitudinal mosquito survey, the total number of mosquitoes collected from each house was obtained by first summing all female mosquitoes per hut per night. Relationships between household or trap room occupancy and mosquito densities were examined also by GLMMs using the *lme4* package [34], and log-linked Poisson error distributions as above. The indoor densities of mosquitoes of different species were modelled as a function of: (a) number of occupants in the mosquito trapping room, (b) total household occupancy, (c) month of mosquito collection, (d) village of collection, (e) whether the eave spaces on the houses were closed or open and (f) distance from nearest water bodies. Date of mosquito collection and house identification codes were incorporated as random variables in the GLMMs. Estimated mean indoor mosquito densities per hut per night and RR of these mosquitoes catches, and associated 95 % confidence intervals (CI), were computed from exponentials of the coefficients generated from the GLMMs.

Identification of spatial patterns of indoor mosquito catches was done using ArcGIS 10 spatial analyst tool (ESRI, USA). The Getis-Ord Gi* statistic [35, 36] in ArcGIS was used to identify locations of households with significant clustering of high indoor densities of disease-transmitting mosquitoes, including the malaria vectors, *An. arabiensis* and *An. funestus*, but also *Culex* species and *Mansonia* species. Clusters depicting both the high vector density foci (i.e. areas with households where the highest densities are most spatially concentrated) and low-vector density foci (i.e. areas with households where lowest densities are most spatially concentrated) were identified. Statistically significant clusters were then determined at a level of Gi* P value ≤ 0.05, and Gi* Z score ≥ 1.96. In this analysis, the conceptual relationship between households was assumed to be inversely related (so that houses far apart were considered more likely to be different, with regard to indoor vector densities, than households near one another), and Euclidean distances between neighbouring features were considered [35, 36].

Ethical consideration

All human volunteer participants were fully informed of the study objectives, benefits and risks involved in the experiment. Participation was only after the volunteer provided written informed consent. All households participating in this study and all volunteers sleeping in the experimental huts were protected by intact LLINs (Olyset® nets), to ensure basic minimum protection. Ethical approval for the study was obtained from Ifakara Health Institute's Institutional Review Board (IHI/IRB/No:10-2013), Liverpool School of Tropical Medicine (Approval No. 01, issued on 10th March, 2014), and the Medical Research Coordination Committee of the National Institute of Medical Research (Certificate No. NIMR/HQ/R.8a/Vol.IX/1816). Permission to publish this manuscript was obtained from National Institute of Medical Research (Ref: NIMR/HQ/P.12 VOL XVII/16).

Results

Relationship between household occupancy and mosquito house-entry in experimental huts

There were significant increases in numbers of all mosquitoes of different taxa, whenever the number of volunteer sleepers (proxy of human biomass) increased in the experimental huts. The increase was observed in the catches of *An. arabiensis*, *An. funestus* and *Culex* mosquitoes. As shown in Figs. 2 and 3, the results indicate that in all huts, with one, three, or six volunteers, there were more mosquitoes than in the controls (i.e. an unoccupied hut, where occupancy is zero) ($P < 0.001$). This observation was valid for malaria mosquitoes, *An. arabiensis* and *An. funestus*, but also *Culex* and *Mansonia* species (Figs. 2 and 3).

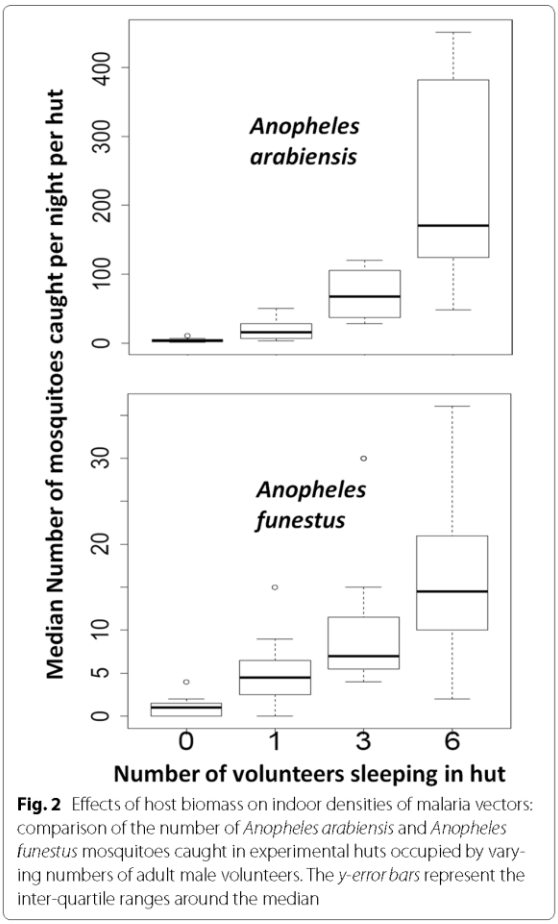


Fig. 2 Effects of host biomass on indoor densities of malaria vectors: comparison of the number of *Anopheles arabiensis* and *Anopheles funestus* mosquitoes caught in experimental huts occupied by varying numbers of adult male volunteers. The *y-error bars* represent the inter-quartile ranges around the median

Relationships between indoor mosquito densities and household or trap room occupancy

The number of malaria vectors caught in houses increased with number of people occupying the house. To match the field observations, where trapping rooms generally had at least one person and households generally had at least two members, the study assessed effects of trap-room occupancy and household occupancies relative to base-line levels of one person versus two persons respectively. The relative rate (RR) of catching *An. arabiensis* mosquitoes in trapping rooms with more than one occupant compared to rooms with one occupant was 1.6 (95 % C.I 1.2–2.3), $P < 0.001$. Similarly, for *An. funestus*, trapping rooms with more than one occupant had higher catches than rooms with just one person [RR = 1.1 (1.0–1.4), $P < 0.001$]. A similar trend was found in densities of non-malaria mosquitoes. With regard to overall household level occupancy, as opposed to just trapping room occupancy, there were also significantly more *An. arabiensis* [RR = 1.8 (1.3–2.7), $P < 0.001$], more *An. funestus* [RR = 1.0 (0.9–1.2), $P < 0.001$] and significantly more *Culex* mosquitoes [RR = 1.3 (1.1–1.7), $P < 0.001$] in houses with more than two occupants, compared to houses with two or fewer occupants.

Having open eave spaces on houses was also significantly associated with indoor vector densities. The number of *An. arabiensis* caught was significantly higher in houses with open eaves, compared to houses with closed eaves [RR = 0.8 (0.4.1–1.4), $P < 0.001$] and the number of *An. funestus* was consistently higher in huts with open eaves compared to huts with closed eaves [RR = 1.2 (1.0–1.5) $P < 0.001$]. Similar trends were observed for *Culex* mosquito species [RR = 0.9 (0.7–1.1), $P < 0.005$], and *Mansonia* species [RR = 3.2 (2.0–5.2), $P < 0.001$]. In this study, the CDC light traps used for sampling mosquitoes were set near human-occupied bed nets in the selected households. Besides, all participating households were

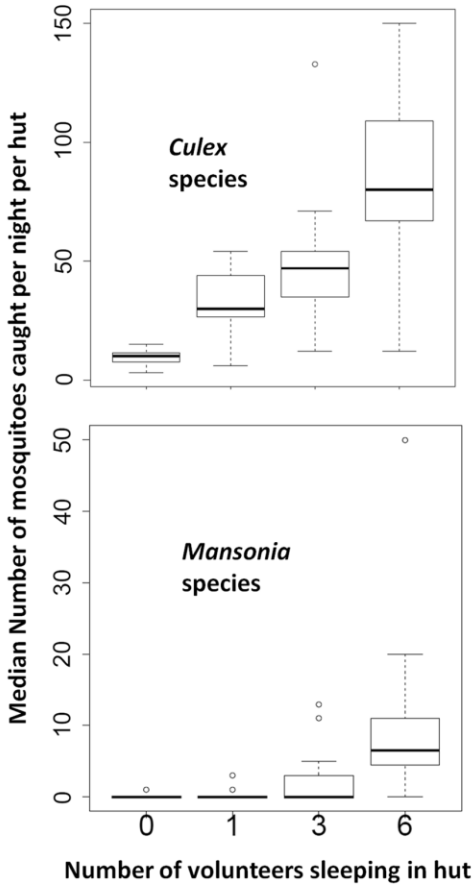


Fig. 3 Effects of host biomass on indoor densities of non-malaria vectors: Comparison of the number of *Culex* and *Mansonia* mosquitoes caught in experimental huts occupied by varying numbers of adult male volunteers. The *y-error bars* represent the inter-quartile ranges around the median

provided with ITNs and encouraged to use these nightly. It was therefore unlikely that this variable would have any effect on overall vector densities, and was excluded in the analyses.

Spatial clustering and correlations between house occupancy and indoor vector densities

There were clearly identifiable and statistically significant clusters of households with high densities of the two main malaria vectors, *An. arabiensis* and *An. funestus* in the central part of the study area (GI* $Z \geq 1.96$, GI* $P \leq 0.005$), but also significant clusters of *Culex* mosquitoes in the northern part of the study area (Figs. 4, 5, 6). Clusters of households with the highest occupancy occurred in these same geographical locations in the study area (GI* $Z \geq 1.96$, GI* $P \leq 0.05$). Since the study obtained the household occupancy data from all the houses where mosquito collections were also conducted, the analysis reveals that geographical clusters of households with the highest occupancy were individually the same clusters of households with highest densities of malaria vector species, but not *Culex* mosquitoes. Clustering of *An. arabiensis* (Fig. 4) and *An. funestus* (Fig. 5) were both geographically coincidental with clustering of household occupancy in the study area, but no such geo-coincidence was observed for *Culex* species, whose indoor densities were on the contrary geographically aligned to areas where houses with the lowest human occupancy were clustered (Fig. 6). The study found no statistically significant clusters of houses with high densities of *Mansonia* species mosquitoes in the study area.

Species composition of malaria vectors and *Plasmodium* infection rates

A total of 39,754 *An. gambiae complex* and 14, 817 *An. funestus* group mosquitoes were assayed in the laboratory. All of the *An. gambiae s.l.* mosquitoes assayed were confirmed to be *An. arabiensis* (100 %), while 94 % of the mosquitoes from the *An. funestus* group assayed were *An. funestus s.s.* The rest of the *An. funestus* mosquitoes were *Anopheles rivulorum* (6 %). Overall, the sporozoite infection rates were 4.04 % in the *An. funestus* mosquitoes and 0.47 % in *An. arabiensis* during the study period.

Discussion

Identification and targeting of high transmission foci particularly at fine scale levels within villages is essential for successful malaria control and eventual elimination [12, 14]. Transmission of malaria pathogens, like many other infectious agents, is heterogeneous over host populations but also over geographical space [21, 37], and this stratification increases significantly in reduced transmission settings [12, 14, 15]. Understanding these dynamics and how they are influenced by the various biotic and abiotic factors is essential to improving planning for interventions of ongoing malaria prevention strategies.

The study hypothesized that household occupancy (being proxy to household-level biomass), would influence not only indoor vector densities as shown in several previous studies [18, 19], but that it also influences mosquito dispersal within communities, and the resulting geographical distribution of human biting risk and pathogen transmission risks across these communities. By extension, it was assumed that overall directional movement of mosquitoes within villages is influenced by spatial distribution and demographic composition of households in these villages. As a result, locations where households with high biomass or occupancy are

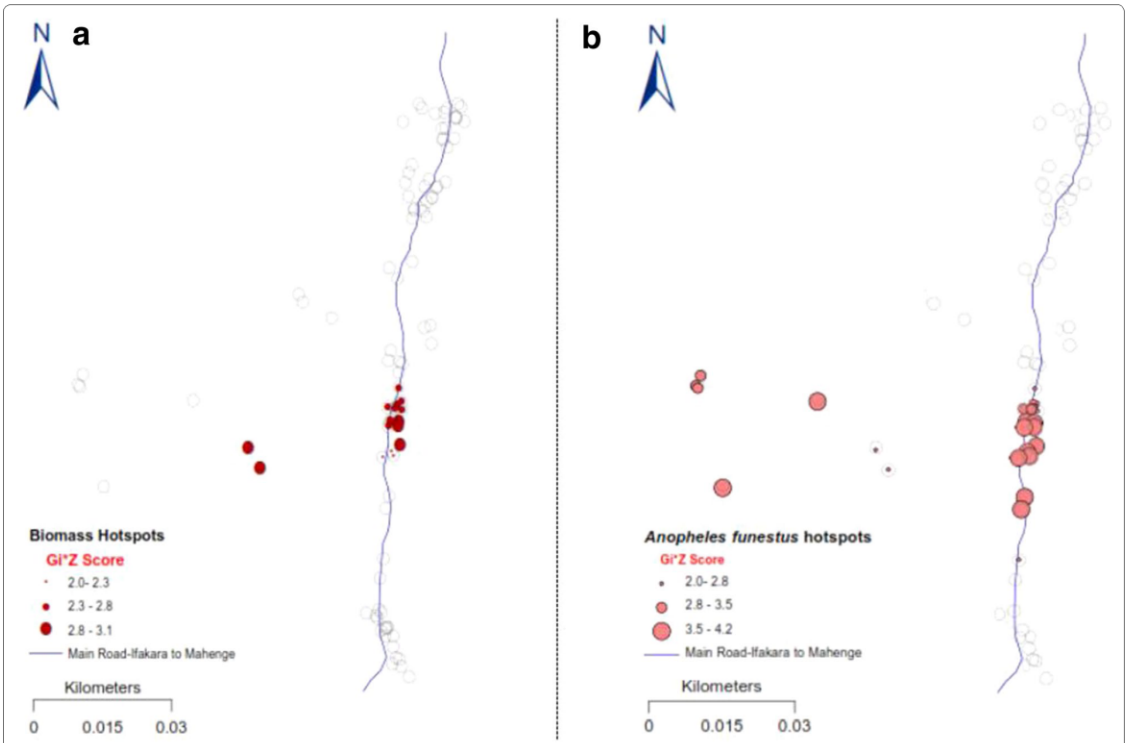


Fig. 4 Maps showing statistically significant clusters of households with the high occupancy (**a**) and statistically significant clusters of households with high densities of *Anopheles arabiensis* in the same study area (**b**). The *grey circles* represent the rest of the households in the study area

clustered would naturally form pockets of high transmission of mosquito-borne diseases, unless there are specific interventions or environmental variables, which significantly modulate such patterns.

Female mosquitoes need vertebrate host blood for reproduction and understanding this host-seeking behaviour would be essential for estimating the transmission of mosquito borne diseases, including malaria [17]. The host-seeking behaviour is influenced by many factors, including host odour cues, host density, dispersal ability of the mosquitoes and host distribution availability [17, 38]. Indeed, where distribution of human populations is heterogeneous, the distribution of adult mosquitoes also tends to be heterogeneous even if the breeding sites are uniformly distributed in the environment [21].

The controlled experimental hut studies verified earlier observations of correlations between vector densities and human biomass [18], but also provided a clear pattern of the seemingly linear relationships between these variables. The design of the experiment, using exit interception traps enabled mosquitoes freely—fly into huts and quantify the densities, by trapping them upon exit. The human volunteers participating in the study were fully randomly assigned to the huts on nightly basis, thereby excluding confounding effects of differential host attractiveness to mosquitoes [38]. Moreover, since the study restricted the age of volunteers to between 18 and 35 years, and relied on a fixed group of ten volunteers for this study, the observed associations between vector densities and volunteer numbers can be considered to represent correlations with human biomass. The experiment therefore provides the first of such datasets obtained under controlled environments in a malaria- endemic community, and lends itself to future use for fitting models that simulate mosquito host seeking and pathogen transmission.

Similarly, the field surveys also showed that houses with higher occupancy tended to have more mosquitoes as compared to houses with low occupancy, even though the indoor vector densities were also modulated by factors such as whether the eave spaces were open or not. In this study, the effects of trap-room and household occupancy were assessed by considering observed base-lines of at least one person per trap room versus at

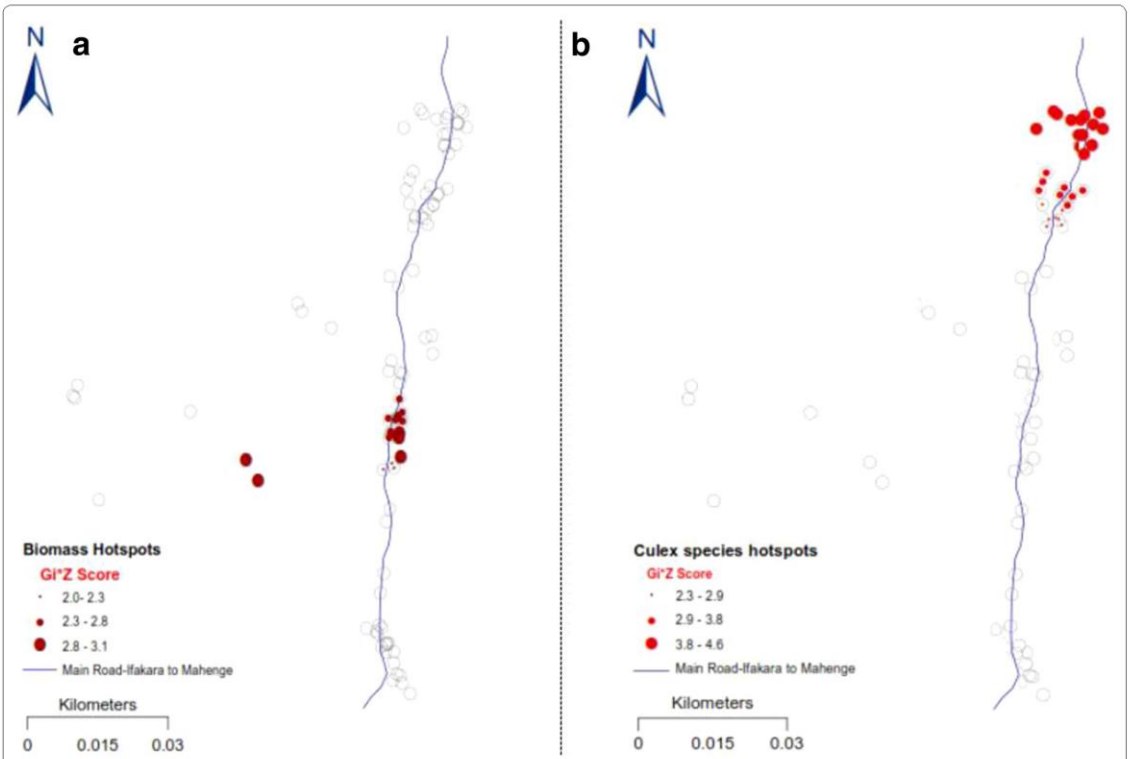


Fig. 5 Maps showing statistically significant clusters of households with high occupancy (**a**) and statistically significant clusters of households with high densities of *Anopheles funestus* in the same study area (**b**). The *grey circles* represent the rest of the households in the study area

least two persons per household. This was because individual trapping rooms generally had at least one person while households generally had at least two members. Although the study observed several other household characteristics other than biomass and eave spaces, the analyses revealed that these were the two most influential variables on indoor vector densities in the study area. A study by Al-Eryani et al. in Yemen has also yielded similar evidence that the number of *An. arabiensis* was positively correlated with the number of occupants in the house [22].

There was a clearly observable geographical overlap in the spatial clustering of houses with high occupancy, and the clustering of houses with high densities of malaria vectors. The study analysed the data for household biomass separately from the data for vector densities, yet in both cases, there were significant clustering. The analysis thus provides a set of possible simple rules, which could be relied upon to predict at fine-scale, the parts of villages where the highest biting risk occurs and where intense, highly focalised vector control efforts would achieve greatest community-level impact. This study was conducted in an area which has historically had very high malaria transmission rates [3, 39], but where LLIN coverage is now evenly very high. Even then, this study suggests that by simply mapping household occupancy and their spatial distribution in the area, one would be able to rapidly identify places with the highest and lowest indoor vector densities, even without any vector trapping. This information is of major significance for spatial targeted interventions, particularly at fine-scale [20, 22], even within small administrative boundaries such as wards and villages.

Visual inspection of Figs. 4, 5 and 6 suggests that both intra- and inter-village variation in indoor mosquito-biting risk would be spatially correlated to household occupancy patterns, readily identifiable by using census and other demographic data in many other malaria-endemic countries. One point of caution is that whereas the assertions could hold true over geographically homogenous areas, and in the absence of any focalized vector control operations that disrupt mosquito-host seeking and density distributions such as IRS [40, 41] or larval source management [42], there are several other features, with

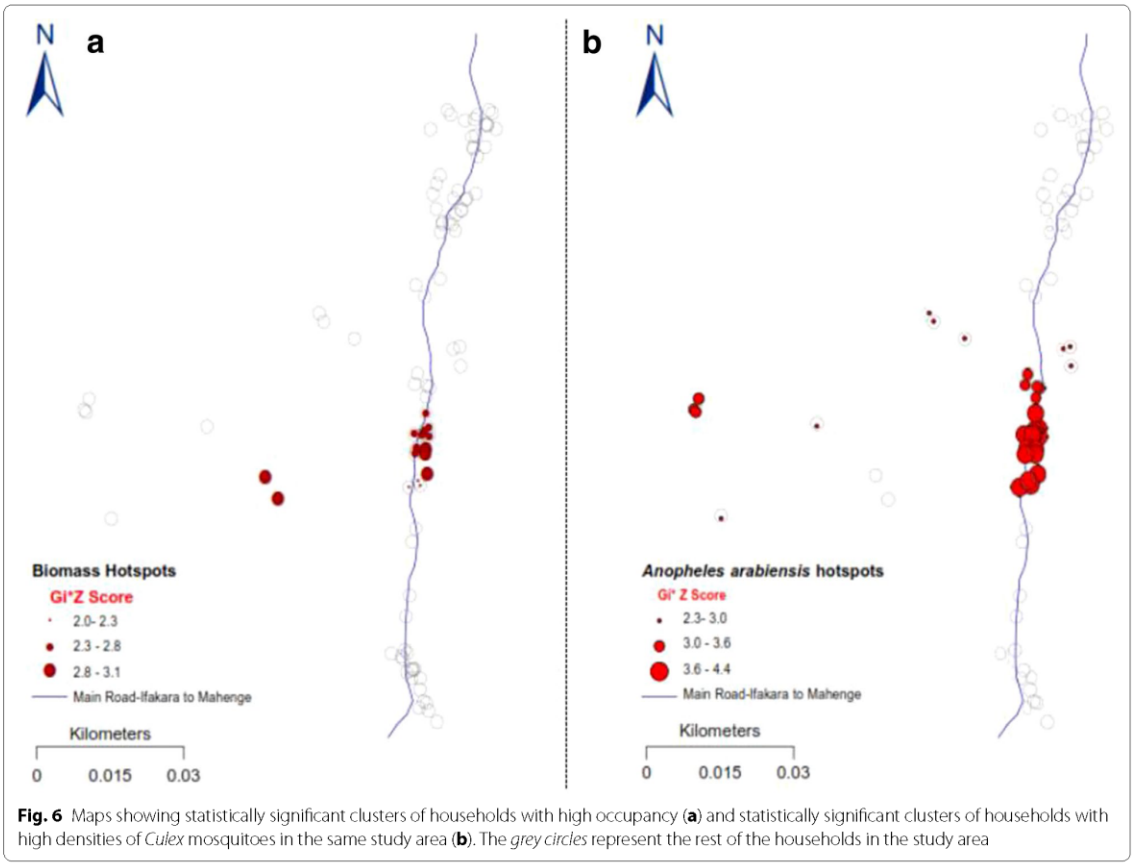


Fig. 6 Maps showing statistically significant clusters of households with high occupancy (**a**) and statistically significant clusters of households with high densities of *Culex* mosquitoes in the same study area (**b**). The *grey circles* represent the rest of the households in the study area

potential to change or eliminate these spatial correlations. Features such as topography [43, 44], ground water and surface water flows [45], growth of urban centres and increased settlement densities [46], as well as agricultural cultivation [47], are examples that could disrupt the geographical coincidences observed. Indeed Thomas et al. recently concluded after analyses of data from The Gambia that mosquito dispersal would likely be landscape specific [44], necessitating that a reasonable level of characterization is conducted in the target communities. Despite these potential sources of discrepancies, the observations and experimental verifications have clearly determined that vector control operations at local district level could rely heavily on readily available household census data to predict basis risk patterns across villages, but that use of other data layers would improve the outcomes and overall predictions.

Since household-level analyses revealed increasing mosquito numbers with increasing number of occupants, the results of these geo-spatial analyses must be interpreted with caution. The increased community-level biting risk implied by these analyses is primarily because the increase in hazard levels, even if the individual level-exposure remained unchanged. Caution should be taken in the interpretation of these results so as not to imply that biting-risk per person was also increased inside household in the areas where host biomass was highest, even if the bite-related hazard was higher. Interpretations of the results should therefore be limited to the understanding that increased concentration of potentially infectious mosquitoes in these areas would enable more effective targeted control, with lower amounts of resources, and also that in such locations, even a low-level exposure, would result in significant risk of malaria infections. For example, it is likely to be more dangerous to sleep without a bed net in these locations with high concentrations of large households, than it is to sleep without a bed net in the rest of the villages. The results should therefore be examined from the perspective of community level protection from the increased biting risk. Since potentially infectious mosquitoes disperse towards, and eventually end up being most abundant in

areas with highest household biomass concentrations, creating opportunities for targeted control of these vectors community-wide mass effect. Moreover, locations with clusters of large households can be considered as providing a significant level of protection to the smaller households elsewhere in the village [20], because mosquitoes are drawn mostly towards these locations, and away from the other areas. The greatest epidemiological value of the results is more on their potential as a way to target community-wide vector control and achieve mass effect on potentially infectious vectors, rather than as a way to predict individual risk.

Other than the experimental studies and community-wide vector surveys, this study also showed that the proportion of *Plasmodium*-infected *An. funestus* was far higher than proportions of *An. arabiensis* infected. The latter species thus plays a much greater role in malaria transmission, contributing up to 87.9 % of potential new infections in the study area, compared to only 12.1 % from *An. arabiensis*. No other infected *Anopheles* species was found during this study. The concern over the increasing role of local *An. funestus* populations remains an important one, given its greater competence as a vector of malaria. Although *An. arabiensis* is still the most prevalent of the vector species in the area, determining that ongoing residual malaria transmission is mostly mediated by *An. funestus* suggests that highly effective household-level interventions that target the indoor-feeding and indoor-resting behaviours of these vector species could still be highly applicable to bring down transmission levels. Such interventions would be greatly enhanced if spatially targeted to the parts of villages where host biomass is most concentrated. Studies by Lwetoijera et al. in southeastern Tanzania [48] and MacCann et al. in western Kenya [49] have also yielded similar evidence of increased role of *An. funestus*. The seemingly growing challenge would be further complicated in areas where the vector species is also increasingly resistant to insecticides commonly used for malaria prevention and control.

Considering both the experimental assays and the entomological survey data, this study indicates that household-level effects of host biomass on host-seeking and indoor vector densities are indeed transferable to community-level patterns. As a result, areas with concentrations of large households tend to have more mosquitoes than areas with sparsely distributed small households. Unfortunately, despite availability of vast quantities of household census and other demographic data regularly collected from large populations in many countries including Tanzania, no efforts have previously been made to triangulate such datasets with the knowledge of how vectors identify, locate and attack humans, so as to map the likelihood of mosquito-borne disease transmission within and between villages. One would propose that such triangulations should be considered as an initial step in the assessments of disease risk. The knowledge is essential for creating baseline estimates of transmission risk and disease burden, which enables actual transmission foci to be easily mapped on fine scales, using simply estimates of human biomass or household occupancy, from regular demographic surveys. In countries where HDSSs have been running for many years, such datasets could also be utilized to provide baseline spatial estimates for risk prediction and prioritization of interventions. Future studies may include modelling of malaria risk from existing datasets such as malaria indicator surveys (MIS) and Demographic Health Surveys (DHS), with the aim of confirming the results observed in this study. An obvious advantage here is that MIS and DHS datasets regularly record numbers and age of people in households and would provide reliable estimates of household-level biomass distribution across communities.

Conclusion

Directly observable household level effects of household biomass on mosquito house entry are manifest at community-level spatial scales. These relationships result in a situation where areas with clusters of large households tend to have most biting mosquitoes, while areas with sparsely distributed small households have least biting risk. Overall, fine-scale and within-village clustering of indoor densities of major malaria vectors in this study area occurred in the same locations where houses with the greatest number of occupants were also clustered. It is hypothesized that in similar communities; the most intense foci of *Anopheles* biting risk could therefore be preliminarily predicted directly from household-level population census data. Where regular census data are available, with records of ages and numbers of people per household, it may be possible to rely on these maps to generate spatially defined high-resolution malaria risk maps, to support disease control programmes. However, since mosquito dispersal over space is often landscape-specific, it may also be necessary to identify what other factors significantly modulate these spatial relationships, and how these observations could be used to improve vector-borne disease mapping and control.

Authors' contributions

EK and FO conceived the study and developed study protocols. EK, GM and FO conducted the experiments and supervised data collection. EK, LKH and FO conducted data analysis and wrote the manuscript. All authors approved the manuscript prior to submission. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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Appendix 2: Original paper II

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Malaria Journal

RESEARCH Open Access

Housing gaps, mosquitoes and public viewpoints: a mixed methods assessment of relationships between house characteristics, malaria vector biting risk and community perspectives in rural Tanzania

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Abstract

Background: House improvement and environmental management can significantly improve malaria transmission control in endemic communities. This study assessed the influence of physical characteristics of houses and surrounding environments on mosquito biting risk in rural Tanzanian villages, and examined knowledge and perceptions of residents on relationships between these factors and malaria transmission. The study further assessed whether people worried about these risks and how they coped.

Methods: Entomological surveys of indoor mosquito densities were conducted across four villages in Ulanga district, south-eastern Tanzania. The survey involved 48 sentinel houses sampled monthly and other sets of 48 houses randomly recruited each month for one-off sampling over 12 months. Physical characteristics of the houses and surrounding environments were recorded. Questionnaire surveys were administered to 200 household heads to assess their knowledge and concerns regarding the observed housing and environmental features, and whether they considered these features when constructing houses. Focus group discussions, were conducted to clarify emergent themes on people's perceptions on relationships between housing or environmental factors and malaria transmission.

Results: The entomological surveys showed statistically higher indoor densities of the malaria vectors (*Anopheles arabiensis* and *Anopheles funestus*) in houses with mud walls compared to plastered or brick walls, open eaves compared to closed eaves and unscreened windows compared to screened windows. Most respondents reported that their houses allowed mosquito entry, at least partially. Participants were aware that house structure and environmental characteristics influenced indoor mosquito densities and consequently malaria transmission. They were concerned about living in poorly-constructed houses with gaps on eaves, walls, windows and doors but were constrained by low income.

Conclusion: In rural south-eastern Tanzania, significant proportions of people still live in houses with open eaves, unscreened windows and gaps on doors. Though they are fully aware of associated mosquito biting and pathogen transmission risks, they are constrained by low-income levels and competing household priorities. The study proposes that community-based house improvement initiatives combined with targeted subsidies could lower the financial barriers, improve access to essential construction materials or designs, and significantly accelerate malaria transmission control in these communities.

Keywords: Community knowledge, Malaria transmission, Housing characteristics, Environmental features, Mosquitoes, Tanzania

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Background

The WHO 2017 World Malaria Report showed significant successes in malaria control in various countries, but with an overall slight increase in malaria cases and deaths relative to the previous year [1]. Over the past decades, LLINs and IRS have been widely used alongside improved diagnosis and treatment, primarily with artemisinin-based combination therapy. These tools have contributed most of the gains accrued against malaria in the past decade [1, 2], but evidence suggests that new interventions will be required to finally move to zero transmission [3, 4]. Unfortunately, novel strategies, such as improved housing [5] and environmental management for malaria control, have only rarely considered [6, 7]. Tanzania has experienced more than 50% reduction in malaria prevalence in children aged 2–10 years across the country since 2000 [8]. It is estimated that less than 2% of people in mainland Tanzania now live in places considered as having intense year-round transmission, while more than 60% of Tanzanians live in areas considered as having low transmission [8]. The 2015–2016 malaria indicator surveys reported an increase in average malaria prevalence to 14.8% prevalence in children under 5 years [9], up from 9.5% in 2012 [10], but the most recent survey has shown much reduced prevalence of 7.3% [11]. Despite these gains, there are still numerous challenges, among them, the rise of insecticide resistance [12], sub-optimal net use each night [9], and the high costs. Greater vigilance and additional interventions are therefore required to consolidate past gains and accelerate efforts towards the current national malaria control targets and eventual elimination.

Community involvement in vector control programmes is essential for successful malaria transmission reduction [13–16]. Existing evidence suggests that acceptance and optimal use of interventions, such as indoor residual spraying (IRS) and long-lasting insecticide-treated nets (LLINs) depends on the level of education and awareness in target communities [17], as well as grass-root organization of such communities to support the health initiatives [16]. Similar successes have been observed with other vector control approaches such as larval source management. For example, in Tanzania, a large-scale community-based larvicide application programme in the city of Dar es Salaam, significantly reduced malaria infection prevalence among city residents [18], despite significant logistical and coverage challenges. In that programme, community owned resource persons supported specific efforts such as mapping, larvicide application and vector surveillance [19, 20]. Elsewhere, in an interesting integration of farmers' experiences, van den Berg and Knols [21] highlighted the importance and potential of using farmer field schools as a method for improving community participation in malaria control. They suggested that malaria control can benefit from complementarities between rural development, farming and pest management and argued for a form of education that uses experiential learning methods to build farmers experiences, thereby achieving improvements in both health and development [21].

Unfortunately, despite the substantial evidence on benefits of community engagement in malaria prevention and various innovations on how to achieve this goal, community participation is increasingly relegated, as malaria-endemic countries adopt vertical campaigns such as universal LLIN distributions and mass drug administration in some settings. Instead, the knowledge and experiences of recipient communities is rarely sought even where such additional knowledge would significantly improve outcomes. In rural Ulanga district, south-eastern Tanzania, recent studies have demonstrated that community members were either at par or more knowledgeable than experts in identifying locations where mosquitoes are most abundant [22] and locations where male mosquitoes regularly swarm [23]. Both examples emphasize the potential benefits of relying on communities for improved vector control.

Another important question is whether individuals understand how malaria transmission risk varies across their communities and how it is affected by factors such as housing characteristics and the environment. Evidence suggests that transmission intensities are rarely uniform between districts and villages or even between households in the same village. Instead some households are always disproportionally more exposed to biting risk and malaria infections than community averages [24]. In a recent study, it was found that there is strong correlations between household occupancy and indoor densities of common malaria vectors (*Anopheles arabiensis* and *Anopheles funestus*), as well as spatial correlations of the two variables within and between villages in rural Tanzania [25]. That study also showed that high indoor vector densities cluster in locations where houses with highest occupancy are also clustered, suggesting the possibility of relying on regular census data to predict malaria transmission patterns. Similarly, Smith et al. [26] illustrated that even though biting risk from malaria vectors may be highest at the edge of the villages near aquatic habitats, infectious proportions of these mosquito populations (tending to be the older ones) are far more likely to be clustered in the middle of the villages, with peak populations of the infectious ones occurring when overall vector densities start declining [26].

Despite the above observations, and other evidence suggesting that house design, geographical location of the house within settlements, and characteristics of

surrounding environments all influence malaria transmission [16–19], several questions remain unanswered, particularly with regard to community awareness of the same factors. For example, it is unclear whether community members fully understand the importance of such factors, whether they have any concerns about them, and what their counter-efforts are during site selection and house construction. Yet, as malaria transmission declines, control efforts aiming at elimination should target locations and possibly households with greatest risk [27–29], while also actively engaging members of those communities and households.

The aims of this study were therefore: (a) to identify common house characteristics that may be associated with increased vector biting risk, and (b) to assess community knowledge and experiences regarding housing and environmental factors that influence malaria transmission in four rural Tanzanian villages and how these experiences can be incorporated in ongoing and future intervention options.

Methods

Study area

The study was conducted in four villages of Kivukoni (8.2135°S, 36.6879°E), Minepa (8.2710°S, 36.6771°E), Mavimba (8.3124°S, 36.6771°E) and Milola (8.2135°S, 36.6878°E) in Ulanga district, south-eastern Tanzania (Fig. 1). The local houses have either mud or bricks walls. The roofs are either grass-thatched or covered with corrugated iron-sheets, and most houses have open eaves (Fig. 2). Mean household size is 4.2 [30]. This area is perennially meso-endemic for malaria, and has high mosquito densities throughout the year, peaking between March and May. Annual rainfall and mean daily temperatures range from 1200 to 1800 mm and 20 to 32.6 °C, respectively.

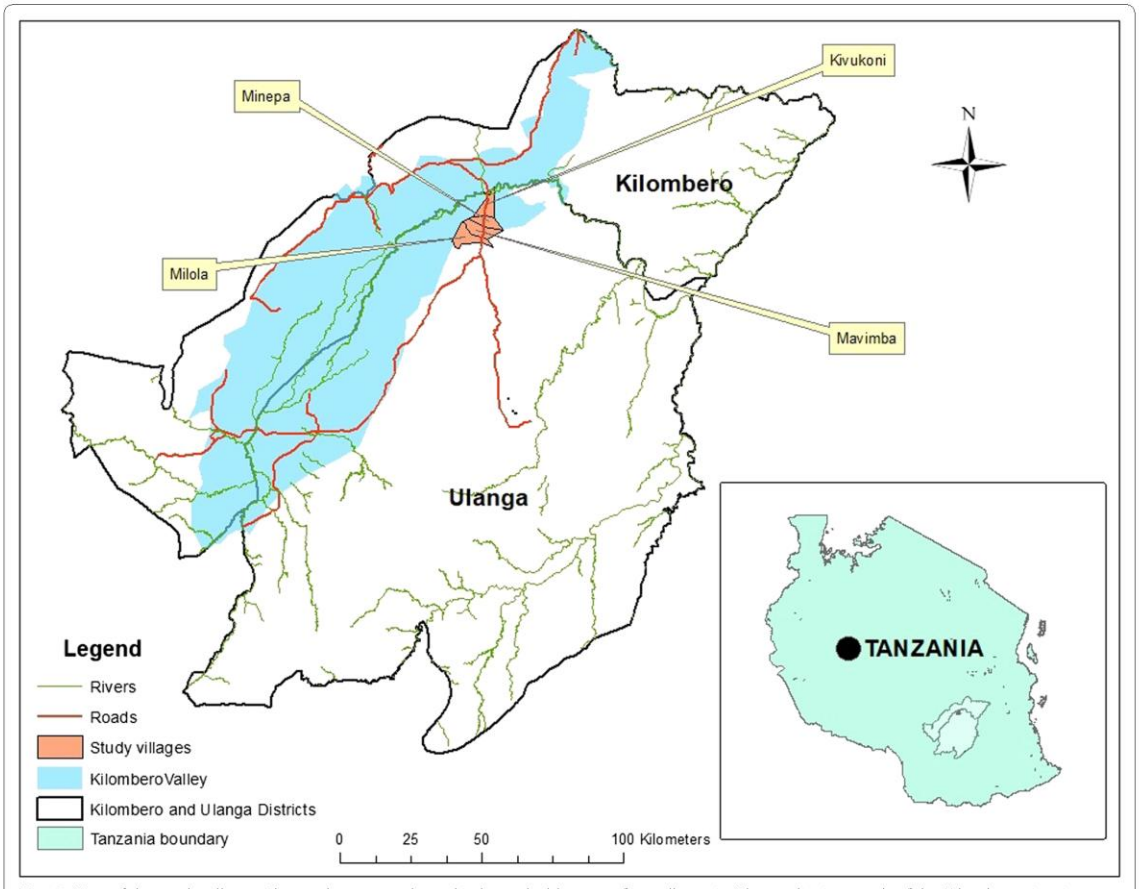


Fig. 1 Map of the study villages. The study was conducted in households across four villages in Ulanga district, south of the Kilombero river in south-eastern Tanzania



Fig. 2 Pictorial representation of typical local house types in the study area. Top left: a house with grass thatch roofing with mud walls. Top right: a house with corrugated iron roof and brick walls not plastered on the outside, and sometime also not plastered on the inside. Bottom left: grass thatch roof with brick walls not plastered on the outside, and sometime also not plastered on the inside. Bottom right: a house with iron sheet roofing and plastered brick walls. A variety of window and door designs and covers are also illustrated

The primary malaria vectors are *An. funestus* and *An. arabiensis,* with minor contributions from *Anopheles rivulorum* [31]. Malaria transmission intensities, i.e. entomological inoculation rate (EIR) was last estimated at 4.2 and 11.7 infectious bites/person/year (ib/p/yr) by *An. arabiensis* and *An. funestus,* respectively, totalling 15.9 ib/p/year [31]. The main vector control method is LLINs, usually distributed through mass campaigns done every 3–4 years, and keep-up campaigns done through reproductive health clinics. The main malaria vectors are resistant to pyrethroids used in the LLINs, but still susceptible to organophosphates [31].

Study procedures

The study used a mixed methods approach with four components (Fig. 3) including: (i) longitudinal surveys of indoor mosquito densities in sentinel and randomly selected households within the Ifakara Health and Demographic Surveillance System (HDSS) area [30] from January 2015 to January 2016; (ii) characterization of the houses and surrounding environmental variables; (iii) a questionnaire survey administered to community members to assess their concerns about household and environmental factors and whether they consider such variables when they are constructing their houses; and (iv) focus group discussions to assess people's knowledge and perceptions on relationships between house characteristics and environmental factors associated with transmission.

Longitudinal surveys of indoor mosquito densities

Random sampling with replacement was done using household listings originally obtained from the Ifakara Health & Demographic Surveillance Systems unit (2433 houses) covering the study area. An initial set of 48 houses were selected and recruited upon consent of household heads, and were set as fixed sentinel houses where vector surveillance was conducted once every month for 12 months. Each month, another set of 48 houses was selected randomly from the same villages (with approximately similar spatial distribution as sentinel houses) for a one-off mosquito collection that month. The following month a new set of 48 houses were selected without repeating those that had previously been sampled. In the end, a total 624 households were studied over the 12-month period, with the 48 sentinels sampled 12 times each.

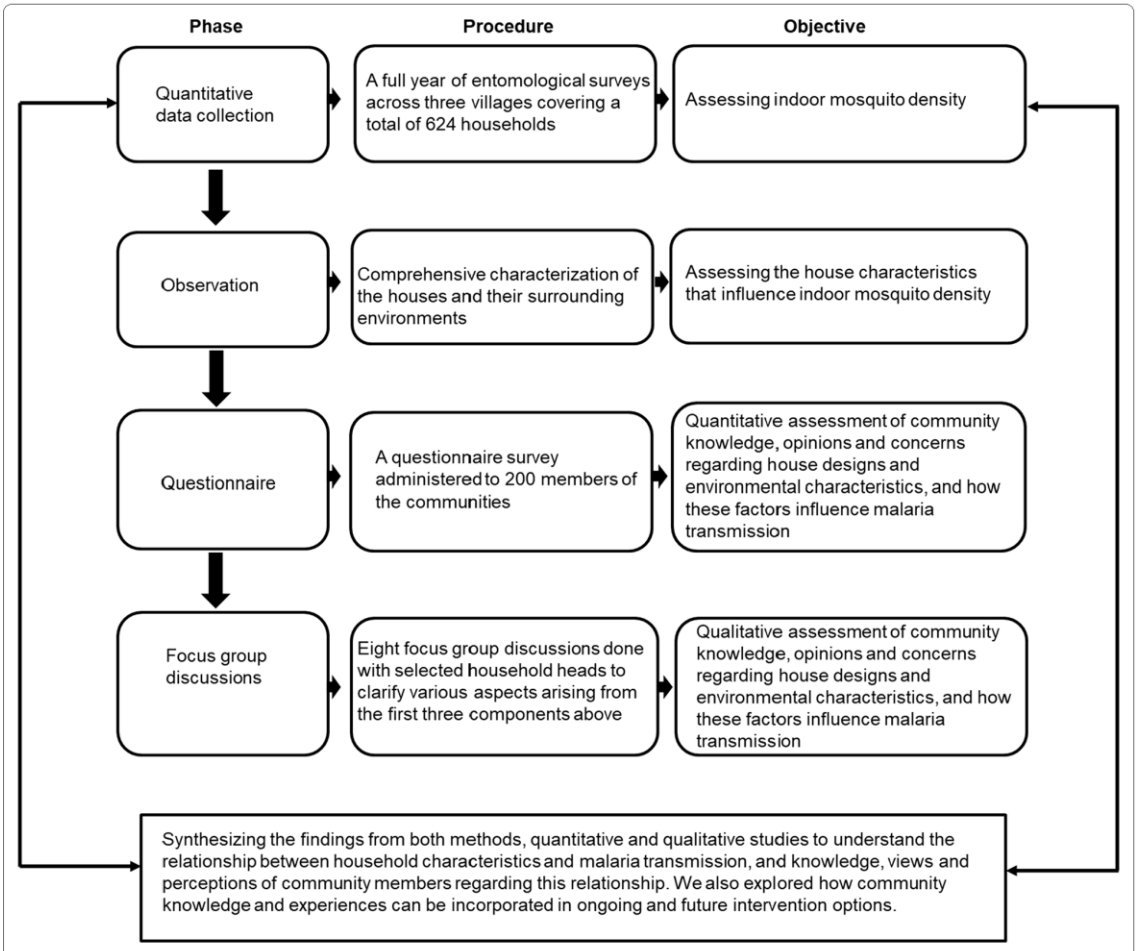


Fig. 3 Mixed methods study design

Since the three study villages were different in size and number of households, the actual selection and distribution of the sampling houses was done in such a way as to cover the full geographical extents of the entire study area. Then stratified random sampling was used. First, 1600 households were randomly identified and spatially assigned to 16 geographical clusters, each having 100 households. The sampling clusters were defined based on household latitudes so that clusters 1–16 were on a North–South direction. On the first month of study, the geographical clusters were visited and six households selected randomly per cluster. The six household heads were requested to volunteer in the study, and half of them requested for permission so their houses could be enrolled as fixed sentinel sampling stations for repeated monthly mosquito collections. Whenever a household head did not consent, the next household in the random listing was selected, so that there were always six households per cluster (three of them being fixed sentinels and another three being random households for one-off sampling). This way, there were 48 fixed sentinels throughout the study period and another 48 new houses newly selected each month across the clusters.

Thereafter, mosquito collections were conducted monthly in each of the study households, each time selecting a new set of three random households from each cluster. A weekly schedule was followed, working for 4 weeks/month. Each week, mosquitoes were sampled in four of the 16 geographical clusters, by visiting all six households per cluster each night, working for four nights per week. This way, all the 16 clusters were covered once every month. In each household, one occupied room was selected for assessing indoor mosquito densities. CDC light traps set near occupied bed

nets were used [32], and were operated from 18:00 h to 06:00 h. Backpack aspirators (CDC model 1412-12 VDC 17 AmpHr Gel-Cell battery) were also used to sample resting mosquitoes each subsequent morning. The collected mosquitoes were killed in a closed container using petroleum fumes, then morphologically identified using taxonomic keys for *Anopheles* Africa, south of Sahara, developed by Gillies and Coetzee [33]. All samples were then sorted by sex, taxa and physiological status. Subsamples of *Anopheles gambiae* complex were further identified by multiplex polymerase chain reaction (PCR), to identify sibling species using protocols developed by Scott et al. [34]. Also a sub samples of *An. funestus* were identified by using PCR, following the techniques developed by Cohuet et al. [35], and Koekemoer et al. [36].

Characterization of the houses and their environments

At each initial visit, all the 624 human houses were characterized from where mosquito collections had been done over the 1-year period. Geo-positions (latitudes and longitudes) of all the households were captured using hand-held GPS receivers (Magellan eXplorist 110) and recorded. In each house, the condition of windows, doors and walls were examined, and whether the houses had closed or open eaves. The surroundings of the households were examined and recorded any nearby water bodies, cattle sheds or other animal sheds. Other environmental variables recorded included whether the houses were located at the edge or in the middle of the villages and whether people kept animals, such as cattle, pigs or chicken.

Survey questionnaires: quantitative assessment of community knowledge, opinions and concerns regarding house designs and environmental characteristics, and how these factors influence malaria transmission

A structured questionnaire was developed, pretested and used for this survey. The questionnaire had three components (Table 1): the first component captured socio-demographic characteristics of participants including variables such as age, gender, education, marital status, household size, household income and expenditure. The second component examined the general knowledge of the participants on malaria, malaria transmission, its symptoms, preventive measures and care-seeking behaviours. The third component of the questionnaire assessed

Table 1 Description of the main themes addressed in the survey to assess community knowledge, opinions and concerns regarding house designs and environmental characteristics, and how these factors influenced malaria transmission

	Concepts investigated	Specific questions asked by the interviewer	Relevance of the concepts
1	Knowledge and perception about house characteristics, mosquito entry and malaria transmission risks	Do you know if the house design can influence mosquito entry?	Assessment of knowledge and perception of malaria transmission risks in relation to house characteristics
		Does your house allow mosquito entry?	
		If your house does not allow mosquito entry, how do you prevent mosquito entry?	
		Why does your house allow mosquito entry?	
		How do mosquitoes enter your house?	
		When was your house constructed?	
		Why did you decide to construct this kind of house?	
		What did you consider during construction?	
2	Knowledge and perception about environmental variables influencing mosquito density	Do you know if the environments surrounding your house influence mosquito density?	Assessment of knowledge and perception of malaria transmission risks in relation to environmental characteristics
		How does the environments surrounding your house influence mosquito density?	
		Mention the common mosquito breeding sites in your area	
		What do you do to prevent mosquito bites?	
3	Knowledge and perception about settlements, mosquito density and malaria transmission risks	Do you think the number of houses in an area can influence mosquitoes and malaria transmission?	Assessment of knowledge and perception of malaria transmission risks in relation to settlement patterns
		Do you think constructing houses near other houses or far from other houses is an important factor in regard to mosquito biting risk and malaria transmission?	
		Why do you think close house have many mosquitoes?	
		What can be done to control mosquitoes in such kind of environment?	

participants' knowledge on how various house characterizes and environmental variables that might influence malaria transmission, how the participants currently deal with those associations and whether the participants usually put in place any measures to address specific malaria risk factors associated with housing and the environment.

The questionnaires were translated and administered in Kiswahili, which is the common language spoken by communities across the study area. After pre-testing and correcting all issues arising, the questionnaire was administered by trained research officers to consenting household heads across three study villages where the entomological surveys had been done and in one village where there was no entomological surveillance. A total of 200 participants responded to the survey.

Focus group discussions: qualitative assessment of community knowledge, opinions and concerns regarding house designs and environmental characteristics, and how these factors influence malaria transmission

Study participants were selected from among the households participating in the entomological survey and recruited upon consent with help from the community leaders. A total of 12 people were recruited for each focus group, ensuring all participants were from the same village.

Eight focus group discussions (FGD) were conducted across the four study villages, each FGD comprised 12 participants recruited from the same village. Overall the study had a total of 96 participants aged between 18 and 70 years. There was an equal representation of men and women in each of the FGD groups (6 men and 6 women). The discussions were held between January and March 2017 and were conducted at local primary schools within the respective villages.

A study guide for the FGD was developed and pretested. The FGD guide followed the themes described in Table 1 for the questionnaire surveys. Adults and youths were separated during the interviews to avoid any chance of intimidation during the discussions. The purpose of the discussions was explained and verbal and written informed consents were obtained from the participants. The discussions were semi-structured: the facilitator asked a question and the participants discussed their knowledge, views and comments. The discussions were facilitated by two social scientists; a note taker and an interviewer, and were tape recorded to ensure that all information was captured. Discussions were all conducted in the local language (Swahili), and lasted between 40 and 60 min each.

The study assessed whether people think that the house designs matter in terms of the risk they are exposed to. The study then assessed if people care about environmental variables and if they worry about these factors when they construct their houses. In cases where people were concerned, the study also assessed how they cope with these concerns. Another concept investigated during the FGDs was whether participants were aware of any relationship between settlement patterns and malaria transmission. For example, the study examined whether the participants thought constructing houses near other houses or far from other houses was an important factor in regard to mosquito biting risk and malaria transmission.

Data analysis

Analysis of quantitative data

Data were analysed using open source software, R version 3.1.0, using the *lme4* package [37]. Associations between house characteristics and indoor mosquito densities were examined by generalized linear mixed effects models (*glmer)* [38], by fitting the data in log-linked Poisson error distributions. The indoor densities of *An. arabiensis*, *An. funestus*, *Culex* and *Mansonia* species were modelled as a function of different house and environmental characteristics. This study assessed effects of (a) wall type, (b) roof type, (c) window type, (d) whether chickens were kept inside or outside the house, and (e) whether the eave spaces were closed or open. Date of mosquito collection and house identification numbers were incorporated as random variables in the *glmer* models. Estimated mean indoor mosquito densities per house per night, the relative rates (RR) of observing these mosquito catches, and associated 95% confidence intervals (CI), were computed from exponentials of coefficients generated from the *glmer* models.

The monthly temporal patterns of biting risk by the two malaria vector species and also the culicine genera, i.e. *Culex* spp. and *Mansonia* spp. mosquitoes were presented graphically.

Data generated through the questionnaire surveys were summarized and presented descriptively in tables to capture: (a) knowledge about risk factors, (b) whether people worry about house and environmental characteristics and (c) whether they consider such variables when constructing their houses.

Processing and analysis of qualitative data

After the FGD session, all recordings were retrieved from the tape recorder and archived on a password-protected computer. A trained behavioural scientist then transcribed and translated the data from Kiswahili to English for further analysis. The scripts were then imported into NVivo software version 13 for organizing and indexing the data. Emergent themes were organized to assess:

(a) whether people worry about the characteristics of the house and if they consider such variables when constructing their houses, (b) whether people care about environmental variables and if they consider such variables when constructing their houses, (c) whether people know effects of seasonality on malaria transmission, (d) whether people know the relationship of settlement patterns and human population on malaria transmission, and (e) how the people cope with these risk factors if they are actually aware of relationships with malaria transmission. Direct quotes from the participants are presented to support their responses on the themes.

Results

Indoor densities of malaria and non-malaria mosquito vectors

A total of 129,052 mosquitoes were collected by CDC light traps and backpack aspirators indoors. Of these, 19.9% were *Anopheles* (n = 25,670) and 80.1% culicines (103,382). The 25,670 *Anopheles* mosquitoes included 20,254 *An. arabiensis* (78.9%) and 4800 *An. funestus* (18.7%), 205 *Anopheles coustani* (0.8%), 128 *Anopheles pharoensis* (0.5%), 103 *Anopheles squamosus* (0.4%), 77 *Anopheles ziemanni* (0.3%) and 103 *Anopheles wellcomei* mosquitoes (0.4%). Of 103,382 culicine mosquitoes collected, there were 100,047 *Culex* spp. mosquitoes (96.7%), 319 *Mansonia* mosquitoes (3.1%) and 138 *Aedes* mosquitoes (0.1%). The highest densities of host seeking mosquitoes were observed in Minepa village, where about 54% and 65% of all *An. arabiensis* and *An. funestus* mosquitoes were collected. The mean density (± se) of *An. gambiae* caught per village per night were, as follows: Minepa, 65 ± 20, Kivukoni, 19 ± 6 and Mavimba, 8 ± 7. Mean monthly densities of the indoor mosquito catches are shown in Fig. 4. Densities of *An. arabiensis* and *An. funestus* peaked between April and June in all

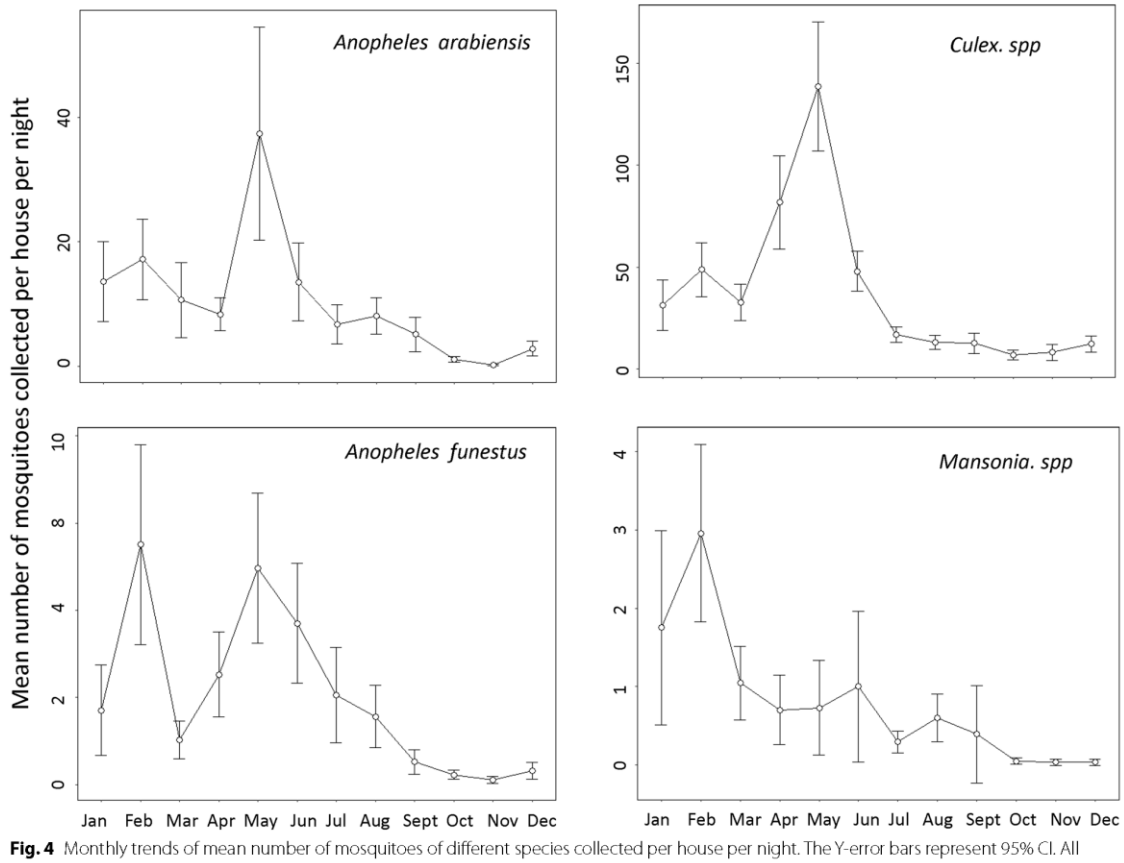


Fig. 4 Monthly trends of mean number of mosquitoes of different species collected per house per night. The Y-error bars represent 95% CI. All the species generally followed same trend peaking between April and June, except *Mansonia* spp., whose densities peaked between January and March

study villages but were generally low between September and November (Fig. 4).

Physical characteristics and microclimate of sampled houses

A variety of construction materials were observed in the local houses (Table 2). Most houses had walls made of mud (50.5%), un-plastered brick (48.6%) or brick and concrete plastering (1.0%). With regard to roofs, a significant proportion had grass-thatched roofing (60.6%), while the rest had corrugated iron sheet roofing (39.4%). At the time when mosquito traps were being set in the evenings, we observed that 64% of the houses had open doors at that time, possibly letting in mosquitoes.

Household risk factors associated with indoor mosquito densities

Houses with mud walls were significantly associated with higher vector densities compared to brick walls. *Anopheles arabiensis* densities were higher in mud-walled houses [RR = 3.9 (2.3–5.6), P < 0.005] and the number of *An. funestus* was also higher in houses with mud walls [RR = 4.0 (2.5–5.3.) P < 0.005]. *Culex* [RR = 2.3 (2.1–2.6), P < 0.001] and *Mansonia* species [RR = 2.5 (1.7–3.8), P < 0.005], were also more abundant in houses with mud walls than brick walls. Similarly, open eaves were significantly associated with higher indoor vector densities. There were more *An. arabiensis* [RR = 1.9 (1.8–2.0), P < 0.001] and more *An. funestus* [RR = 1.2 (0.9–1.6) P < 0.005] in houses with open eaves than houses with closed eaves. This study observed similar differences for *Culex* [RR = 1.9 (0.8–1.0), P < 0.001] and *Mansonia* species [RR = 1.5 (1.4–1.8), P < 0.001] in houses with open eaves relative to closed eaves. Tables 3 and 4 provide parameter estimates for different house characteristics and their significance in relation to mean catches of mosquitoes in the selected households. The number of *An. funestus* was significantly higher in houses with chicken indoors [RR = 2.0 (1.8–2.1), P < 0.001] compared to those without chicken, but no such association was observed with *An. arabiensis* [RR = 1.8 (1.8–1.9), 0.343]. Similarly, there were higher densities of *Culex* [RR = 1.2 (1.1–1.2), P < 0.001] and *Mansonia* mosquitoes [RR = 2.6 (2.4–2.7), P < 0.001] in houses with chicken indoors.

Table 2 Physical characteristics and microclimatic conditions in sampled houses

Variables assessed	Category	Percentage (N)
Wall type	Plastered brick walls	1.0% (6)
	Mud walls	50.5% (315)
	Un-plastered brick walls	48.6% (303)
Eave space	Closed	25.3% (158)
	Open	74.7% (466)
Roof type	Corrugated iron sheet	39.4% (246)
	Grass-thatched	60.6% (378)
Window covers	With netting screen	25.3% (158)
	Without netting screen	74.7% (466)
Door (observed from 6 pm to 7 pm)	Open	63.9% (399)
	Tightly closed	29.5% (184)
	Partially closed	6.6% (41)

Socio-demographic characteristics of survey participants

In the questionnaire survey, the study participants were 40.5% males and 59.5% females, with overall age range between 18 and 95 years (mean age = 36.9 years). The majority had acquired primary education and were farmers. All the demographic characteristics are shown in Table 5.

Results of the questionnaire survey: participants' knowledge regarding indoor mosquito density and how this relates to housing characteristics and environmental variables

Generally, most respondents associated malaria infections with poor house design features and physical conditions (Table 6). More than 90% of participants were aware of different environmental characteristics influencing mosquito density in their area. Similarly, 98.5% of respondents reported that their houses allowed mosquito entry. Houses with open eave space, unscreened windows and mud walls were generally associated with higher mosquito densities in the houses. The age of houses observed was fairly even, with 37% of the houses less than 4 years old, 37% more than 9 years old and the remainder being between 5 and 8 years old.

Focus group discussions: participants' knowledge regarding indoor mosquito density and how this relates to housing characteristics and environmental variables

In the FGDs, participants stated that house structure had an effect on number of mosquitoes entering their houses, and consequently on malaria transmission. The participants were concerned about living in poorly constructed, small houses with poor lighting and gaps on the eaves, walls, windows and doors. They mentioned that these gaps allow mosquito entry into their houses and expressed concern that they are frequently bitten by mosquitoes while indoors when they are not under bed nets. Most participants associated this situation with low household income, expressing concerns that they did not have enough money to construct proper houses.

Table 3 Summary statistics for mosquitoes caught in houses with different characteristics. The mean nightly catches of *An. arabiensis* and *An. funestus* mosquitoes, relative rates and the associated significance levels were calculated from the generalized linear mixed models (GLMMs) at 95% confidence interval

Variables	Category	*Anopheles arabiensis*			*Anopheles funestus*		
		Mean (CI)	RR (95% CI)	P value	Mean	RR (95% CI)	P value
Wall type	Bricks	9.3 (6.9–25.5)	1.0		3.8 (2.4–5.2)	1.0	
	Mud	17.7 (10.1–22.3)	3.9 (2.7–5.6)	0.005	5.0 (2.6–7.4)	4.0 (2.4–5.3)	0.011
Eave space	Closed	9.3 (4.2–14.4)	1.0		2.8 (1.3–4.2)	1.0	
	Open	19.2 (12.9–25.6)	1.9 (1.8–2.0)	< 0.001	4.9 (3.2–6.7)	1.2 (0.9–1.6)	0.005
Roof type	Iron sheets	14.4 (6.6–22.2)	1.0		3.3 (1.9–4.8)	1.0	
	Grass	18.2 (11.8–24.6)	1.5 (1.4–1.7)	< 0.001	5.1 (3.0–7.1)	2.4 (2.0–3.0)	0.318
Doors	Closed	12.6 (0.0–16.8)	1.0		1.6 (0.0–3.0)	1.0	
	Open	36.0 (9.5–42.5)	1.4 (0.9–2.1)	< 0.001	3.8 (2.4–5.2)	1.6 (1.3–2.0)	< 0.001
Window	Screened	15.8 (5.4–23.3)	1.0		4.2 (2.7–5.7)	1.0	
	Unscreened	17.1 (11.5–26.6)	1.9 (1.7–1.9)	< 0.001	4.9 (1.7–8.0)	2.9 (2.3–2.7)	0.130
Chicken indoors	No	15.3 (9.5–21.0)	1.0		4.2 (2.6–5.9)	1.0	
	Yes	20.4 (10.7–29.9)	1.8 (1.8–1.9)	0.343	4.8 (2.4–7.1)	2.0 (1.8 –2.1)	< 0.001

Table 4 Summary statistics for the number of mosquitoes caught in houses with different characteristics

Variables	Category	*Culex species*			*Mansonia species*		
		Mean (CI)	RR (95% CI)	P value	Mean	RR (95% CI)	P value
Wall type	Bricks	51.7 (45.2 – 86.9)	1.0		0.6 (0.3 – 1.5)	1.0	0.805
	Mud	66.2 (43.9 – 88.5)	2.3 (2.1 – 2.6)	<0.001	1.5 (0.4 – 2.5)	2.5 (1.7 – 3.8)	<0.005
Eave space	Closed	57.9 (27.7 – 88.1)	1.0		0.3 (0.1 – 0.6)	1.0	
	Open	67.2 (48.7 – 85.8)	1.9 (1.8 – 2.0)	<0.001	1.5 (0.7 – 2.3)	1.5 (1.4 –1.8)	<0.001
Roof type	Iron sheet	56.7 (33.9 – 79.4)	1.0		0.7 (0.1 – 1.4)	1.0	
	Grass	70.2 (48.7 – 91.7)	2.6 (2.4 – 2.7)	0.139	1.5 (0.6 – 2.3)	2.5 (1.8 – 2.4)	0.705
Doors	Closed	29.5 (0.0 – 79.9)	1.0		0.2 (0.0 – 0.9)	1.0	
	Open	56.2 (43.9 – 88.5)	2.3 (2.1 – 2.6)	<0.001	1.5 (0.4 – 2.5)	2.5 (1.7 – 3.8)	<0.005
Window	Screened	55.4 (23.2 – 86.6)	1.0		0.9 (0.0 – 2.0)	1.0	
	Unscreened	68.4 (50.3 – 86.5)	2.5 (2.4 – 2.6)	<0.001	1.3 (0.6 – 2.0)	1.8 (1.7 – 1.9)	<0.001
Chicken indoors	No	63.8 (46.2 – 81.5)	1.0		1.1 (0.5 –1.8)	1.0	
	Yes	67.4 (34.6 – 100.0)	1.2 (1.1 – 1.2)	<0.001	1.3 (0.0 – 2.7)	2.6 (2.4 – 2.7)	<0.001

The mean nightly catches of *Culex* and *Mansonia* species, relative rates and the associated significance levels were calculated from the generalized linear mixed models (GLMMs) at 95% confidence interval

"Many mosquitoes are found in those houses with smaller windows, in which there is less light because mosquitoes like dark places. Then mosquitoes enter the house and won't leave the area". (46 years old male, Milola village).

Other house characteristics implicated in mosquito biting risk were nearness to mosquito aquatic habitats and also height of the house. The participants suggested that it was necessary to construct houses with greater heights and far from water bodies to prevent this menace. Here are two examples of quotes from one of the participants on this subject.

"It is important to make sure that houses are built as far away from the mosquito aquatic habitats as possible. It is good to know the distance that mosquitoes can fly from their aquatic habitats so that we can know where to build houses". (37 years old male, Minepa village).

"It is necessary to build a house that makes it hard for mosquitoes to get inside. I know that houses that are built lower let mosquitoes in than the houses that have larger height". (26 years old male Milola village).

Table 5 Socio-demographic characteristics of participants who responded to the survey questionnaire to assess people's awareness of how house characteristics affect malaria transmission

Variables assessed	Category	Percentage (N)
Gender	Males	40.5 (81)
	Females	59.5 (119)
Age	18–35	48.7 (97)
	36–50	26.5 (53)
	51–65	15 (30)
	>65	20 (10)
Marital status	Married	66 (132)
	Unmarried	20 (40)
	Widow/widower	4.5 (9)
	Divorced	9.5 (19)
Level of education	No formal education	11.5 (23)
	Primary school	67.5 (135)
	Secondary school	18 (36)
	College/university	2 (4)
	Other trainings	1 (2)
Occupation	Peasant (self-employed in agriculture)	68.5 (137)
	Small scale business	4 (8)
	Formal employment	0.5 (1)
	Unemployed	0.5 (1)
	Other	26.5 (53)

There was also a strong linkage of environmental conditions with mosquito densities in houses. It was commonly mentioned that the proximity of the mosquito aquatic habitat and the state of the environment surrounding the houses had a lot of influence on the mosquito density, and consequently with higher number of malaria cases. Participants stated that mosquitoes liked to rest in dark, damp, grassy and bushy areas. Others said that mosquitoes liked to accumulate in dirty environments as the dirt and trash provided mosquitoes with good hiding places. There were general references to "dirty water", by which participants mostly meant stagnant water pools outdoors ("dirty water" = water not fit for drinking). This type of water, found near dwellings was commonly mentioned as being related to mosquito aquatic habitat. There was however no evidence that the community members knew the specific characteristics of aquatic habitat used by malaria mosquitoes as opposed to other mosquitoes. For example, two of the participants said:

> *"If the environment is dirty mosquitoes increase in number, because they are able to rest in trash. That means if we clean our surroundings then there will be very few mosquitoes. But if our environment is dirty mosquitoes reproduce and increase in numbers"* (58 years old male, Minepa village).

> *"Mosquitoes are found in dirty water around the house, like in water that is in broken containers or cups. They are also found in the ponds, shrubs and in dark places. I believe that is where they accumulate"* (34 years old female, Mavimba village).

An interesting theme that arose during the FGDs was how behaviours of neighbours was thought to influence vector densities and malaria transmission in houses that are in close proximity. Some participants thought that if the behaviours and practices of their neighbours were encouraging mosquito accumulate and survival, then those mosquitoes would also go to the other surrounding houses as well.

> *"When there are many houses together, it means that there is a lot of trash and other things in which mosquitoes can rest. For example, I may keep my surroundings clean, but if my neighbours do not clean their surroundings, and they live close to me, then mosquitoes can easily come from their houses to mine, it will make it easy for mosquitoes to bite me."* (37 years old female, Milola village).

Participants perceived that nucleated settlements encouraged malaria transmission more than dispersed settlements. Most participants expressed that when households were close to each other, mosquitoes could easily move from one house to another, easily transmitting malaria. According to the participants, when households are far apart then it would take time for mosquitoes to travel between the households. Here are comments from some of the participants:

> *"If there are many houses close together then mosquitoes can move from one house to another. But if houses are few and far apart then the mosquitoes cannot really get from one house to another easily, because there can be many obstacles for them on the way, like the wind and rain"* (40 years old female, Milola village).

> *"When there are a lot of houses together then it is a problem for us because this way many mosquitoes come, because they know that they can get a lot of people in close proximity. It is easy for the mosquitoes to transmit malaria" (23 years old male, Kivukoni village).*

> *"If houses are scattered, then the wind will blow mosquitoes to other places where they cannot easily get people"* (51 years old male, Milola village).

Table 6 Summary of community members' knowledge about indoor mosquito density and how these relate to certain housing and environmental characteristics

Variables assessed	Response category	Percentage (N)
Whether participants believe their house let in mosquitoes	Yes	98.5% (197)
	No	1.5% (3)
How participants believed mosquitoes entered their houses	Through open doors	28.5% (57)
	Through windows	32.5% (65)
	Through holes in the wall	3.0% (6)
	Through the eave space	19.5% (39)
	Through both open windows and doors	13.5% (27)
	Through open window doors, holes in walls and eaves	2% (4)
	Others	1% (2)
Age of house (date of construction)	<4 years ago	36.5% (73)
	5–8 years ago	26.5% (53)
	>9 years ago	37.0% (74)
Main reasons for constructing the kind of house	Because it is permanent	36.5% (73)
	Because it prevents animals from entering the house	26.5% (53)
	Because it prevents insects	17.0% (34)
	Others	20.0% (40)
Whether people consider mosquito prevention as a key factor when constructing houses	Yes	58.5% (117)
	No	40.0% (80)
	Does not know	1.5% (3)
Specific practices considered by participants (during construction of their houses) for preventing mosquitoes	Netting on the window	38.0% (76)
	Blocking the eaves	8.0% (16)
	Using bricks on the wall	2.0% (4)
	Using cement on the wall	1 (2)
	Does not know	38.5% (77)
	Others	12.5% (25)
Whether participants knew open eave spaces let in mosquitoes	Yes	96.0% (192)
	No	4.0% (8)
Whether participants knew that surrounding environments influence vector densities in their houses	Yes	97.0% (194)
	No	3.0% (6)

Insecticide-treated bed nets were the main form of protection used against mosquito bites and malaria transmission. All participants reported that they used bed nets regardless of the season. Other means of protection mentioned were cleaning the environment, drying up ponds when possible, covering up with clothing when people are outdoors, using insecticide repellent lotions and indoor sprays and going indoors early. There were however concerns about the integrity of the available nets and how this influences their efficacy. Examples of relevant quotes included:

> *"One thing that most of the people in the village can afford is to use bed nets, because everyone has bed nets here. But in order for these nets to be useful they have to be in a good shape. Sadly not everyone has new nets. I sleep with my children in the same bed because we have one net, but our net is not in a very good state, it has a lot of holes that sometime let mosquitoes in. But it still helps; it is not the same as if we did not use anything"* (31 years old female, Kivukoni village).

> *"We make sure that children are protected from mosquito bites by making sure they go to bed early when it gets dark, and then we make sure they are covered by bed nets. Sometimes when they are sitting outside then we use a piece of cloth to chase mosquitoes away"* (54 years old male, Milola village).

Lastly, there was generally a high level of understanding regarding seasonality and malaria transmission. Participants mentioned that though mosquito numbers were generally high in the area, most biting and malaria transmission occurred in the rainy season, especially February

through May, during the long rains. Here are some of the responses from the participants:

> *"Mosquitoes are found here throughout the year. However, their population is the highest during the rainy season because there is a lot of water around, hence many* aquatic habitat. *During the dry season there are fewer places where mosquitoes accumulate, so their population is lower"* (28 years old male, Milola village).

> *"There are a lot of mosquitoes from February to April because this is the time where there is a lot of water everywhere. There are many pools, ponds and bushes where mosquitoes can accumulate. The mosquito population starts to go down from May and June when the weather starts to get dry and cold"* (24 years old male, Minepa village).

Discussion

Housing design has been shown in different studies to significantly influence malaria transmission [30–32]. Most people in rural and peri-urban areas live in poorly finished houses with open eaves, doors and windows, hence exposure to malaria transmission is very high. Mosquitoes enter the houses easily through eaves and other openings, thus increasing likelihood of biting and pathogen transmission [29, 30]. This study found that the number of mosquitoes were significantly higher in houses with open eaves, grass roofs, Mud walls and unscreened windows. Furthermore, keeping chickens inside the house was also associated with high number of mosquitoes. Unfortunately, these communities cannot afford essential house construction and mosquito-proofing materials. Greater sensitization and enabling financing initiatives to promote better housing should therefore be considered in vector control initiatives to accelerate malaria elimination efforts. This study found statistically higher indoor densities of the main malaria vectors (*An. arabiensis* and *An. funestus*) in houses with mud-walls as opposed to plastered or brick walls, open as opposed to closed eaves, and also in unscreened as opposed to screened windows. Keeping chickens inside the house was similarly associated with higher indoor densities.

There was already very high levels of awareness about how housing characteristics and the surrounding environment influence mosquito densities, and consequently malaria transmission. Another factor identified was the small size of houses (area and height). In additional to poorly-constructed houses with gaps in the eaves, walls, windows and doors. However, people were constrained by low income and could not readily afford construction of properly ventilated and mosquito-proof houses. Nearly all respondents reported that their houses allowed mosquito entry, but they could not afford modern building materials. They expressed that they are also being bitten by mosquitoes while indoors when they are not under bed nets. The relationship between poor housing and low-income is expected in such settings. However, although many households in rural areas are unable to afford modern housing, there are cheaper and simple ways of modifying or screening houses which could readily prevent mosquito entry, especially if the community members are adequately sensitized and financial barriers removed, e.g. through subsidies. Unfortunately, malaria control authorities in sub-Saharan Africa have not invested in house improvement programmes despite the significant available evidence [39, 40].

Community knowledge on mosquito aquatic habitat is another important factor to consider for successful community-based vector control. In this study, participants of the FGD were identified various sources of mosquitoes in their surroundings, such as stagnant water pools, dense vegetation and rice fields. Studies elsewhere have yielded similar findings [41, 42], and such high levels of community awareness can be harnessed to improve disease prevention and control initiatives. In rural south-eastern Tanzania, the nucleated settlement patterns, combined with the previously described spatial distribution of vector densities across the villages, indicate that targeted interventions if supported by local community champions could indeed be highly effective. A study done by Mwangungulu et al. in the same villages yielded evidence that community members are capable of identifying places with low, medium and high mosquito densities [22]. Mosquitoes accumulate in shallow pools of water and puddles [43, 44], which are numerous in rural areas especially during the rainy season. Draining pools of water, levelling land, construction of drains and proper waste management could eliminate mosquito aquatic habitat around homes [45, 46], and subsequently reduce malaria transmission [47]. Given the existing high-levels of awareness, further control initiatives involving environmental management could be improved if community members are considered the main stake holders. Such community-based approaches could also be used to scale-up better housing designs and access to commodities necessary for screening windows, eaves and doors even in rural, low-income areas.

Community knowledge, perception and practices are very important for designing or improving disease control programmes and identifying factors for effectiveness of the interventions [48]. Promoting community based programmes can help to achieve population level change in risk behaviours which in turn results into

positive changes [49]. Unless communities recognize the importance of changing behaviours which influence disease transmission, the best designed interventions might not achieve the intended goals. More broadly in primary health care, it has been demonstrated in places such as Tigray, Ethiopia, that teaching mothers about home-based malaria treatment and management can vastly improve treatment outcomes and reduces child mortality [50]. In this particular programme in Ethiopia, reduction of up to 40% in under-five mortality was achieved in holoendemic malaria areas, by simply training and relying upon local mothers to teach each other how to dispense anti-malarial medicines to their children.

Another important point that emerged from this study was participants' impression that if the houses are close to each other, malaria transmission is likely to be higher because mosquitoes can easily move between houses, and spread pathogens. Though participants were not able to describe settlement patterns, they perceived that closer and congested household are at higher risk of malaria transmission, and were concerned with the distances that a mosquito can move from one house to another. Previous study suggested strong correlations between household occupancy and malaria vector densities [25], but no specific analyses have been done on effects of distance between households on malaria transmission. By combining the new findings from this current study with the observations previously described by Mwangungulu et al. [22] and Kaindoa et al. [25], community driven intervention could be designed, which relies on trained locals to support environmental management and house improvement initiatives for mosquito control [51]. For example, community-based artisans, if provided minimal government support, could create local businesses that support sustainable house-improvement programs with potential benefits against vector-borne diseases.

Conclusion

To improve malaria control and elimination efforts, it is important to develop an understanding of community perspectives on persistent malaria transmission within and around their villages, then engage these communities in identifying and addressing the key factors contributing to the persistent transmission. This study has shown that in rural south-eastern Tanzania, significant proportions of people still live in houses with open eaves, unscreened windows and gaps on doors. Though the people are fully aware of associated mosquito biting and pathogen transmission risks, they are constrained by low-income levels and cannot readily afford better housing or house improvement (e.g. through screening of eaves and windows), alongside other competing priorities. This study concludes that community-based house improvement initiatives combined with targeted subsidies could lower the financial barriers, improve access to essential construction materials or designs, and significantly accelerate malaria transmission control in these communities.

Authors' contributions

EK and FO conceived the study and developed study protocols and supervised data collection. EK, MF, JK, conducted data analysis, EK, MF, JK, AN, MC and FO wrote the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The dataset generated by this study is available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Meetings with local leaders were held in the study areas and the main aims of the study were explained by the research team. A written and signed informed consent was obtained from each study participant. All information was given in Kiswahili, the local language. Ethical approval for the study was obtained from Ifakara Health Institute Institutional Review Board (IHI/IRB/No.: 029-2016), and from the Medical Research Coordinating Committee (MRCC) at the National Institutes of Medical Research (NIMR) (Ref: NIMR/HQ/R.8a/Vol.IX/2428). Approval was also obtained from the Human Research Ethics Committee (Medical) at University of the Witwatersrand (Approval Certificate No.: M160806). Study household were provided with new LLINs as previously distributed by the universal bednets coverage campaign. All personal information that could identify the study participants was omitted from the final report so as to protect identification of respondents. The participants were assured anonymity in the presentation and publishing of the data. Permission to publish this manuscript was obtained from the National Institutes of Medical Research (NIMR) (Ref: NIMR/HQ/P.12/VOLXXV/).

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Appendix 3: Original paper III

PLOS ONE

RESEARCH ARTICLE

Interventions that effectively target *Anopheles funestus* mosquitoes could significantly improve control of persistent malaria transmission in south–eastern Tanzania

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Abstract

Malaria is transmitted by many *Anopheles* species whose proportionate contributions vary across settings. We re-assessed the roles of *Anopheles arabiensis* and *Anopheles funestus*, and examined potential benefits of species-specific interventions in an area in south-eastern Tanzania, where malaria transmission persists, four years after mass distribution of long-lasting insecticide-treated nets (LLINs). Monthly mosquito sampling was done in randomly selected households in three villages using CDC light traps and back-pack aspirators, between January-2015 and January-2016, four years after the last mass distribution of LLINs in 2011. Multiplex polymerase chain reaction (PCR) was used to identify members of *An. funestus* and *Anopheles gambiae* complexes. Enzyme-linked immunosorbent assay (ELISA) was used to detect *Plasmodium* sporozoites in mosquito salivary glands, and to identify sources of mosquito blood meals. WHO susceptibility assays were done on wild caught female *An. funestus* s.l, and physiological ages approximated by examining mosquito ovaries for parity. A total of 20,135 *An. arabiensis* and 4,759 *An. funestus* were collected. The *An. funestus* group consisted of 76.6% *An. funestus* s.s, 2.9% *An. rivulorum*, 7.1% *An. leesoni*, and 13.4% unamplified samples. Of all mosquitoes positive for *Plasmodium*, 82.6% were *An. funestus* s.s, 14.0% were *An. arabiensis* and 3.4% were *An. rivulorum*. *An. funestus* and *An. arabiensis* contributed 86.21% and 13.79% respectively, of annual entomological inoculation rate (EIR). *An. arabiensis* fed on humans (73.4%), cattle (22.0%), dogs (3.1%) and chicken (1.5%), but *An. funestus* fed exclusively on humans. The *An. funestus* populations were 100% susceptible to organophosphates, pirimiphos methyl and malathion, but resistant to permethrin (10.5% mortality), deltamethrin (18.7%), lambda-cyhalothrin (18.7%) and DDT (26.2%), and had reduced susceptibility to bendiocarb (95%) and propoxur (90.1%). Parity rate was higher in *An. funestus* (65.8%) than *An. arabiensis* (44.1%). Though *An. arabiensis* is still the most abundant vector species here, the

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remaining malaria transmission is predominantly mediated by *An. funestus*, possibly due to high insecticide resistance and high survival probabilities. Interventions that effectively target *An. funestus* mosquitoes could therefore significantly improve control of persistent malaria transmission in south–eastern Tanzania.

Background

Malaria continues to be one of the most significant mosquito-borne parasitic diseases, affecting about 212 million people, causing 429,000 deaths annually [1], and adversely affect socio-economic development in sub-Saharan African countries [2]. The World Health Organization (WHO) estimates that there has been a decline in malaria burden, and that morbidity worldwide reduced by 21% and mortality by 29% between 2010 and 2015, but sub-Saharan Africa still accounts for approximately 92% of all malaria deaths and cases [1]

Tanzania has experienced a decline in malaria transmission following the introduction of insecticide treated nets (ITNs) and the scale-up of long-lasting insecticide treated nets (LLINs) [3]. By 2010, the country had made significant progress, and most areas that were experiencing prevalence above 50% in 2000, now had below 10% prevalence [4]. However, the most recent malaria indicator survey has returned an average prevalence of 14.8% prevalence in children under 5 years in Tanzania [5]. Getting to zero transmission therefore remains a major challenge, mainly because the persistent malaria transmission is mediated by mosquitoes that are not adequately responsive to control by existing indoor insecticidal interventions, such as LLINs and indoor residual spraying (IRS). The Tanzanian National Malaria Control Program is aiming at cutting down malaria transmission to 1% by 2020 [6], thus the need for new complementary approaches is even more urgent.

Despite the high coverage of LLINs, high indoor densities of potentially infectious mosquitoes remain common in some places [7, 8]. Evidence suggests that indoor densities of major malaria vectors such as *An. funestus* and *An. arabiensis* depend on house design features, environmental conditions and demographic composition [8, 9]. Previous studies have reported high levels of resistance in *An. funestus* to pyrethroids in Tanzania [10] and elsewhere in East Africa [11–13]. *An. funestus* is one of the major malaria vectors in Africa and is widely distributed across the continent [14, 15]. The species is highly anthropophilic and endophilic, with higher vectorial capacity than most other vector species [14, 16]. The *An. funestus* group includes several sibling species, which are difficult to distinguish morphologically, but can be separated by polymerase chain reaction (PCR) using a cocktail of species-specific primers [17]. Previous studies in Tanzania have reported presence of four member species in the *An. funestus* group, namely *An. funestus* s.s, *An. rivulorum*, *An. leesoni* and *An. parensis* [18]. All the four members of the *An. funestus* group have been found positive for *P. falciparum* though the infection rates are often higher in the predominantly anthropophagic *An. funestus* s.s than the rest. The importance of this vector is increasingly associated with the persistent malaria transmission [8, 10], and it has been suggested that it could be responsible for resurgence of malaria transmission in rural Tanzania [10].

In this study, we assessed contributions of residual populations of major malaria vectors, *An. arabiensis* and *An. funestus*, in endemic villages in rural south-eastern Tanzania, where the last mass distribution of LLINs prior to our study had been completed in 2011 [19]. The study assessed prevalence of *Plasmodium* sporozoites in the vector species, survival rates of these vectors, and their resistance status to examine what factors might be mediating the role of these

two major species in the ongoing malaria transmission. Inferences are made with regard to what would happen if appropriate species-specific interventions were deployed in this and similar settings. More specifically, interventions that effectively target mosquito species mediating most of the remnant malaria transmission should result in extensive declines in the transmission. Where one vector species dominates the transmission system, approaches against such vectors may offer opportunities to sufficiently disrupt local malaria transmission.

Methods

Study area

The study was conducted in three villages in the Kilombero floodplains in Ulanga district, south-eastern Tanzania (Fig 1). This area is perennially meso-endemic for malaria and has high mosquito densities throughout the year, peaking between January and May. Annual rainfall and temperatures range from 1200 to 1800 mm and 20˚C to 32.6˚C respectively. Malaria vector species in the area comprise primarily of the *An. gambiae s.l* (almost exclusively consisting of *An. arabiensis)*, and *An. funestus group*. Several other *Anopheles* mosquitoes such as *An. coustani*, *An. pharoensis*, *An. squamosus*, *An. ziemanni* and *An. wellcomei* are also found, as well as several culicine species, mainly *Mansonia* mosquitoes, *Aedes* mosquitoes and *Culex* species. The major vector control intervention in this area is LLINs [19], and the last mass-distribution of nets, prior to this study had been conducted between 2010 and 2011 [19].

Study procedures

This study was part of a high-resolution longitudinal mosquito surveillance program, where we collected host-seeking and indoor-resting mosquitoes between January 2015 and January 2016, in randomly selected households, and also selected sentinel households within the Health and Demographic Surveillance System (HDSS) maintained by Ifakara Health Institute in the study area [20].

Monitoring indoor densities of host-seeking and resting disease-transmitting mosquitoes. Firstly, 1600 households were randomly selected from the HDSS households database in three study villages of Kivukoni, Minepa and Mavimba, and assigned to 16 geographical clusters each consisting of 100 households. The sampling clusters were assigned based on household latitudes so that clusters 1–16 were arranged in a north-south direction in the study area. From each geographical cluster, six households were selected for sampling. These included three households randomly selected on monthly basis, and another three households that were fixed as sentinel households, visited repeatedly every month. This way, 96 households were studied per month, each time consisting of 48 sentinel households across the study area, and 48 new households randomly selected each month. Geo-positions of all the households were recorded using hand held GPS receivers (Magellan eXplorist 110). Each household was visited only once each month, and mosquitoes were sampled from one room with occupant(s) sleeping under mosquito nets, using miniature Centre for Disease Control (CDC) light traps set near occupied bed nets at the foot end, at approximately 1.5 m from the ground [21, 22]. The traps were operated from18:00 to 06:00 each night.

Indoor resting mosquitoes were collected using a backpack aspirator [23] from the walls, bed nets and floors in same households where the CDC light trap catches were conducted. Aspiration of resting mosquitoes was conducted for approximately 15 minutes in each household. Each morning, the collected mosquitoes were killed in a closed container using alcohol. The mosquitoes were identified morphologically and sorted by taxa and sex before they were identified by PCR. The identification was done following the taxonomic key developed by Gillies and Coetzee, i.e. Keys for the *Anopheles* of Africa, south of Sahara [24].

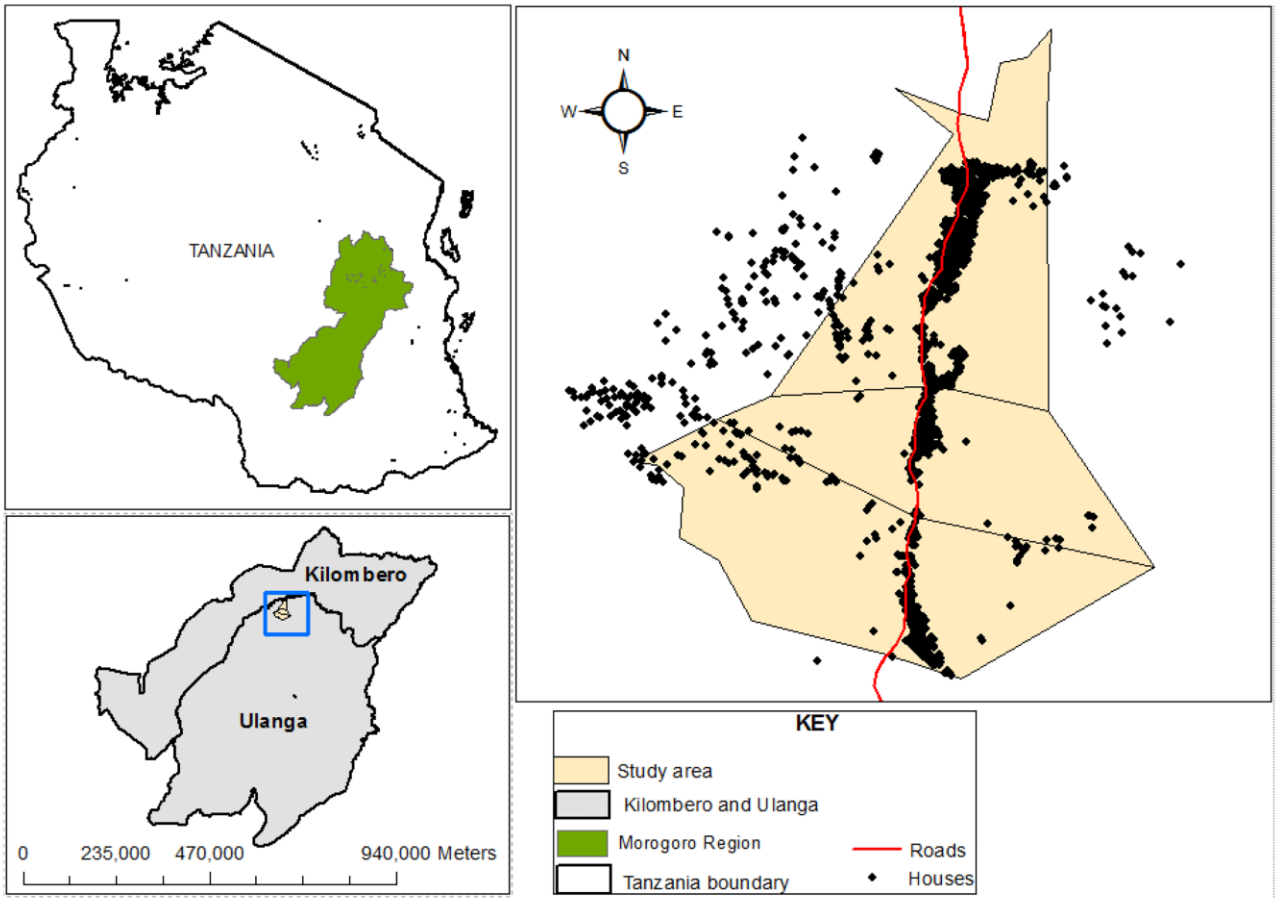


Fig 1. Map of the study area, showing the villages and households in Ulanga district where the study was conducted.

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Identification of sibling species and detection of Plasmodium infection in malaria vectors. All female *An. gambiae* complex mosquitoes, were examined by multiplex polymerase chain reaction (PCR) technique as described by Scott *et al.* [18] to distinguish sibling species. Similarly, all samples of *An. funestus* were examined by PCR, using techniques developed by Koekemoer *et al.* [25] and Cohuet *et al.* [26] to distinguish sibling species. All *Anopheles* mosquitoes caught were segregated by species and examined in pools by enzyme-linked immunosorbent assays (ELISA), to detect *Plasmodium* sporozoites in their salivary glands [27]. To avoid false positives, the ELISA lysates were boiled for 10 minutes at 100˚C, effectively eliminating heat-labile non *Plasmodium falciparum* protozoan antigens [28]. We assessed all the collected *Anopheles gambiae* s.l and *Anopheles funestus* s.l mosquitoes by ELISA, using heads and thoraces. However, only a sub-sample was assessed by PCR for species identification, using legs.

Identification of mosquito blood meals. Blood meal analysis was also done on a sub-sample, working only with the mosquitoes that were blood-fed. Each mosquito abdomen was separated from the thorax and homogenized in phosphate buffer saline (PBS) following the method described by Beier *et al.* [29]. This way, all the blood-fed mosquitoes were screened for human, bovine, chicken, goat and dog blood using ELISA.

Assessment of insecticide susceptibility and parity rates. The insecticide susceptibility tests were conducted using standard WHO guidelines [30]. Insectary-reared *An. gambiae* s.s (Ifakara strain) was used as a reference susceptible population. Because of the difficulties in finding *An. funestus* larvae in the study area, adult female mosquitoes were collected indoors from April to July 2016, by CDC light traps from the same households in three villages. The mosquitoes were transferred into netting cages and maintained on 10% glucose solution. *An. funestus* mosquitoes were then separated from other mosquito species by gently sucking them from the cages into a small-netting cage using mouth aspirators and all other mosquito species were killed.

Non blood-fed adult *An. funestus* s.l females were therefore exposed to 0.75% permethrin, 0.05% deltamethrin, 0.05% lambda cyhalothrin, 4% dieldrin, 4% DDT, 0.1% bendiocarb, 0.1% propoxur, 0.25% pirimiphos methyl, and 1% malathion, as recommended by the WHO. Up to 100 adult females of unknown age were exposed to each insecticide during the experiment, including at least four replicates. In each test, mosquitoes collected from the same villages at the same time were exposed to oil impregnated papers, constituting additional controls. The number of knocked-down mosquitoes in each tube was counted and recorded after 10, 15, 20, 30, 40, 50 and 60 minutes. After one hour of exposure, mosquitoes were transferred to the holding tubes and maintained on 10% sugar solution then monitored for survival and mortality after 24 hours. The post exposure observation was conducted after 80 minutes only if the knockdown was less than 80% [30].

Physiological age of the wild-caught female malaria vectors was approximated based on the status of their ovaries, i.e. whether they had previously laid eggs or not [31]. Abdomens of a randomly selected sub-samples of unfed female *An. funestus* and *An. arabiensis* collected from the selected households were dissected for parity [32]. Each unfed female mosquito was first anesthetized in a refrigerator. A drop of distilled water was added to a slide and each specimen kept still on the slide, then the seventh and eighth abdominal segment was pulled using a fine needle under a stereo dissecting microscope. The ovarial tracheoles were then observed under a compound microscope at 10X magnification, to determine whether they were parous or nulliparous [31].

Data analysis

Statistical analyses were carried out using R statistical software version 3.3.1 [33]. Mosquito abundance was over-dispersed so we modeled it following a negative binomial distribution using the generalized linear mixed model automatic differentiation model builder (glmmADMB) package [34] with log link function. Here, mosquito abundance (nightly total count) were treated as response variable while locality (villages) were treated as fixed effect.

Plasmodium infection rates and blood meal sources of malaria vectors were estimated as percentages of total mosquitoes assayed. Similarly, observed mean mortality after 24 hours of exposure to each insecticide was calculated as percentage of total mosquitoes exposed in all four exposure replicates [30]. The insecticide susceptibility test findings were interpreted according to WHO guidelines [30].

The EIR was calculated as a function of biting rates and proportion of tested mosquitoes infected with sporozoites. Since we initially calculated these biting rates for each trap night, the EIR was adjusted by multiplying the daily EIR values by 365 nights (Annual EIR = Nightly biting rate x Sporozoite rate x 365). We relied on values comparative trap evaluations done in the same study area, where CDC light traps had been directly compared to human landing catches inside households [35]. Those earlier trials had indicated that CDC light traps consistently caught 0.3 times as many *An. gambiae* s.l as human landing catches, and 0.68 times as many

An. funestus mosquitoes as human landing catches [35]. In this current study, we have therefore adjusted the CDC light trap catches using these coefficients, but also presented the unadjusted results.

Ethical considerations

Meetings with local leaders were held in the study areas and the main aims of the study were explained by the scientists. Permission to conduct studies in the separate villages was obtained from respective leaders of each of the villages; Mr. Ayubu Salila, Mr. Kiluka Mdumba and Mr. Baalesa Ligola for Kivukoni, Minepa and Mavimba villages respectively. I confirm that the field studies did not involve endangered or protected species. A written and signed informed consent was obtained from each household head participating in the study. All information was given in Kiswahili, the local language. All the households were provided and protected by intact LLINs (Olyset® nets) as previously distributed by the universal bednets coverage campaign [36]. Ethical approval for the study was obtained from Ifakara Health Institute Institutional Review Board (IHI/IRB/No: 34–2014), and the Medical Research Coordination Committee of the National Institute of Medical Research (Certificate No.NIMR/HQ/R.8a/Vol. IX1903).

Results

Indoor densities of *Anopheles* mosquitoes in the area

A total of 25670*Anopheles* mosquitoes were collected by CDC light trap and back pack aspirator, comprising *An. funestus* s.l (18.7%) and *An. gambiae* s.l, which in turn comprised of *An. arabiensis* sibling species (79.0%), *An. pharoensis* (0.5%), *An. squamosus* (0.4%), *An. ziemanni* (0.3%) and *An. wellcomei* (0.4%). The highest densities, i.e. 54% and 65% of all *An. arabiensis* and 65% of all *An. funestus* were observed in the middle of study area, i.e. Minepa village. Densities of *An.* peaked between May, while the density of *An. funestus* peaked in February and May.one month after onset of the long rainy season, which is the main malaria transmission season. The lowest vector densities were observed between September and November (Fig 2).

Sibling species of malaria vectors and *Plasmodium* infection rates

A total of 20,135 *An. gambiae* s.l and 4,759 *An. funestus* s.l mosquitoes were assayed in the laboratory by PCR or ELISA or both. All the *An. gambiae* s.l. mosquitoes tested by PCR were confirmed to be *An. arabiensis* (100%). On the other hand, the *An. funestus* group consisted of 76.6% *An. funestus* s.s, 2.9% *An. rivulorum*, 7.1% *An. leesoni*, and 13.4% unamplified samples. Monthly variations of proportions of the *An. funestus* sibling species is shown in Fig 3.

The CDC-light trap catches were used to estimate malaria transmission densities as Entomological Inoculation Rates (EIR) as shown in Table 1. Infection rates were higher *in An. funestus* than *An. arabiensis*. Using unadjusted estimates, the overall EIR (bites per person per year) was 9.19, of which *An. funestus* contributed 86.21%, while *An. arabiensis* contributed 13.79% (Table 1). However, when considering estimates adjusted on the basis of relative efficiencies of CDC light traps compared to Human Landing Catches, the overall EIR (bites per person per year) was 15.87, of which *An. funestus* contributed 73.39%, while *An. arabiensis* contributed 26.61% (Table 1). The highest sporozoite rate among *An. funestus* was in April, when 13.79% towards the transmission. Nightly biting rate among *An. funestus* was highest in May, i.e. 344 bites/month, while the highest biting rate in *An. arabiensis* was 2090 bites/month in the same month (Table 2). Of all *An. funestus* s.l mosquitoes that tested positive for malaria

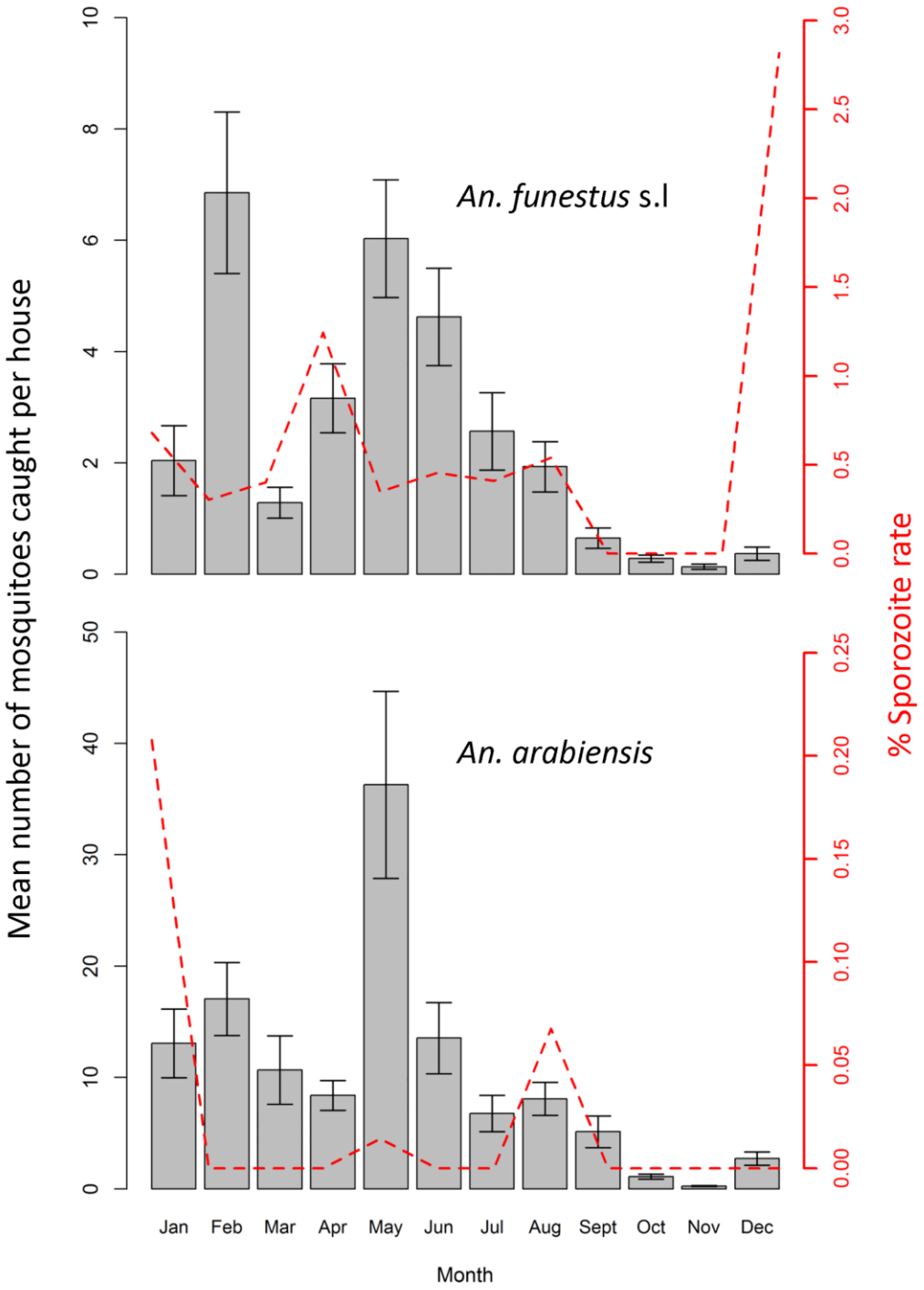


Fig 2. Mean number of *Anopheles funestus* s.l *and Anopheles arabiensis* mosquitoes caught per house per night. Y error bars represent the 2-standard errors of the mean.

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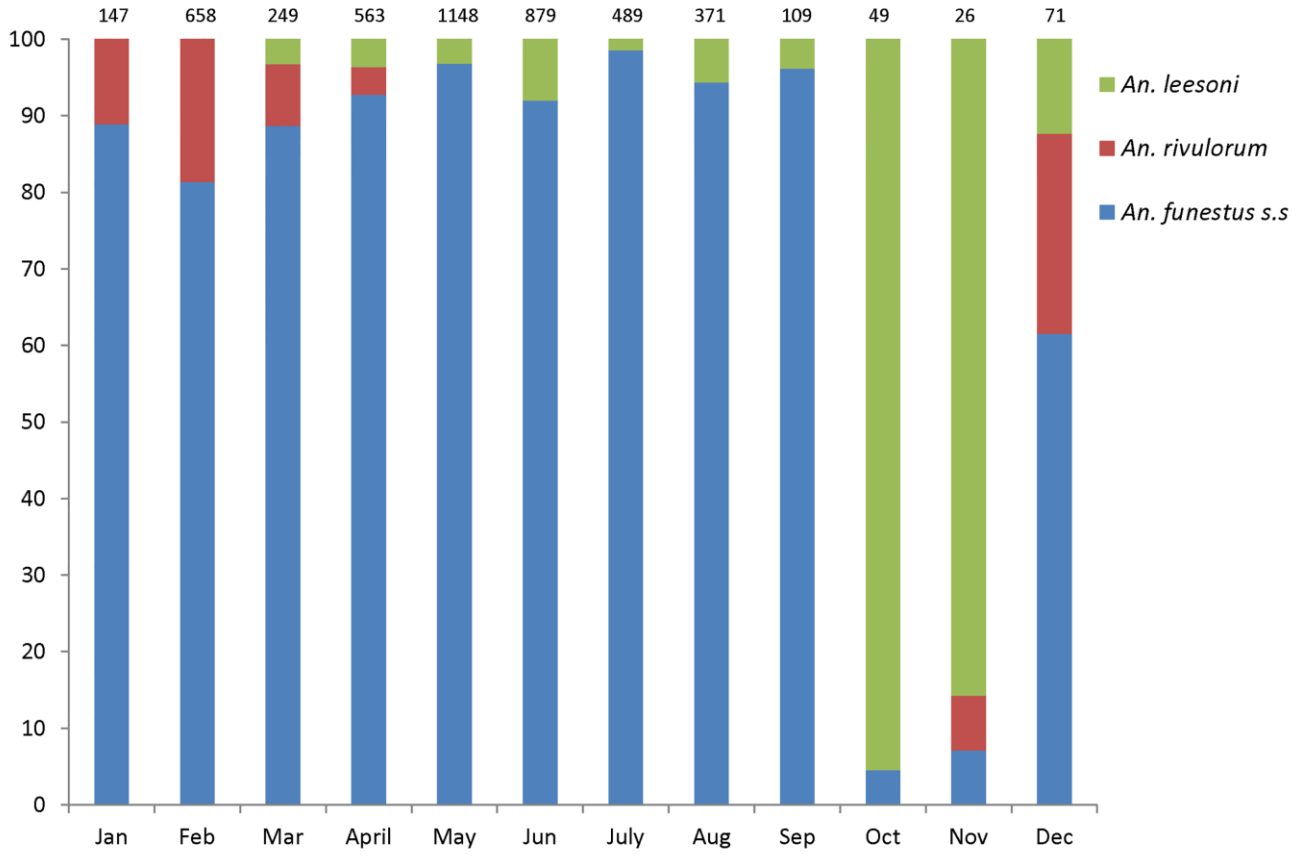


Fig 3. Monthly variations in the composition of sibling species of *Anopheles funestus* group in the study area.

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parasites, 96% were *An. funestus* s.s, the remaining 4% being *An. rivulorum*. None of the *An. leesoni* tested positive for *Plasmodium* sporozoites.

Indoor densities of Culicine mosquitoes in the area

A total of 103,382 Culicine mosquitoes, comprising of 100,047 *Culex* (96.7%), 3197 *Mansonia* (3.1%) and 138 *Aedes* mosquitoes (0.1%). Of all the *Culex* mosquitoes collected, males contributed 76.3%, while females contributed 23.7%. For *Mansonia*, males contributed 58.6% while females contributed 41.4%. Lastly, *Aedes* males contributed 26.1% while females were 73.9%.

Mosquito blood meal sources

A total of 82 mosquitoes that were identified by PCR also had blood meals in their abdomen, so these were assessed by way of blood-meal ELISA. These included 64 *An. arabiensis*, 17 *An. funestus* s.s, and 1 *An. leesoni*. The study identified a broad spectrum of host blood antigens, mostly humans (79.2%), followed by cattle (17.1%), dogs (2.4%) and chicken (1.2%). The analysis did not detect any mixed blood meals. The results for mosquito blood meals are summarized in Table 3.

Table 1. Infectious status of malaria vectors and dominant contribution of *Anopheles funestus* mosquitoes to ongoing malaria transmission, despite their low abundance relative to *Anopheles arabiensis*.

	Anopheles arabiensis	*Anopheles funestus s.l*
Total number of mosquitoes collected by CDC Light Trap (January 2015 to January 2016)	20135	4759
Total number of trap nights	1152	1152
Biting rate per night	17.48	4.13
Relative efficiency (CDC-LT) relative to HLC (Derived from Okumu et al 2008 [38])	0.3	0.68
Corrected biting rate	58.26	6.08
Total number of mosquitoes analysed for *Plasmodium falciparum* circumsporozoite protein (CSP)	20135	4759
Total number of sporozoite positive mosquitoes	4	25
Sporozoite rate	0.0002	0.0053
Annual EIR (Adjusted)**	**4.22**	**11.65**
% EIR Contribution (Adjusted)**	**26.61%**	**73.39%**
Annual EIR (not adjusted)	**1.27**	**7.92**
% EIR Contribution (not adjusted)	**13.79%**	**86.21%**

• Annual EIR (Corrected biting rate x Sporozoite rate x 365)

• Overall EIR (EIR for *An. arabiensis*+ EIR for *An. funestus)* = 18.45

• *86.3% of the mosquitoes from the *An. funestus group* tested were *An. funestus s.s*, 8.4% were *An. leesoni* and 5.2% were *An. rivulorum*. Of all *An. funestus* s.l mosquitoes that tested positive for malaria parasites, 96% were *An. funestus s.s*, the remaining 4% being *An. rivulorum*.

• **Where the estimates are adjusted, these adjustments were done using coefficients computed as the ratio of mosquito catches by CDC-Light Traps to catches Human Landing Catch methods. These coefficients were 0.3 for *An. arabiensis* and 0.68 for *An. funestus* as determined by Okumu et al 2008 [35]

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Spatial distribution of sporozoite infected *An. funestus* and *An. Arabiensis*

Sporozoite infected *An. funestus* were widely distributed across all the study villages, it was also observed that *An. arabiensis* were mostly confined to an area in the middle of the study area (Fig 4). The total number of sporozoite infected mosquitoes during the year was, however, too small to enable detailed analysis of spatial relations.

Table 2. Monthly sporozoite rates and Entomological Inoculation Rates (EIR) (number of infectious bites per person per month) for *Anopheles funestus* and *Anopheles arabiensis* from January to December 2016.

	Anopheles funestus				*Anopheles arabiensis*			
Month	**Number of Mosquitoes Tested**	**Monthly biting rates (adjusted**)**	**Sporozoite Infection Rate**	**Mean Monthly EIR**	**Number of Mosquitoes Tested**	**Monthly biting rates (adjusted **)**	**Sporozoite Infection Rate**	**Mean Monthly EIR**
January	147	44.10	0.0219	0.97	963	288.90	0.0068	1.96
February	658	197.40	0.0107	2.12	1640	492.00	0.0000	0.00
March	249	74.70	0.0129	0.96	2111	633.30	0.0000	0.00
April	563	168.90	0.0413	6.98	1502	450.60	0.0000	0.00
May	1148	344.40	0.0113	3.89	6968	2090.40	0.0005	0.94
June	879	263.70	0.0153	4.04	2576	772.80	0.0000	0.00
July	489	146.70	0.0132	1.94	1283	384.90	0.0000	0.00
August	371	111.30	0.0174	1.94	1478	443.40	0.0023	1.00
September	109	32.70	0.0000	0.00	859	257.70	0.0000	0.00
October	49	14.70	0.0000	0.00	244	73.20	0.0000	0.00
November	26	7.80	0.0000	0.00	51	15.30	0.0000	0.00
December	71	21.30	0.0910	1.94	460	138.00	0.0000	0.00

** Values adjusted based on Okumu et al 2008; see Table 1

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Table 3. Host blood antigens detected in blood-meals obtained from *Anopheles* mosquitoes.

Blood meal source	*Anopheles arabiensis*		*Anopheles funestus* s.s		*Anopheles leesoni*	
	N	%	N	%	N	%
Human	47	73.4	17	100	1	100
Bovine	14	22	0	0	0	0
Dog	2	3.1	0	0	0	0
Chicken	1	1.5	0	0	0	0

https://doi.org/10.1371/journal.pone.0177807.t003

Insecticide susceptibility test results

The insecticide susceptibility test results are summarized in Table 3. There was 100% mortality in the susceptible reference *An. gambiae s.s* colony when exposed to all the candidate insecticides tested. The wild-caught *An. funestus* were also fully susceptible to pirimiphos methyl (100% mortality), malathion (100%), and dieldrin (100%), but they were resistant to permethrin (10.5% mortality), deltamethrin (18.7%), lambda cyhalothrin (18.7%) and DDT (26.2%). In these tests, the *An. funestus* also showed reduced susceptibility to bendiocarb (95% mortality) and propoxur (90.1% mortality). The overall mortality of the *An. funestus* mosquitoes exposed to control papers was 5.4%.

Parity rates of *An. funestus* relative to *An. arabiensis* mosquitoes

Of the 302 *An. funestus* dissected, 65.8% were parous, 28.8% were nulliparous, and 5.2% were pre-gravid. Of the 162 *An. arabiensis* dissected, 49.3% were parous, 37% were nulliparous and 13.5% were pre-gravid.

Discussion

Malaria prevalence is declining across most of African countries [1], due to improved vector control [1] and case management, but also urbanization, improved health care and better living standards [1]. Long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS) contributed 78% of all gains accrued since 2000 [37]. In rural south-eastern Tanzania, where malaria prevalence has reduced by >60% since 2000, low-level transmission still persists despite persistently high LLIN coverage [38]. Our study re-assessed the residual malaria transmission dynamics in rural south-eastern Tanzania, in an area where the last mass distribution of bednets was conducted between 2010 and 2011, four years before our study [19]. This study therefore provides an essential update, of the transmission situation and demonstrates the now dominant role of *An. funestus*. Like in most residual transmission settings in East Africa, our study confirms that populations of formerly notorious *An. gambiae* s.s, have significantly diminished, or completely vanished [10, 39]. *An. arabiensis* now dominates in numbers, but the far more competent vector *An. funestus* carries most of the residual malaria parasites, despite being highly outnumbered by *An. arabiensis*. This study clearly provides a clear lead to the hypothesis that interventions targeting *An. funestus* could dramatically reduce malaria transmission in areas such as the ones where this study was conducted.

This study has also determined that *An. funestus* vector is resistant to pyrethroids used on LLINs (Table 4), and survives unexpectedly longer than *An. arabiensis*, thus requiring new control approaches. Given the extensive resistance in the species population against a wide range of insecticides currently approved for public health use, it appears that we now have only a limited set of options, including organophosphates such as pirimiphos methyl. Thus, one of the main control methods that could be used against this species is focal IRS with long-

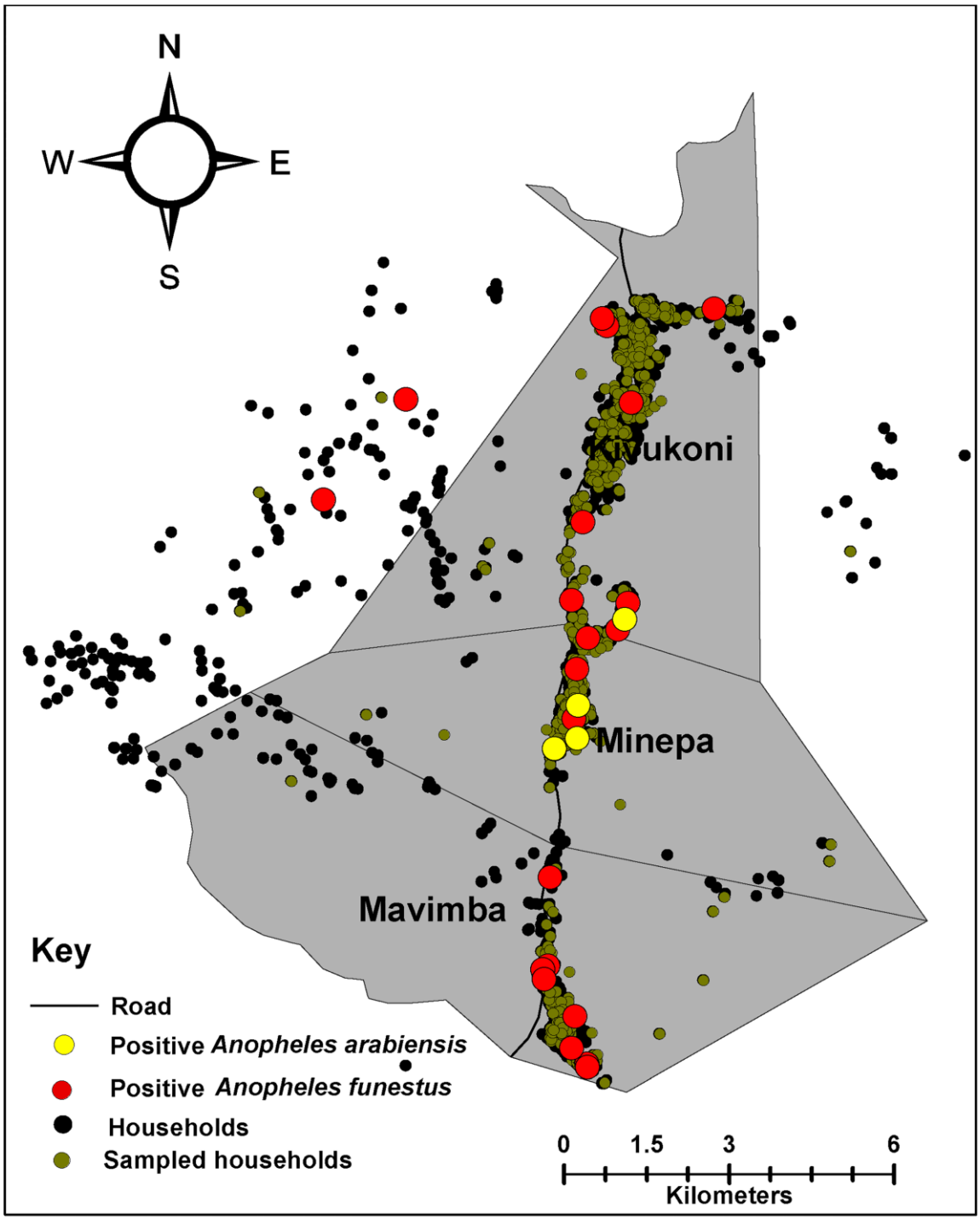


Fig 4. Distribution of *Plasmodium* infected *An. funestus* and *An. arabiensis*.

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Table 4. Summary of data on susceptibility of wild-caught adult female *Anopheles funestus* mosquitoes collected in the study sites.

Insecticide tested	Total. No. Exposed	Number of mosquitoes knocked- down (KD)								Mortality		
		10 min	15 min	20 min	30 min	40 min	50 min	60 min	% KD 60 min	Total No. Dead	% Dead	Status*
Control	129	0	0	0	0	0	0	0	0	7	5.4	-
0.75% Permethrin	95	0	0	0	0	1	1	1	1.1	10	10.5	RR
0.05% Deltamethrin	80	2	2	1	2	3	3	6	7.5	15	18.7	RR
0.05% Lambda cyhalothrin	80	0	0	1	1	1	2	4	5.0	15	18.7	RR
0.1% Bendiocarb	80	0	2	2	6	27	31	35	43.7	76	95.0	RI
0.1% Propoxur	81	21	36	46	67	58	75	77	95.0	73	90.1	RI
0.25% Pirimiphos-methyl	84	6	3	8	46	63	80	80	95.2	84	100.0	SS
5% Malathion	80	7	13	32	55	74	78	79	98.7	80	100.0	SS
4% DDT	80	0	0	4	7	9	8	13	16.2	21	26.2	RR
0.4% Dieldrin	80	0	0	0	0	2	6	14	17.5	80	100.0	SS

*Susceptibility status:

- SS: Indicates susceptibility
- RR: Confirmation of resistance in the tested mosquito population.
- RI: Suggest the existence of resistance and further investigation is needed.

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lasting formulations of organophosphates (e.g. Actellic® CS-300), against which *An. funestus* are still highly susceptible. Other potential interventions may include house improvement and house screening, as well as use of eave baffles treated with organophosphates, which would take far less insecticide, but deliver equal impact on the mosquito populations as IRS (Killeen *et al* unpublished manuscript). Going forward, it may be desirable to conduct new evidence-based studies in well-characterized areas such as these study sites, to demonstrate possible vector elimination strategies leading to malaria transmission control. Approaches may include combinations of organophosphate-based IRS alongside other interventions like LLINs, improved housing and larval source management, especially where rice growing is commonly practiced.

To achieve malaria elimination, we must identify and target all pockets of transmission, including geographically distinct areas of high transmission and demographically high-risk sub-populations [40, 41]. In this study setting in rural south-eastern Tanzania, low-level transmission persists, despite over 80% LLIN coverage [38]. The persistent residual transmission is most definitely mediated predominantly by *An. funestus* s.s, suggesting possibly that targeted, high impact, species-specific interventions may improve control and accelerate efforts towards eventual malaria elimination. Our recent findings suggested that the overall distribution of malaria mosquitoes, including *An. funestus* is strongly influenced by and can be predicted based on numbers and spatial variability of household occupancy [8]. The densities of *Anopheles* mosquitoes were highest in rainy season even though transmission was evidently sustained at low densities even in dry season, mostly by *An. funestus*. Since aquatic breeding habitats for *An. funestus* are not easily identified, it may be necessary to identify other environmental characteristics that affect natural abundance of *An. funestus* within villages and between households. This approach can be feasible by involving the local community and volunteers. The evidence from Mwangungulu *et al* show that scientists can rely on community knowledge and experiences to identify areas where mosquitoes are most abundant [42].

The data presented here would be essential in developing control measures that target primarily the most important residual vector species, in this case *An. funestus*. In these specific study settings in Tanzania, the nucleated settlements, and spatial correlations between human biomass and vector densities [8], indicates this proposed study area renders itself well to spatially-targeted interventions. The study has rigorously demonstrated that *An. funestus* is now the primary vector of malaria in this area in rural-south eastern Tanzania, despite concurrently high densities of other species such as *An. arabiensis*. The area historically had very high malaria transmission, averaged 400 infectious bites/person/year (ib/p/y) until early 2000s [43], with prevalence as high as 70% in 1990s [44], but this study indicates that transmission has now dropped around 20 ib/p/y. Moreover, this study has also demonstrated that *An. funestus* s.s co-exists with *An. rivulorum* and *An. leesoni*, but malaria transmission was mainly mediated by *An. funestus* s.s. It is not possible to determine conclusively if the greater contribution of the *An. funestus* s.s. to malaria transmission is mostly due to its relatively higher abundance than that of the other sibling species, but it is clear that this species mediates most of the residual transmission.

Of all the *An. funestus* mosquitoes that tested positive for malaria parasites, *An. funestus* s. s. comprised 96% of those *An. funestus* s.l. which were positive for *Plasmodium* sporozoites., though their overall relative abundance was 76.6%, while *An. rivulorum* was responsible for 4% of the infections though their relative abundance was only 2.9% of all the *An. funestus* mosquitoes. *Anopheles leesoni*, constituted 7.1% of all mosquitoes in this group, but carried no infections at all. Given that the ELISA lysates were all boiled to eliminate any false positives [28], occasionally associated with non-*Plasmodium* protozoans transmitted by zoophilic mosquitoes [45], we can assume, based on the 4% sporozoites rates, that *An. rivulorum* is indeed an important vector in this setting, and that its role is limited mostly by its low densities, relative to the other species. Other studies in East Africa have previously demonstrated the importance of the species in residual transmission settings [46], its role increasing after the decline of *An. gambiae* s.s populations [47, 48] (Table 5). Our study did not however,

Table 5. Examples of other localities where *Anopheles funestus* mosquitoes have been demonstrated to mediate most of the residual malaria transmission, and where control programs targeting *An. funestus* with effective interventions, could drastically reduce local transmission.

SN	Country	Intervention	Dominant Vectors	Other Vectors	Resistance Status	Sporozoite Rates	Implications for malaria transmission control	Reference
1	Kenya	ITNs and LLINs	*An. funestus*	*An. arabiensis*, *An. gambiae* s.s.	Pyrethroid resistance confirmed	*An. funestus* 4.5%, *An. arabiensis* 0.9%, *An.gambiae* s.s 8.6%	Reduced effectiveness of the current interventions	[52]
2	Burkina Faso		*An. gambiae* s.l.	*An. funestus* s.l, *An. nili*	No pyrethroid detected. *An. funestus* in was highly resistant to dieldrin	*An. funestus* (2.6% -9.7%)	*An. funestus* had higher biting rates and high sporozoite rate compared to *An.gambiae* complex	[53]
3	Tanzania	LLINs	*An. gambiae* s.l.	*An. funestus* s.l	Pyrethroid resistance confirmed	*An. funestus* (0.16% -1.47%)	Resurgence of malaria transmission in Kilombero valley, Tanzania	[10]
4	Senegal	LLINs	*An. funestus*	-	-	*An. funestus* 1.28%	*An. funestus* changed their host seeking behaviour following the introduction of LLINs, thus compromising effectiveness of LLINS.	[54]
5	Cameroon		*An. funestus* s.s	*An. gambiae* s.s	-	*An. funestus* 6.8%, *An.gambiae* s.s 0.6%- 4.1%	*An. funestus* accounts for 88% of the transmission in this setting.	[55]

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identify any *Plasmodium* infections in any of the other *Anopheles* mosquitoes collected, i.e. *An. coustani*, *An. squamosus*, *An. ziemanni* and *An.wellcomei*, at any time during the study, thus these species are likely to be of negligible importance in malaria transmission in this setting. Furthermore, this can be attributed to their low relative abundance in this area. Some previous studies have reported sporozoite infections in *An. coustani* [49], *An. squamosus* [50] and *An. ziemanni* [51], but no such evidence was observed in this current study. Table 5 shows some of the other localities where *An. funestus* mosquitoes significantly contribute to the residual malaria transmission.

The extremely high proportion of *Plasmodium* infections currently mediated by *An. funestus* mosquitoes in this area is compounded by, and is likely also a result of the very high insecticide resistance in this species. This study found that *An. funestus* mosquitoes were not only carrying most of the residual malaria parasites, but they were also very highly resistant to common pyrethroids used for vector control, i.e. deltamethrin, permethrin, lambda-cyhalothrin, as well as to the organochlorine, DDT. Moreover, there was also reduced susceptibility to insecticides like bendiocarb and propoxur, which would normally be candidates for replacing the pyrethroids. An earlier study by Lwetoijera *et a.l* in the neighbouring Kilombero district, also found increasing importance of *An. funestus* in malaria transmission associated with high resistance [10]. Even though the insecticide susceptibility status for *An. arabiensis* was not evaluated in this study, a separate study from these villages by Matowo *et al* (unpublished findings) found *An. arabiensis* to be also highly resistant to pyrethroid chemicals.

While the high human blood index observed in the *An. funestus* clearly suggests that the species could still be best targeted by household-based interventions that protect humans. Observations of increasing outdoor biting by the different *An. funestus* sibling species has been reported in this study area [56, 57], which warrants consideration of complementary interventions that also target outdoor-biting vectors. Also, given the high resistance to the common insecticides for vector control, it appears that use of organophosphates, against which the vector is still susceptible, notably pirimiphos methyl, could provide a temporary solution against residual malaria transmission in this area. Long-lasting formulations of pirimiphos methyl already exist [58], which could be used for IRS in this area to complement LLINs, as it has already been implemented in Zanzibar, northern region and lake zones in Tanzania [59].

Other possible high value interventions could be insecticidal eave baffles (Killeen et al. Unpublished), especially in areas where even outdoor-biting populations forage indoors for their adult life [60]. This study did not assess the role of *An. funestus* on outdoor malaria transmission, but, a separate study conducted in the same villages by Ngowo *et al* (unpublished findings) yield evidence that some *An. funestus* collected outdoors were found to be sporozoite positive. Furthermore, tools should be included such as chemicals with new AIs available for IRS that are being evaluated for consideration by the WHO, including non-pyrethroid active ingredients like neonicotinoids as well as bednets that combine synergists with pyrethroids, or those that combine different insecticide classes to tackle insecticide resistance and lower vector survival.

One limitation of this study is that collections for insecticide susceptibility were conducted on wild caught females of variable age, since we could not find adequate larvae for the assays. WHO recommends where possible to use mosquitoes of the same age cohort (F1 progeny). Using wild mosquitoes that are older than the standard 3–5 days may lead to underestimation of resistance depending on the species distribution and the insecticide being tested. Blood meal analyses were carried out on visually blooded mosquitoes and so may have missed those with small amounts of blood in their abdomens, such as those that had interrupted feeding or had partially digested the blood. Another potential limitation is that parity assessments, such as the ones conduced here are greatly impacted by season, this study conducted parity

assessment for mosquitoes collected for two months, we suggest that future further studies should be done to assess parity status across seasons. Lastly, there was also a significant non-amplification of mosquito samples during the PCR analysis to determine the sibling species. About 13.4% of all sample analyzed in the laboratory did not amplify. An analysis of the sample handling processes, the analysis process, and the control samples, suggests that the laboratory processes were unlikely to be the reason for the non-amplification. Instead, this situation might have arisen due to sub-optimal storage conditions of some samples, or minor discrepancies in the morphological identification. These specific processes therefore deserve greater attention in future analyses.

Conclusion

This study provides an update on the residual malaria transmission situation in rural south-eastern Tanzania, where LLINs were already widely distributed. Though *An. arabiensis* is still the most abundant vector species here, ongoing residual transmission is predominantly mediated by *An. funestus*, possibly due to high resistance and high survival probabilities. Interventions that effectively target *An. funestus* could therefore significantly improve control of residual malaria transmission in the area.

Supporting information

S1 File. Supplementary data. A file containing supplementary data depicting graphs used in this manuscript.
(CSV)

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Funding acquisition: EWK FOO NSM.

Investigation: GM EWK AM MF.

Methodology: EWK FOO MF.

Project administration: EWK.

Resources: FOO.

Supervision: FOO.

Writing – original draft: EWK FOO MF HSN NSM.

Writing – review & editing: EWK FOO MF HSN NSM.

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Appendix 4: Original paper IV

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RESEARCH ARTICLE

New evidence of mating swarms of the malaria vector, *Anopheles arabiensis* in Tanzania [version 1; referees: 1 approved, 2 approved with reservations]

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Abstract

Background: Malaria mosquitoes form mating swarms around sunset, often at the same locations for months or years. Unfortunately, studies of *Anopheles* swarms are rare in East Africa, the last recorded field observations in Tanzania having been in 1983.

Methods: Mosquito swarms were surveyed by trained volunteers between August-2016 and June-2017 in Ulanga district, Tanzania. Identified *Anopheles* swarms were sampled using sweep nets, and collected mosquitoes killed by refrigeration then identified by sex and taxa. Sub-samples were further identified by PCR, and spermatheca of females examined for mating status. Mosquito ages were estimated by observing female ovarian tracheoles and rotation of male genitalia. GPS locations, types of swarm markers, start/end times of swarming, heights above ground, mosquito counts/swarm, and copulation events were recorded.

Results: A total of 216 *Anopheles* swarms were identified, characterized and mapped, from which 7,142 *Anopheles gambiae* s.l and 13 *Anopheles funestus* were sampled. The *An. gambiae* s.l were 99.6% males and 0.4% females, while the *An. funestus* were all males. Of all *An. gambiae* s.l analyzed by PCR, 86.7% were *An. arabiensis*, while 13.3% returned non-amplified DNA. Mean height (±SD) of swarms was 2.74±0.64m, and median duration was 20 (IQR; 15-25) minutes. Confirmed swarm markers included rice fields (25.5%), burned grounds (17.2%), banana trees (13%), brick piles (8.8%), garbage heaps (7.9%) and ant-hills (7.4%). Visual estimates of swarm sizes by the volunteers was strongly correlated to actual sizes by sweep nets (R=0.94; P=<0.001). All females examined were nulliparous and 95.6% [N=6787] of males had rotated genitalia, indicating sexual maturity.

Conclusions: This is the first report of *Anopheles* swarms in Tanzania in more than three decades. The study demonstrates that the swarms can be identified and characterized by trained community-based volunteers, and highlights potential new interventions, for example targeted aerosol spraying of the swarms to improve malaria control.

Open Peer Review

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	Invited Referees 1	2	3
version 1 published 22 Sep 2017	✓ report	? report	? report

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Keywords
Anopheles swarms, mating behavior of mosquitoes, malaria, vector control

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Background

Recent successes in malaria control have been mostly attributed to vector control measures, in particular, long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS)[1]. These tools have contributed to ~78% of all gains achieved since 2000[1,2]. However, effectiveness of these interventions is compromised, partly due to spread of insecticide resistance[3] and increased outdoor exposure to mosquito bites among other challenges[4–6]. Consequently, persistent malaria transmission still occurs in many places, even where high and sustained coverage of LLINs has been achieved.

To develop new options for malaria vector control, it is important to re-examine the overall ecology of the malaria vectors[7], including not only the blood-feeding and resting habits commonly targeted by LLINs and IRS, but also other mosquito habits indoors and outdoors. Mating behavior is one of the most important aspects in the maintenance of species[8], yet it is a widely under-investigated aspect of the mosquito biology. Improved understanding mosquito mating systems could possibly provide new opportunities for expanding vector control options. Most insects mate in swarms, whereby dispersed populations aggregate at specific times and places[9,10]. In mosquitoes, including the malaria vectors, swarming flights are influenced by presence of visual markers on the ground[11,12]. Targeting swarms to deplete mosquito densities indeed offer unrivalled opportunities to drastically reduce mosquito-borne pathogen transmission[13]. Such an approach has proven effective against some *Anopheles* mosquitoes on a limited scale in Burkina Faso[14], but needs to be validated for other vector species in other areas. Besides, mosquito swarms are known to occur perpetually in the same locations at approximately the same time each day[10,15], thus targeting them could be easily achieved by trained community volunteers. The concentrations of males in swarms, predictability and accessibility of the swarming sites and the fact that swarms can be artificially manipulated[16], makes the male mosquitoes vulnerable and an easy control target.

While swarms have been commonly identified in west and central Africa[17–19], observations have rarely been reported in East and Southern Africa, most probably because mosquito swarming has not been as thoroughly investigated in this area, as it has been in West Africa. Prior to this current report, there was only a handful of reports, including the seminal work by R.P. Marchand in Tanzania in the early 1980s[20], the work by J.D. Charlwood in Mozambique in the early 2000s[21], and unpublished observations of *An. arabiensis* swarms in the Kilombero valley in south eastern Tanzania (Dr. Kija Nghabi and Japhet Kihonda, Personal Communication).

This current study was designed to explore the *Anopheles* mating systems in rural south-eastern Tanzania, by assessing occurrence of natural swarming and outdoor nocturnal behaviors of *Anopheles* mosquitoes. The assessment involved accurate identification, mapping and characterizing of mosquito swarms in three study villages in Ulanga district, Tanzania. We assumed that despite lack of previous records, *Anopheles* swarms do indeed occur in the area, and that they can be readily identified and characterized. We used a combination of crowd-sourced community knowledge[22], intensive field surveys and expert advice. Our initial objective was therefore to demonstrate natural occurrence of swarms of malaria vectors in rural, south eastern Tanzania and characterize these swarms.

Methods

Study area

The study was conducted in rural Ulanga and Kilombero districts, in south eastern Tanzania. Initially, the surveys covered multiple villages in the two districts, but we eventually focused on just three villages of Kivukoni (8.2135°S, 36.6879°E), Minepa (8.2710°S, 36.6771°E) and Mavimba (8.3124°S, 36.6771°E) in Ulanga district, on the Kilombero river floodplain (Figure 1). The main malaria vectors in the area include *Anopheles funestus* and *An. arabiensis* with minor contributions from *An. rivulorum*[23]. Overall EIR was last estimated at 4.2 and 11.7 infectious bites/person/year by *An. arabiensis* and *An. funestus* respectively[23]. The main vector control approach used in the area is LLINs, and the villages benefited from universal LLIN coverage campaigns both in 2011 and in 2016. The main malaria vectors are resistant to pyrethroids used in the LLINs, but still susceptible to organophosphates[23,24]. Mean household size is 4.2[25]. Most of the houses are mainly mud and brick walled, with thatched or iron-sheet roofs. The communities rely mainly on substance farming, cultivating rice and maize but also fishing.

Trainings on swarm surveys and characterization

Ten participants were selected to support the project in the three study villages, with the help of community leaders. All participants were male adults aged 18 to 35 years, who had provided written informed consent prior to being enrolled in the study. Initially, the project leaders and research assistants were trained on how to identify mosquito swarms and swarm markers by experienced entomologists from Burkina Faso. The first brief training was conducted in Ifakara, Tanzania, in December 2014, at which stage this project had just been conceived. The second training was done for project leaders during a visit to Burkina Faso in September 2016. All participants in this study, including volunteers searching the swarms, provided written informed consent prior to being enrolled.

In April 2016, five months before the second training of project leaders, exploratory swarm surveys were initiated, as an extension of ongoing entomological studies in the study area. During and after these exploratory surveys, the project leaders trained the local community-based swarm searching volunteers on how swarms are identified and characterized, focusing on major features of both the swarms and their associated markers (Figure 2). This training was conducted over several weeks, and included: i) swarm searching techniques; ii) swarm sampling techniques; iii) swarm characterization and iv) various entomological procedures essential for examining male and female *Anopheles* mosquitoes collected from swarms. All trainings for local community volunteers were conducted in Kiswahili language, which is the common language in Tanzania, and in this specific study area. These volunteers were then relied upon to identify swarms, which the researchers would then follow up for species confirmation.

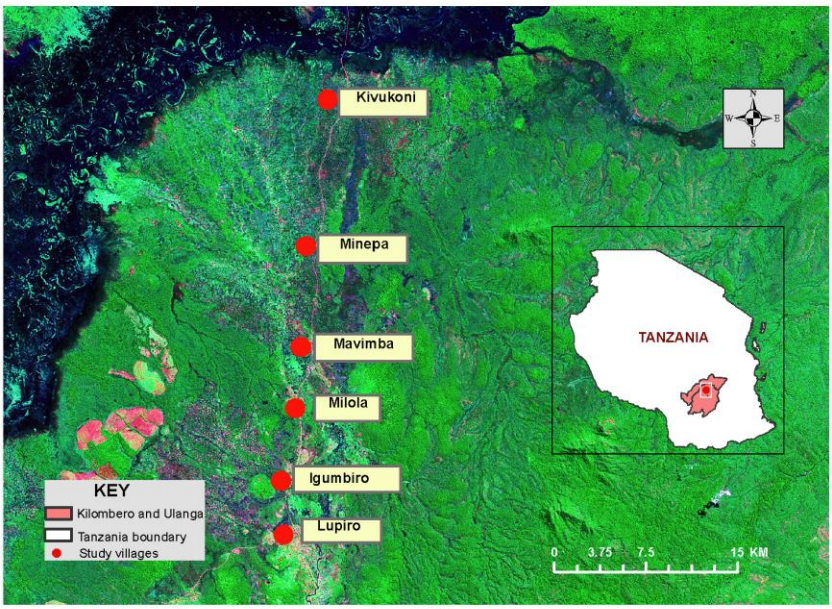


Figure 1. Map of the study area, showing villages in south-eastern Tanzania, where swarm surveys were conducted. Image description: Pan-sharpened mosaic, acquired by Enhanced Thematic Mapper Plus (ETM+) sensor on Landsat 7 Satellite (Courtesy of Donall Cross, University of Aberystwith, UK)).



Figure 2. Pictures of volunteers and researchers learning how to identify and sample mosquito swarms in one of the study villages. All participants in the picture provided consent to being photographed.

Further advanced training on swarm surveys and characterization was conducted in Tanzania by a team of experts from Institut de Recherche en Sciences de la Santé (IRSS), Burkina Faso. This advanced training covered: i) swarm searching techniques in dry and wet seasons; ii) swarm visualization techniques; iii) GIS-based analysis of swarms iv) swarm markers and associated environmental features; v) swarm sampling techniques vi) photographic survey of swarms, vii) swarm characterization (including key features to consider); and viii) identification of mating couples in swarms and examination of male and female mosquitoes to identify mated and non-mated individuals.

Initial exploratory surveys of mosquito swarms

To aid logistical assignment of the swarm searching activities, volunteers were assigned one sub-village to search and report the occurrence of swarms. The trained community volunteers were dispatched to search and report any types of insect swarms in their areas, and report these to the researchers. Every time a swarm was reported by the volunteers, the researchers visited the site immediately (if nearby) or the next day (if in far locations): i) first, to verify whether the swarms were actually present, ii) second, to determine whether they were swarms of mosquitoes as opposed to other insects, and iii) third, to assess whether they were actually *Anopheles* swarms. The first confirmed *Anopheles* mosquito swarm was identified by a community-based swarm searcher in mid-August 2016 in Minepa Village. This provided our initial verified evidence of presence of *Anopheles* swarms in this area. As a result of this initial discovery, the team was reorganized for more intensive swarm searching expeditions. From this point forward, the key objectives now included demonstrating that local community members could be trained to readily identify and characterize *Anopheles* swarms and conduct comprehensive mapping and characterization of *Anopheles* swarms in selected villages. Subsequent efforts were restricted to just three villages (Mavimba, Kivukoni and Minepa) in Ulanga district (Figure 1), to enable detailed swarm studies.

Detailed mapping and characterization of *Anopheles* mosquito swarms and swarm markers in three selected villages

After the initial swarm searching, a comprehensive mapping and characterization of the *Anopheles* swarms was conducted in three selected villages, i.e. Minepa, Mavimba and Kivukoni. We worked with volunteers from the different sub-villages, who had been trained on how to spot mosquito swarms and swarm markers, time of day when swarms typically occur, and how to collect mosquitoes using sweep nets to identify the swarming mosquitoes. We relied on lessons learned during the initial exploratory surveys, and progressively optimized our swarm survey techniques. The training provided by experts from Burkina Faso significantly improved the swarm characterization capabilities of the Tanzania team.

Volunteers were assigned specific area (sub-villages) to search for mosquito swarms, by first identifying potential swarm markers in these areas during the day, and then actively searching of mosquito swarms across the area every evening from 18:00Hrs, with the aim of identifying swarms. During the swarm surveys, the volunteers continued to receive supportive supervision and training on how to identify, locate and sample mosquitoes using sweep nets. This continuous training was important since this is the first time *Anopheles* swarms were being recorded in this area. To facilitate the swarm surveys, we also identified, characterized and recorded all potential swarm markers in the study villages. The swarm markers were classified based on the criteria developed by Diabate *et al.*,[26], into four categories as follows: a) flat-contrast markers, such as bare land and footpaths, b) flat no-contrast markers, such as grasses and rice fields, c) elevated contrast markers, such as anthill, woodpiles, bricks and garbage bin, and d) elevated no-contrast markers such as banana tree. Then these markers were subsequently monitored in the evenings to identify and record those with swarms.

Following the initial identification and mapping, each verified swarm was comprehensively characterized by the expert team, by repeatedly visiting and examining the swarms at least three times in different days. In addition to recording date, month, and name of village, we followed a systematic process and recorded various pre-determined characteristics as follows: a) heights at which the swarms occurred, (measured as a distance between the base of the swarm and the ground level), b) time of day when the swarming started appearing, recorded to the nearest minute, c) time of night when the swarms were completely dispersed, also measured to the nearest minute; d) physiological status of a sample of mosquitoes collected in the swarm, e.g. mating status and parity, e) any other mosquito species and insects occurring in the same swarm, f) exact geo-location of the swarm marker; measured using handheld GPS receiver (Magellan eXplorist GC, USA), g) ratio of males to females collected in the sample; Detailed mapping and characterization of h) number of copulation events observed in the swarm; i) number and proportion of females caught that have evidence of having been inseminated, j) type of swarm marker; k) parity status of female mosquitoes collected in the swarms, including number and proportion of females that were parous or nulliparous; l) morphological and molecular identification of the species of *Anopheles* mosquitoes collected in the swarm; m) any other unique observations made at the swarm site or on the swarms; and n) approximate number of mosquitoes collected in each swarm. Swarm sizes were estimated in two ways: by sampling with a large sweep net (190 cm diameter), approximately 10 minutes after the start of the swarm, and visually, by the trained volunteers counting the flying mosquitoes and approximating the counts. Lastly, we observed other features which may affect the occurrence of mosquito swarms, time of sunset such as human movements and other insects.

Laboratory processing of mosquitoes collected in the swarms

Collected mosquitoes (dead or alive) were aspirated from the sweep nets, kept in separate paper cups and maintained on 10% glucose solution so that those still alive could survive till the next morning. The following morning, all mosquitoes were killed in a closed container by freezing, then identified morphologically by taxa and sex, following keys developed by Gillies and Coetzee[27]. A sub-sample from each collection was further identified by multiplex polymerase chain reaction (PCR) to identify sibling species that were morphologically indistinguishable. The PCR assays were conducted using protocols developed by Scott *et al.*,[28].

Physiological age of the wild-caught female malaria vectors was approximated based on the status of their ovaries, i.e. whether they had previously laid eggs or not, by observing the coiling or uncoiling of the ovariole tracheoles[29]. Each unfed female mosquito was first anesthetized in a refrigerator. A drop of distilled water was added to a slide and each specimen kept still on the slide, then the seventh and eighth abdominal segment was pulled using fine needles under stereo dissecting microscope. The ovarial tracheoles were then observed under a compound microscope at 10X objective lens magnification, to determine whether the mosquitoes were parous (tracheoles uncoiled and stretched) or nulliparous (with tracheolar skeins, i.e. coiled)[29].

Assessment of female insemination status and sexual maturity in males:

To determine the insemination rate, female *An. arabiensis* were dissected under a dissecting microscope and their spermathecae examined for the presence of spermatozoon under a compound microscope using 10X objective lenses[30,31]. To assess whether males had matured sexually, genitalia of *An. arabiensis* males were also observed for signs of rotation[32].

Data analysis

Data analysis was done by using R software version 3.0[33]. Number of mosquitoes per swarm, duration of swarms, proportion inseminated, proportion parous (or nulliparous) or proportion of males with rotated genitalia were modeled as a function of different variables as follows: village, swarm marker type, month, height of swarms, time of swarming, and volunteer identification. Geolocations of the swarms relative to households and other landmarks were visualized using the ArcGIS 10.0 (ESRI, USA). Percentages of parity status, insemination rate, mean heights of swarms above ground, median duration of swarms, and time of day when swarms started or ended at the different months of surveys, were also calculated. Swarm markers were classified following the classification category developed by Diabate *et al.*,[26]. Visualized volunteer estimates of swarm sizes, and sweep net swarm size estimates were compared and their linear correlation coefficients estimated.

Ethics statement

Meetings with local leaders were held in the study areas and the main aims of the study were explained by the research team. Written and signed informed consent were obtained from all volunteers participating in the study. All information was given in Kiswahili, the local language. Ethical approval for the study was obtained from Ifakara Health Institute Institutional Review Board (IHI/IRB/No: 38-2016), and from the Medical Research Coordinating Committee (MRCC) at the National Institutes of Medical Research (NIMR), Ref: NIMR/HQ/R.8a/Vol.IX/2428. Approval was also obtained from the Human Research Ethics Committee (Medical) clearance at the University of the Witwatersrand, where EK is registered for PhD (Ethics approval certificate No: M160806). Approval for publishing the manuscript was obtained from the National Institutes of Medical Research (NIMR), Ref: NIMR/HQ/P.12VOL.XXII/24. Printed copies and web links to the publication will be provided to NIMR after publication.

Results

Results of exploratory surveys: initial evidence of *Anopheles* swarms

The initial exploration, conducted in multiple villages, revealed that *Anopheles* swarms do indeed occur in villages along the Kilombero river valley in rural south-eastern Tanzania. The surveys also revealed that community members can be relied upon to identify these swarms and that by combining their local knowledge with expert knowledge, the *Anopheles* swarm surveys could be effectively characterized. The first *Anopheles arabiensis* swarm was observed on 15th August 2016, in the village of Minepa, by one of the local volunteers. To the best of our knowledge, this is the first recorded *Anopheles* swarm in this specific part of Tanzania, and the first in the country since the last report from north-eastern part of the country in 1983[20]. In the initial exploration, several non-mosquito swarms and also culicine mosquito swarms were incorrectly reported by the volunteers as *Anopheles* swarms, but were dismissed by the expert verification teams. The reliability of the volunteers however increased over the course of the surveys.

Anopheles mosquito swarms in the three selected villages

After the initial exploratory surveys, and confirmation of occurrence of *Anopheles* swarms, subsequent surveys were concentrated in just the three selected villages of Minepa, Mavimba and Kivukoni (Figure 1). A total of 216 swarms were observed from August 2016 to June 2017. The distribution of mosquito swarms in the study villages is shown (Figure 3), and was as follows; 58.3% of the swarms (n=126) were found in Minepa village, 11.1% (n=24) were found in Mavimba village and 30.5% (n=66) were found in Kivukoni village. There were more swarms distributed in areas close to the rice fields, at the edges of the villages, compared to areas near human settlements (Figure 3). All the swarms observed consisted exclusively of *An. gambiae* s.l. We analyzed a sub-sample of 112 *An. gambiae* s.l mosquitoes by PCR to determine actual sibling species, out of which we obtained 97 successful amplifications (86.6%) and 15 non-amplifications (13.4%). All the successful amplifications were determined to be *An. arabiensis* (100%). In one instance in the early days of the surveys, we also collected 13 male *An. funestus* mosquitoes, in an area where we had *An. arabiensis* swarms. Since no repeat observation of *An. funestus*, we are unable to verify presence of swarms of this species here, until further investigations are completed.

Start times, end times, duration and height of *Anopheles* swarms

It was observed that swarms began with two to three mosquitoes congregating after sunset, and flying above a swarm marker. Then the numbers increased over the next 10 to 15 minutes, with multiple males and slowly decreased in size then disappeared after 20 (IQR;15-25) minutes. Flying mosquitoes were observed by viewing them against the sunset. Mosquitoes tended to congregate at the mean height (±SD) of 2.74±0.64m from the ground. The swarm size increased and became compact above the marker but they disappeared when it became dark, which was also when swarms observations became impossible. Furthermore, we observed that the start and end time of swarming periods varied across months.

Swarms were appearing earlier in the period between August 2016 and January 2017, but later in the period between February and June. Specific start and finish times for swarms during each of the sampling months is shown in Figure 4.

According to weather information archived online by timeanddate.com, the latest time of sunset in the study area (Kilombero Valley, Tanzania) varied in a similar pattern as the start and end times of the observed *Anopheles* swarm sessions. We considered sunset times for all the months of our data collection, starting August, 2016 to June 2017. Between August and November 2016, the latest time sunset time ranged between 18:32Hrs and 18:42Hrs. However, between December 2016 and March 2017, sunset occurred much later, with the latest sunset

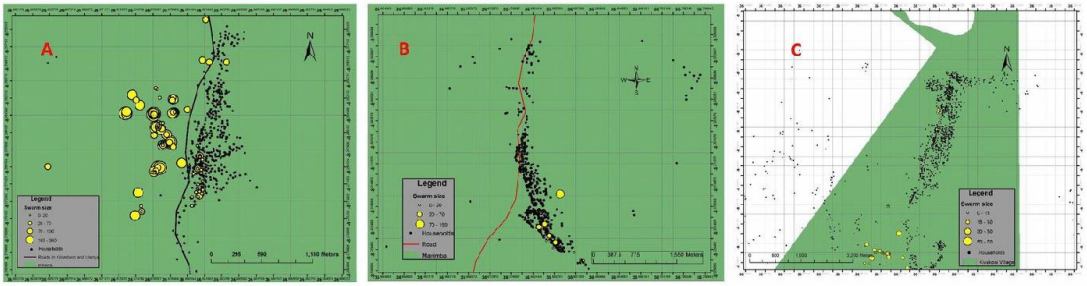


Figure 3. Comparison of swarm distribution between the three study villages (Original figure created by authors of this article).

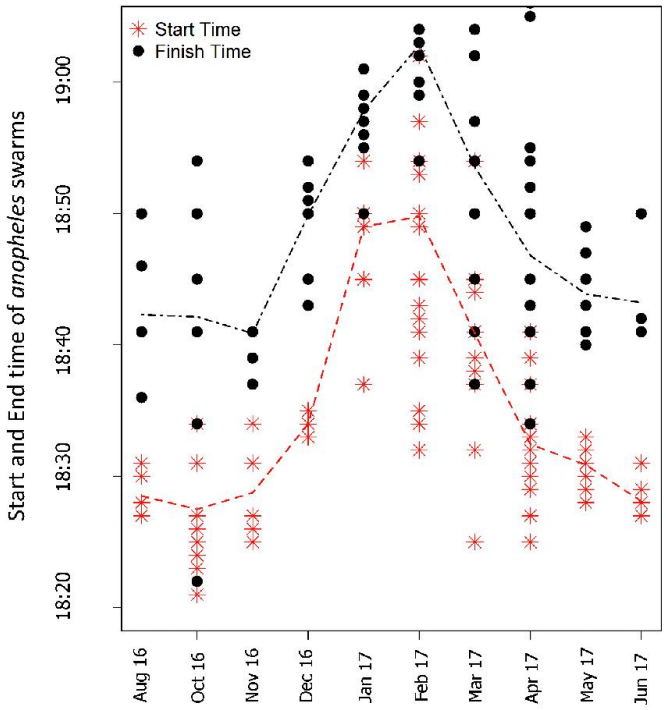


Figure 4. Observed start and end times of *Anopheles arabiensis* swarms in the study villages.

times between 18:56Hrs and 19:03Hrs. The sunset time was early again between April 2017 and August 2017, when the latest sunset times over the valley ranged from 18:26Hrs and 18:35Hrs: https://www.timeanddate.com/sun/@157407.

Estimated swarm sizes: data collected through direct visualization by community volunteers compared to data collected using sweep nets

We estimated swarm sizes from two data sources. First, we used data provided from visual assessments of the volunteers looking at swarms and estimating how many mosquitoes were flying in them at that time. Second, we used data from collections done by the large standardized sweep nets, with which trained volunteers collected the mosquitoes approximately 10 minutes after the start of swarming. Though the values provided from the two data sources were not equal (i.e. visual estimates being consistently higher than sweep net counts), there was a significant linear correlation (Table 1 and Figure 5) between the visual estimates provided by the community volunteers and estimates obtained from sweep net counts ($R^2 = 0.94$; $P < 0.001$). There were

Table 1. Swarm sizes estimated by either visual observations by trained volunteers, or sweep net sampling in the three study villages (a total of 216 swarms were observed). C.I: Confidence Interval.

	Total number of swarms (% of all swarms observed)	Visual estimates by volunteers (No. mosquitoes/swarm)		Sweep net sampling estimates (No. mosquitoes/swarm)	
		Mean	C.I	Mean	C.I
Kivukoni	66 (30.5%)	34.0	31.4 - 36.6	13.1	11.5 - 14.9
Mavimba	24 (11%)	38.7	36.3 - 41. 3	20.6	19.9 - 22.5
Minepa	126 (58.3%)	95.5	93.8 - 97.3	56.1	54.8 - 57.4

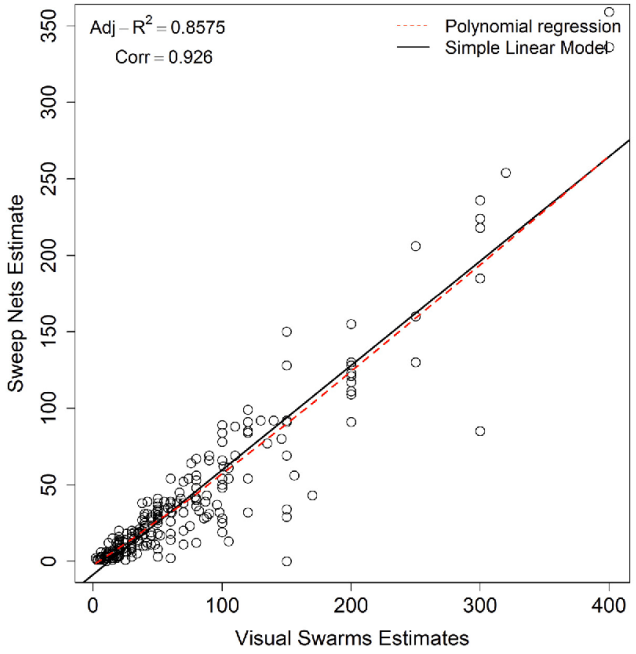


Figure 5. Estimated swarm sizes; and correlation between estimates done by visualization and estimates done using sweep nets.

also variations of swarm sizes observed in different villages, with Minepa village having the largest swarms followed by Mavimba and Kivukoni villages (Table 1).

Results of swarm markers survey

The commonest swarm markers were rice fields (25.6%), burned grounds (17.2%), banana trees (13%), brick piles (8.8%), grass (7.9%) garbage heaps (7.9%), ant hill (7.4%), wood piles (5.6%), demolished houses (1.8%),footpath (1.3%), trenches (1.3%), toilets (1.3) and cowshed (0.5). Table 2 shows the common swarm markers and the frequency of swarms observed in each marker. Most of the swarm locations remained the same, several months after the swarms were first identified and verified.

Copulation events, mating status, sexual maturity and parity rates in mosquitoes collected from the swarms

Throughout the study period, we observed and collected a total of 22 copulation events in the swarms. The proportion of female to male *An. arabiensis* mosquitoes in the swarm was 0.004 (28/7114). Of the 28 female *anopheles arabiensis* mosquitoes dissected, 54% (N=15) were determined as nulliparous. The remaining 46% (N=13) were not examinable since the specimen had dried up. We also assessed the male *An. arabiensis* collected from the swarms for sexual maturity. Of these, 95.4% (N=6787) had rotated male genitalia, indicating sexual maturity. We assessed the 28 females recovered from the swarms, to assess whether their spermatheca were already filled with male sperms or not. Of all females dissected, 42.9% (N=12) had evidence of having been inseminated, while the remaining 57.1% (N=16) were not inseminated.

Discussion

Malaria vector control remains constrained by lack of comprehensive understanding of mosquito ecology, yet such ecological studies are considered a prerequisite for improved control and eventual disease elimination[7]. Though not widely studied, the mating behavior of malaria mosquitoes may presents some unique opportunities for improved vector control[13]. In a recent trial in Burkina Faso, it has been demonstrated that targeting *Anopheles* mosquito swarms with effective aerosol insecticide spraying could rapidly crash vector populations in local communities[14]. Though mating and swarming behaviours of *Anopheles* mosquitoes are among the most important components of the vector reproduction biology[8], there have been limited field studies of mosquito swarming in Tanzania, the last published field observations having been in 1983[20]. This neglect is mainly because most scientist have not considered swarming behaviour as having any potential opportunities for disease prevention and vector control. On the contrary, studies on mating and swarming behaviours of *Anopheles* mosquitoes in West Africa have already demonstrated that it could possibly be relied upon to control mosquito densities and associated pathogen transmission, by killing mosquito swarms with effective insecticides[13,14].

Our study has yielded evidence of occurrence of the swarms of *An. arabiensis* mosquitoes in the rural south-eastern Tanzania. Furthermore, the study proves that trained volunteers are able to successfully identify and locate swarms in their villages. A map of all swarm locations so far identified has been built-up with the help of the locally recruited volunteers (Figure 3). Interestingly, most of the swarm locations remained the same, several months

Table 2. Common types swarm markers observed in the three study villages. In brackets, the actual number of swarm markers that had *Anopheles* swarms over them.

	Flat contrast swarm markers	Flat no-contrast markers	Elevated contrast markers	Elevated no-contrast markers
Minepa Village	Burned ground (17) Well (0) Cowshed (0) Trench (0)	Rice fields (36) Footpath (1) Grass (8)	Bricks (17) Garbage heaps (10) Woodpile (1) Demolished house (2) Ant hill (7) Toilets (3)	Banana trees (24)
Mavimba Village	Burned ground (4) Well (0) Cowshed (0) Trench (2)	Rice fields (1) Footpath (1) Grass (5)	Bricks (0) Garbage heaps (4) Woodpile (1) Demolished house (0) Ant hill (6) Toilets (0)	Banana trees (0)
Kivukoni Village	Burned ground (16) Well (0) Cowshed (1) Trench (1)	Rice fields (18) Footpath (1) Grass (4)	Bricks (2) Garbage heaps (3) Woodpile (10) Demolished house (2) Ant hill (3) Toilets (1)	Banana trees (4)

after they were first identified. This phenomenon of swarms occurring in the same location is widely reported by other scientists, particularly in west Africa[26]. In this current study, we characterized the *Anopheles* swarms repeatedly to obtain an initial profile of malaria vector swarms in this area; first evidence of such swarms in the study area.

The commonest swarm markers are shown in Table 2, and were found on landscapes that were not blocked by either trees or houses. Several other studies have also reported the roles of such markers on mosquito swarming[15], and here, we relied on similar swarm marker classification as previously used in Burkina Faso[26]. Mosquitoes preferred to swarm on areas such as rice fields, burned grounds, banana trees, brick piles, garbage heaps and ant hills. It may therefore be possible to predict the distributions of *Anopheles* swarms based on the spatial distribution of the common swarm markers in the village. In this study, once we had an accurate representation of the main swarm markers, it became easier to conduct training programs for the volunteers, who relied heavily on these swarm markers as a guide to search for mosquito swarms in their assigned areas. Second, with a clear definition of swarm markers, it could be possible, as has been demonstrated in some studies to manipulate swarm markers, or create artificial swarm markers to attract *Anopheles* mosquitoes and enhance mating[16].

Our study also demonstrated that locally recruited and trained non-entomologist volunteers from the study villages are capable of not only identifying the swarm but also estimating the swarm size and the associated swarm markers in the study village. This idea was derived from an earlier study conducted in the villages where community members were able to identify locations where mosquito densities were high[22]. In this current study, we initially guided the volunteers on how to identify mosquito swarms and their associated environmental features. This approach confirmed that such community-based volunteers can be relied upon to identify, characterize and map *Anopheles* swarms across villages, so that these swarms can potentially be targeted for control and ensuring its sustainability. An added advantage of this approach is that it also increases local community knowledge on mosquitoes, and the level of community engagement in research increased. By using such participatory approach, we expect that in the time of targeting *Anopheles* swarms e.g. by aerosol spraying, high levels of acceptability could be achieved, and the volunteers could be relied upon to lower implementation costs.

This study did not observe any *An. funestus* swarms. However, 13 male *An. funestus* mosquitoes were collected near the *An. arabiensis* swarms, suggesting possible presence of *An. funestus* swarms in the area. Unfortunately, this was only one instance and could not be verified during this study. It may be possible that this was a separate distinct *An. funestus* swarm that was formed close to *An. arabiensis*. Since they were all male *An. funestus*, we speculate that *An. funestus* swarms also occur in the area, and that extended surveys could reveal greater details of their swarming behaviours. It is therefore recommended that more detailed surveys should be conducted to understand the swarming behaviours of *An. funestus* in the area. Evidence by other studies in Africa suggest that swarms of *An. funestus* appear in a similar way to those of *An. gambiae*[21]. Since it is know that *An. funestus* contributes to the significant amount of the remaining malaria transmission in this area[23], it is therefore suggested that any control measures targeting *An. gambiae* without affecting *An. funestus* will have little impact on the ongoing malaria transmission in this area. More attention should be put on how to identify swarms of *An. funestus* and their associated environmental features.

Consequently presence of dragonflies, though not initially targeted for observation, was an indication of swarm presence in many locations, though in this case we have not considered it as a physical swarm marker. Similar observations were also reported by Sawadogo *et al.*,[15] in their studies in Burkina Faso. Furthermore, human movements, sounds, strong wind and rainfall were found to affect mosquito swarming behaviours. For instance, in some cases when swarm sampling was conducted during the rainfall, we rarely observed swarms even in the previously confirmed swarms. Dragon flies and other predators could thus be considered good indicators of presence of mosquito swarms in certain areas. Lastly, in this study, most of the swarms were found and confined at the edge of the village and in the rice fields, this could mostly be attributed to the fact that swarms prefers to the location that are not disturbed by human activities such as movements.

Overall, the swarming behavior of *An. arabiensis* in the valley is now clearly tractable. Further investigations should explore opportunities on whether new interventions exploiting this phenomenon, such as targeted aerosol spraying of swarms, could significantly disrupt malaria transmission.

Conclusions

This is the first report of *Anopheles* swarms in Tanzania, in more than three decades. The study provides evidence that the swarms can be identified, characterized and quantified by trained community-based volunteers; and opens potential new opportunities for targeting male malaria mosquitoes to improve disease control. Further investigations are recommended to further characterize the swarms in the area and to verify whether other residual malaria vector populations such as *An. funestus* also form similar mating swarms. More importantly, future studies should seek to demonstrate whether new interventions exploiting this phenomenon, such as targeted aerosol spraying of swarms, could significantly disrupt malaria transmission.

Data availability

The data used in this manuscript have been uploaded online to the Ifakara Health Institute, DOI, 10.17890/ihi.2017.06.99[34], under a CC0 1.0 license.

The terms and conditions that users need to agree to in order to download the data, can be viewed here.

Competing interests

No competing interests were disclosed.

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This paper represents a lot of work. The problem with studying swarming behavior (as opposed to mating behavior) is the difficulty of having sensible questions to ask. I have watched and filmed swarms of anophelines for many hours (mainly in Mozambique) and still am not sure what the most relevant ones, at least without sophisticated equipment, might be. There is always more perspiration than inspiration. This paper describes the basics of swarms of *Anopheles arabiensis* but I have a number of questions regarding some of the information in the paper. For example, having recently observed swarms of *An. arabiensis* in Eritrea (but only over a period of two weeks and in a single village) I remain at a loss regarding definitions of swarm markers for this species. The swarms I observed occurred over a very slightly darker area of sand in a dry riverbed but the mosquitoes did not alter their behavior when either a light cloth or dark T-shirt was placed under the swarming mosquitoes. I am therefore of the opinion that I have no real clue what the insects were using to maintain station. (This is unlike the situation with *An. coluzzii,* which will follow a dark cloth or *An. pharoensis* which follow light ones). I am, therefore, not convinced that the so-called markers described in the present study were actually being used by the *An. arabiensis.* Their wide variety indicates that they were just in the area and that something else was the actual 'marker'. Only by manipulating apparent markers and seeing their effect on the swarms can we be sure that they are for real.

In Eritrea males left their diurnal resting sites once light levels had fallen to around 520 Lux (which occurred at different times according to whether it was cloudy or not) and immediately started swarming. There was no delay. As the authors indicate swarming did not last long but at the same time, as they also point out, the cessation of swarming was coincident with difficulty of actually observing the insects so it is difficult to be sure that activity had ceased. Swarms of *An. funestus,* monitored with a video camera with infra-red capabilities in Mozambique, continued beyond the time that they could be seen with the naked eye. So a caveat regarding ending time should be included. The change in start times according to sunset times was also recorded in *An. coluzzii* from São Tomé and should perhaps be referenced.

Sweep netting, of course, does not remove all of the insects from a swarm. A better alternative is to take a flash photograph and then use a program like imageJ to count objects of different sizes in the image (which can be compared to counts done by hand). Since most pictures are taken from below the swarm the only objects in such pictures are the mosquitoes. Should two insects overlap then their combined size is larger than individual insects and so they are considered by the program to be a separate category. It may even be possible to use the camera on a smartphone, now widely available, to take such pictures.

Similarly I saw (perhaps) just a single pair in copula in Eritrea. These are normally very easy to see since the pair is larger and flies more slowly than individual insects (the male and female flying in different directions). They almost invariably leave the swarm (the female dragging the male behind her. It is only perhaps when a small female mates with a larger male that they continue in the swarm, the male in this case pulling the female behind him). In all their observations the authors report 'observing and collecting 22 copulation events'. (Difficult to know how you collect an event). I think that this begs the question as to whether, like 'love and marriage' these phenomena really go together (and the ones in Eritrea) really were 'mating swarms'. In São Tomé pairs dropped out of the swarms 'like flies' (if you'll excuse the pun). Something more is going on – but we don't know what it is. I think it is a misnomer to call them mating swarms. Indeed it is worth discussing the fact that so few mating pairs were seen. It is possible that mating was largely taking place in swarms elsewhere.

To watch swarms and note their behavior does indeed not require that much professional input. In São Tomé the swarm with mating was discovered by a two-year-old child who had to really persist to get the 'professionals' attention!

The authors also found that 12 of 28 females collected from swarms had been inseminated. Like one of the other reviewers I also find this very strange. Did these insects have mating plugs? What were they doing there? Elsewhere, as mentioned above, pairs in copula leave the swarm before the male ejaculates. The females do not return (as shown by the absence of multiple mating plugs in the many hundreds of females that have been examined). My own suspicion would be that the volunteers were being bitten and included these females in the sample.

It is also interesting that males with un-rotated terminalia were collected. Since these males would probably have ecloded the previous evening they would have been almost 24hrs old at the time of the swarm it implies that rotation takes longer than a day. But I was of the impression that that was all the time that was needed. Some comment on this might also be informative.

Mosquitoes swarm close to where they rest. Rather than attempting to target swarms per se a better and more sustainable method of control would be to find the resting site (and here the swarming site could be used as an indicator of nearby resting sites) and intervene there. If, as is likely, there are common characteristics between resting sites (a dark background perhaps) then it might also be possible to develop and deploy artificial resting sites that might be treated with a non-repellent insecticide or a factor that the males might transfer to the females that they mate with to affect them. Since newly emerged females also often share the same resting site as the males such an intervention would work against them too.

I agree with the comments of the other referees in that the article could be a lot shorter, that the figures could be improved and the text be made more concise.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Referee Report 20 October 2017

doi:10.21956/wellcomeopenres.13490.r26301

Tovi Lehmann
Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Rockville, MD, USA

This is a valuable description of A. arabiensis mating swarms in Tanzania based on one year swarm survey in 3 villages. The motivation of the study, as stated by the authors is that after the pioneering work of Marchand and later of Charlwood in East Africa, there have been no studies of swarming behavior of African anophelines in that region. The absence of observations on swarms of A. gambiae s.l., despite some efforts, led some to doubt the relevance of swarms to mating in these species, especially in that region. By replicating older results, the authors confirmed the role of swarming behavior to mating across Africa. Thus, the results, mostly replicate previous studies including: swarm formation time after sunset, swarm size range, and association with ground markers. The study relied on trained local village observers/collectors following earlier studies in Mali and Burkina Faso (cited). The paper reads easily (and pleasantly) but it could be improved by a substantial reduction in length, adhering to concise and more precise scientific writing, and possibly addressing specific questions/hypotheses, as suggested below.

Major comments:

1. Because this study is a descriptive survey of swarms that mostly replicate previous studies (it could cite other studies for details on the Methods), it would better fit a “Research Note” or a much shorter article. Suggestions to accomplish that include replacing Fig. 1 with a brief text and removal of Fig. 2, which is not informative. The Methods section could be reduced to 1/4 of its current length. The detailed description of the training for swarm spotters/collectors, should be summarized in 3 sentences including the description of training in standard entomological and field ecology methods, which would better rely on citations. The first section of the Results “Results of exploratory surveys: initial evidence of Anopheles swarms,” (which repeats information given in the Methods) could be removed (also from the Methods section) or replaced by a single sentence such as: “The initial exploration, confirmed that Anopheles swarms do occur in the study area.” Additionally, there are repetitions and unnecessary details that dilute the main message.

Certain terms must be defined accurately. For example a "swarm" may represent a single observation of males flying stationary above a marker in a single evening or to the site, where such aggregations were observed on multiple evenings. When stated that 216 swarms were observed, it is not clear which definition was used. Likewise, the term "sub-village" is vague. Please define its area.

Despite the long Method section, I could not find a summary of the sampling effort i.e. the number of swarm collectors operating throughout the year in every village, which is essential. Did the same team passed between villages in different times or they were divided between the villages? How were the swarm spotter/collectors been evaluated in terms of quality and efficiency of work?

2. Interestingly, large differences were found among villages in the number of swarms and their sizes. Could these differences reflect different density of A. arabiensis overall in these villages (it would be helpful to include average indoor density if such data exist)? Were the sampling effort the same between villages in terms of the number of collectors/observers, duration of activity, and their work-quality, or hospitality of local residents? Were the sampling conducted at the same time across all villages? For example, if the survey in one village occurred in a dryer period, it might account for the lower number and sizes of the swarms. What other factors might explain that variation (e.g., availability of suitable markers)?

Minor/Specific points:

Background

- "Most insects mate in swarms, whereby dispersed populations aggregate at specific times..." – this statement might meant to refer to diptera or to culicids. I doubt it holds for all insects. The references given focused on dipterans. Please check and confirm.

- "Targeting swarms to deplete mosquito densities indeed offer unrivalled opportunities to drastically reduce mosquito-borne pathogen transmission" – in what way it is unrivalled?

- "Such an approach has proven effective against some Anopheles mosquitoes on a limited scale in Burkina Faso14, but needs to be validated for other vector species in other areas. ..thus targeting them could be easily achieved by trained community volunteers. … easy control target." I afraid the scalability of this approach for regional, country and continental control is a heavy challenge that cannot be described as "easy". There are other control strategies that can be effective in a single village or few villages, but can't be effectively scaled up. For example draining or insecticide-treating all larval sites in certain villages to drastically reduce transmission, but unless the scalability issue has been addressed, the promise if this strategy is doubtful. Therefore the promise of the proposed new approach should be considered seriously with its limitations.

- " ..a combination of crowd-sourced community knowledge22, intensive field surveys and expert advice" – specifically what "crowd sourced community knowledge" was obtained.

Results:

- Figure 3 – the legend is very small and nearly impossible to read. It would help to have on large-font and uniform legend for all 3 villages. Please clarify if (i) all houses in the area depicted are shown, ie., there are no other houses (not shown) regardless if they "officially belong" to the

village, gpsed etc.. Also, what is the reason that the background to C is different? The landscape around the villages may be better conveyed using Google Earth (than a green color). Figure 3 summarizes large body of spatial information. It might be interesting to extract measures of inter-swarm distances, its seasonal variation, and effect of swarm size, as well as the variation between villages.

- Figure 4. The authors explain the variation in the swarming time by the change in sunset time. Thus, please add the corresponding line (curve) showing the sunset time and include the correlation between these variables in the Figure.

- Figure 5. Unless many observations are on top each other, the number of swarms observed appear to be 4-6/month, which amount to mere ~60 observations total rather than 216 as understood from the top of the results. If there are on top of each other, the author can consider jittering observations and changing their symbol to better appreciate the weight of each value. Please clarify.

- Table 2 shows variation in swarm occurrence on different markers. I suggest that authors reshape the table by listing all the swarm markers along the rows (in groups based on their classification) and have the columns show the percent (and total number) of swarms above each marker across villages. This will allow the reader to compare the ranking of the markers. Moreover, it might be helpful to know how many such markers exist in the surveyed area. For example if there are no rice fields or banana trees in one village and many in another, it will be difficult for the mosquitoes to express their preference for banana tees, etc. The interpretation of this table can be expanded in the discussion.

- The authors may want to comment on whether the collection of a proportion of inseminated females in swarms as well as some funestus specimens might reflect collection of mosquitoes that are not related to the swarming activity.

Is the work clearly and accurately presented and does it cite the current literature?
No

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Referee Expertise: Vector ecology

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Referee Report 28 September 2017

doi:10.21956/wellcomeopenres.13490.r26445

Edward D Walker
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This quite interesting and pleasantly written article summarizes a useful study combining observational science, citizen science, community engagement, Anopheles mosquito swarming and mating behaviour, and empirical analysis. The study is mensurative, that is, depending upon various methods of various degrees of sensitivity in measuring and recording observations and events. The distribution of locations of mating swarms of male Anopheles arabiensis is presented quite well and reliably. One problem is the issue of recorder bias; how likely are the observers to go to (say) sites most convenient for them to visit as opposed to sites difficult to visit. But this problem seems to have been surmounted here and the authors are not unaware of it. The observations on swarm composition (e.g., sex ratio), timing of formation and deformation, and density (number of mosquitoes comprising them) are all useful. This study might lead to new tools for vector control based upon these observations and related data.

Conclusions from data are again based on observational inferences but this kind of study is useful precisely for that reason.

Certain matters remain unclear and these have to do in particular with the location of swarms and what is meant by swarm markers. For example, a category of swarm marker is rice field and another is banana tree and another is toilet by which we must mean latrine shed. These are all entirely different environmental features with very different shapes, sizes, compositions, origins, and so on. From the observations one cannot make a strong conclusion about the particular configuration of what is meant by "swarm maker" typologically. That is, are swarm markers typically of a certain configuration or do swarms form just about anywhere the initial males starting them begin to fly at a particular spot? The authors should clarify this issue as a point of discussion. Secondly is the problem of what is meant by contrast or no contrast swarm markers. I do not know what this means. Do they mean contrast with a horizon, contrast between two hues or shades, ... ? As a a means of providing a particular attribute of a typical swarm marker, the use of this descriptor of contrast is vague.

The writing style is generally quite good and the manuscript needs little adjustment for editing. The caption legends for Figure 5 are awkward. The X axis could simply read Visual Estimate and the Y axis could read Sweep Net Estimate. I see no reason to include a polynomial fit to the data on this figure because the relationship looks plainly linear. There seem to be two data points tucked up high in the upper right corner but they are obscured by the regression information.

In the last paragraph of the Results section, the following sentence should be revised: "Of the 28 female anopheles arabiensis mosquitoes dissected, 54% (N=15) were determined as nulliparous. The remaining 46% (N=13) were not examinable since the specimen had dried up." It should read as follows: "of the 28 female An. arabiensis dissected, fifteen were nulliparous and the remainder were too dry to determine

parity status."

In Table 2, the structure of the table needs some changes reflecting the clarification of the contrast and no contrast comment above. By cowshed must be meant the area of land where cows are stanchioned at night. This is not a 'cowshed' in the usual sense but rather a flat piece of ground where the animals are kept at night. If it is a kraal type of situation, there may be some living or nonliving fence in place in which case it cannot be flat. Otherwise, explain how a cowshed is flat. Under elevated contrast markers, I would like to know if by ant hills is meant termite mounds. Please clarify. My experience is east Africa is that ant hills are quite low to the ground and indiscernible whereas termite mounds are of course tower like structures and clearly elevated.

Some banana trees are quite tall. How did the citizens with the sweep nets manage to sample over them?

It is interesting that Figure 3 shows that in some locations the swarms form independently or distance from houses whereas in other locations the swarms form mostly amongst dense clusters of houses. Compare panel 1 and panel 2, for example. Why is this?

Thank you for giving me this opportunity to review this interesting and useful manuscript.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Appendix 5: Original paper V

Kaindoa *et al. Malar J* (2019) 18:29
https://doi.org/10.1186/s12936-019-2660-y

Malaria Journal

RESEARCH Open Access

Swarms of the malaria vector *Anopheles funestus* in Tanzania

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Abstract

Background: *Anopheles funestus* mosquitoes currently contribute more than 85% of ongoing malaria transmission events in south-eastern Tanzania, even though they occur in lower densities than other vectors, such as *Anopheles arabiensis*. Unfortunately, the species ecology is minimally understood, partly because of difficulties in laboratory colonization. This study describes the first observations of *An. funestus* swarms in Tanzania, possibly heralding new opportunities for control.

Method: Using systematic searches by community-based volunteers and expert entomologists, *An. funestus* swarms were identified in two villages in Ulanga and Kilombero districts in south-eastern Tanzania, starting June 2018. Swarms were characterized by size, height, start- and end-times, presence of copulation and associated environmental features. Samples of male mosquitoes from the swarms were examined for sexual maturity by observing genitalia rotation, species identity using polymerase chain reaction and wing sizes.

Results: 581 *An. funestus* (98.1% males (n = 570) and 1.9% (n = 11) females) and 9 *Anopheles gambiae* sensu lato (s.l.) males were sampled using sweep nets from the 81 confirmed swarms in two villages (Ikwambi in Kilombero district and Tulizamoyo in Ulanga district). Six copulation events were observed in the swarms. Mean density (95% CL) of *An. funestus* caught/swarm/village/evening was 6.6 (5.9–7.2) in Tulizamoyo and 10.8 (5.8–15.8) in Ikwambi. 87.7% (n = 71) of the swarms were found in Tulizamoyo, while 12.3% (n = 10) were in Ikwambi. Mean height of swarms was 1.7 m (0.9–2.5 m), while mean duration was 12.9 (7.9–17.9) minutes. The PCR analysis confirmed that 100% of all *An. funestus* s.l. samples processed were *An. funestus* sensu stricto. Mean wing length of *An. funestus* males was 2.47 mm (2.0–2.8 mm), but there was no difference between swarming males and indoor-resting males. Most swarms (95.0%) occurred above bare ground, sometime on front lawns near human dwellings, and repeatedly in the same locations.

Conclusion: This study has demonstrated occurrence of *An. funestus* swarms for the first time in Tanzania. Further investigations could identify new opportunities for improved control of this dominant malaria vector, possibly by targeting the swarms.

Background

Global statistics indicate that malaria morbidity and mortality have declined mostly as a result of scaling up vector control interventions [1, 2], but that the gains are stagnating in some countries [3]. This decline has also been observed in Tanzania with more than 50% reduction of malaria burden recorded since the year 2000 [4]. The 2016–2017 Tanzania malaria indicator survey demonstrated reduction of prevalence in children under five, from 18.0% in 2008 to 7.3% in 2017 [5–7] in the mainland. These successes may be attributed to scaling up of vector control tools, such as long-lasting insecticide-treated bed nets [8] and indoor residual sprays [9], as well as socio-economic developments and urbanization [10] and treatment with artemisinin-based combination therapy (ACT) [11].

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Full list of author information is available at the end of the article

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In a recent study, it was demonstrated that just one malaria vector species, *Anopheles funestus,* contributes to more than 85% of malaria transmission in south-eastern Tanzania, despite occurring at far lower densities than the other major vector, *An. arabiensis* [12]. Unfortunately, the *An. funestus* populations are resistant to pyrethroid insecticides commonly-used on bed nets [12, 13], survive longer than *An. arabiensis,* and feed almost exclusively on humans [12, 14]. Other studies in different African countries have also documented *An. funestus* resistance to pyrethroids: Malawi [15, 16], Mozambique [17, 18], South Africa [19], Zambia [20, 21], Zimbabwe [21], Cameroon [22, 23] and Senegal [24], a situation that compromises effectiveness of current vector control options [25], and perpetuates residual transmission even in communities where bed net coverage is more than 90% [26]. Given its dominance in Tanzania, it has been suggested that interventions that effectively target *An. funestus* could have a high impact on residual transmission here [12]. One potentially effective approach involves suppressing mosquito populations by identifying and directly targeting *Anopheles* swarms with highly effective insecticides [27], before the mosquitoes enter houses.

Swarming behaviours have previously been intensively studied, mostly in Western and Central Africa [28–30]. However in East and southern Africa, there have only been a few studies in Zambia and Mozambique [20, 31] in addition to an old set of observations in northern Tanzania in the 1980s [32]. *Anopheles* swarms are naturally difficult to find since they occur at dusk when visibility is poor and last only for a few minutes. In fact, the rarity of *Anopheles* swarms in East Africa has led some to hypothesise that they mate primarily indoors, such as previously observed in experiments by Dao et al. [33]. Nevertheless, a recent study by the Ifakara Health Institute, which relied primarily on community volunteers rather than experts, demonstrated natural occurrence of swarms of *An. arabiensis* in villages across south-eastern Tanzania [34], more than three decades after the last records of this species swarming anywhere in the region [32]. In addition to providing detailed characterization of over 200 *An. arabiensis* swarms, the study also demonstrated that trained volunteers were able to identify and locate mosquito swarms in their villages [34]. Follow-up studies have since demonstrated potential for localized control by targeting the swarms using aerosol spraying (Kaindoa et al. unpublished data).

An interesting revelation in that last study on *An. arabiensis* swarms was a single incidence where 13 *An. funestus* males were caught in a sweep net [34], providing earliest indications that this species too formed swarms in the valley, but that these swarms were certainly more elusive than those of *An. arabiensis*. The aim of this current study was, therefore, to identify and characterize swarms of *An. funestus*, which is now the dominant malaria vector species in rural south-eastern Tanzania.

Methods

Study area

The study was done in the two districts of Ulanga and Kilombero in south-eastern Tanzania. In Ulanga, collections were done in Tulizamoyo village (8.354497°S, 36.705468°E), while in Kilombero, the collections were in Ikwambi village (7.98244°S, 36.82167°E), both of them in the Kilombero river valley (Fig. 1). The villages were selected because previous adult mosquito surveys had yielded high densities of *An. funestus*. The area is characterized by perennial meso-endemic malaria transmission, with mosquito densities peaking between February and May [35]. Malaria vectors comprise primarily *An. arabiensis* and *An. funestus*, the former being more abundant but the latter more dominant in transmission.

Observation and characterization of swarms

Following the successful training of village volunteers during the *An. arabiensis* swarm identifications in 2016 and 2017, a refresher training course was provided in early 2018 to the same volunteers, using the same procedures for swarm searching [34]. In this approach, the study villages were divided into sub-sections where volunteers had to first identify potential swarm markers, and then search for swarms later in the evening. Whenever a swarm was seen, volunteers reported to the supervisors and after physical expert verification, swarm sampling and characterization was performed the following day. Sampling was done 10–15 min after the start of swarming using standardized sweep nets (190 cm diameter attached to a 2 m long stick) as describe by Kaindoa et al. [34] and the sweeping was done only once per swarm. Similar sampling procedure was done across all the swarming events to allow comparison. Using a standard observation sheet, all the identified swarms were characterized as shown in Table 1.

Morphological and molecular identification of *Anopheles* mosquitoes collected in swarms

All collected mosquitoes were aspirated from the sweep nets, placed in paper cups and maintained on 10% glucose solution. The following morning, the mosquitoes were killed in a closed container by freezing, then identified morphologically by taxa and sex following keys by Gillies and Coetzee [36]. All samples from each collection were further identified by multiplex polymerase chain reaction (PCR) to sibling species [37].

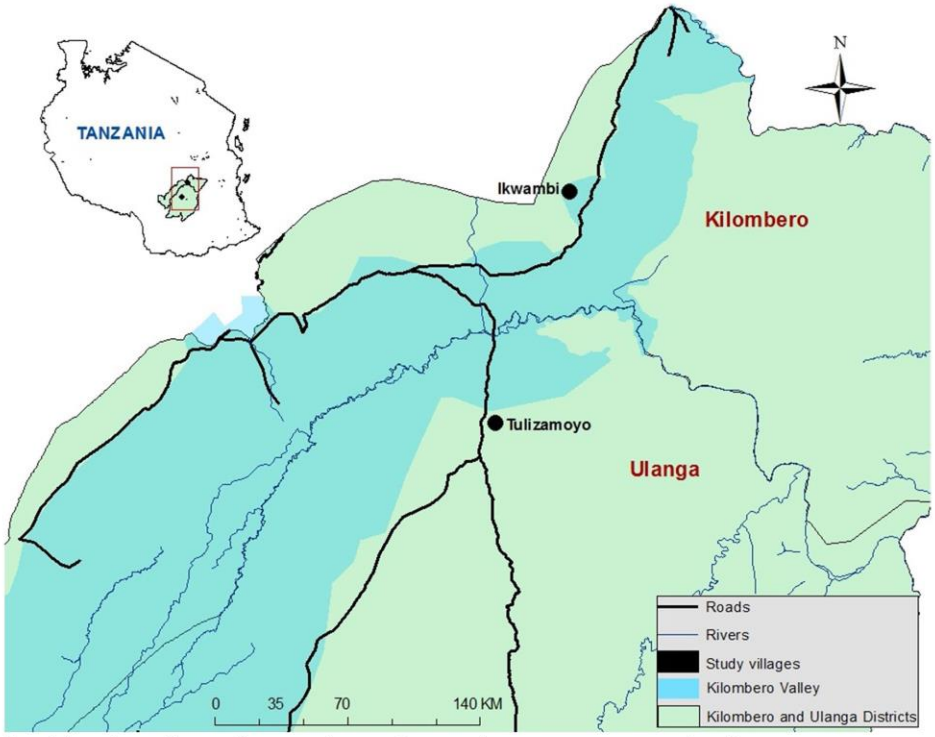


Fig. 1 Map of the study area, showing villages in south-eastern Tanzania, where swarm surveys were conducted

Table 1 Descriptive variables assessed during field surveys of *Anopheles funestus* swarms

	Variables	Methods used and indicators measured
1	Swarm size and copulation events	Visual estimates of swarm sizes: approximate number of mosquitoes in swarm, as estimated visually to the nearest 5 mosquitoes (observations were made between 10 and 15 min after start of the swarm)
		Sweep net estimates of swarm sizes: approximate number of mosquitoes in the swarm, as estimated using standardized sweep nets, collected once by experienced collectors. Swarm size was a measure of density of mosquitoes per collection by sweep net per instance
		Copulation: number of copulation events observed in the swarm after 10 min of observation
2	Location, time and height of swarms	Geo-location of the swarm measured using handheld GPS receivers
		Unique ID of compound owner (each swarm was uniquely identified on this parameter)
		Time of day when the swarm begins appearing, recorded to the nearest minute
		Time of day when the swarms completely disperse, also measured to the nearest minute
		Height measured as distance between the base of the swarm and the ground level in meters
3	Molecular identification and characteristics of sampled mosquitoes	Morphological and molecular identification of the species of *Anopheles* mosquitoes collected in the swarm
		Proportion of males caught that have evidence of being capable of mating (determined by observing the rotation of male genitalia)
		Measurements of wing size (mm)
4	Important landmarks and potential swarm markers	A record of important landmarks, at or near which swarms occur such as vegetation, house, mosques, markets, schools, water pumps, houses, cowshed, banana tree and cemeteries

Assessing sexual maturity and wing sizes of sampled mosquitoes

All collected *An. funestus* males were assessed for maturity through observations of genitalia rotation as previously described by Dahan et al. [38]. Wing lengths of male mosquitoes sampled from swarms or resting indoor surfaces, were measured under a dissecting microscope. An additional 70 mosquitoes were collected from indoor surfaces of local houses using mouth aspirator and used for comparative assessment of wing size. These samples were collected in the same period as the samples from swarms. One wing of each specimen was cut and placed onto a slide, then its length measured from the alula notch to wing tip following procedures by Lyimo et al. [39] and Charlwood et al. [31].

Data analysis

Data analysis was done by using R software version 3.3.2 [40]. Swarm sizes were calculated as median number of mosquitoes sampled in each swarm (using a sweep net when the swarming was at its peak), and compared between the two study villages. The swarm size was, therefore, a measure of mosquito densities in each sweep net collection. Swarm duration was obtained based on the difference between start and end times of each swarm in minutes. Mean height of swarms above ground, and mean duration of swarms were also calculated. Wing sizes of swarming and resting *An. funestus* males were compared. Predicted means from generalized linear model (glm) were used to produce different figures showing variations in catches between study sites. In this model, the number of mosquitoes caught in the swarms was modelled as count data following a Poisson distribution while study sites were considered as a predictor variable. Student's T-test was used to compare the mean wing length of swarming and resting *An. funestus*. Geolocations of the swarms were visualized in ArcGIS 10.4 (ESRI, USA). Swarm sizes estimated visually were compared to those estimated by using sweep nets and their correlation coefficients computed.

Results

Mean density of *Anopheles funestus* and copulation events in swarms

A total of 570 *An. funestus* males and 11 females were sampled using sweep nets, from 81 swarms observed in the two villages. A total of 6 copulation events were observed in the swarms, following the standardized observation period of 10 min per swarm (Table 1). Mean density of *An. funestus* caught per swarm per village per evening was 6.6 (5.7–7.5) in Tulizamoyo and 10.8 (5.8–15.8) in Ikwambi (Fig. 2). Overall, 87.7% [n = 71] of the swarms were found in Tulizamoyo, while only 12.3% [n = 10] were in Ikwambi. Four instances of mixed swarms were identified in Tulizamoyo village which contained 2, 3, 1, and 3 *Anopheles gambiae* sensu lato male mosquitoes found together in the same sweep nets with 2, 2, 3 and 7 *An. funestus* males, respectively, suggesting either mixed swarms or swarms of different species appearing in close proximity.

Correlations between visual and sweep net estimates of swarm sizes

Anopheles funestus swarm size was estimated by visual assessments of approximate numbers of mosquitoes forming the swarm prior to sampling with sweep nets (as described in Table 1) to estimate mosquito densities per sweep. Regression models revealed significant but weak correlation between visual estimates and sweep net estimates (Linear regression analysis: $R = 0.4518$, P-value < 0.001; Polynomial regression: Adjusted $R^2 = 0.2041$, P-value < 0.001) (Fig. 3).

Location, time and height at which the swarms occurred

The identified swarms were all characterized in the months of June and July 2018 and are shown in a georeferenced map in Fig. 5. Most swarms started at approximately 6:40 pm. Mean (± SD) duration of the swarm was 12 (± 5) minutes. The mean height of swarms above

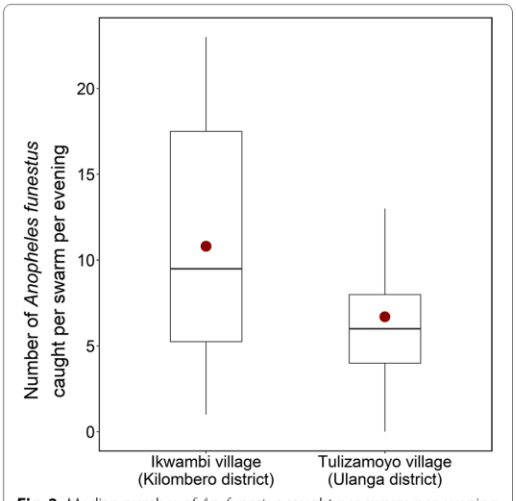


Fig. 2 Median number of *An. funestus* caught per swarm per evening in the two study sites. The fitted model was generalized linear model (glm) fitted to obtained the predicted means used to plots the graphs showing the means catch between study villages

ground was 1.7 (±0.8) meters. It was observed that most *An. funestus* swarms occurred close to human houses.

Environmental features associated with swarm occurrence

There were no obvious physical features being used as markers as previously described for *An. arabiensis*. However, assessment of all the swarm locations resulted in four different categories of places where swarming by *An. funestus* occurred. The most common feature was bare ground near the houses (Fig. 4), usually dusty but no obvious textural discontinuation and sometimes in the front lawns of human dwellings (91.4% (n = 74) of the swarms occurred in such sites) (Fig. 5). Two swarms were observed on small cleared farms (2.5% of the swarms; (n = 2), one swarm under of a teak tree, and one over a demolished house (Table 2).

Molecular identification, wing lengths and sexual maturity of sampled *An. funestus*

A total of 413 *An. funestus* males were analysed for species identification in the laboratory, 93.9% (n = 388) of which yielded successful DNA amplifications. All amplified samples were confirmed as *An. funestus* s.s. A total of 109 and 70 swarming and resting *An. funestus* males, respectively, were examined for size, using wing length as proxy. The wing length of *An. funestus* males ranged between 2.00 and 2.80 mm with a mean of 2.47 mm (Minimum = 2.00 and Maximum = 2.80) as shown in Fig. 6. There was a very small insignificant difference in wing size between the swarming and resting male *An. funestus* (t = 2.301, df = 214.38, P = 0.022). Nearly all (97.3%) of the male *An. funestus* had rotated genitalia, suggesting sexual maturity.

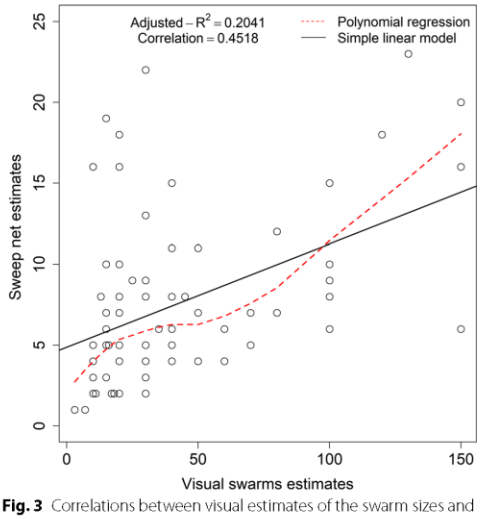


Fig. 3 Correlations between visual estimates of the swarm sizes and the swarm densities per sweep net collection per evening

Table 2 Environmental features of locations where *Anopheles funestus* swarms were observed in the study villages

	Environmental features	Total marker (%)	No. mosquitoes caught
1	Bare ground near dwellings	77 (95.0)	535
2	Demolished house	1 (1.2)	18
3	Cleared farms	2 (2.5)	10
4	Teak tree	1 (1.3)	18
	Total	81 (100)	581

Discussion

Improved understanding of malaria vector ecology and behaviour is crucial for achieving the ambition of malaria elimination through vector control tools [41]. The swarming and mating behaviours in particular are components of mosquito behaviour that has been neglected for a long time [30, 31, 34]. Given that *An. funestus* contributes to more than 80% of the ongoing malaria transmission in the area, it is important to identify and characterize swarms of *An. funestus,* so as to explore complimentary tools, possibly targeting swarming behaviour.

Furthermore, it is important to understand mating behaviour for implementation of certain control methods, e.g. use of sterile insect techniques and release of genetically modified males. This is the first verified report of *An. funestus* swarms in Tanzania, though the earlier study confirmed natural occurrence of *An. arabiensis* swarms in the same area [34]. Together, the two reports confirm widespread occurrence of swarms by the main malaria vectors, even though they are generally elusive and require dedicated teams with local knowledge to map.

An attempt to characterize the swarm sites did not yield any obviously distinctive physical markers of the swarm stations. Instead, most of the swarms were observed to occur above bare ground, sometime on the front lawns of human houses (Fig. 4). This is very similar to observations by Charlwood et al. who suggested that *An. funestus* mosquitoes in Mozambique formed swarms close to the houses used for resting and that the swarming sites could be used as indicators of houses to be targeted for vector control interventions [31].

Proximity to houses is therefore an essential condition for *An. funestus* mating swarms, and the possibility that some degree of mating happens indoors cannot be excluded. Relative to previous observations in the same area [34], this current study has demonstrated that *An.*



Fig. 4 Pictorial illustration of commonest locations where swarms of *Anopheles funestus* mosquitoes were observed

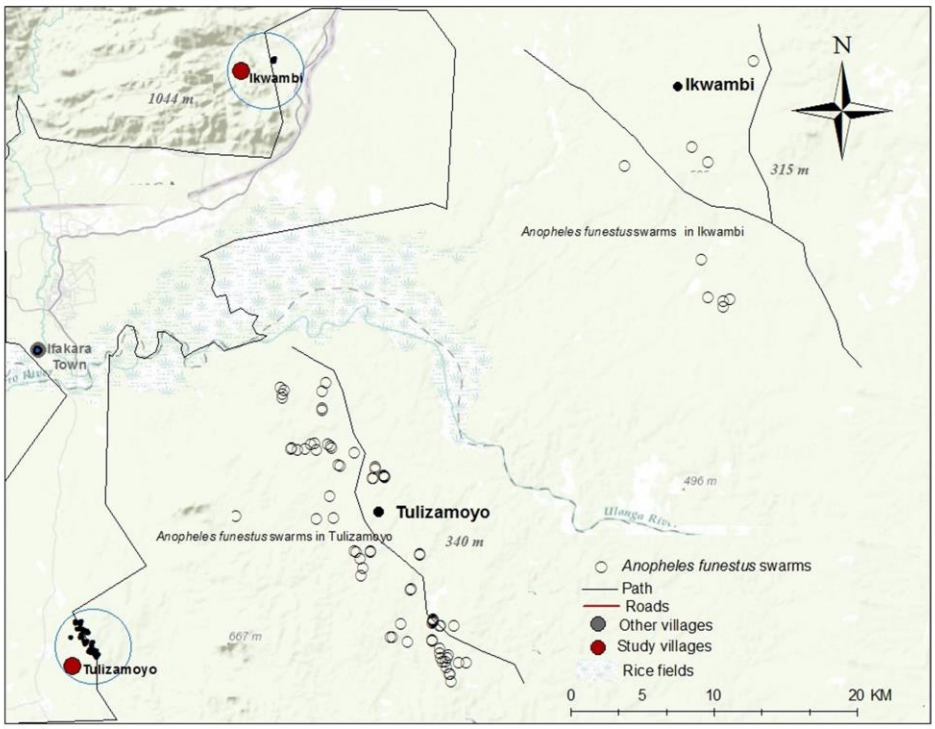


Fig. 5 Distribution of *Anopheles funestus* swarms observed in study sites

funestus swarms differ from those of *An. arabiensis* in terms of height, swarm size and location. For example, while *An. arabiensis* males were observed swarming at mean heights of 2.5 m, swarms of *An. funestus* occurred at much lower heights averaging just 1.7 m above ground, with several swarms lower than 1 m. In terms of location and markers, *An. funestus* preferred to swarm very close to human houses, unlike *An. arabiensis* swarms, which were mostly at the edge of the villages [34], possibly due to the greater anthropophily of the former than the latter species. The *An. funestus* swarms were small and generally consisted of less than 15 mosquitoes, as collected by sweep nets, while *An. arabiensis* swarm sizes ranged from approximately 10 to 60 mosquitoes [34]. It is possible that these differences in vector densities may be associated with season (this *An. funestus* study was conducted between June and July 2018 while the *An. arabiensis* study was conducted between August 2016 and June 2017). Nonetheless, the natural differences in population sizes as previously observed in the valley [12].

While the work with community volunteers certainly increased the ability of research team to identify the *An. funestus* swarms, visual estimates of the swarm sizes by the volunteers did not strongly correlate with the sweep net estimates (R = 0.4518). Though the volunteers were able to locate *An. funestus* swarms, either they could not accurately estimate the number of mosquitoes in the swarm, or the flying males were able to avoid the sweep nets. In the previous study however, there was a strong correlation between sweep nets estimates and visual estimates of the number of *An. arabiensis* in the swarm, indicating that visual estimates could possibly be relied upon to estimate swarm sizes [34]. Given that this current study was not done across multiple seasons, the discordance between estimates may indicate density dependence of such relationships, or that the correlations are non-linear (Fig. 3). Besides, one limitation of the approach is that the approximation of the peak swarming time and the collections using sweep nets may have been imprecise, even though the standardization allowed comparison across all the swarming events.

The molecular analysis confirmed that 100% of all amplified samples of *An. funestus* s.l. mosquitoes were *An. funestus* sensu stricto (s.s.). Although *Anopheles rivulorum* and *Anopheles leesoni* have also been recorded from this area [12], the present study did not find any other sibling species in the swarms apart from *An. funestus* s.s. However, in a recent study in Zambia, a mixed swarm of *An. funestus* and *An. leesoni* was found [20]. Though rare, *An. arabiensis* and *An. funestus* mosquitoes may also swarm either in very close proximity or together. Indeed, in the previous study of *An. arabiensis* swarms, one instance of 13 male *An. funestus* mosquitoes

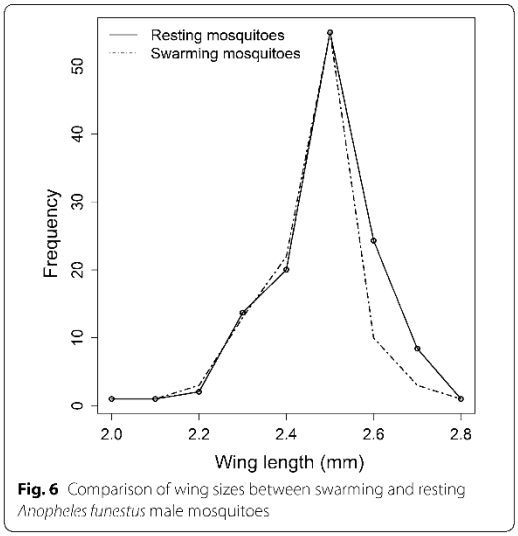


Fig. 6 Comparison of wing sizes between swarming and resting *Anopheles funestus* male mosquitoes

was observed in a sweep net targeting the former species [34]. Also, in this current work, four instances of 2, 2, 3 and 7 *An. arabiensis* males were caught in a sweep net targeting *An. funestus*. Previously, mixed swarms of *Anopheles coluzzii*, *An. gambiae* and *An. funestus* have also been reported from other areas in Africa [20, 42].

Genitalia rotation is a physiological change that occurs when male mosquitoes become sexually mature [20]. Dahan and Koekemoer indicated that these are visible a few hours after emergence, but the rotation rate can increase with the increase in temperature [38]. During this process, the genitalia turn clockwise or ant-clockwise until sexual maturity is reached at 180 degrees full rotation. This current study showed that 100% of all sampled males had complete genitalia rotation, suggesting that only sexually mature males participate in the swarming activity.

It was also observed that there was no special selection based on size of mosquitoes entering the swarms. The wing lengths of *An. funestus* males ranged between 2.0 and 2.8 mm, with no statistically significant differences observed in mean sizes between the mosquitoes caught in the swarms and those caught resting indoors. The results are comparable with those of Charlwood et al. [31] on *An. funestus* in Mozambique.

The elusive nature of *Anopheles* swarms in East Africa is confirmed the paucity or complete lack of reports on such swarms by previous entomologists working in the region. However, this study adopted the approach of working with trained community members to search for swarms as previously described by Kaindoa et al. [34],

and also in Burkina Faso [27]. Similar approaches have also been used by community members to accurately identify places with low, medium and high mosquito densities [43]. This has implications for vector control strategies using community participation in targeting mosquito swarms. Indeed, there are several examples where community participation in vector control programs have been successfully relied upon for disease control [44–46]. Even though aerosol spraying could be used to target swarms, additional surveys are needed given that *An. funestus* swarms occur very close to human houses and are generally smaller than *An. arabiensis* swarms. Moreover, additional safety precautions would be required to protect humans. Alternatively, improved technologies such as small robotic drones could potentially be used to target the identified swarms of *An. funestus* mosquitoes, and apply small but targeted insecticide doses.

The challenge of identifying *An. funestus* swarms in the study area was associated with the unexpected low height of the swarms, given that this study had used previous knowledge of *An. arabiensis* swarms when searching for *An. funestus* swarms. *Anopheles funestus* swarms occurred in close proximity to the houses, places that could not be predicted or associated with mosquito swarming. Additional research and exploration of technologies such as the use of unmanned aerial vehicles fitted with high-resolution infrared cameras could help to locate swarms in areas that are inaccessible by humans. Moreover, though the characterization here was restricted to one season, future studies should consider assessments across multiple seasons to assess whether climatic factors may have an influence on the characteristics of these *An. funestus* swarms. There is also a need to develop methods for prediction and estimation of *An. funestus* swarms, which could help to improve the control of malaria in rural areas.

Conclusion

This study has demonstrated for the first time the occurrence of *An. funestus* swarms in south-eastern Tanzania. Based on available evidence, the study team believe that this is also the first report on swarms of *An. funestus* in the country. More intensive studies should be conducted so as to map and characterize *An. funestus* swarms and assess the factors which influence swarming and mating behaviours of this species as well as how these swarms could best be targeted for control. Approaches aimed at *An. funestus* swarms could be one of the complementary tools used alongside existing interventions, such as long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) to control malaria transmission, to target the mosquitoes while outdoors.

Abbreviations

ACT: artemisinin-based combination therapy; GLM: generalized linear model; IRS: indoor residual spraying; LLINs: long-lasting insecticide-treated nets; PCR: polymerase chain reaction.

Authors' contributions

EWK and FOO conceived the study and developed study protocols. EWK, HSN, ALJ, EH, SA, JK and MT supervised data collection. EWK, HSN, AJL, SA conducted data analysis, EWK, HSN, AJL, EH, SA, AM, RM, GM, HB, MC, and FOO wrote the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The dataset generated by this study is available from the corresponding author upon request.

Ethics approval and consent to participate

Meetings with local leaders were held in the study areas and the main aims of the study were explained by the research team. A written and signed informed consent was obtained from volunteers who participated in swarm searching. All information was given in Kiswahili, the local language. Ethical approval for the study was obtained from Ifakara Health Institute Institutional Review Board (IHI/IRB/No: 038-2016), and from the Medical Research Coordinating Committee (MRCC) at the National Institutes of Medical Research (NIMR) (Ref: NIMR/HQ/R.8a/Vol.IX/2428). Approval was also obtained from the Human Research Ethics Committee (Medical) at University of the Witwatersrand (Approval Certificate No: M160806). Permission to publish this manuscript was obtained from the National Institutes of Medical Research (NIMR) (Ref: NIMR/HQ/P.12 8VOL.XXVI/25).

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PROJECT TITLE:	Assessing Relationship between Human Settlement Patterns and Malaria Risk in a Residual Transmission Setting in South-Eastern Tanzania
DATE CONSIDERED:	26/08/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Fredros O. Okumu and Prof Maureen Coetzee
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	05/12/2016

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in August and will therefore be due in the month of August each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

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IHI/IRB/No: 029 - 2016

INSTITUTIONAL CLEARANCE CERTIFICATE FOR CONDUCTING HEALTH RESEARCH

On 2nd September, 2016, the Ifakara Health Institute Review Board (IHI-IRB) reviewed from study titled: ***"Assessing relationships between human settlement patterns and malaria risk in a residual transmission setting in south-eastern Tanzania"*** **submitted by P.I Emmanuel W. Kaindoa.**

The following documents were reviewed:

1. Protocol
2. Informed Consent Forms English and Kiswahili
3. Budget
4. Data collection tools in English and Kiswahili
5. CVs

The study has been approved for implementation after IRB consensus. This certificate thus indicates that; the above- mentioned study has been granted an Institutional Ethics Clearance to conduct the above named study in (Ifakara Health Institute, and in Communities in Ulanga and Kilombero Districts) Tanzania.

The Principal Investigator of the study must ensure that, the following conditions are fulfilled during or after the implementation of the study:

1. PI should submit a six month progress report and the final report at the end of the project
2. Any amendment, which will be done after the approval of the protocol, must be communicated as soon as possible to the IRB for another approval
3. All research must stop after the project expiration date, unless there is prior information and justification to the IRB.
4. There should be plans to give feedback to the community on the findings
5. Any publication needs to pass through the IRB
6. The approval is valid until 1st September, 2017

The IRB reserves the right to undertake field inspections to check on the protocol compliance

Deputy Chairperson
Dr Saidi Mpendu

Acting IRB Secretary
Bakar Fakih

Appendix 8: Household observation sheet

House characterization form

Responsible Scientist: Emmanuel Kaindoa

Other investigators: Halfan Ngowo, Arnold Mmbando, Nancy Matowo, Stephen Mwangungulu, Salum Mapua, Gustav Mkandawile and Fredros Okumu.

House characteristics data recording form

Date	
Cluster number	
1. Name of household head	
2. Wall material (mud or bricks or any other materials)	
3. Roof material (iron sheets or grass or any other material)	
4. Eaves (Open, blocked, partially blocked, or screened)	
5. Size of the eaves in cm	
6. Window (closed, partially closed, open, screened, unscreened)	
7. Average size of window (cm)	
8. Doors (Tightly fitted, open, partially closed)	
9. Why do you keep your door open?	
10. Cattle around the house (within 50m)	
11. Pigs around the house (within 50m)	
12. Goats around the house (within 50m)	
13. Distance from cowshed (measured within 30m)	
14. Distance from the nearest water bodies (m)	
15. Distance from the toilet (in m)	
16. Total number of people in the house	
17. Total number of people in a trapping room	
18. Total number of adults in a trapping room	
19. Total number of children in a trapping room	
20. Mean humidity (%)	
21. Maximum humidity	
22. Minimum humidity	

23. Mean temperature (^{0}c)	
24. Maximum temperature (^{0}c)	
25. Minimum temp (^{0}c)	
26. Latitude	
27. Longitude	
28. Data logger code	
29. Total bednets	
30. Bednet Brand (Olysetnet, Safinet, permanet or unlabeled)	
31. Total no of treated nets	
32. Total number of untreated nets	
33. Number of intact nets	
34. Number of damaged nets	
35. Number of windows on a sleeping room	
36. Number of windows on the whole house	
37. Number of doors on the house	
38. Presence of chicken in the house	
39. At what time do you sleep?	

By_____

Signature _____

Appendix 9: Questionnaire

Identifying, characterising and mapping malaria vector swarms in rural Tanzania

Responsible scientist: Emmanuel Kaindoa

1. Researcher information

No.	Variable	Response
1	Name of researcher:	
2	Date	
3	Consent obtained?	○ Yes ○ No
4	Volunteers Name:	
5	Village:	
6	Kitongoji:	
7	Name of owner of the compound:	
8	Swarm code (Swarm Number)	
	SWARMS questions follow next	
9	Location of the swarm Latitude:	
	Longitude:	
10	Approximate number of mosquitoes in swarm (estimated visually)	
11	Approximate number of mosquitoes in swarm (estimated using standardized sweep nets)	
12	Approximate number of mosquitoes in swarm (estimated using light camera)	
13	Total males (*An. gambiae*)	
14	Total females (*An. gambiae*)	
15	Number of copulation events observed in the swarm	
16	Proportion of females caught that have evidence of having been inseminated	
17	Proportion of males caught that have evidence of having mated (determined by observing the inversion of male genitalia)	
18	The parity status of female mosquitoes collected in the swarms	
19	Other mosquito species and insects occurring in the same swarm	a) b)

		c) d)
20	Time of day when the swarm begins	
21	Time of day when the swarms completely disperse (end)	
22	How long did the swarm take place?	
23	Height of the swarm from the ground (m)	
24	Environmental features (landmarks)	○ Rice field ○ Bare land ○ Grassland ○ River ○ Football ground ○ Road ○ Cemetery ○ Bush ○ Banana ○ Farm ○ Public tap Other (mention)
25	Any other unique observations made at the swarm site or on the swarms.	

Signed by : _____

Date : _____

Appendix 10: Plagiarism report

a0051271:Kaindoa-PhD_Thesis-revised-25092019_(1).pdf

ORIGINALITY REPORT

15%	9%	9%	10%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	malariajournal.biomedcentral.com Internet Source	5%
2	Submitted to University of Liverpool Student Paper	5%
3	wellcomeopenresearch.org Internet Source	1%
4	data.ihi.or.tz Internet Source	<1%
5	link.springer.com Internet Source	<1%
6	Submitted to University of Salford Student Paper	<1%
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